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FUTURE CHALLENGES IN THE POULTRY INDUSTRY: BIOLOGICAL AND NUTRITIONAL RESPONSE LIMITS.

M. SIFRI[1]

Summary

Future challenges in the poultry industry will be caused by numerous factors. Such factors may be biological, social, political, economic, environmental, health and others or a combination of some or all of them. The main focus in this article is the biological challenges in the poultry industry. These challenges are symbolized by exploring the area of limits to theoretical maximum responses. The impetus to deal with this subject is the continuous influx of technical, marketing and sales push and promotions for unlimited products to deliver a perceived economic improvement by the poultry industry. A straight and simple mathematical calculation for the projected, perceived and accumulated benefits and improvements may lead a person to draw the erroneous conclusion that the return on any investment is "magical" and unlimited. The reality is that these issues are so complex that they defy the ability of modeling experts to resolve. In order to have a better appreciation of the complexity of response limits, the subsequent write-up is presented as a simplified outline in order for the readers to design their own customized evaluation with better knowledge to lead them to a more realistic and quantitative answer. Consequently, a few areas are selected that are of pertinence to make the point for limited responses. The list includes genetics, immunity, additional amino acid fortification, increasing fiber content and embryo nutrition. It is evident that limitations of responses to genetics, immunity and nutrition exist with different magnitudes. Our challenge is to maximize the response in a balanced approach in order to truly quantify the value of any effector quantitatively and determine its total and comprehensive impact on the enterprise.

I. LIMITATIONS TO GENETIC POTENTIAL

During the 2011 PSA Informal Nutrition Symposium, Leeson (2012) reported individual broiler growth rate of 95.23 grams/ day at 42 days of age and individual white egg layer production of 345 eggs in 365 days. Such results have not been achieved under commercial conditions. It is clear that the limitations are within the commercial setting. The answer might be related to the following list of variables:

1. Ingredient matrix
2. Nutrient matrix
3. Environment (temperature, humidity, pressure, altitude)
4. Health
5. Light
6. Feed Texture (meal, crumbles, pellets)
7. Particle size of ingredients
8. Presence of modifiers such as medications, prebiotics, probiotics, exogenous enzymes, herbs, extracts, vaccines, toxicants and others
9. Life cycle stage (age)
10. Feeding system and structure
11. Animal density (space, floor, cage, colony, volume)

[1] ADM Alliance Nutrition, USA

12. Ingredient availability and cost
13. Feed intake pattern (Frequency, feed level, feeder space, color and other stimuli)
14. Feed management and waste
15. Appetite
16. Animal physiological status
17. Species (Type and stage: chicken broiler, chicken layer, turkey, duck, geese, breeders)
18. Interaction(s)

II. LIMITATIONS TO IMMUNITY POTENTIAL

Theoretically, maximum immunity responses can be achieved; however, there are many compromises and complications that should be dealt with. Selvaraj (2012) addressed this issue during the 2011 PSA Informal Nutrition Symposium. For the immunity system to respond, certain nutrients or components such as carbohydrates, amino acids, fatty acids and vitamins are required. With that in mind, it is essential that a balanced approach is used in order to attain the desirable response with a measure of acceptability for certain limitations. Otherwise the results could be disastrous. Supplying a specific nutrient or certain component might put the immunity system in overdrive where other critical nutrients are depleted and rendered unavailable for other essential metabolic functions where performance can be compromised. Some nutrients might have biphasic effects where at low doses the immunity response is desirable; however, at higher doses, they might illicit a contradictory impact. Examples of those could include lutein, arginine and tryptophan. Other complicated issues could involve an improper replacement of a nutrient with another nutrient that can lead to unexplained responses. Such nutrients could include certain fatty acids, amino acids, minerals or vitamins. Depending on the level of fortification and the physiological condition of the animal, it is necessary to understand the potential benefit or damage that can be caused by certain interactions of specific nutrients.

III. LIMITATIONS TO NUTRITIONAL POTENTIAL

The scientific literature of nutritional limitations is vast. In order to make the points in a simplified manner, three pertinent and new aspects are addressed in this section. These will include some of the limitations that can be caused by increasing dietary amino acid and fiber levels. The third area of interest will focus on the challenges of providing nutrients to the chicken embryo during incubation.

1. Vieira and Angel (20l2) addressed the amino acid issue in broilers during the 2011 PSA Informal Nutrition Symposium. In spite of the fact that general growth can be affected by many of the variables listed in the preceding sections, the impact of increasing some of the amino acids such as lysine and/ or other amino acids has limitations whereby there is a plateau when only live weight is considered. However; different plateaus of responses can be attained if feed efficiency or component yield are the parameters to measure. Their findings demonstrated that the impact of response to amino acid levels will vary with the age of the animal and the specific components such as breast meat yield.
2. Mateos et.al (2012) addressed the fiber issue in extensive details in poultry during the 2011 PSA Informal Nutrition Symposium. It is fascinating to realize the impact and the limitations of fiber on performance, health and metabolism in poultry. Their findings demonstrated that response potential and limitations are seriously affected by the fiber source. Poultry diets that are low in fiber are excellent candidates to show the

beneficial effects of including low dietary fiber levels (1-3%) on the development of the different organs of the GIT, nutrient digestibility, gut health, and productive performance. The impact of additional fiber is also dependent on the solubility and other physico-chemical properties of the fiber source.

3. Uni et.al (2012) addressed the embryo nutrition limitations in poultry during the 2011 PSA Informal Nutrition Symposium. They explored in an elegant way the nutrient sources for the embryo that are part of the egg components; egg shell, egg white and egg yolk. They concluded that the yolk sac membrane plays a critical role that might be similar to how the intestinal cells express their impact on carbohydrate, peptide and mineral digestion and absorption in the chicken.

Understanding the opportunities and limitations in such metabolic processes might lead to unlocking the secrets of how the biological system can reach to its potential under different stages of life and different conditions.

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CROSSROADS FOR GROWTH: CHANGING COMMODITY MARKETS URGE POULTRY INDUSTRY TO CHANGE

N-D. MULDER[1]

Challenging global fundamentals in food and fuel demand and supply will urge the global poultry industry to change. One of the major challenges is a change in input costs with higher and more volatile grain and oilseed prices. As these costs represent 50 percent to 70 percent of total production costs, the impact on the industry will be huge. Poultry players will need to adjust their business models to the new situation. In the wake of the changing input costs, global supply and demand of poultry meat will also change. The importance of production in countries such as the Americas and probably the Black Sea region, will increase as the world's major growth areas in Asia face increasing difficulties in meeting supply and demand and therefore turn to imports. This will lead to stronger linkages between Asian countries and the Americas with investments in both directions. Differences between winners and losers in the industry will increase, where winners will be the ones who have adequately implemented the new realities in their business models.

I. CHANGING FUNDAMENTALS AS DRIVING FORCE FOR CHANGE

The world food industry will face big challenges in the years ahead. FAO expects world food demand to grow by 70 percent until 2050; world population will grow from the current 7 billion to 8 billion in 2030 and to 9 billion by 2050. Income levels are expected to increase, which will stimulate meat consumption, especially in lower income countries. The growing demand for food (and especially meat) will have a huge impact on the supply chain. Competition for land, water and energy will intensify as resources are limited. Global per capita land availability has declined in the past decades, and areas which face growth in food demand in Asia have already a very high percentage of arable land in cultivation. Expansion of big cities in emerging countries will also compete with agriculture. Water availability is expected to increasingly become a competitive advantage, and again Asia has disadvantages compared to more competitive production areas in the Americas and the Black Sea region.

In such a changing global food scenario it is very likely that the focus of the industry will have to change. The industry is dealing with a situation in which resources are limited and, although there is still room to expand land reservoirs for agricultural supply - especially in Brazil, Russia and Africa, but also in the EU and the United States (US) because of set-a-side programmes - the ease of expansion will be more difficult and slower. Therefore, the emphasis has to change to further focus on output with higher yields, better efficiency and more focused product development to adjust to changed consumer demand. The global agriculture industry has a great challenge here as better efficiency and yields can only be achieved by better farm management - both for are-able and animal production - by using better genetics, better feed (fertilisers or animal nutrition, equipment (including housing) and by using better disease protection (agricultural chemicals and animal health). Better agronomics and farm management would play a key role in optimising inputs for the best performance in the given circumstances in each region. The entire process of improving all inputs, preferably in an integrated way, will help to reach the challenging target for 2050.

[1] Associate Director Animal Protein, Rabobank International

II. ROAD TO NEW BALANCE WILL BE A BUMPY ONE: HIGH AND VOLATILE FEED PRICES

Experiences from the past have shown that the process of adjusting to the new realities is not an easy road. The increase in feed prices since 2006 can be attributed to the challenge of meeting a growing demand for food, feed and fuel, with limited resources. Limited resources are illustrated by historically low stocks-to-use ratios in grains, a shift in world trade with a growing importance of the volatile Black Sea region as well as a move in Asian markets, especially net imports of corn in China. Any change in supply and demand in these markets has a huge impact on market prices and also attracts speculators. This has resulted in a trend of increasing and more volatile prices in international grain and oilseed markets.

III. GROWING MEAT DEMAND AS A MAIN DRIVER FOR CHANGE

A growing meat demand, together with a growing demand for biofuel and other food products, is one of the main driving forces behind the increase in demand for grains and oilseeds. Global meat demand is expected to grow 45 percent in the next twenty years, while poultry demand will grow 60 percent as the world population expands and average incomes increase. The poultry industry will benefit from competitive costs of production as well as its health and convenience image. Unlike pork and beef, poultry has no cultural consumption limitations and the efficiency of poultry production ensures a relatively good sustainability footprint compared to other meat proteins.

It will be a big challenge for the global poultry industry to keep up with the 30 percent global demand growth over the next decade; growth which is not evenly spread around the globe. Countries in Asia and large parts of Africa, with no natural competitiveness in poultry production, need to reconsider their supply strategies. It is clear that most of the supply will continue to be produced in local areas as fresh product demand remains strong. However, scarcity in global markets will increase the importance of international trade.

In the slipstream of growing meat trade, companies in developed markets and in exporting countries will come under strong pressure to benefit from global growth. US companies are facing a more challenging local production and trade environment and are also facing pressure from shareholders to internationalise their business model. The success of the Brazilian model is going to be a base for further internationalisation of the poultry industry and companies will further move (with the support of national investment funds) to multinational structures in which the three directions of internationalisation - access to low-cost production, synergy in distribution and access to local market growth - will be all exploited.

IV. GLOBAL CHALLENGES, REGIONAL DIFFERENCES

Not all poultry companies will make the move to internationalise; companies in many regions still have plenty of opportunities in their local markets as demand might grow, modern distribution is still in an early stage of development, the level of fragmentation is low and/or export potential is still not being utilised.

This is why poultry companies in the EU will do well to first utilise the great opportunities in the internal EU market by better integrating the local industry and moving to a regional, and also later, pan-European business model. Companies in Eastern Europe still have plenty of growth opportunities in their domestic markets, although the growth potential in Russia might slow down somewhat after 2015 when Russia will be fully self-sufficient in poultry. Ukrainian companies still have significant local growth potential, and there is potential for additional growth as the industry may become an exporter of poultry products in the medium to long term. A probable opening of the EU market in medium term might present local industries with a great growth opportunity here.

The position of Asian poultry companies will change in the medium term as efforts to increase local market growth with limited resources will raise awareness about the importance of food supply security. It can be expected that more Asian countries, such as China, will follow the Japanese model regarding import security via local joint ventures. China's import position will be forced to change due to its limited availability of resources for grains and oilseeds. And although most of the poultry supply

will continue to be produced in China, import companies will start to acquire companies or set up joint ventures with exporting countries in Asia and Latin America to secure supply. This might force other importers such as Middle East countries and the EU to react and follow a similar strategy.

The strategic moves in the global poultry landscape, which will occur over the next decade, will be beneficial for companies in exporting countries and will stimulate consolidation among companies in these countries. It will also be a great base for newcomers in the global poultry landscape. Countries like Ukraine and Argentina have great long-term growth potential as by responding to the need of importers they will become less dependent on exports to only a few companies in a small number of countries. Nevertheless, Brazil will remain the dominant player in the export poultry meat market, especially for whole birds, white meat and bulk processed meat. Thailand and China will remain dominant suppliers of labour-intensive poultry products while the US and the EU remain more 'access' supply exporters. However, cost competitiveness in raw meat might give the US opportunities to develop a more value-added strategy.

Internationalising companies will shift their focus to the Asian region where growth in local markets and the early stages of the development of a modern distribution system will present great growth potential. China, India and South East Asia will be relatively 'hot' for foreign investors and may also offer trade synergies with existing business.

V. GLOBAL BUSINESS MODELS NEED TO CHANGE

The global poultry industry needs to prepare for a period with a much more volatile business environment, especially from an input (feed costs) and exchange-rate perspective. Companies who are not adequately positioned in the market and lack value-chain efficiency and flexibility encounter difficulty in times of volatility.

Sustainability is going to be a much more important topic in the new market environment of the next decade. Growth in poultry demand with limited resources will require more emphasis on existing resources throughout the value chain. In such a situation, a good corporate social responsibility policy should be a key factor among industry players in the poultry industry. NGOs and clients will be more concerned than before about the sustainability of poultry production. Companies need to be more proactive in this sort of issue. At the same time, animal welfare will become more important, especially for companies operating in developed markets where suppliers also need to be active in the debate, while retailers and QSRs might offer opportunities to set up good farming product chains.

The global poultry industry has much going for it here and its efficiency has led to a more sustainable production system compared to other animal proteins. Proactive approaches, marketing of standards and joint approaches between clients and industries will become more important.

The opportunities for the global poultry industry are considerable, with demand growth in all regions of the world, increased international trade, growth in modern distribution and a good competitive position for the poultry industry compared to other proteins. Industry players are well positioned to benefit from these challenges but they need take the right strategic direction at the current crossroads. If they take the right direction and shape their business models to be ready to deal with the challenges as well as the much greater turbulence in the global market environment, they should be well positioned to become winners in the next decade.

GASTROINTESTINAL TRACT DEVELOPMENT: IMPLICATIONS FOR FREE-RANGE AND CONVENTIONAL PRODUCTION

B. SVIHUS[1]

Summary

By allowing birds access to an outdoor area in free-range poultry production, they will be stimulated to consume forage such as fibrous plant materials and grit stones. This will facilitate gizzard development, potentially resulting in better nutrient utilisation and a bird more resistant to enteric diseases. The fibrous components and the fine grinding due to an active gizzard will facilitate caecal development and result in increased fermentation, which could have a beneficial effect on energy and protein utilisation. It is also possible that access to an outdoor area will increase retention time in the crop, and thus potentially improve efficacy of the digestion process.

I. INTRODUCTION

Modern poultry production encompasses a severe, as in caged layer hens, or a mild, as in broiler chickens, restriction of movement of the birds. This has been met with criticism by some consumer groups on an ethical basis, and thus alternative production systems have been developed. Most of the alternative systems do not allow the birds to range freely as the word "free-range" implies, but rather is a description of a production system where there is significantly more space available, and which usually includes an outdoor area. In this context, free-range can be defined as a system where there is very little restriction of movement. It is, in this context, interesting to note that many birds to not take the opportunity to use an outside range when given the opportunity (Dawkins et al., 2003). This is particularly true for broiler chickens, where Weeks et al. (1994) found that these birds made very limited use of the outdoor area.

Free-range production would mainly have an effect on gastrointestinal tract development due to ingestion of material present in the extended environment of the free-range bird. The wild ancestor to commercial chickens, the red jungle fowl, has been shown to spend a very large amount of its time on foraging behaviour, with pecking behaviour observed 60 % of the time and scratching behaviour observed 35 % of the time, despite the fact that the birds were fed (Dawkins, 1989). In the wild, the omnivorous class of birds to which the chicken and the turkey belong, will choose among nutritious foods such as other animals, seeds and fruits. More fibrous foods such as young leaves and shoots are also eaten, but the limitations in absolute size of the digestive tract of flying birds prohibits developments to allow for a very efficient digestion of roughages.

Kirk Klasing has published an excellent review on feeding strategies of chickens and turkeys (Klasing, 2005). Although a quantitative analysis of diets for red jungle fowl surprisingly enough has not been undertaken, studies of crop contents have revealed that the diet of red jungle fowl include fruits and berries from trees and herbaceous shrubs, seeds (especially from bamboo), nuts, young shoots of bamboo and other grasses, leaves, petals, tubers, termites and ants (and their eggs and pupae), earthworms, roaches, grasshoppers, spiders, moths (and their caterpillars), beetles, small crabs, snails, centipedes and lizards (Klasing, 2005). Foods of plant origin are present in quantitatively greater amounts than those of animal origin. Although the structure of the digestive tract is very similar between

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Gallus gallus and *Meleagris gallopavo*, analysis of feeding behaviour shows that the wild turkey feeds on a much larger proportion of vegetable feed materials than the red jungle fowl. The diet composition of turkeys will obviously vary greatly with season and habitat, but the proportion of vegetable ingredients will never be below 60 % and can be as high as 95 %. Although the turkey is feeding mainly on plant material, it is certainly an omnivorous bird. Klasing (2005) reports on an experiment where 524 wild turkey crops and gizzards were analysed. More than 350 plant species and 300 invertebrate species were identified. Turkeys are reported to feed heavily on food from trees, such as acorn and beechnuts. They are also reported to feed on vegetative parts of grass, where they bite the long leaf off and swallow it without further mastication.

In addition to foods, wild birds are known to ingest large quantities of small stones from the environment to aid grinding in the gizzard. In a survey of 1440 gizzards from 90 American bird species, grit stones were found in the gizzard of 69 % of the species, with highest prevalence for granivorous birds (Gionfriddo and Best, 1996). For example, all the 37 gizzards from ring-necked pheasants contained grit stones, with median number of stones being as high as 88 and average diameter being 2.3 mm. Similarly, Norris et al. (1975) found the gizzard of wild ptarmigans to contain an average of more than 100 gizzard stones during the fall.

II. CHARACTERISTICS OF FREE-RANGE FORAGING

The ultimate example of free-range is birds that roam unrestricted, as is often the case in many developing countries. In African villages, for example, such free-range production has been estimated to produce over 70 % of the poultry products and 20 % of the animal protein eaten (Aboe et al., 2006a). These birds are usually being fed surplus feeds such as maize and cereal by-products (Aboe et al., 2006b), but the quantitative contribution of surplus feed compared to feed consumed due to scavenging is uncertain. It is reasonable to assume that a large quantity of the feed consumed comes from scavenging. Mwalusanya et al. (2002) reported that birds were only fed occasionally, and that the majority of the feed was found in the surroundings of the village. Analysis of crop contents taken at sunset revealed that between 40 and 75 % of the diet, depending on season, consisted of green forages, insects/worms and kitchen waste, with green forages being the quantitatively most important (Mwalusanya et al., 2002). The average proportion of green forages and insects/worms in the contents of the crop were 29 and 12 %, respectively. Gunaratne et al. (1993) found household refuse to be the quantitatively most important component of the diet of scavenging chickens in four Sri Lankan villages. These data show that scavenging birds can obtain a large quantity of their diet from the environment, and that composition of the diet under such conditions is not very different from the diet of the red jungle fowl, with the difference that household waste is a significant contributor to the diet of village hens.

In a more restricted version of the free-range production where the outdoor area is fenced, such as systems commonly used in organic poultry production in Europe, the limitations on roaming allows for less feed to be procured from the environment. Usually, birds reared in such systems are fed complete diets *ad libitum*, and thus will not have any large nutritional need for scavenging. However, it is well known that, despite this, birds will consume large quantities of foraging material in the environment when present. Wood (1956) assessed crop contents and found that 5 % of the dry matter in cockerels and pullets fed a mixture of grain and pellets came from the Kentucky bluegrass on which they were allowed to forage. When being allowed to select among several pastures, birds consumed up to 9 g forage material per day. Although a large variation in forage consumption was observed (the crop from one bird contained 48 % forage), only one out of 72 birds had eaten no forage.

Wood et al. (1963) found similar variations in forage intake, and noted that clover was consumed in larger quantities than grasses, particularly late in the season. It was also noted that the chickens consumed significant amounts of dead grass. More recently, Horsted et al. (2007) investigated foraging activity in hens which had access to approximately 10 m^2 of grass and forb-covered ground per hen and which were fed either a pelleted layer diet or whole wheat *ad libitum*. The average amount of plant material found in the crops in the evening was between 2 and 10 g, with significantly higher amounts for wheat-fed birds than for layer diet fed birds. The amount of invertebrates found was small and usually less than 1 g. Grit stones were found in the crops, with significantly larger amounts for hens fed whole wheat than for hens fed the layer diet. Steenfeldt et al. (2007) gave layer hens access to roughage, and also found that they consumed large amounts of roughage under such conditions. As already mentioned, broiler chickens have been reported to use free-range outdoor area to a lesser extent than layer hens. Despite this, Ponte et al. (2008a,b) found that 5 to 6 % of the crop dry matter consisted of forage, thus indicating a significant free-range area use.

It is clear that free-range production will affect dietary composition and thus potentially digestive tract development if the free-range environment contains forage material or grit stones. To what extent this occurs, depends on the size of the outdoor area, type of soil, climate and season, but usually some foraging material will be available, not the least plant materials, soil and small stones. Thus, free-range production may affect digestive tract macrostructure and development mainly through ingestion of large, fibrous and hard materials. The increased need for grinding activity will affect gizzard size and functionality (Svihus, 2011), and the increased fibre content of the diet will affect caeca size and functionality (Clench and Mathias, 1995). In addition, it is possible that limited access to free-range will affect the use of the crop as an intermediate storage organ for foods procured in the free-range environment.

III. GIZZARD DEVELOPMENT

The digestive tract is a dynamic organ which rapidly responds to changes in the diet. This is particularly true for the gizzard, where the structure of the diet will have a large impact on gizzard size. This issue have been extensively reviewed before (Svihus, 2011), and will only briefly be outlined herein.

Structure can be defined as the size and internal binding strength of feed particles, more specifically particle size and internal binding strength of the particles remaining after the feed has been dissolved following consumption, i.e. the microstructure of the diet. A rapid and conspicuous enlargement in size of the gizzard is observed when structural components such as hulls, wood shavings or large cereal particles are included in the diet. This was nicely demonstrated by Starck (1999), where a large increase in the size of the gizzard with contents was observed after a high-fibre diet was given to quails for 14 days, and a similarly large decrease in size of the gizzard was observed after switching to a low-fibre diet for 14 days. The increase in size of the gizzard is a logical consequence of an increased need for particle size reduction, due to the stimulative effect of the increased grinding activity on size of the two pairs of gizzard muscles. Gizzard size may increase to over 100 % of its original size when structural components are added to the diet. It has also been shown that the volume of the gizzard increases substantially when diets with whole cereals or insoluble fibre are fed (Hetland et al., 2003; Bjerrum et al., 2005; Amerah et al., 2008). In fact, the magnitude of increase in weight of gizzard contents is usually much larger than the increase in size of the gizzard, with more than two-fold increases in weight of contents frequently observed. Thus,

structural components do not only increase the size of the gizzard, but also results in a large increase in holding capacity of the gizzard.

A number of studies have shown improved nutrient utilisation when birds are switched from diets lacking structure to diets with structural components such as coarsely ground cereals or coarse fibre material (an overview of published literature can be found in Svihus, 2011). It has been shown that particle size of material entering the small intestine is smaller for a diet containing large amounts of structural components than for the same diet containing a smaller amount of structural components (Hetland et al., 2002, 2003; Amerah et al., 2009). This apparently counterintuitive result may simply be due to the fact that a well-developed gizzard will result in an improvement in grinding capacity which will outweigh the increased need for grinding due to a larger number of coarse particles.

It has been shown repeatedly that, when structural components such as whole or coarsely ground cereals or fibre materials such as hulls or wood shavings are added, pH of the gizzard content decreases by a magnitude of between 0.2 and 1.2 units (see Svihus, 2011 for a complete reference list). One possible logical explanation for this is an increased gizzard volume and thus a longer retention time which allows for more hydrochloric acid secretion, combined with a stimulative effect of gizzard activity on acid secretion. In addition to the indirect potentially beneficial effects of a reduced pH due to less pathogenic microflora in the digestive tract, a reduced pH may also contribute to an improved gastric digestion. An increased amylase activity and bile acid concentration has also been observed, and may be part of the cause for improvements in nutritive value associated with structural components.

Both forage and grit stones are structural components which strongly stimulates gizzard development, as shown by Steenfeldt et al., (2007) for a foraging in the form of silage, and by Hetland et al. (2003) for grit stones. Thus, it is logical to assume that birds in a free-range system will benefit from a more developed gizzard which will potentially improve nutrient utilization and gut health.

IV. CAECA DEVELOPMENT

A characteristic of free-range poultry production is that significant amounts of fibre-rich materials are consumed. These carbohydrates cannot be digested by enzymes produced by the bird, but can to some extent be fermented in the caeca. The extent to which fibres can be fermented in the caeca and be used as an energy source for turkey and chickens is uncertain. Clench and Mathias (1995) placed chickens and turkeys among the birds with a long intestinal caeca of the most developed and functional type. The opening of the caeca contains numerous villi which act as a filter that prevents large particles from entering. Particles entering the caeca will be attacked by numerous microorganisms, and thus be digested to produce volatile fatty acids which can be absorbed and used as an energy source. As reported by Clench and Mathias (1995), it has been estimated that between 6 and 30 % of the energy requirement of the Willow grouse in the winter comes from caecal fermentation. The caeca have been shown to be highly adaptive, and the length of the caeca in the Willow ptarmigan thus increases 30 % during the winter season (Pulliainen and Tunkkari, 1983). Since it has been estimated that more than 90 % of the fibre entering the caeca is digested (Clench and Mathias, 1995), a sufficiently fine grinding appears to be the minimum factor for fibre digestion by the caeca.

To what extent there is a potential for a significant fibre digestion in modern chickens and turkeys is uncertain, but Steenfeldt et al. (2007) found fibre digestibility to vary from 24 to 29 % in layer hens fed large amounts of roughage, thus indicating that caecal fibre digestion can be significant in layer hens. The above-mentioned strong adaptive capacity of the caeca may also result in significant increases in fermentative capacity of layer hens when

kept for long periods in an environment with access to forage. Under such conditions, it is possible that fermentation may play a significant role in providing energy through volatile fatty acids. Jorgensen et al. (1996) fed broiler chickens varying levels of fibre of different types, and observed a 50 % increase in caeca weight within 20 days. It was estimated that between 3 and 4 % of the metabolisable energy of broiler chickens could come from caecal fermentation.

In addition, increased fermentation will also potentially result in a higher vitamin synthesis and thus reduce the risk of vitamin deficiency, particularly for B-vitamins and vitamin K. Lastly, stimulation of caecal fermentation may also contribute to a more effective nitrogen utilisation through re-synthesis of amino acids from uric acid in the caeca. As discussed by Karasawa (1999), nitrogenous compounds can be refluxed from the cloaca into the caeca, where they may be used for amino acid synthesis by the microflora, potentially followed by absorption through the caecal wall.

V. STIMULATION OF CROP USE

As shown in free-range village hens, the crop is used extensively as an intermediate storage organ for food under such conditions (Gunaratne et al., 1993; Mwalusanya et al., 2002; Mekonnen et al., 2010). An extensive crop use has also been demonstrated for free-range layer hens under European conditions with *ad libitum* access to a layer diet, where the crop contained between 20 and 30 g sun-dried material in the evening (Horsted et al., 2007). Since a majority of the crop contents consisted of layer diet, it is uncertain whether access to free-range has had any particular stimulatory effect on crop use. An extensive use of the crop, at least in the afternoon as a preparation for the dark period, has been reported for layers without access to free-range (Harlander-Matauschek and Bessei, 2005). In broiler chickens, *ad libitum* fed birds have been shown to not use the crop to any considerable extent (Boa-Amponsem et al., 1991; Svihus et al., 2010). Observations of commercial broilers on *ad libitum* feeding have shown that they eat in a semi-continuous way (Nielsen, 2004), and that the crop is not used to its maximal capacity under such conditions (Denbow, 1994). Although data on crop use by free-range broiler chickens appears sparse, significant amounts of forage (3 to 5 % of DM) in the crop has been observed for broiler chickens with access to pastures (Ponte et al., 2008a,b). It is possible that access to an outdoor area containing foraging materials results in an increased desire to consume food, and therefore stimulates crop use. Non-continuous access to an outdoor area will possibly have the same effect.

There may be some nutritional benefits to an increased use of the crop as an intermediate storage organ for food. Retention time in the crop will facilitate the first step which is a prerequisite for digestion, namely moistening of the feed. Water content of a diet exceeds 40 % after 30 minutes retention in the crop (Svihus et al., 2010). Retention time in the crop is also associated with a considerable fermentation activity dominated by lactic acid producing bacteria (Hilmi et al., 2007), but also production of considerable quantities of other short-chain fatty acids (Huang et al., 2006), which lowers pH. An overview of literature reporting pH in crop contents can be found in Svihus (2010), and shows that pH will decrease to between 4.5 and 5.5 after a prolonged crop retention. In poultry, passage through the digestive tract is rapid, and thus may represent a limiting factor for efficacy of exogenous enzymes. Since temperature, moisture content and pH in the crop appears optimal for many of the enzymes added to the feed, an increased retention time due to crop use by free-range birds may thus have a beneficial effect on nutrient availability. Svihus et al. (2010) showed that phytic acid content was halved after 100 minutes retention time in the crop when a phytase was added to the diet.

VI. CONCLUSION

The stimulation of feeding behaviour when birds have access to free-range will most likely affect digestive tract development, in particular the gizzard and the caeca. In addition, birds will possibly be stimulated to a more extensive use of the crop as an intermediate storage organ. These changes could result in improvements in nutrient utilisation.

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NUTRITIONAL IMMUNITY: POSSIBLE CHALLENGES IN FREE RANGE PRODUCTION

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I. INTRODUCTION

The response of the innate immune system to changes in the amount or frequency of pathogens has implications on how dietary nutrients are used for productivity. Given the diffuse nature of the immune system and inability to quantify nutrient requirements using traditional empirical approaches, nutritionists often attribute nutritional costs of immunity to increased maintenance costs. While this approach has helped to conceptually understand the impact of an immune response on nutrient utilization for growth, more clarity in this field will allow for further improvements in nutrient-driven approaches to influence immunity. By understanding altered nutrient metabolism during an immune response, we can then begin to use hypothesis-driven approaches to understand how to better provide optimum nutrient profiles for desired immune responses and performance. With the continued development and utilization of alternative production systems, such as free-range production systems, reassessment of some of these nutritional approaches will need to be considered, given the potential unique attributes of these management systems on immunity. The objective of this paper is to provide a basic understanding of nutritional immunology, with a special emphasis on how use of free range production systems may influence applicability of this approach to influence animal productivity and welfare.

II. ACTIVATION OF THE IMMUNE SYSTEM RESULTS IN ALTERED NUTRIENT USE FOR GROWTH

Microbial pathogens are typically utilised in experimental models used to examine altered nutrient metabolism during an immune response. The innate immune system provides the initial response to these pathogens and this initial response plays an important role in coordinating the appropriate immune response to the pathogen (cellular or humoral) as well as the degree of nutrient repartitioning within the host. Depending upon the severity of microbial challenge, the innate immune system elicits a systemic response to help coordinate the immune response to the pathogen. The systemic response is manifest through behavioral changes in the animal in addition to decreased nutrient use for growth; however these changes are transient and may last hours to days.

From an amino acid perspective, activation of an innate immune response results in skeletal muscle degradation and a negative nitrogen balance. Catabolism of skeletal muscle releases amino acids into the plasma and these substrates are available for use by tissues and cell types involved in host defense (Klasing and Austic, 1984a,b). Consequently, activation of the innate immune system results in the repartitioning of amino acids from growth toward immunity (Humphrey and Klasing, 2004). The shift in amino acid consumption from growth to immunity results in a shift in the amino acid composition of proteins being synthesized. These proteins differ in their amino acid composition and this change in amino acid profile may also be reflected in the diet. Reeds (Reeds et al., 1994) first hypothesized that the high rates of skeletal muscle catabolism during periods of infection are due to the high demand for specific amino acids whose proportions are particularly high in acute phase proteins. By

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comparing amino acid profiles of several acute phase proteins to skeletal muscle, aromatic amino acids appear to be in greatest demand due to their low proportion in skeletal muscle and high proportion in acute phase proteins (Reeds et al., 1994). Consequently, skeletal muscle is catabolized to supply amino acids for acute phase protein synthesis in an amount sufficient to supply aromatic amino acids, specifically phenylalanine, while the surfeit levels of other amino acids liberated from skeletal muscle are excreted and contribute to the negative nitrogen balance of the animal (Reeds et al., 1994). Thus, catabolism of skeletal muscle is an important metabolic alteration during infection since this process provides amino acid substrate for the liver to synthesize acute phase proteins. Rather than implementing nutritional support to prevent skeletal muscle degradation, efforts may be best served by providing the composition of amino acids that are ideal for the synthesis of protective factors, such as acute phase proteins. As an example, Faure et al. (Faure et al., 2007) have shown in rats that threonine utilization for synthesis of acute phase proteins and intestinal mucins is increased during infection.

III. NUTRIENTS OF IMPORTANCE TO THE IMMUNE SYSTEM

The immune system has specific energy and amino acid substrates that are important for fueling the proliferation of lymphocytes and the synthesis of protective factors associated with host defense.

Energy metabolism is of particular importance to lymphocytes since their development and activation require cell proliferation. Leukocytes primarily utilize glucose and glutamine as an energy source (Ardawi and Newsholme, 1985). Glucose is the fuel of choice for lymphocytes, and in mammals glucose is actually an essential nutrient for lymphocytes (Greiner et al., 1994). Glucose is also important for generating reducing equivalents through the pentose phosphate pathway. These reducing equivalents are essential for producing killing compounds involved in the macrophage respiratory burst (Newsholme et al., 1996). Second to glucose, glutamine is also a major energy substrate for leukocytes. Like glucose, glutamine metabolism can also generate reducing equivalents necessary for the production of reactive oxygen species (Newsholme, 2001). Glutamine conversion to glutamate may also aid in the transport of amino acids since glutamate is a substrate for many amino acid transport exchange systems (Aledo, 2004).

Arginine can be metabolized to produce nitric oxide involved in inflammatory responses and polyamines involved in wound healing. In mammals, arginine plays an integral role in the development of B lymphocytes (de Jonge et al., 2002) and also regulates the signaling ability of T lymphocytes (Rodriguez et al., 2002). Since arginine can be synthesized in ureotelic species, immune cells, particularly macrophages, are capable of synthesizing and recycling arginine for use in nitric oxide production. In uricotelic species and strict ureotelic carnivores that are incapable of arginine synthesis, this amino acid is not only essential, but cannot be recycled (Sung et al., 1991). Consequently, this amino acid is of particular importance in these species during periods of infection since increased endogenous synthesis of arginine cannot compensate for increased utilization of this amino acid by the immune system.

A considerable amount of research has been conducted in chickens examining the effect of arginine on the immune system (Kidd et al., 2001; Kwak et al., 1999; Takahashi et al., 1999), and the provision of this nutrient to the immune system appears most important for activation. For example, avian macrophage activation results in increased synthesis of nitric oxide. Since arginine is required for nitric oxide production, it is not surprising that these cells increased the expression of genes involved in arginine uptake and decrease the expression of genes involved in arginine export (Moulds and Humphrey, unpublished). The

net result is increased arginine intake to help supply the increased flux of this amino acid through the nitric oxide synthesis pathway.

Glutamine has long been recognized as an important metabolic fuel for the immune system (Ardawi et al., 1985). Glutamine is primarily metabolized to generate energy for leukocytes, as well as reducing equivalents for synthesizing reactive oxygen intermediates (Newsholme, 2001). Glutamine can also be metabolized to arginine in murine macrophages for NO synthesis (Murphy and Newsholme 1998). Though glutamine can be synthesized endogenously, differences in glutamine metabolism between uricotelic and ureotelic species may have implications on glutamine use by cells of the immune system between animals with these nitrogen excretion strategies.

Cysteine can be metabolized to produce glutathione (GSH). GSH is one of the major intracellular antioxidants and its production is regulated by the availability of cysteine. GSH production increases during periods of inflammation (Malmezat et al., 2000), and consequently a greater proportion of cysteine metabolism is directed toward GSH synthesis (Malmezat et al., 1998). GSH plays an important role in leukocyte function and these cells have a strong ability to obtain cysteine (Droge et al., 1991). Increased cysteine utilization for GSH synthesis results in taurine production, and inflammatory responses result in increases taurine levels in the liver and kidney. However, taurine levels were reduced in the gastrointestinal tract (Malmezat et al., 1998), and this reduction may have implications on bile salt formation and lipid absorption in carnivores.

IV. NUTRIENTS REGULATE THE TYPE OF IMMUNE RESPONSE

The immune response is organized to protect the host from intracellular and extracellular pathogens. Dependent upon the location of the pathogen, the immune system coordinates a specific response that is tailored toward eliminating that pathogen (Goldsby et al., 2003). For example, extracellular pathogens, such as *E. coli*, elicit an innate inflammatory response as well as an adaptive antibody response, whereas intracellular pathogens, such as viruses, elicit a cell mediated immune response. The release of cytokines by immune cells in response to pathogen recognition provides instruction for coordinating the appropriate type of immune response to eliminate the extracellular or intracellular pathogen. T helper 1 and 2 (Th1 and Th2, respectively) lymphocyte populations and macrophages are important cell types involved in the production of cytokines in response to pathogen; therefore, these cells function to direct the type of immune response elicited to a particular pathogen.

Nutrients can regulate the type of immune response, and this is, in large part, attributed to their ability to alter cell communication pathways and/or gene expression. Altered cell communication pathways and gene expression provide instruction on how, when and for how long to respond which are all important criteria for mounting an immune response. Examples of immunomodulatory nutrients include vitamin A, E and polyunsaturated fatty acids (PUFAs). We have provided a summary in Table 2 of some of the published reports that have examined the effect of these nutrients on immune function in broilers, layers and turkeys. The mammalian literature is much more replete with reports on the immunomodulatory properties of nutrients and helps to provide basic information that can aid in the understanding of how these nutrients may modulate the immune response in poultry.

The impact of these nutrients on immune function is dependent upon nutrient dose, type of pathogen challenge, dose of pathogen challenge and immune parameter measured (Table 2). Often times these immunomodulatory properties are realized when these nutrients are included in the diet above NRC recommendations. However, the effect of some of these immunomodulatory properties is curvilinear and higher dietary concentrations can have

adverse effects. The majority of studies have also utilized broilers and there is limited information on how these and other nutrients may modulate immune function in layers and broilers. Given the divergence in immune response between broilers and layers (Leshchinsky and Klasing, 2001), applying results obtained in broilers to layers may not be justified. More research examining the impact of nutrition on the immune response of turkeys is needed.

As nutrients continue to be examined for their immunomodulatory properties, it is important to carefully consider the selection criteria utilized to evaluate their effectiveness or response (Fulton, 2004). Specifically, it will be important for future studies in this area to select assays that are accurate in their estimation and are relevant and/or related to disease resistance (Cunningham-Rundles, 1998). As mentioned previously, it is not clear how changes in relative immune organ weights relate to disease resistance. Furthermore, it is assumed that lymphocyte proliferation in vitro to a common mitogen is similar to the proliferation of any signal that triggers proliferation of lymphocytes in vivo, such as antigen. Additionally, in vitro assays commonly use standardized cell numbers as well as isolated cell populations and this does not reflect the cellular environment in vivo. This requires careful selection of measurements of immune response and the development of new methodology in this area that fit these criteria may be constructive. These are important steps toward understanding the ability of nutrients to modulate the immune system in a relevant context.

V. POTENTIAL IMPLICATIONS OF FREE RANGE PRODUCTION ON IMMUNITY

The amount of information in the literature on the impact of free range production on immunity in poultry is very limited at this stage. The majority of the work published to date has evaluated feeding behaviors of free range birds, optimization of feeding programs for free range birds as well as impacts on product quality, such as taste or enrichment in specific vitamins (Ipek et al., 2009; Ponte et al., 2008a,b). Therefore, the impact of free range production on immunity is a bit immature and implications at this point can only be inferred. However, providing poultry with access to pasture has implications on the type of genetics utilized in the production system and exposure to pasture provides the bird with an opportunity to consume vegetation which results in un-controlled intake of nutrients and potential displacement of nutrients from the complete feed. The impact of using different genetics as well as consuming vegetation may have implications on immunity and will be discussed briefly.

Utilization of free range production systems typically results in the use of genetic strains that differ considerably from those used in conventional housing systems. Use of dual purpose breeds, or local breeds, that have a much slower growth response and longer time to market are typically used due to increased hardiness and ability to tolerate free range conditions. Consequently, genetic differences in the chickens used in free range systems is a major differentiating factor and understanding the impact of genetics on immunity is important to consider. The modern day meat and egg-type bird have been intensively selected for increased performance and efficiency of gain. The divergent selection of meat and egg-type birds for specific production traits has also resulted in a divergent immune system. For example, meat-type birds have a blunted febrile response to LPS as compared to egg-type birds, indicating differential immune responses between these two genetic lines (Leshchinsky and Klasing, 2001). Even within a genetic line there are notable differences in immune response as animals have been selected to have increased efficiency of production. For example, chickens selected for high antibody response had lower mature body weights and reduced egg production compared to chickens selected for low antibody response (Martin et al., 1990). Consequently, the slower growing genetic strains used in free range systems are likely to have divergent immune response as compared to faster growing genetic lines used in

conventional systems. These differences would be hypothesized to result in different immune responsiveness, and therefore nutritional approaches to influence immunity should be carefully considered.

Access to pasture provides the bird with an opportunity to consume vegetation. The contribution of vegetation consumption to the diet is highly variable across production systems, but potential impacts are beginning to be realized. It is important, therefore, to gain an understanding of the amount of vegetation consumed and to identify the types of vegetation consumed to understand the different types of nutritional and anti-nutritional factors that the bird may be consuming. Depending on the type of nutrients contained in the vegetation there may be potential for them to impact immune responsiveness. Nutrients of particular importance are fiber, phytate, essential oils, fatty acids and carotenoids. All of these nutrients can influence the immune response and will be briefly discussed in the presentation. It is critical in free range operations to understand the amount of vegetation intake and the amount of complete feed that this is displacing to begin to understand the potential impact of these nutrients on immunity.

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INFLUENCE OF WHOLE WHEAT INCLUSION AND PELLET DIAMETER ON THE PERFORMANCE AND GIZZARD DEVELOPMENT OF BROILERS

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Summary

The aim of the present study is to determine whether pellet diameter will influence the performance of broilers fed pre-pelleted whole wheat diets. The experimental design was a 2 x 3 factorial arrangement of treatments, which included three diet forms; namely ground wheat (GW), 200g/kg whole wheat (WW) replacing GW before or after pelleting and two types of pellet dies (3.0 or 4.76 mm diameter). Diets were offered *ad libitum* from day 11 to 35 post-hatch. The bigger pellet diameter increased ($P < 0.05$) the weight gain and lowered ($P < 0.05$) the feed per gain of birds fed diets with GW and post-pellet inclusion of WW. However, in birds fed diets with pre-pelleting inclusion of WW, increasing the pellet diameter lowered ($P < 0.05$) weight gain and increased ($P < 0.05$) feed per gain. Increasing the pellet diameter with pre-pelleting inclusion of WW increased ($P<0.05$) gizzard weights, but pellet size had no effect ($P > 0.05$) on the relative weight of gizzard in diets containing GW or post pellet inclusion of WW.

I. INTRODUCTION

The ever-rising cost of feed is a major challenge to the growth of poultry industry. In recent years, whole wheat (WW) feeding of broilers has received renewed attention as a means of lowering feed manufacturing costs and because of its reported positive effects on production performance and nutrient utilisation (Wu *et al*., 2004; Wu and Ravindran, 2004). These positive effects are often attributed to the impact of larger particle size on gizzard development which could impact nutrient digestion. However, studies examining effects of including WW pre-pelleting have produced equivocal results. Wu *et a*l. (2004) found that pre-pelleting inclusion of WW at 200g/kg of feed improved feed per gain. On the other hand, Jones and Taylor (2001) used the same level of WW inclusion and found no effect on broiler performance. The aim of the present study was to determine whether using a pellet die with a larger diameter (4.76 versus 3.0 mm) during the pre-pelleting of WW will be beneficial to broiler performance. The intention was to produce larger wheat particles during the pelleting process and maintain the beneficial effect of whole wheat feeding on the performance of broiler chickens.

II.MATERIALS AND METHODS

The experiment design was a 2 x 3 factorial arrangement of treatments, which included three diet forms, namely ground wheat (GW), 200g/kg WW replacing (w/w) GW before or after pelleting and two pellet dies (3.0 or 4.76 mm diameter). The diets were pelleted at 70 ^{0}C. The six treatment diets were formulated to be isocaloric (13.0 MJ/kg apparent metabolisable energy [AME]) and isonitrogenous (203 g/kg crude protein), but differed either in wheat form or pellet size. All diets were supplemented with a commercial xylanase (Avizyme 1502; Danisco Animal Nutrition, Marlborough, UK)

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Day-old male broilers (Ross 308) were obtained from a commercial hatchery and a wheat-soy based diet starter diet (12.4 MJ/kg AME and 226 g/kg crude protein) was fed to all birds from till day10 post-hatch. On day 11, birds were individually weighed (average weight, 309 ± 1.8 g) and allocated to 36 cages (8 birds per cage). Each cage was then assigned randomly to one of the 6 dietary treatments. Diets were offered *ad libitum* from day 11 to 35 post-hatch. Body weights and feed intake were recorded on day 11 and 35. On day 35, two birds per cage were killed and gizzard weights were recorded. The data were analysed by two-way analysis of variance using the General Linear Models procedure of SAS (2004). Differences were considered to be significant at $P < 0.05$ and significant differences between means were separated by the Least Significant Difference test.

III.RESULTS AND DISCUSSION

The influence of treatments on the performance of broilers and gizzard weight is summarised in Table 1. Significant ($P<0.05$) interactions between the method employed for wheat inclusion and pellet diameter were observed for weight gain, feed intake and feed per gain. Increase in pellet size to 4.76 mm from 3.0 mm sized pellet increased ($P < 0.05$) the weight gain of birds fed diets with GW and post-pellet inclusion of WW but this trend is reversed in birds fed diet with pre-pelleting inclusion of WW, where it decreased ($P < 0.05$) the weight gain. Increasing the pellet size to 4.76 mm from 3.0 mm increased ($P < 0.05$) feed intake of GW diets, but reduced ($P < 0.05$) that of diets with pre-pelleting inclusion of WW. However, feed intake was similar in broilers fed with post pelleting inclusion of WW. Feed per gain was lowered ($P < 0.05$) by increased pellet diameter in diets with GW and post pelleting inclusion of WW, while no effect was seen in diets with pre-pellet inclusion of WW. Two way interactions were significant ($P < 0.05$) for the relative weight of gizzard. With pre-pelleting inclusion of WW, 4.76 mm sized pellet increased ($P<0.05$) gizzard weight compared to 3.0 mm sized pellet. However, in GW or post pellet inclusion of WW diets, increasing the pellet size had no effect on the relative weight of gizzard.

In the present study, the influence of pellet diameter on broiler performance differed depending on the method of wheat inclusion. In GW and post-pelleting addition WW treatments, increasing the pellet diameter improved weight gain and lowered feed per gain. On the other hand, when WW was added pre-pelleting, increasing pellet diameter resulted lower weight gain and poorer feed efficiency. It is difficult to provide a reason for this unexpected result with pre-pelleting addition of WW. The lower weight gain with larger pellet diameter in the group was associated with reduced feed intake. One possibility may be that there were differences in pellet quality, causing the birds to consume less of the larger pellets. Pre-pelleting inclusion of WW using the 4.76 diameter die may have only partly crushed the WW or resulted in uneven breakage of the WW, reducing the pellet quality. Thus it is possible that the effect of particle size may have confounded the effect of pelleting (Behnke and Beyer, 2002). The way whole grain is crushed while pelleting and its effect on pellet quality needs to be addressed in future studies. This may be responsible, in part, for equivocal results reported for pre-pelleting inclusion of WW in the literature.

Table 1. Influence of method of whole wheat inclusion and pellet diameter on weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) and relative gizzard weight (g/kg BW) for broilers (11-35 days post-hatch)

Method of wheat inclusion	Pellet diameter (mm)	Weight gain	Feed intake	Feed per gain	Gizzard weight
GW	3.0	1665^{c}	3249^{b}	1.956^{a}	7.13^{c}
	4.76	1999^{a}	3377^{a}	1.689^{cd}	6.90^{c}
Pre-pellet WW	3.0	2004^{a}	3371^{a}	1.688^{cd}	6.72^{c}
	4.76	1776^{b}	3065^{c}	1.725^{bc}	8.97^{b}
Post-pellet WW	3.0	1625^{c}	2893^{d}	1.781^{b}	12.75^{a}
	4.76	1794^{b}	2972^{d}	1.656^{d}	11.85^{a}
SEM		22.87	31.98	0.020	0.448
Main effects					
Method of wheat inclusion					
GW		1832	3313	1.822	7.01
Pre-pellet WW		1890	3218	1.707	7.85
Post-Pellet WW		1710	2932	1.719	12.3
Pellet diameter					
3.0		1765	3171	1.809	8.87
4.76		1856	3138	1.690	9.24
Probabilities ,P ≤					
Method of inclusion		0.0001	0.0001	0.0001	0.001
Pellet diameter		0.0001	0.21	0.0001	0.31
Method of inclusion x Pellet diameter		0.0001	0.0001	0.0001	0.001

[a,b] Means in column not sharing a common superscript are significantly different (P<0.05).
[1] Each value represents the mean of six replicates.

The observed influence of pellet diameter on the feed intake and weight gain of broilers fed GW diets is interesting and difficult to explain. These findings appear to suggest that when all the grain is milled then a larger pellet is the better option. Presumably this has to do with better feed intake due to greater physical density of larger vs. smaller pellets.

In the present study, increasing pellet size had no effect on relative gizzard weight in birds fed GW and post-pellet WW diets, but increased the weight in pre-pellet addition of WW. Although the positive effects of WW on performance are often attributed to the impact of larger particle size on gizzard development and the resultant improvements in nutrient utilisation, there was no association between performance responses and gizzard development in the current study. Similarly, Wu *et al*. (2004) found that pre-pelleting inclusion of whole wheat at 200g/kg of feed improved feed per gain, but had no effect on gizzard weight whereas several other studies showed significant increases in gizzard weight with no effect on performance (Jones and Taylor, 2001; Taylor and Jones, 2004; Svihus *et al*., 2004).

Overall, the current data suggest that the effect of pellet diameter on broiler performance varied depending on the form of wheat and method of WW inclusion. When all the grain is milled or when WW is added post-pelleting, a larger pellet is beneficial. On the other hand, when WW is added pre-pelleting, a smaller pellet resulted in better broiler performance.

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THE EFFECT OF INSOLUBLE FIBRE AND INTERMITTENT FEEDING ON GIZZARD DEVELOPMENT, GUT MOTILITY, AND PERFORMANCE IN BROILER CHICKENS

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Summary

A trial was conducted to test the following hypothesis; broiler exposure to coarse insoluble fibre in the diet or litter will result in enhanced gizzard function and performance, improved adaptability to an intermittent feeding program and an increase in the occurrence of reverse peristalsis. Ross 308 broiler chickens were either intermittent or ad libitum fed a basal diet, a basal diet diluted with 15 % coarse hulls (barley and oats) or a basal diet diluted with 15 % finely ground hulls in a 2x3-factorial experiment (n = 17 birds/treatment). From 18 days of age, the birds were transferred to individual cages. Birds on intermittent feeding had restricted access to feed from 11 days of age. From 18 days of age, the restrictive feeding program consisted of four one-hour meals and one two-hour meal per day. AME value and faecal starch digestibility were determined by quantitative collection of excreta. At 31 and 32 days of age, birds were inoculated with CrEDTA via the cloaca. Weights were recorded and digesta samples collected from the gizzard, duodenum, jejunum, and ileum. There was no interaction between diet and feeding regime for any of the parameters measured. The addition of coarse oat and barley hulls resulted in birds with fuller, heavier gizzards ($p < 0.001$). Intermittently fed birds raised on the coarse hull diet exhibited an improved starch digestibility compared to birds not exposed to hulls ($p < 0.001$). The presence of chromium in all intestinal tract sections of birds from the six treatment groups, confirms that reflux occurs along the entire length of the gastro intestinal tract, irrespective of insoluble fiber content of the feed or feeding regime.

I. INTRODUCTION

In commercial broiler production feed is available to birds ad libitum often under continuous or near continuous lighting programs. This approach to husbandry is associated with potential overconsumption (Svihus et al., 2010). Restrictive feeding, by the use of intermittent lighting programs, has been shown to reduce such problems and improve feed efficiency (Decuypere et al., 1994). Intermittent feeding requires the bird to retain ingested feed for greater lengths of time than ad libitum feeding by storing ingesta in the crop and proventriculus/gizzard (Buyse et al., 1993). By stimulating gizzard development with the dietary inclusion of barley and oat hulls, it is possible that the resulting increase in holding capacity, heightened gizzard function and occurrence of reverse peristalsis will enable the broiler to thrive under intermittent feeding.

The purpose of this study was to investigate the effect of a dilution of broiler diets with hulls on bird performance, reverse peristalsis in the digestive tract, and ability to adapt to an intermittent feeding regime.

II. MATERIALS AND METHODS

Day-old male broiler chickens (Ross 308) were raised on a commercial pelleted starter diet in a multilevel brooder. At 11 days of age the birds were moved to 12 group cages.

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Two cages were assigned to each of 6 treatments in a 2-feeding regime × 3-diet experimental design. At 18 days of age, 17 birds were randomly selected from each of the six groups (102 in total) and transferred to individual cages. Temperature was reduced from 31 °C to 28 °C at 7 days of age and to 25 °C at 16 days of age.

Diets were steam-pelleted through a 3 mm die, and consisted of a basal control diet, a basal diet diluted with either 15% unground coarse, or fine ground hulls (equal weights of hulls from oats and barley). Irrespective of feeding regime, ad libitum (AL) or intermittent feeding (IF), birds experienced complete darkness between the hours of 02.00 and 08.00. From 11-18 days of age, the IF groups had access to feed from 08.00 to 09.00, 12.00 to 13.00, 16.30 to 17.30, and from 21.00 until the light went off at 02.00. From 18 days of age, these birds had access to feed from 08.00 to 09.00, 12.00 to 13.00, 16.00 to 17.00, 20.00 to 21.00, and from 24.00 until the light went off at 02.00.

Feed intake and weights for individual birds were recorded twice a week from 18 days of age until the end of the study. Quantitative excreta collection for AME was carried out between 26 and 29 days of age. At 32 and 33 days of age, after one hour off feed, 2 ml of Cr-EDTA marker was administered into the cloaca of the bird. Birds were then returned to their cages and given access to feed as determined by their normal feeding program for two hours then euthanized and dissected. Weights were recorded for gastrointestinal sections, contents were dried then digested in nitric acid using a closed chamber microwave, then analysed for chromium content using inductively coupled plasma emission spectroscopy (ICP). Excreta samples were frozen, mixed, dried and then analysed for gross energy content and starch along with feed samples. Data was analysed using a 2 × 3 factorial arrangement followed by pair-wise comparisons using the Duncan procedure with $P < 0.05$ as significance level.

III. RESULTS

There was no significant interaction between feeding regime and diet for any parameter measured (Table 1). The AL birds tended to consume more feed than IF birds ($P = 0.07$). Diet affected feed intake ($P < 0.05$) due to an increased feed intake for diets with coarse hulls added. No significant effect of feeding regime on weight gain was observed. However a reduced ($P < 0.01$) weight gain for birds consuming the finely ground hull diet was observed. Dilution of the diet with hulls reduced gain:feed ($P < 0.001$). Feeding regime did not affect AME or starch digestibility. Dilution with hulls, however, improved starch digestibility ($P < 0.001$). The amount of dry matter in the crop was higher ($P < 0.05$) in IF groups than in AL groups. Both IF and dilution with hulls reduced ($P < 0.001$) gizzard pH (Table 1). The addition of hulls to diets resulted in fuller and larger gizzards, with birds exposed to coarse hulls yielding the largest ($P < 0.001$) gizzards. Chromium at levels considerably higher than the background level was present in all sections of the gastrointestinal tract, with no significant difference between treatments (Table 2). However, chromium levels tended to be higher in the gizzards ($P = 0.07$) and the jejunum ($P= 0.06$) of the AL fed birds.

IV. DISCUSSION

Access to coarse hulls stimulated gizzard development, in agreement with previous studies, highlighting the stimulatory effect of structural material on the gizzard (Svihus et al., 2010). The large particle size and hardness of the coarse hulls explains why birds consuming that diet developed the heaviest gizzards. The coarse hull particles are retained in the gizzard until they are ground to a certain critical size that allows them to pass through the pyloric sphincter (Hetland et al., 2003). This leads to an increase in the volume of the organ's contents and a muscular adaptation to meet the greater demand for grinding.

Table 1. Performance and digestive characteristics of birds (17- 32/33 days of age)

	Ad libitum			Intermittent			P-values		
	Control	Coarse hull	Fine[1] hull	Control	Coarse hull	Fine[1] hull	Feeding	Diet	√MSE
Feed intake (g)	1724.0^{ab}	1803.2^{a}	1706.5^{ab}	1646.4^{b}	1716.2^{ab}	1649.0^{b}	0.07	*	46.6
Weight gain (g)	1075.8^{ab}	1113.9^{a}	1020.9^{bc}	1050.5^{abc}	1057.6^{abc}	989.4^{c}	NS	**	104.7
FCR	1.61^{ab}	1.62^{ab}	1.68^{a}	1.57^{b}	1.63^{ab}	1.68^{a}	NS	***	0.1
AMEn (MJ/kg diet)[2]	12.6^{a}	11.9^{b}	12.2^{ab}	12.7^{a}	11.9^{b}	12.4^{ab}	NS	***	0.8
Starch % excreta	5.5^{a}	1.1^{b}	1.1^{b}	4.3^{a}	0.8^{b}	1.0^{b}	NS	***	2.9
Crop content (g)	4.1	2.3	3.3	3.7	2.3	4.9	NS	NS	6.1
Crop DM (g)	1.5	0.9	1.8	1.7	2.3	4.1	*	NS	138.6
Gizzard Ph	3.6^{a}	2.27^{c}	2.28^{c}	3.01^{b}	1.96^{c}	1.97^{c}	***	***	0.6
Rel. gizz. empty [3](g)	1.2^{c}	2.4^{a}	1.8^{b}	1.1^{c}	2.3^{a}	1.7^{c}	NS	***	0.3
Gizzard DM (g)	0.3^{b}	4.6^{a}	4.6^{a}	1.1^{b}	3.8^{a}	4.0^{a}	***	NS	1.7
J&I[2] full (g)	3.9^{a}	3.7^{ab}	3.2^{bc}	3.5^{abc}	3.3^{abc}	3.1^{c}	0.09	*	7.0
J&I[2] empty (g)	2.3^{ab}	2.4^{a}	2.1^{b}	2.2^{ab}	2.3^{a}	2.1^{c}	NS	*	0.4

a-c = values in a row with unlike superscripts differ significantly where; *p<0.05, **p<0.01 and ***p<0.001.
[1]Fine hulls were produced by grinding the oat and barley hulls through a 1mm sieve.
[2]Calculated AME_n (MJ/kg diet) for the basal diets, and basal+coarse/fine hulls, 12.61 and 10.97 respectively (NRC, 1994)
[3] Relative weights are calculated as a percentage of BW

Table 2. Levels of chromium detected in the digestive tracts of birds

	Ad libitum			Intermittent			P-values		
Amount of Cr in section (µg)	Control	Coarse hull	Fine hull	Control	Coarse hull	Fine hull	Feeding	Diet	√MSE
Gizzard	4.3	32.5	34.8	3.3	8.5	11.4	0.07	NS	38.6
Duodenum	6.1	11.7	5.1	22.0	4.4	8.9	NS	NS	26.5
Jejunum	41.8	14.9	30.7	9.4	3.2	4.2	0.06	NS	58.7
Ileum	11.9	17.9	25.0	16.2	25.2	7.0	NS	NS	39.7

Birds fed the fine hull diet also exhibited heavier gizzards than the control group, although less so than the coarse hull group, suggesting that the fine particles were retained and partly induced the same response. In accordance with previous findings, the increase in volume and weight of gizzards in birds was accompanied by a reduction in pH in the caged bird (Nir et al., 1994). The strong effect of feeding regime on pH in the control birds may be explained by the increase in the dry matter content of the crop and gizzard with IF leading to increased fermentation, resulting in more acidic digesta flowing into the gizzard.

These gains in gizzard function coupled with a longer retention time have been shown to result in improvements in nutrient digestibility and feed utilization (Svihus, 2011). This was confirmed by starch digestibility data from the current experiment, and also by the considerably higher AMEn of the hulls-diluted diets than what would be expected based on calculated energy value of the control diet and the hulls (NRC, 1994). The lack of interaction between feeding system and diet structure indicates that the increase in volume of the gizzard does not influence the bird's ability to adapt to meal feeding.

The tendency for higher levels of chromium to be present in the gizzards and jejunal contents of AL birds fed the hull diets is contrary to what was expected. Reflux has been characterized as an organized motility response to feed shortage, resulting in temporary re-establishment of normal motility along the digestive tract (Clench and Mathias, 1992). It was assumed that reverse peristalsis, especially the gastroduodenal reflux, would be one method by which the IF birds would improve feed utilization. In view of this, the intermittent feeding system was perhaps not severe enough to induce an anti-peristaltic response. Conversely, reflux could be viewed as characteristic of optimal mixing of the lumen contents of satiated birds. However, Cr-EDTA is a liquid phase marker and retention time in the gizzard is dependent on particle size (Vergara et al., 1989). If digesta was refluxed back into the gizzard, the marker would be the part of the fraction to pass first through the pylorus sphincter. Chromium levels measured in the gizzard may not be wholly representative of the extent of the gastroduodenal reflux and explains why the chromium results do not concur with the pH levels in the gizzard and the large amount of DM present, from birds fed on the hull diets. Previous research has suggested the stimulating effect of coarse fibre on gizzard function, in particular more frequent and powerful contractions and the subsequent intraluminal pressure changes that they induce, will lead to an increase in the occurrence of gastric refluxes (Hetland et al., 2003). In the current study, Cr levels in the gizzard appeared higher in birds with access to the hulls, though this was not significant due to the high degree of variability in results. The aforementioned limitation of the liquid phase marker and increase in gizzard contents in birds exposed to the hull treatments, suggest that further work should be carried out investigating the potential for reflux of solid particles and possible interaction with improvements in nutrient digestibility.

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COMPARISON OF PERFORMANCE OF COMMERCIAL CONVENTIONAL AND FREE RANGE BROILERS

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Summary

Comparisons of the performance of free range and conventionally housed broiler chickens are scarce. A new free range operation was recently established and an opportunity to compare its progressive performance with conventional broilers under similar management practices and nutrition was taken. Growth, mortality and feed conversion of 8 paired batches of broilers from the same breeder flocks and hatchery in a similar geographical location over 16 months was compared. Although the initial batch performance of the free range sheds was similar to conventional, the free range performance demonstrated a continuous decline, with slower growth, higher mortality and deteriorating feed conversion efficiency over time. The implications for the industry and consumer and the need to better understand the reasons for this poorer performance are discussed.

I. INTRODUCTION

Farm animal welfare is an important subject in higher income countries particularly in the EU, Switzerland, Canada, Australia, New Zealand and the USA especially for commercial poultry production. It has been demonstrated that there is a strong relationship between Gross National Income and Animal Welfare Legislation (Van Horne and Achterbosch, 2008). According to Bennett's (1996) study, 21% of respondents were "very concerned" and another 60% were "concerned" about the welfare and mistreatment of farm animals in food production. Harper and Makatouni (2002) reported that the two main reasons behind the purchasing of free range products were animal welfare and a perceived health benefit. Consumers identify free range products as more advanced than conventional products in terms of health benefits. Free range broiler production is in its infancy in Australia but is growing rapidly. In the mid-1990s in Victoria, it was estimated that free range broiler production per week was around 1000 birds, with total broiler production around 171,000 per week (less than 1% of total broiler production) (Dixon, 2002). In 2006, free-range broiler production accounted for 4% of total broiler production and today it is around 15% of total broiler production (ACMF, 2011). Free-range broiler production is associated with poorer bird performance, higher feed conversion and higher mortality compared with conventional broiler production. This 'performance gap' is not well understood but is thought to be as a result of poorer digestive health, coccidiosis and dysbacteriosis challenge, nutritional inadequacy and variable pasture consumption. These performance challenges contribute to poor economic sustainability in the industry. It is the purpose of this paper to describe performance of free-range and conventionally-reared broilers at the same location, under commercial production constraints. Comparative benchmarking is an important prerequisite to further exploratory empirical or mechanistic research.

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II. MATERIALS AND METHODS

Two farms from the same geographical area (1.5 km apart) were selected in order to compare flock performance over time. Placements of day old chicks were synchronized as much as possible using the same donor flocks. Both farms received an identically formulated diet from the same feed mill differing only in that the free-range diet did not contain in-feed antibiotics as per the requirements of Free Range Egg & Poultry Australia (FREPA, 2009). Dead birds were collected and recorded daily. Birds on both farms were weighed at 7, 14, 21, 28 and 35 days. Feed conversion was calculated at the end of each batch for each farm and corrected to 2.45 kg live weight to be able to compare different killing age and final weights. Data were entered into an Excel spreadsheet and exported to JMP v.8 (SAS Software). Production system and age were used as leverage terms in a least square model to explore the main effects of both and interactions between the two on body weight and mortality. As FCR was only known for a whole batch (not by age) the main effect of production system only was explored. Significance was set at $P < 0.05$ and where differences existed means were separated using Tukeys HSD.

III. RESULTS

The effects of age and production system on mortality rate and body weight are shown in Tables 1 and 2. There was a significantly higher mortality for free-range compared with conventional production. Mortality rates for the first week did not differ between production systems. However mortality rates from the second week and thereafter were significantly different.

Differences in mortality especially in the second week were mostly the result of yolk sac infection. Antibiotic treatment is not allowed in free range broiler production due to FREPA regulations. On the other hand, in a few batches, predator attacks occurred (mostly chicken hawks), and resulted in high mortality due to pack-up in the free range broiler farm when free range broilers had range access.

Table 1. Mortality rates by age and production system

Production System	Cumulative Crude Mortality Rate (%)				
	0-7 d.	0-14 d.	0-21 d.	0-28 d.	0-35 d.
Free Range	1.262	2.025	2.806	3.819	5.281
Conventional	1.032	1.482	1.798	2.180	2.893
P value <	0.06	0.01	0.001	0.001	0.01
RMSE	0.2245	0.3087	0.3922	0.7076	1.2547

Differences between growth rates for each production system were significant only from d 28-35, resulting in an interaction ($P < 0.01$) between age and production system.

Table 2. Bird live weights by age

Production System	Body Weight (gram)				
	7 d.	14 d.	21 d.	28 d.	35 d.
Free Range	151	398	803	1283	1776
Conventional	156	419	816	1318	1917
P value	NS	NS	NS	NS	0.001
RMSE	10.776	25.674	43.674	45.485	65.129

FCR corrected to 2.45kg body weight was higher ($P < 0.05$) in the free range production compared to the conventional production (Table 3). Growth rate was significantly slower in the free range system than in the conventional production system. Birds in the free range production system required 2 more days to reach 2.45 kg body weight than those in the conventional production system.

Table 3. Effect of production system on feed conversion rate and growth rate

Production System	FCR Corrected to 2.45kg	Age Corrected to 2.45kg (days)
Free range	1.975	43.25
Conventional	1.874	41.20
P value <	0.05	0.05
RMSE	0.067	1.551

IV. DISCUSSION AND CONCLUSIONS

That free range production systems return poorer performance in broiler chickens has been previously observed. Weeks et al. (1994) showed that conventionally reared broilers had heavier (4.49 ± 0.08kg) body weight than free range broilers (4.08 ± 0.08kg) at ten weeks of age. This performance gap was attributed to the fact that free range broilers perform walking, running and ground pecking behaviours more often than conventionally reared broilers and so the poor performance was associated with increased activity. The results of the study by Weeks et al. (1994) are comparable to the results presented herein where, at 35d, body weights of the conventional birds were around 7.5% greater than their free-range counterparts (Table 2).

Extrapolating the results from the current study to the free range broiler industry in Australia, the impact of this higher FCR in free range production would cost around $8,000,000 per year. Further, the higher mortality in free-range systems may be indicative of stronger disease challenges and/or metabolic disorders.

Considerable demand exists for chicken meat that has been produced under free-range systems and this is expected to grow in the foreseeable future. This demand is partially

emotive and linked to anthropomorphic interpretation of intensive animal practice. However, the performance gap is substantial and may not be sustainable in the long-term. The reasons for this performance gap are obscure and further research is required to delineate the effects of the absence of antibiotic growth promoters and the effects of range access. A greater appreciation for the challenges that free-range broilers face, whether immunological, nutritional or behavioural, will allow more appropriate and strategic intervention by producers on one or all of these axes.

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DOES THE COMPOSITION OF INTESTINAL MICROBIOTA DETERMINE OR REFLECT FEED CONVERSION EFFICIENCY?

J. APAJALAHTI[1], T. RINTTILÄ[1] and A. KETTUNEN[1]

Summary

The importance of intestinal microbiota for the performance of broiler chickens has been studied for decades. Today most research groups analyse intestinal microbiota by DNA-based methods, which have brought fresh insights into the intestinal system of the broiler chicken. There is growing evidence of connection between the apparent metabolisable energy of the diet and microbiota composition in the lower intestine of the host. This connection can be due to direct conversion of some dietary components into high energy metabolites by specialised bacteria. This would provide more energy for the host, which could be measured as improved feed conversion efficiency. However, it is also possible that caecal microbial profile is a reflection of feed digestion and nutrient absorption efficiency in the proximal intestine. Disorders in these functions cause nutrient bypass to the lower intestine, providing new easily available substrates for bacteria that could otherwise not compete in that habitat. The two causal links described are likely to co-exist in real-life situations, but their relative dominance may vary case-by-case. Irrespective of the mechanism, rapid diagnostic determination of Performance-linked Microbial Index (PMI) can help develop more advanced feed ingredients by allowing rapid screening of a wide variety of potential concepts.

I. INTRODUCTION

Intestinal microbiota of man are currently being very actively studied. It was reported recently that human individuals can be divided into three major enterotypes according to the structure of the colon microbial community (Arumugam et al., 2011). No correlation was found between enterotype and phenotypic characteristics such as sex, body mass index and ethnicity. However, long-term dietary preferences affected the assignment of individuals into enterotypes. High consumption of animal fat and protein was associated with *Bacteroides* enterotype, whereas a low-fat/high-fibre diet yielded an enterotype characterised by *Prevotella* spp. (Wu et al., 2011).

Separate studies carried out with germ-free mice and human volunteers showed that obesity was associated with the capability of the colon microbiota to harvest energy from the diet. It was shown that the tendency to become obese could be transferred from an obese mouse to a germ-free individual by transferring microbiota from the former to the latter (Turnbaugh et al., 2006). The ratio of Firmicutes to Bacteroidetes was higher in individuals with efficient energy capture than in those characterised by higher energy bypass to faeces.

Knowledge on the intestinal microbiota of man is mainly motivated by health applications, one sideline of which is obesity. Intestinal health is also highly important for production animals. However, when productivity is not threatened by disease, the most important driver of performance is the capability of the animal to convert feed into carcass as efficiently as possible.

Based on the human studies reviewed above, the question arises as to whether the microbiota associated with obesity in humans would improve energy harvesting and feed conversion ratio (FCR) in production animals. Human individuals have a highly variable genetic background, a unique microbiota inherited from the mother and variable dietary

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preferences. Therefore, finding significant associations between the structure of intestinal microbiota and various phenotypic traits is likely to be more challenging than finding such associations in flocks of broiler chickens with a uniform genetic background, diet and bacterial pressure from the environment. In spite of this apparently easy experimental setting, there is no consensus regarding the type of microbiota associated with ideal performance.

In studies involving microbiota analysis, the complexity of interpretation increases with the use of applied methods, all of which have inherent biases of varying degrees. No method is ideal, and even with the most advanced PCR-based techniques it is possible that microbial groups representing significant fraction of the total microbial community escape analysis. Consequently, it is possible that a link between microbiota and performance can be found using one method but not confirmed using some other method, even if the trial setup is identical.

This paper reviews studies that have investigated bacterial communities in broiler chickens and discusses the potential implications of microbial community for the industry.

II. CHICKEN INTESTINAL MICROBIOTA

Microbiota in the intestine of an animal species has evolved together with the host. This co-evolution has produced specific host-microbe combinations called superorganisms with the best possible fitness in a given environment. One of the preconditions for such long-term co-adaptation is the uninterrupted transfer of maternal intestinal bacteria to the next generation. If this lineage breaks, the superorganism ceases to exist and the adapted microbiota are replaced by any bacteria from the environment. It is worth considering whether such natural inoculation is achieved in today's commercial hatchery systems and whether the characteristics evolved are desirable in animal production. Microbiota beneficial for the laying hens may not be ideal for the health and performance of short-lived broiler chickens.

Bacterial community in the proximal intestine of broiler chickens differs significantly in density and species distribution from that in the distal intestine. In general, bacteria in the small intestine utilise the same simple nutrients that are being absorbed and utilised by the host. Therefore, the small intestine is a site where microbiota and the host clearly compete for dietary nutrients. The host can recover part of the energy lost to microbes by absorbing and metabolising bacterial fermentation products, lactic and volatile fatty acids (VFAs).

The bulk of digestible organic nutrients are likely to be absorbed in the small intestine. Therefore, the substrates available to the caecal microbiota are dietary components that have escaped digestion in the host, or secretes of the intestinal epithelium. As a result, intense bacterial fermentation in the caecum does not lead to energy losses for the host, since these bacteria scavenge energy and nutrients from feed residues that are already beyond the endogenous digestion system of the host. Accordingly, any bacterial metabolites produced from such residues that have any value for the host will, improve the yield of overall energy and nutrient capture from the diet. It is possible that with poorly digestible diets, a considerable proportion of total dietary energy comes from bacterial fermentation.

With methods based on sequencing of specific DNA fragments, there is relatively good consensus on the composition of the microbial community in the chicken small intestine (Knarreborg et al., 2002; Lu et al., 2003; Apajalahti and Kettunen, 2006; Bjerrum et al., 2006; Dumonceaux et al., 2006; Torok et al., 2008). By far the most dominant bacteria are lactobacilli, which represent 80 to 90% of the total microbiota, the remainder mainly consisting of enterobacteria and enterococci. The most dominant caecal bacteria are also found in the distal ileum. It is possible that these bacteria enter the ileum during caecal emptying or reverse peristalsis and are not in fact metabolically active in the small intestine.

Caecal microbiota in healthy chickens is dominated by representatives of the order Clostridiales. For a long time representatives of this order were grouped according to their 16S rDNA sequence homology to Clostridial clusters I to XIX. During the last few years several bacterial phylotypes that previously could not be assigned to proper taxonomic clusters have now found a place in newly described families and genera. In the chicken caecum the most common families within the order Clostridiales are Lachnospiraceae and Ruminococcaceae (Torok et al., 2011). These appear to represent more than 50% of the caecal bacteria, the remainder belonging mainly to the orders Bacteroidales and Lactobacillales (Apajalahti and Kettunen, 2006). The genus *Clostridium* is well known to those working in the poultry industry, mainly because of one pathogenic species, *Cl. perfringens*. Unfortunately this species has affected the reputation of the entire order Clostridiales, even though the genus *Clostridium* is only one of 123 genera within the order and the species *Cl. perfringens* is only distantly related to the majority of the 129 species in the genus *Clostridium*. Therefore the generic term clostridia should not be automatically associated with poor health and performance in broiler chickens. In fact, all the existing evidence suggests that the reverse is true, namely that clostridia are good for-animals and for farm profits.

III. CAECAL MICROBIAL COMMUNITY AND METABOLISABILITY OF THE DIETARY ENERGY

Microbes in the chicken gastrointestinal tract colonise the intestine and reach their full density within a couple of days of hatching (Apajalahti et al., 2004). The composition of the microbial community is likely to be affected by the source of the microbes that first meet the virgin intestine and by the diet. Indeed, it appears that the first inoculum reflects the intestinal microbiota composition during the entire life of the broiler chicken. If hatching takes place in an environment where microbial load has been minimised, individual chicks may pick up different inocula from their surroundings and this may lead to differences in the intestinal physiology of individual birds in a flock. It is worth noting that intentional inoculation of the chicks might render a flock more uniform, not only with regard to microbiota composition, but also to FCR.

In our studies on microbiota of the chicken intestine over the years, it has become apparent that on farms with good health and FCR history, the between-bird variability is small, whereas on problem farms the flock is less uniform. Figure 1 clearly illustrates this. Ten commercial Finnish farms were randomly sampled for microbiota analysis three weeks after arrival of the chicks. Three pools of three birds per farm were analysed for caecal microbiota structure by a PCR-independent DNA-based method that fractionates total bacterial chromosomes according to their guanine+cytosine content (%G+C). Three weeks later the flock was taken to slaughter and the carcass weights were recorded for each farm. Final FCR was calculated and compared against the G+C profiles. The results clearly demonstrated that on farms with better overall FCR, the variability in the caecal microbiota composition three weeks before the slaughter was much lower than on farms with poorer FCR.

It is intriguing to think that on any farm there may be individual birds that have highly different efficiency to convert diet into body mass. The flock may appear uniform in size, but some animals may consume much more feed than others. The farm and flock-specific situation can be revealed by determining a variability index by G+C profiling.

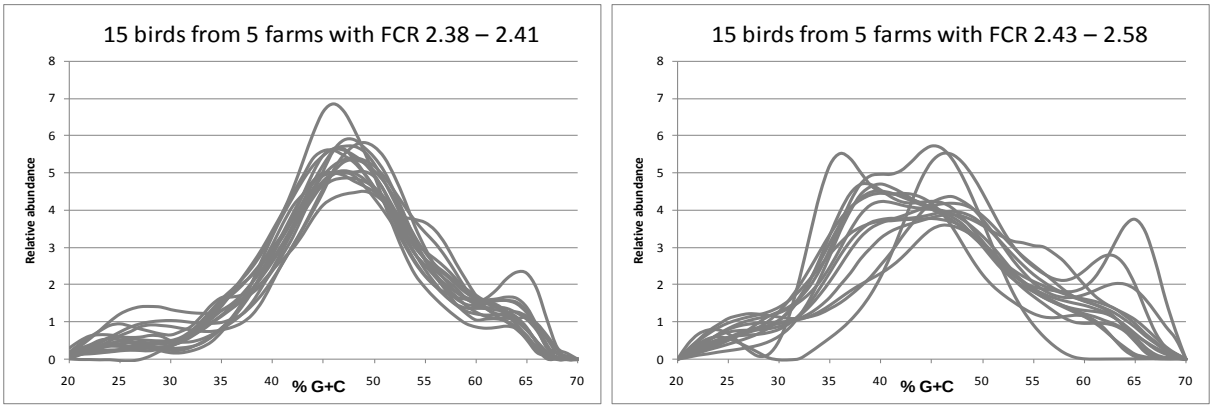

Figure 1. Chicken caecal microbial profiles arranged according to the FCR of the farm from which they originated. Bacterial profiles from five farms with the lowest FCR are on the left and those from five farms with the highest FCR on the right.

In a study carried out by McCracken at Queen's University (Belfast, Northern Ireland), broiler chickens were individually caged, fed with the same wheat-based diet and analysed for metabolisable energy from 7 to 28 days. Caecal bacterial profile was determined by G+C profiling and compared against the ability of the individual chicken to harvest energy from the diet. It was found that metabolisable/gross energy was significantly different between individual birds, ranging from less than 60% to more than 80%, and there was no good correlation between FCR and body weight gain (McCracken et al., 2006). Figure 2 shows the microbial profiles of the eight most efficient birds in terms of energy harvesting and the eight least efficient birds. There was a highly significant correlation between caecal microbial community structure and the energy harvesting efficiency of the host. Caecal bacteria from the most efficient birds were dominated by bacteria with G+C content around 45%. In inefficient birds, this peak was replaced by two major peaks, representing bacteria with G+C content around 37% and above 60%, respectively.

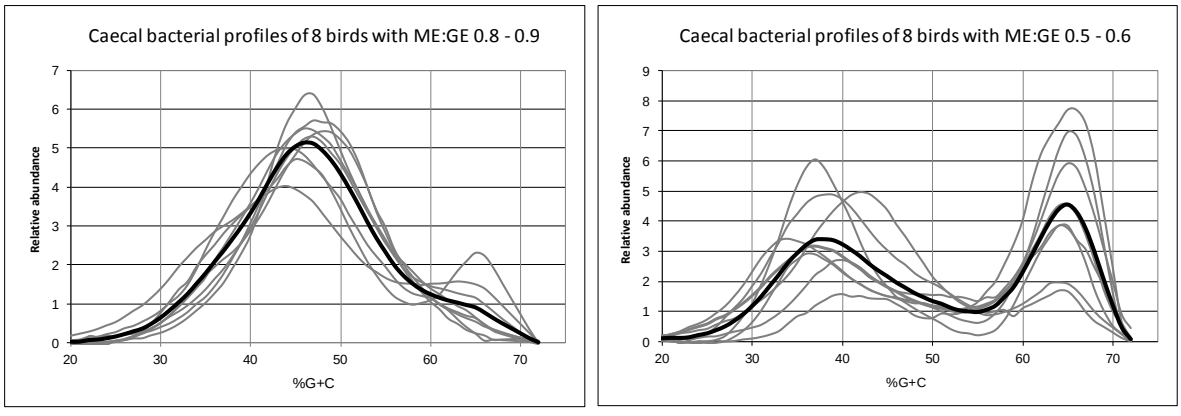

Figure 2. Chicken caecal microbial profiles of individually caged birds arranged according to diet metabolisability. The profiles on the left are from the eight birds with the highest metabolisability and those on the right from the eight birds with the lowest metabolisability. The black line in each diagram represents the mean of the eight profiles.

These two totally independent studies demonstrated that caecal bacterial composition was similarly related to FCR on commercial farms in Finland and to metabolisable energy at the experimental facility in Northern Ireland. In both cases bacteria with G+C ~45%

represented birds with good performance, whereas an increased proportion of bacteria with G+C ~37% and ~65% represented broiler chickens with reduced efficiency to harvest energy from the diet and convert feed into carcass mass.

Chromosomal DNA representing the diagnostics peaks of the %G+C profiles was collected and analysed in more detail by 16S rDNA sequencing to identify the actual bacterial phylotypes behind the shifts in the %G+C profiles. Eight taxonomic units were identified and a rapid quantitative real-time PCR assay was designed for their quantification. The abundance of these bacterial groups was used to design an algorithm that correlated with performance. The algorithm provided a value referred to as Performance-linked Microbial Index (PMI), which was first applied to all samples from the two studies. In Figure 3, the PMI values are plotted against FCR and ME/GE. As expected, FCR and PMI showed a highly significant negative correlation, whereas ME and PMI showed a positive correlation.

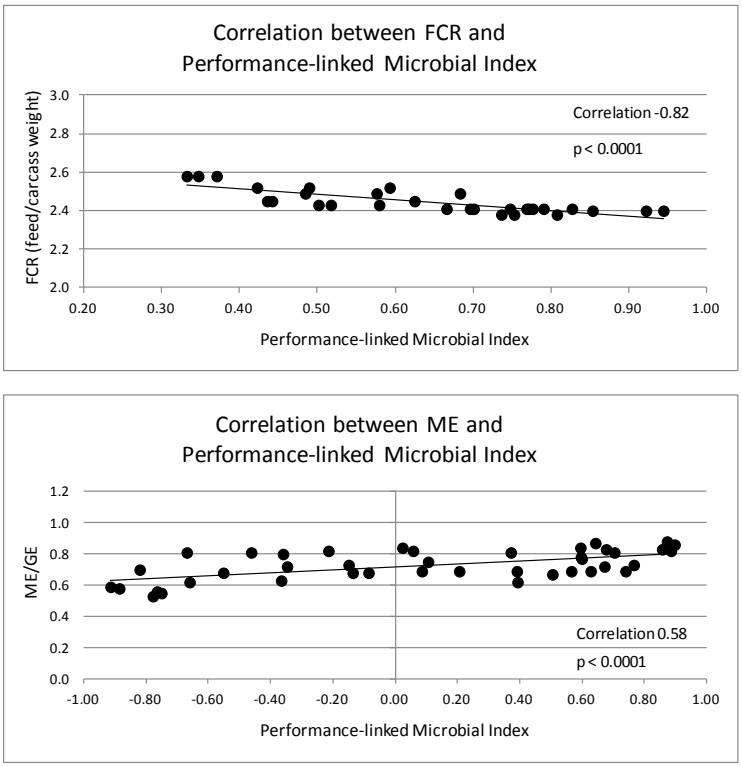

Figure 3. Relationship between Performance-linked Microbial Index and feed utilisation efficiency expressed as FCR (upper panel) and ME (lower panel).

IV. POTENTIAL EXPLANATIONS FOR THE MICROBIOTA/FCR CORRELATION

As exemplified by the trials described above, there is a correlation between caecal microbiota composition and the efficiency of the host to retain dietary energy in the body. The existence of this correlation may mean that specific microbes provide the host with this ability. On the other hand, it may be the case that host efficiency to digest feed and absorb nutrients in the upper gastrointestinal tract reflects the microbial community structure of the caecum.

It has been demonstrated that the transfer of microbes from a conventional mouse to a germ-free individual leads to different energy harvesting efficiency depending on whether the inoculum comes from an obese or a lean mouse (Turnbaugh et al., 2006). This suggests that

specific microbes can provide the host with an energy-efficient phenotype.

In broiler chickens, it is possible that dietary components that cannot be hydrolysed by the digestive enzymes of the host can be hydrolysed and metabolised by bacteria in the caecum. For example, various non-starch polysaccharides (NSPs) such as arabinoxylans and glucans can be metabolised by specialised caecal bacteria, with concomitant production of VFAs. VFAs are a valuable energy source for the host, with butyrate being especially important as a preferred energy source for the intestinal epithelium (Velazquez et al., 1996). It is worth noting that ruminants obtain nearly all their energy from VFAs produced in the rumen. Pigs may also obtain as much as 30% of their energy from VFA-producing hindgut fermentation. Soluble resistant protein that enters the caecum not only yields VFAs, but also several amines as fermentation products. VFAs and polyamines produced by gut bacteria have stimulatory effects on the proliferation rate and secretory activity of intestinal mucosa (Sakata, 1987; Osborne and Seidel, 1989; Furuse et al., 1991).

It is obvious that caecal microbiota converts dietary components into: i) VFAs that serve as an energy source for the chicken, and ii) many other biologically active compounds. It is also clear that there are differences in the efficiency of different bacteria to utilise resistant dietary compounds and in the spectrum of beneficial and harmful substances they produce. Therefore, the abundance of different bacterial types present cannot be passive and are likely to affect the overall metabolisability of the diet. One of the most important bacterial metabolites is butyric acid, which helps maintain good epithelial condition, thus ensuring that dietary nutrients and high energy VFAs are taken up by the host at best possible efficiency. Accordingly, butyrate-–producing representatives of the bacterial order Clostridiales can be expected to be especially beneficial.

An alternative explanation for the connection between FCR and the composition of caecal microbiota is that digestive disorders or compromised absorption of nutrients in the small intestine lead to abnormal nutrient bypass to the lower intestine. Dietary components that under normal circumstances would have been digested and absorbed in the upper intestine fail to do so for some reason. This could be a consequence of epithelial damage caused by coccidiosis, necrotic enteritis or a high level of anti-nutritional lectins in the diet. It is also possible that an exceptionally high passage rate of digesta leads to poor nutrient absorption and high nutrient bypass (Hughes, 2007). Poor nutrient absorption reduces FCR and, simultaneously, the overflow of nutrients to the lower intestine favours bacteria that grow rapidly on easily available substrates. In such situations, a correlation between FCR and caecal microbiota is apparent but the favoured bacteria do not directly affect FCR. Instead, the shift in microbiota composition is a reflection of reduced nutrient absorption.

It is most likely that both of the above-described causal links exist in real-life situations. Even though there are two clearly different mechanisms, they are not mutually exclusive and most likely co-exist. In the ideal situation, the main dietary nutrients, starch and protein, are effectively digested and absorbed in the small intestine. The only dietary components that reach caecum are NSPs, which are hydrolysed and metabolised by caecal bacteria to high energy VFAs, with a sufficient proportion of butyrate. In this situation the proportion of bacteria capable of utilising NSPs is high and butyrate-producing bacteria of the order Clostridiales are well represented.

V. HOW CAN WE USE THE KNOWLEDGE ON IDEAL BACTERIAL COMPOSITION IN THE CAECUM?

Knowing the bacteria associated with good performance is highly interesting from an academic viewpoint. However, if poor bacterial profile cannot be transformed into a good profile, with a concomitant improvement in animal performance, this knowledge has no practical value. If optimisation of caecal microbiota leads to good performance, we should try to find ways to provide a competitive advantage for representatives of taxa identified as being beneficial or taxa providing similar functions. There are many potential ways of achieving this. One way could be to develop starter cultures that directly provide the most important bacterial species. This is challenging because the desirable bacteria are strict anaerobes. Such products are available in the marketplace (*e.g. Megasphaera elsdenii*), but for poultry applications significant development work is required. An alternative to early inoculation is to provide dietary ingredients that favour the growth of the desirable bacteria. In principle, it is possible to affect microbial balance by appropriate selection of the main feed raw materials. In addition, various special ingredients such as prebiotics, feed enzymes and other products that potentially provide substrates for the NSP-digesting, VFA-producing caecal bacteria, could serve as efficient microbiota modulators. Today, there are good *in vitro* methods for pre-screening a wide range of dietary ingredients under laboratory conditions for the above-described properties. Furthermore, a number of potential ingredients could be tested in small-scale trials with a small number of birds, if the parameter studied is caecal bacterial composition instead of animal performance.

In some cases it is possible that caecal microbiota composition does not directly affect FCR, but only reflects feed digestibility, conditions in the proximal intestine and consequent nutrient bypass to the caecum. In such cases, the potential use of knowledge obtained is different. One way to use the information could be in diagnostic testing on the farm to determine the intestinal condition and uniformity of the flock. In wider farm surveys, common factors that lead to a lack of between-bird uniformity and ways to improve the situation could be identified. With early diagnosis, it could even be possible to take corrective actions before the birds are slaughtered.

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EFFECT OF LITTER MATERIAL AND DIETARY FIBRE ON GUT DEVELOPMENT, GUT MICROFLORA AND PERFORMANCE IN BROILERS

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Summary

A feeding study was conducted to compare the effects of litter material, dietary fibre and sex on growth performance, organ development, mucosal morphometry and gut microbial communities in broilers. Seven hundred twenty day old Cobb chicks were allocated to 24 floor pens in a 2 x 2 x 2 factorial design with 3 pens of 30 birds per replicate (3 pens of males and 3 pens of females). Factors were: litter material, paper or hardwood shavings; dietary fibre, low or high and sex. Diets consisted of wheat, soybean meal, meat meal, expeller canola meal, poultry fat, vitamin and minerals. Birds and feed were weighed on days 7, 21, and 35. Low fibre groups contained no oat hulls whereas high fibre groups contained 70 g/kg oat hulls. Birds grown on hardwood shavings had larger gizzards ($P < 0.01$) and a more favorable FCR ($P < 0.03$) than those grown on paper litter at 35 d. Dietary oat fibre was more beneficial in birds reared on paper litter than hardwood shavings as evidenced by significant fibre by litter interactions at 35 d for FCR ($P < 0.02$) and caecal *C. perfringens* counts ($P < 0.01$). Dietary oat fibre improved body weight at 7 d ($P < 0.04$), and lowered gizzard pH ($P < 0.02$) at 35 d. The results suggest that the combination of clean paper litter with high oat fibre diet may be beneficial in enhancing gut health in broilers.

I. INTRODUCTION

In many areas, wood shavings are becoming scarce and expensive. Processed newspaper was found to be a suitable alternative to hard wood shavings as a litter material in broilers (Malone and Chaloupka, 1983). No significant effects on feed efficiency, litter caking or total disease condemnations were found between treatments. A broiler study conducted by Ali *et al.,* (2009) found no differences in 42 d performance ($P > 0.05$) or gut microflora ($P > 0.05$) of birds reared on rice hulls, softwood sawdust, pine shavings, reused single batch litter, hardwood sawdust, shredded paper or chopped straw. Other investigators have concluded that litter material does not significantly impact broiler performance (Lien et al., 1992; Brake *et al.,* 1993; Martinez and Gernat, 1995; Anisuzzaman and Chowdhury, 1996; Swain and Sundaram, 2000). Dietary fibre as soy or oat hulls was found to increase gizzard size and total tract nutrient retention in broilers suggesting a requirement for fibre in chick diets (Gonzalez-Alvarado et al., 2007). The current study was conducted to investigate the effects of litter type (paper or hardwood) and dietary oat hulls on gut physiology, broiler performance and gut microflora.

II. MATERIALS AND METHODS

Seven hundred twenty feather sexed Cobb 500 day old broiler chicks were randomly assigned to 24 sex separate pens in Ingham Enterprises environmentally control broiler research facility, 30 birds per floor pen. Birds were fed the diet compositions given in Table 1 in three

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phases. The ratio of protein, essential amino acids and minerals to metabolisable energy were constant between the low and high fibre diets for each phase. On day 35, four birds were randomly chosen from each pen for sample collection. The birds were euthanized by cervical dislocation as per UNE animal ethics protocol. Total body weight and various organ weights were measured and expressed as relative g/100g body weight. Contents of gizzard, ilea and caeca were collected for pH determination and microbial culture. The duodenum from a single bird per treatment was collected and fixed in 10% buffered formalin for histological examination after sectioning and staining with hematoxylin and eosin. A Leica DM LB microscope and Sony Exwave (SSC-DC83p) video camera was used to capture images and morphometric indices were determined using image processing software (VideoPro 32). Villus height and crypt depth were measured in 15 vertically, well-oriented, intact villi and crypts. Lactose-negative *Enterobacteriaceae* were counted on MacConkey agar (Oxoid, CM0115) after aerobic incubation at 39^{o}C for 24 h. *C. perfringens* were counted on tryptose-sulfite-cycloserine and Shahidi-Ferguson Perfringens agar base (Oxoid, CM0587) mixed with egg yolk emulsion (Oxoid, SR0047) and Perfringens (TSC) selective supplement (Oxoid, SR0088E) as described by Engberg *et al.*, 2004. Bacterial numbers were expressed as log10 CFU/g digesta. All data were analyzed using the univariate general linear means procedure of SPSS (Ver 19.0.0).

Table 1. Diet composition

Nutrients	ME,MJ/kg	Protein, g/kg	Fat, g/kg	Fibre, g/kg
Starter				
Low fibre	12.8	235	62	30
High fibre	12.1	222	62	50
Grower				
Low fibre	12.9	210	62	30
High fibre	12.2	200	62	50
Finisher				
Low fibre	13.1	200	62	30
High fibre	12.4	190	62	50

Ingredients (starter)	Low fibre, g/kg	High fibre, g/kg
Wheat	646	594
Soybean meal	181	164
Meat meal	66	66
Canola meal (expeller)	50	50
Oat hulls	0	70
Poultry oil	38	38
Vitamins, minerals	19	18

III. RESULTS AND DISCUSSION

A significant litter type by dietary fibre interaction was observed for body weight and FCR at 21 d ($P < 0.01$) and 35 d ($P < 0.02$). Performance was favored in birds fed diets containing oat hulls and reared on paper litter and low fibre diets reared on hardwood litter. The highest 35 d weight was achieved in birds fed high fibre diets on paper, while the best FCR was obtained feeding the low fibre diet to birds on hardwood shavings (see treatments means Table 2). Body weight was greater in birds fed oat hulls at 7 d ($P < 0.04$), and 21 d (921 vs. 903g; $P < 0.01$) and tended to be greater at 35 d ($P < 0.07$) as compared to the low dietary

fibre treatments. As expected, males had higher body weights and better FCR than females at 21 and 35 d ($P < 0.001$).

Relative empty gizzard weight (g/100g BW) was 20% greater in birds reared on hardwood shavings than those reared on paper litter ($P < 0.01$) and 30% greater in birds fed oat hull diets relative to those fed low fibre diets ($P < 0.01$). Gizzard content pH was significantly lower in birds fed oat hull diets than those fed low fibre diets ($P < 0.02$). Litter type had no impact on gizzard pH ($P > 0.27$). Results appear in Table 3. Small intestinal crypt depth tended to be greater in birds grown on hardwood litter (339 vs. 281 μm; $P < 0.08$ while villus height was significantly greater in birds grown on paper litter (1707 vs. 1529 μm; $P < 0.04$.

There were significant litter by dietary fibre interactions for caecal *C. perfringens* counts ($P < 0.01$) and a tendency for an interaction for caecal *Enterobacteriaceae* counts ($P < 0.08$). Dietary oat hulls were more effective in reducing caecal *C. perfringens* and *Enterobacteriaceae* counts in birds reared on paper litter than on hardwood shavings (Table 3). Birds reared on hardwood litter had significantly higher counts of caecal *Enterobacteriaceae* than those grown on paper litter ($P < 0.009$). High dietary fibre tended to decrease *Enterobacteriaceae* numbers in caecal contents ($P < 0.060$).

Table 2. Bird performance on days 7 and 35

Main effect means		BW7, g	FCR7	BW35, g	FCR35	Mort35, %
Litter	Paper	179	0.818	2125	1.530[b]	3.2
	Hardwood	177	0.803	2153	1.503[a]	3.7
Dietary fibre	Low	175[a]	0.824	2124	1.510	3.7
	High	180[b]	0.798	2154	1.523	3.2
Sex	M	180	0.807	2285[b]	1.482[a]	3.9
	F	176	0.814	1993[a]	1.551[b]	2.9
P > F statistic	Litter	0.27	0.38	0.10	0.03	0.73
	Fibre	0.04	0.14	0.07	0.26	0.73
	Sex	0.08	0.68	0.01	0.01	0.50
	L x F	0.06	0.01	0.07	0.02	0.19
	L x S	0.48	0.85	0.03	0.97	0.11
	S x F	0.90	0.14	0.58	0.84	0.74
	L x F x S	0.54	0.07	0.91	0.51	0.75
Treatment means						
	Paper, low fibre	175	0.860	2094	1.538	4.4
	Paper, high fibre	183	0.777	2156	1.522	1.9
	Hardwood, low fibre	176	0.788	2153	1.481	2.9
	Hardwood, high fibre	177	0.819	2153	1.525	4.4

[a,b] means with different superscripts within a main effect are different ($P < 0.05$)

IV. CONCLUSION

Results indicate a performance benefit for dietary oat hulls in young broilers especially those reared on paper litter. Litter type by dietary fibre interactions indicated performance benefits for dietary oat hulls in finishing broilers when reared on paper litter but not hardwood shavings. Higher counts of potentially pathogenic *C. perfringens* and *Enterobacteriaceae* (includes the genus *Salmonella*) were observed in the caecal contents of birds reared on hardwood litter compared to paper litter suggesting hardwood litter may be a potential source of inoculum. Dietary oat hull fibre was effective in reducing caecal *C. perfringens* and

Enterobacteriaceae in birds reared on paper but not hardwood litter. This warrants further investigation.

Table 3. Gizzard weight and pH, caecal *C. perfringens* and *Enterobacteriaceae* counts

Main effect means		Gizzard g/100 g bw	Gizzard pH	Caecal *C. perf* $\log_{10}$	Caecal *Ent ero* $\log_{10}$
Litter	Paper	1.44[a]	3.8	4.42[a]	4.20[b]
	Hardwood	1.73[b]	4.0	6.55[b]	7.67[a]
Dietary fibre	Low	1.37[a]	4.2[b]	6.09[b]	7.59
	High	1.80[b]	3.6[a]	4.87[a]	7.27
Sex	M	1.56	3.8	5.40	7.51
	F	1.61	4.1	5.56	7.36
P > F statistic	Litter	0.01	0.27	0.01	0.01
	Fibre	0.01	0.02	0.01	0.06
	Sex	0.49	0.21	0.53	0.38
	L x F	0.34	0.33	0.01	0.08
	L x S	0.60	0.72	0.08	0.46
	S x F	0.95	0.72	0.29	0.61
	L x F x S	0.99	0.84	0.04	0.12
Treatment means					
	Paper, low fibre	1.20	6.9	5.57	7.51
	Paper, high fibre	1.68	6.8	3.26	6.89
	Hardwood, low fibre	1.55	6.3	6.62	7.68
	Hardwood, high fibre	1.91	6.6	6.48	7.65

[a,b] means with different superscripts within a main effect are different ($P < 0.05$)

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SEMDURAMICIN AND NUTRITIONAL RESPONSES: THE EFFECTS OF PROTEIN SOURCE AND CONCENTRATION

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Summary

An experiment was conducted to determine whether feeding semduramicin affects the broiler chicken's response to dietary protein, and whether protein source (vegetable vs. animal protein) would affect the response to semduramicin. In a 42-day floor pen study, three protein concentrations (16, 20 and 24%) sourced from animal (12% of total protein) or complete vegetable ingredients were fed to broilers in the presence and absence of semduramicin (25 ppm). At 42 d, growth rate was affected by protein concentration of the diet and birds fed animal protein diets were slightly heavier than those fed control (vegetable) diets. No interactive effects of protein concentration or source with semduramicin were observed. Semduramicin did not affect feed consumption throughout the study and overall feed conversion ratios were identical.

I. INTRODUCTION

Ionophores have been widely used in broiler production for more than 40 years because they are effective in controlling the coccidial species that infect chickens. The major anticoccidial ionophores, monensin, lasalocid and salinomycin, are all known to influence nutritional responses of broilers (Austic and Smith, 1980). Thus, as semduramicin, an additional anticoccidial ionophore has gained usage around the world, questions about possible dietary changes have arisen.

Bartov and Jensen (1980) determined that a significant interaction between dietary protein source and monensin may affect growth of broilers. When they fed a mixture of menhaden fish meal, poultry by-product meal, and meat and bone meal, growth was depressed more in the presence of monensin that in a corn-soybean control diet. However, other investigators could not confirm these results (Willis and Baker, 1981). Welch et al. (1986) then demonstrated that dietary protein clearly affects the monensin-protein source interrelationship, thereby explaining the conflicting reports of other researchers.

Parsons and Baker (1982) had earlier demonstrated that the growth-depressing effects of monensin are dependent on the protein content of the diet. Salinomycin was also shown to depress growth when fed with relatively low protein levels (Welch et al., 1986). In addition to these reports, monensin had also been associated with poor feathering and increased feather picking (Charles and Kiker, 1974). Patel et al. (1980) demonstrated that both monensin and lasalocid influence floor feather score and weight of feathers remaining on birds.

The experiment reported herein was conducted to determine whether semduramicin (25 mg/kg) influences the performance of broilers fed various protein concentrations and affects the response to diets containing high concentrations of animal protein ingredients.

II. MATERIALS AND METHODS

One thousand five hundred eighty-four Ross x Ross male broilers were obtained from a local hatchery. Prior to their placement, the facility was thoroughly cleaned and heated in order to

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minimize the chance of coccidial infection occurring during the study. Thirty-three chicks were placed in each of 48 floor pens containing pine shavings as litter. The facility was divided into blocks with one treatment replicate allocated within each block. Treatments were assigned to pen within block at random. Chicks were maintained on a 24-hr light schedule, and feed and water were provided *ad libitum*. Chicks were given the same starter diet for 18 d; the diet was formulated to meet all NRC (1994) requirements. At day 18, birds were given experimental diets with two protein sources (all vegetable or containing 12% high animal protein ingredients) and three protein concentrations (16, 20 or 24% protein). These rations supplied 80, 100 or 120% of NRC (1994) recommendations for protein and amino acids. Specifics of these rations are presented in Table 1. Two levels of semduramicin (0 or 25 mg/kg) were also evaluated.

At 42 d, feed intake and performance variables were determined. In addition, 10 birds per pen were randomly chosen for feather scoring. Birds were scored 1 (best) to 4 (worst) on the basis of feather cover. Also, the number 8 primary feather from the left wing was removed, dried, and weighed. Data were analyzed using the General Linear Models procedure of SAS (SAS Institute, 1985), and when main effects were significant, means were separated by Duncan's New Multiple Range Test. $P < 0.05$ was considered significant.

Table 1. Dietary formulations for experimental diets[1] administered during the growth period.

	All Plant Products			Animal Protein Ingredients		
	16% CP	20% CP	24% CP	16% CP	20% CP	24% CP
Corn %	72.3	60.2	48.0	80.9	68.7	56.4
Soybean meal 48 %	20.3	30.6	41.0	4.8	15.3	25.6
Poultry fat %	3.4	5.3	7.2	0.9	2.8	4.7
Poultry Meal %	-	-	-	4	4	4
Meat & Bone Meal %	-	-	-	4	4	4
Fish Meal (Menh.) %	-	-	-	4	4	4
Estimated Amino Acid Composition:						
Met + Cys %	0.58	0.72	0.86	0.58	0.72	0.86
Lysine %	0.82	1.12	1.42	0.80	1.05	1.35

[1]All diets were formulated to contain 3200 kcal/kg, 1% Ca, and 0.5% aP. Vitamin and trace mineral premixes exceeded standard requirements.

III. RESULTS

The growth performance of broilers fed either the all-plant protein ration or the 12% high protein animal ingredients was comparable, and there were no significant interactions between protein source and semduramicin in performance. Protein content was found to have a significant effect on body weight at day 42 of the study (Table 2). Semduramicin did not affect feed consumption during the growing period when it was fed, and overall body weights and feed conversion ratios were identical to controls for this period. On day 42 no interaction between semduramicin and protein concentration was observed.

Protein source had no effect on final performance or feed intake, and no protein source x semduramicin interactions in performance were observed. However, increasing

protein content decreased feed consumption and thereby improved feed conversion. Significantly different feather weights and feather scores were observed in birds receiving the lowest level of protein fortification; semduramicin had no effect on these measurements (Table 3).

Table 2. Effect of dietary protein source and concentration on performance and feed intake of male broilers fed semduramicin during the growth phase.

Variable	n	BW gain	Feed Intake	FCR (feed:gain)
		---------- kg ----------		g:g
Protein Source				
All Plant	24	2.36	4.51	1.91
Animal Products	24	2.40	4.52	1.89
Dietary Protein				
16%	16	2.30[b]	4.59[a]	2.00[a]
20%	16	2.42[a]	4.52[a]	1.87[b]
24%	16	2.43[a]	4.43[b]	1.82[c]
Semduramicin				
0 mg/kg	24	2.39	4.52	1.90
25 mg/kg	24	2.37	4.51	1.90

[a-c] Values with no common superscript differ significantly (P <0.05).

Table 3. Effect of dietary protein source and concentration on feather score and feather weight of male broilers fed semduramicin during the growth phase.

Variable	n	Feather Score[1]	Feather Weight[2]
Protein Source			
All Plant	24	1.55	0.212
Animal Products	24	1.54	0.214
Dietary Protein			
16%	16	1.43[b]	0.207[b]
20%	16	1.51[ab]	0.213[ab]
24%	16	1.69[a]	0.218[a]
Semduramicin			
0 mg/kg	24	1.57	0.211
25 mg/kg	24	1.52	0.215

[a-b] Values with no common superscript differ significantly (P <0.05). Values represent the means of n replicate pens of 33 cockerels.
[1] 1 = best; 4 = worst. [2] Means for n replicate pens of 10 observations each, per treatment.

IV. DISCUSSION

Previous research has shown that ionophores are capable of producing a variety of performance responses when administered in lower protein diets. Parsons and Baker (1982) demonstrated that the growth depressing effects of monensin are largely dependent upon the protein content of the diet, with low protein diets causing a greater reduction of feed intake. Similar results were reported by Welch et al. (1986), who also showed that salinomycin produced responses of a similar nature in low protein feeds. In contrast to these reports, Izquierdo et al. (1987) demonstrated that narasin produced no adverse effects in growth performance or feed intake when administered in a low protein feed. As a result of these findings, these authors described a spectrum of responses for ionophores in diets of suboptimal protein content – from monensin, which produces the greatest adverse effects, to narasin whose negative responses are minimal.

The results of the present study indicate that semduramicin produced no adverse effects in feed intake or growth performance when administered in diets of different protein sources or concentrations. Likewise, no adverse effects of this ionophore on feathering were observed. These facts suggest that semduramicin produces no measurable negative effect on feed intake or performance when administered in diets of suboptimal protein content. Results of this study also indicate that that no special adjustments of protein nutrition are necessary when semduramicin is fed.

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EXPLOITING THE CALCIUM SPECIFIC APPETITE OF BROILERS

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Summary

A total of 144 Cobb 500 broilers were used to investigate if modern broilers can regulate calcium intake using choice feeding. Birds were fed diets containing 0.25, 0.50, 0.75 or 1.00% total Ca and a separate Ca source ($CaCO_3$) from 1-21d. Consumption of the separate Ca source increased with decreasing Ca concentration of the mixed ration ($P < 0.0001$) indicating that modern broilers retain a Ca-specific appetite. Further, weight gain and FCR was inferior in the diets with the highest Ca concentration and this is interpreted as a Ca-induced reduction in phytate-P utilisation.

I. INTRODUCTION

Phosphorus (P) in plants and seeds is stored as phytate (*myo*-inositol hexaphosphate) which is poorly available to monogastric animals (Tamim and Angel, 2003), mainly due to the simultaneous supply of calcium (Ca) as part of the mixed ration. Phytate has 12 reactive sites which range from being strongly acidic (pK 1.5-2.0) to weakly acidic (pK 9.0-11.0) and in the digestive tract of poultry, phytate carries a strong negative charge that is capable of binding di- and trivalent cations such as Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Fe^{2+} and Ca^{2+} (Angel et al., 2002). Despite Ca having one of the lowest affinities for phytate, due to its high concentration in poultry diets Ca potentially has the greatest effect. Excess dietary Ca has been reported to impede the availability of other minerals such as P, Mg, Mn, Zn as well as reduce the efficacy of phytase through the formation of Ca-phytate complexes. (Driver *et al.*, 2005; Selle *et al.*, 2009). Reducing the concentration of dietary Ca has been reported to improve phytate-P availability, but this has also been shown to reduce skeletal integrity and have implications for the health and welfare of poultry.

Poultry have been shown to possess specific appetites for nutrients and are able to select a diet from a variety of sources to meet their nutritional requirements. Seminal work in layer hens by Kempster (1916) and Rugg (1925) showed that hens produced more eggs when they were able to choice feed when compared to those fed a single mixed ration. Specific appetites in poultry have also been shown for lysine (Newman and Sands, 1983), methionine (Steinruck *et al.*, 1990), total protein (Forbes and Shariatmadari, 1994), selenium (Zuberbuehler *et al.*, 2002) and Ca (Wood-Gush and Kare, 1966; Hughes and Wood-Gush, 1971; Joshua and Mueller, 1979). Of the available literature, work assessing the Ca specific appetite in poultry predominantly evaluates the consumption of a separate Ca source when fed in combination with either a Ca deficient diet or Ca adequate diet. Few reports detail the response to multiple basal diets formulated with different Ca concentrations in the one experiment. Furthermore, the majority of work using Ca choice feeding models describes work in layer hens. Therefore, the study reported herein investigated whether a modern commercial broiler still possesses a Ca specific appetite as well as the ability to consume

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sufficient Ca to meet its requirement. The effect of this complementary feeding approach on the digestibility of P and other minerals was also assessed.

II. MATERIALS AND METHODS

A total of 144 Cobb 500 day-old male broilers were obtained from a commercial hatchery, weighed and randomly allocated to one of four dietary treatments in a completely randomised design. Each treatment was replicated six times with six chicks per replicate cage. Broilers were kept at a temperature of 31 °C for days 1-4 and thereafter this was reduced by 0.5 °C/day to 24 °C. The lighting regime for the study consisted of 23L:1D for the first 4 days and then 18L:6D for the remainder of the experiment. Diets were based on maize and soybean meal and consisted of 0.25, 0.50, 0.75 and 1.00% total calcium (Table 1). Available P was formulated at 0.25% in order to assess the effect of Ca feeding strategy on phytate-P digestibility. Maize/soy diets were chosen intentionally to minimise possible confounding effects of endogenous phytase e.g. from wheat. All birds had free access to the mixed ration, a separate source of calcium ($CaCO_3$ grit, 38% Ca and 2mm mean particle size) and water. Feed and calcium source intake were recorded daily and body weight weekly. Apparent ileal digestibility (AID) coefficients for crude protein (CP) and minerals were calculated using acid insoluble ash as an indigestible marker. On day 22, all birds were euthanised and ileal contents collected and pooled for each cage. Toe ash measurements were recorded from samples that were obtained by severing the middle toe through the joint between the 2nd and 3rd tarsal bones from the distal end. Toes were collected from individual birds and pooled by treatment replicate. Samples were dried to a constant weight at 105 °C and then ashed in a muffle furnace at 500 °C for 12 hours.

The gross energy of diets were determined using a Parr 1281 adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL, USA). The diets and digesta were also analysed for selected minerals (P, Ca, Cu, K, Mg, Mn, Na, Sr, Fe and Zn). Samples were wet acid digested using nitric acid and hydrogen peroxide prior to the determination of mineral concentration by Inductively Coupled Plasma-Optical Emission Spectroscopy using a Perkin Elmer OPTIMA 7300 (Perkin Elmer Inc, Waltham, MA, USA). Nitrogen concentration of samples was determined by the Dumas method using a FP-428 nitrogen analyser (LECO® Corporation, St. Joseph, MI, USA) as described by Sweeny (1989). The acid insoluble ash component of dried diets and ileal digesta samples were determined according to the method of Siriwan et al. (1993). Data were analysed for significance by one-way ANOVA using JMP v 8.01 software (SAS Institute, Cary, NC, USA). Treatment differences were considered significant at $P < 0.05$. If significance was determined, a Tukey's HSD was performed to differentiate between treatments.

III. RESULTS AND DISCUSSION

The analysed concentration of Ca in diets was greater than formulated and is most probably due to higher than expected concentrations of Ca in the grains used (see Table 1). The influence of dietary Ca on feed intake, consumption of the separate Ca source and performance is shown in Table 1. Birds fed the basal diet containing 1.00% total Ca consumed significantly less feed and gained significantly less body weight during the experiment when compared with birds from the other treatment groups ($P < 0.05$). These results are in agreement with those of Rama Rao et al. (2006) who showed increased levels of dietary Ca depressed feed intake and weight gain in broilers. The feed efficiency of birds from the 1.00% Ca group was also significantly poorer than birds from the other groups ($P < 0.05$). There was no effect of treatment on the toe ash percentage.

Table 1. The effect of dietary calcium (Ca) concentration on the intake of a separate Ca source, total Ca intake and broiler performance (1- 21d of age)

	Calcium concentration (%) of mixed ration					
	0.25	0.50	0.75	1.00	SEM[1]	*P* value
Determined dietary Ca (%)	0.35	0.71	1.08	1.23		
Mixed ration intake (g/b/d)	48.4[a]	50.1[a]	45.1[a]	37.8[b]	1.41	< 0.0001
Separate Ca source consumed (g/b/d)	0.80[a]	0.58[b]	0.35[c]	0.30[c]	0.04	< 0.0001
Total feed intake (g/b/d)[2]	49.2[a]	50.6[a]	45.5[a]	38.1[b]	1.40	< 0.0001
Total Ca intake (g/b/d)	0.45[a]	0.56[b]	0.61[b]	0.57[b]	0.02	< 0.0001
Ca intake as a proportion of total feed intake (%)	0.93[a]	1.11[b]	1.35[c]	1.51[d]	0.04	< 0.0001
Final body weight (g)	780.5[a]	778.2[a]	703.2[a]	594.9[b]	22.9	< 0.0001
Live weight gain (g)	736.3[a]	745.1[a]	676.6[a]	563.9[b]	24.3	0.0001
FCR (g:g)	1.49[a]	1.49[a]	1.50[a]	1.58[b]	0.02	0.002
Toe ash (%)	12.7	13.0	12.7	12.5	0.21	0.317

[1] Pooled standard error of mean of choice feeding treatments
[2] Combined mixed ration and separate Ca source intake
[a-c] Within rows, values with different superscripts are statistically different (P < 0.05)

The results from this current study show that the specific appetite for Ca is still active in modern commercial broilers and these were able to consume sufficient Ca to meet their requirement. There was a significant relationship between the Ca concentration of the mixed ration and intake of the separate Ca source (P < 0.0001) and this is in keeping with the results reported by Joshua and Mueller (1979). Birds fed diets containing 0.25% Ca consumed significantly more of the separate Ca source when compared to birds fed diets containing either 0.50, 0.75 and 1.00% Ca (P < 0.05). Birds offered diets with 0.50% Ca also consumed more of the separate Ca source than those fed 0.75 and 1.00% Ca diets (P < 0.05). The source of Ca as a proportion of the total Ca intake was influenced by the Ca concentration of the mixed ration (P < 0.0001). Calcium intake from the separate source accounted for approximately 63% of the Ca intake of birds fed the 0.25% Ca mixed ration which is in contrast to birds fed the 1.00% Ca diet where 19% of their total Ca intake was provided from the separate source (P < 0.05). Total Ca intake as a proportion of total feed increased with increasing dietary Ca concentration (P < 0.001). Birds fed the low Ca diet consumed significantly less Ca than the other treatment groups (P < 0.05).

The influence of Ca concentration of the mixed ration on the apparent ileal digestibility of minerals is summarised in Table 2. Calcium digestibility was significantly lower for birds fed 0.25% Ca when compared to birds fed 0.50 and 0.75% Ca diets (P < 0.05). Dietary calcium concentration affected the digestibility of Cu with birds fed 0.75% Ca showing a significantly greater digestibility coefficient than birds from the other three treatment groups (P < 0.05). The ileal digestibility of P and K and Mg showed an inverse relationship to the Ca concentration of the basal diet. As Ca concentration increased, the digestibility of P, K and Mg decreased. Manganese and Zn digestibility were significantly greater in birds reared on 0.50% Ca diets when compared to those formulated with 0.25 and 1.00% Ca (P < 0.05). There was no effect of diet on the digestibility of Fe, Na and Sr.

Increasing the utilisation of phytate-P by broilers would reduce the dependence on inorganic sources of P and lower the total P currently used in poultry diets (Tamim and Angel, 2003). By reducing the concentration of dietary Ca and therefore limestone inclusion in the mixed ration may provide an opportunity for greater phytate-P utilisation through

increased phytase (endogenous and exogenous) efficacy (Angel et al., 2002). The results from this study show that lowering the Ca concentration of the basal diet while providing a separate source of Ca had no adverse effect on bird performance or bone mineralisation (toe ash %). In contrast, birds that were fed diets with 1.00% Ca consumed significantly more total Ca and had poorer performance than their counterparts and is most probably a result of the dietary Ca:P ratio. Further research investigating the use of phytase, dietary P concentration and commercial application are planned.

Table 2. Influence of basal dietary Ca concentration on the apparent ileal digestibility coefficients of selected minerals for broilers offered a separate source of Ca

	0.25% Ca	0.50% Ca	0.75% Ca	1.00% Ca	SEM[1]	P-value
Ca	0.208[a]	0.511[b]	0.456[b]	0.421[ab]	0.062	0.013
Cu	0.084[b]	0.073[b]	0.350[a]	-0.042[b]	0.038	< 0.0001
Fe	0.448	0.472	0.497	0.509	0.018	0.107
K	0.921[a]	0.893[ab]	0.867[b]	0.855[b]	0.011	0.002
Mg	0.335[a]	0.321[ab]	0.219[ab]	0.174[b]	0.038	0.017
Mn	-0.015[ab]	0.153[c]	0.042[bc]	-0.089[a]	0.033	0.0004
Na	0.148	0.081	0.194	-0.007	0.078	0.316
P	0.614[a]	0.584[a]	0.480[b]	0.446[b]	0.026	0.0004
Sr	0.222	0.334	0.270	0.214	0.039	0.125
Zn	0.072[a]	0.191[b]	0.098[ab]	0.013[a]	0.025	0.0007

[1]Pooled SEM value

[a-c] Within rows, values with different superscripts are statistically different ($P < 0.05$)

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SMALL SCALE VILLAGE CHICKEN PRODUCTION IN TIMOR-LESTE: IMPORTANCE, PRODUCTION CHARASTERISTICS AND MAJOR CONSTRAINTS

E.A. SERRAO[1], R. COPLAND[2], R.A.E. PYM[1], J. HENNING[1] and J. MEERS[1]

Village chickens are a socially and economically significant livestock species owned by 73% of rural families in Timor-Lesté. Despite these roles, the productivity and constraints to production of village chickens in this region have not been scientifically studied. Therefore, the objective of this study was to describe production characteristics and constraints to productivity of these chickens. A baseline questionnaire was used to record household details, management status (feeding and housing), productivity, mortality, health and marketing of village chickens in 308 households in twenty villages, from December 2008 to February 2009.

The study revealed that rearing village chickens was considered important for 77% (n=238) of families. Some 55% of farmers replied that their chickens and eggs were less utilised for domestic consumption while the cash from selling live chickens and eggs was important for approximately 92% and 36% of households, respectively. Almost 66% of flock management tasks (i.e. looking after the chickens, taking care of newly hatched birds and cleaning trough and shelters) were undertaken by women, while men were mostly responsible for nest and simple shelter preparation. Women and men had similar roles in trading the chickens. An average of 10.6±5.1 min/day were spent on feeding chickens, 4.4±6.4 min/day for looking after newly hatched chicks, 1.3±0.4hrs/month on nest preparation and 4.4±1.1hrs/month spent in cleaning troughs.

Supplementary feed and water was provided by 98% (n=301) of households. Approximately 84% (n=258) of families did not provide any shelter for their chickens. The average flock size in each family was 17.5±13.5 chickens. Each hen produced an average of 12.2±1.5 eggs/clutch, and about 3 batches /year. An average of 10 eggs was incubated in each clutch with the hatching rate of 85%. Only about 42% of chicks reached 6 wks of age, with predators and exposure to extreme climate being the major causes of loss. Mortality in grower and adult birds was estimated to be about 56.3% and 39.1% respectively; disease and predators were considered the most common cause of loss. Disease outbreaks were common in almost 55% of flocks and about 48% (n=148) claimed that this was caused by Newcastle disease (ND). Most disease outbreaks occurred during the dry season, particularly between August and November. Losses during a disease outbreak were approximately 90% of the flock. Lack of ND vaccination was reported throughout households where 95% did not have access to veterinary services. With this situation, ethnoveterinary practices were developed in some farmers to prevent and cure of their sick chickens.

Village chickens, contribute a critical supply of dietary protein to families in the country. In addition they provide immediate cash income for rural households, business opportunities for traders, and are used in religious and cultural ceremonies in rural and urban areas of Timor-Lesté. Disease, predators, lack of shelter and supplementary feeding, lack of veterinary services, poor management practices, and high mortality rate were the major constraints to the improvement of productivity of village chickens in the country.

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POTENTIAL FEED INGREDIENTS FOR SMALL SCALE POULTRY PRODUCTION IN TIMOR-LESTE

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Timor-Lesté is a developing country with an increasing population. The availability of animal protein especially from chickens is desirable. Presently, the demands for chicken meat are met mostly from overseas. There is a need for small-scale broiler production but the high price of imported feedstuffs is a significant constraint to the development of a local broiler industry. The aim of this study was to identify and characterise the nutritional quality of feedstuffs available locally. Feedstuffs were purchased from markets and farmers in Timor-Lesté. The samples were sun dried and subsequently analysed for dry matter, crude protein (CP), crude fat (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), minerals, tannins and phytate.

Three varieties of tuberous roots (cassava, sweet potato and taro), legumes (soya, mungo and red beans), nuts (candle nuts, coconuts and peanuts), five agricultural residues and wastes (cassava peels, banana peels, coffee skins, rice bran and rice husk) and a palm tree product (sago) were identified as possible broiler feedstuffs. Analyses, summarised in Table 1, revealed that most of the ingredients contained levels of CP, CF, NDF, ADF and minerals (Ca, P, and Fe) in the range reported in standard poultry nutritional references.

Table 1 - Composition of local feedstuffs

Ingredient group	Crude protein (g/kg)	Crude fat (g/kg)	Fibre (NDF and ADF) (g/kg)	Minerals (Ca, P and Fe) (mg/kg)
Tuberous roots (cassava, sweet potatoes and taro)	9.9-38.2	0.80-1.90	37-78 and 9-26.80	331-782, 725-1328, 11.6-38.8
Sago	2.2-28.0	1.00	205 and 69.8	2623, 776, 93.8
Legumes (soy, mungo and red beans)	192-404	3.8-42.7	129-286 and 67.5-142.8	883-3262, 2923-5362, 40.51-92.63
Nuts (peanuts, coconuts and candle nuts)	74.7-314	97.2-111	136-447 and 97-287	210-1489, 1703-6803, 47.05-50.03
Agricultural by-products (rice bran, rice husks and coffee skins)	15.4-100.6	1.1-7.3	365-816 and 256-654	502-5227, 215-4867, 323-817
Wastes (cassava and banana peels)	52.4-70.6	4.0-15.3	374-377 and 227-235	1396-6217, 1439-2083, 49.42-174

Tannin levels ranged from 0.2-0.33g/kg in tuberous roots, were 2.99g/kg in sago and below detection levels in beans and nuts. Coffee pulp, cassava peels and bananas peels contain tannins of about 5.5g/kg, 1.1g/kg and 1.3g/kg, respectively. All bean samples contained phytate ranging from 15.6 to 32.2mg/kg. Phytate levels in candle nuts and peanuts were similar (44.05 and 44.42mg/kg, respectively) and about double that in coconuts (26mg/kg).

Characterisation of these feedstuffs demonstrates that small scale broiler industries can be developed in Timor-Lesté without relying on imported feed. Problems such as availability of feedstuffs, low protein content, anti-nutritional factors, digestibility and effects on feed intake need to be considered when designing feeding strategies. Importantly, further research, especially digestibility studies, is required to allow the maximum utilisation of these potential broiler feed ingredients.

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EFFECT OF DIFFERENT ENZYME PREPARATIONS ON *IN VITRO* VISCOSITY OF WHEAT

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Wheat is a common raw material in Australian broiler diets because of its high starch and protein content. However, the main concern with wheat is the presence of soluble non starch polysaccharides (NSPs), especially arabinoxylans. Arabinoxylans may account for up to 61 g/kg of wheat dry matter (Choct and Annison, 1990). These NSPs are difficult to digest and may cause digesta to become viscous thereby reducing nutrient digestion and absorption. Many NSP degrading enzyme products are commercially available and are used in the poultry feed industry. This study was undertaken to compare the effect of different enzymes preparations on *in vitro* viscosity of different wheat varieties.

Samples of five varieties of wheat were ground and treated with 4 different enzyme preparations plus an untreated control. Enzymes were added at twice the recommended dose for mixed feed. Aliquots of these samples were heated at 65^{o}C in an oven for 16h to deactivate the endogenous glycanases as described by Fengler et al., (1990). Duplicate samples were incubated in water at 40^{o}C for 2 h and centrifuged and relative viscosity of the supernatant was measured.

Table 1. Viscosity (mpa.s) of 5 wheat samples subjected to different treatments[1]

Treatment	Wheat A	Wheat B	Wheat C	Wheat D	Wheat E
Control	3.24±0.04^{a}	6.17±0.68^{ab}	6.08±0.33^{d}	5.93±0.01^{a}	5.67±0.13^{c}
Heated sample	4.69±0.11^{d}	6.58±0.25^{b}	5.58±0.01^{c}	9.02±0.18^{c}	5.00±0.37^{b}
Wheat plus xylanase[2]	3.40±0.14^{a}	5.30±0.42^{ab}	3.59±0.13^{a}	7.49±0.01^{b}	4.70±0.06^{b}
Wheat plus phytase[3]	3.26±0.08^{a}	4.75±0.14^{a}	3.39±0.02^{a}	6.41±0.31^{a}	3.91±0.06^{a}
Wheat plus enzyme I[4]	3.97±0.07^{c}	13.4±0.42^{c}	4.31±0.02^{b}	8.44±0.47^{bc}	8.80±0.28^{d}
Wheat plus enzyme II[5]	3.71±0.09^{b}	12.7±1.13^{c}	4.64±0.14^{b}	9.31±0.76^{c}	9.58±0.10^{e}

[1] Mean ± SD, means within a column with different superscripts are different ($P < 0.05$)
[2]Porzyme 9310 ®, Danisco. [3]Phyzyme XP5000®, Danisco. [4]Enzyme I: Rovabio XL®, Adisseo. [5]Enzyme II: Rovabio Max®, Adisseo

Different wheats responded differently to heat treatment and enzyme supplementation, with the viscosity of some samples increasing ($P < 0.05$) with heat treatment and that of others decreasing. Of these, the most noticeable difference occurred with wheat D where there was a large difference in viscosity values with and without heat treatment. This was likely to be due to the presence of endogenous glycanases in this wheat which were deactivated by heat. Surprisingly, the phytase was as effective in reducing viscosity as the xylanase, the reason for which is yet to be elucidated. Enzymes I and II led to an increase in viscosity of wheats B, D and E. It is postulated that these enzymes may have affinity for the insoluble parts of the cell wall architecture, including the phytate complex in the aleurone layer, leading to solublization of previously insoluble NSPs in cell walls.

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ASSESSING AME AND DIGESTIBLE AMINO ACIDS OF DIFFERENT SOYBEAN MEALS BY NIRS AND BROILER PERFORMANCE

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Summary

It has long been realized that the nutritive values of major feed ingredients vary considerably not only among different feedstuff but also the same ingredients of different origins and production batches. Rapid screening to assess true nutritive value has been a great challenge in the feed industry. This study investigated soybean meal (SBM) samples of four different origins using both near infrared reflectance spectroscopy (NIRS) and broiler experiments. In Broiler Test 1, 4 SBM samples were assigned an identical "book values", so that the four dietary formulations were the same except different sources of SBM; In Broiler Test 2, individual NIRS prediction values were used for formulation so SBM inclusion levels varied to ensure diets were iso-energetic and iso-nitrogenous. The broiler studies consisted in eight treatments with 6 replicates using 16 male birds each, from day 1 to 40 of age. The results showed that when using the common values for the four SBM, broiler performed very differently in terms of live weight gain and feed conversion ($P<0.05$); whilst formulating based on individual NIRS values, the birds grew very similarly. The results clearly demonstrate that the nutritive values of the four SBM varied considerably among origins and largely differed by their levels of metabolisable energy (ME) and digestible amino acids (DAA) rather than crude protein level, and that the NIRS estimates for AME and DAA appeared to be largely correct.

I. INTRODUCTION

Numerous works have shown substantial variation in nutritional value of soybean meals (SBM) due to several factors such as genotype, crushing, anti-nutritional factors, implying a need for better and rapid characterization (De Coca-Sinova *et al.,* 2008; Karr-Lilienthal *et al.,* 2004). During the past two decades, Adisseo has been conducting *in vivo* studies to evaluate digestibility of amino acids (Green *et al.,* 1987) and energy (Bourdillon *et al.,* 1990) of SBM, the data were used to develop prediction models using the Near Infrared Reflectance Spectroscopy (NIRS), and validated by both *in vitro* (high performance liquid chromatography or HPLC) and *in vivo* tests. These NIRS models can be used to predict nutritional value of SBM with a reasonably good level of accuracy (Tang *et al.*, 2008).

The objective of the present study was to assess whether the NIRS is a more practical and accurate approach of nutritional assessment of SBM than average table values on nutrient composition of these ingredients.

II. MATERIALS AND METHODS

Through a collaboration with America Soybean Association (ASA), we collected four samples of SBM from 4 major producing countries (non-dehulled SBM from India, dehulled SBM from Argentina, Malaysia and USA), and predicted its nutritive values (Table 1) using

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NIRS equations for proximate nutrients, total and digestible amino acids and apparent metabolisable energy (AME).

Two sets of broiler performance experiments were carried out at Bangkok Animal Research Centre (BARC, Thailand) to evaluate the values of those 4 SBM, using corn-cassava-soybean meals-based diets. Besides SBM, all the other ingredients were identical and at the same inclusion levels in the formulations. The specifications of SBM, on the other hand, were based on either common SBM specifications (USA) or individual NIRS specifications.

The experiment used 768 newly hatched Arbor Acres FSY male broiler chicks allocated to a completely randomised design, each treatment consisted in 6 replicates and 16 male birds per replicate. The experiment was conducted in a closed house with tunnel ventilation and evaporative cooling system. Birds were raised on concrete-floor pens using rice hull as bedding material. Feed and water were provided *ad libitum*. The diets were provided in crumble form during the first 10 days and pellet form thereafter. Total pen feed consumption was recorded weekly. Body weight and feed intake as pen basis was measured for growth and feed conversion ratio calculation at 18 and 40 days of age. Mortality was recorded daily. Body weight gain, feed intake, feed conversion ratio and livability were calculated and were subjected to analysis of variance in randomized complete block design.

All formulations were iso-energetic and iso-nitrogenous, based on digestible amino acids. The starter diets contained ME 12.74 MJ/kg or 2950 kcal/kg, protein 211 mg/kg and digestible lysine 11.5 mg/kg (Table 2) and the grower diets had ME 12.96 MJ/kg or 3,000 kcal/kg, crude protein 190 mg/kg and digestible lysine 10.0 mg/kg (formulations not shown).

Table 1. Nutrient values (mg/kg) of the four soybean meals as estimated by NIRS

SBM source	India	Argentina	USA	Malaysia
Moisture	104.4	106.1	106.6	111.3
Fat	13.6	21.2	26.3	25.5
Crude fibre	60.5	37.5	39.4	22.8
Crude ash	80.4	68.1	62.2	55.4
ME (MJ/kg)	9.34	10.11	10.26	11.02
ME (kcal/kg)	2162	2340	2376	2550
Protein	460	465	475	475
Dig. Lys	23.6	24.3	24.6	26.9
Dig. Met	5.5	5.7	5.6	5.9
Dig. M+C	10.3	11.2	11.3	11.8
Dig. Trp	5.6	5.9	6.0	6.4
Dig. Thr	15.6	16.4	16.1	17.5
Dig. Arg	30.6	31.6	31.0	33.9

III. RESULTS AND DISCUSSION

Results presented in Table 3 illustrate a generally good performance. Overall performance in this trial was excellent; with the best treatment achieving live weight of 2908 g and FCR 1.624 at 40 days (Ross 308 standard for males is 2669 g and 1.664 FCR at 40 days), with a precision found at 2% variation for most performance parameters.

When all four soybean meals were formulated with the same soybean meal specification (US values, Treatments 1-4), broilers performed quite differently among treatments ($P<0.05$), ranking SBM values from low to high in the following order: India, Argentina, USA and Malaysia origins. The Indian SBM showed the poorest quality with 14 points higher in FCR and 100 g lower live weight as compared to the highest quality SBM of Malaysia origin.

On the contrary, when formulating with NIRS specifications (Treatments 5-8), the differences among the 4 SBM samples were minimised, all birds achieved similar live weight and FCR among the four treatments. These results suggest that the NIRS values be closer to the true nutritive values of the SBM than simply using book values since little difference among treatments resulted from formulating using the NIRS values.

Table 2. Composition of starter diets (1-18 days) *

	US soybean meal specs				NIRS soybean meal Specs			
SBM Source	Indian	Argentina	USA	Malaysia	India	Argentina	USA	Malaysia
Corn	406.0	406.0	406.0	406.0	383.6	406.0	406.0	456.5
Cassava	150	150	150	150	150	150	150	150
SBM India	345	-	-	-	352	-	-	-
SBM Argentina	-	345	-	-	-	343	-	-
SBM USA	-	-	345	-	-	-	345	-
SBM Malaysia	-	-	-	345	-	-	-	309
Full fat soybean	30	30	30	30	30	30	30	30
Soybean oil	22	22	22	22	37	24	22	7
Limestone	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.3
MDCP 21	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.8
Salt	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Na bicarbonate	2	2	2	2	2	2	2	2
Ch. chloride 60	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.3
L Lysine HCL	2.0	2.0	2.0	2.0	2.2	2.1	2.0	2.0
DL Met	3.0	3.0	3.0	3.0	3.3	3.0	3.0	3.1
L Thr	0.4	0.4	0.4	0.4	0.5	0.3	0.4	0.4

Other ingredients include (kg, for all diets): Maduramycin 1% 0.5; Mycotoxin binder 0.5; Pelleting binder 5; vitamins and minerals premixes 1. Nutrients:
ME 12.744 MJ or 2,950 Kcal/kg; Protein (actual mg/kg) 211, 210, 216, 201, 211, 211, 211, 211.
Lysine (actual, mg/kg): 13.3, 13.4, 13.8, 12.8, 13.3, 13.3, 13.3, 13.3.
Calcium 8.6 mg/kg; Available P 4.3 mg/kg; Sodium 2.2 mg/kg; Choline, ppm 1850
Digestible amino acids (mg/kg): Lys 11.5, Met 4.26; M+C 8.28; Try 1.84; Thr 7.13; Arg 12.08; Iso 7.48; Val 8.86.

Table 3. Performance of male broilers (0-40 days) fed on different soybean meals based on common (US) and NIRS specifications

SBM	Final weight, g	Weight gain, g	Feed intake, g	FCR	Livability %
Formulation based on common (US) specs					
India	2812^{b}	2771^{b}	4762	1.765^{d}	92.71
Argentina	2837^{b}	2797^{b}	4675	1.672^{b}	98.96
USA	2869^{ab}	2830^{ab}	4765	1.693^{b}	96.88
Malaysia	2908^{a}	2869^{a}	4656	1.625^{a}	98.96
Formulation based on NIRS specs					
India	2814^{b}	2775^{b}	4771	1.739^{cd}	96.88
Argentina	2815^{b}	2776^{b}	4710	1.711^{bc}	96.88
USA	2865^{ab}	2825^{ab}	4736	1.676^{b}	100.00
Malaysia	2831^{b}	2792^{b}	4686	1.690^{b}	98.96
Pooled SEM	20.54	20.55	30.73	0.008	1.82
C.V. %	1.77	1.80	1.91	1.13	4.56

^{abcd} means in the same column without a common superscript letter differ ($P<0.05$)

The economic values of the 4 soybean meals were estimated based on market prices of the feed ingredients and broiler carcasses. Results showed their values were quite different,

with as much as U$120/t difference between the top and bottom quality meals, of which over half came from the difference in AME values.

IV. DISCUSSION

This study result demonstrated that the 4 commercial soybean meals used are very different in their nutritive quality, as shown by NIRS prediction and their performances in the broiler diets. The data also confirmed that the quality of a soybean meal is largely defined by its levels of metabolizable energy (ME) and digestible amino acids, rather than crude protein levels as routinely judged in the most commercial practice.

When soybean meals of different qualities are formulated with their correct specifications of energy and digestible amino acids, they would be expected to produce similar levels of live performance. It means a lower quality soybean meal (with lower levels of energy and digestible amino acids) are able to produce good broiler performance if it is formulated with its correct nutrient values.

The NIRS determined estimates for energy and digestible amino acids as conducted for this trial appear to be largely correct, because when these values were used in formulation, the birds performed similarly.

A rather surprising finding from this trial was the large differences in energy between the soybean meals and the economic value of the energy differences. For example, between Malaysia and Indian meals there was almost 1.674 MJ/kg difference in ME, and the US and Argentine meals had about 0.84 MJ/kg ore ME than the Indian meal. This was picked up by NIRS, and confirmed by the performance results when these soybean meals were fed, indicating that the energy value of a soybean meal can vary greatly and has a large impact on its economic value.

V. CONCLUSION

In conclusion, the results clearly demonstrated that the nutritive quality of soybean meals is not the same, and their values are largely defined by their levels of metabolisable energy (ME) and digestible amino acids (DAA). This means that if the levels of ME and DAA of any soybean meal are known, then the quality and economic value of that meal can be calculated. A lower quality soybean meal (with lower levels of ME and DAA) may still produce good broiler performance if it is formulated with its correct nutrient values. The NIRS technology for ME and DAA estimation as conducted for this trial is promising.

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NUTRIENT DIGESTIBILITY OF SORGHUM AND WHEAT BY BROILERS: EFFECT OF AN ENZYME COCKTAIL

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Sorghum and wheat are the most important cereals used in the Australian poultry industry; both contain anti-nutritional factors and are usually incorporated into diets together. Feed enzyme cocktails are used to target a range of anti-nutritional factors and enhance the availability of nutrients. This study investigated the efficacy of a commercial enzyme cocktail on the nutrient digestibility of sorghum and wheat by broilers.

Two sorghum and two wheat diets (918g cereal/kg diet) were prepared and to one of each cereal diet, an enzyme cocktail (Natuzyme®, 0.35g/kg) was added. The diets were fed as mash to four replicate pens of 35-day-old broilers (8 birds/pen) in an environmentally controlled room. After a week (day-42), all birds were euthanased and intestinal contents collected and pooled/pen. Nitrogen, energy and acid insoluble ash (AIA) in feed and ileal samples, and phosphorus in excreta were analysed using standard scientific procedures and digestibility coefficients were computed (Table 1). Feed intake and excreta output were measured and apparent metabolisable energy (AME) was calculated.

Treatment	Ileal digestibility		AME (MJ/kg DM)	Digestibility coefficient
	IDE (MJ/kg DM)	Crude protein		Phosphorus
Sorghum	13.83^{ab}	0.776^{b}	14.38^{ab}	0.205
Sorghum + EC*	14.23^{a}	0.784^{b}	14.73^{a}	0.253
Wheat	13.81^{b}	0.788^{b}	13.92^{b}	0.190
Wheat + EC*	14.02^{ab}	0.813^{a}	14.18^{ab}	0.191
Pooled SEM	0.04	0.01	0.05	0.02

*EC: Enzyme cocktail; ^{ab} Means in column followed by different superscript are significantly different ($p<0.05$)

Enzyme addition increased ileal digestible energy (IDE) of sorghum and wheat by 2.9 and 1.5%, respectively. Similarly, the AME of sorghum and wheat was also increased significantly by 2.4 and 1.9%, respectively. Sorghum protein digestibility was only numerically increased (1.13%) while that of wheat was significantly increased (3.17%) with enzyme supplementation. Overall, the impact of cereal type was significant on protein digestibility (P=0.003) and AME (P=0.004), while that of the enzyme was significant for IDE (P=0.01), protein (P=0.008) and AME (P=0.041) but not phosphorus. No interaction between the cereal type and enzyme was recorded.

It can be concluded that the application of enzymes to both sorghum and wheat based diets can enhance nutrient utilization by broilers. However, to optimise the strategic application of feed enzymes further investigations are required.

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THE EFFECT OF DIGESTA VISCOSITY ON TRANSIT TIMES AND GUT MOTILITY IN BROILER CHICKENS

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Summary

The hypothesis that an increase in luminal viscosity would result in reductions in performance parameters, digesta passage rate and frequency of reverse peristalsis was tested. Chickens were fed diets containing varying quantities of guar gum, alginic acid or corn starch to influence digesta viscosity. The two guar gum treatments yielded the highest intestinal viscosities mirrored by very high feed conversion ratios, low starch digestibility and, for birds exposed to the highest level of guar gum, very low weight gain compared to the control birds. An association between a rise in ileal viscosity and low transit times was recorded; however at the highest viscosity, transit rates were significantly less than the control. Cloacally administered Cr-EDTA was retrieved from the gizzards of birds in all treatments. The marker was recovered in greater amounts in birds exhibiting lower ileal viscosities, with the exception of birds from the high level guar gum group, displaying faster passage rates and lower ileal viscosities.

I INTRODUCTION

The presence of viscous chyme in the lumen, due to diets high in soluble non starch polysaccharide (NSPs), results in longer retention times than diets low in NSPs or containing exogenous enzymes (Almirall and Esteve-Garcia, 1994; van der Klis and van Voorst, 1993). Variations in retention times may influence the performance of the broiler by affecting the time of exposure of digesta to the digestive enzymes and absorptive sites, as well as shaping the microflora composition present in the gastrointestinal tract GIT (Choct, et al., 1996).

The occurrence of reverse peristalsis will influence transit times. Reverse peristalsis, or reflux, has been identified in fasted and fed chickens, and is viewed as a mechanism by which the bird can maximise nutrient availability in the feed by prolonging the exposure of digesta to the digestive and absorptive sites along the GIT (Clench and Mathias, 1992; Clench and Mathias, 1995; Dziuk and Duke, 1972; Sacranie, et al., 2008). It is not known how a change in the flow dynamics of digesta, by an increase in viscosity, might affect its occurrence.

The following trial was conducted to investigate the inter-relationships between, intestinal viscosity, digesta passage rates and the occurrence of reverse peristalsis.

II MATERIALS AND METHODS

Two hundred and ten day-old male Cobb broiler chicks (Baiada Poultry Pty. Ltd., Kootingal, NSW, Australia), balanced for weight, were randomly divided into 7 groups and assigned to a separate level of a multi-brooder. A mash commercial diet plus one of three carbohydrates, alginic acid (A, a low viscosity NSP, 1.38 cP in 1% solution), guar gum (G, galacto-mannan, 4000 cP in 1% solution) and corn starch (S, approximately 73% amylopectin and 27%

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amylase, 340 cP in 1% solution); at either a 1 or 2 % dilution, was presented to six groups. The control birds received the commercial diet without a carbohydrate additive. An initial room temperature of 34^{o}C was maintained for the first 5 days and gradually decreased to 21^{o}C by 21 days of age. At 21 days of age, 12 birds from each group were moved and placed in individual cages for transit rate estimation and motility measurements. The remaining birds were weighed and randomly assigned to slide-in cages, 3 birds per cage. Birds had *ad libitum* access to both feed and water. At 27 days of age, the broilers were given access to feed for one hour. After feeding, six birds from each treatment group were weighed and a gelatine capsule containing approximately 0.15 g ferric oxide was placed on the oro-pharynx for spontaneous deglutition, without any previous habituation. The time was recorded in minutes from administration of the marker until first appearance in the faeces. At 28 days of age, 2 birds from each cage across all seven treatments were euthanized by CO_2 asphyxiation. Birds were dissected; the weights of the empty gizzard, pancreas and empty small intestine were recorded. Contents of the ileum were collected for starch digestibility and viscosity analysis.

At 30 days of age, one individually caged bird from each of the treatment groups was denied access to feed for one hour. After fasting, the chickens received a 1 mL injection of Cr-EDTA (2.66 mg of Cr) into their cloaca via a crop needle. Birds were placed back in their cages and given access to feed. After two hours post injection, the chickens were euthanised by CO_2 asphyxiation, dissected and digesta samples quantitatively collected from the gizzard, duodenum, jejunum and ileum. The process was repeated across replicates and treatments.

Digesta samples were weighed, freeze dried, dry matter (DM) calculated, digested in perchloric acid and hydrogen peroxide, and measured for mineral content using an inductively coupled plasma spectrophotometer (Binnerts et al., 1968). Total Cr in each section was calculated by multiplying the DM of a given section by the concentration of Cr recovered. The seven treatments were analysed using one way ANOVA, means were separated using Fisher's test at $p \leq 0.05$ significance level.

III RESULTS

The least desirable feed conversion ratio (FCR) was observed in birds from the G-2% treatment group, at 1.87, the average FCR was significantly higher ($p<0.001$) compared to chickens from the other six treatments, as shown in Table 1. The A-1% and C broilers displayed the lowest ($p<0.001$) FCR, 1.62 and 1.63, respectively.

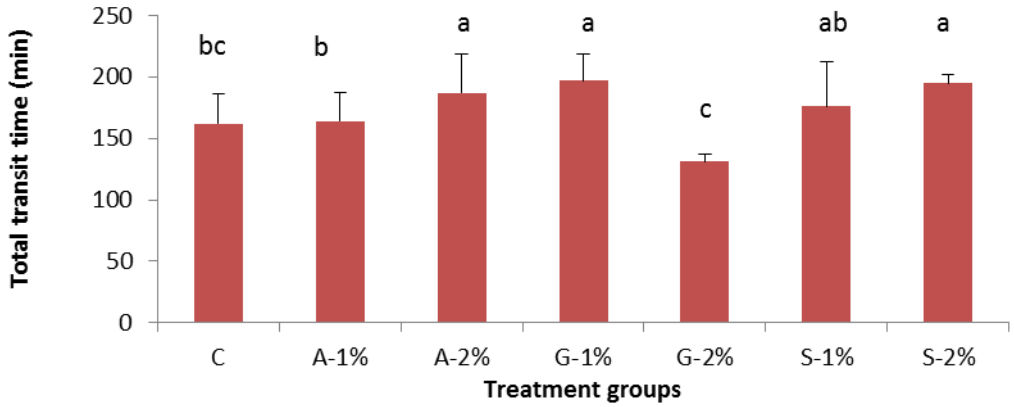

Figure 1. **Total transit time estimation with Fe_2O_3** a-c = values with no common letter differ significantly ($p<0.05$).

Analysis of the ileal contents of birds from the G-2% group revealed a strong effect on viscosity, with significantly higher ($p<0.001$) values (12.34 cp) than those recorded in the digesta of broilers from the other six treatments. Chickens fed the G-1% and S-2% diets had considerably higher ($p<0.001$) readings for ileal viscosity than the control (the least viscous). Starch was most readily ($p<0.001$) utilised in birds from the C, A-1%, S-1% and S-2% treatment groups. Chickens raised on the G-1% and G-2% feeds yielded the lowest ($p<0.001$) digestibility values for starch (0.81 and 0.79, respectively).

The longest retention time, 197 minutes, was recorded in the G-1% birds, significantly higher ($p<0.05$) than the C, A-1% and G-2% and comparable to the S-1%, S-2%, and A-2% treatment groups, as shown in Figure 1. The quickest rate of passage of the visual marker was observed in birds from the G-2% group, at 131 minutes, it was considerably faster ($p<0.05$) than birds from the other six treatment groups.

The soluble marker, Cr-EDTA, was retrieved from the gizzard contents of chickens from all seven treatment groups, as shown in Table 2. The highest ($p<0.001$) amount of Cr in the gizzard chyme was observed in birds from the G-2% group, comparable to levels in C, A-1% and A-2% treatments. The lowest ($p<0.001$) amounts of Cr in the gizzard digesta were recorded in broilers from the S-1% and S-2% groups. Compared to gizzard contents, levels of Cr decreased in the duodenum of birds across all the treatments. There was a tendency ($p=0.074$) for higher levels of Cr in the duodenum of birds from the A-1% group, similar to those observed in birds from the C treatment.

IV DISCUSSION

In general a negative relationship was observed between viscosity and performance parameters. The two guar gum treatments yielded the highest intestinal viscosities; the dramatic rise from a 1 to 2 % tended to initiate exponential rises in viscosity with increases in guar gum concentration. The high ileal viscosities in birds from these two treatments were mirrored by very high feed conversion ratios, low starch digestibility and for birds exposed to the highest level of guar gum; very low weight gain compared to the control birds in concurrence with previous studies (Bedford and Classen, 1992; Choct and Annison, 1992).

A longer retention time of digesta is associated with high intestinal viscosities. Slow moving intestinal contents provide a greater opportunity for microbial proliferation, poaching nutrients that could otherwise be used for growth (Choct, et al., 1996). There was an association between higher ileal viscosities and slower rate of passage of Fe_2O_3. The transit rates for the control and A-1% birds were in the range of expectation for broiler chickens exposed to low-viscous diets, at that age (Danicke, et al., 1999; Hughes, 2008). The longest retention times were observed in chickens from the A-2%, G-1% and S-1% treatment groups.

The results from the A-2% and S-1% treatment groups, in this study, suggest a possible positive relationship between a longer retention time of digesta in the lumen and performance. As already stated, starch digestibility was slightly lower in birds from both groups (significantly so, in the A-2% group) compared to chickens raised on the commercial diet, however, FCR remained relatively unaffected. In addition chickens from these two treatments had the highest body weights. While starch digestion was obviously retarded by the slight rise in viscosity, it was perhaps not enough to hinder absorption of its digestion products and other nutrients.

Interestingly, the G-2% birds displayed a significantly increased rate of passage of the marker, compared to chickens from the other six treatments, even though they possessed the highest ileal viscosity. Digesta are propelled along the intestines by migrating myoelectric

Table 1. Performance and digestive physiology characteristics at 28 days of age

Observations	Diets							P-values	√MSE
	Control	Alginic 1%	Alginic 2%	Guar 1%	Guar 2%	Starch 1%	Starch 2%		
Body weight (g)	966.8^{b}	1064.1^{ab}	1108.6^{a}	964.9^{b}	796.0^{c}	1011.9^{ab}	952.6^{bc}	**	118.79
Weight gain 21-28 days (g)	416.75^{ab}	432.73^{ab}	440.82^{a}	412.7^{ab}	352.69^{cd}	392.98^{bc}	339.74^{d}	**	40.42
Feed intake	680.2^{ab}	697.52^{ab}	726.96^{a}	704.6^{ab}	658.11^{abc}	642.21^{bc}	592.25^{c}	*	64.03
Feed conversion ratio (FCR)	1.63^{d}	1.62^{d}	1.65^{cd}	1.71^{bc}	1.87^{a}	1.64^{cd}	1.77^{b}	***	0.055
Ileal viscosity (cp)	1.55^{d}	1.56^{d}	1.91^{cd}	3.90^{b}	12.34^{a}	1.66^{d}	3.15^{bc}	***	0.98
Starch digestibility coefficient	0.93^{a}	0.93^{a}	0.87^{b}	0.81^{c}	0.79^{c}	0.88^{ab}	0.92^{ab}	***	0.04

a-d = values in a row with unlike superscripts differ significantly where; *p<0.05, **p<0.01 and ***p<0.001.

Table 2. Recovery of Cr-EDTA from the gizzard and small intestines at 30 days of age

Total Cr in sections (µg)	Diets							P-values	√MSE
	Control	Alginic 1%	Alginic 2%	Guar 1%	Guar 2%	Starch 1%	Starch 2%		
Gizzard	7.071^{a}	5.84^{ab}	4.89^{bc}	4.05^{bc}	7.48^{a}	3.13^{c}	2.72^{c}	***	2.91
Duodenum	1.68	2.75	0.78	1.31	1.02	0.76	1.36	0.074	1.69
Jejunum	2.32	2.72	2.27	1.79	1.55	1.47	1.19	NS	1.99
Ileum	5.36	40.10	5.10	22.72	8.81	14.38	18.94	NS	46.74
Sum	9.36	45.57	8.15	25.82	11.38	16.61	21.49		

a-c = values in a row with unlike superscripts differ significantly where; ***p<0.001

complexes in the smooth muscles of the intestines. Smooth muscle contraction on the orad side induces a rise in intraluminal pressure which is then absorbed by the corresponding relaxation of muscle on the aborad side, as the viscosity of the digesta increases so does the pressure required for propulsion (Lentle and Janssen, 2008). The pressure required to transport highly viscous digesta, as observed in the lumen of G-2% birds, may be reduced by the formation of a less viscous peripheral layer close to the mucosal surface if the intestines, this reduces friction and promotes flow (Lentle and Janssen, 2008). It is possible that the luminal contents of birds from the G-2% treatment, characterised by a highly viscous solid suspension fraction and a less viscous fraction containing the visual marker. The increased pressure of the smooth muscular contractions may have been partly absorbed by the fluid fraction; inducing a fast flow of this fraction and resulting in the rapid transit rates recorded in these birds, while ineffective in displacing the highly viscous solid suspension fraction.

The highest recovery of Cr was recorded in A-1% birds, equivalent to 1.7% of the total Cr introduced to each chicken, compared to 0.3% in control birds. Previous research conducted by the same group observed detectable levels in the crop of birds and that the majority of cloacally administered Cr-EDTA flows immediately into the ceca, and in addition, refluxed Cr from proximal sites collects in the ceca over time (Sacranie, et al., 2008). In the current trial samples for Cr analysis were not collected from the ceca or crops of birds or crop, due to laboratory limitations most likely contributing to the low total recoveries. The Cr results from the gizzard suggest an association with the transit times recorded in birds from this trial. The highest levels of Cr recovered from the DM of gizzard contents and the fastest rate of passage of the Fe_3O_2 through the GIT, was observed in the G-2%, C and A-1% birds, in that order. A very fast passage rate through the lumen as seen in the G-2% birds may reduce satiety, triggering a reverse motility response similar to what has been observed in fasted chickens, and often attributed by unique motility patterns referred to as rhythmic oscillating complexes or ROCs (Clench and Mathias, 1992; Clench and Mathias, 1995; Jimenez, et al., 1994; Sacranie, et al., 2008). The results from the G-2 % birds could represent an adjustment to the normal motility conditions along the GIT as a result of the fast transit times. Under these conditions, the occurrence of reflux may be up regulated.

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USE OF PROTEASE AND XYLANASE IN BROILER DIETS CONTAINING DISTILLERS' DRIED GRAINS WITH SOLUBLES

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Summary

Individual or combined effects of xylanase and protease on nutritive value of diets containing sorghum distillers dried grains with solubles (sDDGS) and fed to broiler chickens were investigated. A total of 480 day-old male broiler chickens were assessed in a 3 x 2 x 2 (0, 150 or 300 g sDDGS/kg diet, with or without xylanase, and with or without protease) factorial design. Each of the 12 treatments was replicated 5 times accommodating 8 birds per replicate. Feed intake (FI) and body weight gain (BWG) of the birds were increased by inclusion of sDDGS to the diets independent of enzyme supplementation. Feed conversion ratio (FCR) deteriorated as sDDGS was incorporated into the diets at both levels. Regardless of sDDGS and protease, xylanase significantly improved FCR. Digestibility of protein and most amino acids were adversely affected by inclusion of 150 and 300 g/kg sDDGS. While protease, individually, improved amino acid digestibility in birds offered diets containing the highest amount of sDDGS (300 g/kg), an admixture of xylanase and protease did not result in further improvement in amino acid digestibility. Addition of xylanase reduced the concentration of insoluble non-starch polysaccharides (NSP) in the ileum. Noticeably, the response of birds to xylanase supplementation on the concentrations of arabinose, xylose and total insoluble NSP was compromised when xylanase and protease were added to the diet simultaneously. To conclude, xylanase and protease in combination were effective for the growth performance of the birds on sDDGS, in particular improving FCR.

I. INTRODUCTION

The incorporation of DDGS in poultry diets is limited mainly by the presence of large amounts of NSP, lower nutrient digestibility and wide nutrient variation between grain sources and production batches. Results of our previous study (Barekatain et al., 2011) revealed that increasing levels of sorghum DDGS (sDDGS) in broiler diets adversely affected feed efficiency and protein digestibility. In addition, other reports indicate poor amino acid digestibility of DDGS in the diet of broilers compared to diets without DDGS (Kim et al., 2010). Thus far, little work has been done on the application of protease and its interaction with carbohydrases, xylanase in particular, on broiler diets containing DDGS. Hence, this experiment aimed to evaluate the interaction between exogenous protease and xylanase in broiler diets containing sDDGS with an emphasis on amino acid and protein digestibilities as well as NSP degradation.

II. MATERIAL AND METHODS

A 21-day experiment was conducted using 480 male day old Cobb-500 broiler chicks in a 3 x 2 x 2 factorial arrangement to evaluate the effectiveness of xylanase and protease and their combination in the diets containing three levels of sDDGS (0, 150 and 300 g/kg). Birds were allocated to 12 dietary treatments; each treatment was replicated 5 times with 8 birds per

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replicate cage. Diets were formulated to be isocaloric (13.01 MJ/kg as fed) and isonitrogenous (224.8 g/kg). Xylanase (Ronozyme WX, DSM Nutritional Products, Ltd.) and protease (Ronozyme ProAct, DSM Nutritional Products, Ltd.) were added at the recommended revels of 0.25 and 0.2 g per kg of diets, respectively. Feed and water were provided *ad libitum* throughout the study. At day 21, three birds per replicate were euthanised by cervical dislocation and ileal samples were collected and immediately frozen to measure the apparent ileal digestibility (AID) of protein, starch and amino acids. Insoluble NSP and free sugar contents of ileal digesta were quantified using gas chromatography according to the method described by Englyst and Hudson (1987). Data were analysed using general linear model in SAS (SAS/STAT 9.1) as a three-way ANOVA to assess the main effects of DDGS, xylanase, protease and their interactions. Fisher's LSD test was used to separate differences of variables at levels of $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Feed consumption ($P < 0.001$) and BWG ($P < 0.001$) were increased as sDDGS was incorporated in the diets (Table 1). While xylanase, independently, reduced FI of the birds, addition of protease increased feed consumption ($P < 0.001$) and BWG ($P < 0.01$) irrespective of sDDGS and xylanase inclusion. Poorer FCR was noticed as sDDGS was incorporated in the diet at both 150 and 300 g/kg. However, xylanase significantly improved ($P < 0.01$) FCR when assessed as a separate factor from sDDGS and protease. Higher FI and BWG in the birds fed sDDGS are in accordance with results reported by Olukosi et al., (2010). It is of importance to note that xylanase and protease had a degree of synergy towards the improvement of FCR in the birds fed diets containing sDDGS which was more pronounced at the highest inclusion of sDDGS.

Inclusion of sDDGS adversely affected ($P < 0.001$) the digestibility of crude protein (CP) and availability of all amino acids measured in this study, except lysine which remained unaffected by dietary treatment (data not shown). Xylanase supplementation did not influence amino acid digestibility and no interaction between xylanase and sDDGS was observed. Supplementation of protease to the diets significantly improved ($P < 0.001$) ileal digestibility of methionine (Met) and tyrosine regardless of sDDGS and xylanase addition. Moreover, combination of the two enzymes did not have a synergy in AID of amino acids. In contrast to CP digestibility values, response to protease was more pronounced for AID of amino acids showing 2 to 9 % improvement for Met digestibility at different levels of sDDGS inclusion. Despite the lack of effect of protease on AID of most amino acids, interaction between sDDGS and protease for most amino acids indicate that exogenous enzymes, protease in particular, are probably more efficient on the less digestible diets (Cowieson, 2010). In the current study, it is obvious from the results of AID of amino acids that protease was more effective in the form of sole treatment than in combination with xylanase. This may highlight a possible interference of xylanase through the action of protease that could prevent synergy between two enzymes in terms of AID of amino acids. Furthermore, the lack of effect of protease on protein and amino acid digestibility in the control and 150 g/ kg inclusion of sDDGS may suggest an effect other than simply improving degradation of protein which warrants further investigations.

As shown in Table 2, incorporation of sDDGS to the diets significantly ($P < 0.001$) increased the total concentration of insoluble NSP in ileal digesta of the birds, with the highest values on the 300g/kg sDDGS diet. Independently, there was a reduction ($P < 0.05$) in the concentration of insoluble NSP in the presence of xylanase in diets.

Table 1 Growth performance and ileal protein and starch digestibilities of the birds

Treatment			Growth performance (0-21d)			Ileal digestibility	
sDDGS (g/kg)	Xyl	Pro	BWG	FI	FCR	Protein	Starch
0	-	-	732.8^{d}	1020.8^{de}	1.394^{ef}	0.76^{ab}	0.94
	+	-	713.9^{d}	960.1^{e}	1.347^{f}	0.78^{a}	0.94
	-	+	764.6^{cd}	1103.9^{c}	1.447^{cde}	0.77^{a}	0.93
	+	+	769.4^{cd}	1068.4^{cd}	1.391^{ef}	0.75^{ab}	0.92
150	-	-	844.7^{ab}	1249.0^{ab}	1.480^{bc}	0.71^{c}	0.94
	+	-	844.8^{ab}	1220.8^{b}	1.447^{cde}	0.72^{c}	0.92
	-	+	899.5^{a}	1289.5^{ab}	1.436^{cde}	0.73^{bc}	0.93
	+	+	877.2^{ab}	1235.9^{ab}	1.409^{def}	0.73^{bc}	0.93
300	-	-	820.7^{bc}	1293.4^{ab}	1.577^{a}	0.67^{e}	0.93
	+	-	844.3^{ab}	1285.4^{ab}	1.523^{ab}	0.68^{de}	0.93
	-	+	865.2^{ab}	1297.7^{a}	1.502^{bc}	0.70^{cd}	0.94
	+	+	838.1^{ab}	1228.8^{ab}	1.467^{bcd}	0.68^{d}	0.93
SEM			7.409	6.537	0.0107	0.003	0.002
Mains effects and interactions							
DDGS			<.0001	<.0001	<.0001	<.0001	NS
Xylanase			NS	0.0071	0.0035	NS	NS
Protease			0.0083	0.0366	NS	NS	NS
DDGS × Xylanase			NS	NS	NS	NS	NS
DDGS × Protease			NS	0.0074	0.0239	NS	NS
Xylanase × Protease			NS	NS	NS	0.0492	NS
DDGS × Xylanase× Protease			NS	NS	NS	NS	NS

Means in a column not sharing a superscript are significantly different. NS = non significant
Xyl = Xylanase, Pro = Protease, SEM = Pooled standard error of means

Supplementation of protease did not significantly alter the concentration of insoluble NSP regardless of sDDGS and xylanase addition. However, an interaction between xylanase and protease was noticed for the amount of total insoluble NSP ($P < 0.01$), arabinose ($P < 0.05$) and xylose ($P < 0.05$). In this regard, there have been some reports on the negative impact of protease on the effectiveness of other exogenous enzymes (Ghazi et al., 2003, Naveed et al., 1998). Naveed et al., (1998) reported that the effect of carbohydrases was compromised when broilers were fed diets containing lupins supplemented with a mixture of carbohydrases and protease, thus they proposed a possible inactivation through the degradation of carbohydrases by the exogenous protease. The analysis of free sugar contents of ileal digesta revealed that a significant interaction ($P<0.05$) between xylanase and protease resulted in higher residual free sugars when xylanase and protease were added to the diets simultaneously. A likely explanation may lie in a possible inactivation of xylanase by exogenous protease which could result in higher undigested amount of free sugars in addition to any alteration in the soluble part of NSP as a result of enzyme and dietary treatments which was not studied in the present work. It can be concluded that a mixture of protease and xylanase may be beneficial to birds fed sDDGS in particular improving FCR, whereas individual application of protease is more effective for the BWG of the birds.

Table 2 Insoluble NSPs and free sugar contents of ileal digesta in day 21 of age

Treatment			Insoluble NSPs composition (g/kg TiO_2)			Free sugars (g/kg TiO_2)
sDDGS (g/kg)	Xyl	Pro	Arabinose	Xylose	Total	
0	-	-	3373.7^{cde}	2854.2^{f}	10242.7^{bcd}	3375.8^{d}
	+	-	2879.4^{fg}	2340.8^{g}	8873.2^{ef}	3404.2^{d}
	-	+	2775.7^{g}	2385.4^{g}	8603.8^{f}	3550.9^{bcd}
	+	+	3032.2^{efg}	2576.3^{fg}	9600.4^{def}	4174.2^{ab}
150	-	-	3661.8^{abc}	3910.7^{cd}	10748.7^{abc}	3565.8^{abcd}
	+	-	3224.3^{def}	3431.3^{e}	9710.1^{cde}	3758.3^{abcd}
	-	+	3464.2^{bcde}	3662.4^{de}	10177.0^{bcd}	3248.6^{d}
	+	+	3477.4^{bcd}	3496.4^{de}	10130.4^{bcd}	4084.6^{abc}
300	-	-	3845.5^{ab}	4704.6^{a}	11435.9^{a}	3434.7^{cd}
	+	-	3576.0^{abcd}	4145.8^{bc}	10591.0^{abcd}	3826.2^{abcd}
	-	+	3994.2^{a}	4772.3^{a}	11400.8^{a}	3271.0^{d}
	+	+	3824.7^{ab}	4414.8^{ab}	11117.3^{a}	4230.1^{a}
SEM			112.15	251.35	263.65	100.95
Mains effects and interactions						
DDGS			<.0001	<.0001	<.0001	NS
Xylanase			0.0446	0.0007	0.0467	0.0006
Protease			NS	NS	NS	NS
DDGS × Xylanase			NS	NS	NS	NS
DDGS × Protease			NS	NS	NS	NS
Xylanase × Protease			0.0185	0.0227	0.0034	0.0322
DDGS × Xylanase × Protease			NS	NS	NS	NS

Means in a column not sharing a superscript are significantly different. NS = non significant
Xyl = Xylanase, Pro = Protease, SEM = Pooled standard error of means

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BETAINE HCL IMPROVES CARCASS YIELD IN BROILERS

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Betaine hydrochloride (HCl) was fed in diets which were reduced in methionine, choline and energy (oil) in three replicated broiler trials. The PC (Positive Control) was a high specification corn- soybean meal diet. Levels of standardised ileal digestible (SID) lysine and methionine+cysteine were 1.20 and 0.84% in the starter (1-18 days) and 1.10 and 0.803% respectively in the grower (17-38 days). ME levels were in the range of 12.55-12.97 MJ/kg. The NC (Negative Control) was as PC with removal of 1.05 kg/t dl methionine, all added choline and 10 kg/t oil (0.209 MJ/kg). These modifications were achieved by a least cost formulation with lower levels of digestible methionine, digestible m+c, choline and ME. Betaine HCl was added to the NC in amounts of 1-2.5 kg/t. The NC plus betaine HCl diets were about $10/t lower cost than the PC and gave live performance equal to or slightly better than the PC. Measurements of carcass yield were taken and are shown in Table 1.

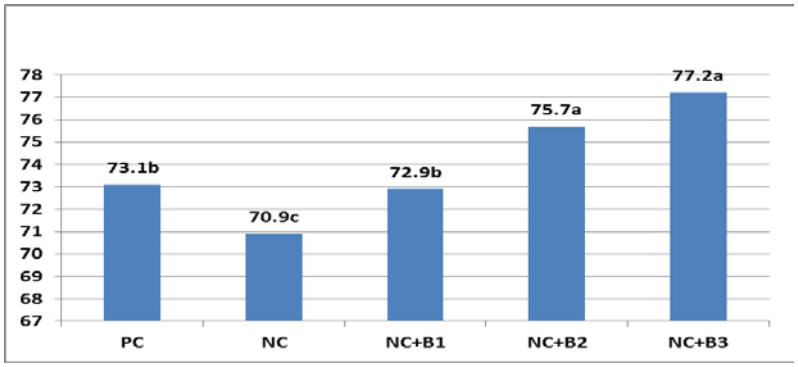
Table 1. Effect of betaine HCl on carcass yield (%) in three trials

Trial No.	PC	NC	Betaine HCl level			Significance
			1	2	3	
1 (Cobb 500)	66.8	65.7	66.2	67.6	-	NS
2 (Cobb 500)	73.3[b]	72.9[b]	74.2[ab]	75.1[a]	-	P<0.05
3 (Ross 308)	73.1[b]	70.9c	72.9[b]	75.7[a]	77.2[a]	P<0.05

[abc] P<0.05

A consistent finding in the 3 trials was reduced carcass yield in the NC, and then followed by improved carcass yield with added betaine HCl. The clearest effect was seen in Trial 3 which used Ross 308 strain (Figure 1). In all trials, carcass yield with the highest level of added betaine HCl was higher than in the PC. The reduced carcass yield in the NC would be due to a nutrient deficient diet giving a heavier viscera (Neoh and Ng, 2004). Addition of betaine HCl produced a lighter viscera. The osmolyte effect of betaine HCl allows for maintenance of ion and water balance in cells at a lower energy cost, reducing the energy cost of maintaining the digestive tract, leading to a lighter intestinal tract.

Figure 1. Effect of betaine HCl on carcass yield (%)



In summary, betaine HCl appears to be an economic way of improving carcass yield.

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PHYTASE SUPPLEMENTATION OF SORGHUM-BASED BROILER DIETS WITH REDUCED PHOSPHORUS LEVELS

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Summary

An average reduction of 1.74 g/kg nonphytate P in broiler diets from 1-42 days post-hatch substantially depressed feed intakes and weight gains but phytase supplementation essentially counteracted the reductions. This confirms phytase liberated phytate-bound P in sorghum-based diets; however, phytase responses in N retention and AID of amino acids were equivocal. The possibility that this is due to limited interactions between phytate and sorghum protein, particularly kafirin, is discussed.

I. INTRODUCTION

The application of exogenous enzymes to counteract anti-nutritive properties of targeted dietary components and improve broiler growth performance is routinely practiced. The performance of broilers offered sorghum-based diets may be inconsistent and is not usually enhanced by non-starch polysaccharide-degrading enzymes. However, amongst cereal grains, sorghum contains relatively high phytate concentrations (Selle et al., 2003) and it follows that broilers on sorghum-based diets should be advantaged by phytate-degrading enzymes. Nevertheless, there are indications that, in poultry, responses to phytase with sorghum are less than with other cereals (Wu et al., 2004; Pourreza and Ebadi, 2006). To investigate this aspect, the present study determines the effects of phytase in sorghum-based broiler diets with substantially lower phosphorus (P) concentrations generated by reduced dietary inclusion levels of meat-and-bone meal.

II. MATERIALS and METHODS

Positive control (PC) diets formulated to commercial standards were offered to an initial total of 840 male Cobb chicks on deep litter, from 1-14, 15-28 and 29-42 days post-hatch. The diets contained averages (g/kg) of 571 sorghum, 197 soybean meal, 100 canola meal, 58 meat-and-bone meal (MBM) and 25 rice bran. The PC diets contained 76, 54 and 44 g/kg MBM which was reduced to 20 g/kg in the starter diets and eliminated in the grower and finisher negative control (NC) diets. Compensation was not made for the analysed 36 g/kg P in MBM; consequently nonphytate-P levels were reduced from 4.40 to 2.51, 3.62 to 1.79 and 3.21 to 1.72g/kg in the three dietary phases. The NC diets were supplemented with 500 and 750 FTU/kg *Escherichia coli*-derived phytase (Phyzyme® XP) and each of the four dietary treatments were offered to six pens of 35 birds from 1-42 days post-hatch to determine their effects on growth performance. From 15-28 days post-hatch, five birds from each pen were relocated in cages in an adjacent facility for total collection of excreta to determine apparent metabolisable energy (AME) and nitrogen (N) retention. At day 28, the birds were

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euthanised and ileal digesta samples collected to determine apparent ileal digestibility (AID) of amino acids. More details for the bioassay methodologies adopted in the resent study are outlined by Selle et al. (2006).

III. RESULTS and DISCUSSION

Treatment effects on growth performance and nutrient utilisation are shown in Tables 1 and 2. From 1-42 days, reduced dietary specifications in the control diets significantly depressed weight gain by 20.3% and feed intake by 17.9% and tended to depress feed efficiency. On average, phytase inclusions in NC diets significantly improved weight gains by 20.7% and feed intakes by 21.9% but not feed efficiency. However, on the basis of gain-corrected feed conversion ratios (FCR), phytase improved feed efficiency by 8.5%. Phytase significantly increased AME by 0.29 MJ (2.0%); however, dietary treatments did not influence N retention. Also, phytase reduced mortality rates of birds offered NC diets from 6.7 to an average of 1.9%, which may have been related to the liberation of phytate-bound P.

Table 1 Effects of phytase supplementation (500, 750 FTU/kg) on growth performance of broilers offered modified (NC) sorghum-based diets

Treatment	1-14 days post-hatch			1-28 days			1-42 days post-hatch		
	Gain	Intake	FCR	Gain	Intake	FCR	Gain	Intake	FCR
PC	362^{a}	493^{a}	1.362^{a}	1384^{a}	2089^{a}	1.509^{a}	2644^{a}	4378^{a}	1.656
NC	286^{c}	431^{b}	1.508^{c}	1026^{c}	1748^{b}	1.704^{b}	2107^{c}	3595^{b}	1.707
NC + 500	343^{b}	503^{a}	1.468^{bc}	1293^{b}	2123^{a}	1.643^{b}	2553^{b}	4410^{a}	1.727
NC + 750	339^{b}	491^{a}	1.449^{b}	1303^{b}	2110^{a}	1.620^{b}	2533^{b}	4353^{a}	1.719
SEM	3.05	6.32	0.0201	11.73	34.82	0.0319	27.62	57.31	0.0184
Significance	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.053
LSD ($P < 0.05$)	9.01	18.6	0.0594	34.6	102.7	0.0941	81.5	169.1	-

Table 2 Effects of phytase supplementation on AME, N retention, gain-corrected FCR and mortality rates of broilers on modified (NC) sorghum-based diets

Treatment	Nutrient utilisation		Gain-corrected FCR (1-42 days)[1]	Mortality rate (%)
	AME (MJ/kg DM)	N retention (%)		
PC	14.48^{a}	62.0	1.582^{a}	3.89^{ab}
NC	14.23^{b}	62.7	1.847^{c}	6.67^{b}
NC + 500	14.52^{a}	65.3	1.690^{b}	2.22^{a}
NC + 750	14.52^{a}	62.1	1.690^{b}	1.67^{a}
SEM	0.0752	1.070	0.0231	1.1249
Significance	0.032	0.138	0.000	0.022
LSD ($P < 0.05$)	0.222	-	0.0682	3.319

[1]Corrected to the average gain of 2459 g on the basis that 100 g = 0.04 points in FCR

The effects of phytase on AID coefficients of 15 amino acids where acid insoluble ash was used as the dietary marker are shown in Table 3. It is evident that amino acid digestibility was higher in the NC than PC diets which may reflect the lower amino acid digestibilities of MBM in the NC diets. Phytase linearly increased ($P < 0.05$) the digestibility of isoleucine, methionine, valine and tyrosine and tended to enhance ($P < 0.10$) the digestibility of arginine, histidine, leucine, phenylalanine, threonine, alanine and glutamic acid.

Table 3 Effects of phytase supplementation AID coefficients of amino acids in broilers offered modified (NC) sorghum-based diets

Treatment	Arg	His	Ile	Leu	Lys	Met	Phe
PC	0.851^{b}	0.796^{b}	0.806^{c}	0.805^{b}	0.868^{b}	0.913^{b}	0.782^{b}
NC	0.887^{a}	0.837^{a}	0.836^{b}	0.836^{a}	0.907^{a}	0.911^{b}	0.815^{a}
NC + 500	0.892^{a}	0.845^{a}	0.853^{ab}	0.851^{a}	0.904^{a}	0.929^{a}	0.828^{a}
NC + 750	0.898^{a}	0.852^{a}	0.858^{a}	0.857^{a}	0.905^{a}	0.939^{a}	0.835^{a}
SEM	0.0043	0.0066	0.0064	0.0083	0.0055	0.0040	0.0089
Significance	0.000	0.000	0.000	0.001	0.000	0.000	0.002
LSD ($P < 0.05$)	0.0126	0.0195	0.0188	0.0244	0.0163	0.0177	0.0262
Linear phytase effect (P =)	0.091	0.089	0.021	0.084	0.766	0.000	0.078

Thr	Val	Ala	Asp	Glu	Gly	Ser	Tyr
0.711^{b}	0.793^{c}	0.794^{b}	0.767^{b}	0.836^{b}	0.761^{b}	0.717^{b}	0.789^{c}
0.770^{a}	0.823^{b}	0.826^{a}	0.825^{a}	0.869^{a}	0.803^{a}	0.811^{a}	0.828^{b}
0.788^{a}	0.841^{a}	0.839^{a}	0.835^{a}	0.881^{a}	0.810^{a}	0.815^{a}	0.846^{ab}
0.787^{a}	0.843^{a}	0.842^{a}	0.839^{a}	0.886^{a}	0.810^{a}	0.815^{a}	0.854^{a}
0.0075	0.0063	0.0091	0.0051	0.0060	0.0056	0.0080	0.0077
0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000
0.0221	0.0187	0.0267	0.0152	0.0176	0.0165	0.0236	0.0226
0.071	0.036	0.079	0.197	0.068	0.355	0.623	0.025

As a result of lower MBM inclusions in NC diets the average reduction in non-phytate P levels across the three diets was 1.74 g/kg, which is substantial. This is reflected in the marked depressions in weight gain and feed intake between PC and NC diets to 42 days; however, phytase supplementation of NC diets essentially reversed these reductions. NC diets contained an average of 3.15 g/kg phytate-P (or 11.2 g/kg phytate) of which 41% was derived from sorghum. Assuming phytase degraded 45% of dietary phytate this would release 1.42 g/kg P to compensate for non-phytate P reductions. This confirms the capacity of phytase to degrade phytate in sorghum-based diets and, under *in vitro* conditions, Chivandi et al. (2010) reported that phytase released fractionally more inorganic P from sorghum than maize.

While the capacity of microbial phytase to degrade phytate and liberate phytate-bound P is fundamental, the negative influences of phytate on nutrient availability are not limited to P. Indeed, they extend to amino acids, energy and other minerals and this forms the basis for the so-called "extra-phosphoric" effects of phytase (Ravindran, 1995). While AME was enhanced in the present study, phytase did not significantly improve the AID of the majority of amino acids or N retention. This raises the possibility that the "protein effect" of phytase was muted. The negative impact of phytate on protein/amino acid digestibility and its attenuation by phytase has received considerable attention in poultry (Selle and Ravindran,

2007) and is probably pivotal to the extra-phosphoric effects of phytase. In sorghum, the dominant protein fraction is kafirin and it may represent up to 70% of total protein (Hamaker et al., 1995). However, kafirin is hydrophobic, deficient in basic amino acids, with extensive disulphide linkages in the β-and γ-fractions located in the periphery of protein bodies in sorghum endosperm (Selle, 2011).

Logically, the extent to which phytate interacts with kafirin protein is crucial to the magnitude of any protein-related, extra-phosphoric responses to phytase in sorghum-based diets. While an alternative has been proposed (Cowieson and Cowieson, 2011), the accepted mechanism for direct protein-phytate interactions was initially outlined by Cosgrove (1966). Proteins carry a net positive charge under acidic conditions when pH is less than the isoelectric point of the protein. The negatively-charged phytate molecule may bind protein in binary protein-phytate complexes via basic amino acid residues (arginine, histidine, lysine). Moreover, proteins bound in insoluble protein-phytate complexes are refractory to pepsin digestion at very low pH levels (Vaintraub and Bulmaga, 1991). However, the paucity of basic amino acids in kafirin suggests that phytate may not readily bind kafirin in binary complexes. Thus, if phytate interactions with kafirin are limited, the magnitude of extra-phosphoric responses to phytase in sorghum-based diets would be less pronounced as a consequence. This would not, however, impact on the phytase-induced liberation of phytate-P in sorghum-based diets. It is possible that phytase supplementation of sorghum-based diets would be advantaged by the simultaneous inclusions of proteases and/or reducing agents to target the kafirin fraction of sorghum protein.

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PROBIOTIC AGENTS TO PREVENT REPRODUCTIVE TRACT INFECTIONS IN FREE-RANGE HENS

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The hen oviduct is the site for egg formation, and defence against pathogens in this organ is essential for the health of birds and production of safe eggs. Hens held under free-range conditions are exposed to various microbes that might influence the types of bacteria that colonise their intestinal and reproductive tract. Pathologies such as oophoritis, salpingitis, peritonitis, and metritis are frequently encountered at onset and during the laying period, causing increased mortalities and decreased egg production. One key problem facing the egg industry is that there are virtually no suitable agents for use against infections of the reproductive tract. In recent years, probiotics have been often proposed as a natural and useful choice. It has been recognised that probiotics can change the bacterial community structure in the avian gastro-intestinal tract, and the presence of lactobacilli in the cloaca and uterus of hens has been seen as an important factor in maintaining the microbial ecosystem and preventing the growth of pathogens, such as *Salmonella* (Van Coillie et al., 2007).

The objective of this study was to investigate the ability of two commercial probiotics applied in drinking water for 4 weeks (from 18 to 22 weeks of age) in reducing the occurrence of reproductive tract pathologies in laying hens, and improving their general health and performance. Six hundred and thirty, 17-wk old brown layers were transferred to a freshly cleaned free-range laying facility and randomly divided into three groups with three replicates of 70 birds each. Protexin® (IAHP, Australia) and Biomin® Poultry5Star (Biomin, GmBH, Austria) were administered in the drinking water of group 1 and 2 on a daily basis and at a dose of 1g/L and 20 g/1000 hens for 4 weeks, respectively. Group 3 was left untreated. Statistical analyses were performed using the GLM procedure of SAS (SAS Institute, 1996). Data on performance were based on a replicate basis and subjected to one-way ANOVA. If treatments were found to be significantly different, the Duncan's multiple range test was used to determine the statistical significance between treatment least-square means. For pathological findings, an unpaired t-test was used comparing two means (control and probiotic 1 or 2 treated birds) and determine the p-value. A 99% confidence interval for the true difference between the means was set. Differences among treatment effects were considered significant at $P < 0.05$. At 38 weeks of age the results demonstrated that treatment with both probiotics significantly reduced the occurrence of reproductive tract pathologies (control vs. probiotics, 66% vs. 33%; $P < 0.01$) and mortalities (control vs. probiotics; 3.3% vs. 1.5 and 1.9%; $P < 0.01$), and increased the performance of hens, for 20 weeks post-treatments (HDP control vs. probiotics 75% vs. 90%; $P < 0.01$). Birds treated with probiotics maintained their BW and egg weights at standard ranges, while untreated birds did not perform at this level. The results of this study provided some initial evidence that the use of a probiotic could be beneficial to control reproductive tract infections in hens. Overall, the data showed that probiotics not only improved the resistance of laying hens to reproductive tract infections, but also increased their general health, performance and liveability.

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A SYSTEMATIC GEOMETRIC APPROACH TO THE PREDICITION OF FEED ENZYME EFFICACY IN BROILERS

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The concentration of ileal undigested nutrients in feed ingredients has been shown to influence the effect of exogenous enzymes on nutrient digestibility and so it is possible to predict the magnitude of the effect of an exogenous enzyme by assessment of inherent digestibility of diets. However, the expense of *in vivo* assays is high and data output relatively slow and so the development of *in vitro* systems to simulate conditions in the chicken intestine can be useful if validated with *in vivo* data. A common problem with correlating *in vivo* data to *in vitro* response is that often there is inadequate variance in a given system to be able to generate accurate prediction equations. In order to overcome this, a total of 15 broiler diets were formulated using 7 commonly-used feed ingredients which were systematically titrated at varying inclusion concentrations. These 15 diets were geometrically related in that as, for example, corn inclusion was reduced in one diet from 22.5% to 7% in another diet, the inclusion of another ingredient e.g. sorghum, was increased proportionally. Importantly, the objective was not to examine the relative nutritional value of corn compared with sorghum but rather to use a geometric approach to create 15 diets with a wide range of chemical composition and *in vitro* characteristics. Once each diet was prepared it was balanced with essential amino acids and then mixed with a fixed component of oil, acid insoluble ash and minerals to minimise variance in macronutrient composition, divided into two equal batches and to one batch an enzyme admixture (xylanase, amylase and protease; Danisco Animal Nutrition, Marlborough, UK) was added. The resulting 30 diets were fed to a total of 1080 (6 replicate cages of 6 birds per cage) 17d broiler chickens for a period of 7 days. Up to d17 all birds received a common starter diet that contained a blend of all 7 test ingredients to aid physiological acclimatisation. Feed intake, weight gain and FCR were recorded from d17 to d24, on d23 excreta were collected for total tract fibre retention and on d24 all birds were humanely euthanized and ileal contents were collected for digestibility of starch, fat and protein. Each diet was analysed for starch, protein, fat, fibre and various *in vitro* parameters such as rate of starch digestion, viscosity, solubility, water holding capacity, bulk density and others. A least square model was generated using JMP v.8.0 (SAS) where the *in vitro* diet characteristics were used to predict the inherent digestibility of the diets as well as the response to the enzyme admixture for ileal and total tract nutrient digestibility. Feed intake ranged ($P < 0.05$) from 885.5 g/bird to 953.8 g/bird over the 7d experimental period. Body weight gain varied ($P<0.05$) from 475.2 g/bird to 538.2g/bird and FCR varied ($P<0.05$) from 1.69-1.97. The *in vitro* digestibility of starch at 30, 60, 120 and 240 minutes varied considerably by treatments (approx. 50-90%) and similar variance was observed in several other *in vitro* parameters, suggesting that the geometric formulation approach had been successful. Several correlations were evident between the ileal digestibility of the various diets and *in vitro* starch digestibility between 240 and 60 min negatively correlated with the ileal dry matter digestibility in response to feed enzymes ($R^2 = 0.717$, $P<0.01$) and the implications of these are being explored. It can be concluded that the inherent digestibility of starch, fat and protein in broiler diets can be predicted from *in vitro* measurements and that the magnitude of response to exogenous enzymes does depend on the characteristics of the diet to which they are added. Further analysis will reveal more about the complex interactions between the characteristics of the diet and enzyme response, however, it is apparent that a geometric and systematic approach is valuable when attempting to predict the bioefficacy of feed enzymes.

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PREDICTION OF *IN VIVO* STARCH DIGESTIBILITY RESPONSES TO PHYTASE SUPPLEMENTATION BY *IN VITRO* STARCH HYDROLYSIS

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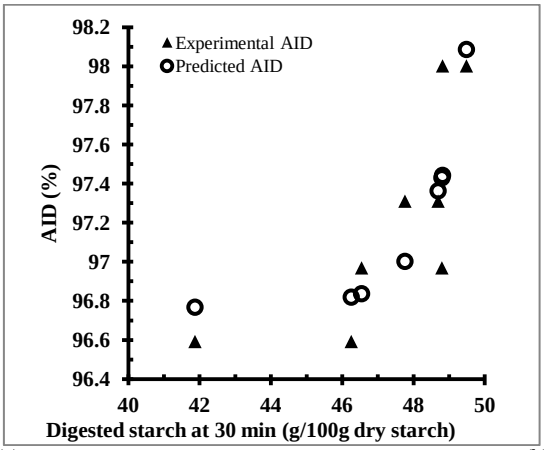
While *in vitro* techniques have obvious advantages, their validity is entirely dependent on the accuracy with which they translate into the *in vivo* situation. The effects of graded phytase inclusion levels (0, 500, 1000, 2000 FTU/kg) on apparent ileal digestibility of starch was determined in broiler chickens with six replicates per treatment. The birds were offered wheat-soybean meal diets containing acid insoluble ash that had been steam-pelleted at 85°C. At 21 days post-hatch 5 ex 35 birds from each pen were randomly selected and euthanized to collect digesta from distal ileum to determine apparent starch digestibility coefficients by standard methods. An adaptation of the Sopade and Gidley (2009) method was used to determine *in vitro* starch digestibility. Following pepsin treatment for 30 minutes at pH 2 starch was hydrolysed by pancreatin and amyloglucosidase at pH 6 and glucose concentrations were measured at 0, 10, 20, 30, 45, 60, 90 and 120 minutes from which starch digestibility was calculated. A first-order kinetic model (Liu and Sopade, 2011) was used to estimate starch hydrolysis rates. A comparison of *in vitro* starch digestibility at 30 minutes and apparent ileal starch digestibility (AID) is shown in Figure 1.



(a) (b)

Figure 1. prediction of *in vivo* apparent starch digestibility in distal ileum by *in vitro* starch hydrolysis at 30 min, $AID_{distal} = 96.767461 + 4.264e - 22 \times \mathrm{Exp}(D_{30})$, p=0.0142, Rsq=0.66

There is a significant relationship ($P < 0.015$) between 30 minute *in vitro* starch hydrolysis rates and the apparent digestibility of starch at the distal ileum of broiler chicks. Thus, this *in vitro* technique may prove valuable for the rapid estimation of starch digestibility of feed ingredients and complete diets in poultry.

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FURTHER IMPROVEMENT OF THE UNE NECROTIC ENTERITIS CHALLENGE MODEL IN BROILER CHICKENS

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Summary

A necrotic enteritis (NE) challenge model in broiler chickens has been developed and modified at the University of New England. However, detail on the roles of predisposing factors and the causative agent (*Clostridium perfringens*) on the performance and mortality of birds have not been thoroughly investigated. In this study, we examined the effects of fishmeal feeding, *Eimeria* inoculation and *C. perfringens* challenge on the growth performance and NE-associated mortality of the birds using a 2 × 2 × 2 experimental design. Fishmeal addition significantly elevated body weight gain during the second week, but this effect was not evident thereafter. *Eimeria* inoculation significantly reduced body weight gain in weeks 2, 3 and 4, but not in week 5. In contrast, *C. perfringens* challenge only reduced body weight gain of the birds in week 4. Additionally, interactions among the factors on body weight gain of the birds were detected in weeks 3 and 4. The combination of *Eimeria* inoculation and *C. perfringens* challenge significantly increased the NE-related mortality of the birds whereas 25% fishmeal addition in the starter diet did not show such an effect. We speculate that this may be due to the high crude protein level in the basal starter diet which reduced the effect of fishmeal in the corresponding treatment groups. It is suggested that the minimal level of crude protein required to induce necrotic enteritis would be an interesting topic for further investigation.

I. INTRODUCTION

Necrotic enteritis is one of the major diseases threatening the poultry industry, with outbreaks costing the world's broiler industries over $2 billion annually (Dahiya *et al.*, 2006). Challenge models utilised to reproduce the disease under experimental conditions have been used and under development for more than thirty years (Al-Sheikhly and Truscott, 1977; Baba *et al.*, 1992; Branton *et al.*, 1997; Cowen *et al.*, 1987; George *et al.*, 1982; Hofacre *et al.*, 2003; Prescott, 1979). However, reproducibility of the models to induce clinical NE has been highly sporadic with mortality and morbidity rates varying between 0% and over 60% (Baba *et al.*, 1992; Hofacre *et al.*, 2003). At the University of New England, an NE challenge model was developed (Kocher *et al.*, 2004) and subsequently modified to increase reproducibility of the model (Wu *et al.*, 2010). The important contributing factors to introduce consistent infections of birds in the models are the predisposing factors such as dietary protein level and *Eimeria* infection. Yet the detailed investigation into the roles of these predisposing factors as well as the causative agent of the disease, (the bacterium *C. perfringens*), has not been described extensively in the literature. In this study, we aimed to examine the roles of dietary fishmeal inclusion, *Eimeria* inoculation and *C. perfringens* challenge on the growth performance and NE infection, to further improve the model.

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II. MATERIALS AND METHODS

Forty eight floor pens bedded with pine shavings each housed 28 one day-old broilers (1344 male Cobb 500; 40.8±0.1g: Baiada Country Road Hatchery, Tamworth, NSW, Australia) for the first 7 days of the experiment and were then culled to 25 per pen on day 7. Thus, 1200 birds were then used for the remaining 4 weeks in a temperature-controlled room (33–34^{o} C during days 1 and 7, decreased by 3^{o} C each week to 21–23^{o} C by the third week) at Kirby research station at the University of New England. The birds were subjected to artificial fluorescence illumination of 16 hr per day. The treatments were arranged in a 2 × 2 × 2 factorial design with or without: 25% fishmeal (F±) feeding from day 8 to 14, *Eimeria* inoculation on day 9 (E±), and oral gavage *C. perfringens* challenges on days 14 and 15 (C±). Each pen was assigned to one of the 8 treatment groups with six replicates per treatment in a blocked design to minimise cross-contamination of pathogens between treatments. All the birds were raised and handled humanely, and the animal trial was approved by the Animal Ethics Committee of the University of New England. Nutrient and dietary composition of the starter and finisher diets followed a previous trial (Wu *et al.*, 2010) except that an additional 7% soy bean meal was added to bring the diet up to acceptable nutrient specifications for growing broiler chicks (21.5% CP). Birds had *ad libitum* access to feed and water throughout the experiment. Live weights of the birds were determined on days 7, 14, 21, 28, and 35 of the experiment. Bird mortality was recorded daily. The NE challenge was performed based on previous experiments (Wu *et al.*, 2010) except that only two inoculations of *C. perfringens* were given. One ml of *C. perfringens* type A strain EHE-NE18 (Keyburn *et al.*, 2006) at a concentration of 10^{8}–10^{9} CFUs/ml was administered by oral gavage to birds in the appropriate cages. Performance data were analysed using the statistical package IBM® SPSS® Statistics package version 19 (IBM Corporation). The main effects of fishmeal addition, *Eimeria* inoculation and *C. perfringens* challenge, and their interactions on bird performance were examined by analysis of variance using the General Linear Model. As mortality data were not normally distributed, these were analysed by the nonparametric Kruskal-Wallis test.

III. RESULTS

Performance of the broiler chickens was analysed for the whole period of treatment and on a weekly basis to detect the effects of fishmeal addition, *Eimeria* inoculation and *C. perfringens* challenge and their interactions. Fig. 1 shows the responses of body weight gain during the whole five weeks of the experiment to these three factors. Overall, fishmeal addition in the starter diet during the second week significantly elevated ($P < 0.05$) body weight gain, whereas *Eimeria* inoculation on day 9 and *C. perfringens* challenge on days 14 and 15 significantly reduced body weight gain ($P < 0.001$ and 0.05 respectively). No interactions were observed. When the responses were investigated weekly, fishmeal addition only significantly increased body weight gain during week 2 ($P < 0.001$). *Eimeria* inoculation significantly reduced body weight gain during weeks 2, 3 and 4 ($P < 0.001$, $P = 0.001$, and $P < 0.001$, respectively), but not in week 5. In contrast, *C. perfringens* challenge only reduced body weight gain of the birds in week 4 ($P < 0.01$).

The mortality caused by NE is shown in Fig. 2. While the birds were gavaged with *Eimeria* on day 9 and challenged with *C. perfringens* on days 14 and 15, the NE mortality was significantly higher than other groups regardless of fishmeal feeding. However, the individual *Eimeria* or *C. perfringens* inoculations did not significantly lead to an increase in NE mortality.

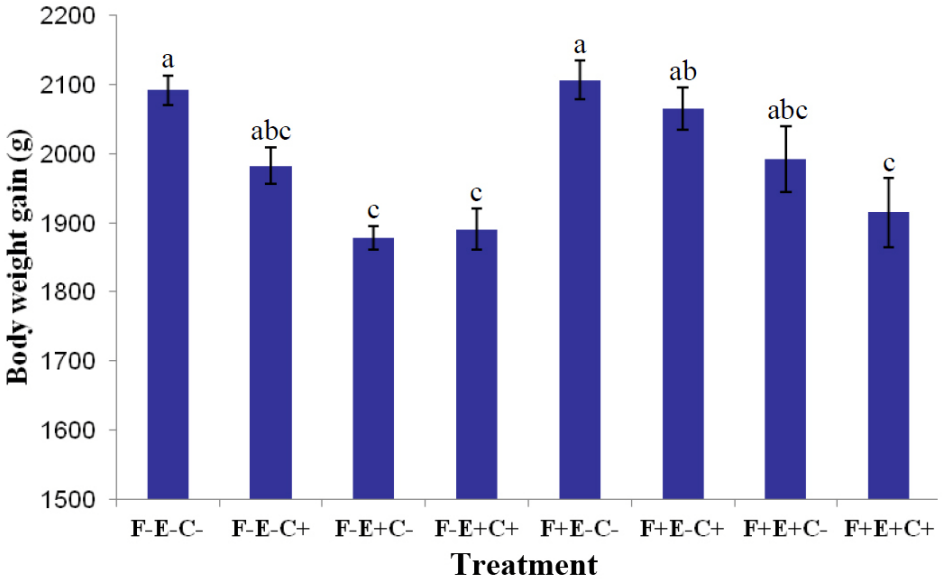

Figure 1. Body weight gain of chickens in groups with or without 25% fishmeal addition in the starter diet during days 8 to 14, *Eimeria* inoculation on day 9, and *C. perfringens* challenge on days 14 and 15 of the trial. Columns not sharing a same letter are significantly different ($P < 0.05$). The abbreviations are: F, fishmeal addition; E, *Eimeria* inoculation; C, *C. perfringens* challenge; +, the treatment applied; -, no such treatment.

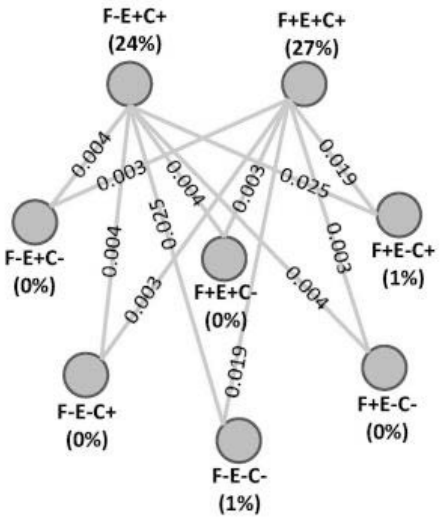

Figure 2. The NE-caused mortality of the birds in groups with or without 25% fishmeal addition in the starter diet during days 8 to 14, *Eimeria* inoculation on day 9, and *C. perfringens* challenge on days 14 and 15 of the trial. The levels of mortalities (%) are shown in the brackets underneath the names of treatments. The mortalities are significantly different between two treatments if the pair is linked by a line on which P value is shown. The data were analysed using nonparametric Kruskal-Wallis test.

IV. DISCUSSION

Predisposing factors are critically important in NE outbreaks and thus for the successful introduction of clinical and/or subclinical symptoms of the disease in an experimental model. At the University of New England, a NE challenge model was established about a decade ago (Kocher *et al.*, 2004), and subsequent modifications have been made (Wu *et al.*, 2010). However, the individual roles of predisposing factors as well as the dietary nutrient influence were not yet fully understood. In this study, we examined the effects of fishmeal feeding, *Eimeria* inoculation and *C. perfringens* challenge on bird performance and mortality caused by NE.

Surprisingly, we found that 25% fishmeal addition in the starter diet during the second week did not contribute to the occurrence of the disease. This seems contradictory to the long-held belief that high animal protein is one of the essential factors predisposing poultry to NE

(Cowen *et al.*, 1987; Drew *et al.*, 2004; Prescott, 1979). The result obtained in this study is also different from what we have shown in our previous report regarding the role of fishmeal addition in the starter diet (Wu *et al.*, 2010). In the previous trial, nutrient analysis showed that the crude protein level in the starter diet was 19%, which is lower than the level of industry recommendation. Therefore, in this study, we adjusted the crude protein level up to 21.5% by adding soybean meal. We cannot yet conclude that fishmeal effect was compensated by higher crude protein level in the starter diet, it will be interesting to understand the minimum level of crude protein in the diet that can induce NE together with the challenge by both *Eimeria* and *C. perfringens*. Therefore, further investigation into this topic is warranted.

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THE INTERACTIVE EFFECTS OF VITAMIN D, PHYTASE, CALCIUM, AND PHOSPHORUS IN BROILER PERFORMANCE AND SKELETAL INTEGRITY

L.C. BROWNING[1], C. ANTIPATIS[2] and A.J. COWIESON[1]

Summary

Vitamin D is essential for calcium (Ca) and phosphorus (P) absorption and utilization within the chicken. Higher vitamin D levels in the diet may increase Ca and P absorption and utilization and improve bone strength and subsequently leg health. In this experiment, increased vitamin D3 (5,000 vs 10,000 IU/kg) levels did not influence Ca or P retention nor did higher vitamin D change bone health. However, exogenous phytase significantly improved FCR on those diets low in Ca and available P. Calcium and strontium (Sr) retention showed a direct linear correlation (R^2=0.98) suggesting they are perceived similarly by nutrient transport infrastructure.

I. INTRODUCTION

The metabolism of vitamin D, calcium and phosphorus within the chicken is uniquely integrated. The absorption of intestinal calcium depends on many factors but one of the most important is vitamin D (Ameenuddin, Sunde et al. 1985). There is obscurity regarding the exact mechanisms of calcium absorption across the intestine, however it is known that vitamin D is essential for the synthesis of calcium binding protein (CaBP) in the cells of the intestinal wall and CaBP actively transports calcium across the epithelial wall into the plasma (Wasserman and Taylor 1966). Recent research on the modern broiler genotype indicates there is an increased physiological demand for calcium (Whitehead, McCormack et al. 2004). Unfortunately, high levels of calcium in the diet interfere with the availability of other minerals such as P, magnesium (Mg), manganese (Mn) and zinc (Zn) (Vandepopuliere, Ammerman et al. 1961). One way to minimize the negative effects of Ca on P availability in the chicken may be to increase vitamin D concentration in the diet to escalate calcium absorption and retention rather than increase calcium levels *per se*. The addition of higher levels of vitamin D may also increase the rate of absorption of phosphorus thereby also reducing its availability to form insoluble calcium salts.

Vitamin D has also been shown to increase phytate digestibility and reduce the rachitogenic nature of low calcium and high phytate diets in chickens (Mellanby 1950; Steenbock and Herting 1955). It has been shown that low levels of dietary calcium fed with elevated levels of vitamin D permit greater utilization of calcium and phytate-P and reduce the requirement for inorganic P (Mohammed, Gibney et al. 1991). Subsequently, it was demonstrated that vitamin D and exogenous phytase have a synergistic effect when fed together to broiler chickens (Edwards 1993). This synergistic effect of vitamin D when fed in combination with exogenous phytase was shown to improve leg health by significantly increasing tibia strength and tibia ash (Philipps, Aureli et al. 2007).

Finally, vitamin D and strontium (Sr) have a close physiological relationship following similar metabolic pathways to calcium absorption, bone deposition, and renal re-sorption. In poultry feeding, the major sources of Sr in the diet are the bulk mineral ingredients such as limestone powder or chips, dicalcium or mono-calcium phosphate. Excessive Sr has been shown to produce rickets because strontium inhibits vitamin D metabolism in the kidney (Corradino and Wasserman 1970). Conversely, small amounts of Sr

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(in combination with 800 IU vitamin D + 1000mg Ca per day) significantly reduced the number of fractures in older women patients with osteoporosis (Meunier, Roux et al. 2004). The exact role of Sr in poultry nutrition requires further investigative research. It was therefore the purpose of the experiment reported herein to explore the interactive effects of vitamin D, Ca, P and phytase in broiler chickens and to elucidate the effects of these on the retention of Sr and other minerals.

II. MATERIALS AND METHODS

A total of 288 day old male Cobb broiler chicks were fed for 28 days in an experiment with 8 treatments and 6 replicates in a complete 2x2x2 factorial design (36 birds per treatment) (Table 1). All diets were fed *ad libitum* in a mash form with the starter diet being fed to 14 days of age followed by the finisher diet to 28 days of age. The principal ingredients in both diets were wheat, soybean and canola meal. On the 20th day of the trial, a 48 hour total collection procedure was undertaken to measure mineral retention. On day 28, all birds were humanely sacrificed and measurements of bodyweight (BW), feed intake (FI), toe ash, tibial length, tibial dyschondroplasia (TD), and the retention of the minerals calcium, phosphorus, magnesium, strontium and sodium were undertaken. The Ca:AvP ratio of 2:1 was used based on the recommendation of the NRC (1994) and current commercial practice.

Table 1. Summary of treatments

Treatment	1	2	3	4	5	6	7	8
Ca /Av.P g/kg	9/4.5	9/4.5	9/4.5	9/4.5	6/3	6/3	6/3	6/3
Phytase, FYT	0	1000	0	1000	0	1000	0	1000
Vit D_3 IU/kg	5000	5000	10000	10000	5000	5000	10000	10000

III. RESULTS

The addition of higher levels of vitamin D did not produce a significant difference in BW, feed conversion ratio (FCR) or mineral retention (Table 2). However, exogenous phytase improved FCR when added to the low Ca/P diets but not in diets with high or adequate levels of Ca/avP, resulting in a diet*phytase interaction ($P<0.05$). The addition of exogenous phytase increased P retention in the low Ca/avP diet but not in the high Ca/avP diet resulting in a significant diet*phytase interaction. The diet with reduced Ca/avP returned poorer ($P<0.01$) FCR but superior ($P<0.01$) Ca, P and Sr retention compared with the high density diet. Furthermore, there was a direct linear correlation between Ca and Sr retention (R^2 = 0.98). Bone mineralization was not changed in any treatment group as measured by toe ash, nor was there any difference in TD prevalence (data not shown), growth rate and FCR.

IV. DISCUSSION

The lack of response to the addition of vitamin D was not expected and is contrary to the results of Whitehead et al (2004) who found that 10,000 IU D3/kg in broiler diets maximized growth and tibia ash. BW and FCR were close to Cobb500 breed standard of 1324g and 1.45 respectively at 28 days of age. Similarly, the fact that higher levels of vitamin D did not produce an additive or synergistic response with exogenous phytase is in contrast to the findings of Edwards (1993) who found the highest retention of phytate-P (79.4%) was

Table 2. Summary of Bird Performance and Mineral Availability

Ca/avP (g/kg)	*Phytase (FYT/kg)*	*Vitamin D (IU/kg)*	*D28 BW (g/b)*	*FCR (g:g)*	*Ca Ret (%)*	*P Ret (%)*	*Sr Ret (%)*	*Toe Ash (%)*
9/4.5	0	5000	1322	1.48[a]	47.02[a]	56.05[ab]	32.93[a]	14.5
9/4.5	1000	5000	1313	1.51[ab]	43.94[a]	50.32[a]	29.69[a]	15.0
9/4.5	0	10000	1258	1.54[ab]	46.87[a]	55.99[ab]	32.77[a]	14.6
9/4.5	1000	10000	1285	1.53[ab]	46.35[a]	55.35[ab]	31.83[a]	14.5
6/3	0	5000	1286	1.59[ab]	58.30[b]	56.30[ab]	43.22[b]	13.8
6/3	1000	5000	1296	1.54[ab]	62.10[b]	58.80[b]	47.15[b]	15.2
6/3	0	10000	1273	1.60[b]	58.19[b]	56.66[ab]	43.48[b]	14.0
6/3	1000	10000	1286	1.54[ab]	61.06[b]	58.00[b]	43.96[b]	14.1
P			*NS*	*<0.05*	*<0.01*	*<0.01*	*<0.01*	*NS*
Pooled SEM			*27.067*	*0.024*	*1.872*	*1.585*	*1.788*	*0.380*
Main Effects								
9/4.5			1295	1.51	46.05	53.68	31.80	14.7
6/3			1287	1.57	59.91	57.44	44.45	14.3
P			*NS*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*NS*
	0		1286	1.55	52.60	56.25	38.10	14.2
	1000		1297	1.53	53.36	54.87	38.16	14.7
	P		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
		5000	1304	1.53	52.84	55.37	38.25	14.6
		10000	1277	1.55	53.36	55.75	38.01	14.3
		P	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Interactions								
Ca/avP*Phytase			*NS*	*<0.05*	*NS*	*<0.01*	*NS*	*NS*
Ca/avP*Vitamin D			*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Phytase*Vitamin D			*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Ca/avP*Phytase*Vitamin D			*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

**Within individual columns means not connected by same letter are significantly different.*

obtained when both phytase and additional vitamin D were present in the diet. An explanation for the response in this experiment to extra vitamin D may be that the differential between 5,000 IU D3/kg and 10,000 IU D3/kg was insufficient to detect differences in Ca and phytate-P utilization. In future research higher levels of vitamin D will be investigated.

There was a significant improvement in FCR by the addition of phytase to low Ca/avP diets. This positive effect of exogenous phytase is of great economic advantage because FCR is one of the most critically important values in commercial broiler production. The addition of phytase to the low density diet also improved both Ca and P retention which is interpreted as an improvement in phytate-P digestibility and a reduction in the antinutritive effect of dietary phytate on cation solubility. This result is in line with many researchers who have clearly demonstrated that exogenous phytase improves Ca and P retention (Denbow, Ravindran et al. 1995).

Reduced dietary Ca/avP concentrations were associated with increased efficiency of Ca retention as compared to the four high Ca/avP diets which no doubt indicates a physiological response by the chicken to overcome a Ca deficiency by up-regulating its nutrient transfer and deposition infrastructure. Sr retention was also significantly improved when Ca and available P levels were reduced. The improved retention of Sr was directly

proportional to Ca, presumably because these minerals are elementally similar. To the authors knowledge this is the first time that this relationship has been demonstrated. It maybe hypothesized that Sr uses similar metabolic pathways to Ca for intestinal absorption and bone mineralization. There is no current data on the strontium content of feed ingredients or on the effects of small amounts of strontium on basic parameters such as BW, FCR and toe ash. There is a need for future research into strontium in broiler nutrition in lieu of its close interrelationship with calcium and vitamin D.

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THE EFFECT OF MARINE CALCIUM SOURCE ON BROILER LEG INTEGRITY

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Summary

Leg weakness and lameness are important welfare issues in the poultry industry and may be influenced by nutrition in its broadest sense. Dietary calcium is commonly provided by limestone. However when included at high concentrations limestone can reduce phosphorus digestibility and this may lead to reduced skeletal integrity. Provision of an alternative highly digestible calcium source at lower dietary concentrations may circumvent this problem. The study reported herein aimed to explore the potential of a highly soluble marine calcium source (Calcified Seaweed) compared to limestone, with and without phytase, on broiler performance and leg health. High body weight was found to negatively ($P < 0.01$) influence leg health as determined by a Latency-to-Lie test (LTL). It can be concluded that feeding broilers diets with reduced total calcium concentrations (0.77% vs 1.0%) (particularly if digestible calcium concentrations are maintained) can improve leg strength. Modification of diets to include excess levels of the more digestible marine calcium source (0.77%) and lower inclusion concentrations of limestone (0.60%) increased LTL times and risks of reduced leg health.

I. INTRODUCTION

The modern broiler chicken has been genetically selected for rapid growth, increased muscle mass and heavier breast weight (Garner et al., 2002). This increase in production may also be associated with poor leg health and lameness and is often linked to skeletal abnormalities due to reduced bone mineralisation. Poor leg health and lameness affects millions of broiler chickens worldwide, with lame birds having significantly reduced performance and altered behaviour patterns (Weeks et al., 2002; Berg and Santora, 2003). Poor leg health in the broiler industry is often attributed to reduced bone quality. Studies have shown that the dimensions of the tibiotarus of the modern broiler are the appropriate size for weight bearing, however, the bone itself is weak (Sherlock et al., 2010). The cortical bones of broilers have lower mineralisation which is attributed to rapid deposition in the outer layers to increase cortical width to support the increased weight of the bird (Sherlock et al., 2010).

Calcium (Ca) and phosphorus (P) are two of the most abundant minerals in bone and form the majority of the inorganic matrix. The structure and composition of bone vary according to the age and nutritional status of the bird, with the extent of bone mineralisation influencing bone strength (Waldenstedt, 2006). Calcium, provided in the form of calcium carbonate ($CaCO_3$) grit or flour is a major source of Ca in broiler diets. High concentrations of $CaCO_3$ may increase the pH in the proximal gastrointestinal tract due to its high acid binding capacity leading to a decrease in P and amino acid digestibility (Selle et al., 2009). Further, due to its potent net negative charge at intestinal pH, phytate from vegetable ingredients chelates Ca in the small intestine, reducing the bioavailability of both minerals. Diets with lower $CaCO_3$ concentration in the presence of phytase are therefore desirable to enhance P digestibility, feed conversion efficiency and weight gain. However, low levels of dietary Ca may lead to poor skeletal integrity. Displacement of limestone with a more

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digestible Ca source may be beneficial for reducing total dietary Ca concentration, whilst maintaining intestinal absorption of Ca. One such source is novel calcified seaweed that is mined sustainably from the ocean floor near Iceland and Ireland. Due to a unique porous architecture, this $CaCO_3$ source is highly soluble and may meet the digestible Ca requirement at a lower total dietary Ca concentration. In addition to benefits in Ca and P retention, this creates 'space' in least cost formulation, increasing diet energy density and reducing the reliance on added fat sources. Therefore, the aim of the research presented here was to assess the efficacy of a marine Ca source on skeletal integrity and bird behaviour.

II. MATERIALS AND METHODS

A total of 1820 male Cobb-500 broilers were obtained as day olds from a commercial hatchery. Chicks were randomly allocated to 91 deep litter floor pens (1.5 m x 1.5 m) of 20 broilers. Birds received experimental diets from d 1 to d 40 (d 1 to 14 starter and d 15 to 40 grower), with temperature and lighting controlled according to Cobb broiler breeder instructions. The thirteen experimental treatments (6 replicate pens/treatment) were arranged as a 2 x 2 x 3 + 1 factorial, including calcium source (limestone or marine), two dietary calcium concentrations (0.77 or 0.60% in the starter and 0.57% or 0.40% in the grower), phytase (0, or 500 FTU/kg from either QPT2 or Quantum) and an industry standard reference diet containing 1% total Ca and 0.50% available P (avP) in the starter and 0.85% total Ca and 0.42% available P in the grower. All 12 factorially-arranged diets contained 0.35% av.P in the starter and 0.25% avP in the grower. Both QPT2 and Quantum are enhanced phytases from Escherichia coli (AB Vista Feed Ingredients, Marlborough, UK). All diets were based on corn and soybean meal, were isocaloric and isoenergetic and were formulated to meet breeder targets for all nutrients other than Ca and avP as dictated by the experimental design. All diets were steam pelleted at 80°C and provided, with water on an *ad libitum* basis.

On d 24 and 39 four focal birds, randomly selected from each pen, were marked for identification for use in the LTL test that was conducted on d 27 and 42. The LTL procedure was performed as described by Berg and Santora (2003). Individual focal birds were removed from the home pen and taken into the area outside the rearing compartment. Birds were individually placed into plastic tubs filled with 3 cm of tepid water (30 to 33 °C). All birds were placed into the tub in a standing position and their time spend standing was recorded. When the bird made an attempt to sit down timing was stopped. If after five minutes no attempt had been made to sit down the test was terminated. Once the test was over, birds were taken out of the tubs, their body weight was recorded and the bird was returned to their home pen.

Statistical analyses were carried out using GenStat (GenStat 14th ed). The GenStat survival analysis model was used for modelling time against the proportion of birds which remained standing for each diet. Treatment differences were considered significant at $P < 0.05$. The hazard risk ratio used reflects the analysis of time survived to the bird sitting when indexed against the control "industry standard" reference diet.

III. RESULTS AND DISCUSSION

As the purpose of the present paper is to explore leg integrity and bird behaviour the full performance effects will not be presented in detail. However, in order to set the context, birds fed the "industry standard" diet had mean terminal body weights (d 40) of 3.17kg and an FCR of 1.98. There was an overall mortality rate of 2.91% that was unrelated ($P > 0.05$) to dietary treatment. Inclusion of phytase from both sources significantly increased terminal body weight and reduced FCR ($P < 0.05$). Higher Ca concentrations enhanced ($P<0.05$)

terminal body weight and reduced (P<0.05) FCR. However, there was a significant Ca level*phytase interaction for FCR where phytase enhanced FCR only in the low Ca diet. The marine Ca source tended (P = 0.08) to improve FCR compared to limestone.

Latency-to-lie results were significantly correlated (P < 0.001) with terminal body weight (Table 1). Overall, for every 100g increase in body weight the chance of the bird sitting in the water increased by 12%. As bodyweight was influenced by dietary treatment (data not shown) this factor was included in the model to remove the effect of bodyweight *per se* and to allow conclusions regarding the absolute diet effects. Diet C and Diet D were the only diets that were statistically different from the "industry standard" reference diet (Diet A; Table 1). Birds that were fed these diets were found to be 59% and 54% respectively less likely to sit in the water at any given point during the test, as indicated by the Hazard risk ratio. Though not statistically significant, only diet E tended to increase the likelihood of birds to sit compared with Diet A (+28%; P=0.285). Diet E was the novel marine Ca source included at a high concentration and this suggests that high dietary concentrations of very soluble Ca sources may be unwise (especially given the significant benefit at a lower level) due to precipitation with P possible exacerbation of a P deficiency.

Table 1. Dietary comparison and composition

Parameter	Calcium source	Calcium concentration	Phytase	Average body weight (g) d 40	P Value	Hazard Risk Ratio
Body Weight	-	-	-	-	0.000*	-
Diet B	Limestone	0.60	0	3489	0.734	0.92
Diet C	Limestone	0.77	0	3516	0.044*	0.59
Diet D	Marine	0.60	0	3467	0.019*	0.54
Diet E	Marine	0.77	0	3553	0.285	1.28
Diet F	Limestone	0.60	Quantum	3413	0.465	0.83
Diet G	Limestone	0.77	Quantum	3585	0.390	0.81
Diet H	Marine	0.60	Quantum	3497	0.095	0.66
Diet I	Marine	0.77	Quantum	3570	0.119	0.68
Diet J	Limestone	0.60	QPT2	3589	0.418	0.82
Diet K	Limestone	0.77	QPT2	3579	0.441	0.83
Diet L	Marine	0.60	QPT2	3528	0.142	0.70
Diet M	Marine	0.77	QPT2	3568	0.174	0.72

* P < 0.05 versus Industry standard reference diet A containing 1.0% total Ca and 0.50% avP from d 1 to 14 and 0.85% total Ca and 0.42% avP from d 14 to 40.

Behavioural responses of broilers in the LTL test are highly correlated with gait scores, which is a widely used, but subjective method for assessing leg weakness and lameness in broilers (Weeks et al., 2002). The results of this study show a significant correlation between the body weight of broilers and the standing times during the LTL test. Body weight may significantly influenced lameness as indicated by a strong correlation among heavier broilers having shorter standing times (Table 1). Kestin et al. (2001) was the first study to show a clear relationship between body weight and lameness, across a wide range of genotypes including fast and slow growing birds. The previous study showed a threshold weight of 1.25 kg at 54 days of age, with birds becoming increasingly more lame in a linear relationship as body weight increased. The results of the present study are consistent with Kestin *et al.* (2001) where weight gain was associated with lameness and leg weakness. Leg weakness and lameness in broilers is also attributed to the rapid growth of the birds as at slaughter age skeletal maturity has not been reached resulting in softer skeletal bones. To

compensate for this, there is an increased deposition in the outer layers of the cortical bones resulting in cortical bones that have high porosity and low mineral content (Venalainen et al., 2006; Sherlock et al., 2010)

Individual diet LTL analysis revealed birds fed Diets C (limestone, high calcium concentration) and Diet D (marine, low calcium concentration) were statistically superior compared with the "industry standard" reference diet, Diet A (Table). The results show that a lower total calcium concentration in general is desirable and that a low concentration of a more digestible calcium source performed comparably with a higher total calcium concentration of limestone. These findings suggest that a more digestible calcium source may be effective when used at low concentrations.

Further research is required to better understand the usefulness of a novel marine Ca source on broiler leg health, bone mineral content and bone histology. Although this research is a pilot study only it provides a starting point for understanding the effect of total Ca concentrations and Ca sources on broiler leg health. Further evaluation (economic and nutritional) of the novel marine Ca source is required before it can be considered for commercial use.

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EFFECTS OF DRINKING WATER ACIDIFICATION ON BROILER PERFORMANCE

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Summary

An experiment was conducted to determine the influence of organic acid water supplementation on the performance of broiler chickens. The trial was conducted at a commercial broiler farm. Ten thousand two hundred day-old commercial Cobb-500 broiler chicks were randomly distributed into three treatment groups (4 replicates each) using 850 chicks in a pen as an experimental unit. Control (C) birds were offered non-supplemented water, treatment 1 (T1) was supplied a commercially available organic acid[3] product at 0.5 mL/litre during weeks 1, 3 and 5 and treatment 2 (T2) supplied the organic acid product at 0.5 mL/litre on a continuous basis. Chickens that received supplemented water continuously had significantly increased live weight gain between 0-14 days ($P < 0.05$) and 0 - 35 days compared with control group. Chickens that received acidified water every other week had a significantly improved gain ($P < 0.05$) for the overall trial period (0-35 days) compared with non-supplemented chickens. No significant differences between treatments were noted for feed intake during any phase. T1 and T2 chickens that received organic acid every other week or on a continuous basis had improved FCR relative to C between 28 – 35 days and overall 35 ($P < 0.05$). T2 chickens had higher final body weights at 14, 28 and 35 days of age compared with C ($P < 0.05$). T1 and T2 chickens had greater body weight than C on day 35 ($P < 0.05$). It is concluded that acidification of drinking water for broiler chickens improves weight gain and FCR.

I. INTRODUCTION

With the growing concern and regulations in various regions of the world that antibiotics should no longer be used as animal growth promoters, there has been widespread interest in the use of safer alternatives for inhibiting detrimental bacteria. Products such as organic acids, essential oils, prebiotics, competitive exclusion cultures, probiotics and phytogenic products are becoming more appealing to the industry as the consumer is increasingly demanding use of more natural products in broiler production. Antibiotics kill both detrimental and beneficial bacteria while organic acids suppress pathogenic bacteria and enhance growth of lactic acid-producing bacteria (LAB) and acid tolerant bacteria due to lowering of intestinal pH (Percival 1997). This is a "Eubiotic" approach which overcomes digestive disorders ensuring a healthy gastro-intestinal microflora. Organic acids can demonstrate eubiotic effects by lowering the pH of the feed and the animal gut. Pathogenic bacteria such as *Salmonella* and certain strains of *E. coli*) stop growing at a pH lower than 4.5 whereas lactic acid producing bacteria such as *Lactobacilli*, *Streptococci*, and *Bifido* are enhanced. The antibacterial activity of short chain fatty acids (SCFAs) is also well known (Foegeding and Busta, 1991). Commercially available organic acid preparations improved feed efficiency and growth of animals (Meeusen and Hayat 2011). Contaminated drinking water (DW) can be a potential source of microbial infection in chickens (Pearson *et al.*, 1993) because certain microbes can survive in water for a long period (Kazwala *et al*., 1990). Previous trial work revealed that SCFAs are potential microbial inhibitors in water (Chaveerach *et al*., 2002). It has also been reported that organic acids in drinking water administered during feed withdrawal reduced Campylobacter contamination in the crop and carcass (Byrd *et al.*, 2001). Drinking water

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supplementation of organic acids has been effective in reducing pathogens in the water and the digestive tract, regulating gut microflora, increasing feed digestion and improving growth performance (Philipsen, 2006). Providing organic acids in the water for broilers resulted in increased body weight compared to the broilers offered normal drinking water (Pesti et al. (2004).

The objective of the present study was to determine the effects of organic acid administration in the drinking water on performance of male broilers under commercial conditions in the Asia Pacific region.

II. MATERIALS AND METHODS

The trial was conducted on a commercial broiler farm located in the Eastern region of Thailand. Ten thousand two hundred 1-day old Cobb 500 male broiler chicks were housed in a closed-sided house with tunnel ventilation incorporating an evaporative cooling system. Birds were raised on solid-concrete-floor pens using rice hulls as bedding material. Each pen measured 6.0 m x 12.0 m and was equipped with 32 self feeders and 96 nipple water drinkers. Broilers were randomly allocated to 3 treatments with 4 replications using 850 chicks in a pen as an experimental unit with a stocking density at market weight of 23 kg/m^2. Acidified water containing a commercial organic acid product was provided in the drinking water (normal cool water through nipple drinker lines) as detailed in the treatment design: Control: Drinking water without supplement-ation); Treatment 1: Organic Acid based product was provided in drinking water at 0.5 ml/L Day 1 – 7, Day 14 – 21, and Day 28 – 35; Treatment 2: Organic Acid based product was provided in drinking water at 0.5 ml/L drinking water from Day 1 to 35.

Broilers were fed a commercial starter diet from 0 to 17 days of age and a grower diet from day 18. Commercial feed contained a coccidiostat, Sacox®.Water and feed were provided *ad libitum* throughout the 35-day test period. All birds were provided continuous lighting from 0 to 7 days followed by 23L:1D (20 lux) over 8-35 days. The average max/min temperature and relative humidity in the broiler house were 33.6/28.0 ^{o}C and 82.3/62.4 % over 0-7 days, 31.1/26.0 ^{o}C and 82.1/54.3 % over 7-14 days and 27.6/23.2 ^{o}C and 85.4/53.4 % over 14-35 days of age, respectively. Total pen body weight and feed intake on a pen basis were measured while growth and feed conversion ratio were calculated at 14, 28 and 35 days of age. Dead and culled birds were recorded daily. Water pH was measured weekly using Digicon Model PH-201 and coliform count (APHA, 2005) was also monitored weekly. Feed intake, body weight gain, feed conversion ratio and liveability data were subjected to analysis of variance using SAS (2002) for a randomized complete block design.

III. RESULTS AND DISCUSSION

Continuous drinking water acidification at 0.5 ml/liter improved body weight (BW) and body weight gain (BWG) from 0 to 14 days of age ($P < 0.05$). There was no significant ($P < 0.05$) effect on feed intake (FI), feed conversion ratio (FCR) and mortality during that period due to water acidification either continuously or every other week. Supplementation of the drinking water every other did not impact BW and BWG from 0 to 14 days (Table 1). A significant improvement in BW was observed from14-28 days of age ($P < 0.05$); likewise with continuous water supplementation. However water supplementation either continuously or every other week had no significant ($P < 0.05$) impact on any other parameter during this period (Table 2). The feed conversion ratio for the period 28-35 days of age was better ($P < 0.05$) in the acidifier treatments compared with the non-treated group and the same response was observed for BW and BWG (Table 3). The overall BW, BWG and FCR for the period of 0-35 days of age were

significantly improved (P < 0.05) when broilers were provided with organic acids in the drinking water; however no effect on FI was observed (Table 4).

Table 1. Effects of acidifier on the performance of broilers (0 - 14 days of age)

Treatment		Initial body weight	Final Body weight	Body weight gain	Feed intake	FCR	Mortality[1]
Group	Diet	(g)	(g)	(g)	(g)		(%)
1	Control	42	433 [b]	391 [b]	488	1.246	1.09
2	T1(wk 1,3,5)	42	436 [b]	394 [b]	487	1.235	1.00
3	T2 (wk 1-5)	42	446 [a]	404 [a]	495	1.225	1.00

[1]Mortality including dead and culled birds.

Table 2. Effects of acidifier on the performance of broilers (14-28 days of age)

Treatment		Initial body weight	Final Body Weight	Body weight gain	Feed intake	FCR	Mortality[1]
Group	Diet	(g)	(g)	(g)	(g)		(%)
1	Control	433 [b]	1,334 [b]	901	1,552	1.722	1.43
2	T1(wk 1,3,5)	436 [b]	1,351 [ab]	915	1,563	1.708	1.28
3	T2 (wk 1-5)	446 [a]	1,360 [a]	915	1,552	1.698	1.16

[1]Mortality including dead and culled birds.

Table 3. Effects of acidifier on the performance of broilers (28-35 days of age)

Treatment		Initial body weight	Final body weight	Body weight gain	Feed intake	FCR	Mortality[1]
Group	Diet	(g)	(g)	(g)	(g)		(%)
1	Control	1,334 [b]	1,974 [b]	640	1,362	2.129 [b]	0.81
2	T1(wk 1,3,5)	1,351 [ab]	1,990 [a]	638	1,340	2.099 [a]	0.81
3	T2 (wk 1-5)	1,360 [a]	1,999 [a]	639	1,334	2.088 [a]	0.69

[1]Mortality including dead and culled birds.

Table 4. Effects of acidifier on the performance of broilers (0-35 days of age)

Treatment		Initial body weight (g)	Final Body weight (g)	Body weight gain (g)	Feed intake (g)	FCR	Mortality[1] (%)
Group	Diet						
1	Control	42	1,974 [b]	1,932 [b]	3,397	1.758 [b]	3.29
2	T1(wk 1,3,5)	42	1,990 [a]	1,948 [a]	3,385	1.738 [a]	3.06
3	T2 (wk 1-5)	42	1,999 [a]	1,957 [a]	3,377	1.725 [a]	2.82

[1]Mortality including dead and culled birds.

The positive effect of providing acidified drinking water on growth performance of broiler chicken may be due to the relatively healthy intestinal tract of the chickens. The drinking water has been suggested a prominent risk factor for the spread of microbial infection in broiler flocks (Kapperud *et al.,* 1993; Pearson *et al.,* 1993; Gibbens *et al.,* 2001). Indeed Chaveerach *et al.,* (2004) reported that drinking water supplemented with organic acids could prevent bacterial infection in chickens as the organic acids in drinking water were able to keep the water free from pathogenic organism. Griggs and Jacob (2005) also confirmed that the organic acids have the potential to reduce bacterial colonization in the gut of poultry. There is a possibility that the improvement may be associated with improvement in nutrient digestibility as organic acid supplementation has been attributed to a positive effect on the ileal digestibility of nutrients (Hernndez *et al*., 2006)

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INTERACTION BETWEEN GRAIN CHARACTERISTICS AND XYLANASE SUPPLEMENTATION IN WHEAT-BASED DIETS FOR BROILERS

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Summary

The objective of the study was to define the impact of grain characteristics (viscosity and endosperm texture) on performance and nutrient utilization in broilers fed wheat-based diets supplemented with xylanase. Two hundred an eighty eight day-old broilers were randomly allocated to 8 dietary treatments (with 6 replicates cages and 6 birds per cages), following a 4x2 factorial design. Diets were formulated using 4 different pre-characterised wheat cultivars (high or low viscosity and hard or soft endosperm texture), with or without addition of xylanase. Wheat samples were ground, steam-pelleted and incorporated into mash diets. Feed was offered over two growing phases: 1-21 (starter) and 22-40 (finisher) days. Individual bird performance was measured for the starter, finisher and overall periods. Total excreta was collected and feed intakes recorded for 3 days during both the starter and grower/finisher phases in order to determine nitrogen (N) retention and diet AMEn. At day 40, the birds were euthanized and ileal digesta samples taken for calculation of apparent ileal digestibility (AID) coefficients of crude protein and energy. Results showed that wheat type influenced ($P < 0.05$) AMEn (starter phase), N retention (finisher phase) and AID of crude protein. Xylanase addition improved ($P < 0.05$) overall performance, as well as N retention (starter and grower/finisher phases) and AMEn (finisher phase). Though interactions between wheat characteristics and xylanase addition were not confirmed statistically, it appeared that high viscosity wheat was generally more responsive to xylanase than low viscosity wheat, as evidenced by a generally superior response in terms of FCR, weight gain and AMEn. Endosperm texture did not appear to have any major influence on performance responses to xylanase addition, but its effect was somewhat more pronounced when considering AID responses.

I. INTRODUCTION

The inclusion of non-starch polysaccharide (NSP) degrading enzymes, with predominantly xylanase activity, in wheat-based broiler diets is now almost invariably practiced to counteract the anti-nutritive properties of soluble NSPs in wheat. The degradation of NSPs, including arabinoxylans, by exogenous enzymes reduces gut viscosity and releases 'encapsulated' nutrients, thereby improving growth performance and nutrient utilisation in broilers. The magnitude of these responses is mediated by wheat quality and they are most evident in poor quality 'low-ME' wheat (Mollah et al. 1983). However, as reviewed by Bedford (1997), an array of factors may govern the magnitude of responses to NSP-degrading enzymes. It would be beneficial if influential factors in wheat could be identified in advance. For example, *in vitro* methods to determine the viscosity of wheat have been developed to differentiate more viscous varieties (Bedford and Classen, 1993) and grain texture (hardness) can be determined rapidly by NIR spectroscopy. Thus the aim of the present study was to define the impacts of viscosity and endosperm texture of four pre-characterized wheats on

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response in growth performance and nutrient utilization to xylanase supplementation in broilers.

II. MATERIALS AND METHODS

A total of 288 day-old Ross chicks were allocated to 48 cages. Birds were assigned to 8 dietary treatments, with 6 replicate cages per treatment (6 birds per cage), following a 4 x 2 factorial design. The experiment was conducted in compliance with specific guidelines approved by the Animal Ethics Committee of The University of Sydney. Four wheat types were identified with an array of hard and soft textures coupled with high and low viscosities. The protein contents and energy densities (AME) of the four wheats were also determined (Table 1). Practical starter and grower/finisher diets were formulated specifically for each of the four wheats, so they contained similar levels of nutrients. Celite was included as an insoluble acid ash marker in the finisher diets. The four wheat types were hammer-milled and steam-pelleted at approximately 80°C separately, then re-ground and incorporated into mash diets. The diets were offered to broilers with or without addition of exogenous xylanase (Porzyme® 93010, providing a guaranteed minimum of 2,000 xylanase U/kg feed). Chickens were offered starter diets from 1-21 days and finisher diets from 22-40 days. Individual body weights and feed intakes per cage were recorded at 1, 21 and 40 days to determine weight gain and feed intake for the appropriate intervals, and feed conversion ratios (FCR) calculated where the body weight of any dead or culled birds were taken into account. Total excreta was collected and feed intakes recorded for 3 days during both the starter and grower/finisher phases in order to determine apparent metabolisable energy (AME expressed as MJ/kg), nitrogen (N) retention and N-corrected AME (AMEn). At 40 days post-hatch the birds were euthanized and samples of digesta taken from the terminal ileum and freeze-dried. This was in order to determine the apparent ileal digestibility (AID) coefficients of crude protein (N) and energy. The various determinations were made following standard procedures and calculations as outlined by Selle et al. (2003) and AMEn was calculated by allowing 36.54 kJ per g N retained (Hill and Anderson, 1958). The experimental data was analysed as a 4 x 2 factorial array of treatments by analyses of variance using the general linear model procedures of SPSS® Inc.

Table 1. Characteristics of the four wheat cultivars used in the feeding trial

Wheat cultivar	Crude Protein (g/kg, as fed)	Viscosity (cP)	NIR hardness	Auscan NIR AME (MJ/kg, as fed)
Kidman	133	11.4 (high)	106 (hard)	13.4
QualBis	92	13.0 (high)	27 (soft)	12.6
Sun 571G	147	6.7 (low)	130 (hard)	13.7
QUAL2000	101	6.7 (low)	53 (soft)	12.8

III. RESULTS AND DISCUSSION

Growth performance of Ross broilers (Table 2) was satisfactory with a 1-40 days weight gain of 2,657 kg/bird and FCR of 1.76. Overall, wheat type did not influence ($P > 0.05$) weight gain or feed intake but tended ($P = 0.059$) to affect feed efficiency which ranged from 1.72 for the soft texture/high viscosity wheat to 1.79 for the hard texture/low viscosity wheat. Xylanase supplementation did not influence ($P > 0.05$) feed intake but improved ($P < 0.05$) weight gain and feed efficiency by 4.0 and 3.4%, respectively. There were no significant treatment interactions between wheat type and xylanase supplementation. However, it would appear that high viscosity wheat was generally more responsive to xylanase than low

viscosity wheat, as evidenced by a generally superior response in terms of weight gain (5.4% vs. 2.5%) and FCR (5.1% vs. 1.7%). Endosperm texture did not appear to have any major influence on growth performance, however, soft wheat seemed to be slightly more responsive to xylanase addition than hard wheat (4.8 vs. 3.2% and P = 0.25 for weight gain; 3.9 vs. 3.4% and P = 0.33 for FCR). This observation is different from previous research where hard wheat-based diets were shown to be more responsive to xylanase addition than soft wheat-based diets (Singh et al., 2008; Amerah et al., 2009; Péron et al., 2010). Results for nutrient utilization are presented in Table 3. During the starter phase, wheat type influenced (P < 0.05) AMEn, but not N retention. Unlike wheat type, xylanase addition improved (P < 0.05) N retention, but did not affect AMEn significantly. During the finisher phase, wheat type influenced (P < 0.05) N retention, but had no significant effect on AMEn. Xylanase addition improved (P < 0.05) both N retention and AMEn. Further data analyses (not shown here) also suggest that the magnitude of xylanase AMEn responses were greater with high viscosity wheats than with low viscosity wheat (1.9 vs. 0.0% for starter phase, and 5.0 vs. 0.9% for finisher phase). Determination of ileal digestibilities at 40 days of age revealed that wheat type influenced (P < 0.05) the AID coefficient of crude protein. Data also showed that the AID coefficient of energy tended to be improved (P = 0.069) by xylanase addition. Although no significant interactions between wheat type and xylanase addition were noted for any of the digestibility parameters, it would appear that AID responses to xylanase supplementation were more pronounced in hard wheat than in soft wheat (5.5 vs. -2.5% for crude protein, and 5.7 vs. 1.4% for energy). In this study, AMEn values of hard wheat-based diet were numerically higher than those of soft wheat-based diet during the starter phase (13.45 vs. 13.30 MJ/kg DM) but the trend was reversed during the finisher phase, with soft wheat-based diet exhibiting numerically higher AMEn values than hard wheat-based diet (12.18 vs. 12.31 MJ/kg DM).

Table 2. Effect of wheat type and xylanase supplementation on overall growth performance of broiler chickens

Treatment		Overall performance (1-40 days)		
Wheat type Texture / Viscosity	Xylanase Nil/Plus	Feed intake (g)	Weight gain (g)	FCR (g/g)
Hard / High	Nil	4648	2612	1.78
Hard / High	Plus	4693	2748	1.71
Soft / High	Nil	4597	2589	1.78
Soft / High	Plus	4564	2735	1.67
Hard / Low	Nil	4647	2625	1.77
Hard / Low	Plus	4623	2657	1.74
Soft / Low	Nil	4618	2593	1.81
Soft / Low	Plus	4784	2696	1.78
SEM		85.9	50.3	0.027
Significance (*P*-value)				
Wheat type		NS	NS	0.059
Xylanase		NS	0.001	0.001
Interaction		NS	NS	NS

NS: not statistically significant (P > 0.05)

Table 3. Effect of wheat type and xylanase supplementation on nutrient utilization of broiler chickens

Treatment		Starter		Finisher		40 days of age	
Wheat type / Texture / Viscosity	Xylanase Nil/Plus	AMEn (MJ/kg DM)	N retention (%)	AMEn (MJ/kg DM)	N retention (%)	AID coefficient of crude protein	AID coefficient of energy
Hard / High	Nil	13.4	61.1	11.8	64.4	0.58	0.70
Hard / High	Plus	13.6	65.3	12.5	68.0	0.61	0.75
Soft / High	Nil	12.9	58.4	12.1	63.3	0.57	0.73
Soft / High	Plus	13.2	63.0	12.6	63.1	0.56	0.74
Hard / Low	Nil	13.5	62.4	11.9	63.2	0.53	0.70
Hard / Low	Plus	13.3	63.8	12.5	65.7	0.56	0.73
Soft / Low	Nil	13.4	62.2	12.4	65.7	0.62	0.74
Soft / Low	Plus	13.6	64.9	12.1	66.6	0.60	0.75
SEM		0.15	1.25	0.32	0.95	0.022	0.017
Significance (*P*-value)							
Wheat type		0.003	NS	NS	0.007	0.019	NS
Xylanase		NS	<0.0001	0.014	0.015	NS	0.069
Interaction		NS	NS	NS	NS	NS	NS

NS: not statistically significant (P > 0.05)

IV. CONCLUSION

The addition of xylanase to wheat-based diets improved overall growth performance of broilers. Though interactions between wheat characteristics and xylanase addition were not confirmed statistically, it would appear that high viscosity wheat is generally more responsive to xylanase than low viscosity wheat as evidenced by a generally superior response in terms of FCR and weight gain. However, more work would be required in order to determine whether endosperm texture should be considered as a parameter of interest when predicting xylanase responses in wheat-based diets for broilers.

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THE AVIAN TASTE SYSTEM: AN UPDATE

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Summary

Taste has evolved largely as a response to identify adequate nutritious foods and is important for detecting carbohydrates, amino acids and toxic compounds. Species differences in the taste system are intimately related to ecological niche and food availability. It has been argued that birds have a lower taste acuity compared to mammals due to their low taste bud numbers. In addition, birds seem to have fewer taste receptor genes: the sweet taste receptor is missing in chickens and their bitter taste receptor repertoire, the T2R family, is very small consisting of only three members. Furthermore, chickens are tolerant to dietary capsaicin while most mammals are not, and compared to pigs show a lower sensitivity to glucosinolates (a bitter plant metabolite). However, compared to ruminants chickens show higher aversion to glucosinolates. Emerging knowledge shows that birds have a well-developed taste system. Preliminary data indicate that the umami taste receptor is functional in chickens and that the T2R repertoire in some bird species is as broad as in mammals. Additional putative taste genes are present in the chicken genome. Moreover, most birds (including commercial chicken breeds) seem to perceive dietary calcium. The presence and function of taste sensing cells in the avian gastrointestinal tract is an area mostly unexplored that merits further investigation.

I. INTRODUCTION: THE TASTE SYSTEM IN MAMMALS

Taste genes show one of the strongest signatures of positive selection in vertebrates, suggesting that taste can play a critical role in the survival and adaptation of a species (Shi and Zhang, 2006; Kosiol et al., 2008). The peripheral taste system functions as a net of nutrient sensors that uncovers the nutritional value of foods. Sweet taste is related to dietary carbohydrates, such as sugar. Umami taste is related to dietary protein and senses some L-amino acids (L-aa), such as glutamic acid (or monosodium glutamate –MSG). Bitter taste detects potentially toxic molecules present in the diet. Salt and sour tastes are triggered by sodium and acids (H^+) respectively. In addition to the classical primary tastes, fat, calcium, complex carbohydrates, or water may also be perceived through taste mechanisms (McCaughey and Tordoff, 2011).

Taste buds are epithelial structures present in the oral cavity that consist of clusters of ca. 100 sensory cells (TSC). In mammals the taste buds are grouped in papillae, on the tongue and throughout the oral cavity. Each bud is composed of at least three different functional TSC types: type I for sensing sour, type II for sensing sweet, umami and bitter, and type III for transferring the signal to sensory neurons (DeFazio et al., 2006). The stimulation of TSCs is mediated through taste receptors. Some of the receptors belong to the super-family of G

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protein-coupled receptors and have been divided into two families: T1Rs and T2Rs. The T1R sub-family consists of three genes that form two heterodimeric receptors which sense umami (T1R1/T1R3) and sweet (T1R2/T1R3) (Li et al., 2002). The T2R is the bitter receptor sub-family consisting of around 20 to 40 genes in mammals (Shi and Zhang, 2006).

Nutrients and toxins present in food are monitored as they advance through the digestive system. In mammals, the chemosensing system may use the same sensors in the oral cavity and along the gastrointestinal (GI) tract (Wellendorph et al., 2010). For example, the TSCs present in oral taste buds are part of a family of chemosensory cells found also in the non-lingual epithelia of the GI mucosa. Some TSCs seem to be part of the enteroendocrine system that orchestrates motility and absorptive and secretory processes (Roura, 2011). For example, gut T1Rs are part of an integrated network that modulates absorption of sugars, peptides and amino acids (Mace et al., 2009). The T2Rs (bitter sensors) have also been implicated in non-taste tissues and may have a relevant role in gastric motility and defence mechanisms (Wu et al., 2002; Janssen et al. 2011). In addition, other receptors including amino acid, fatty acid and calcium sensors, seem to be expressed along the GI tract in addition to the oral cavity (Wellendorph et al., 2010). Recently, the expression of the T1R members in the hypothalamus has been reported, where they are thought to monitor glucose and L-aa levels (Ren et al., 2009). Overall, the potential role of the taste system in the hunger-satiety cycle is obtaining growing recognition.

II. TASTE IN BIRDS

The structures and genes underlying the sense of taste are mostly conserved across vertebrates. Fish, amphibians, birds and mammals perceive taste through taste receptors that are expressed by TSCs that are found in epithelial clusters known as taste buds. In addition, as is the case in mammals, the *Chorda tympani* and the *Glossopharyngeal* nerves carry the taste information from the oral cavity to the brain (Kare and Mason, 1986). The fact that birds are believed to have inferior taste acuity compared to mammals reflects their relatively low numbers of taste buds (Table 1), lack of mastication and low saliva secretion (Berkhoudt 1985; Klasing, 1998).

Unlike most mammals, the majority of avian species examined do not have taste papillae or taste buds in the dorsal surface of the tongue (Ganchrow et al., 1993). Taste buds in avian species are found mainly around salivary glands in the soft epithelium of the palate, the base of the tongue and the pharynx (Kurosawa et al., 1983). However, within the class *Aves*, taste bud distribution and overall taste sensitivity is variable and reflects the variety of feeding regimes and feeding strategies among birds. This variation is also seen in the differing number of taste buds (Table 1) and the size of the bitter TR gene repertoire (Table 2). Furthermore, differences in the number of taste buds between commercial chicken breeds have been reported. Recently, it has been shown that White-Leghorn chicks have lower number of taste buds than Rhode Island chicks which, in turn, have fewer buds than broiler chicks (192±12, 253±9 and 312±13 taste buds for White-Leghorn, Rhode Island and broiler chicks respectively). Across these three breeds, the number of taste buds is positively correlated with their bitter taste sensitivity (Kudo et al., 2010).

Table 1. Number of taste buds in the oral cavity of avian and mammalian species (Sources: Kare and Mason, 1986; Travers and Nicklas, 1990; Roura et al., 2008).

	Number of taste buds		
Birds		**Mammals**	
Blue Tit	24	Rat	1438
Pigeon	56	Dog	1706
Quail (Jap.)	62	Cat	2755
Starling	200	**Human**	**7902**
Broiler	**316**	Rabbit	17000
Parrot	350	Pig	19904
Duck	375	Cow	20000

Table 2. Family 1 (T1R) taste gene repertoire and size of family 2 (T2R) in birds and mammals and T1R1 homologies with human (Sources: Shi and Zhang, 2006; Roura et al., 2008; Davies et al., 2010).

	T1R genes	T2R n^{er}	Homol.(1) (%)
Broiler	**T1R1, 3**	**3**	**60**
Zebra finch	T1R1, 3	7	n/a
White-throat. sparrow	n/a	18	n/a
Dog	T1R1, 2, 3	15	83
Cow	T1R1, 2, 3	18	79
Human	**T1R1, 2, 3**	**24**	**100**
Rat	T1R1, 2, 3	36	79

(1) Nucleotide sequence homology (expressed as %) between each specific T1R1 and the human T1R1.

In addition to the differences in anatomical structures, birds and mammals have also distinctive genetic and behavioural traits related to taste. The advent of the genomic era has brought relevant insight in taste perception. A few studies on comparative genomics that include avian taste genes have become available in recent years (Shi and Zhang, 2006; Lagerström et al., 2006; Dong et al., 2009). For example, the bitter TR repertoire may differ significantly in birds compared to mammals. The general assumption that birds share the same primary tastes as mammals should be reviewed. In order to define our current knowledge on avian taste, some of the classical taste categories, as well as putative novel tastes will be discussed.

a) Sweet

None of the three avian genomes available through public data bases (i.e. chicken, turkey and zebra finch) have a gene homologous to the mammalian sweet taste receptor T1R2. Lagerström et al. (2006) found no traces of the T1R2 in the chicken genome. Similarly, Shi and Zhang (2006) came to the same conclusion, and identified the two flanking genes on chromosome 21 (at the predicted site for the T1R2) concluding that chickens lack the gene and that the apparent loss was not merely an artefact of the genome assembly. If T1R2 is necessary for sweet taste, its absence would make chickens a sweet insensitive vertebrate. The genomic data is consistent with more extensive behavioural data showing that omnivorous and granivorous birds do not respond positively to sugars, although nectivorous and frugivorous species do (Kare and Mason, 1986; Klasing, 1998; Matson et al., 2001). For example, many behavioral studies in chickens show no positive responses or avoidance to simple sugars or saccharin (Gentle, 1972; Ganchrow et al., 1990; Forbes 1998). In contrast,

Japanese quail showed a preference for sucrose (Harriman and Milner, 1969) suggesting that sensing sugars may be important in the quail's feeding strategy. However, studies of sugar preference, especially those involving longer feeding bouts instead of brief-access designs, can be confounded by post-ingestive effects and should be viewed with caution.

b) Umami

The umami taste receptor dimer (T1R1/T1R3) has been identified and appears functional in all mammalian genomes available (except the giant panda bear, where it has been pseudogenized), as well as birds and fish (Shi and Zhang 2006; Roura et al., 2008). Such a broad distribution across vertebrate species makes the umami taste an essential and highly conserved taste. Efforts to confirm the integrity of the chicken T1R1/T1R3 via cloning and sequencing are ongoing. Despite a gap in the present version of the chicken genome, preliminary data indicate that the umami taste receptor may be functional (Baldwin and Klasing, unpublished). Furthermore, the expression of T1R1 has been reported in hypothalamus, liver and abdominal fat using c-DNA microarrays (Byerly et al., 2010) suggesting, like in mammals, that taste receptors are involved in the orchestration of post-ingestive and metabolic events.

The existence of at least one taste-related amino acid sensor in birds is consistent with previous findings of behavioral preferences. For example, the blackbird was shown to be able to taste L-alanine (Werner et al., 2008). The human umami taste receptor dimer is narrowly tuned to L-glutamic (and MSG) and L-aspartic acids. However, pig and laboratory rodents have a much broader umami sense and show strong preferences for L-alanine among other L-aa (Roura et al., 2011). Interestingly, in a choice feeding scenario broiler chicks showed an immediate preference for a balanced diet containing synthetic amino acids compared to the same diet but deficient in lysine, methionine and tryptophan (Picard et al., 1993) suggesting that oral sensing might be occurring also for these L-aa. However, whether the oral sensor in *Aves* could be the umami receptor remains to be seen.

c) Bitter

The size of the vertebrate T2R gene repertoires varies greatly, presumably linked to evolutionary adaptations to dietary habits (Shi and Zhang, 2006; Dong et al., 2009). Thus, differences across species in bitter taste sensitivity are intimately related to a species' trophic group (Glendinning 1994). The same principle applies to birds. Chickens appear to have a very small T2R gene family represented by only three members as opposed to most mammals which exhibit more than 15 functional T2Rs (Table 2). This could suggest that chickens may have adopted a feeding strategy that eliminates the need of a large array of bitter receptors by avoiding the ingestion of large amounts of potential food-derived toxins. In addition, the sensitivity to some bitter plant metabolites, such as glucosinolates, is less marked than in pigs (Tripathi and Mishra, 2007).

The dozens of taste buds in birds pale in comparison to the thousands found in mammals, yet the relative deficit does not preclude birds from perceiving bitter compounds effectively. In

passive avoidance learning trials chickens respond well to methyl anthranylate (Burne and Rogers, 1997). According to Matson and co-workers (Matson et al., 2004) cockatiels are equally sensitive to quinine compared to humans and more sensitive than some other mammals. In addition, chickens appear to have a higher sensitivity to glucosinolates than ruminants (Tripathi and Mishra, 2007). Bitter taste perception in birds can vary within- and between-species. For example, the effect of glucosinolates on intake is more severe in laying hens and turkeys than in broilers (Fenwick and Curtis, 1980) and this, in turn, may be a consequence of commercial genetic selection programs. To date there have been very few genetic studies characterizing the T2R gene family in birds. However, recently, Davies and co-workers (2010) showed that the variation in T2R repertoire among birds is similar if not wider than among mammals (Table 2). White-throated sparrows, have more functional T2Rs (18) than either the chicken (3) or zebra finch (7) suggesting the possibility that they are more finely tuned in their perception of a wider array of bitter compounds.

d) Calcium

Calcium is the most abundant mineral in vertebrates and dietary calcium requirements are particularly high during egg laying (Klasing, 1998). Preferences by many avian species for calcium rich items such as shells, bones, grit, and even mortar have been commonly observed (Reynolds and Perrins, 2010). Post-ingestive effects may partially explain how animals are able to adjust calcium intake to meet changing requirements. However, recent work suggests that the sense of taste is important in fulfilling calcium appetite (Tordoff et al., 2008). Thus, the “calcium-like” sensing may represent a distinct taste quality (McCaughey and Tordoff, 2011). Recently the T1R3 and the so-called Ca++ Sensing Receptor (CaSR) have been identified as calcium sensors (Conigrave and Brown, 2006; Tordoff et al. 2008). However, it is yet uncertain if they mediate calcium appetite or taste. Both orthologous genes have been identified in the chicken genome as well (Roura, unpublished).

Birds seem to have a well-defined appetite for calcium. For example, under calcium deprivation chickens offered two dietary choices preferred the calcium-rich diet (Hughes et al., 1971; Wood-Gush and Kare, 1966). Furthermore, when chickens were offered free access to supplementary calcium carbonate, the consumption of the supplement was inversely affected by the calcium content of their diet (Taher et al., 1984). Similarly, when offered diets differing in calcium concentration, broiler chickens are capable of converging on a common calcium intake target by selecting between the basal ration and a complementary calcium source (Wilkinson et al., 2011). However, an assessment of calcium appetite across multiple individuals shows that all birds are not equally attracted to the calcium sources (Joshua and Mueller, 1979) suggesting that there might be allelic variations in calcium sensors across the broiler chicken population. Further research is warranted regarding calcium appetite in chickens.

e) Salty and sour

Birds have been reported to respond to salt and acid, indicating that these tastes are also highly conserved across vertebrates. Salt-free diets are likely to result in a craving behavior,

causing the animal to seek sodium. However, high salt solutions (i.e. 2% or more) are toxic to birds without nasal salt glands (Kare and Mason, 1986). Salt-deficient chickens in a choice situation will select salt solutions up to 0.9% while higher sodium concentrations are rejected. Sourness is related to the acidity of food, which is often caused by bacterial fermentation, and typically evokes a rejection response. Chickens show aversion to 0.2N or higher hydrochloric and acetic acid solutions (Gentle, 1972) as well as to some acid blends (Viola et al., 2008), however, contrasting findings related to preferences for acidic pH may require revisiting (Kare and Mason, 1986).

Regarding the salty and sour taste machinery it is known that sodium present in the oral cavity can cross the TSC membrane through the Epithelial Na+ Channel (ENaC) triggering an action potential. However, the evidence that this protein is the sole receptor for salt taste is not compelling (Lin et al., 1999). The receptors for sour taste are similarly thought to be transmembrane channels, but ones that are selective for hydrogen ions. Several of these channels have been identified (DeSimone et al. 2001; Ishimaru et al. 2006). Both sodium and hydrogen ion channels are quite ubiquitous, thus not specific to taste tissues, and the orthologous genes appear to be present in the chicken genome (Roura, unpublished).

f) Other

Chemosensory research in mammalian species points at novel tastes and candidate taste receptors. For example receptors linked to water, calcium, complex carbohydrates or fat sensing are currently under scrutiny (McCaughey and Tordoff, 2011). Some of the candidate mammalian taste receptors have putative avian orthologous genes (Roura, unpublished). Furthermore, published data suggest that gustation plays a key role in determining oil preferences in chickens (Furuse et al., 1996) suggesting that fat taste might be relevant to poultry feeding practices.

Finally, although not part of the taste system but part of the oral somatosensing, chickens are also very tolerant to dietary capsaicin and other spices while most mammals are not (Brenes and Roura, 2010). It is known in mammals and birds that the receptors of the pain pathway, the transient receptor potential family, play a major role as somatosensors but their role in *Aves* is far less understood.

III. FUTURE RESEARCH ON AVIAN TASTE

Taste receptor research in avian species is far behind that in mammals. A thorough validation of the sequence and expression patterns of taste-related avian genes orthologous to those known in mammalian systems is needed. A detailed analysis of the ligands for each taste receptor is also important in order to understand the fine-tuning of each taste modality in chickens and of the ways in which they may differ from mammals. As a result, avian scientists will be able to address topics that could potentially greatly impact our current knowledge in avian nutrition such as fat, calcium or amino acid sensors and the prediction of the palatability of diets based on their chemical makeup.

Finally, recent work has shown that some of these taste receptors are expressed in non-taste tissues such as the hypothalamus, liver and adipose tissue suggesting that, in birds as in mammals, they play a fundamental role in nutrient metabolism and potentially the hunger-satiety cycle. In addition, differential taste receptor expression between fat and lean lines of chickens could become a unique model to study genetic obesity (Byerly et al., 2010).

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FEED ADDITIVE MYTHBUSTERS: HOW SHOULD WE FEED SYNTHETIC AMINO ACIDS?

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Summary

Myths that have erroneously limited feed-grade amino acid inclusion in poultry diets have been dispelled in these proceedings by presentation of a historical perspective. In addition, recent research has been presented which has allowed for aggressive feed-grade amino acid inclusion that maintains good bird performance in diets with reduced costs and environmental impact. Namely, in the past 10 to 15 years numerous research reports have delineated threonine, valine, and isoleucine needs in various genetic strains and time periods of bird growth. Indeed, optimization of feed-grade amino acids requires a continual evaluation of lysine needs for a reference as bird genotypes continually change in terms of food intake and tissue accretion patterns. Moreover, as dietary ingredients, growth promotants, and enzymes programs evolve, the dietary threonine need should also be evaluated for optimal industrial protein synthesis and health.

I. INTRODUCTION

These proceedings accompany a presentation addressing the question: How should we use feed-grade amino acids in commercial poultry diets? References dating back to the 1950s are used herein to highlight key factors that have impacted the use of feed-grade amino acids. High emphasis is placed on amino acid research in broilers conducted during the past ten years as much of this work is focused on predicting the dietary needs of the branched chain amino acids. Hence, accurate ratios of the branched chain amino acids to lysine, coupled with an understanding of minimum nutrient levels of the less limiting amino acids for a non-essential nitrogen pool, are critical for future advances in the use of feed-grade amino acids.

II. THE EARLY YEARS

The theme of the feed additives section of this symposium, of which these proceedings fall under, is “mythbusters.” This term is well-suited for feed-grade amino acids in poultry. A few common past myths in amino acid nutrition of poultry are provided. In the 1950s many nutritionists believed protein needs could never be replaced by feed-grade amino acids. In the 1980s, many nutritionists said broiler diets should not contain more than one pound of L-lysine per ton. In the 1990s, many nutritionists said L-threonine would never enter a broiler diet formulation. Clearly, these myths have been dispelled.

Amino acid feed-additive use began in the mid-1950s with the introduction of both feed formulation and dry methionine hydroxy analogue calcium. The first diet formulated using linear programming was a dairy ration (Waugh, 1951). Thereafter, a group at the Pennsylvania State University Experiment Station led an effort in formulating poultry diets (Hutton et al., 1958) by publishing a series of seven articles in Feedstuffs magazine. The first issue on acceptance of feed-grade amino acids in poultry diets arose with linear programming, as poultry industry nutritionists and many university scientists could not afford computers. Later that year, Hutton (1958) published an article on least-cost poultry diets that included corn, soybean meal, and a methionine source. Because diet cost was $70.95 USD/2000 pounds and the methionine source represented $5.73 USD ($2.18 USD/pound) of

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total diet cost, few accepted any efficacy of a feed-grade methionine source. The second issue arose with the dietary inclusion of feed-grade methionine, because reduced protein and amino acid balance were as foreign as computer formulation. As a result, the Monsanto Chemical Company invested in a computer system and formulated feed as a service to its customers in the US.

Bray and Garlich (1960) were the first to document large responses to amino acids in low-protein diets. They supplemented DL-methionine, L-lysine HCl, DL-tryptophan, DL-isoleucine, and DL-valine to a 9% crude protein diet (corn- and soybean-meal-based) and documented improved egg production and nitrogen retention similar to a diet higher in crude protein. They attributed their amino acid responses, and the lack of responses in previous research (Thornton et al., 1957), to the extent to which crude protein was reduced.

Subsequent research in the 1960s was conducted to determine the efficacy of feed-grade methionine and lysine sources. Harms et al. (1962) conducted studies in laying hens fed corn and soybean meal diets and found an improvement in laying performance from methionine hydroxy analogue calcium in the presence of reduced dietary protein. Additional research from the same laboratory (Harms and Waldroup, 1963) evaluated both methionine and lysine in Hy-Line pullets fed diets based on corn and soybean meal. They demonstrated the nutrient limitation of both methionine and lysine by supplementing lower-protein diets with methionine hydroxyl analogue calcium and L-lysine HCl. The former research and other reports of the like allowed for more accurate formulation with feed-grade methionine and lysine throughout the 1970s and 1980s. But it was not until the 1990s that commercialization of L-threonine allowed for a more full optimization of lysine in diets reduced in crude protein.

III. 1990s: THE THREONINE YEARS

A myth that was in place by some nutritionists in the 1990s was a dietary restriction on the amount of feed-grade L-lysine that could enter dietary formulation. This restriction, sometimes 400 to 500 grams/ton, allowed nutritionists to solve for total sulfur amino acids and lysine while other amino acids were in excess. Research throughout the 1990s provided threonine estimates (Kidd, 2000) which aided nutritionists in dispelling the feed-grade L-lysine restriction and closer meeting the birds' needs for essential amino acids. The 1990s was an important decade for threonine because it represents the third limiting amino acid in most commercial poultry feeds and little prior research existed in growing and finishing broilers (Table 1). However, commercial production of L-threonine in the 1980s created interest and some laboratories delineated requirement estimates. Much of the threonine research conducted focused on needs of chicks (Thomas et al., 1979, 1986, 1987; Uzu, 1986; Smith and Waldroup, 1988; Robbins, 1987). The limiting factors for commercial L-threonine use in the early 1990s were price and lack of requirement estimates in the growing and finishing periods. The latter limitation required research, since the threonine need for bird maintenance is substantial relative to lysine. In a past Australian Poultry Science Symposium (Kidd, 1999) it was pointed out that the inclusion of wheat or sorghum cereals rather than corn will place more constrains on formulation to meet dietary threonine needs in broiler chickens.

Four laboratories in the 1990s published threonine needs between 20 and 42 days of age and generated estimates of digestible threonine between 0.60 and 0.61% of diet (Webel et al., 1996; Leclercq, 1998; Penz et al., 1997; Kidd and Kerr, 1997). In older broilers (42 to 56 days of age), threonine estimates vary. Webel et al. (1996) determined that the digestible threonine need for 42- to 56-day-old broilers was 0.52%, whereas Dozier et al. (2000) and Kidd et al. (1999) estimated digestible needs of 0.59 and 0.58%, respectively. The later

estimates may be higher than that of Webel et al. (1996) because trials were carried out in floor pen environments with built-up litter.

Wu (1998) published an article delineating amino acid catabolism of intestinal mucosa. It was hypothesized that 30 to 50% of arginine, proline, isoleucine, valine, leucine, methionine, lysine, phenylalanine, threonine, glycine, and serine are utilized for enzyme and tissue needs and are not available to extraintestinal tissues. Regarding threonine, Bertolo et al. (1998) indicated that piglets fed intragastrically have a threonine requirement 45% less than piglets fed orally, indicating the importance of threonine in intestinal functionality. Last year at the Australian Poultry Science Symposium meeting, Moran (2011) discussed the importance of meeting the mucin amino acid need in diets lowered in crude protein. Hence, reduced performance in broilers fed low crude protein diets may be more of a need for mucin, rather than a direct function of amino acid digestibility or availability.

Research by Kidd et al. (2003) and Corzo et al. (2007[b]) evaluated dose titrations of dietary threonine in birds from 42 to 56 days of age placed in pens with new or old litter. Although both studies predicted threonine needs of birds raised on old litter of 0.61% and 0.65% digestible respectively, it was shown that birds raised in the pens with old litter had threonine needs at least 4% higher than those in pens with clean litter. Therefore, it must be pointed out that consideration of environment and litter type should be taken into consideration for threonine research trials for resultant industry applicability.

How much does the dietary threonine requirement vary in birds differing in intestinal environments? Proteins in the intestinal mucus layer, an important non-immune intestinal barrier, have been shown to contain 30% threonine (Neutra and Forstner, 1987). Azzam et al. (2011) provided Babcock Brown laying hen's threonine in excess of published standards (NRC, 1994) and noted increases in jejuna and ileal mucin2 in RNA expression. Trends were also observed for increases in jejuna and ileal IgA with threonine levels in excess of needs for egg production. The extent to which threonine needs should be increased in birds with intestinal compromises (all vegetable-based diets and/or intestinal bacteremia) should be researched further for accurate threonine predictions that can be used in industry feed formulation. Moreover, threonine estimates that accurately reflect needs of birds in practice will allow for optimization of the 4th limiting amino acid. The most likely candidates for the 4th limiting amino acid in broiler diets are valine, isoleucine, tryptophan or arginine, but will vary relative to the ingredients used in formulation (Kidd and Hackenhar, 2005).

Table 1. Research on digestible threonine estimates in male broilers

Day of age	% threonine[1]	Strain	Reference
14-28	0.75	Ross x TP-16	Corzo et al. (2009[a])
20-40	0.60	Numerous[2]	Leclercq (1998)
21-42	0.61	Ross x Hubbard	Webel et al. (1996)
21-42	0.61	Peterson x Arbor Acres	Penz et al. (1997)
28-42	0.68	Ross x TP-16	Everett et al. (2010)
30-42	0.61	Ross x 308	Kidd and Kerr (1997)
42-56	0.52	Ross x Hubbard	Webel et al. (1996)
42-56	0.58	Ross x Hubbard	Kidd et al. (1999)
42-56	0.59	Ross x 308	Dozier et al. (2000)

[1]Average of body weight gain and feed conversion estimates.
[2]Mathematical model predictions from selected published research reports.

IV. THE 2000S: VALINE AND ISOLEUCINE YEARS

In an effort to maintain adequate levels of a pool of amino acid nitrogen for non-essential *de novo* synthesis, the past few years have given rise to numerous reports on the dietary needs for the essential branched chain amino acids: valine, isoleucine and leucine. As leucine is considered to be less limiting in practical diets, the bulk of the emphasis has been placed on valine and isoleucine. A review paper of recently published reports on valine and isoleucine requirement estimates for broilers has been made (Tillman, 2011) and a summary from that paper is presented in Tables 2 & 3, respectively. Research with feed grade L-valine has increased as it is completely available to the bird (Rodehutscord and Fatufe, 2005) and became commercially available in 2008. Corzo et al. (2011) showed that L-valine could be successfully used at an inclusion level above 0.05%, in practical broiler diets, which also contained DL-methionine, L-lysine HCl and L-threonine. Several published papers (Corzo et al., 2007[a], 2009[b], 2010), have noted in all vegetable based broiler diets, with corn and wheat as the primary grain, valine is the fourth limiting amino acid.

In broiler diets, which contain animal by-products, research has suggested the existence of a co-limitation between valine and isoleucine (Corzo et al., 2009[b], 2010; Dozier et al., 2011). From these papers, it seems as though diets containing meat & bone meal tend to be 4th limiting in valine while those containing poultry by-product meal are more likely to be 4th limiting in isoleucine. However, in a paper by Corzo et al. (2008[b]) it was suggested that isoleucine was fourth limiting in a meat and bone meal diet. As such, it is clear that due to the co-limitation between the these two branched chain amino acids, minor changes in the minimum constraint of either, within feed formulation software, can change the dynamics and thus the order of limitation. In reality, and because of the co-limitation issue, in order to fully optimize broiler performance, such as body weight gain and feed conversion, levels for both digestible valine and digestible isoleucine need to be near their requirement. Corzo et al. (2004[a,b], 2010) has shown the requirement for both amino acids to be higher than NRC (1994) recommendations.

Table 2. Research on digestible valine estimates in male broilers

Day of age	% valine[1]	Strain	Reference
1-14	0.91	Ross x 308	Corzo et al. (2008[a])
1-21	0.90	Cobb x 500	Goodgame et al. (2011)
7-21	0.84	Cobb x 500	Campos et al. (2009[b])
14-28	0.86	Ross x 308	Corzo et al. (2008[a])
21-35	0.84	Cobb x 500	Goodgame et al. (2011)
21-42	0.65	Ross x 508	Thornton et al. (2006)
21-42	0.72	Ross x 708	Corzo et al. (2007[a])
28-40	0.77	Cobb x 500	Campos et al. (2009[b])
28-42	0.78	Ross x 308	Corzo et al. (2008[a])
42-56	0.67	Ross x 308	Corzo et al. (2004[b])

[1]Average of body weight gain and feed conversion estimates.

An observation of particular note across several publications is the response in breast meat weight and/or yield from an increased or optimal isoleucine level (Kidd et al., 2000; Kidd et al., 2004; Campos et al., 2009[a]; Berres et al., 2010; Corzo et al., 2010; Helmbrecht et al., 2010; Dozier et al., 2011; Mejia et al., 2011). Therefore, in markets where white meat is the primary goal of broiler production, emphasis should not only be placed to assure

methionine, lysine and threonine requirements are met, but also that levels for digestible valine and perhaps especially digestible isoleucine are met as well. If the concept of ideal protein is used in formulation, setting appropriate ratios on these next two limiting amino acids will assure an adequate pool of amino nitrogen is available for *de novo* synthesis.

In the near future it may become common practice for commercial broiler diets to contain L-valine and L-isoleucine, in addition to methionine, lysine, and threonine sources. As more supplemental amino acids enter formulation a concomitant decrease in soybean meal occurs. In this scenario, and in particular all vegetable diets, glycine seems to be semi-essential deeming a glycine + serine minimum to be set in least cost formulation. Chick glycine + serine needs range from just over 2% (Waguespack et a., 2009) to almost 2.5% (Dean et al., 2006). This effect does not seem to necessitate a nonessential nitrogen need as the efficiency of glycine + serine in low crude protein diets in chicks was not spared by dietary addition of L-glutamic acid or nitrogen (Waguespack et a., 2009). In addition to protein pool supply, glycine is a precursor of glutathionine peroxidase , nucleic acids, creatine, heme, bile, and it serves as a methyl source Reeds and Mersmann, 1991). In order to effectively reduce crude protein with the commercially available amino acids, glycine interrelationship to threonine, betaine, and enzymes warrants attention.

Table 3. Research on digestible isoleucine estimates in male broilers

Day of age	% isoleucine[1]	Strain	Reference
7-21	0.68	Cobb x 500	Helmbrecht et al. (2010)
7-21	0.72	Cobb x 500	Campos et al. (2009[a])
18-30	0.62	Ross x 308	Kidd et al. (2004)
22-42	0.65	Ross x Hubbard	Kidd et al. (2000)
28-40	0.69	Cobb x 500	Campos et al. (2009[a])
28-42	0.65	Ross x 708	Mejia et al. (2011)
30-42	0.59	Ross x 308	Kidd et al. (2004)
30-43	0.66	Cobb x 500	Helmbrecht et al. (2010)
42-56	0.55	Ross x 308	Kidd et al. (2004)

[1]Average of body weight gain and feed conversion estimates.

V. PRACTICAL USE OF COMMERCIALLY AVAILABLE AMINO ACIDS

As each synthetic amino acid has reached the marketplace, there has been a period of uncertainty towards its' full adoption within practical formulation. Some of this is driven from economics of inclusion based upon least-cost feed formulation, while other reasons have been based upon lack of requirement knowledge and/or concern of reducing the crude protein of the diet excessively. This scenario existed for DL-methionine, L-lysine HCl and most recently L-threonine. It has taken approximately 25 to 30 years for L-threonine to reach a significant market penetration and it will most likely take a decade or more before L-valine is fully adopted as well. With feed grade L-tryptophan already being commercially available, perhaps there will come a day when L-isoleucine and L-arginine are also economical to incorporate into broiler diets. From an environmental standpoint, the inclusion of each subsequent amino acid allows for formulating closer to the bird's requirement, which reduces amino acid overages. This leads to a reduction in nitrogen pollution and a likely reduction in the amount of nitrous oxide produced, which provides a significant portion of the CO_2 equivalence in greenhouse gas output.

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MYTHBUSTERS-ENZYMES IN THE SPOTLIGHT

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Summary

Over the past twenty years, the use of enzymes has been refined in practice to the point that in most cases they are used successfully and with a good return on investment. Some grey areas remain however, whereby problems occur apparently as a result of their use, or new findings suggest that a long held tenet has to be questioned. One such example of the former is the association of wet litter with phytase use. It is argued that it is more likely that it is dietary changes implemented to accommodate the phytase rather than this enzyme per se which is responsible for this problem. An example of the latter is the effect of NSP'ases on cell walls. The currently held tenet is that they degrade cell walls directly but recent evidence suggests that this may be more indirect through hormonal signalling from the large intestine. This delays gastric emptying thereby accelerating degradation of endosperm cell walls. In both examples, the effect is real but the common explanation is likely incorrect. Knowledge of the actual mechanisms enhances our ability to solve the problems at hand.

I. INTRODUCTION

The topic of exogenous enzyme use in monogastric animal feed has generated many thousands of papers over the past 25 years (Rosen, 2002) and as a result, and as would be expected, the level of knowledge in this field has risen dramatically. In the field of phytase research, there are observations in the field from commercial users that suggest that not all of the consequences of phytase application have been identified in academic research. For example, in many parts of the world there is an association between the use of phytase and wet litter. The potential causes for this are beginning to unravel in such a way that it is possible that it is not the enzyme which is responsible, but the changes, or lack of them, made in dietary composition to accommodate the enzyme. This is the first topic to be covered in this paper.

It is remarkable that many of the mechanistic theories propagated in the early literature as to how the NSP enzymes functioned *in vivo* are still supported today. However, some long standing tenets, with more recent scrutiny, may prove to be incorrect. Although the commercial consequences for such new explanations may not be significant, the development of new and more efficacious products may benefit from such enhancements in our understanding. Two areas of interest are highlighted in this paper, one relating to phytase use and one to non-starch polysaccharide (NSP) enzymes.

II. PHYTASES CAUSE WET LITTER

Although phytases have been in commercial use for over 20 years, some problems associated with their commercial use have only recently become apparent. Almost a decade ago in the UK and other EU countries, and recently in Australia, commercial users of phytase were reporting increased incidence of wet litter in broilers which they were attributing to the use of the enzyme. This was "proven" empirically in the UK (to the authors' knowledge) by some producers who removed the phytase and observed a decrease, if not a cessation, of wet litter almost immediately. The question then arises as to why such an event should occur and why

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it is not a universal problem, which it clearly is not. There are many possible reasons but the following are offered as potential points to consider;

a) Phytase releases minerals which increase osmolarity of the diet

It is well known that phytate is a potent chelator of metal ions and as such will remove cations from solution that otherwise would contribute to the acid base balance of the ration (Viveros et al. 2002; Maenz et al. 1999). Whilst it could be argued that phytase releases phosphate (anions) as well as other minerals (cations) when phytate (IP6) is sequentially degraded, ultimately to IP1, they are not released in proportion. It is suggested that during the initial stages of IP6 degradation, the principle products are IP5 and IP4. Such moieties have significantly reduced capacity to bind cations under gastro intestinal conditions compared with IP6 (Persson *et al.* 1998), and under such circumstances more cations than anions are released. Since the ability of IP4 and lower phospho-esters to chelate and precipitate metal ions is vastly reduced given their lesser and weaker binding capacity coupled with increased solubility (Luttrell, 1992, Persson et al., 1998, Schlemmer, 2001), subsequent dephosphorylation events will continue to yield anions without substantial release of cations. Thus it is suggested when low phytase dosages are employed, more cations than anions are released, which may contribute to excess urine production and water loss. As more and more phytase is added, proportionally more phosphate than cations are released and the cation/anion imbalance becomes redressed somewhat.

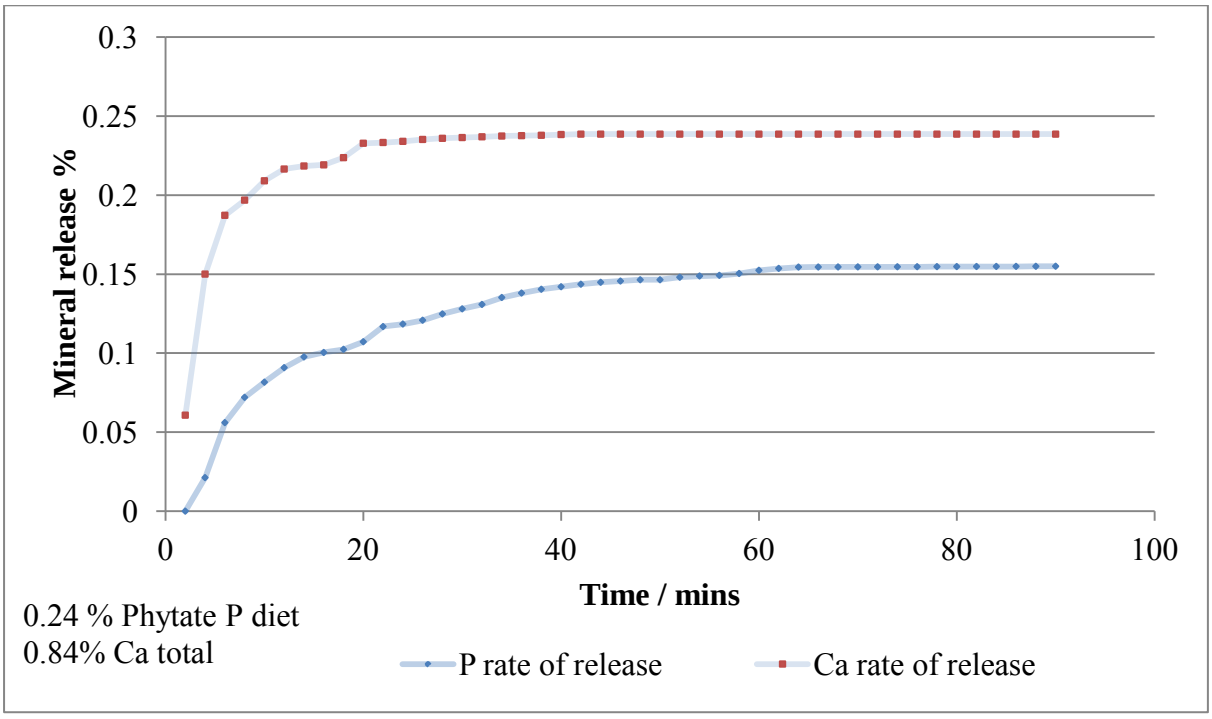

Figure 1. Relationship between in vitro rate of release of P and Ca (% of diet)by treatment of a corn-soy broiler starter diet with 500 FTU /kg of an evolved E coli phytase.

Figure 1 shows the rate of release of calcium and phosphorus *in vitro* from a corn soy diet treated with 500 units of an evolved *E.coli* phytase. It is clear that in the first few minutes, where the bulk of the activity of the phytase will focus on IP6 degradation to IP5 and IP4, Ca release is very rapid and reaches an asymptote after 15-20 minutes. Phosphate release, on the other hand, is continuous up to, and until 60 minutes of incubation. Whilst this graph has time on the x-axis, it would be equally valid to replace it with dose of phytase, with higher dosages of phytase releasing proportionately more phosphate than Ca. The consequence of this is that use of low, or very low levels of phytase (e.g. 100 FTU/kg) in diets, which are not

significantly reduced in major cation concentrations, may result in an increase in the cation:anion ratio and thus create a more alkalogenic ration. Indeed recent data modelling has suggested that the Vmax and Km of a phytase, coupled with stomach residence time are all instrumental in determining the degree and extent of phytate hydrolysis (Letourneau-Montminy et al. 2011). From the data above it appears that such an imbalance can be corrected by feeding incremental doses of phytase.

b) Phytases change ingredient usage in the diet

Additional to the problems highlighted above are the consequences of formulating a phytase into the ration. In some diets, particularly all vegetable protein diets, the act of reducing Ca and P in the ration to accommodate the phytase matrix, results in a reduction in the inclusion of inorganic phosphate and an increase in the inclusion of limestone, especially if a full Ca matrix is not applied. This affects the acid base balance of the diet as captured by the dietary undetermined anion (DUA) calculation, which takes into account Ca, Mg, Na and K as cations and Cl, SO_4 and phosphate as anions, all of which are ignored by the classical Mongin equation.

Table 1. Consequences of formulating a phytase with a 0.13AvP and 0.14 Ca matrix into a broiler starter on DUA and Meq of ration

Ingredient	Starter	Full Ca + P	Full P, limit 0.1 %Ca	Equivalent DUA
Corn	59.37%	60.82%	60.60%	60.85%
Soybean meal 48	34.45%	34.22%	34.25%	34.21%
Poultry Fat	2.16%	1.63%	1.71%	1.62%
Salt	0.46%	0.46%	0.46%	0.46%
DL Methionine	0.30%	0.30%	0.30%	0.30%
Lysine HCl	0.23%	0.24%	0.24%	0.24%
Threonine	0.03%	0.03%	0.03%	0.03%
Limestone	0.96%	1.14%	1.25%	0.96%
Dicalcium Phos	2.04%	1.16%	1.16%	1.33%
Crude protein %	21.40	21.40	21.40	21.40
Poult ME kcal/kg	3,060.00	3,060.00	3,060.00	3,060.00
Calcium %	0.95	0.81	0.85	0.78
Phos %	0.80	0.63	0.63	0.66
Avail Phos %	0.48	0.35	0.35	0.35
Me+Cys %	0.99	0.99	0.99	0.99
Lys %	1.35	1.35	1.35	1.35
Thr %	0.85	0.85	0.85	0.85
Arg %	1.44	1.44	1.44	1.44
Phytate P %	0.23	0.23	0.23	0.23
Na %	0.20	0.20	0.20	0.20
Cl %	0.36	0.36	0.36	0.36
K %	0.93	0.93	0.93	0.93
Na+K-Cl	225	224	224	224
DUA	417	431	457	417
Magnesium	0.19	0.19	0.19	0.19

It is clear from Table 1 that as long as a full, or near full, Ca matrix is applied, there is little change in either the Meq or DUA of the ration. Nevertheless, even under such conditions, there is still almost 1kg/tonne more limestone in the phytase diet compared with the control. If, however, a 0.1% Ca and a 0.13% AvP matrix is applied (i.e. larger P than Ca matrix), whilst the Meq is unaffected, the DUA increases by almost 10% and the inclusion rate of limestone (a significant buffer in its own right) increases by 3kg / tonne compared with the control. An additional problem to consider is the content of magnesium carbonate in limestone sources which can vary from almost zero to a maximum permitted level of 5% (Canadian Border Services Agency brief D10-14-33). Some limestone sources can thus be a significant source of Mg (1.37%), and since Mg is present in the DUA equation, it contributes to the base excess of the ration. Moreover, an increase in Mg has been shown to precipitate wet litter (Enting *et al.* 2009). A further interest with Mg is that in diets which have been depleted in P but not Ca, plasma levels of Ca and Mg have been shown to increase 15 and 28% respectively and their retention to fall by 30 and 11%, respectively, suggesting that inadequate balancing of Ca and P reductions on formulation of a ration for phytase can put the bird in a severe base excess (Viveros et al. 2002) and result in significant urinary losses.

Evidence that even marginal errors in Ca and P formulations can lead to significant wet litter problems is shown in Figure 2 (Bedford *et al.* 2007). Broilers that were fed a control corn-soy-meat and bone meal diet containing 1% Ca and 0.45% AvP (0.74% total phosphorus) produced litter which was considered dry (32% DM) at 28 d of age. When the phosphorus content of the diet was reduced by 0.13% but the Ca maintained at 1%, litter moisture increased dramatically to almost 50%. Reduction of the Ca level in this problem diet to 0.9% reduced litter moisture to 44%, clearly an improvement, but not to the level of the positive control. Addition of 500 and 1000 FTU of either of the two phytase sources investigated in this study resulted in significant reductions in litter moisture, suggesting that phytase *per se* is not the problem, rather, it is the adjustments made to the diet to accommodate the enzyme. Indeed the addition of incremental levels of phytase actually reduced the incidence of wet litter.

A further cautionary note with regards to adjustments to calcium and limestone inclusion levels with the use of a phytase enzyme relates to the fact that limestone is a buffer and will increase the pH of the gastric phase if fed in excess. Such an effect may impair pepsin function and, as a result, excess nitrogen may enter the large intestine, resulting in growth of putrefactive and potentially pathogenic bacteria. The incidence of necrotic enteritis in a recent study more than halved when the calcium concentration of the diet was reduced from 0.9 to 0.6%, presumably as a result of reduced buffering capacity of the diet (Walk et al, 2011, unpublished). This suggests that optimal gastric protein digestion may play a pivotal role in limiting nitrogen supply to the caeca. Whilst necrotic enteritis can be the result of an acute presentation of nitrogen to the large intestine, wet litter can be driven by a more chronic presentation of such a problem. Thus an inadequate adjustment to the calcium content of the diet with the use of a phytase may precipitate wet litter through excess buffering of the proventriculus/gizzard which subsequently results in an oversupply of nitrogen to the caeca. Further to this issue is the fact that the buffering capacities of different sources and grind sizes of limestone differ markedly (Green 1991) and consequently the problems encountered with dietary adjustments made with the use of a phytase may or may not precipitate an episode of wet litter.

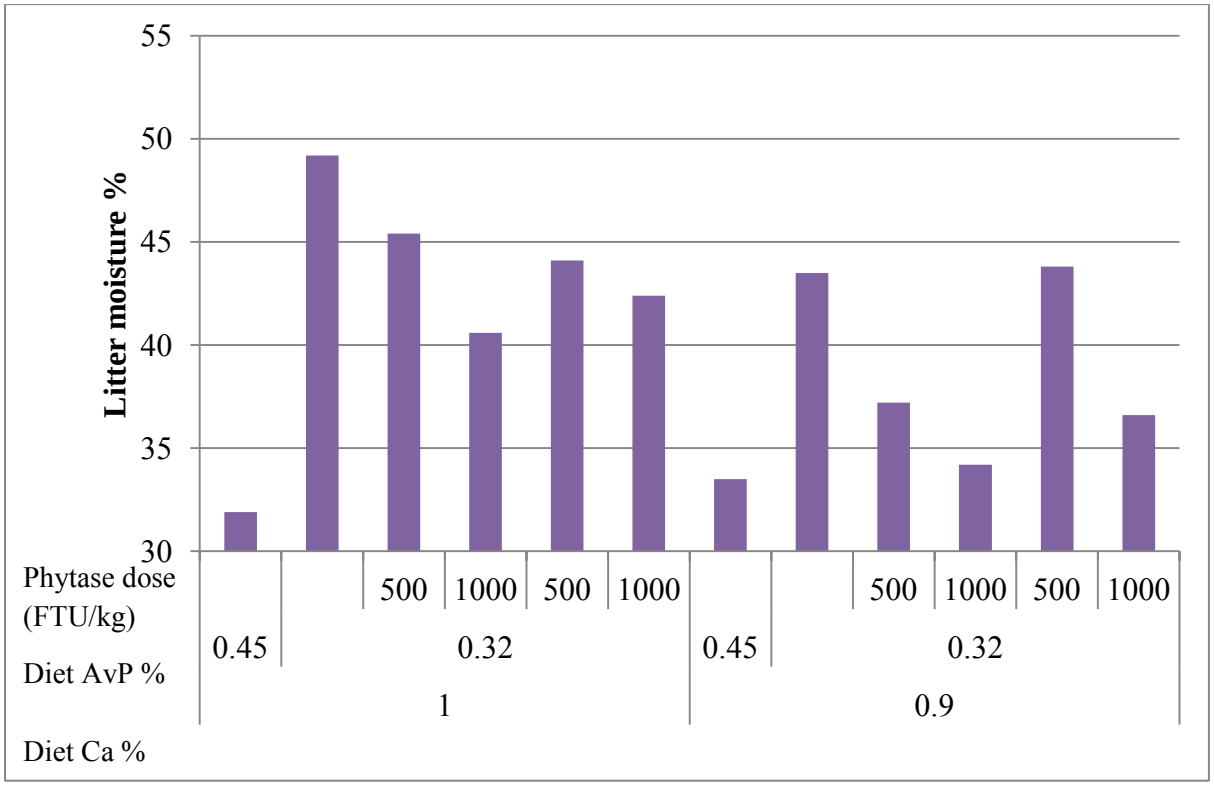

Figure 2. The effect of phytase dose, dietary AvP and Ca on litter moisture content.

c) Other cations – Sodium

The involvement of sodium should also be noted. The use of phytase has been shown to significantly reduce sodium losses for several reasons which are beyond the scope of this paper but are explained in detail elsewhere (Cowieson et al. 2011a; Cowieson et al. 2011b; Ravindran et al. 2008). The sodium content of the diet should be reduced when using phytase if excesses are to be avoided. The choice of anion is especially important since it has been suggested that water to feed intake is greatest with use of sodium bicarbonate, followed by sodium carbonate and lastly sodium sulphate (Enting et al. 2009). There are several commercial anecdotal incidents where reductions in dietary Na content subsequent to the use of a phytase have resulted in a resolution of a previous wet litter problem, suggesting that such an adjustment may be necessary to avoid such problems. For each 0.01% reduction in dietary sodium there is a 4.3 meq reduction in dDEB (provided the anion is metabolisable).

It is clear that the application of phytase can precipitate pressures on the acid-base balance of the bird through a variety of mechanisms. With phytase use influencing dietary cation/anion balance in a multitude of ways, it is important to accommodate its effects if wet litter is to be avoided.

III. NSP ENZYMES DIGEST CELL WALLS THUS EXPOSING THEIR CONTENTS

In the late 1980's and early in the 1990's, a series of papers attempted to explain the mode of action of NSP enzymes used in barley and wheat/rye based rations (Annison 1993; Simon 1998; Pettersson and Aman 1989). The viscosity reduction and cell wall mechanisms were proposed as the principal modes of action of xylanases and glucanases, with the former being possibly more relevant in rye and barley-based rations on account of their extremes in viscosity. The cell wall mechanism was based on the premise that the NSP enzymes degraded the structural integrity of the cell walls of those endosperm cells which remained intact despite feed processing and subsequent grinding in the gizzard. This results in perforation of

these intact cells such that digestive enzymes could gain access to their contents and digestion was thus more efficient and complete. Such a proposal, however, ignores some digestive constraints. It is well known that the structural integrity of endosperm cells is indeed significantly compromised by NSP enzymes by the time digesta flows into the duodenum (Bedford and Autio 1996). However, the time and pH constraints placed on feed entering the duodenum are such that there is insufficient opportunity for the feed enzymes to digest endosperm cell walls to the extent noted *in vivo*. In a simulated crop/gastric/small intestinal incubation, incubation of wheat with a xylanase at 15-20 times the commercially used rates did indeed result in significant cell wall destruction, but more commercial rates of application were far less effective (Parkkonen et al. 1997; Tervila-Wilo et al. 1996). Given that this work allowed for a 60 minute small intestinal phase and the *in vivo* work suggested cell wall destruction had occurred by the duodenum, it seems that there is probably a further mechanism involved as the exogenous enzymes likely have not had enough time under the right conditions (pH) to bring about this effect. A possible explanation for this conundrum is that the NSP'ase is encouraging the bird to retain feed in the gizzard until it is more completely ground and digested prior to release into the duodenum. It was appreciated in the 1990's that fermentation in the large intestine could influence the rate of gastric emptying by encouraging release of gastro-intestinal hormones in the rat (Gee et al. 1996) and that incorporation of a xylanase in a wheat based diet could significantly enhance caecal fermentation rates (Choct et al. 1999). Presumably, this effect on caecal fermentation is brought about through the production of fermentable xylo-oligomers during transit of the enzyme through the small intestine. Feeding such oligomers has been shown to be as effective as the enzyme itself in both wheat and maize based diets (Courtin et al. 2008), suggesting this mechanism may be dominant in some cases. It may be that glucanases and other NSP'ases effect the same response through provision of fermentable oligosaccharides related to their own activity. Recent work (Masey O'Neill and Haldar, 2011, personal communication) has shown that feeding a xylanase to broilers results in elevated plasma levels of the peptide hormone PYY which has been shown to be involved in the control of gastric emptying. Further recent work (Masey O'Neill et al, 2011) has shown that the breakdown of particulate matter in a simulated gastric environment is highly pH dependent, apparently following the pH optimum of pepsin. If this is correct, then there is clearly an exquisite control of gastric emptying and pH and this is to some degree under the influence of caecal fermentation. Given that the development of the caeca is age related, it seems that such control is likely developed over a period of time. In other words, the benefit of an NSP'ase may well increase with age as a result of the development of the caeca and thus the value of such enzymes may be greater in an older bird compared with a younger bird. Indeed a recent holoanalysis of our database on xylanase involving over 300 data points includes the term 'duration' as a positive factor in the model which supports the above mentioned theory.

If such a proposition is correct, it does not change the fact that the use of NSP'ases reduces the size of particulates in the small intestine, but that this is effected through manipulation of gastric emptying rather than through direct action upon the cell walls prior to evacuation into the small intestine. Such an understanding of the mechanism will result in better implementation of the enzyme products in practice, as it will be evident that factors which influence caecal fermentation patterns will likely influence the response to NSP'ases. Indeed the work of Rosen (Rosen 2001) has shown that antibiotics diminish the response to NSP'ases which infers a microbially mediated interaction. Feed processing methods, prebiotics, probiotics, organic acids and many other ingredients and additives which have been shown to empirically influence performance of an NSP'ase now have a mechanism by which such an interaction may be effected. More work is needed to confirm whether such a mechanism is important in eliciting the response to an NSP'ase, but it is clear that should this

be proven, a new line of research investigating this response may yield enzyme products even better suited for purpose than current NSP enzymes.

IV. CONCLUSIONS

Whilst both phytases and xylanases have been used commercially for more than twenty years, some effects and consequences are still debated and their causes unclear. Recent work suggests that one "effect" of phytase, viz wet litter, may be more due to the formulation changes made to accommodate the enzyme rather than the use of the enzyme *per se.* In another field, the mechanism of action of NSP has long been thought to be due in part to direct cell wall dissolution. Recent work suggests this is driven more through physiological effects of the enzyme as a result of its activity in the lower intestine. Whilst this information does not alter the performance benefit achieved through use of the enzyme, knowledge of how it works improves our ability to optimise the search for potentially better candidate enzymes.

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MODERN PROBIOLOGY - DIRECT FED MICROBIALS AND THE AVIAN GUT MICROBIOTA

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Summary

Direct fed microbials (DFM - microorganisms which when fed exert beneficial effects on poultry performance, health, and immunity) routinely demonstrate efficacy in enhanced feed conversion and growth performance that is comparable to that obtained with subtherapeutic antibiotic usage. The mechanistic basis of the probiotic is largely unknown. Our laboratory has used a microbial ecology approach to understanding gut microbial communities, and host immune response from DFM feeding in broilers, layers and turkeys. A unique DFM strain selection and formulation process is presented which is based on an understanding of the genetic diversity and levels of *C. perfringens* and avian pathogenic *E. coli*. Changes in microbial diversity and profile or balance of the avian gut are associated with disease and poor performance; examples of this are the cases of clostridial dermatitis, and focal duodenal necrosis and the concomitant changes in gut microflora. Probiology is the study of probiotics and their interaction with the host. The probiotic concept is evolving and a new generation of DFMs, for different feedstuffs, climates and genetic lines of poultry is potentially on our horizon.

I. INTRODUCTION

Reduction or elimination of subtherapeutic antibiotics in poultry production is a formidable issue with the potential for significant losses in production efficiencies. Considering alternate rearing strategies, Bedford (2000) emphasized how antibiotic withdrawal, and high variability in feedstuffs will require nutritional control to help mitigate negative impacts of increased intestinal pathogens and parasites. Feed ingredient variation greatly influences gut microstructure, microflora, and tissue enzyme activities which are linked to performance (Amerah et al., 2009; Shakouri et al, 2008). Dietary and management practices for clostridial and *Eimeria* control will include multiple technologies, in combination and on rotation (Dahiya et al., 2006).

Progress and growth of the field of probiotic biology is strongly tied to the development of molecular microbiological techniques and bioinformatics which have greatly freed the biologist from the once arduous tasks of microbial community assessment. DFM use has increased in the last ten years and is growing in light of what is becoming a post-antibiotic era of food animal production. DFM usage will increase as more poultry scientists are trained in the use of DFMs and the scientific and economic basis for their inclusion in feeds.

Probiotic biology is a two-sided system of probiotic microorganisms intertwined with the host. The gut microbiome and its host conduct active chemical communications within and between each other (Corthesy et al., 2007; Deplancke and Gaskins, 2001; O'Flaherty and Klaenhammer, 2009). Knowledge of the relationship between the gut microflora and its host provides a framework from which to understand how controlling and manipulating this system by DFM feeding will be understood at the biochemical level.

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II. A PRIMER ON DIRECT FED MICROBIALS

Probiotics for livestock, are termed direct fed microbials or DFMs. Three definitions of probiotics are [i] 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host' (FAO,2002,ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00 .pdf), [ii] 'A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance' (Fuller, 1989) and this has been expanded to [iii] 'A preparation or a product containing viable, defined micro-organisms in sufficient numbers, which alter the micro-flora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host' (Callaway et al., 2008). DFMs can be bacteria, yeast, fungi, or even viral agents, this paper is limited to bacterial DFMs, mainly those which are *Bacillus*-based. A comprehensive list of DFMs can be found at www.efsa.europa.eu/en/efsajournal/doc/2497.pdf.

Which bacteria are accepted for use and can be generally recognized as safe (GRAS) differs across countries. In the United States, the US-Food and Drug Administration Center for Veterinary Medicine regulates the usage of DFMs in feeds. In Australia, the pertinent regulatory for DFMs is the Australian Pesticides and Veterinary Medicines Authority (www.apvma.gov.au/publications/guidelines/gl9_microbial.php). Within the European Union, DFMs are regulated by the EFSA (2011).

Bacterial DFMs are either sporulating (spore-forming) or non-sporulating (asporogenous) Sporulating DFMS can be administered through the watering system or in heated extruded feed, whereas non-sporulating DFMs are limited to water delivery or non-heated feeds.

Sporogenous DFMs administered as spores are bacteria confined to the bacterial genus *Bacillus*. Bacilli are grown in large-scale industrial fermentors to where sufficient proportions of cells have developed endospores (spores and endospores are synonymous). Then, a concentrate of bacterial spores is made by concentrating the cell/spore mass with centrifugation, and spray-drying or freeze-drying then milling into the "pure culture form" ranging from ~ 10^{10} to 10^{12} cells or colony forming units (CFUs) per gram of powder. At this point, *Bacillus* spore DFMs are stable for many years when held under dry conditions and ambient temperatures; refrigeration of *Bacillus* spore preparations is not required. The spore concentrate is subsequently blended into a pre-mix with carrier materials such as soy hulls or limestone to ~ 10^6 to 10^{11} CFU/g and supplied to mills for blending into feeds. At his point, feed mills generally will add the pre-mix at an inclusion rate of ~1 lb per treated ton of feed giving ~ 10^5 CFU per gram of feed. The standard in broiler and turkey production is extruded pelleted feeds, Extrusion subjects the DFM to temperatures of 82 to 88˚C for 30 to 40 seconds. This treatment does not significantly affect viable *Bacillus* DFM cell counts.

Proficient manufacturers of *Bacillus*-based DFMs conduct quantitative microbial assays for viable cell counts and purity at two stages of production; the concentrated product/pre-mix and the final feed. In addition, genetic fingerprint analysis of the *Bacillus* product assures identity and is an analytical step which, though not required (e.g. in the United States), is used by some manufacturers of *Bacillus* DFMs., Due to the environmental stability of *Bacillus* spores, fermentation facilities that produce *Bacillus* on a large fermentation scale are usually dedicated facilities which do not produce other types of DFMs outside of the *Bacillus* genus.

Within the current US guidelines *Bacillus* species allowed for feed use in poultry are: *Bacillus subtilis, B. licheniformis, B. pumilus,* and *B. coagulans*. Others with EFSA qualified presumption of safety (QPS) are: *B. amyloliquefaciens, B. atrophaeus, B.clausii, B. fusiformis, B. lentus, B. megaterium, B. mojavensis, B. vallismortis* and *Geobacillus stearothermophilus (EFSA, 2011.)*

Asporogenous DFMs include several genera of bacteria mainly classified as lactic acid-producing bacteria or "lactics" (LABs or LAB). This group is not confined to the *Lactobacillus* genus. *Lactobacillus,* for example, is produced in fermentation-requiring complex production media. Following fermentation, cells are concentrated and separated from the spent fermentation medium and either freeze dried or spray dried and stored frozen or under refrigeration until used. Because LABs are heat labile, they must be fed in non-extruded feeds or through water. Much like techniques for feed vitamin protection, encapsulation and coating have all been proposed for DFMs. Yet, efficacious and cost effective protective technologies for asporogenous DFMs have not been developed. This is an area in need of continued research.

The genus *Lactobacillus* is generally a major constituent of the healthy gut within the maturing animal. The nemesis group of gut bacteria largely consists of type A *Clostridium perfringens (Cp)* and avian pathogenic *Escherichia coli* (APEC) groups. In the diseased or dysbiotic avian gut, Cp and APEC can be at levels directly inverse to the resident lactobacilli. The DFM user should understand the significance of taxonomic subtypes also called strains, , within the genus and species nomenclature. First, bacteria are generally classified and named according to an accepted system of classification based roughly on Linnaean taxonomy (www.bacterio.cict.fr/). Within a single species are different subtypes each with distinct genetic and functional traits. Subspeciation occurs within all species of bacteria. A good example is *Bacillus subtilis*, a species commonly used in DFMs. At the species level, all are classified as *B. subtilus*, yet within the species are a collection of different subtypes commonly known as strains. The same holds true for species within the DFM genera of *Lactobacillus, Enterococcus, Pediococcus, Bifidobacterium, Propionibacterium* and others.

Pulsed field gel electrophoresis (PFGE) is a technique to separate different strains by a DNA fingerprinting. PFGE is recognized and accepted by the European Food Safety Agency (EFSA) for registration of DFMs.

From country-to-country there are differences among DFM registration processes. Also, for the DFM user, it is worth stating that the naming of bacterial species based on classical systematics (*Kingdom, Phylum, Class, Order Family, Genus, Species, Subtype*) is still in use and is the accepted nomenclature. However, as subtyping data accrue, the naming will eventually be modified to accommodate new names. Here, the regulatory and DFM production sectors will have to convene to harmonize with current microbiological taxonomy.

Some questions common to DFM users arebriefly presented in the following paragraphs.

Does the DFM have to be alive or viable to work or can it work as a dead cell? There is evidence that nonviable cell DFMs can have biological effects (Adams, 2010); however at this stage, most probiotic biologists still assume viability is required. Feeding components of dead bacterial cells can result in immune stimulation. This concept is not covered in this review. Dead cell DFMs and components might offer superior stability to live cells. By current definition, probiotics are live and viable cells.

Are *Bacillus* normal inhabitants of the gut? Yes, they are frequently isolated from poultry. Species of the genus *Bacillus* are found at high levels in the intestines of animals and poultry (Cutting, 2008).

What are traits of an ideal probiotic? Patterson and Burkholder (2003) summarized as follows: probiotics should be of host origin, non-pathogenic, resist gastric pH and bile, resistance to processing, stable in storage, adherent to gut epithelium, persist in the gastrointestinal tract, produce inhibitory compounds, modulate immune response and alter other microbial activities in the gut. Some of these apply more to some DFMs than others, they are a good generalized set. As caveats, it is important to state that not all DFMs persist or colonize and therefore require continual feeding.

Will feed antibiotics, both subtherapeutic and therapeutic impact DFM efficacy? This would depend on the antibiotic, its dosage or inclusion rate, and the DFM itself. In some cases, DFMs can be inhibited by antibiotics *in vitro,* but it would appear that DFM efficacy on performance is equivalent whether subtherapeutic growth promoting antibiotics are used or not. The potential for a synergism between DFMs and subtherapeutic antibiotics is possible. The current reality is that, with the exception of the EU and Korea, growth promoting antibiotics are still widely used. Again, as probiotic biology is taught to new generations of nutritionists, veterinarians, and poultry scientists DFM usage will rise.

Once ingested, do spores germinate in the bird's gut? Cartman et al. (2008) demonstrated germination of *Bacillus subtilis* spores in the avian chick gut and, post spore ingestion, vegetative forms of the *Bacillus* DFM outnumbered spore forms. This is evidence that *Bacillus* DFMs function by mechanisms which are linked to their metabolic activity.

How do DFMs work? DFMs are capable of one or all of the following activities; better nutrient conversion, lower mortality immune stimulation, anti-inflammatory action and protection from enteric pathogens. To accomplish these duties, DFMs make or are a source of volatile fatty acids, antimicrobials and bacteriocins, competitive exclusion, cell wall components, small molecular weight antimicrobial and bioactive metabolites, enzymes, bile salt deconjugation enzymes, mycotoxin inactivation, mucin stimulation and others (Patterson and Burkholder, 2003; Rehman et al., 2007). Herein, I will present a unique DFM selection process with a synopsis of probiotic research, and future speculations on this exciting field of biology. Much of what is presented from our laboratories is the outcome of fundamental and applied scientific research conducted in controlled and production conditions over the last decade. The perspective is from the standpoint of the gut microbial community in health and disease as well as functional impacts of DFM usage in poultry immunity.

III. CUSTOMIZING DFMS FOR POULTRY - THE MULTI STRAIN APPROACH

The complexities of poultry production and the interconnectivity of all of the contributing factors is well described by Williams (2005), who penned the term the 'intercurrent coccidiosis-necrotic enteritis syndrome', abbreviated here as ICNES. As gut microbial ecologists, it has been our working hypothesis that the ICNES is a major driver and that, specifically, the levels of *C. perfringens* as well as avian pathogenic *E. coli* (APEC) often are a major driver of health and performance. It is obvious that these factors do not function in isolation of all others, but it is becoming generally accepted that, in the post-subtherapeutic antibiotics era, subclinical and clinical levels of Cp and APEC with a coccidial overlay can dictate routine bird performance. Necrotic enteritis (NE) is the final end of a spectrum of symptoms generally derived from ICNES. More the norm is subclinical NE and the continuous synthesis of several toxins in the gut including Cp α and NetB toxins (Keyburn et al, 2010; Cooper and Songer, 2010) all of which are a likely source of gut leakage and pathogenesis (Lovland et al., 2004).

In our lab we routinely use RAPD (Randomly Amplified Polymorphic DNA) as a tool to genotype and generate pathogen profiles of the populations of Cp and APEC in poultry intestinal tracts taken from farms and whole operations. From each intestinal tract, it is possible to isolate hundreds of different isolates, and using RAPDs we create family trees of isolates and pick representative members that are highly related. These selected isolates are representative of the dominant genotypes of pathogens in an operation. It became evident that the levels of Cp and APEC differ widely in operations as do their genotypes or genetic fingerprints. We concluded that, not only were there resident populations of APEC and Cp,

but that subtypes differed from operation to operation and farm-to-farm and within the sane operation over time (Gebert et al., 2006).

How does pathogen fingerprinting relate to DFMs and specifically *Bacillus*-based DFMs? The answer seems to be the difference in susceptibility of different genotypes of pathogens to the inhibitory effects of different strains of *Bacillus*-based DFMs; also that pathogen subtype populations are not static. Finally, based on those data, a set of *Bacillus* strains is formulated from the mixture of bacilli offering the highest level of inhibition for a specific set of pathogens obtained from a specific farm. This entire process (pathogen isolation, genotyping, subtype selection, susceptibility to *Bacillus* DFMs and selection of combination of strains to formulate a custom DFM) is known as CSI (Customer Specific Inoculant) and serves as a core basis for formulating DFMs which are not generic but tailored to pathogen populations. A typical outcome of a CSI-derived *Bacillus* DFM on the levels of toxigenic C. perfringens in the broiler gut can be seen in Table 1.

Antimicrobial activities of *Bacillus* strains in poultry have been previously documented against Cp, APEC and *Salmonella* in pen-reared broiler trials (La Ragione and Woodward, 2003). *Bacillus coagulans* as a poultry DFM, resulted in performance comparable to that of virginiamycin supplementation (Cavazzoni et al., 1998) and Wu et al. (2011), in feeding *B. subtilis* to broilers rates of 10^9, 5×10^9, and 10^{10} bacilli/kg of feed reported increases in lactobacilli and concomitant decrease in *Escherichia coli* versus controls.

A typical Cp reduction control response from a CSI-derived *Bacillus* DFM used in commercial broiler production is presented (Table 1). Final performance outcomes however are perhaps the more important measures of multi-strain *Bacillus* DFM efficacy. An example will be provided which illustrates a successful application in a US broiler flock experiencing high levels of gut *E. coli* as well as a viral disease challenge. CSI-DFM *Bacillus* usage in this operation resulted in regaining performance goals as well as reportedly shorter time to final weight. Achieving target weights sooner permitted the producer longer inter-flock down periods and 2-3 days more litter drying time. Increased drying times result in lower litter water activity (A_w) and subsequently lower litter *Salmonella* isolation rates (Hayes et al., 2000).

Bacillus DFMs are commonly applied as a single strain of probiotic, however multi-strain *Bacillus* DFMs are not only warranted, but essential for broader pathogen control of highly diverse Cp and APEC populations (Gebert et al., 2006).

IV. GUT MICROFLORA IN HEALTH AND DISEASE

What is the normal composition of the intestinal microflora of domestic poultry? Is there actually a core microbiome of the domestic chicken? In a healthy state, the microbiome is a community of hundreds of microbial genera and species which changes from birth to death, and is influenced by diet , age, environment and genetics. In comparative terms, diet is perhaps the single factor contributing the most to the profile (Apajahlati et al., 2001). In a healthy state, the relative proportions of major groups will change, but, are dominated by bacteria classified as lactobacilli, enterobacteriaceae, clostridia, bacteroides, enterococci and many other groupings both anaerobic and facultative (Ewing, 2008). In a healthy or balanced state lactobacilli dominate, but under conditions of disease or imbalance, termed dysbacteriosis, other populations will dominate and relative proportions change. The case of NE is a suitable example; here the proportion of *C. perfringens* and clostridia enlarges with a concomitant decrease in the proportion of the lactobacilli. Such an inverse relationship appears, from consistent reports in the scientific literature, to be a repeated theme in the avian gut system (Bjerrum et al., 2006). Using techniques ranging from microbiological

culture (Barnes et al., 1972) to molecular biology (Apajalahti et al., 2001; Lu et al., 2003; Torok et al, 2008; Wise and Siragusa, 2006; Nordentoft et al, 2011; Qu et al., 2008; Zhu and Joerger, 2003), the resulting microbial profiles are remarkably similar.

Attempts to correlate gut microbiota with high levels of performance have been attempted (Apajahlati et al, 2004; Torok et al, 2008). Here are presented examples of avian gut microbial ecology and observations from our labs applied to problems of poultry production over several years using different techniques of assessment. All of these methods rely upon using a species specific bacterial signature gene known as the 16S-rRNA-DNA (16S) ribosomal RNA-DNA gene.

a) Microbial profiling of turkeys with clostridial dermatitis - cloning and sequencing

Clostridial dermatitis (a.n.a. gangrenous dermatitis, or turkey cellulitis) is a lethal condition of intestinal origin caused by the histolytic clostridia *C. septicum* and *C. perfringens.* (Clark et al., 2010). Our lab characterized the gut microflora of turkeys in the same flock, symptomatic and asymptomatic for clostridial dermatitis. Gastrointestinal tracts (GITs) were harvested from approximately 12-15 week old turkeys fed a standard corn-soy diet, from a flock afflicted with clostridial dermatitis. DNA was isolated from the duodenal, illeal, and jejunal sections of the GIT mucosa. Derived 16S sequences were analyzed and profiles constructed. Figure 1 (top) displays community assessments obtained by using 16S cloning and sequencing of DNAs from healthy turkeys and the same number from diseased flock mates. In the diseased turkey samples, the major portion (82%) of the diseased bird microbial profile were clostridia, whereas microbes comprising 85% of the profile of the healthy bird belonged to the lactobacillus group.

b) Microbial profiling of turkeys with clostridial dermatitis - TRFLP

Terminal restriction length polymorphism (TRFLP) profiling is a powerful technique useful for assessing overall microbial community structures. The same DNA samples used in the above section were subjected to TRFLP. Each TRFLP peak corresponds to a group of bacterial species, or in some cases, a single species. It can be seen (Figure 1, bottom) that the general pattern of the avian gut microbial community is again evident. Comparing the healthy to the diseased GIT profiles, we observe several lactic acid bacterial peaks not present in the diseased GIT, and vice versa for the levels of clostridia and enterobacteriaceae (the group containing *E. coli*).

Another finding from our lab is the first description of the putative clostridial origins of the layer condition termed focal duodenal necrosis or FDN. Denaturing gradient gel electrophoresis or DGGE was used in conjunction with TRFLP to substantiate the hypothesis that the putative causative agent in FDN was *Clostridum colinum* and, conversely in FDN-negative flockmates, a TRFLP peak specific to *Lactobacillus* was identified. (Baltzley et al., 2008).

c) Community assessment of healthy broilers – Pyrosequencing

Litter is a significant part of the total mass intake by broilers. An experiment to profile the gut microbiomes of broilers reared on used litter from either diseased or healthy flocks (Neumann et al., 2011) was conducted. Over time, GITs were harvested at points day 14, 28,and 42. Mucosal DNA was isolated and, following 16S gene amplification, the amplicons were subjected to high throughput automated DNA sequencing using pyrosequencing

technology (Hume et al., 2011). Each community profile is represented in a pie chart format (Figure 2). Each profile is based on at least 3,000 bacterial signature sequences per mucosal DNA sample. Again we observe the progression of the gut bacterial community from mainly clostridial to a more lactobacilli dominated population. It is noteworthy that the dominant sequence reported at day 14 is a clostridial organism known as *Candidatus* Arthromitus, also known as segmented filamentous bacteria or SFBs. This group of bacteria are members of the clostridial group and, to date, are non-culturable. *Candidatus* Arthromitus is reportedly the most potent stimuli of the gut immune system (Talham, et al., 1999).

Analysis using high throughput pyrosequencing offers the greatest range of resolution to date in the shortest return time. Using the same technique, a study was recently initiated and preliminary data reported on differing microbial community profiles of healthy heavy and light flock mate turkeys (Benson, 2011).

V. DIRECT FED MICROBIALS AND AVIAN IMMUNE EFFECTS

The gut microbiota exerts a significant influence on the host's immune function starting at birth and throughout life (Corthesy et al., 2007; Klasing, 2007). DFM feeding has been demonstrated to influence the gut, systemic, and tissue adaptive and innate immune responses. In a series of experiments Lee et al reported *Bacillus*-based DFMs enhanced humoral antibody in response to *Eimeria* infection and lower lesion scores, macrophage augmentation, differences in cytokine gene expression and lymphocyte profiles, and subdued acute phase protein levels in broilers fed *Bacillus* based DFMs, singly and combined, through 22d (Lee et al., 2010a, 2010b, 2011a, 2011b) Also from our lab, a study of farm-reared, ionophore and antibiotic-fed turkeys, Novak et al. (2007) reported differences in $CD4^+$, $CD8^+$ and dual $CD4^+CD8^+$ peripheral blood lymphocytic cells in turkeys pulse fed *Lactobacillus brevis* for three days compared to controls not fed the DFM. These same authors found that in addition to altering immune development, in the early life stage (up to 16d) a growth enhancement effect was reported which was not observed at 37d. In this instance and as previously reported, there is a distinct difference in immune effects between different subtypes of the same species of DFM.

VI. NEXT GENERATIONS OF DFMS

Using molecular microbial ecology techniques for DFM strain selection, we have applied DFMs to a portion of the US poultry population. Our strategy was to acquire knowledge of the microbiology specific to individual operations but which could be extrapolated to production in general. From that knowledge there are several areas which should be a focus of further research and development for the next generation of DFMs.

Understanding the microbial succession which occurs in the avian gives a more coherent understanding of gut microbiomes across production systems (see *Big Science* below). DFM strain selection can be for the level of gut maturity at feed change points. In general, broiler production feeding regimens give six points for different DFM addition; maternal flock, hatchery, starter, grower, finisher, withdrawal.

Multi-functional DFMs will mimic the heterogeneity of the natural gut microbiota. Just as the gut microbiota is a widely diverse community with each member serving some function, multi-functional DFMs, and mixtures thereof, would ideally have greater species strain diversity, heterogeneity in metabolic products and enhanced ability to induce a favorable immune response relative to inflammation. Promoting a balanced and diverse core gut microbiome should be an over-riding goal for the probiologist. Whether through using probiotic mixtures with multiple activities or through feeding prebiotics or botanicals

to achieve such a profile; multi-function DFMs will function in the specific region of the avian gut to which they are adapted, and, as per above, are tailored for the specific age of the poultry species.

DFMs should be selected for pharmacological activities in the host bird including hormone release, and neuroactive activities. For example, a specific strain of *Lactobacillus acidophilus* induced expression of intestinal opioid and canabanoid receptors in the mammalian gut, thereby lowering the neural pain threshold and imparting an analgesic effect similar to that of morphine. (Rousseaux et al., 2004). Feeding a probiotic–mannan combination to broilers reduced the effects of high levels of cortisol in birds under heat stress (Sohail et al., 2010). Hypothetically, DFMs specifically selected for anti-inflamatory properties could be applied to the avian in face of stress-induced hormonal levels and chronic inflamation. Chronic inflammation and elevated hormone levels outside of overt disease, shunt energy from growth and development (Klasing, 2007) and hurt flock performance.

VII. OPPORTUNITIES TO ELUCIDATE PROBIOTIC MECHANISMS

Current tools readily available to the microbial ecologist allow the study of microbial communities and their activities in ways unprecedented both in speed and low costs. Our ability to achieve high throughout rapid gene sequencing for gut profiling and analysis of those datasets using open source software has eliminated a major hurdle to understanding DFM effects. Several paths for progress are suggested.

Linking gene expression to metabolic response. Metabolomic analysis through GC-mass spec and NMR; coupled with gene-expression microarrays could provide an understanding of the host's response to gut microflora and DFM induced effects not only at the gene expression level, but at the biochemical expression level by identifying previously unknown chemical markers and signals resulting from DFM feeding.

Bacterial communications in situ. Understanding the chemical languages of resident gut bacteria, through cell-cell signaling molecules (also known as quorum sensing), are potential targets for interruption of signals that would otherwise enhance pathogen growth and, conversely, promote high density growth of beneficial intestinal organisms.

Probiotic lifestyle in the gut. Understanding the details of probiotic bacterial lifestyles in situ and how host stress hormone levels influences gut microflora and pathogen excretion (Humphrey, 2006; Lyte, 2011) will direct efforts to discover and develop neuroactive probiotics to mitigate the stress response of the host (Rousseau et al., 2004). Bacterial gene expression can now be studied at the epigenetic level (Veening et al., 2008), an approach well suited for Bacillus DFMs *in situ*.

Selectable markers for poultry breeding. Using gene markers derived from expression analysis of DFM fed high performing birds will give selectable markers for geneticists for use in breeding programs. Ample evidence exists that the composition of the gut microflora is highly correlated with MHC and other heritable factors (Khachatryan et al., 2008; Vaahtovuo, 2003).

Big science. The time has come for a multi-national project based on high throughput DNA sequencing to establish a repository and reference database of core microbiomes and microviromes of the avian in reference to life stage, feedstuffs, genetic line, and climate. Similar to the human genome project, such an effort will require government-academic and industry cooperation.

VIII. POULTRY PROBIOTICS – MYTH NO MORE

Although used for over a century (Vila et al., 2010), it was not long ago that DFMs were frequently disparaged by skeptics. From these very humble beginnings has evolved a scientific foundation to understand the biological mechanisms and improve the technology of probiotics for livestock. Used properly, the efficacy of modern DFMs is rarely debated. Probiotics are not antibiotics; they are a different approach to achieve efficient poultry production. Through a close and continued partnership of producers, and scientific research, we will begin to change the previously skeptical perceptions of DFMs. This author considers the myth that probiotics could not replace subtherapeutic antibiotics to be no more, i.e. *myth busted*. Much remains to be understood about this technology and that knowledge will only be derived from a multi-disciplinary scientific approach. DFMs offer great potential as a technology for producing poultry meat in the post-antibiotics era, and assure a sustainable livelihood for farmers and poultry producers.

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Table 1. Levels of intestinal mucosal *C. perfringens* in commercial broilers fed a CSI-derived multi-strain *Bacillus* DFM compared to control (Pre-CSI) fed no DFM. Means (top) and categories analysis (bottom) bare log_{10} CFU/g of *C. perfringerns* mucosal homogenate. Means separation indicated by different superscripts, P<0.05.

Parameter	**Untreated**	***Bacillus*-DFM Fed**
mean	2.85[a]	0.88[b]
n	52	16

	Frequency		**Proportion (%)**	
log_{10} CFU/g	**Untreated**	***Bacillus*-DFM**	**Untreated**	***Bacillus*-DFM**
0-1	18	11	34.6	68.8
1-2	3	2	5.8	12.5
2-3	5	1	9.6	6.3
3-4	7	1	13.5	6.3
4-5	9	0	17.3	0.0
5-6	4	1	7.7	6.3
6-7	3	0	5.8	0.0
>7	3	0	5.8	0.0
Total	*52*	*16*	*100*	*100*

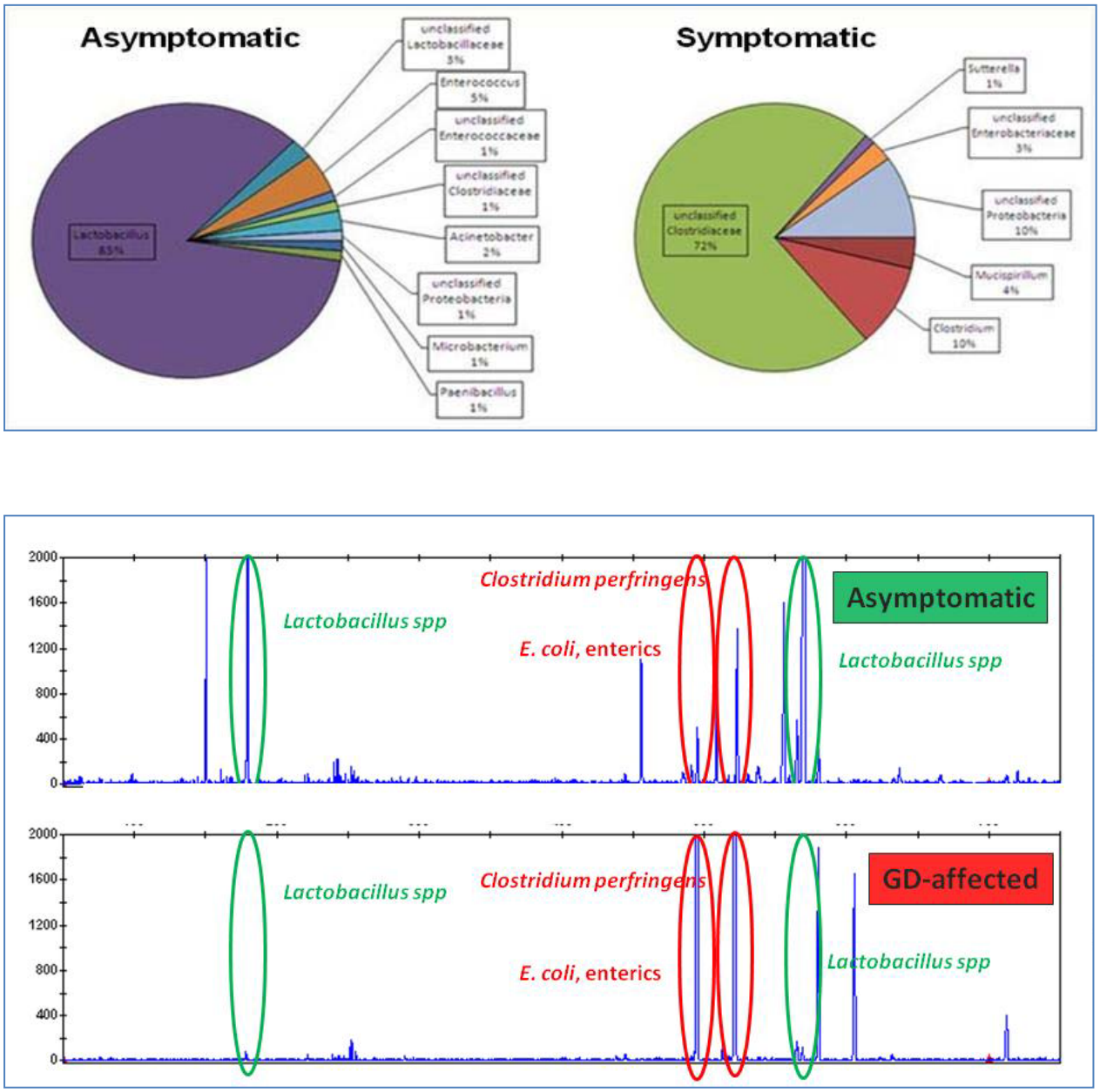

Figure 1. Microbial (bacterial) community profiles of asymptomatic and clostridial dermatitis afflicted flockmate turkeys. Top panel: 16S cloning and sequencing library (n=96 clones per sample). Bottom Panel: TRFLP analysis of the same samples.

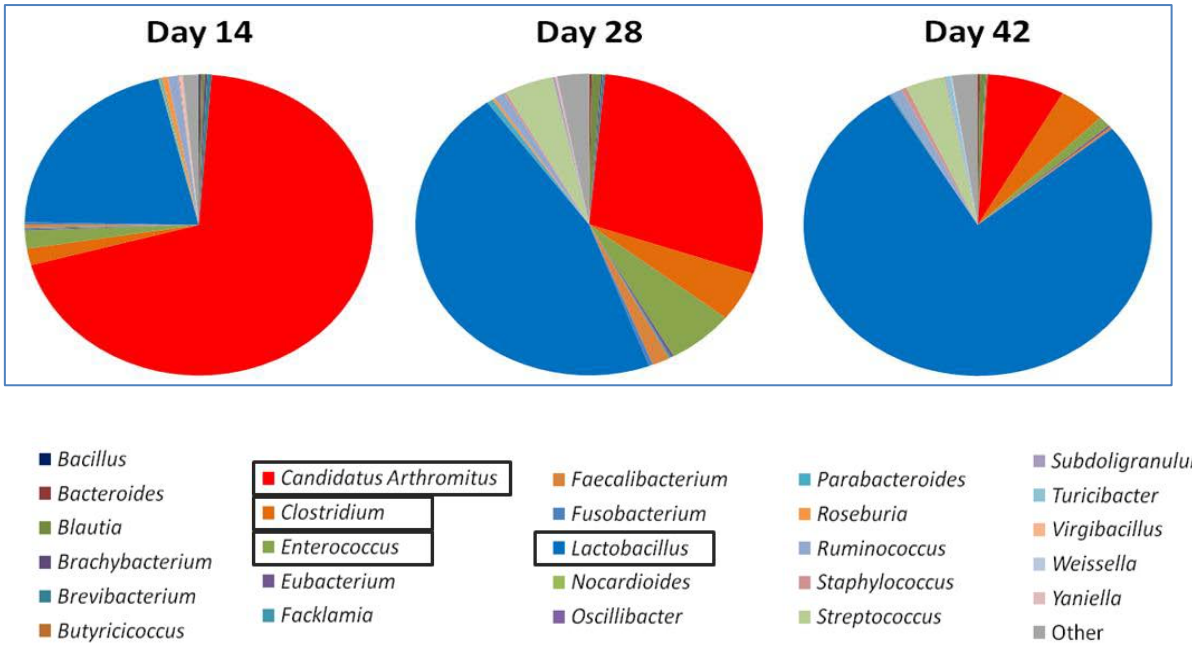

Figure 2. Mean relative abundance of major bacterial genera in non-cecal mucosa DNA extracts during broiler maturation as determined by pyrosequencing determined over time. All genera reported are > 0.1% of the total 16S bacterial signature gene sequences.

EFFECTS OF PROTEASE SUPPLEMENTATION ON BROILER PERFORMANCE AND *IN VITRO* PROTEIN DIGETIBILITY

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Summary

Two studies were conducted to determine the effects of protease enzyme supplementation on broiler performance and *in vitro* protein digestibility. For the performance study, four treatments in a 2 x 2 factorial arrangement were evaluated; two levels of crude protein (CP), positive control and 7.5% reduction, with or without protease supplementation at 0.05%. The positive control diet was formulated with corn soybean meal and 14% Distillers Dried Grains with Solubles (DDGS) to contain 22, 21, and 18.5% CP for starter (1-21 d), grower (22-35 d), and finisher (36-46 d) periods, respectively. Each treatment had 7 replicate pens of 50 Ross 308 males. Bird weight, feed intake, feed conversion ratio (FCR), and mortality were determined at 7, 14, 21, 28, 35, and 46 days of age. For the protein digestibility study, soybean meal, canola meal, cottonseed meal, DDGS, corn, wheat, lupin, poultry meal, meat and bone meal, fish meal, feather meal, and blood meal were evaluated using a modified Novus Immobilized Digestive Enzyme Assay (IDEA®) assay. Body weights were reduced by lowering dietary CP at each weigh day ($P< 0.05$), and protease supplementation increased body weights at 35 d ($P < 0.05$) regardless of dietary CP levels. At 35 and 46 d of age, protease significantly improved FCR by 5.2 and 4.3 points respectively ($P < 0.05$), irrespective of dietary CP level. For the *in vitro* study, protease demonstrated proteolytic activity for all ingredients tested ($P < 0.0001$). It was most effective in blood meal protein and less efficient for corn and feather meal proteins. Use of a protease in a corn-soy-DDGS based diet improved FCR of broilers throughout the trial, and the effect was independent of dietary CP level at 35 and 46 day of age. Proteases evaluated in this study were shown to have a broad substrate spectrum.

I. INTRODUCTON

With feed representing the most significant portion of overall poultry production cost, formulating feed with an optimal protein level is becoming an increasingly difficult task due to the rising cost of protein meals and the consequences of insufficient protein intake. Even with high quality corn-soybean meal based diets, amino acid digestion is not complete and there is still room for improvement. Protease has been shown to be effective in improving feed conversion ratio and/or increasing body weight for broilers fed corn soybean based diets or corn soybean cottonseed meal based diets (Wang et al., 2006; Wang et al., 2008). Novus IDEA® *in vitro* assay is an effective tool to monitor quality and predict digestibility of amino acids for commonly used protein meals, such as soybean meal, DDGS, and meat and bone meal (Schasteen et al. 2007). In this study, a performance trial was conducted to evaluate the effect of protease supplementation on broilers fed corn, soybean meal and DDGS based diets. Furthermore, the effects of protease on protein digestibility from various feed ingredients were evaluated using a modified IDEA®.

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II. MATERIALS AND METHODS

Protease A (CIBENZA™ DP100, Novus International Inc.) was evaluated *in-vivo* and *in-vitro*. It has a minimum of 600,000 units protease activity per gram and the recommended dosage for poultry is 0.05%. Protease B was purchased and used for the purpose of comparison for the *in vitro* study only.

In the performance trial, a total of 1400 Ross 308 male broilers were randomly distributed to 28 floor pens with 50 birds per pen for a 46 d feeding trial. The trial consisted of four treatments in a 2 x 2 factorial arrangement of two levels of CP (positive control and 7.5% reduction) with or without Protease A supplementation at 0.05%. The positive control (basal) diet was formulated with corn-soybean meal and 14% DDGS of known composition to contain 22%, 21%, and 18.5% CP for starter (1-21 d), grower (22-35 d), and finisher (36-46 d) diets respectively. The negative control was achieved by reducing the CP and limiting amino acids Lys, Met, and Thr content of the basal diet primarily through decreasing the amount of soybean meal and adjusting supplemental synthetic amino acids. Each diet was fed to 7 replicate pens of birds. Broilers were weighed by pen at 7, 14, 21, 28, 35, and 46 day of age; feed intake, mortality, and feed conversion ratio (FCR) adjusted by weight of mortality were also determined at each weigh day. Data were subjected to ANOVA as 2 x 2 factorial arrangement with GLM procedures of SAS®. Pen was the experimental unit. Statements of significance were based on P value < 0.05.

In the *in vitro* study soybean meal, canola meal, cottonseed meal, DDGS, corn, wheat, lupin, poultry meal, meat and bone meal, fish meal, feather meal, and blood meal were ground and analyzed for CP content, which was used to standardize protein concentration. Each ingredient was solubilized in a phosphate solubilization solution and kept at pH 2.4 for 4 hours. Protein concentration was then adjusted to 8 mg/ml and buffered to pH 7.5 using phosphate buffer for each of the samples. Protease A and Protease B were solubilized in deionized water for 1.5 hrs. A 0.25 mL of the solubilized and buffered ingredient solution was then transferred to a separate digester tube to which either Protease A or Protease B solubilized solution was added. The concentration of the enzyme in each tube was adjusted to a level that mimicked the concentration of the same enzyme expected in the digesta *in vivo* when chickens consume feed supplemented with the recommended level of these commercial enzymes. For the negative control, deionized water was added. Samples were incubated at 37^{o}C on an end-to-end rotator for 18 hrs. At the end of the reaction, o-phthaldialdehyde (OPA) analysis with UV-vis spectroscopy (340 nm) was performed to measure newly formed α-amino groups. All tests were performed in quadruplicate. Data were analyzed by one-way ANOVA using GLM procedures of SAS® with each reaction tube treated as an experimental unit. When ANOVA was significant, means were separated by Fisher's protected least significant difference (LSD) test. Statements of significance were based on P value < 0.05.

III. RESULTS AND DISCUSSION

a) Broiler performance trial

Body weights were significantly reduced by lowing dietary CP at 14, 21, 28, 35 and 46 d (P < 0.05). Protease supplementation significantly increased body weights at 35 d (P < 0.05) regardless of dietary CP level. Performance data for d 35 and 46 are shown in Table 1. A significant interaction was observed between dietary CP and protease for FCR at 14 and 21 d, where protease improved FCR when it was added to the control CP diet (P < 0.05) but not to the 7.5% reduced CP diet. At 35 and 46 d of age, protease significantly improved FCR by 5.2 and 4.3 points respectively (P < 0.05), irrespective of dietary CP level (Table 1). Regardless of protease supplementation, reducing dietary CP level increased mortality. In summary, use

of a protease in a corn-soy-DDGS based diets improved FCR of broilers throughout the trial, and the effect was independent of dietary CP level at 35 and 46 day of age.

Table 1. Effect of dietary CP level and Protease A supplementation on performance of broilers at 35 and 46 d

		35 d				46 d			
CP	Protease A	BW (kg)	FCR (kg:kg)	FI (kg)	Mort (%)	BW (kg)	FCR (kg:kg)	FI (kg)	Mort (%)
Control	-	1.907[a]	1.575[b]	2.940[a]	2.59[b]	2.986[a]	1.711[b]	5.040[a]	2.60[b]
-7.50%	-	1.836[b]	1.601[a]	2.876[b]	6.50[a]	2.856[b]	1.744[a]	4.911[b]	7.00[a]
P-value		0.0004	0.0687	0.0063	0.005	<.0001	0.0031	<.0001	0.0018
SEM		0.012	0.01	0.015	0.90	0.011	0.007	0.014	0.89
-	No	1.854[b]	1.614[a]	2.927	4.60	2.920	1.749[a]	5.035	4.93
-	Yes	1.890[a]	1.562[b]	2.889	4.50	2.922	1.706[b]	4.916	4.67
P-value		0.0456	0.001	0.0843	0.9398	0.9306	0.0004	<.0001	0.8336
SEM		0.012	0.010	0.015	0.90	0.011	0.007	0.014	0.89
Control	No	1.879	1.611	2.960	2.86	2.990	1.725	5.089	2.86
Control	Yes	1.936	1.540	2.919	2.33	2.983	1.696	4.991	2.33
-7.50%	No	1.829	1.618	2.893	6.33	2.851	1.772	4.981	7.00
-7.50%	Yes	1.843	1.585	2.858	6.67	2.861	1.716	4.841	7.00
P-value		0.2129	0.1704	0.8955	0.7343	0.559	0.1889	0.2853	0.8336
SEM		0.017	0.014	0.021	1.27	0.016	0.010	0.02	1.25
Model P-value		0.0012	0.0025	0.0158	0.0406	<.0001	0.0003	<.0001	0.0168
CV		2.24	2.14	1.81	69.38	1.31	1.45	0.97	65.09

b) Protein digestibility study

Soybean meal, canola meal, cottonseed meal, DDGS, corn, wheat, lupin, poultry meal, meat and bone meal, fish meal, feather meal, and blood meal used in this study were analyzed and found to contain 46.3, 35.6, 39.8, 23.4, 9.2, 9.7, 28.6, 58.9, 52.5, 61.7, 83.9, and 91.9% CP, respectively. Both Protease A and Protease B demonstrated proteolytic activity against all ingredients tested ($P < 0.0001$), indicating broad substrate specificity. The proteases analyzed were most effective in hydrolyzing blood meal proteins and less efficient for corn and feather meal proteins (Table 2). Except for corn where no difference was observed between the two enzymes; Protease A outperformed Protease B for all ingredients (from 7.3 to 59.4% depending on the ingredient).

Table 2. Effect of proteases on *in vitro* protein digestibility quantified by absorbance at 340 nm after incubation at 37°C for 18 hrs[1]

Feed Ingredients	Negative Control	Protease A	Protease B	SEM	CV	% Increase Protease A over Protease B
Soybean meal	0.040[c]	0.394[a]	0.328[b]	0.011	8.31	23.0
Canola meal	0.071[c]	0.339[a]	0.281[b]	0.003	2.80	27.3
Cottonseed meal	0.039[c]	0.270[a]	0.202[b]	0.004	3.83	42.0
DDGS	0.051[c]	0.338[a]	0.231[b]	0.006	7.47	59.4
Corn	0.058[b]	0.166[a]	0.168[a]	0.008	11.67	0
Wheat	0.048[c]	0.346[a]	0.262[b]	0.003	2.81	39.5
Lupins	0.076[c]	0.412[a]	0.298[b]	0.011	8.57	51.6
Poultry meal	0.071[c]	0.297[a]	0.270[b]	0.006	5.63	13.4
Meat and bone meal	0.048[c]	0.281[a]	0.241[b]	0.008	8.30	20.9
Fish meal	0.070[c]	0.378[a]	0.343[b]	0.005	3.43	12.8
Feather meal	0.017[c]	0.210[a]	0.197[b]	0.003	4.70	7.3
Blood meal	0.026[c]	0.555[a]	0.426[b]	0.013	7.75	32.1

[1]P-value for overall F test is <0.0001 for all ingredients

IV. CONCLUSION

While matrix values of Protease A will need to be eventually tested and confirmed *in vivo*, the results from this *in vitro* digestibility study can provide some guidance in choosing reasonable matrix values for *in vivo* testing. Our studies with poultry fed corn soybean meal based diets have indicated that Protease A supplementation can spare approximately 7.5% dietary protein without negative impact on performance. This can be used as a reference to derive the matrix values for Protease A when diets other than corn soybean meal based are used.

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INFLUENCE OF PRE-PELLETING INCLUSION OF WHOLE MAIZE ON PERFORMANCE, GIZZARD WEIGHT AND ENERGY UTILISATION OF YOUNG BROILERS

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The need to lower feed costs has renewed interest in the feed industry in whole grain feeding. To our knowledge, no published data are available on the inclusion of whole maize in poultry diets. The objective of the present study was to examine the effects of pre-pelleting inclusion of whole maize in broiler starter diets on performance, gizzard weight and energy utilisation. Five diets, containing 600 g/kg ground maize or 150, 300, 450 and 600 g/kg whole maize replacing (w/w) ground maize, were formulated and pelleted at 65°C. Each diet was offered *ad libitum* to six replicate cages of broilers (8 birds per cage) from day 1 to day 21 post-hatch. Body weight and feed intake were recorded weekly. Between days 17 and 20, total excreta collection was carried out for the determination of apparent metabolisable energy (AME). On day 21, two birds per cage were euthanised and gizzard weights were recorded. Ileal digesta was collected from two birds per cage and apparent ileal nitrogen digestibility coefficient was calculated using the titanium marker ratios in the diet and digesta. Pellet durability index (PDI) was determined using a Holmen Pellet Tester. The data were analysed by orthogonal polynomial contrasts to examine whether the responses to increasing levels of pre-pelleting inclusion of whole maize was of linear or quadratic nature. The results are summarised in the table below.

Parameters	Whole maize, g/kg[1]					
	0	150	300	450	600	SE
Weight gain (g/bird)[1]	1005	990	919	933	857	19.8
Feed intake (g/bird)[1]	1303	1312	1214	1226	1136	25.6
Feed per gain (g/g)[1,2]	1.304	1.324	1.334	1.341	1.341	0.005
Gizzard weight (g/kgBW)[1,2]	11.9	14.4	15.0	15.7	15.5	0.56
AME (MJ/kg DM)[2]	14.23	14.42	14.50	14.33	14.33	0.053
Ileal nitrogen digestibilty[1]	0.791	0.780	0.802	0.811	0.810	0.010
PDI, %[1,2]	64.4	77.9	77.5	81.9	83.5	0.42

[1] Linear effect (P<0.05); [2] Quadratic effect (P<0.05).

Feed intake and weight gain were linearly (P<0.05) decreased with increasing pre-pelleting inclusion levels of whole maize. A quadratic (P<0.05) effect was observed for feed per gain, with feed per gain increasing as the inclusion level of whole maize level increased to 300 g/kg and then plateauing with further inclusions. Relative gizzard weight (quadratic effect, P<0.05), AME (quadratic effect, P<0.05) and ileal nitrogen digestibility (linear effect, P<0.05) increased with increasing inclusion of whole maize. Pellet quality, measured as PDI, also increased (quadratic effect, P<0.05) with increasing whole maize inclusion levels. The present data showed that bird performance was poorer, despite gizzard development, nutrient utilisation and pellet quality being improved with pre-pelleting inclusion of whole maize. Poor performance was due largely to reduced feed intake. Reasons for the observed intake reductions are unclear, but it is plausible that selective ingestion of larger maize particles from whole maize diets may be partly responsible.

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ENVIRONMENTAL IMPLICATIONS OF AMINO ACIDS IN BROILER FEEDS[1]

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Summary

Incorporation of feed-grade amino acids (AA) in broiler diets is generally recognized as a tool to lower dietary crude protein level and connected with reduced nitrogen excretion and improved health status of animals in intensive broiler production. The effect of dietary AA supplementation (Lysine, Threonine and Methionine) on other environmental parameters has not yet been widely investigated. Therefore, the impact of feed AA on climate change, eutrophication, acidification, terrestrial ecotoxicity, cumulative energy demand and land occupation was studied for broiler feeds under different feed formulation scenarios. Feeds were formulated to provide only methionine or methionine, lysine and threonine or to minimize greenhouse gas emissions (GHG). Other investigated variables were the origins of soybean meal and corn. Feeds formulated with three supplemental amino acids reduced in most scenarios, values for climate change, eutrophication, terrestrial ecotoxicity, and cumulative energy demand. For climate change and acidification, improvements by AA supplementation were dependent on the origin of feed ingredients. Certain ingredients favour AA supplementation to reduce these parameters. Feeds formulated to minimize greenhouse gas emissions had the lowest values for climate change and land occupation. Feeds formulated to minimize GHG emissions were < 1% more expensive compared to feeds with three amino acids used and ~ 7% less expensive than feeds formulated with methionine only.

I. INTRODUCTION

Emissions from intensive livestock production may affect air, water and soil quality (Steinfeld et al., 2006). Intensively raised broilers are fed with high protein and energy feedstuffs imported from outside the farm. The production of these complete feeds contributes to the overall environmental footprint of broiler production (Katajajuuri et al., 2008).

The amount and the composition of feed protein are key factors for growth and carcass quality in chicken. Methionine, lysine and threonine are the most limiting AA for protein deposition. The incorporation of supplemental AA to diets results in reductions of protein-rich ingredients such as soybean meal in chick diets. Hence less soybean meal would be needed per kg broiler meat production, reducing the extension pressure for new cultivation area. Cultivation of soy is specified as the major contributor to global warming in Life Cycle Assessment (LCA) for soybean meal, dominated by N_2O emission from degradation of crop residues (Dalgaard et al., 2008).

The objective of this study was to assess the environmental implications of using supplemental lysine, threonine and methionine in broiler feeds applying LCA.

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II. MATERIALS AND METHODS

a) Feed formulation

Feeds were formulated using linear programming. Main ingredients were wheat, maize, barley, peas, rapeseed and soybean meal. Costs of ingredients were averages for 2009 (IFIP, 2009). Three different diets (phases) for broilers were studied from 1- 45 d of age. Feeds were formulated to provide only methionine or methionine, lysine and threonine, or to minimize greenhouse gas emissions (GHG). For each situation, two calculations were performed using the two types of soybean meal and the two types of maize from different areas in France (Aquitaine, where mineral fertilizer and irrigation were applied; and, Britanny, where fertilization was based on pig manure).

b) Crop and feed ingredients production – LCA

Environmental impacts per kg of feed were calculated from the ingredient values delivered at the feed factory. Inputs for crop production and delivery were taken from Nemecek and Kägi (2007). Soybeans were assumed to be imported from Brazil, distinguishing between Centre West (extension planting area) and from the South of Brazil (traditional planting area). Inputs for other crops were based on French government statistics (AGRESTE, 2006). For feed manufacturing inputs see Mosnier et al. (2011).

c) Calculation of emissions

Emissions to air were estimated for NH_3, N_2O and NO_x. Emission factors for NH_3 volatilization following application of mineral fertilizer and NO_x were based on Nemecek and Kägi (2007). Emission factors for N_2O were based on IPCC (2006). Losses of NO_3 to groundwater were based on Basset-Mens *et al.* (2007). Phosphate emissions to water were estimated according to Nemecek and Kägi (2007). Emissions of Cd, Cr, Cu, Ni, Pb and Zn to the soil were calculated according to a balance approach, considering input by synthetic and organic fertilizers and output via harvested produce.

d) Impact categories

For calculation of the different impact categories see Mosnier et al. (2001). The following impact categories have been assessed:

- Climate change (GHG-emissions) (g CO_2-eq)
- Eutrophication (g PO_4-eq)
- Acidification (g SO_2-eq)
- Terrestrial ecotoxicity (g 1.4-DCB-eq)
- Cumulative energy demand -CED (MJ)
- Land occupation ($m^2 \cdot yr$)

III. RESULTS AND DISCUSSION

a) Impacts of feed ingredients

Climate change impact was 50% larger for soybean meal from Center West compared to meal from the South of Brazil. Also acidification, terrestrial ecotoxicity and Cumulative Energy Demand (CED) were greater for such meal. For maize from Aquitaine, climate change was 26% larger than for maize from Brittany. Eutrophication and CED were also larger for maize from Aquitaine than from Brittany. Conversely, maize from Aquitaine had lower values for acidification, terrestrial ecotoxicity and land occupation than maize from Brittany.

b) Impacts of broiler feeds

Results on environmental parameters are shown in Table 1. Feeds formulated with more supplemental AA than only methionine decreased in most cases climate change, eutrophication, terrestrial ecotoxicity and cumulative energy demand. For feeds containing soybean meal from Centre West, supplement AA decreased climate change values to a greater extent compared to feeds with soybean meal South Brazil.

Table 1. Potential environmental impacts and cost of broiler feeds based on cereals and soybean meal of different origin (Mosnier et al., 2011)

Scenario	Climate Change g/CO_2eq/kg	Eutrophication g PO_4 eq/kg	Acidification g SO_2 eq/kg	Terrestrial Ecotoxicity g1,4-DCB /kg	CED MJ/kg	Land Occupation M^2 * yr/kg	Cost €/t
SBM CW *Maize - A*							
+ M	719	5,2	5,0	3,5	7,9	1,43	220,1
+ MLT	683	4,7	5,0	2,9	7,0	4,45	205,5
Min GHG	682	4,8	4,9	3,0	7,1	1,41	206,1
Maize - B							
+ M	640	4,8	6,1	5,9	6,8	1,52	220,1
+ MLT	642	4,5	5,6	4,2	6,5	1,50	205,5
Min GHG	598	4,5	5,8	6,0	6,3	1,44	207,3
SBM SB *Maize - A*							
+ M	620	5,3	4,4	3,0	6,7	1,48	220,1
+ MLT	611	4,8	4,5	2,6	6,2	1,48	205,5
Min GHG	609	5,0	4,1	2,9	6,6	1,38	206,5
Maize - B							
+ M	541	4,8	5,5	5,5	5,6	1,57	220,1
+ MLT	570	4,5	5,1	3,9	5,6	1,53	205,5
Min GHG	522	4,5	5,3	5,7	5,4	1,48	207,3

SBM CW - Soybean Meal Center West; SBM SB – Soybean Meal South Brazil; Maize A – from Aquitaine; Maize B – from Brittany; +M – Methionine added; +MLT – Methionine, Lysine and Threonine added

Utilization of AA allowed the reduction of acidification values for feeds based on cereals and soybean meal in combination with maize from Brittany. Whatever the base ingredients, supplement AA decreased the values for eutrophication, terrestrial ecotoxicity, and CED of feeds, as well as cost of feeds.

Feeds formulated to minimize GHG emissions had lowest climate change and land occupation values in all investigated scenarios but were more expensive under the given ingredient price conditions during the study. Feeds formulated to minimize GHG emissions were less than 1% more expensive compared to feeds with three amino acids used and about

7% less expensive than feeds formulated with methionine only. That means that feeds can be formulated containing three supplemental AA without substantial increase in feed cost and meeting objectives in terms of environmentally friendly feeds.

Supplemental AA exhibit a higher impact value in the different environmental impact categories compared to protein meals. However, due to their low dietary inclusion level, their contribution to climate change ranged only from 1.0 to 3.4% for the investigated broiler feeds. AA incorporation was associated with a decrease in the use of protein-rich ingredients and an increased use of cereals.

IV. CONCLUSIONS

The incorporation of AA in broiler feeds varied with the objective of the formulation and with the nature of the feed ingredients used. Supplemental AA reduced incorporation of protein-rich ingredients, decreased feed cost and reduced environmental impact of the feed for most investigated parameters. The positive environmental effect was more pronounced in feed formulations using ingredients with high environmental impacts.

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CEREAL TYPE AND LIPID SOURCE INTERACTIONS IN BROILER STARTER DIETS

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Summary

An experiment was conducted to examine the interaction between cereal type and lipid source on the performance, apparent metabolisable energy and fat digestibility of broiler starters (day old to 21d). The experimental design was a 3 × 2 factorial arrangement of treatments, which included three cereals (wheat, maize or sorghum) and two lipids (soybean oil or tallow). The results demonstrated that the effect of lipid source on the weight gain of broiler was dependent on the cereal base used in diet formulations. Weight gain was not affected ($P > 0.05$) by lipid source in sorghum-based diets, but increased ($P < 0.05$) with soybean oil supplementation compared to tallow supplementation in wheat- and maize-based diets. Total tract fat retention and ileal fat digestibility were higher ($P < 0.05$) and FCR was lower ($P < 0.05$) in birds fed diets supplemented with soybean oil compared to those fed tallow supplemented diets.

I. INTRODUCTION

Current broiler strains require high daily energy intake to achieve their rapid growth potential and this often necessitates the dietary inclusion of lipids (fats and oils), the energy value of which is at least twice as those of carbohydrates and protein (NRC, 1994). Tallow and soybean oil are the most widely used lipid sources in the poultry industry. Tallow contains a high proportion of saturated fatty acids, which are non-polar and need emulsification for absorption. On the other hand, soybean oil contains a high proportion of unsaturated fatty acids and is better utilised by poultry than tallow (Krogdahl, 1985).

Globally, maize is the most commonly used cereal in poultry diets, followed by wheat and sorghum. Some reports suggest that the type of cereal may influence the digestion and absorption of lipids and that this effect is more evident when tallow is used as the lipid source. Danicke et al. (1997) reported that broilers fed rye-based diets supplemented with soybean oil had higher body weights compared to those fed diets containing tallow. These effects were attributed to the presence of high concentrations of non-starch polysaccharides (NSP) in rye and their interaction with saturated fats. The adverse effects of NSP in increasing intestinal viscosity and depressing the utilisation of nutrients are well documented (Choct, 1997). The aim of the present study was to investigate the interaction between cereals (wheat, maize and sorghum) and dietary lipids (tallow and soybean oil) on the energy utilisation and performance of broilers to 21d of age. Wheat contains relatively high concentrations of NSP compared to maize and sorghum (Choct, 1997) and it was hypothesised that the effect of dietary lipid source will differ in birds fed diets based on different cereals.

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II. MATERIALS AND METHODS

The experimental design was a 3x2 factorial arrangement of treatments evaluating three cereal types (wheat, maize and sorghum) and two lipid sources (soybean oil and tallow). Determined fatty acid composition of soybean oil and tallow is presented in Table 1. Soybean oil and tallow have the unsaturated to saturated fatty acids ratios of 4.80 and 0.63, respectively. Six diets, based on one of the cereals supplemented with either tallow or soybean oil (60 g/kg), were formulated to meet the Ross 308 strain recommendations for major nutrients for broilers to 21d of age. All diets were formulated to be isocaloric and isonitrogenous. Day-old male broilers (Ross 308) were individually weighed and allocated on the basis of body weight to 36 cages in electrically heated battery brooders. Each of six diets, in mash form, was then randomly assigned to six cages, each housing eight birds. Feed was offered *ad libitum* and water was freely available. Body weights and feed intake were recorded on a cage basis at weekly intervals throughout the 21-day trial. Mortality was recorded daily and feed conversion ratio (FCR) was corrected for the body weight of any bird that died during the course of the experiment. Feed intake and excreta output were quantitatively measured from day 17 to 20 post hatch for the determination of apparent metabolisable energy (AME). On day 21, four birds per cage were euthanised and the contents of the lower ileum were collected, pooled within a cage and lyophilised. Diet and digesta samples were analysed for fat and titanium. Apparent ileal fat digestibility coefficient was calculated using the titanium marker ratios in the diet and digesta.

Table 1 Fatty acid composition of soybean oil and tallow (g/kg)[1]

	Soybean oil	Tallow
Saturated fatty acids		
C14:0 Myristic	1.3	29.8
C16:0 Palmitic	105.3	225.7
C17:0 Margaric	1.7	18.3
C18:0 Stearic	44.4	237.5
Unsaturated fatty acids		
C14:1 Myristoleic	-	4.5
C16:1 Palmitoleic	0.8	18.9
C18:1 Oleic	216.9	257.5
C18:1 Vaccenic	11.1	26.4
C18:2 Linoleic	476.9	5.8
C18:3 Linolenic	67.1	6.6
Total saturated fatty acids	161.9	517.5
Total unsaturated fatty acids	776.8	323.8

[1] Only the major fatty acids are shown

III. RESULTS AND DISCUSSION

Weight gain was not affected by lipid source in sorghum-based diets, but increased with soybean oil supplementation compared to tallow supplementation in wheat- and maize-based diets (Table 2), as indicated by the cereal type x lipid source interaction ($P < 0.05$). The main effect of cereal type was significant ($P < 0.001$) for feed intake. Birds fed wheat-based diets consumed more ($P < 0.001$) feed than those fed maize and sorghum-based diets. The main effect of lipid source was significant ($P < 0.001$) for FCR, with birds fed diets supplemented with tallow having higher FCR compared to those fed diets supplemented with soybean oil.

Neither the main effect of cereal nor the interaction between cereal type and lipid source was significant for FCR.

The main effect of cereal type was significant ($P < 0.001$) for AME (Table 1), with higher AME values determined for sorghum-based diets compared to wheat- and maize-based diets. The main effect of lipid source was also significant ($P < 0.001$) for AME. Birds fed tallow supplemented diets showed higher AME than those fed soybean oil supplemented diets. The main effects of cereal type ($P<0.01$) and lipid source ($P<0.001$) were significant for total tract retention of fat. Birds fed wheat-based diets had a lower ($P < 0.01$) fat retention coefficient compared to those fed maize- and sorghum-based diets. Birds fed diets supplemented with soybean oil had higher total tract retention of fat than birds fed diet supplemented with tallow. The main effect of lipid source was significant ($P < 0.001$) for ileal fat digestibility coefficient. Ileal fat digestibility coefficient of soybean oil supplemented diets was higher than that of tallow supplemented diets. No interaction ($P > 0.05$) was observed between cereal type and lipid source for AME, fat retention and ileal fat digestibility coefficient.

In the present study, the hypothesis that the performance, AME and fat digestibility of broilers fed diets based on different cereals will be influenced by the type of supplemental fat was tested. However, a significant cereal type x lipid source interaction was observed for the weight gain. The observed interaction in wheat-based diets might be explained by the high concentrations of NSP in wheat, which increase the intestinal digesta viscosity (Choct, 1997) and lower the digestion and absorption of saturated fatty acids in tallow. As reported by Danicke (2001) the long chain saturated fatty acids present in tallow are digested and absorbed more poorly than unsaturated fatty acids in the presence of NSP (Danicke, 2001). The lower weight gain of birds maize-based diets supplemented with tallow, however, cannot be explained on the basis of NSP concentrations.

Viscous grains, such as wheat, with relatively high levels of NSP are known to have lower AME and inhibit the digestion of saturated fats than non-viscous grains such as maize and sorghum which consist of low level of NSP (Hughes and Choct, 1999). In the present study, however, total tract fat retention and ileal fat digestibility were lower in tallow-supplemented diets irrespective of the cereal type. Higher AME values of diets supplemented with tallow was unexpected and, contrary to the better retention and digestibility of fat determined for the soybean oil-supplemented diets. One possible explanation may be that the classical excreta-based AME measurements do not reflect the actual responses because of the modifying effects of caecal microorganisms on energy utilisation and the contribution of microbial mass to energy output in the excreta. In conclusion, the present data demonstrated that the effect of lipid source on the weight gain of broilers to 21d was dependent on the cereal base used in diet formulations. The current study also showed that soybean oil supplemented diets had higher fat retention, ileal digestibility of fat and better FCR compared to tallow supplemented diets.

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Table 2 Influence of cereal type and lipid source on the weight gain (g/bird), feed intake (g/bird) and FCR (g feed/g gain), AME ((MJ/kg DM), total tract fat retention coefficient and ileal fat digestibility coefficient of broilers to 21d[1].

	Fat source	Weight gain (g)	Feed intake (g)	FCR	AME	Fat retention coefficient	Ileal fat digestibility coefficient
Wheat	Soybean oil	880[a]	1118	1.262	14.37	0.819	0.877
	Tallow	819[bc]	1093	1.336	14.62	0.672	0.717
Maize	Soybean oil	860[ab]	1055	1.239	14.45	0.850	0.892
	Tallow	782[c]	1054	1.338	14.61	0.697	0.666
Sorghum	Soybean oil	809[c]	1009	1.259	14.92	0.852	0.906
	Tallow	813[bc]	1052	1.300	15.11	0.685	0.694
SEM[2]		16.4	15.3	0.014	0.07	0.007	0.016
Main effects							
Cereal type							
Wheat		850	1106[a]	1.299	14.49[b]	0.746[b]	0.797
Maize		821	1054[b]	1.289	14.53[b]	0.774[a]	0.779
Sorghum		811	1030[b]	1.280	15.01[a]	0.768[a]	0.800
Lipid source							
Soybean oil		850	1060	1.254[b]	14.58[b]	0.840[a]	0.892[a]
Tallow		804	1066	1.325[a]	14.78[a]	0.685[b]	0.692[b]
Probabilities, P ≤							
Grain type		0.06	***	NS	***	*	NS
Lipid source		***	NS	***	***	***	***
Cereal type x Lipid source		*	NS	NS	NS	NS	NS

[a,b,c] Means in a column not sharing a common superscript are significantly different (P < 0.05).

NS, not significant; * P < 0.05; *** P < 0.001.

[1] Each value represents the mean of six replicates (eight birds per replicate).

[2] Pooled standard error of mean.

THE USE OF HIGH EFFICIENCY JUNCEA CANOLA MEAL AND FULL FAT JUNCEA CANOLA MEAL IN BROILER FEEDING

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Summary

Juncea canola (*Brassica juncea)* is a drought tolerant canola variety with low glucosinolates and erucic acid. It has a yellow seed coat and generally has higher protein content as compared with canola. It has the potential to replace canola meal (CM) and other protein meals in poultry feeding. Canola meal has a lower metabolizable energy and digestible amino acid coefficients when compared with soybean meal. Classen et al. (2004) identified that the traditional processing methods for canola may destroy more than 10% of digestible amino acids particularly lysine. When a superior processing method is used, a high efficiency juncea CM is produced. We have incorporated this meal into broiler diets to measure bird performance in two 34 day feeding trials. In the first trial, up to 15% juncea CM was tested in a soybean meal control diet. The results showed no statistically significant difference for weight gain, feed conversion ratios and mortality among the diets but increasing levels of juncea CM gave numerically higher weight gains.

The juncea canola was then processed without the extraction of oil to produce a high efficiency full fat juncea (FFJCM). This FFJCM was then used in a 34 day broiler feeding trial to compare with a full fat dehulled soybean meal (FFSBM) and a full fat canola meal (FFCM) produced using the same processing method. FFJCM and FFCM were incorporated at 5% for starter feed and 7.5% for the grower feed. The results showed no statistical differences for weight gain, feed conversion ratios and mortality.

These trials demonstrate that good results can be obtained when well processed juncea canola, canola meals and their full fat meals partially replaced other protein meals such as soybean meal in broiler feeding.

I. INTRODUCTION

Juncea canola (*Brassica juncea)* was developed as a drought and heat tolerant alternative oilseed to canola in Australia. Its oil and meal qualities are similar to canola. The level and types of glucosinolates in the juncea canola meal meet the specification of canola meal with total glusinolates of less 30 umoles/g. (NSW DPI, 2009). Juncea canola oil is low in erucic acid (<2%) and moderate (57-63%) in oleic acid (Pritchard, 2009).

Canola meal (CM) has been used in poultry diets. A previous study using CM at levels higher than 10% in broiler diets showed that growth rate decreased, with poorer feed intake and conversion efficiency (Hickling, 1997). However Perez-Maldonado (2003) found that CM can be used up to 20% during the starter phase and up to 30% during the finisher phase in broiler diets formulated on a digestible amino acid (DAA) basis. This evidence demonstrated that CM can be used as a protein supplement in poultry diets.

Previous work has shown that the metabolisable energy and digestible amino acids of soybean meal were increased by about 10% when using an improved processing method (Creswell & Swick, 2009; Neoh, 2009). The studies reported here were aimed to determine if an improved processing method for juncea CM can increase its protein digestibility/availability and metabolisable energy and to determine if performance of

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properly processed full fat juncea CM and full fat CM are comparable to soybean meal with added vegetable oils and full fat soybean meal in broiler feeding.

II. METHOD AND MATERIALS

Two broiler trials were conducted at Bangkok Animal Research Center (BARC), Thailand. The first trial examined broiler performance when the high efficiency juncea CM (HEJCM) replaced 5%, 10% and 15% of soybean meal in broiler diets. The second trial compared correctly processed full fat juncea CM (FFJCM) and full fat canola meal (FFCM) with dehulled full fat soybean meal (FFSBM) and dehulled soybean meal with added palm oil (SBMPO). Starter and grower diets in both trials were formulated following the ideal protein concept to satisfy the minimum nutrient requirements of birds as listed in Table 1. The nutrient matrices of the juncea CM, full fat juncea CM, full fat CM, and FFSBM used for these two trials are presented in Table 2.

A total of 240 day old male Arbor Acres Plus broiler chicks were used in each trial. Chicks were assigned to four treatments with six replicates per treatment. Chicks were allocated equally over 24 pens at ten chicks per pen. Chicks were raised on rice hull bedding material over concrete floor pens.

Starter diets were offered to the birds from 0 to 16 days of age and grower diets were offered from 17 to 34 days of age. All diets and water were provided *ad libitum* throughout the 34 day experimental period. Body weight was determined at 0, 16 and 34 days of age. Total feed consumption was measured at 16 and 34 days of age. Faecal moisture was scored at day 35. Mortality was recorded daily.

Table 1. Minimum nutrients content of the diets (as is basis)

Diets	ME	Digestible amino acids (g/kg)						
	MJ/kg	Lysine	Meth	M+C	Tryp	Thr	Arg	ILL
Starter	12.34	12	4.44	8.4	1.92	7.44	12.6	7.8
Grower	12.97	10.5	3.99	7.67	1.78	6.72	11.34	7.04

Table 2. Nutrient matrixes of juncea canola meal, dehulled SBM, full fat SBM, full fat juncea canola and full fat canola (as is basis)

Ingredient	Juncea canola meal	Dehulled SBM	Full fat SBM	Full fat canola/Juncea
ME, MJ/kg	10.46	11.09	16.53	18.62
Protein, g/kg	350	465	360	210
Fat, g/kg	100	15	200	400
Crude Fibre, g/kg	90	35	30	60
Dig. Lysine, g/kg	16.06	27.3	21.84	10.70
Dig. Met, g/kg	6.23	6.10	4.88	4.15
Dig. M+C, g/kg	13.42	12.19	9.76	8.94
Dig Thr, g/kg	12.23	16.27	13.01	8.15
Dig Tryp, g/kg	4.18	6.34	5.07	2.79
Dig Arg, g/kg	18.74	33.67	26.07	12.49
Dig Isoleucine, g/kg	11.47	20.11	16.34	7.65

III. RESULTS AND DISCUSSION

Broiler performance for Trial 1 is shown in Table 3. There were no differences in body weight gain or feed conversion ratio (FCR) among diets with different inclusion rates of juncea CM.

At day 34, broilers offered the diet with 10% high efficiency juncea CM had the highest feed intake (3745g) which was higher (P<0.05) than those fed 0% juncea CM. Other studies have shown no reduction in feed intake when CM was used up to 15% (Rojas *et al*., 1985 & Leeson *et al*., 1987). In all treatments, birds presented similar liveability and faecal score.

Table 3. Performance of high efficiency juncea canola meal in broiler feedings (0-34 days of age).

Juncea CM %	Initial BW (g)	Final BW (g)	WG (g)	Feed intake, (g)	FCR	Liveability (%)	Faecal score
0	43	2385	2343	3632[b]	1.578	96.67	2.33
5	43	2398	2355	3715[ab]	1.578	100	2.00
10	43	2409	2366	3745[a]	1.599	98.33	2.33
15	43	2396	2353	3661[ab]	1.578	98.33	2.33
P value		0.815	0.820	0.048	0.880	0.529	0.496
Pooled SEM		17.423	17.398	27.909	0.022	1.552	0.183
CV%		1.78	1.81	1.85	3.37	3.87	19.88

[a,b,c] Means within column with no common superscript differ significantly (P<0.05).
Faecal score 1 = hard, 2 = soft, 3 = watery.

Trial 2

There were no differences (P<0.05) in body weight gain, feed intake and FCR amongst the diets (Table 4) andthe liveability and faecal score were similar among diets. Numerically, the FFSBM diet had the highest body weight gain and lowest FCR. In general, the diets containing FFSBM, FFCM or FFJCM had numerically lower feed conversion ratios when compared to the diet using SBM with added palm oil. These results showed that juncea CM and CM when processed into full fat meals performed similarly to FFSBM or SBM with added palm oil.

Table 4. Broiler performance (0-34 days) when using full fat juncea canola, canola meal, full fat soybean meal and soybean meal with added oil.

Diets	Initial BW (g)	Final WG (g)	BWG (g)	Feed intake (g)	FCR	Liveability (%)	Faecal Score (at 34 d)
SBM +PO	42	2376	2335	3633	1.556	100.0	2.50
FFSBM	42	2465	2423	3711	1.533	96.7	2.33
FFCM	42	2377	2336	3595	1.540	98.3	2.17
FFJCM	42	2415	2374	3647	1.537	100.0	2.00
P-value		0.1958	0.1945	0.3658	0.5668	0.5686	0.1692
Pooled		31.397	31.367	45.432	0.012	1.912	0.155
C.V.%		3.19	3.25	3.05	1.92	4.74	16.89

[a,b,c] Means within column with no common superscript differ significantly (P<0.05).
Faecal score: 1 = hard, 2 = soft, 3 = watery

IV. CONCLUSION

Trial 1 suggests that juncea CM can be used in the broiler diets at a level of up to 15% without affecting growth performance. It is interesting to note that despite formulating with a higher metabolizable energy of 10.46MJ/kg for the high efficiency juncea CM and 5% higher digestible amino acid levels than normal CM, the high efficiency juncea CM performed equally to the diet using only SBM.

Trial 2 demonstrates that juncea canola and canola can be processed into full fat meals with high metabilizable energy and digestible amino acids which are 5% higher than normal CM. With an oil content of 40%, these full fat meals can completely replace FFSBM or SBM with added vegetable oil in broiler diets.

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ENHANCING POULTRY PRODUCTION: THE WORLD VETERINARY POULTRY ASSOCIATION (WVPA) AND AUSTRALIA TO PRESENT AND TOWARDS 2020

T. J. BAGUST[1]

Summary

For the decade 2011-2020, the only certainty about avian health-related challenges (referred to as *avian health* in this paper*)* for the global poultry industry is that problems will continue to arise. The World Veterinary Poultry Association (WVPA) is the professional body which links the national avian specialist veterinary associations of some 40 countries. WVPA organises major international scientific congresses focussed on avian health each second year while fostering regular communication among all of these national branches. WVPA's scientific journal *Avian Pathology*, which communicates the results of scientific research internationally in avian health, has now been publishing for 40 years. By promoting avian health research and continuing professional education, the WVPA will continue to support avian veterinary scientists worldwide in their work toward better control of poultry diseases and poultry-related aspects of human health.

I. INTRODUCTION

a) The WVPA – what and whom?

The WVPA is the umbrella organisation which joins together the specialist national poultry veterinary associations that have formed independently in some 40 countries to date - As the WVPA letterhead says ***Linking poultry veterinary scientists worldwide.*** While our professional thrust and major expertise areas are closely related to avian health, a more accurate description might I suggest is avian health-in-production. The days of production animal vets being just interested in preventing/diagnosing diseases are over! So while health is certainly an important element to be able to assure, avian health must take its place alongside and will interact directly with genetics, housing & management, nutrition and animal welfare so as to achieve efficient productivity, hence sustainability in modern intensive poultry production. This is the goal which will involve us all.

The WVPA scientific journal *Avian Pathology* considers original material relevant to the entire field of infectious and non-infectious diseases of poultry (i.e. the common production bird species) as well as other bird species e.g. wild and captive birds.

b) WVPA membership

WVPA membership comprises veterinarians involved in avian science and relevant subjects, and non-veterinarians who hold recognised scientific qualifications and are engaged in research, advisory work or teaching concerned with avian science. The WVPA currently has some 2200 ordinary members. Analysis of the membership of this region's national branch, the Australasian Veterinary Poultry Association (AVPA) shows that, in 2011, of the 128 members 96 (75%) are veterinarians. This is likely to be the case for most national branches of WVPA.

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II. THE OBJECTIVES OF WVPA

The objectives of WVPA have remained essentially unchanged since the WVPA was formed in 1960. They are:

- To organise **meetings** to study disease conditions and control relating to avian species
- To encourage **research** in this field
- To promote the **exchange of information and material** for study between individuals and organisations in the avian fields
- To establish and **maintain liaison** with other bodies having related interests.

a) Towards meeting WVPA's objectives

In broad terms, what does the WVPA do for its membership? Our first Objective is the **organising of meetings** for studying field and laboratory developments in avian health, both for recognised diseases (i.e. those known to have *specific* causes) and for those disease conditions which will "emerge" from time to time in a poultry industry. While time, field experience backed up by poultry disease diagnostic investigations and research will usually unite these two categories of diseases, other forces such as the continuing evolution of pathogens (disease-causing microorganisms) and also those complex dynamic interactions which occur between the host animal - production environment will be in motion. In short "*Intensivism is the friend of Disease"*. The outcome is that health problems will continue to challenge each poultry industry nationally and, for the majority of infectious poultry diseases, globally. Communication in avian health across the world is therefore of the first importance because there are few exceptions indeed to there being but *"One World of Poultry Diseases"*.

Within each country, a local WVPA Branch such as the Australian (now Australasian) Veterinary Poultry Association will be organising locally for their members including scientific meetings usually twice each year. At the international level, the WVPA is responsible each second year for mounting what is the largest avian health congress in the world. Attendances are now regularly in the order of 1000-1500 delegates and the venues will move around the world. Because most of WVPA's current membership of 2200 is resident in the European region, the WVPA Congress each 4th year is now being held in a European location. In the intervening 2 years, the venue will be "Rest of the World".

The venues for future Congresses are decided by a (sometimes intense!) process of country representatives bidding through their making a formal presentation at the WVPA's Bureau Meeting, being the representatives of all major national Branches, four years ahead of the next WVPA Congress. Australia (Sydney) was the host for WVPC–10 in 1993, WVPC-17 at Cancun, Mexico most recently took place in August 2011, the next will be in Nantes, France during 19-23 August 2013 and WVPC-19 is scheduled for South Africa (Capetown) during August 2015. The 2017 venue will be decided by the WVPA Bureau during the WVPA Congress in 2013. It should be noted here that the conduct of a WVPA Congress requires organisation of a high standard scientific program and also the social activities for delegates and those accompanying them. Emphasis is also given to maximising opportunities for the sponsors to interact with those attending. Commercial activities and associated sponsorship now contributes some 60+ % of the funding needed to operate such an event. The scope of Topics and associated Keynotes Lectures for the 2013 WVPA Congress in France will be illustrated in the presentation.

For our second objective, **encouraging research in avian diseases** and conditions, the scientific Journal of the WVPA is *Avian Pathology*. This peer-reviewed Journal is now in its 40th year of continuous publication. *Avian Pathology* has over the years become a

foundation stone for new publications and scientific communications in international avian health research, whilst also providing a means for WVPA to be able to actively encourage the exchange of scientific information worldwide (our third objective). *Avian Pathology* is published every second month i.e. six issues per year, with each issue containing a dozen or more original scientific papers and usually a short review of the scientific aspects of a subject which is recognised as being of major importance. Each year's volume contains some 700 pages of new science, and each paper will have been examined by at least 2 peer reviewers and overseen by the Editor-in-Chief of *Avian Pathology.* By policy, there are no page charges to authors whose papers are accepted for publication.

Ratings published in 2011 for the major 10 journals for poultry science gave *Avian Pathology* the highest scores for Impact Factor (1.967) and Article Influence (0.521).The nature and range of the topics of the scientific papers that are being published in *Avian Pathology* will relate to all countries with developed or developing poultry industries.

For the final of the objectives i.e. **maintaining liaison with other bodies having related interests,** WVPA has direct relationships and communicates with our sister organisation in the global poultry industry, the World's Poultry Science Association (WPSA). For the 2008 WPSA World's Poultry Congress which was held in Brisbane, by invitation, WVPA through the AVPA Branch here was pleased to be able to assist by organising and presenting the avian health stream for that very successful WPSA Congress. For APSS in 2012, WVPA is also pleased to accept your invitation to participate and contribute.

III. WHAT ELSE IS WVPA DOING FOR ITS MEMBERS?

1) The organising of international scientific Congresses each second year is supported by WVPA through maintaining the Website <www.wvpa.net>. This is designed to operate both as a communications hub and a source of information on the WVPA as an organisation e.g. WVPA Office Bearers of the Executive as well as each of the contacts on the Bureau's 40+ country Corresponding Secretaries, the WVPA Constitution, membership, grants and awards, publications, postgraduate education contacts, future meetings and the links to related websites. This website is currently undergoing a process of systematic upgrading, which is to be completed by mid-2012.

   WVPA's newsletter *Aerosols* is published each 6 months and is circulated to all Branches. This newsletter has a major role to play in maintaining communications in avian health around the world as it keeps the numerous national branches and their members informed on what is happening within the associations of other countries. It is a requirement for each country that we have a news item from them at least once a year. Professional activities and especially brief reports from their scientific meetings, current major disease problems, poultry production summaries and pressure points e.g. feed/climatic problems, costs of production, exotic disease outbreaks and future national meetings are being regularly featured. While still being produced in hard copy, the WVPA is encouraging national Branches increasingly to now undertake their distribution by electronic means.
2) WVPA BRIEFINGS – These communiqués are now being produced quarterly. They are designed to provide detailed backgrounding for the executives of each national Branch by summarising the initiatives and activities which are currently being undertaken by the WVPA's Executive.
3) AWARDS & GRANTS - In research, apart from publication of scientific papers in *Avian Pathology*, the WVPA has for some decades now hosted the BART RISPENS RESEARCH AWARD. Named in memory of the Dutch researcher who first

developed the Rispens strain of Mareks Disease vaccine, this prestigious prize of a medal and a significant financial sum is awarded at each WVPA Congress to the author(s) of the scientific paper judged to be the most meritorious of those published in *Avian Pathology* during the previous two years. Interesting in the context of the present symposium, is that this award has been won several times already by avian health research scientists working from Australia, including Clive Jackson (Marek's Disease virus), Kevin Fahey (infectious bursal disease virus recombinant vaccine) and Jagoda Ignjatovic (infectious bronchitis virus). In the last few years, Schering Plough-Intervet has kindly undertaken to support this Award.

4) WVPA YOUNG AVIAN VETERINARIAN OF THE YEAR - To encourage the further professional development of poultry veterinary practitioners, WVPA has now teamed up with Pfizer Animal Health Global Poultry to launch a new annual global award for poultry veterinarians who are in practice or extension. This award is open to qualified veterinarians who are under the age of 35 on 1 January 2012 and will be judged by a panel of global experts. The winner will receive US$ 5,000 towards travel and accommodation expenses for attending an international scientific/technical or continued professional education meeting of their choice. The first award will be made in February 2012.
5) SUPPORT FOR YOUNG SCIENTISTS – This is provided through the Houghton Trust, UK-based and administering the royalties from *Avian Pathology* independently but in association with the WVPA. Financial assistance in part is awarded to meritorious younger scientists to assist their participation in the WVPA Congresses.

IV. AVIAN HEALTH IN POULTRY PRODUCTION

a) Avian veterinarians in intensive poultry production

We should now look into the current roles of avian veterinarians as the health professionals of the poultry industry, and in the context of commercial production. Referring to Figure 1:

1) The avian veterinarian has two key roles - the optimisation of *poultry health*-in-production and also *public health* i.e. assuring that the product which is to be eaten by humans is safe for consumption – hence the deep blue circle located at the apex of the pyramid representing production and processing. Failure in *either* of these areas will have grave commercial consequences for any poultry production company.

2) The special disciplines in which a veterinarian will have received training as an undergraduate are shown at the centre of this pyramid. It is these disciplines, along with field/laboratory experience in their practical application, which will enable veterinarians to be animal health professionals capable of understanding, diagnosing, preventing and controlling the many infectious diseases of poultry. Continuing professional education will further strengthen their abilities and range of competencies and include performing even more specialised tasks, examples being shown here as interfaces.

3) The base of this structure illustrates the high levels of interdependency and synergism that are needed to be established between the avian veterinarian and other poultry professionals, e.g. housing and poultry management experts, geneticists and nutritionists. These functions must be occurring positively as an integral part of the

production operations of any medium - large integrated operation, so as to ensure success in maintaining profitable poultry production over time

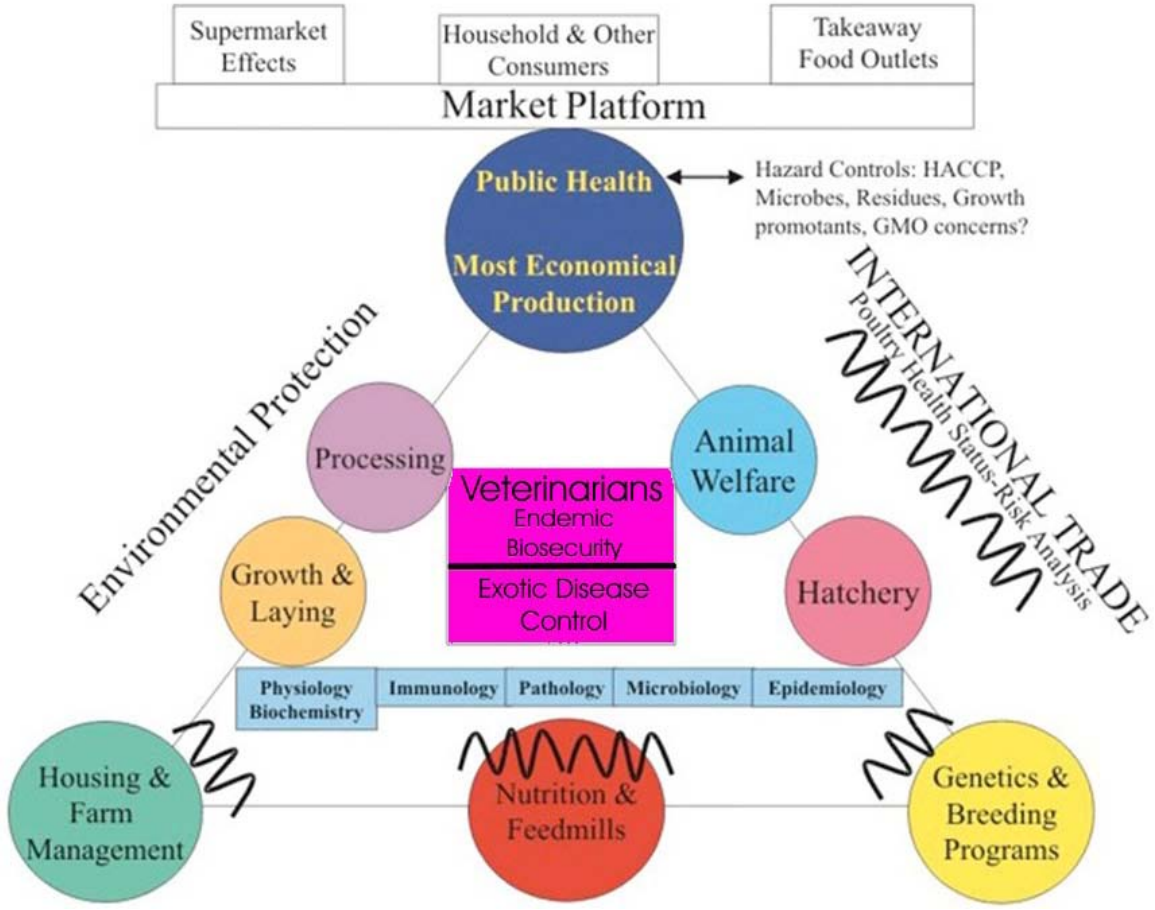

Figure 1. Roles of veterinarians in intensive poultry production (Source:Avian Health Online)

b) Current major poultry disease problems in Australia

In line with findings obtained in other advanced intensive poultry industries, the levels of economic losses to the Australian poultry industry from poultry diseases will be in the order of 10% of industry GVP (Biggs, 1982; Burns Grogan 2007). This figure includes the costs of vaccinations and medications as well as the costs of frank mortalities from diseases, primarily those from infectious causes. It also contains a component at least twice as large which will reflect the effects of subclinical disease which causes suboptimal production during the life of these poultry flocks.

Disease problems being caused by single pathogens are seen far less now in developed intensive poultry industries, a partial exception being some free-range poultry. However mixed infections, complex interactions with the production environment and poor production from disease complexes are common - particularly respiratory diseases, enteric disorders and immunosuppressive infections - which will predispose to infection by other

pathogens whether viral, mycoplasmas or bacterial. Across the poultry industries of many countries, low levels of mycotoxins in contaminated poultry feed ingredients are also now being recognised as potential causes of immunosuppression and subclinical disease in their commercial flocks.

In the Australian poultry industry the most important causes of significant economic losses, to be illustrated further during presentation of this paper are:

- ***E.coli*** **-** a major secondary pathogen in poultry, but which by itself can also kill chicks.
- **Infectious bronchitis virus**- difficult to control because of the potential of its RNA genome to undergo mutations. Multiple vaccinations needed for control. IBV has a major capacity to establish respiratory infections which predispose to secondary invasion by bacteria e,g *E.coli* and Mycoplasma; Also is an important cause of drops in egg production and with some strains, nephritis.
- **Coccidiosis (***Eimeria* spp) and additionally, a role in necrotic enteritis (*Clostridium*).
- **Mycoplasma gallisepticum –** a major respiratory pathogen in conjunction with infectious bronchitis virus and also secondary infections by bacteria (*E.coli*);
- **Infectious laryngotracheitis** - an old enemy which was relatively well controlled, apart from sporadic outbreaks in started pullets. However in the last several years since 2007, ILTV has shown resurgence in disease importance especially on broiler sites .While the likely root cause has been genetic recombination occurring between those ILTV strains which have been circulating in the field in recent years, complicating and even predisposing factors involved include periodic shortages experienced by industry in supply of commercial ILT vaccines in the mid-2000's, and some practices in our modern intensive poultry industry which will assist the persistence of ILTV e.g. the high densities of poultry stocks in production areas and also arrangements used for transport of poultry. While the unacceptable levels of ILT-associated disease losses on broiler sites appear to be coming under control in later 2011, it is now apparent that at least two new molecular classes of ILT viruses, each being more aggressive and invasive than Australian vaccine strains of SA2 and A20, have emerged in Australia in recent years.

Other poultry pathogens which must always be kept in mind and monitored closely:

- **Marek's Disease** – While quite well controlled at present, MDV has a high threat potential for the emergence of new pathotypes, which the evolutionary history of MDV to date indicates will almost certainly be of enhanced virulence;
- **Hypervirulent infectious bursal disease virus** (vvIBDV) – another of the major exotic poultry pathogens which occurs in countries in this region and is of high pathogenicity. This virus is relatively resistant to physical inactivation. Although not egg-transmitted, vvIBDV presents a continuing threat through contamination persisting whether in carcasses, on inanimate objects, wrappings or even on clothing.
- **Avian influenza H5N1** which emerged from 2003, not only as a major pathogen but also presenting a major potential threat to human health. H5N1 remains endemic in poultry flocks in Indonesia, Thailand, China and Vietnam. While vaccine use will suppress the disease effects in poultry, it does not however prevent H5N1 infection from occurring. Australia has already experienced and eradicated some 5 separate outbreaks of avian influenza (H7), the sources being migratory birds which inadvertently contaminate the groundwater supplies in use for commercial poultry.

Avian Pathology is continually publishing research papers on each of the agents listed above. It has also published contemporarily on each of the three major infectious disease challenges which Australia's poultry industry has faced in the last decade, these being :

- **Newcastle Disease Virus** - Molecular evolution led to emergence of virulent neurotrophic NDV **(vNDV)** from mild "lentogenic "Australian strains (1998 - 2000)

- **Avian Leukosis Virus (Subtype –J) -** detection and eradication from elite broiler breeder stocks. After development of in Australia of technology for detection of ALV-J, industry testing of breeding stock at grandparent level was followed by removal of ALV-J positive flocks. Breeding stocks supplied by overseas primary breeding companies were eventually able to be certified free of ALV-J (2000 - 2005)

- **Infectious laryngotracheitis virus (ILT) –** See above (2007- present).

For completeness and the recent occurrence of the problem, should also be included **Paramyxovirus (PMV-1) infection of pigeons** (August – December 2011)
A paramyxovirus not previously reported in Australia was detected in hobby pigeons on a number of properties in Victoria causing high mortality, associated with lethargy, gastrointestinal and neurological signs. 57 outbreaks were reported in Shepparton and in suburbs of Melbourne. Controlled by quarantine of pigeon flocks to their home sites. While natural infection by this virus has not been detected in chickens, this PMV-1 occurrence may have complicated Australia's international poultry health status in relation to assuring industry freedom from virulent Newcastle Disease Virus.

The other key activity of veterinarians working in the poultry industry, maintaining public health, requires them to be working to reduce the threat potential of several bacterial agents known to infect humans and cause illness. The problem of zoonotic food-borne bacterial pathogens particularly **Campylobacteria**, **Salmonella** species serotypes and **Listeria monocytogenes** can present huge threats to the economic viability of commercial poultry companies in both the egg and meat sectors of a poultry industry. Each of these pathogens can cause food-poisoning outbreaks, but the last is particularly problematical because it can replicate in cooked/processed product even when being stored under refrigeration, then cause severe disease and even deaths in humans.

In the next decade, for poultry production around the world, and specifically for Australia, the specific details of future poultry and human health-related challenges are not able to be predicted with any precision as there are many variables operating. What is known, however, is that avian health-related problems and challenges will be occurring! So our best approach then lies in forward defence, by ensuring we have **well-trained professionals** in place in the poultry industry and supporting them **with accurate information** from past and current occurrences. The WVPA along with its national Branches worldwide will be working to contribute to strengthening of the state of avian health preparedness in both of these areas.

V. WVPA AND GOING FORWARDS

During 2011-12 we are undertaking the development of Strategic Planning for WVPA operations through to 2020. Some of the key points of activity will include:

- WVPA will be continuing its advocacy for avian health research and continuing education.
- Communications upgrading to continue two-ways for Executive – National Branches.
- WVPA while research-driven is to monitor so as to ensure that our scientific Congresses are also meeting the current needs of practitioner , industry and government based members.
- WVPA- commercial interfacing in mutual- benefit activities are to be further encouraged
- Promoting a program for scientific fostering & mentoring which will be focussed on assisting those poultry veterinarians who are working in countries with developing industries.
- Furthering Membership: Focus to be on developing new Branches for Asia, South America and Eastern Europe, particularly in those countries developing large poultry industries.
- Organising future WVPA Congresses – Protocols are now to provide for 2000+ delegates.

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WPSA: 100 YEARS OF SERVICE TO THE WORLDWIDE POULTRY INDUSTRY

R.A.E. PYM[1]

Summary

WPSA has played a pivotal role over the past 100 years in facilitating the development of the global poultry industry through the organisation of branches in member countries and forums to identify and discuss issues, problems and their solution, as well as structures to disseminate that information. In this manner, WPSA has contributed significantly since 1956 to the development of the Australian poultry industry. The Australian poultry industry has benefitted greatly over the years as a result of a well-supported and high quality national poultry research program, facilitated through close cooperation between the poultry industry itself, national and state governments, and the country's research and education institutions.

In response to the major projected increase in poultry meat and egg production and consumption in developing countries, WPSA has focussed increasing attention on facilitating efficient, sustainable and socially equitable poultry production in these countries. Associated with this, WPSA and WVPA are working towards a closer degree of cooperation and collaboration.

I. HISTORY AND STRUCTURE OF WPSA

The World's Poultry Science Association (WPSA) celebrates its 100th anniversary in 2012. The International Association of Poultry Instructors was founded at a meeting in London in July 1912; Australia was represented at that meeting. The organisation later became known as the World's Poultry Science Association in 1928 but it was not until 1946 that national branches (UK and USA) were formed. The Australian branch was formed in 1956. There are now some 82 country branches, with an additional four countries presently in the process of forming a branch, and more than 7500 members of the Association.

There are two regional Federations of the Association viz the European Federation and the Asian Pacific Federation. One of the important activities of the Federations has been the development of working groups in specific discipline areas. The European Federation has 11 working groups viz. Economics and Marketing, Nutrition, Breeding and Genetics, Egg Quality, Poultry Meat Quality, Reproduction, Hygiene and Pathology, Poultry Welfare and Management, Turkeys, Education and Information, and Physiology, and the more recently formed Asian Pacific Federation has three working groups viz. Small-Scale Family Poultry Farming, Water Fowl, and Ratites. All of these working groups organise periodic symposia.

The motto of the Association is "Working together to feed the world" and its main focus is on the promotion of poultry science through the dissemination of information under the pillars of Research, Education and Organisation. To this end, country branches organise local, regional and national meetings on a wide variety of topics and for different participant groupings according to topic and level of presentation. Federation working groups organise symposia, usually every two or four years, and the Federations organise their multi-

[1] President, WPSA

disciplinary regional poultry conferences every four years. The most recent of these were the European Poultry Conference in Tours, France in September 2010 and the Asian Pacific Poultry Conference in Taipei, Taiwan in March 2011.

The flagship meetings of the Association are the multidisciplinary World's Poultry Congresses (WPC), held every four years. In addition to the large and comprehensive technical program, WPCs also include a major poultry trade exposition. Country branches bid for the right to host these meetings at the WPC staged eight years prior to the subject WPC. The Australian branch hosted the 12th WPC in Sydney in 1962 and, following its successful bid at the 2000 Montreal WPC, the Australian branch hosted its second WPC (23rd) in Brisbane in July 2008. The next WPC (24th) will be held in Salvador, Brazil in August 2012.

The association produces a quarterly journal viz. *The World's Poultry Science Journal (WPSJ)*, which publishes review articles covering a wide variety of discipline areas associated with poultry production. The journal, which does not publish original research papers, is highly regarded by the poultry industry and research community as indicated by its high *Impact Factor* (1.48 in 2010). Members of WPSA receive either a hard or electronic copy of the journal every three months. To reduce costs, WPSA is keen to increase the number of members electing to receive the electronic copy, but is mindful of the need to maintain supply of hard copy to those electing this in developing countries where Internet access is problematic. In addition to *WPSJ*, WPSA produces an electronic quarterly Newsletter which can be accessed on the WPSA website www.wpsa.com.

II. WPSA IN AUSTRALIA.

Following the establishment of the Australian branch of WPSA in 1956, in recognition of the great distances and the need for regional branches to service the rapidly developing industry, sub-branches of WPSA were established in all of the States, with three (sub)-branches in NSW (NSW (essentially Sydney), Gosford/Newcastle, and Tamworth/ Armidale). The branches hold regular meetings to provide technical information to poultry farmers and industry service personnel. In addition to these local and regional meetings, in the 1960s the Australian branch of WPSA was a foundation sponsor together with the Australian Chicken Meat Federation (ACMF) and the Stock Feed Manufacturers Association of Australia (SFMAA) in the establishment and continuing organisation of the triennial Australian Poultry Conventions. The Australian Egg Corporation Ltd (AECL) replaced SFMAA as a sponsor in 1999. The last of these Conventions (13th) was held on the Gold Coast in 2005, and the 2008 meeting was subsumed into the 23rd World's Poultry Congress in Brisbane.

In 1989, the Australian branch of WPSA combined with the University of Sydney to establish the first of the annual Australian Poultry Science Symposia. This initiative was championed by Bruce Sheldon, the then Australian branch President, and the joint symposium proposal was developed from the annual University of Sydney Poultry Husbandry Foundation Symposium. The Organising Committee membership comprised four representatives from each organisation. Since its inception, the Chair of the Organising Committee has been retained by the University of Sydney, but the position of Editor has alternated between the two organisations every three years. A technical sub-committee was

also established with wide representation from research and industry-based scientists. Although the structure of the committees has changed over the years, the same basic arrangement persists. WPSA is very pleased with the way that the symposium has developed over the years to now be an important and highly regarded regional poultry science meeting with international speakers and participants.

WPSA in Australia has been very well served by its officers, all acting in an honorary capacity. Officers who have made an outstanding contribution include: Otto Moll, principal instigator in the establishment of the Australian branch in 1956 and Secretary /Treasurer of the branch from 1956 to 1974, a major contributor to the development of the Australian poultry industry over this period, Managing Director of Multiplo Pty Ltd, and instrumental in securing and organising the 12th World's Poultry Congress in Sydney in 1962; Jeff Fairbrother, President of the Australian branch from 1979 to 1983, Executive Director of the Australian Chicken Meat Federation where he played a crucial role in the development of the meat chicken industry in Australia, a much valued member of the Egg and Chicken Meat Research Councils, and organiser of many of the Australian Poultry Conventions; and Bruce Sheldon, President of the Australian branch from 1983 to 1992, much admired and revered Senior Vice President on the Board of the World's Poultry Science Association, Senior Principal Research Scientist in poultry genetics at CSIRO and strong supporter of Australia's role in assisting the development of the poultry industry in the developing countries of our region. Otto Moll and Bruce Sheldon were respectively inducted into the *WPSA Poultry Hall of Fame* in 1996 and 2004. Many other officers and members have made exceptional contributions to the organisation and to the Australian and global poultry industries.

III. POULTRY RESEARCH IN AUSTRALIA

There has been a mutually beneficial relationship between the Australian poultry industry, the Australian and State governments and the country's poultry research and education institutions. The industry has recognised the importance of research and has been a willing partner, if not instigator, of structures to facilitate that research. The establishment of the Australian Egg and Chicken Meat Research Councils was an important step in securing ongoing poultry research capability in the country. These Councils have been through a variety of name and structural changes, but they still play an important role in maintaining the high level of poultry research capability which has benefitted the Australian poultry industry since the inception of the Councils.

State governments provided very significant funds and facilities for poultry research in all of the states from the 1950s through until the mid 1980s (NSW) or later (2008, Queensland), but due to a change in State Government priorities, most of these facilities are no longer available. The loss of these in many cases excellent facilities has had considerable implications for the nature of the research capable of being conducted, and also the future cost of research if the same sort of facilities require to be constructed. It is most regrettable that the exceptional large scale broiler and layer production research facilities at the Queensland Department of Primary Industries (QDPI) Poultry Research Centre at Alexandra Hills were lost to the Australian poultry industry following the closure of the Centre in 2008. These were undoubtedly the most modern, best designed and equipped large scale poultry

production research facilities in Australia, particularly for work on poultry nutrition. Following discussions with QDPI in 2008, the Australian branch of WPSA attempted to generate interest from stakeholders in taking on the Centre as a jointly managed and funded Australian poultry research centre, without success.

The establishment of the Australian Poultry CRC in 2003 made a most valuable contribution to poultry research capability in the country and the recent approval of its phase 2 application in 2010, indicates the high regard with which the research program is held by the poultry industry and the Australian government.

The principal impact of WPSA on research and on the application of that research, is through dissemination of information through the organisation of meetings, workshops, seminars, symposia, conferences and Congresses, involving farmers, industry technical and other personnel, extension specialists, researchers, educators and/or government personnel. As a not-for-profit association, WPSA is not set up for, or capable of, running or funding a research program. What the Association does very well, is bring people together in appropriate forums to discuss solutions to problems.

There are many Australian researchers and others who have made very significant contributions over the past sixty or more years and over a wide array of discipline areas, to the advancement of poultry science for the benefit of the industry, both here in Australia and globally. It is not my intention here to identify them or their contribution. This is the subject of another paper and for someone braver and more fully acquainted with past Australian poultry research than I. In this paper I would like to share with you some of the initiatives and activities of WPSA since the election of the present Board in Brisbane in July 2008.

IV. SUPPORT FOR POULTRY PRODUCTION IN DEVELOPING COUNTRIES

The very large majority of the projected increase in global poultry meat and egg production and consumption over the next 20 or more years will take place in developing countries (Farrell, 2008). It is thus appropriate that WPSA's focus should be on facilitating efficient and sustainable poultry production in these countries. This was the subject of an earlier paper to APSS (Pym, 2010).

In considering the facilitation of efficient, sustainable and socially equitable poultry production in developing countries, it would seem sensible to define the poultry production systems in use in the these countries, the present impact of these on the people, the sustainability of the systems and the nature and drivers of transition from one system to another. For purposes of this discussion, the FAO classification that was originally developed for categorizing the different biosecurity systems (and later lost importance for this purpose) is used here. Applying a similar classification on the husbandry and feeding aspects rather than biosecurity, *sector 1* is large scale commercial production ("improved" genotypes, large scale, sophisticated facilities and equipment with automated systems and feeding with compounded diets); *sector 2* medium scale commercial (similar to sector 1 but smaller and somewhat less sophisticated, less automation); *sector 3* small-scale commercial (small flocks of "improved " genotypes mostly local materials in facilities, no automation but provision of compounded diets often formulated from local materials); and *sector 4* (small semi-

scavenging flocks of indigenous birds, typically given household scraps and a small amount of grain daily).

The structure of the poultry industry with regards to sector proportion varies widely between the different developing countries and there is considerable variation between the drivers for transition from one sector to the other. In the poorer developing countries, many families in rural regions have a household flock of indigenous scavenging chickens (sector 4 production system) which are kept to improve their nutrition and livelihoods (Alders and Pym, 2009). In some countries, these flocks constitute as much as 90% of the country's total poultry population, although due to low productivity, their contribution to national production of poultry meat and eggs, is proportionally much lower (Pym et al., 2006). The poultry meat and eggs produced by their own or their neighbours' flocks, are the only poultry meat and eggs that the majority of these mostly very poor families ever eat. As they are not dependent upon supplies of poultry feedstuffs or improved genotypes from outside, they are essentially unaffected by changes in the other sectors of production. Very few of them aspire to becoming larger scale commercial producers, and indeed there are limited opportunities to do so. The system is very low input and output, but because the food supplied to the birds is essentially "free", it can be an economically efficient production system, particularly with small cost-effective inputs into management and disease control which reduce chick attrition and bird mortality generally (Henning et al., 2009).

It goes without question that the provision of affordable and good quality poultry meat and eggs for the urban dwellers in developing countries, is dependent upon efficient production systems. In terms of domestic production, this essentially involves the use of "improved" meat or egg genotypes and the provision of compounded feeds. Given that feed accounts for about 70% of the cost of production, a critical element in this is ready access to affordable and high quality feed ingredients, whether they be locally produced or imported. Importation undoubtedly has benefits, but also significant drawbacks in relation to the establishment of a viable and sustainable poultry industry in the country. Due to the effects on global availability and price of animal feedstuffs of climate change, past unsustainable agricultural practices and urban encroachment through the availability of arable land, combined with increased competition with requirements for human food and the increasing demand for biofuels, there is a very good argument for developing countries to become as self sufficient as possible in the production of these feedstuffs (Hindricks and Steinfeld, 2008).

In October 2011, I visited a number of southern and eastern African Countries (South Africa, Mozambique, Kenya and Ethiopia) in relation to the establishment of WPSA branches and the development of the African Poultry Network. Most of these countries are presently not self sufficient in poultry feedstuff requirements, but many countries throughout Africa have the potential to be so (Lovell, 2011), given the incentive and direction combined with technical and financial support from government, NGOs and global funding organisations, at least initially.

Availability of feed ingredients is a fundamental requirement, but to maximise the efficient use of these resources in the form of balanced diets requires technical expertise and good information on feed composition. This is one area where a very significant input is required in many developing countries. Frequently diets are compounded with little

information on nutrient composition with the result that performance outcomes are compromised, often quite severely. There is an undoubted role for WPSA in facilitating more effective use of feed resources in developing countries through the establishment of branches and the concommitent focus on information exchange through meetings, workshops etc.

Closely associated with this, in sector 4 semi-scavenging village poultry flocks, the contribution to household food security and poverty alleviation could in many cases be dramatically increased if the chicks could be kept alive; mortality to six weeks of age is frequently in excess of 60%. One very important intervention to dramatically reduce mortality is the provision of relatively small amounts of high quality starter diet for about two weeks (preferably in a creep feeder and in concert with protection from predation, disease and the climatic elements). Thus the establishment of feed mills providing good quality balanced diets in these countries, impacts in a very meaningful way on both the commercial production of poultry meat and eggs as well as the production of meat and eggs from the indigenous semi-scavenging village birds. I believe this to be a very important challenge to all of us as members of WPSA and particularly to those involved in poultry nutrition and poultry feed manufacture.

Over the past ten years or so, WPSA has been actively involved in supporting poultry science and education in developing countries through: the incorporation of the International Network for Family Poultry Development (INFPD) as a global working group of WPSA; the establishment of the Asian Pacific Federation Working Group on small-scale family poultry farming; the organisation at numerous poultry conferences in developing countries of workshops and symposia focussed on defining constraints to development of the national poultry industry; the establishment of the Mediterranean Poultry Network and; more recently, the establishment of the (sub-Saharan) African Poultry Network. The latter network was established at a workshop organised specifically for this purpose immediately following the European Poultry Conference in Tours in September 2010.

V. SOCIALLY EQUITABLE AND SUSTAINABLE POULTRY PRODUCTION

Despite the obvious enormous success of the poultry industry in the efficient production of poultry meat and eggs for the burgeoning global human population, concerns have emerged on a number of fronts. From society and consumers in both developed and developing countries there have been expressions of concern about health threats from diseases (principally HPAI), food safety and quality, animal welfare and the impact of poultry production on the environment. In more recent times concerns have also been expressed about the loss of biodiversity and the marginalization and disenfranchisement of small-scale commercial producers in developing countries arising from competition with large-scale commercial operations recently established in the country.

In his keynote address *"Emerging boundaries for poultry production: Challenges, opportunities and dangers"* at the 23rd World's Poultry Congress in Brisbane in July 2008, (Hodges, 2009), Dr John Hodges challenged the poultry industry to examine its practices from sustainability and social equity perspectives. The challenge was taken up and a *think-tank* meeting to discuss sustainability and social equity issues relating to developments in global poultry production, was organised in Freising, Germany in June 2009 with

representation from WPSA and FAO and from a number of the large global poultry breeding companies.

An important driver for industry participation in the think tank, was the acknowledged need by industry to identify genuine areas of societal concern that require to be addressed or rectified. There is a generally recognized need by industry for better and more open communication with consumers, ideally through a mechanism that is perceived generally by society as being authoritative and objective, for providing the public with accurate information about industry practices, to publicise positive features and developments within the industry as well as measures taken to overcome problems, and to de-bunk misinformation.

At the think tank, participants were initially asked to list their own and perceived societal *"concerns"* about poultry production. From the responses, the main focus of societal concern would appear to be directed towards large-scale poultry production systems in both developed and developing countries, as these are seen as the main contributors to production of poultry meat and eggs globally and as the "models" adopted by the industry in developing countries. There was good evidence for societal concern about the impact of replacement of existing production systems in developing countries and about practices in all production systems.

The following specific concerns were listed: (1) diseases and food safety, (2) welfare of animals, (3) environmental impact, (4) loss of biodiversity, (5) the impact of Intellectual Property Rights and patents, (6) impact on small producers, and (7) concentration of ownership. This was followed by an in depth discussion of the reasons for the concerns expressed by society. It was generally accepted that irrespective of the objectivity of the reasoning behind such expressions of concern, the industry needed to deal with these issues. It was noted and acknowledged that significant improvements have been made over the years and continue to be made by the poultry industry in many of the areas, e.g. in bird health and welfare, environmental impact and in product safety, but these are all still areas of ongoing societal concern. The challenge here is to address and rectify the areas that require attention, and to counter unwarranted criticism and complaint with objective and reasoned argument.

One of the outcomes from the *think tank*, was a recognition of the need for input from a much wider array of stakeholders than present at Freising. The FAO representatives at the meeting undertook the task of arranging this and organized a special one-day session at the European Poultry Conference in Tours, France in August 2010, entitled *"Guidance for the poultry sector-issues and options"*. The outcomes of that meeting will be published shortly.

This process is continuing with involvement from WPSA. There is a current proposal for WPSA and the Global Challenges Forum (GCF) to co-sponsor a global summit where the business leaders in the worldwide poultry industry would be invited to participate and address current and emerging issues related to equity and sustainability. The GCF is committed to exploring challenges jointly with the leaders of significant worldwide activities with the aim of promoting sustainability and ethics for all stakeholders. The suggestion is that the summit take place in April/May 2012 so that the outcomes can be presented at the World's Poultry Congress in Brazil in August. Discussions are presently underway between GCF and WPSA.

VI. WPSA PROGRAMS AND AWARDS

WPSA has developed a number of programs and awards in keeping with its mandate of facilitating poultry research and education globally.

The *Travel Grants Scheme* is set up to provide support for young researchers, students and others to attend WPSA-sponsored meetings in other countries. The awardees require to be aged 40 or under and to have been a member of WPSA for a specified period. The award is open to applicants from all countries. Details of the award and application procedures are provided on the WPSA website www.wpsa.com

The *Speakers Bureau Scheme* has been set up to provide funding to cover travel costs of speakers at meetings organised by WPSA branches in developing countries. The airfares of the approved speakers (up to two speakers per meeting), is covered under the scheme. At its annual meeting in Cesme, Turkey in November 2011, the WPSA Board voted to extend the scheme to include meetings in "developed" countries. The revised guidelines for the scheme and application procedures will shortly be included on the WPSA website.

There are a number of awards established by WPSA which recognise exceptional contribution to the organisation, poultry science and/or the world-wide poultry industry. These include: the MacDougall Medal, induction into the WPSA International Poultry Hall of Fame, and the Research, Education and Organisation awards made by the Foundation for Promoting Poultry Science of the Netherlands branch of WPSA. Details of these awards are included on the WPSA website. The winners of the Netherlands branch awards and the inductees into the International Poultry Hall of Fame, are announced at each World's Poultry Congress.

VII. RELATIONSHIP WITH WVPA

Over the past four or more years, there has been ongoing dialogue between WPSA and WVPA about bringing the Associations closer together. It was felt by some that there would be considerable merit in running joint meetings, even joint world congresses. A particular benefit of this would be the opportunity to run multidisciplinary sessions focussed on multifactorial problems.

One of the particular constraints to combining the world congresses of the two organisations is the inherent structure within each organisation associated with the determination of the country and venue of forthcoming meetings. In WPSA, a very significant section of the Constitution and By-laws deal with the processes associated with the selection and election of the host country of forthcoming Congresses.

There are, however, good examples from here in Australia, of how the organisations can work together to achieve desirable outcomes. At WPC2008 in Brisbane, the poultry health and disease stream of the Congress was organised by the Australian Veterinary Poultry Association (AVPA) in the form of the 6^{th} *Asia Pacific Poultry Health Conference.* This ran concurrently with the other streams of the Congress. The other example of such collaboration is the shared session with AVPA on the third day of APSS, where multidisciplinary health and disease issues and problems are usually discussed.

Issues relating to poultry heath are not the exclusive province of WVPA. Most health issues and problems are multi-factorial and WPSA historically has demonstrated a keen interest and involvement in promoting discussion of these problems. The involvement of poultry health professionals is, of course, fundamental to this. In many developing countries, the issue which stimulates producers and others to seek assistance and advice, is frequently related to bird health problems. This collective action often results in the formation of a branch of either WVPA or WPSA in the country. This argues for close collaboration and cooperation between the two Associations.

In northern Africa, WVPA is relatively well represented in the countries bordering the Mediterranean Sea, whereas apart from Egypt, WPSA is not. One of the aims of establishing the Mediterranean Poultry Network within WPSA, was to facilitate the development of WPSA branches in that region through collaboration with WVPA. This has been somewhat interrupted by recent developments there with the "Arab Spring" uprisings.

There are other ways in which the two organisations can interact and collaborate and I look forward to discussions with my good friend and colleague, Dr Trevor Bagust, the new President of AVPA, to explore opportunities for a closer relationship between the two organisations.

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THE RELATIONSHIP BETWEEN PRE-LAYING ACTIVITY AND CORTICOSTERONE CONCENTRATIONS, AND THE INTERPRETATION FOR LAYING HEN WELFARE

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Summary

Some opponents of cage housing for laying hens argue that nest boxes are important for hen welfare. Evidence to support this argument is that most hens choose to lay in a nest box if available (i.e. hens show preference), and hens are more active in the absence of a nest box and may perform pacing before egg laying (i.e. hens show frustration). In this experiment, in which hens were housed in groups of 2, 4 or 8 in 8-bird cages with and without a nest box, we investigated the relationship between pre-laying activity and basal stress levels, measured via corticosterone in eggs and plasma (~4-5 h post-laying). The posture and behaviour of 58 Hy-line Brown hens during 2-h pre-laying were collated from video records for a total of 135 egg-laying events. Hens that displayed more bouts of sitting posture, or that spent less time sitting, had higher plasma corticosterone concentrations. Further, hens that displayed more pre-laying activity had lower plasma corticosterone concentrations, although the occurrence of walk/run behaviour was not associated with plasma corticosterone. Lower activity, increased frequency of sitting and reduced time spent sitting prior to oviposition may reflect increased stress as indicated by the higher plasma corticosterone concentrations. While the evidence is limited, the finding that some pre-laying activities of hens were related to plasma corticosterone concentrations is contrary to expectation, and highlights a lack understanding of the relevance of pre-laying activity and sitting posture to layer hen welfare.

I. INTRODUCTION

Pre-laying behaviour in domestic hens appears to involve two phases (Sherwin and Nicol, 1992). About two hours prior to oviposition, hens become more active in a phase of behaviour termed 'searching'. Locomotion increases and hens perform behaviours such as inspection of potential nests. In wild populations this phase seems to facilitate hens seeking an appropriate site for egg laying and subsequent incubation of the eggs (Duncan *et al*., 1978). Once hens have selected their nest site, the 'sitting' phase commences; this includes the adoption of a sitting posture interspersed with 'nest-building' activities such as scratching the floor/litter, rotating the body on the nest and collecting litter if available.

In furnished cages, the majority of hens chose to lay in a nest box, indicating that nest boxes are an important resource for hens (Barnett *et al*., 2009). However, providing a nest box in the cage was associated with elevated plasma corticosterone around the time of onset of lay (23 weeks) compared to hens without a nest box (Cronin *et al*., 2008). The latter authors suggested an acute stress response was associated with competition for the nest box. While the motivation for hens to lay in a nest box has been measured (e.g. Appleby and McRae, 1986; Duncan and Kite, 1989), Freire *et al*. (1997) indicated that motivation may only increase close to the start of the sitting phase. In the absence of a nest box, hens were reported to be more active (Cooper and Appleby, 1995) and performed more walking (Cronin *et al*., 2005). Duncan and Wood-Gush (1972) described this increased ambulatory behaviour as stereotyped pacing, which Wood-Gush and Gilbert (1969) interpreted as a sign of frustration in hens. Brantas (1980) amongst others, concluded that increased activity

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performed in the absence of a nest box was a behavioural indicator of a welfare problem. Yue and Duncan (2003) compared the pacing behaviour of hens aged 28, 32 and 36 weeks, in cages with a nest box, without a nest box and when access to the nest box was blocked. Hens with access to the nest box spent significantly less time pacing in the hour before egg laying (7%) compared to hens that had no experience of a nest box (23%) or that had their nest box blocked (20%), and there was no change in behaviour as birds aged suggesting that hens did not adapt, at least behaviourally, to the lack of a nest box. Few studies have specifically examined the effects of nest boxes on hen physiology: Guesdon and Faure (2004) and Barnett *et al.* (2009) found no effects of the presence of 'furniture', including a nest box, and Beuving (1980) found no effects of a nest tray with litter, on adrenal responsiveness of laying hens. There are no reported studies investigating the relationship between pre-laying behaviour, nest box use and physiological stress response (and thus welfare). The objective of this experiment, therefore, was to investigate the relationship between hen posture and behaviour in the pre-oviposition period and stress physiology, measured by corticosterone concentrations in plasma and eggs around the time of the video-recorded egg-laying event.

II. MATERIALS AND METHODS

The activity of 58 focal hens during the 2 h preceding oviposition was collated from video records. The birds were part of a larger experiment previously reported by Cronin *et al.* (2008) involving a total of 112 Hy-Line Brown hens housed from 16 weeks of age in Victorsson 8-bird furnished cages (Sweden). The experiment used six cages in each of two adjacent climate-controlled rooms. The perch and dust bath had been removed from the cages and half of the cages had a nest box. The present experiment had a 2x3 factorial design; the main effects were Nest box (present vs absent) and Group size (2, 4 vs 8 birds/cage). There were two replicates in time, and in each replicate, each Nest box x Group size treatment combination was represented in each room and was allocated to cages at random. Cages measured 1.2 m wide, 0.5 m deep and 0.45 m high at the rear. The nest box (if present) was 0.24 m wide, 0.5 m deep and 0.27 m high and was positioned on the right side of the cage. Nest box floors were 'astro turf'. Feed and water were available *ad libitum*.

A continuous video record was made of all experimental hens from 16 weeks. Cameras were positioned above and below each cage and inside nest boxes. Each hen was identified by a unique combination of white and black leg bands, and head and back feathers marked with carbon-based ink. A total of 135 egg-laying events were analysed from the video record, over four occasions (sampling events): the tenth egg laid by the hen (Egg10) and around age 22, 29 and 36 weeks. Approximately equal numbers of hens were observed at each sampling event (n=35, 34, 33 and 33 hens, respectively). Hens were only observed once per sampling event and the activity of 22, 13, 5 and 18 hens, respectively, were collated in 1, 2, 3 and 4 sampling events. Within sampling events, focal birds were selected at random if possible, but the objective was to balance the number of focal hens according to Nest box x Group size treatment combinations. In addition, there were three Oviposition-site categories: (1) oviposition occurred in the nest box, (2) a nest box was provided but oviposition occurred on the cage floor, and (3) no nest box provided and oviposition occurred on the cage floor. The number of focal hens observed in each oviposition-site category, averaged across the six treatment and sampling event combinations, was 4.6 (±0.67), 2.0 (±0.95) and 4.7 (±0.98) hens, respectively (mean (±SD). The frequency and duration of each focal hen's posture (stand, sit) and behaviour (inactive, walk/run, other) were quantified using the continuous recording technique via the Observer XT program (Noldus, The Netherlands). Corticosterone concentrations in egg albumen and plasma were measured for each hen (Barnett *et al.*, 2009). Within sampling events, hen video data were collated on day of egg collection for

corticosterone, which tended to occur a few days before plasma collection. Plasma was collected between 1300-1400 h using the method described by Barnett *et al*. (2009).

Statistical analysis using linear mixed models in REML (GenStat release 11.1, VSN International Ltd) was used to examine the effects of the Nest box, Group size and Sampling event on the frequency and duration of pre-laying posture and behaviour, and the relationships between posture and behaviour and corticosterone concentrations. Egg corticosterone data were available for all four sampling events, whereas plasma corticosterone data were only available for three sampling events (weeks 22, 29 and 36). The effects of Oviposition site, Group size and Sampling event on the frequency and duration of visits to the nest box were also analysed. Where necessary, data were normalised using the log or square-root transformations and back-transformed means (BTM) are presented. To facilitate comparisons, least significant differences (LSD) have also been calculated.

III. RESULTS

Hens spent more of the 2 h before oviposition standing compared to sitting (67.7% vs 32.3% of the time, respectively; se 2.85%) and, on average, hens changed posture between standing and sitting on 10.4 occasions (se 1.06). Group size affected the time spent sitting (and its complement: standing), with birds in 2-bird cages spending more time sitting (P = 0.009) than birds in 4- and 8-bird cages (mean: 43.5%, 27.7% and 25.1% of observation time; LSD = 11.9%) and less time standing (mean: 56.3%, 72.0% and 74.8% of observation time; LSD = 1.88%). In addition, the frequency of sitting was higher at Egg10 and 22 weeks compared to 29 and 36 weeks (log: 2.257, 2.268, 1.810 and 1.924; LSD = 0.348, P = 0.013; BTM: 9.6, 9.7, 6.1 and 6.9 bouts). The predominant behaviour of hens was ‘inactive’, which accounted for 71.1% (se 2.67) of hens’ time budget. The complement of ‘inactive’ was ‘total activity’ (mean 28.9% of the time), and there were no differences due to the main effects on ‘total activity’. However, of the behavioural components of ‘total activity’, the duration and frequency of walk/run differed due to the Nest box and Sampling event main effects. In cages with, compared to without, a nest box, hens spent less (P = 0.007) time in locomotion (log: 1.400 and 1.926, LSD = 0.377; P = 0.007; BTM 4.1% and 6.9%) and there were fewer bouts of walk/run behaviour (log: 1.400 and 1.926, LSD = 0.377; P = 0.007; BTM 46.0 and 77.7 bouts). Hens at 22 and 29 weeks spent less time in walk/run than at 36 weeks (log: 2.257, 2.268, 1.810 and 1.924; LSD 0.348, P = 0.013; BTM: 9.6%, 9.7%, 6.1% and 6.9%, for Egg10, and Weeks 22, 29 and 36). The frequency of walk/run behaviour was lower at 22 weeks than at 36 weeks (log: 4.095, 3.899, 4.050 and 4.320; LSD = 0.286, P = 0.031; BTM: 60.0, 49.4, 57.4 and 75.2 bouts for Egg10, and Weeks 22, 29 and 36). Plasma corticosterone concentrations decreased as total pre-laying activity increased (b = -0.01354; P = 0.039), time spent sitting increased (b = -0.01562; P = 0.013) and the frequency of sitting decreased (b = 0.03544; P = 0.034). However, there were no relationships between pre-laying behaviour and egg albumen corticosterone concentrations.

In cages with a nest box, while there was a tendency (P = 0.052) for nest-box layers to visit the nest box more than non-nest box layers (3.1 vs 1.7 visits, LSD = 1.36), the duration of nest box occupancy was strongly related to both Oviposition site (31.7 vs 3.9 min, LSD = 8.08; P < 0.001) and Sampling period (17.4, 15.3, 15.7 and 22.9 min, LSD = 3.96; P = 0.001).

IV. DISCUSSION

Hen posture in the 2 h pre-laying was affected by Group size, with hens in 2-bird cages sitting more (and conversely standing less) than in 4- or 8-bird cages. While standing could be considered an indicator of activity, walking/running are obviously ‘active’ behaviours.

Walk/run was reduced in the presence of a nest box, supporting previous reports (Cronin *et al.*, 2005), and was greater at 36 than 22 or 29 weeks of age. Nevertheless, 'total activity', derived as the complement of 'inactivity', was not affected by any of the fixed effects.

Three indicators of activity in the pre-lay period, the frequency and duration of sitting and total activity, were related to plasma corticosterone concentrations which was spot-sampled a few hours later in the day but on a different day to the collated video. Hens with higher total activity in the pre-laying period had lower plasma corticosterone concentrations later on. Although standing was not related to corticosterone concentrations, there were relationships between plasma corticosterone with both the frequency of sitting bouts (positive) and the time spent sitting (negative). Thus, more bouts of sitting was related to higher plasma corticosterone and increased time sitting was related to lower plasma corticosterone. Increased frequency of sitting bouts and reduced time spent sitting prior to oviposition may reflect either more disturbance or more restlessness and in turn increased stress as indicated by the higher plasma corticosterone concentrations. While sampling of plasma corticosterone was limited, the measurements are more likely to reflect basal concentrations rather than acute effects around oviposition since the samples were collected in the early afternoon and not around oviposition. It is unknown whether these relationships are causal or simply associations, e.g. due to the individual nature of birds.

In conclusion, sitting posture and total activity, but not walking/running, in the 2 h pre-laying were predictive of plasma corticosterone concentrations measured in spot samples obtained a few hours after egg-laying on a different day to the collated video. Without further, more-comprehensive examination, the implications for hen welfare are unknown.

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SERUM ANTIBODY RESPONSES FOLLOWING *SALMONELLA* VACCINATION AND CHALLENGE WITH *SALMONELLA* TYPHIMURIUM

S.M SHARPE[1,2], J.M. COX[2] and P.J. GROVES[3]

Summary

During evaluation of various live and inactivated *Salmonella* vaccines, commercial brown egg layer birds were bled at various ages. An evaluation of the correlation of *Salmonella enterica* serovar Typhimurium (ST) antibody titres with protection in vaccinated and unvaccinated birds was included in a larger study. The results reported here concern humoral antibody responses following vaccination and challenge with a field strain of ST. *Salmonella* vaccination induced an antibody response which was stronger following two vaccinations. It was concluded that there was an association between high antibody level and protection against *Salmonella* colonisation.

I. INTRODUCTION

Salmonella stands out as the most commonly reported microbiological agent responsible for foodborne illness where eggs have been implicated as the cause (Van Immerseel et al., 2005). In Australia, it has been estimated there are about 12,800 cases of egg-related salmonellosis per year, costing $44 million, and this appears to be rising. (FSANZ, 2009). Success with an autologous trivalent *Salmonella* vaccine has resulted in the incorporation of this vaccine into a commercial broiler breeder production system (Pavic et al., 2010).

In this study, four vaccination programs were selected based on the outcome of work conducted previously (Groves et al., 2011), with the aim of evaluating serum antibody development to ST. The vaccines used were the Australian Bioproperties Vaxsafe® *Salmonella* Typhimurium (ST) live vaccine, which has the *aro*A gene deleted, and the Intervet inactivated multivalent *Salmonella* vaccine (containing inactivated cultures of *S.* Typhimurium, *S.* Infantis, *S.* Montevideo and *S.* Zanzibar). Serologic responses were monitored throughout the experiment.

II. MATERIALS AND METHODS

This report describes a portion of a larger trial using 600 day old Hyline (Rhode Island Red x White) layer chickens which were obtained from a commercial hatchery in Victoria and were floor pen reared. They were randomly allocated into 10 pens (n = 60 birds / pen) and assigned into five experimental groups (n = 2 pens / treatment). The birds were *Salmonella* vaccinated using four different vaccination combinations, utilizing live and killed vaccines (Table 1). Environmental drag swabbing was regularly conducted in each pen throughout the trial. Unchallenged birds in each group were bled from the wing vein at intervals from four weeks of age, until 25 weeks. Serum *Salmonella* Typhimurium antibody levels were measured by enzyme-linked immunosorbent assay (x-Ovo Flockscreen ST ELISA). For the portion of the experiment reported here, at 16 weeks of age 12 birds per group were removed from their rearing pens and placed in cages by group at The University of Sydney's Poultry Unit and challenged with a recent field isolate of ST (phage type 108) by oral gavage of approximately

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10^8 cfu per bird. Each of these challenged birds was bled individually at the time of challenge.

Table 1. Experimental design

Group Code	Vaccination regime
C	No vaccination - Control
N6 N12	Killed[2] vaccine at 6 and 12 weeks
V6 V18	Live[1] vaccine by subcutaneous injection at 6 weeks and live[1] vaccine by oral gavage at 18 weeks
V6 N12	Live[1] vaccine by subcutaneous injection at 6 weeks and Killed[2] vaccine at 12 weeks
V0 V6	Live[1] vaccine by coarse spray at day old and live[1] vaccine by subcutaneous injection at 6 weeks

[1] Bioproperties Vaxsafe ST at 10^8 CFU/bird
[2] Intervet *Salmonella* vaccine at 0.5ml /bird by intramuscular injection

Following challenge at 16 weeks, the birds were held for 21 days and then euthanized and their caeca were cultured for the presence of *Salmonella*. On the basis of the culture results, birds were categorised as either having no Salmonella detected (NSD) or having ST detected (Positive). These two categories were used as independent variables in a retrograde Student's t-test comparison of their ELISA titre at the time of challenge (16 weeks).

III. RESULTS

All vaccination programs induced significant ($P < 0.05$) seroconversion compared to the unvaccinated birds, as measured by ELISA. Interestingly birds that received injected live vaccine at 6 weeks and inactivated vaccine at 12 weeks (group V6 N12) showed an increase in antibody titre to a higher and longer lasting level than the other three groups, including group N6 N12 (Figure 1). Antibody titres in control birds (group C) did not appreciably change up to 16 weeks but thereafter showed an increase in titre which remained below the ELISA cut-off positive point (Figure 1). This declined by 22 weeks of age.

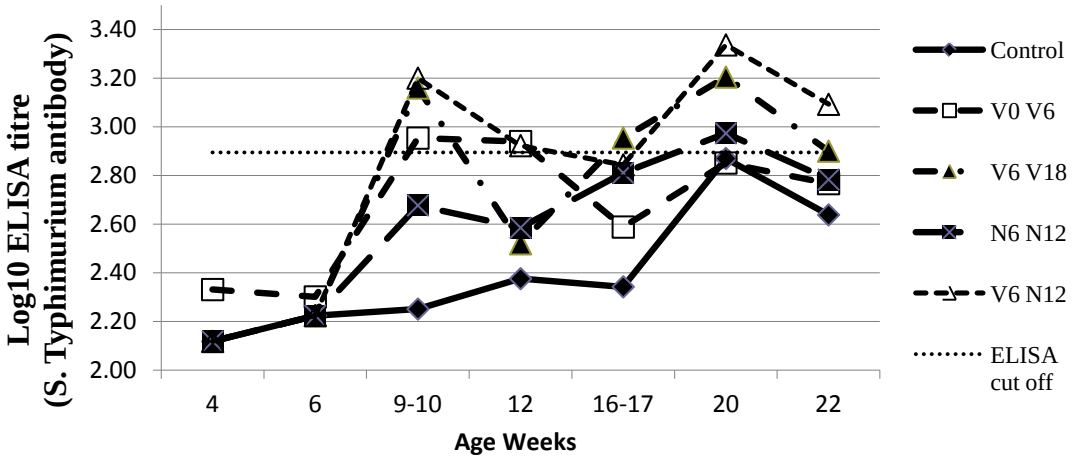

Figure 1. Unchallenged birds: Serological results: 4 – 22 weeks.
V0V6= live vaccine by spray at day old and live vaccine s/c at 6 weeks; V6V18= live vaccine s/c at 6 weeks and live vaccine orally at 18 weeks; N6N12= inactivated vaccine i/m at 6 and 12 weeks; V6N12= live vaccine s/c at 6 weeks and inactivated vaccine i/m at 12 weeks.

Following the 16 week ST challenge, subsequent caecal culture revealed a statistically significant association (Student's t-test, $P = 0.002$) between higher serum antibody pre-

challenge (at 16 weeks) and subsequent inability to recover ST in caeca 21 days later (Figure 2). If birds had anti-*Salmonella* Typhimurium antibody titres greater than the ELISA positive cut-off value of 785 ELISA units (log_{10} 2.89) at the time of challenge, they were significantly less likely to be colonised 21 days later.

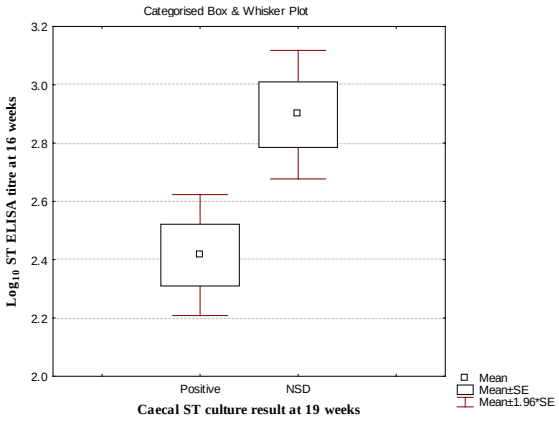

Figure 2. Challenge Study: 16 weeks *S.* Typhimurium. Mean Log $_{10}$ ST ELISA titres of birds at 16 weeks in regard to their 19 week caecal culture result (NSD = no *Salmonella* detected). Whiskers represent 95% confidence limits for the mean.

IV. DISCUSSION

A single administration of the live attenuated vaccine produced higher and more rapid seroconversion than a single dose of the inactivated vaccine, the latter requiring a second dose to reach a similar serum antibody level. The use of the live vaccine parenterally at 6 weeks, followed by an inactivated booster at 12 weeks, produced the strongest and longest lasting antibody level of any of the comparisons in this study. A live vaccine given orally as a booster also provided an extended elevated titre.

The unvaccinated group (C) exhibited an increase in antibody titre below that considered positive for the ELISA test between 17 and 22 weeks of age. Environmental swabs had not detected the presence of salmonellae in the pen environments for these groups of birds. This may be consistent with the findings of McMullin et al. (1997) who, in a wide ranging field survey, found occasional strong serological reactions in birds with no evidence of *Salmonella* infection. Wigley et al. (2005) describe a marked decline in cell mediated immune responsiveness associated with the onset of lay (typically 17-20 weeks of age in this bird strain) and a subsequent increase in overall antibody production as a result of less retrained control of bacterial multiplication. Barrow (1992) also draws attention to the risk of false positive results using LPS-based ELISA tests for salmonellae. A similar rise in control birds was also observed in our previous study (Sharpe and Groves, 2011) at the same age and it is suspected that this reflects a non-specific reaction associated with physiologic change at sexual maturity.

A strong association between anti-ST antibody ELISA titre prior to ST challenge at 16 weeks and the subsequent ability to culture ST from the caeca was observed. Birds with a serum titre above the positive cut off point for the x-OvO ELISA were significantly less able to be colonised than those with titres below this level at 16 weeks.

V. CONCLUSION

Vaccination of laying hens has been shown to significantly increase the levels of circulating antibodies and decrease the colonisation of the caecum, as shown by the data presented here. Therefore, vaccination is undeniably a useful tool and, along with good husbandry and environmental conditions, will aid in reduction of the infectious pressure from salmonellae especially *Salmonella* Typhimurium which is the main serovar of concern for the Australian poultry industry. A recommended vaccination program using a combination of live at 6 weeks and inactivated at 12 weeks, as shown by these results, could be adopted by the layer industry to improve bird resistance to caecal colonisation by salmonellae.

ACKNOWLEDGEMENTS

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EFFECT OF *SALMONELLA* VACCINES IN COMMERCIAL LAYER CHICKENS AGAINST VARIOUS *SALMONELLA* SEROVARS

S.M. SHARPE [1,2], J. COX [2] and P.J. GROVES [3]

Summary

Live attenuated (Bioproperties Vaxsafe ST) and inactivated (Intervet) vaccines against *Salmonella* were administered, alone or in combination using four different vaccination regimes, to Hyline brown egg laying chickens. Vaccines were applied up to 12 or 18 weeks of age. The birds were challenged with *Salmonella* Typhimurium, *S.* Infantis or *S.* Virchow at ~ 10^8 colony forming units (cfu) per bird of at 10, 16 and 22 weeks of age. After three weeks, the challenged birds were euthanised and their caeca cultured for presence of salmonellae. Proportions of birds positive by caecal culture were compared at each age and protective index calculated (against unvaccinated birds). Protection was demonstrated against *S.* Typhimurium and *S.* Infantis by both vaccines with some protection afforded against *S.* Virchow by some vaccine combinations, from 16 weeks. The best overall protection was delivered by the combination of subcutaneous injection of the live vaccine at six weeks followed by an intramuscular injection of the inactivated vaccine at 12 weeks of age.

I. INTRODUCTION

Salmonellae are common members of the normal flora of many animals, including chickens, cattle and reptiles (e.g. pet turtles). The strains that cause gastroenteritis are usually transmitted by chicken meat, eggs and dairy products (Engleberg et al., 2007). However, the mechanisms of pathogenicity of *Salmonella* infection are poorly understood. The transmission of *Salmonella* to humans by contaminated eggs has been a prominent international public health issue for more than two decades (Gast et al., 2011). *Salmonella* Enteritidis, which is of major concern to the food industry globally, is not considered to be prevalent in Australian poultry flocks. However, Cox et al. (2002) reported that *Salmonella* Infantis was the predominant *Salmonella* serovar in the Queensland egg industry. Furthermore, the ever-increasing rate of overseas travel, along with the prevalence of *S.* Enteriditis in many overseas countries means that the risk of infection of Australian layer flocks is increasing (AECL, 2000). However other serovars, including *S.* Typhimurium, are often implicated in food-borne disease outbreaks and OzFoodNet (2009) reported it was the most common *Salmonella* serotype notified in Australia. *S.* Typhimurium resembles *S.* Enteriditis with regard to the known virulence mechanisms central to mammalian cell invasion, survival and growth in the host (Guard-Petter, 2001). The objective of the current research was to evaluate the protection afforded layer chickens by vaccines containing homologous and heterologous *Salmonella* serovars measured as *Salmonella* colonisation of the caeca.

II. MATERIALS AND METHODS

Hyline (Rhode Island Red / White) day old chicks (650) were obtained from a commercial hatchery in Victoria and were floor pen reared at a trial farm facility. They were randomly

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allocated into 10 pens (n = 60 birds per pen) and assigned into five experimental groups (two pens per treatment). The birds were vaccinated using four different vaccination regimes, utilising live and inactivated vaccines. The inactivated vaccine is an autogenous multivalent killed vaccine, which contains serovars *S.* Typhimurium, *S.* Infantis, *S.* Zanzibar and *S.* Montevideo. The live vaccine is an *aro*A gene deleted *S.* Typhimurium mutant. Group 1 was not vaccinated, Group 2 received one dose of killed vaccine by intramuscular injection (i/m) at 6 and 12 weeks, Group 3 received live subcutaneous (s/c) at 6 weeks and oral live vaccine at 18 weeks, Group 4 received live s/c at 6 weeks and killed i/m at 12 weeks and Group 5 were sprayed with live vaccine at day old and live s/c at 6 weeks. These were all compared to a control unvaccinated group. The birds were experimentally challenged at 10, 16 and 22 weeks of age with ~ 10^8 cfu per bird of *S.* Typhimurium, *S.* Infantis or *S.* Virchow and three weeks later their caeca were cultured for *Salmonella*.

III. RESULTS

The protective index was calculated [PI= (% positive in controls - % positive in vaccinated group)/(% positive in controls)] for each vaccination regime and each of the three different serovar challenges (Figures 1, 2 and 3). At the 10 week challenge, the birds had only received one vaccination, except Group 5 which received the live vaccination by spray at day old, as well as the live s/c at 6 weeks. The results for this challenge demonstrate that vaccination with a single dose of either vaccine appears to give insufficient protection. However, following two vaccinations, the results from the 16 and 22 week challenges indicate protection, especially from the combination of the live vaccine at 6 weeks followed by the inactivated vaccine at 12 weeks. The results indicated that birds with elevated *S.* Typhimurium serum antibody levels had lower amounts of *Salmonella* colonisation. The vaccination program that was significantly effective in providing protection was the live vaccinated at 6 weeks, followed by the killed vaccine at 12 weeks. There was a good association between anti-*S.* Typhimurium antibody titres greater than 785 and protection.

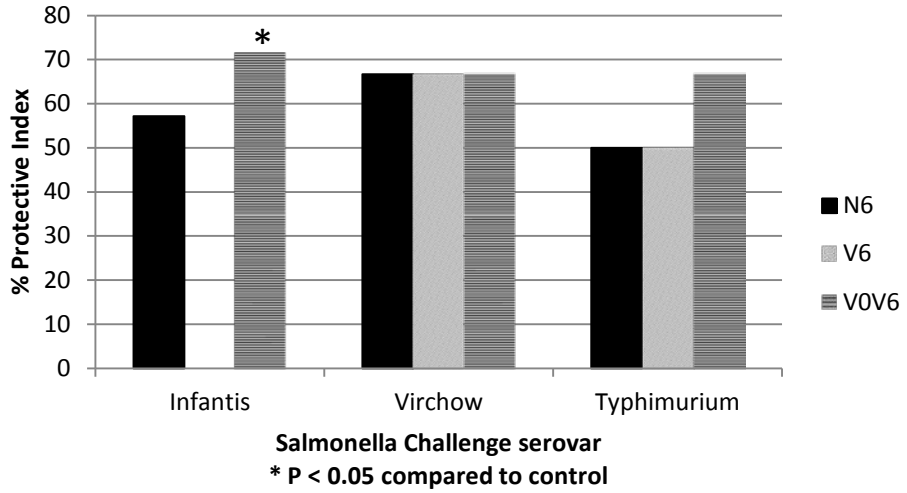

Figure 1. Protective indices from vaccination following *Salmonella* challenge at 10 weeks of age (N6= inactivated vaccine at 6 weeks, V6= live vaccine at 6 weeks s/c; V0V6= live vaccine by spray at day old and live vaccine s/c at 6 weeks).

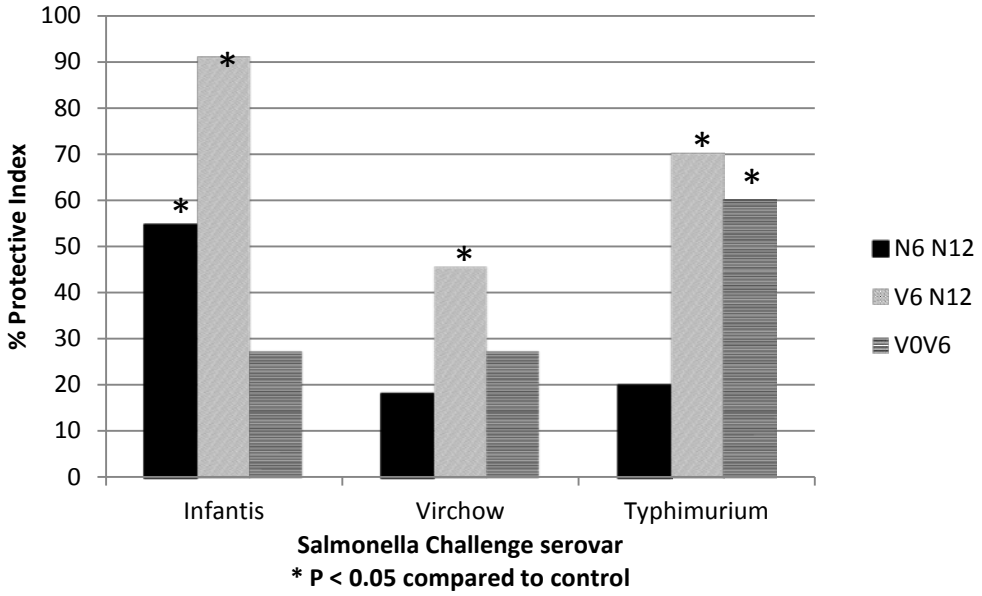

Figure 2. Protective indices from vaccination following *Salmonella* challenge at 16 weeks of age (N6N12= inactivated vaccine at 6 and 12 weeks; V6N12= live vaccine at 6 weeks s/c and inactivated at 12 weeks; V0V6= live vaccine by spray at day old and live vaccine s/c at 6 weeks).

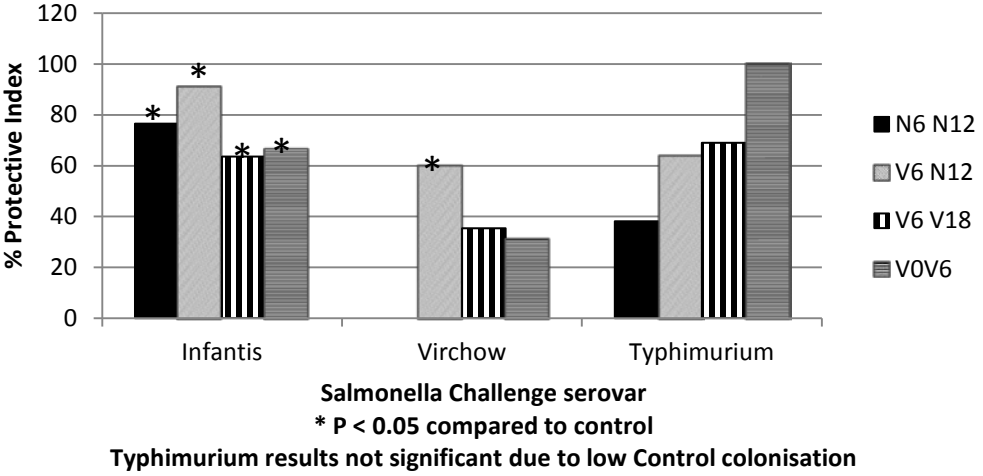

Figure 3. Protective indices from vaccination following *Salmonella* challenge at 22 weeks of age (N6N12= inactivated vaccine at 6 and 12 weeks; V6N12= live vaccine at 6 weeks s/c and inactivated vaccine at 12 weeks; V6V18= live vaccine s/c at 6 weeks and live vaccine orally at 18 weeks; V0V6= live vaccine by spray at day old and live vaccine s/c at 6 weeks).

IV. DISCUSSION

This work replicated previous findings (Groves et al., 2011), demonstrating that subcutaneous administration of the live vaccine appeared to elicit a humoral response in chickens, similar to the response seen with intramuscular injection of the killed vaccine. Overall, the most effective protection against caecal colonisation was provided by the combination of the subcutaneous injection of the live vaccine at 6 weeks followed by an intramuscular injection of the inactivated vaccine at 12 weeks of age. Protection was demonstrated against *S.* Typhimurium and *S.* Infantis by both vaccines with some protection afforded against *S.*

Virchow by the combined live and inactivated regime from 16 weeks of age. The 22 week challenge results with S. Typhimurium were not significantly different, due to the low colonisation of the control birds.

The results also indicated that the combination of the live and inactivated vaccine, gave better cross protection against *S.* Infantis, which is contained in the inactivated vaccine, than *S.* Virchow which is heterologous to both vaccines. *S.* Virchow however belongs to the same serogroup (C_1) as *S.* Infantis and *S.* Montevideo.

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OVIPOSITION FEEDING COMPARED TO NORMAL FEEDING: EFFECT ON PERFORMANCE AND EGG SHELL QUALITY

J. DE LOS MOZOS[1], A. GUTIERREZ DEL ALAMO[1], T. VAN GERWE[1], A. SACRANIE[1] and P. PEREZ DE AYALA[1]

Summary

The following trial was conducted as part of an on-going project investigating the effect of an oviposition determined feeding system on hen performance. This study was conducted to assess whether protein, energy and calcium utilization in laying hens could be improved by providing the nutrients at the time of day when there is a greater physiological requirement during the various stages of egg development. Individually housed hens were fed a diet high in energy and protein (2900 ME/kg, 18.5% CP) and low in calcium (1.6%) from two hours before expected oviposition to two, four, six, eight or ten hours after recorded oviposition. From this moment to two hours before expected oviposition the next day, hens were fed a diet high in calcium (4.5%) and low in energy and protein (2323 ME/kg, 13.3% CP). Hens who received the calcium rich diet four or six hours post oviposition were unable to maintain egg shell quality parameters compared to the control birds. Hens who received the calcium rich diet eight and ten hours post oviposition consumed less energy, calcium and protein than the control group without negatively affecting the eggshell quality parameters. This study illustrates the benefits of providing individual laying hens with energy and protein rich diets during the hours of albumen production and a calcium rich diet during the hours of shell calcification. Hens display improvements in nutrient utilization without negatively affecting egg shell quality, because of a more balanced nutrient intake in accordance with the physiological needs of production.

I. INTRODUCTION

The protein, calcium and energy requirements of laying hens do not remain constant, but vary during different hours of the day depending on the hen's physiological requirements for the various stages of egg formation. The current method of feeding laying hens with one diet with a constant level of nutrients may not result in optimal utilization of nutrients (Chah, 1972, 1985, Leeson and Summers, 1997). Commercial hen lines lay the majority of their eggs during the morning (Etches, 1987, Leeson and Summers, 1978, Larbier and Leclercq, 1994). The interval between two successive ovipositions in a cycle is about 24 hours or slightly greater (Keshavarz, 1998a and 1998b). During the first four hours the egg white is formed. Thereafter, the egg moves to the shell gland and the shell is deposited around the albumen during, approximately, the next 20 hours (Larbier and Leclercq, 1994). Shell formation takes place mainly during the evening and night. As a result, hens have higher protein and energy requirements during the morning and a higher calcium requirement during the evening and night. According to Farmer et al. (1986), hens utilize significantly more calcium for egg shell formation from the diet in the afternoon as compared to the morning. Hens provided with calcium in the afternoon will be less dependent on their bone reserves.

When birds are offered diets that allow self-selection of nutrients, there is an increased intake of protein and energy observed in the morning around the peak of egg production (Chah, 1972). The intake of calcium is higher during the latter part of the day. In free choice feeding, birds displayed an increased weight gain compared to the control group, while egg size was similar (Leeson and Summers, 1997). This suggests that the hen is

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utilizing energy and protein more efficiently by consuming these nutrients at moments of the day when requirements are high. Therefore, the current practice of providing hens with only one diet might not be an ideal approach for optimal utilization of nutrients.

Choice feeding does not have practical applications under current feeding practices in commercial egg laying farms (Keshavarz, 1998a). Keshavarz (1998b) was unable to show daily Ca requirements could be reduced by providing the hens with adequate levels of Ca during the afternoon and low levels during the morning. In addition, providing adequate protein in the morning, followed by low protein levels in the evening impacted negatively on egg production performance. However, in these experiments nutrient manipulation was not based on the hen individual physiological status.

The current study was conducted to assess whether the protein, energy, and calcium utilization can be improved by providing these nutrients during the hours the hen has a specific physiological requirement due to egg formation.

II. MATHERIALS AND METHODS

One hundred and forty-four 40 weeks old Lohmann Brown Classic laying hens, individually caged, with a laying percentage of 96% were used. Water and feed were available *ad libitum* and hens were exposed to 16 hours of continuous light per day. Temperature and ventilation were controlled by means of an Ecorel environment regulator. The barn was provided with cooling and heating system and with an air extractor of with 7.000 m^3/h of maximum flow.

Two experimental diets were produced; diet 1 was low in calcium and high in energy and protein (approximately 6% and 10% higher than the control diet), and diet 2 was low in protein and energy and high in calcium (approximately 21% higher than the control diet). The control diet consisted of a commercial diet based on corn, wheat and soybean meal with 2750 kcal ME layer/kg, 168 g CP/kg and 37 g Ca/kg.

Six experimental treatments were studied differing in composition or in the time of day the diet was made available to birds (Table 1). Each treatment group consisted of 24 hens. Each day the moment of oviposition was visually recorded and the moment of oviposition the next day was estimated. Two hours before this expected moment of oviposition the hen received diet 1. Two, four, six, eight or ten hours after the real moment of oviposition diet 2 was provided (treatments 2, 3, 4, 5 and 6, respectively). The daily consumption of each diet was recorded. The feed intake of hens receiving the control diet all day (treatment 1), was recorded from two hours before the expected moment of oviposition till 6 hours after the real moment of oviposition and from this moment till 2 hours before the expected moment of oviposition the next day.

Egg weight per treatment was determined daily. Furthermore, various indices of shell quality were measured during the last four days of the experiment. Shell weight was determined after breaking the egg and drying the shell in the oven at 105ºC for 6 hours. Shell strength was assessed using a QC-SPA Shell analyser (Technical services and supplies, York, England) and shell quality with the SWUSA (Shell Weight per Unit of Surface Area) method.

Data were analysed using the general linear models (GLM) procedure of SAS® (SAS Institute, 1999) to determine the effect of each treatment on all the studied parameters.

III. RESULTS

Hens in the control group (treatment 1) consumed 44% of their daily feed intake from two hours before oviposition till six hours after oviposition approximately (Table 1). The feed intake of hens in treatment group 4 was assessed at the same time as the control group, however the group 4 hens received the experimental diets. They consumed 42% of their daily intake from two hours before oviposition until six hours after oviposition. The total feed

intake of this group was not significantly different from the control group; however intake of energy, calcium and protein was significantly reduced (P<0.05). These hens consumed approximately 5% less metabolisable energy, 10% less calcium and 13% less protein.

Table 1. The effect of different feeding regimes on daily feed and daily nutrient intake

Treat.	Feed Intake (g/d)			Energy Intake	Calcium Intake	Protein Intake
	Diet 1	Diet 2	Total	(kcal/d)	(g/d)	(g/d)
1	54.30	68.30	122.6 ^{ab}	337.23 ^{a}	4.54 ^{b}	21.71 ^{a}
2	22.10	105.6	127.7 ^{a}	309.36 ^{bc}	5.10 ^{a}	17.70 ^{b}
3	39.50	82.20	121.7 ^{ab}	305.61 ^{bc}	4.33 ^{bc}	17.80 ^{b}
4	52.20	71.90	124.1 ^{a}	318.52 ^{b}	4.07 ^{c}	18.75 ^{b}
5	61.60	54.70	116.3 ^{bc}	305.63 ^{bc}	3.45 ^{d}	18.19 ^{b}
6	75.60	34.00	109.6 ^{c}	298.20 ^{c}	2.74 ^{e}	18.03 ^{b}
P value	-	-	<0.0001	<0.0008	<0.0001	<0.0001

a-e values in a column with no common superscripts are significantly different (P<0.05)

Treatment groups 2 and 3 received diet 1 for a shorter period than group 4. Birds from treatment group 2, who received diet 1 from two hours before oviposition until two hours after and group 3, who received diet 1 from two hours before oviposition till four hours after, consumed 17% and 33% of their daily intake, respectively, during these feeding periods. Treatment groups 2 and 3 received diet 2 for four and two hours longer, respectively, than treatment group 4. There was no significant difference in feed intake between these treatment groups compared to the control group. They consumed significantly (P<0.05) less metabolisable energy (respectively 8% and 9%) and protein (both 18%) per day. The consumption of calcium was not significantly different between birds from the control group and treatment group 3. However, laying hens in treatment group 2 consumed significantly (P<0.05) more calcium than those from the control group and treatment 4.

Treatment groups 5 and 6 received diet 1 for the longest period. The consumption of this diet was, respectively, 53% and 69% of their daily feed intake. For both treatment groups, the total feed intake was less than for the other treatment groups. Both treatment groups showed a significantly (P<0.05) lower intake of metabolisable energy compared to the control group (9 and 12% respectively). The consumption of calcium was, for both treatment groups 5 and 6, significantly lower than for the other treatment groups and the control group (24 and 40%, respectively). There was also a significantly (P<0.05) lower intake of protein than the control group (16 and 17%, respectively).

Table 2. The effect of different feeding regimes on egg shell quality

Treat.	Eggshell weight (g)	SWUSA, (mg/cm^2)	Breaking strength (g)	Egg weight (g)
1	6.56 ^{a}	85.52 ^{a}	5316 ^{a}	66.32
2	6.45 ^{ab}	84.02 ^{ab}	5275^{a}	66.37
3	6.43 ^{ab}	84.63 ^{a}	5481 ^{a}	65.47
4	6.48 ^{a}	84.39 ^{a}	5333 ^{a}	66.43
5	6.27 ^{b}	82.25 ^{b}	5189 ^{a}	65.65
6	5.76 ^{c}	75.22 ^{c}	4517 ^{b}	66.06
P value	<0.0001	<0.0001	<0.0001	0.7200

a-c values in a column with no common superscripts are significantly different (P<0.05)

No significant differences on eggshell quality parameters were observed between the control treatment and treatments 2, 3 and 4 (Table 2). Treatment group 6 showed significantly ($P<0.05$) lower shell weight, shell quality and shell strength compared to the other treatment groups. Treatment group 5 showed significantly ($P<0.05$) lower shell weight and shell quality compared to the control group. This treatment had also a significantly ($P<0.05$) lower shell weight and shell quality than treatment group 4 and it had a lower shell quality than treatment 3.

IV. DISCUSSION

Compared to the control group, hens consumed less energy, protein and calcium (except treatment group 2 for calcium). In a study of Chah (1972) where hens were offered diets that allow self-selection of nutrients, a 8% lower total intake of energy, a 11% lower intake of protein and 26% lower intake of calcium was observed. Based on this experiment the diets for the current study were formulated with diet 1 approximately 6% higher in energy, approximately 10% higher in crude protein and diet 2 approximately 21% higher in calcium. In contrast to the study of Chah (1972) the hens were not offered diets that allow self-selection, but they were provided with diets containing high levels of energy, protein or calcium during the moments of the day that the physiological need for these nutrients was high. This trial showed that not only choice feeding can reduce the amount of energy, protein and calcium consumed, also oviposition feeding with providing the nutrients based on the moment of oviposition will reduce the consumption of these nutrients.

This study has shown that giving laying hens an energy and protein rich diet during the hours of albumen production and a calcium rich diet during the hours of shell calcification will improve nutrient utilization without affecting egg shell quality, because of a more balanced nutrient intake in accordance with the needs for production. Further research is necessary to assess whether the same results will be obtained when the feed is not provided at the moment based on the individual hen physiological needs, but on a group mean moment of oviposition.

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DETERMINATION OF THE VIRULENCE AND PATHOGENICITY OF AUSTRALIAN FOWL ADENOVIRUS FIELD STRAINS IN CHICKENS

P.A. STEER[1], A.H. NOORMOHAMMADI [1], P. SCOTT [1], and G. BROWNING [1]

Fowl adenoviruses (FAdVs) cause inclusion body hepatitis (IBH) in chickens, a disease that has caused significant problems for the Australian broiler industry. Recent research has identified serotypes FAdV-8b and FAdV-11 in association with the re-emergence of IBH over the past decade (Steer et al., 2011). In addition, evidence suggests that immunosuppression caused by chicken anaemia virus (CAV) or infectious bursal disease virus (IBDV) is not a contributing factor in these outbreaks, indicating IBH may be a primary disease in Australia

To determine the pathogenicity and primary disease-causing status of these two Australian FAdV field strains, specific pathogen free (SPF) chickens were inoculated with identical quantities of plaque purified FAdV-8b, or FAdV-11, or with sterile saline solution (negative controls), at 1-day-old. Inoculation was via the ocular route and the three groups of birds were housed in separate isolators. Clinical signs were observed throughout the study. On days 1 to 7, and day 14 post inoculation (PI), birds were randomly selected from each group, gross pathological (GP) signs of disease observed in each bird, and portions of liver, thymus, bursa, kidney and gizzard taken for virus detection. Tissue tropism was assessed by DNA extraction from individual tissues, followed by PCR-HRM genotyping, as described previously (Steer et al., 2011).

Minimal clinical signs were observed during the study. The negative control group exhibited no GP signs of disease throughout the study. GP signs of disease were observed in the FAdV-8b group from day 4 PI to day 7 PI, with increased severity on days 5 and 6 PI, and one mortality on each of these days. On day 14 PI, signs were less severe and indicated early stages of recovery from disease. All observations in the liver were typical of IBH in field cases, together with swollen pale kidneys. Slightly flaccid bursa and atrophy of thymic lobes were observed in some birds. In the FAdV-11 group, occurrence and GP signs of disease were similar to those observed in the FAdV-8b group, with increased severity however on days 6 and 7 PI, and no mortalities. Tissue tropism in the FAdV-8b group indicated the virus first appeared in the bursa on day 1 PI, and then also in the liver on day 2 PI. On days 3 to 4 PI the virus was present in all tissues except the thymus, then in all tissues of all birds on days 5 to 7 PI, and persisted in the gizzard on day 14 PI. FAdV-11 was first detected on day 2 PI in all tissues types except the thymus, in one bird only. By day 6 and 7 PI the virus was present in all tissues of all birds, and persisted mainly in the gizzard on day 14 PI.

It may be speculated from the results that FAdV-11 has a slightly longer incubation period than FAdV-8b, as the peak GP signs of disease were observed on days 5 and 6 PI in the FAdV-8b group, and on days 6 and 7 PI in the FAdV-11 group. Additionally, these GP results were consistent with the tissue tropism results, in that for both groups the days when peak GP signs were observed were also the days when the virus was detected in all five tissues from all birds tested. This study indicates that the Australian FAdV-8b and FAdV-11 field strains are primary disease causing pathogens, capable of inducing typical signs of IBH in the absence of immunosuppressive agents such as CAV and IBDV.

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SEROLOGICAL SURVEY OF *ERYSIPELOTHRIX RHUSIOPATHIAE* EXPOSURE IN NEW ZEALAND POULTRY

A.KURIAN[1], E. NEUMANN[1], W. HALL[2] and D. MARKS[3]

Summary

A cross-sectional serological survey of a convenience sample of 545 New Zealand broiler, breeder, and layer chickens from 55 flocks was conducted in 2010-2011. The birds ranged from 5 to 83 weeks of age, were housed under various conditions (free range, shed, caged, and unknown), and were located in seven geographical regions of New Zealand. All samples came from flocks which had no previous history of erysipelas or vaccination against erysipelas. The likelihood of seropositivity based on flock age, housing type, and geographical location was estimated using logistic regression.

The overall prevalence of *E. rhusiopathiae* exposure as measured by the presence of anti-*E. rhusiopathiae* serum antibodies in chickens was found to be 40%; 84% of farms that were tested had at least one positive sample. The prevalence of seropositive birds was highly variable amongst different housing types and geographical regions. A significant increasing trend ($p<0.001$) in the prevalence of seropositive birds was identified from birds less than or equal to 12 weeks of age (2%), through 13-24 weeks (29.13%), 25-36 weeks (47%), 37-48 weeks of age (75%), and greater than 48 weeks 63.78% of age. Neither housing type nor geographical location had a significant effect on the likelihood of being seropositive for *E. rhusiopathiae* antibodies.

The results of this study indicated the seroprevalence of erysipelas under New Zealand field conditions may be higher than expected and that the seroprevalence is significantly associated with age. Housing type and geographical location appeared to be unrelated to seropositivity.

I. INTRODUCTION

The epidemiology of erysipelas in chickens is not well understood. While large outbreaks of erysipelas have been infrequently reported in chickens; the disease is rare under intensive commercial conditions. Commercial producers and researchers from various parts of the world have reported outbreaks more frequently in recent years, coincident with the expanded use of free-range housing systems (Takahashi et al., 2000; Wang et al., 2010). To assess the prevalence of erysipelas exposure in chickens under field conditions and to elucidate some information about the epidemiology of erysipelas in New Zealand chickens, a serological survey using a convenience sample of birds from different flocks was conducted.

II. MATERIALS AND METHODS

A cross-sectional survey was conducted on serum samples from breeders, layers and broilers collected between May 2010 and January 2011 as part of a routine national surveillance programme for infectious bursal disease virus in the New Zealand poultry industry. Serum samples from all major genetic lines, from different age groups, and across several types of housing systems were included in this study. A total of 545 serum samples representing seven distinct geographical regions were tested (approximately 10 samples from each of 55 flocks

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were used in this study). All samples came from flocks which had no known clinical history of erysipelas or vaccination against erysipelas.

An indirect ELISA for detection of antibodies (IgG) against *E. rhusiopathiae* in domestic chickens was developed by modifying a similar assay that had been developed for use in pigs. After optimization for use in New Zealand laying hens, the test had an estimated diagnostic sensitivity of 93%, diagnostic specificity of 98%, and an overall accuracy of 96% (Kurian et al., 2011).

The prevalence of anti-*E. rhusiopathiae* serum antibodies (stratified by housing systems, flock age, and geographical locations) were calculated based on results of the ELISA assay. The prevalence of exposure *E. rhusiopathiae* based on the different type of housing systems was determined by classifying the housing system of each sampled flock at the time of sample collection into either "free-range", "shed-based", or "caged". Seventy-eight samples were received with no information about the housing system and were classified as "unknown".

Each sample was also classified based on the age of the bird at the time of sampling. For convenience, five age group strata were created: less than or equal to 12 weeks of age, 13 to 24 weeks of age, 25 to 36 weeks of age, 37 to 48 weeks of age, and greater than 48 weeks of age. Thirty-seven samples were received for which no information on bird age was provided; these samples were classified as age "unknown".

A logistic regression mixed model was developed to predict the probability of flock seropositivity using geographical region, housing system, and flock age at time of sampling as fixed effects; farm identification was included in the model as a random effect. Geographical location was effect coded using the "Taranaki" region as the reference as it presented an adequate number of samples for accurate approximation of mean seroprevalence and included flocks from every age group. Housing system and flock age were dummy coded using "caged housing" and "unknown age" respectively for reference groups, as they presented a suitable basis for establishing comparisons.

III. RESULTS

The mean prevalence of anti-*E. rhusiopathiae* serum antibodies in New Zealand chickens was 39.8% (CI= 35.6-44.1%; n=545). Forty-six of 55 flocks tested (84%, 95% CI=71-93%) had at least one positive sample.

Data on the seroprevalence of anti-*E. rhusiopathiae* antibodies was stratified by housing type and the mean seroprevalence for each of the four types of housing was determined: free range 44.2%, shed 23.7%, caged 73%, and unknown 50%.

There was a strong tendency for the seroprevalence of anti-*E. rhusiopathiae* antibodies to increase with age. The mean seroprevalence of flocks that were sampled when birds were 12 weeks of age or less was 2%, 13-24 weeks of age was 29%, 25-36 weeks of age was 47%, 37-48 weeks of age was 75%, greater than 48 weeks of age was 64%, and of unknown age was 89%. Figure 1 depicts the mean seropositivity for each age group and shows a significant trend of increasing seropositivity to *E. rhusiopathiae* with increasing age (Cochrane-Armitage χ^2 test for trend = 97.12, $p < 0.001$).

Exposure to *E. rhusiopathiae* was detected in all seven geographical regions from which the samples originated and mean seroprevalence ranged from 8% to 86%.

A mixed model logistic regression approach was developed in order to explore the relationship between sample seropositivity and the potentially explanatory variables of geographical region, housing system, and flock age at time of sampling. To evaluate which variables to retain in the final model, a Wald χ^2 test was used and flock age was retained as the only independent variable in the final model. A summary of final model coefficients is

presented Table 1. Results of the modelling presented indicated that birds 12 weeks of age or less were 0.03 times as likely to be seropositive when compared to birds of unknown age ($p < 0.001$), birds 13-24 weeks of age were 0.45 times as likely to be seropositive as birds of unknown age ($p = 0.003$), birds 37-48 weeks of age were 2.71 times as likely to be seropositive as birds of unknown age ($p = 0.04$), and birds greater than 48 weeks of age were 1.90 times as likely to be seropositive as birds of unknown age ($p = 0.009$).

IV. DISCUSSION

The prevalence of exposure to *E. rhusiopathiae* amongst birds housed in the caged housing system was very high (73%). Given the limited exposure of caged birds to soil and bedding material, the seroprevalence within-flocks was expected to be near zero in the absence of any known history of clinical erysipelas. The convenience sampling used in this study resulted in an under-representation of caged birds and limited the certainty of the seroprevalence estimate for this population. However, all samples from cage-housed birds happened to be collected from birds that were older than 48 weeks of age, consistent with previous research suggesting older birds may be more susceptible to effects of the disease (Mazaheri et al., 2005).

The seroprevalence of *E. rhusiopathiae* exposure in this study was notably higher in those age categories that included birds older than 12 weeks of age as compared to the age category that only included birds 12 weeks of age or less. One possible explanation for this is related to a hypothesis that the common red mite of chickens might serve as a vector for *E. rhusiopathiae* (Chirico et al., 2003; Mazaheri et al., 2005) and the probability of older birds being exposed to the parasite for a greater period of time both of which may contribute to increasing seroprevalence with age.

The high prevalence of exposure to *E. rhusiopathiae* in this study (84% of flocks tested were positive) is consistent with the findings from previous studies in Japan that determined that nearly all poultry included in the studies were contaminated with *Erysipelothrix* spp. (Nakazawa et al., 1998).

This limited study of the seroprevalence of *E. rhusiopathiae* serum antibodies in New Zealand poultry demonstrated a high level of exposure to the organism, even in caged bird populations expected to have only a minimal chance of contact with *E. rhusiopathiae*. Further studies of erysipelas in New Zealand poultry should be undertaken to determine the epidemiological features of *E. rhusiopathiae* infection most relevant to the control of erysipelas.

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Table 1. Effect of different flock housing types on the likelihood of New Zealand poultry having a positive ELISA titre for anti-*E. rhusiopathiae* antibodies (mixed model logistic regression).

	Estimate	p-value[a]	Odds Ratio	95% CI[b]
Fixed effects:				
(Intercept)	0.5462	0.218	-	-
12 weeks of age or less	-3.4951	<0.001	0.03	0.01-0.10
13-24 weeks of age	-0.7886	0.003	0.45	0.27-0.76
25-36 weeks of age	0.3897	0.258	1.48	0.75-2.90
37-48 weeks of age	0.9989	0.040	2.72	1.04-7.06
Greater than 48 weeks of age	0.6418	0.009	1.90	1.17-3.08
	Variance	Standard deviation		
Random effects:				
Farm identification (Intercept)	0.8967	0.94694		

[a] Statistical significance relates to the contrast between each age group and the reference age group (flock age = "unknown")
[b] 95% confidence interval around the calculated Odds Ratio

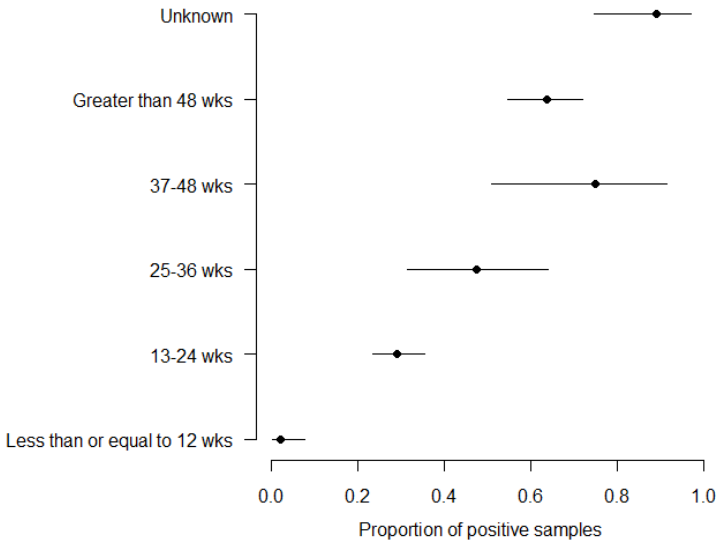

Figure 1. Prevalence of New Zealand poultry positive for the presence of anti-*E. rhusiopathiae* antibodies based on age at time of sample collection (error bars indicate 95% CI around the mean value).

EFFECTS OF BLOOD SAMPLE MISHANDLING ON ELISA TEST RESULTS FOR INFECTIOUS BRONCHITIS VIRUS, AVIAN ENCEPHALOMYELITIS VIRUS AND CHICKEN ANAEMIA VIRUS

A.KURIAN[1], E. NEUMANN[1], W. HALL[2] and D. MARKS[3]

Summary

This study was conducted to determine the effect of sample mishandling on the performance of ELISA tests for detection of antibodies against infectious bronchitis virus (IBV), avian encephalomyelitis virus (AEV), and chicken anaemia virus (CAV) in chicken serum. The effects of five different sample mishandling treatments were assessed: heat treatment, repetitive freezing and thawing, and three levels of haemolysis severity. These mishandling treatments were designed to simulate different conditions that might occur during routine blood collection, transport, or storage in a clinical practice setting. Each mishandling treatment was experimentally applied under laboratory conditions then samples were assayed for antibodies against IBV, AEV, and CAV using commercial ELISA kits. Severe haemolysis had the most consistent adverse effect on ELISA performance, producing results that were significantly different from the reference standard (serum that was not mishandled from the same bird) in all three brands of ELISA test kits. Moderate levels of haemolysis had a similar, yet less consistent effect, to severe haemolysis, producing results that were significantly than the reference standard only for the IBV and AEV ELISAs. Repetitive freeze-thawing also produced a significant adverse effect on ELISA results for IBV and AEV. The IBV ELISA appeared to be most susceptible to the effects of serum maltreatment. The magnitude and direction of the changes in test results for the five different serum sample treatments were compared to reference sera. The findings from this study suggested that unpredictable variation in the results of ELISAs can occur due to different sample mishandling treatments.

I. INTRODUCTION

Serum analysis is an important tool in disease diagnosis and for use in the epidemiological study of diseases. The availability of high quality analytical standards, use of standardised testing protocols and the wide-availability of commercial assays has reduced systematic error in diagnostic laboratory serological testing. However, non-standardised procedures for sample collection, handling and storage remain as important factors that can affect the accuracy and reliability of test results in a clinical, non-experimental setting. To understand the effect of poor sample quality on antibody quantification, commercial ELISAs for IBV, AEV and CAV were conducted on poultry serum samples that were purposefully mishandled (excessive heating, repetitive freezing and thawing, and three levels of haemolysis) and then compared with results obtained from matched reference samples collected, handled, and processed under optimum conditions.

II. MATERIALS AND METHODS

Whole blood samples were collected from 30 white spent breeder hens in a commercial abattoir. All sampled birds were known to originate from flocks that had been previously vaccinated against IBV, AEV and CAV.

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Six individual blood samples were collected from each bird. Four blood samples were collected in serum separator tubes (BD Vacutainer SSTII Advance serum separator tubes; Becton Dickinson and Company); and two samples were collected in tubes containing ethylene diamine tetraacetic acid (EDTA; BD Vacutainer K2E 7.2 mg EDTA; Becton Dickinson and Company).

Five treatments simulating common blood sample mishandling practices that occur under field conditions were applied to the whole blood or serum samples similar to work reported previously (Neumann and Bonistalli, 2009).

One sample from each bird was designated as the reference standard (treatment RS) and immediately placed into storage at -80 °C. A second sample from each bird was placed into storage at -10 °C, then thawed to 25 °C (held for 2 h) and refrozen at -10 °C repeatedly every day for 14 consecutive days (treatment FT). The third sample from each bird was used to create aliquots with standardised levels of haemolysis, as described below. The fourth sample from each bird was placed in a 50 °C water bath for 2 h prior to centrifugation and serum harvest (treatment HT). This treatment was established to simulate sample collection under hot summer conditions when the risk of mishandling as a result of storage in an enclosed vehicle during transport to a laboratory is high.

The two blood samples collected in EDTA tubes were immediately frozen at -10 °C to lyse the red blood cells. Aliquots from the third serum tube (described above) were combined with appropriate volumes of haemolysate created from the EDTA samples, to create 10%, 50% and 100% V/V dilutions (treatments H10, H50 and H100, respectively).
Fourteen days after collection, all samples were removed from their respective storage conditions and thawed at 4 °C. Haemolysis 10% and 50% V/V dilutions were created 14 days after sample collection, just prior to conducting the ELISA.

Three commercially-available ELISA kits were used for this study: IBV (Infectious Bronchitis Antibody Test Kit CK119; BioChek), AEV (FlockChek AE; IDEXX Laboratories) and CAV (FlockChek CAV; IDEXX Laboratories). The ELISA procedures were carried out as per the recommendations of the manufacturers.

III. STATISTICAL ANALYSIS

The S/P and S/N results from ELISA were assessed for normality by the Shapiro-Wilk test. All the samples from IBV and AEV data met the test for normality after square root transformation; and all of the CAV data were $\log_{10}$ transformed to meet the test for normality. A repeated measures ANOVA (RM-ANOVA) was performed on the transformed data to determine the effect of any of the five serum maltreatments on ELISA results (Green, 1993); separate analyses were conducted for IBV, AEV and CAV results. Contrast analysis (Tukey's Honestly Significant Difference) of all pair wise comparisons was performed to identify any treatment groups that showed significant differences from the reference standard. Mauchly's test for sphericity was applied to the data to ensure the assumption of homoscedasticity were met; when the assumption was not met Huynh - Feldt's correction was applied to reduce the likelihood of a Type 1 error.

The study was designed with 80% power to detect a medium effect size (0.3) amongst the six treatment groups, with the significance level (alpha) set at 0.05.

IV. RESULTS

The main effect across serum maltreatments for the one-way RM-ANOVA of IBV square root transformed ELISA results was significant ($F(5, 29) = 18.21$, $P < 0.001$). The contrast

comparison of each treatment showed that all other treatments, except HT, were significantly than the RS value ($P < 0.05$).

The one-way RM-ANOVA of AEV square root transformed ELISA results was significant ($F(5, 29) = 20.68$, $P < 0.001$). The contrast comparison of each of the treatments showed that FT, H50 and H100 were significantly than RS ($P < 0.05$).

The one-way RM-ANOVA of CAV $\log_{10}$ transformed ELISA results was significant ($F(5, 29) = 17.65$, $P < 0.001$). The contrast comparison of each of the treatments showed that HT and H100 were significantly lower than the RS value ($P < 0.05$). The results of the analyses are shown in Figs. 1a-c.

V. DISCUSSION

Severe haemolysis (H100) had the most consistent negative effect on ELISA performance, producing results that were significantly lower than the reference standard on all three ELISAs. Moderate levels of haemolysis (H50) had a similar, yet less consistent effect than the severe haemolysis, producing results that were significantly different from the reference standard for only the IBV and AEV ELISAs. Repetitive freeze-thawing (FT) also produced a significant adverse effect on ELISA results for IBV and AEV. The IBV ELISA appeared to be most susceptible to the effects of serum maltreatment, with 4/5 treatments (FT, H25, H50, and H100) having a significant adverse effect on ELISA performance. The CAV and AEV tests were more robust to sample maltreatment with only two or three of the maltreatments, respectively, having a significant effect on the results.

The magnitude in change of the median ELISA values as a result of sample maltreatment was not pronounced in most cases. The most significant maltreatment effects appeared to reduce the reported ELISA values (i.e. were more negative), in addition to a more general effect of increasing the range of reported values. There were an insufficient number of samples of both positive and negative status to formally assess the likelihood of a change in sample state (i.e. from positive to negative) as a result of sample maltreatment. However, the magnitude of observed changes in ELISA values suggest that only those samples near a positive cut-off value would be likely to change state. Interpretation of the test results at a flock level would be unlikely to change due to sample maltreatment, but a change in state for at least some individual birds would be expected. As a practical example, data from this study suggests that the H100 treatment effect on the CAV ELISA might be considered as a “worst-case” scenario for flock-level interpretation of serological testing results using maltreated samples. If one assumes that under perfect conditions, the CAV ELISA is 100% sensitive and specific, a 30-bird sample of a 5000 bird flock (presumed CAV negative status, but accepting that if the infection is present, 20% of birds would likely be positive) would provide 99.0% certainty that at least one positive bird would be identified. The estimated effect of H100 treatment on serum for the CAV ELISA is to reduce the test sensitivity by approximately 10%. Using the same testing scenario described above but with test sensitivity set at 90% (and leaving specificity at 100%), the certainty in finding at least one positive bird in a sample of 30 severely haemolysed samples would only be reduced to 97% (analysis completed using FreeCalc Version 2; Toowoomba, Queensland, Australia).

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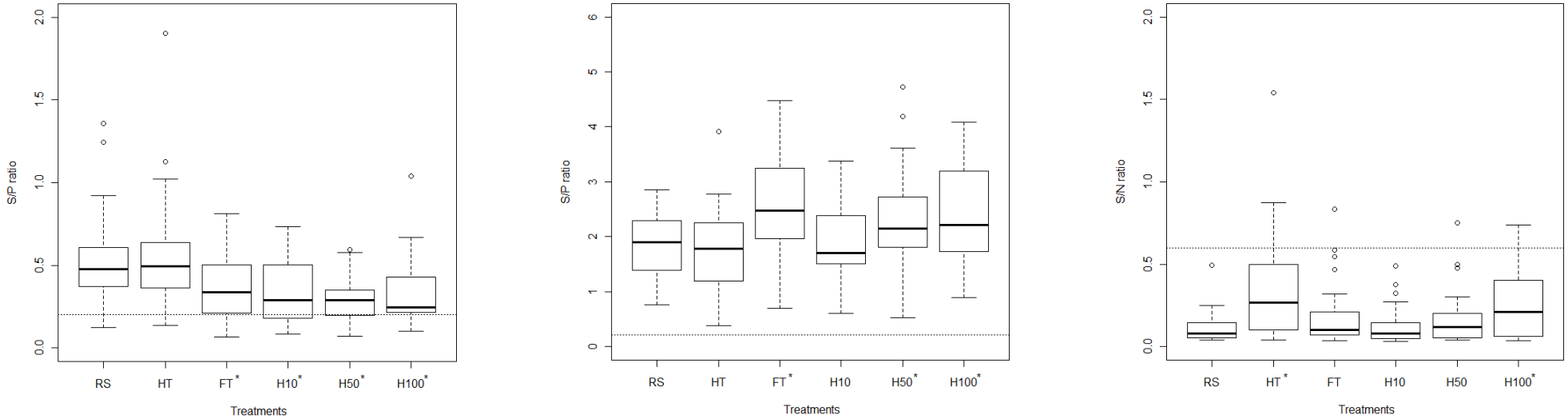

Figure 1. Variation in ELISA results due to sample mishandling. (a) Infectious bronchitis virus. (b) Avian encephalomyelitis virus. (c) Chicken anaemia virus. RS, reference standard; HT, heat treatment; FT, repetitive freezing-thawing; H10, 10% V/V haemolysis; H50, 50% V/V haemolysis; H100, 100% V/V haemolysis. Dotted line represents the positive cut-off value. Box plot represents the median and interquartile range; whiskers identify the 5^{th} and 95^{th} percentile range; dots represent the values lying beyond the 5^{th} or 95^{th} percentile range. An asterisk (*) denotes a treatment effect that is significantly different from RS ($P<0.05$).

ROLE OF INFLAMMATION IN THE PATHOGENESIS OF FATTY LIVER HAEMORRHAGIC SYNDROME IN LAYING HENS

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Fatty liver haemorrhagic syndrome (FLHS) is a metabolic disorder of commercial laying hens characterized by excessive fat accumulation in the liver and abdominal cavity, and sudden death due to liver rupture and internal bleeding. The aetiology and pathogenesis of FLHS still remain elusive. Recent evidence in humans indicates that obesity reflects a generalized proinflammatory state with increased inflammatory markers (acute phase proteins and cytokines) leading to chronic low-grade inflammation and development of metabolic syndrome (Gregor and Hotamisligil, 2011). This state seems to play a determinative role in many chronic metabolic diseases, including non-alcoholic fatty livers. In laying hens the liver has a central role in lipid metabolism, and fatty livers are common during egg production. However, hepatic lipid accumulation does not universally result in FLHS indicating that additional secondary insults are important and lead to progressive hepatocellular damage and liver rupture.

Experiments have been undertaken to study FLHS under field and experimental conditions. Here we report initial data on inflammatory responses of hens experimentally induced with FLHS. Hens were injected with oestradiol, 5mg/kg/body weight, every 4-5 days for 4 weeks. To evaluate the inflammatory status of birds, total and differential leukocyte counts and fibrinogen concentration were measured together with other circulating metabolites and liver histopathology. At 24 h, 1 wk and 2 wk, oestradiol-treated hens showed a significant increase in peripheral leukocytes, particularly lymphocytes ($P < 0.001$). Plasma fibrinogen levels were also elevated ($P < 0.001$). Thereafter, leukocytes and fibrinogen levels decreased, indicating chronic liver damage. It should be noted that as in mammals, fibrinogen levels in birds demonstrate increased systemic inflammation and tissue repair. Fibrinogen, an acute-phase protein, rises in response to high cytokine levels, showing that prothrombotic and proinflammatory states may be metabolically interconnected. Whereas, elevated lymphocyte numbers in birds can be seen in inflammation associated with infectious and non-infectious causes, including, haemorrhages into a body cavity (Campbell, 1995). Histologically, livers showed that all birds had some lipid vacuolation. However, livers of oestradiol-treated hens comprised a morphologic spectrum of lesions ranging from lipid vacuolation to portal inflammation and haemorrhage. There was a presence of macrovesicular fat in hepatocytes of treated hens with displacement of the nucleus to the edge of the cell wall. Additional features present were lobular and perisinusoidal heterophilic inflammation and in some cases lymphocytic portal inflammation and congestion.

These findings suggest that hepatic lipid accumulation and an inflammatory state characterized by increased production of circulating inflammatory markers and liver inflammation were sufficient to induce FLHS. A greater understanding of the pathogenesis of FLHS will assist in developing strategies for early detection and reduction of this disease in hens.

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EFFECT OF AGE ON FERTILITY IN THE JAPANESE QUAIL (*COTURNIX JAPONICA*).

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Summary

Egg break-out and *in vivo* sperm-egg membrane interactions were used to study the effect of age on egg fertility and sperm supply in Japanese quail breeders. Whole day egg production was sampled five times between 7-46 weeks of age and examined for fertilization status of the germinal disc, the numbers of sperm trapped and holes made in the vitelline membrane above the germinal disc of non-incubated eggs. Egg fertility increased ($P<0.05$) from 79% (Mean ± SEM) at Week 7 reaching a maximum 84% by Week 26, and then declined to 64% by Week 46. The sperm numbers were high and comparable between Weeks 7 (23 ± 1) and 16 (24 ± 1). Then the numbers reduced ($P<0.05$) by Week 26 (17 ± 1) and 36 (16 ± 1), and even further by Week 46 (8.3 ± 1). The numbers of holes were low at Week 7 (38 ± 1) and comparable to Week 36, reaching a maximum by Week 16 (61 + 1.6) and then gradually declining ($P <0.01$) over Weeks 26 to 46. Those patterns were similar to the egg fertility pattern. We conclude that low sperm and hole numbers at a young age and low sperm numbers after the maximum fertility has passed are responsible for reduced egg fertility.

I. INTRODUCTION

Japanese quails (*Coturnix japonica*) are grown for egg and meat production worldwide. Demand for improved performance is on-going but the genetics of reproduction has been little investigated (Minvielle, 2004). Some efforts have been made to address the problem of fertility in breeder quails and those few reports available are still based on conventional methods of fertility assessment such as after hatch break-out analysis (Woodard and Alplanalp, 1967; Narahari et al., 1988).

One of the critical steps in the fertilization process is binding of the sperm to the inner perivitelline layer (IPVL) and the subsequent acrosomal reaction. The result is a hydrolyzed hole through which sperm enter the ovum. Spermatozoa that fail to enter the egg cytoplasm become trapped under the outer perivitelline layers (OPVL). Spermatozoa interacting with the egg are identified in laid eggs in the perivitelline membrane (Wishart, 1987, Bramwell and Howarth, 1992; Wishart and Wood, 1994; Bramwell et al., 1995). Estimating individual egg, hen and flock fertility can be performed quite accurately by combining the information regarding appearance of germinal disc (GD) area, number of sperm attached ($Sperm_{OPVL}$) and holes made ($Holes_{IPVL}$) in the IPVL of freshly laid eggs (Bramwell et al., 1995; Wishart and Staines, 1995, 1999; Staines et al., 1998). As a first step towards determining true fertility in Japanese quail breeding flocks, we used the egg break-out approach and the perivitelline techniques to explore the in *vivo* sperm-egg interactions as the age of the Japanese quail flock changes.

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II. MATERIAL AND METHODS

Eggs were supplied by Game Farm Pty Ltd. (Galston NSW, Australia). The house environment of the breeder quails was maintained at 22-26°C temperature, 16-h light with adequate ventilation flow and *ad-libitum* feed and water supply. The quails were fed quail breeder diet containing 20.0% CP and 11.5 MJ/kg ME.

A whole day of egg production was collected from approximately 30% of the breeding population consisting of 5 flocks maintained in cages. Cages were randomly selected and the same numbers of cages in each flock were repeatedly sampled at 7, 16, 24, 36 and 46 weeks of age. Eggs were stored in a cold room for 3 days at the farm and then sent by air to the University of Western Australia.

All the eggs were opened, appearance of the GD was captured with a DP70 digital camera (Olympus Australia Pty. Ltd., Mt Waverley, VIC, Australia), and the PVL membranes were collected for sperm-hole counts. The fertility status of eggs was determined by viewing the GD under a low power magnification aided by lateral illumination from a light source before the photograph was taken. A piece (1.5 x 1.5 cm) of PVL was collected around the germinal disc using a paper filter ring (Whatman, Grade 1, Sigma- Aldrich Co., Castle Hill, NSW, Australia). The collected membrane was washed with phosphate buffered saline (PBS; pH 7.4) to remove the adhering yolk and then placed on a microscope glass slide. For sperm counting, the membrane was stained with Hoescht dye 0.01 mM solution (Sigma-Aldrich Co. Castle Hill, NSW, Australia). The sperm nuclei were visualized with a fluorescence microscope (Olympus BX60-FL, Olympus Australia Pty. Ltd., Mt Waverley, VIC, Australia) using a 'U' filter cube with 372 nm excitation and 456 nm emission wavelengths. For counting $Holes_{IPVL}$, Schiff's reagent (Sigma-Aldrich Co. Castle Hill, NSW, Australia) was used for staining the IPVL section as described by Bramwell et al. (1995). Counting was carried out in six fields (5.7 mm^2 total area) along the horizontal axis passing through the GD region.

The egg fertility, sperm and hole numbers data were analyzed by One-way ANOVA using PASW Statistics 18, Release Version 18.0.0 (© SPSS, Inc., 2009, Chicago, IL, www.spss.com).

III. RESULTS

Egg fertility increased between Weeks 7 and 16 reaching a maximum at Week 26 and then reduced between Weeks 36 and 46 (Fig. 1). Fertility was significantly higher ($P<0.05$) at Weeks 16 and 26 of age as compared to Weeks 7, 36 and 46. At weeks 16 and 26, fertility was comparable ($P>0.05$).

The mean $Sperm_{OPVL}$ numbers were the same between Weeks 7 and 16 (Fig. 1), reaching a maximum (24 ± 1). The numbers then decreased ($P<0.05$) by Week 26 and that mean was comparable to Week 36. A further decrease was observed between Weeks 36 and 46 of age. The mean sperm numbers were significantly higher ($P<0.05$) at Weeks 7 and 16 as compared to Weeks 26, 36 and 46 of age. The mean number of $Holes_{IPVL}$ was lower ($P<0.05$) at Week 7 than Week 16 and was comparable ($P>0.05$) to that in Week 36. The maximum number of $Holes_{IPVL}$ (61 ± 2) was recorded at Week 16 of age. Numbers decreased ($P<0.05$) by Week 26 and through to 46. There was a significant ($P<0.05$) difference in sperm hole numbers between all studied ages except between Weeks 7 and 36.

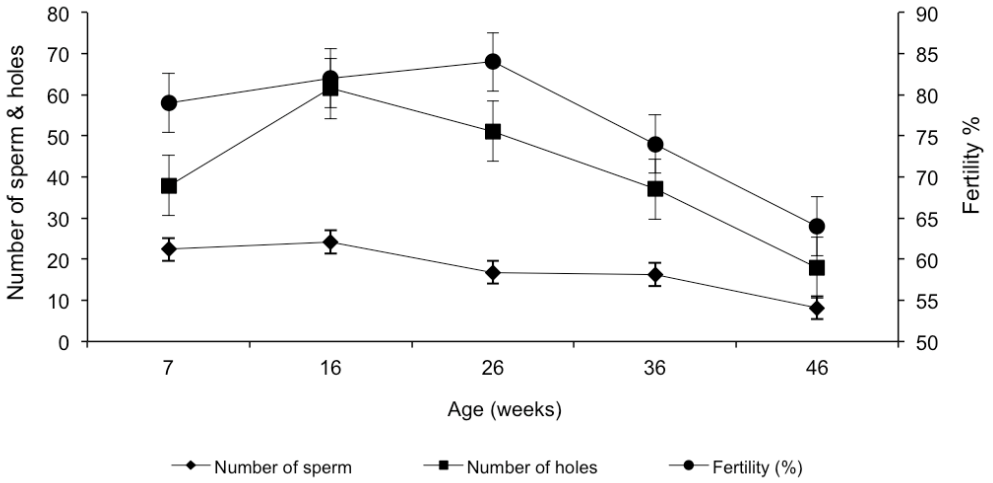

Figure 1. Changes in mean (± SEM) egg fertility, $Sperm_{OPVL}$ and $Holes_{IPVL}$ numbers with quail age.

IV. DISCUSSION

The pattern of fertility agrees in general with poultry species where the age-related fertility decline commences around the middle of the reproduction period and is associated with reduced retention of sperm by females (Bakst et al., 1994; Brillard, 2003). In Japanese quail, studies of age-related fertility indicate high egg fertility between weeks 10 and 20 of age with a peak at the age of 14-16 weeks (Woodard and Alplanalp, 1967; Insco et al., 1971, Narahari et al., 1988). In contrast, higher fertility beyond 20 weeks of age was observed in our study with a peak at the age of 26 weeks. Moreover, high levels of fertility observed in this study (90 %) had never been reported for quail breeder flocks. This could be due to different methods used for estimating flock fertility, true fertility in our study, or genotype differences. However, fertility decline has not been well described in the previous studies. In our study, the first 20 weeks of production egg fertility ranged 80-90% but in the next 20 weeks egg fertility declined sharply (by about 20%). The decline in fertility is, however, rapid (approx. 10% every 10 weeks). While the changes in egg fertility are underpinned by changes in numbers of sperm in the perivitelline membrane, the egg fertility pattern appears to correspond more with the sperm hole numbers in the IPVL than with the sperm trapped in the OPVL suggesting the ability to fertilize eggs is being lost with age. The underlying causes should be further investigated.

V. CONCLUSION

Fertility in Japanese quail is age-dependent. While fertility loss due to age is associated with reduced numbers of supplied sperm, the underlining cause appears to be a loss in the rate of sperm penetration of the inner perivitelline layer.

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THE QUALITY OF THE PEKIN DUCK (*ANAS PLATYRHYNCHOS DOMESTICA*) EJACULATES

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Summary

Ducks that delay ejaculation produce more ejaculate because of high secretions of lymphatic fluid that is added to semen at ejaculation. We tested the hypotheses that the ejaculate quality and sperm motility will be affected by reaction time to ejaculation. Nine males were trained to ejaculate into an artificial cloaca using a teaser female. A total of 34 ejaculates were collected and their quality (volume, sperm concentration and sperm viability) and sperm motility were determined. The reaction time (time delay to ejaculation), ejaculate quality and sperm motility parameters (sperm kinematics) varied between males but correlations between reaction time and ejaculate parameters were not significant. Negative trends though might suggest that as the time to ejaculate increases the quality of the ejaculate may decrease. Thus lymphatic fluids may contribute to the loss of motile and viable sperm in an ejaculate.

I. INTRODUCTION

Numerous factors affect fertility in poultry. In breeding, the ratio of females to males is high and for this reason the male can have a disproportionate effect on flock fertility (Wilson *et al*. 1979). The quality of the male ejaculate will influence flock fertility with natural mating as well as the efficiency of the artificial insemination (AI) or semen cryopreservation. Drakes are selected for their physical characteristics such as body size and shank length and not on their fertility attributes. In flock mating the competition between sperm from different drakes fails to guarantee that males of a high genetic merit actually contribute to the gene pool.

Quality tests for sperm concentration, viability, and motility may be time consuming and labor intensive but they can be good predictors of semen quality and fertility (McDaniel, 1998) None of these predictive measures have been evaluated in the Pekin duck. There is also - little information on the relationships between semen characteristics and libido or between libido and fertility. Gvaryahu *et al.* (1984) reported that ducks that have longer reaction time to ejaculation produced more ejaculate volume. This could be associated with the lymphatic fluids being added to semen because of extended courtship and stimulation (Fujihara and Nishiyama 1976).

In this preliminary study we tested the hypotheses that the ejaculate quality and sperm motility characteristics of the drake will depend on the reaction time to ejaculation.

II. MATERIALS AND METHODS

The ducks used in this study were the commercial strain of the Pekin duck (*Anas platyrhynchos domestica*) provided by the Luv-a-duck Pty. Ltd (Victoria, Australia). The study was conducted with nine male and 4 female ducks (age 30 weeks) brought from the farm to the Native Animal Facility of the University of Western Australia Research Station in

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Shenton Park (Western Australia). Ducks were trained according to the method developed by Tan (1980). The drakes and teaser females were maintained in individual wire cages. At collection the -drake was placed in a mating cage and the teaser female was then introduced. When the drake mounted the teaser and seized the nape of the neck, the artificial cloaca (AC) was placed close to the male's cloaca and the phallus was guided into the AC. Before experiment commenced, semen was collected every day from each drake for five days to accustom males to regular collection procedures.

The reaction time to ejaculation was measured using a stopwatch as the time the male commenced courtship to ejaculation. Ejaculate volume was measured using an automatic pipette by carefully drawing semen from the collection vial into a tip. Sperm concentration (10^9/ml) was estimated by a spectrophotometer using a pre-determined standard curve. The percentage of membrane intact and normal sperm was estimated in nigrosin-eosin smears on 2 replicate slides. In every smear, at least 300 spermatozoa were counted. Sperm motility parameters were measured by a computer-aided sperm analysis (CASA) system (Sperm Class Analyser, Microptic S.L., Spain). Semen from each male was diluted to 20 x 10^6/ml with motility buffer (DMEM, Dubecco's Modified Eagle Medium) and recorded at 40°C on a thermo plate mounted on the microscope stage. We estimated the curvilinear velocity (VCL), average path velocity (VAP), straight-line velocity (VSL) tracking the path of individual sperm over five randomly chosen fields. A minimum of 500 sperm per ejaculate (100 per field) was recorded and a mean value in each field was used as a single observation for analysis. Data were subjected to General Leaner Model using SPSS (ver. 18). The results are presented as means ± S.D. and $P<0.05$ is considered significant. In order to determine correlation between reaction time and sperm parameters, Pearson's correlation analysis was performed.

III. RESULTS

The semen collection was attempted five times from each male and 34 ejaculates were collected (75.5% success rate). There was no significant difference among males in the percentage of membrane intact and normal sperm ($P>0.05$) whereas motility parameters, VCL, VSL, and VAL, reaction time to ejaculation, ejaculate volume, and sperm concentration differed between males (Table 1). The reaction time was not correlated with any of the ejaculate characteristics.

Table 1. The mean (± S.D, min-max) reaction time to ejaculate, the ejaculate and sperm motility parameters in the Pekin duck.

Reaction time (sec)	Ejaculate volume (ml)	Sperm concentration ($x10^9$/ml)	Membrane intact sperm (%)	Membrane intact normal sperm (%)	VCL	VSL	VAP
57.4±30.7* (15.0-146.0)	0.8±0.5* (0.15-2.50)	4.3±1.6* (1.75-7.29)	92.4±2.2 (84.4-96.0)	88.5±3.0 (79.2-93.8)	68.3±16.5* (39.6-108.8)	58.6±13.2* (36.1-89.0)	65.0±15.7* (38.9-104.1)
Correlation	0.04	-0.23	-0.14	-0.23	-0.23	-0.24	-0.24
P - Value	0.82	0.19	0.42	0.18	0.19	0.18	0.17

Ejaculates (n) = 34, 2-5 ejaculates/male, * Denotes significant difference between males ($P<0.05$)

IV. DISCUSSION

The ejaculate quality was independent of the reaction time but was affected by the male. Although, negative correlation was recorded between the reaction time and most parameters measured. While the hypothesis is not supported by our results, it is within general expectation that time delay to ejaculation may lead to excessive addition of the accessory fluids and poor quality of an ejaculate (Nishiyama *et al*., 1976). In out study, the male affected the ejaculate output. Thus factors such as genotype, temperament, excitement and stress, or the time being handled by operator may also contribute to the volume of accessory fluid. As the ejaculate did not come into contact with the female cloaca, the secretions from the male reproductive system may contribute to the loss of motile and viable sperm in an ejaculate. Further studies need to explore how the quality of the drake ejaculate can be influenced and can be related to the natural mating success in the commercial flock.

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META-COMPARISON OF THE WELFARE OF LAYING HENS IN CONVENTIONAL CAGES AND ALTERNATIVE SYSTEMS

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Summary

Quantitative reviews include meta-analysis type approaches that combine the results of several studies that address similar research hypotheses are particularly useful when there are multiple studies with conflicting results. Here, we conducted a meta-comparison of the effect of conventional cages and alternative systems on measures of production, behaviour and physical and physiological condition in laying hens. Statistical limitations of our identified papers did not allow usual methods of meta-analysis, so we used a vote counting approach based on the treatment means and compared these counts with a Binomial test. Egg production was higher in conventional cages than in alternative systems though this effect was probably mostly confined to the comparison to perchery systems. Bones were significantly stronger from hens from alternative systems than from conventional cages. Mortality, which perhaps was expected to be higher in alternative systems, was not found to differ between systems. We confirmed previous reviews that birds show more comfort and dustbathing behaviour in alternative systems, but surprisingly feather and aggressive pecking did not differ between systems. The absence of a significant difference in feather score or body wounds between systems supports the physical condition and mortality meta-comparison results indicating no difference between systems in injurious pecking. In conclusion, the meta-comparison undertaken here suggests that the chance of an injurious pecking and cannibalism outbreak may be no greater in alternative systems than in cage systems. Instead, the often-reported higher incidence of injurious pecking and mortality in alternative systems may indicate the magnitude of the problem once outbreaks of cannibalism have occurred.

I. INTRODUCTION

The welfare of laying hens in conventional cages has probably attracted more debate than any other intensive husbandry system. The ability to perform specific behaviours, absence of unwanted behaviours, specific physiological responses, health measures, physical condition and injuries and production parameters have all been recorded and considered in assessing welfare of hens in cages in multiple experiments, and form the basis of many reviews (e.g. Appleby and Hughes, 1991; Lay et al., 2011). Such qualitative reviews of the literature are a fundamental scientific activity which reduces large quantities of information in palatable pieces, is efficient in avoiding the need for a further study and can lead to the generalisation of scientific findings. In these instances, the choice of welfare criteria reported and relative importance paid to each involves a certain degree of subjectivity. Within other disciplines the issue of subjectivity has led to qualitative reviews being criticised as haphazard and biased, subject to the impressions and ideals of the reviewers (Murlow, 1987).

Quantitative reviews include meta-analysis type approaches that combine the results of several studies that address similar research hypotheses. The technique is particularly useful when there are multiple studies with conflicting results, or where there may be conflicting interests. Quantitative reviews have been considered more objective than qualitative reviews and better able to precisely identify effects (Mulrow, 1994).

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It is often recognised that a combination of qualitative and quantitative reviews is essential in ensuring that bias is limited and that reliability and accuracy of recommendations are maximised (Mulrow, 1994). Our aim in the meta-comparison presented here was to synthesise the results of studies comparing differences in the effects of conventional cages and alternative systems on measures of production, behaviour and physical and physiological condition in laying hens.

II. METHODS

Information regarding the effects of conventional cages on behaviour, physical condition, physiology and production was collected from studies published in peer-reviewed journals between 1974 and 2010. The Institute for Scientific Information (ISI) web of knowledge online-database was used with a final viewing date of the 20/3/2011. The search terms hen/hens, cage/cages and welfare/well-being were used. Reference lists from all identified papers were then viewed to identify additional papers for consideration.

In order to numerically summarise these papers we first counted the number of papers in which conventional cages or alternative systems had a higher mean for the various response variables (primary analysis). Results in each paper for different systems were averaged so that each paper contributed only one count. The number of counts for conventional cage and alternative systems were analysed using a Binomial test. Further (secondary) analysis was undertaken to examine differences between current conventional cage and alternative systems. A vote counting approach was again used, in which the number of comparisons in which conventional cages or alternative systems had higher means was scored. Conventional cage systems in which birds had less than 550cm^2 per bird were excluded as this is the current local requirement (SCARM report 83, 2002). Additionally, floor systems that provided less than 666cm^2 per bird of useable area were excluded, based on the current regulation of 30kg/m^2 for 2kg birds (SCARM report 83, 2002).

III. RESULTS

Primary analysis indicated that egg production was significantly greater in cages, particularly when cages were compared to perchery systems (P=0.041, Table 1). Comfort behaviour was higher in alternative systems than conventional cages, and was also higher in furnished cages than conventional cages (Table 1). There was also more dustbathing behaviour in alternative systems than there was (sham-) dustbathing in conventional cages. Birds in alternative systems (in fact furnished cages and percheries) had significantly stronger bones than hens in conventional cages (Table 1). Perhaps unexpectedly, housing system was not found to have any effect on mortality (Table 1). Curiously, activity levels did not differ between conventional cages and alternative systems (Table 1). No difference between systems was found in aggressive and feather pecking (Table 1). Feather score and body wounds were not affected by housing (Table 1).

IV. DISCUSSION

In summary, egg production was higher in conventional cages than in alternative systems. We also confirmed previous reviews that birds show more comfort and dustbathing behaviour in alternative systems, and that these birds have stronger bones.

Mortality, which perhaps was expected to be higher in alternative systems, was not found to differ between systems. Mortality, in particular due to cannibalism, has been considered to be in general similar in conventional cages and alternative systems unless there

is an outbreak of cannibalism, in which case it can be more severe in alternative systems (e.g. Appleby & Hughes, 1991; Lay et al., 2011). Indeed, beak trimming is usually justified as a means to control mortality due to cannibalism in large-group systems, and is a routine management operation in many countries. Approximately half of the studies included in this meta-comparison did not beak trim, so we expected large-group systems in particular to be strongly impacted by cannibalism and mortality. In fact, this was not found to be the case-mortality did not differ between systems.

In addition, the absence of a significant difference in the severity of body wounds between housing systems further supports that cannibalism was not affected by housing system. Feather pecking and the associated deterioration of feather condition have previously been considered to be linked to cannibalism, yet no effect of housing system was found on feather pecking and feather score. Hence, the mortality scores, behaviour and physical condition of the birds indicate that mortality and cannibalism were not differing between systems, even though half the studies did not beak trim which might have been expected to increase mortality in large-group systems. Clearly, there is a need to revisit our assumption that mortality and cannibalism are worse in alternative systems. It may be that cannibalism outbreaks lead to more deaths in alternative systems than in conventional cages, but the evidence presented here suggests that outbreaks are no more likely in alternative systems than they are in conventional cages. Management has been found to be an important tool in controlling cannibalism in all systems, and it may be that the control of cannibalism and mortality should focus on management rather than the type of system.

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Table 1. Primary and secondary analysis of the number of times that cage systems and alternative systems had higher means. Probability obtained from binomial test of hypothesis that the probability that the proportion of papers with cage mean higher equals ½ is provided.

Analysis	**Primary**			**Secondary**		
Mean	**Cage higher**	**Alternative higher**	**P**	**Comparison**	**Cage higher**	**Alternative higher**
Egg production	**15**	**5**	0.041	CC-LI	**4**	**2**
				CC-FC	**4**	**2**
				CC-FR	**1**	**1**
				CC-PE	**8**	**2**
Mortality	**8**	**11**	0.648	CC-LI	**2**	**4**
				CC-FC	**2**	**3**
				CC-FR	**1**	**2**
				CC-PE	**4**	**5**
Comfort	**0**	**8**	0.008	CC-LI	**0**	**1**
				CC-FC	**0**	**6**
				CC-FR	**0**	**1**
				CC-PE	**0**	**4**
Dustbathe	**0**	**5**	0.062	CC-LI	**0**	**1**
				CC-FC	**0**	**4**
				CC-FR	**1**	**0**
				CC-PE	**0**	**1**
Activity	**4**	**6**	0.754	CC-LI	**0**	**2**
				CC-FC	**2**	**3**
				CC-FR	**0**	**2**
				CC-PE	**1**	**2**
Feather peck	**4**	**2**	0.687	CC-LI	**1**	**0**
				CC-FC	**0**	**3**
				CC-FR	**1**	**0**
				CC-PE	**2**	**1**
Aggressive peck	**3**	**3**	1.000	CC-LI	**1**	**0**
				CC-FC	**1**	**3**
				CC-FR	**0**	**0**
				CC-PE	**1**	**1**
Breaking strength-Tibia	**0**	**12**	<0.001	CC-LI	**0**	**0**
				CC-FC	**0**	**6**
				CC-FR	**0**	**0**
				CC-PE	**0**	**5**
Breaking strength-Humerus	**0**	**8**	0.008	CC-LI	**0**	**0**
				CC-FC	**0**	**6**
				CC-FR	**0**	**0**
				CC-PE	**0**	**4**
Feather score	**9**	**2**	0.065	CC-LI	**1**	**0**
				CC-FC	**3**	**1**
				CC-FR	**2**	**0**
				CC-PE	**4**	**1**
Foot score	**5**	**3**	0.727	CC-LI	**0**	**0**
				CC-FC	**3**	**2**
				CC-FR	**0**	**2**
				CC-PE	**3**	**2**

CC= conventional cage, LI=litter, FC= furnished cage, FR=free range and PE=perchery.

PREVALENCE OF *MYCOPLASMA SYNOVIAE* IN EGGS FROM LAYING HENS USING ELISA

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Summary

Mycoplasma synoviae (*M. synoviae*) can cause respiratory disease, synovitis, peritonitis, egg apical abnormalities or a subclinical infection. The importance of *M. synoviae* is well established in broilers but only a few studies have been conducted in layers. In the present study, the prevalence of *M. synoviae* in commercial layer flocks was determined by ELISA using egg yolk antibodies. Subsequently, a possible correlation between the serological status of *M. synoviae* and egg shell quality was also studied. In the flocks under study, seroprevalence of *M. synoviae* was found to be 69 % (95 % confidence interval (CI) = 47 to 91). Statistical analysis showed that the vaccinated group (3.0 ± 0.1) had the highest translucency score as compared to infected (2.4 ± 0.1) and uninfected (2.5 ± 0.1) groups, whereas % shell reflectivity was highest in the infected group (31.41 ± 0.3) as compared to the other two groups. Shell breaking strength (39.5 ± 0.5 Newtons) and shell deformation (298.7 ± 3.8 μm) values were significantly lower in the infected group than in the uninfected and vaccinated groups. There was no significant difference among these three groups for egg quality parameters egg weight, egg shell weight, % egg shell, shell thickness.

I. INTRODUCTION

Mycoplasma synoviae (*M. synoviae*) can cause respiratory disease, synovitis, peritonitis, egg apical abnormalities or a subclinical infection (Feberwee et al. 2009a,b). Mycoplasma species are well-known pathogens of domestic poultry, causing significant economic losses (Lierz et al., 2007). The importance of *M. synoviae* is well established in broilers but only a few studies have been conducted in layers (Hagan et al., 2004). *M. synoviae* is known to be transmitted vertically through eggs (Board and Fuller, 1994). The prevalence of egg *M. synoviae* antibody in egg yolk has been found to be a suitable approach to assess the flock prevalence of *M. synoviae* infection in layer hens (Hagan et al., 2004) and found comparable with serum antibodies (Mohammed *et al*., 1986 a,b). Earlier, the Dutch strain of *M. synoviae* was found to be one of the factors causing egg shell translucency (Feberwee et al., 2009a,b). However, there is little information available regarding the effects of Australian strains of *M. synoviae* on egg shell quality. In the present study, the prevalence of *M. synoviae* in commercial layer flocks was studied by ELISA using egg yolk antibodies. Finally, correlations between egg shell quality parameters and the presence of *M. synoviae* in eggs was investigated.

II. MATERIALS AND METHODS

Eggs were randomly collected from 19 different commercial layer flocks. Of these 19 flocks, three flocks were vaccinated. In general, 30 eggs from each flock were used for seroprevalence studies and 30 eggs from each flock were collected for determining egg quality parameters such as translucency score, shell reflectivity, egg weight, shell deformation, shell weight, % shell and shell thickness.

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The method for extracting antibodies from egg yolk was adapted from Mohammed et al. (1986b). For a saline extraction, 3 mL of egg yolk was collected from each of the 570 eggs (n=30 from 19 flocks) and mixed with 3 mL saline, vortexed and left for 48 h at 4°C. For the chloroform extraction, 0.5 mL saline extraction and 1 mL chloroform were vortexed to a thick paste. This was allowed to stand for 30 min at room temperature before being centrifuged at 850 x g for 20 min. The upper aqueous layer was removed and used in the ELISA. These extracted yolk antibodies were stored at -20°C. Each extracted antibody sample was diluted 1:50 ratio in PBS and was then used in the ELISA. The BioChek *Mycoplasma synoviae* antibody kits (BioChek B.V., Holland) were used in this study according to the manufacturer's instructions in order to study the prevalence of *M. synoviae* in the sampled commercial layer flocks. Absorbance of controls and test samples was recorded at 405 nm. Dilutions of chloroform-extract egg yolk antibody were prepared from the pools of known positive (*M. synoviae* vaccinated) and known negative eggs and tested for the following titres; 1:10, 1:50, 1:100, 1:500 and 1:1000. From the curve produced, the linear part was expanded. Reading the known positive and negative samples individually at the selected dilution produced a cut-off point for the test. The cut off values were determined using the model described by Greiner et al. (1995). The results were used to calculate the optimum sensitivity (Se) and specificity (Sp). The Se and Sp for each threshold value were calculated as the proportion of positive results in the positive reference population and negative results in the negative reference population, respectively (Greiner et al., 1995). Depending upon ELISA results, the flocks were divided into three groups: infected, uninfected and vaccinated. The flocks with more than 10% positive reactions were considered positive serologically based on the method of Kleven and Bradbury (2008). Using the ELISA results, seroprevalence of *M. synoviae* was determined at 95% confidence with a Binomial exact confidence interval model. ANOVA of the S-PLUS statistical software was used to compare egg shell quality parameters of infected, uninfected and vaccinated group.

III. RESULTS

Using the chloroform-extracted egg yolks, a dilution factor of 1:50 was chosen as it was on the linear part of the standard curve produced. It was observed that the optimized optical density cut-off point was 0.390 with 90 % Se and Sp. Out of the 19 flocks screened under this study, numbers of serologically positive (infected) and negative (uninfected) flocks were found to be 11 and 5, respectively, and the remaining 3 flocks were vaccinated. Thus, the prevalence *of M. synoviae* serologically positive flocks in commercial layers was high {11/16 (69 %) , 95 % CI = 47 to 91}. Table 1 shows the individual flock-wise seroprevalence of *M. Synoviae.* Table 2 shows the relationship between *M. synoviae* serological status and different egg shell quality parameters (Translucency, shell reflectivity, egg weight, shell breaking strength, shell deformation, egg shell weight, % egg shell and shell thickness). Statistical analysis showed that the vaccinated group (3.0 ± 0.1) had the highest translucency score as compared to infected (2.4 ± 0.1) and uninfected (2.50 ± 0.1) groups whereas the infected group (31.4 ± 0.3) had the highest % shell reflectivity as compared to the other two groups. Shell breaking strength (39.5 ± 0.5 N) and shell deformation (298.7 ± 3.8 μm) values were significantly lower in the infected group than in the uninfected and vaccinated group.s However, there was no significant difference among the three groups for other egg quality parameters such egg weight, egg shell weight, % egg shell, shell thickness.

Table 1. Individual flockwise seroprevalence of *M. synoviae* in unvaccinated flocks

Flock number	Sample size	*M. synoviae* serologically positive	Prevalence (95% CI) of seropositive farms (%)
1	30	11	37 (20 to 54)
2	30	15	50 (32 to 68)
3	30	12	40 (22 to 58)
4	30	20	66 (49 to 83)
5	30	0	0
6	30	1	3 (0 to 9)
7	30	29	97 (91 to 100)
8	30	4	13 (1 to 25)
9	30	8	27 (11 to 43)
10	30	3	10 (3 to 26)
11	30	1	3 (0 to 9)
12	30	11	37 (20 to 54)
13	19	12	63 (41 to 85)
14	30	10	33 (16 to 50)
15	30	0	0
16	30	0	0

Table 2. Relationship between *M. synoviae* serological status and different egg shell quality parameters

Variables	Infected	Uninfected	Vaccinated
Number of Flocks	11	5	3
Translucency	$2.4^{a} \pm 0.1$	$2.5^{a} \pm 0.1$	$3.0^{b} \pm 0.1$
% Shell reflection	$31.4^{a} \pm 0.3$	$28.7^{b} \pm 0.5$	$29.7^{b} \pm 0.5$
Egg weight (gm)	$61.5^{a} \pm 0.4$	$60.2^{a} \pm 0.9$	$59.9^{a} \pm 0.5$
Shell breaking strength (N)	$39.5^{a} \pm 0.5$	$43.2^{b} \pm 1.0$	$45.9^{b} \pm 1.3$
Shell deformation (μm)	$298.7^{a} \pm 3.8$	$321.9^{b} \pm 10.1$	$310.5^{b} \pm 8.8$
Egg shell weight (gm)	$5.8^{a} \pm 0.1$	$5.69^{a} \pm 0.1$	$5.6^{a} \pm 0.1$
% Egg shell	$9.4^{a} \pm 0.1$	$9.5^{a} \pm 0.1$	$9.3^{a} \pm 0.1$
Shell thickness (μm)	$390.9^{a} \pm 2.5$	$385.6^{a} \pm 3.1$	$383.9^{a} \pm 3.4$

Means with the different superscript in the same row are statistically significantly different ($P \geq 0.05$) from each other.

IV. DISCUSSION

The present study was conducted in order to determine the seroprevalence of *M. synoviae* infection in commercial layer flocks by ELISA. A high seroprevalence of *M. synoviae* in commercial layer flocks was found. This finding is in agreement with data of other research groups. The study of Hagan et al. (2004), which was also based on the detection of *M. synoviae* antibodies in eggs, reported a prevalence of 78.6% in commercial layer flocks in East England. In another study (Mohammed et al., 1986), a *M. synoviae* prevalence of 87% was found in commercial layer flocks in Southern California. In the present study, sample size is relatively small compared to earlier studies and study is ongoing. The high prevalence and persistence of *M. synoviae* infections in layer stock have been explained by the frequent occurrence of multiple-age housing and lower biosecurity standards in this sector (Stipkovits & Kempf, 1996; Kleven, 2003). *M. synoviae*-infected commercial layer stocks therefore pose a significant epidemiological risk for other categories of poultry. Feberwee et al. (2009a,b) reported that a Dutch strain of *M. synoviae* was associated with formation of egg apex abnormalities (EAA) and also reported synergism between *M. synoviae* and infectious bronchitis virus. In the present study, it was found that shell breaking strength and shell deformation were significantly lower in the infected group as compared to the uninfected and

vaccinated groups. The vaccinated group had the highest translucency score as compared to infected and uninfected groups, whereas the infected group had lighter coloured shells as compared to the other two groups. Findings of the study are in contrast to earlier findings by Lott et al. (1978) who found that *M. synoviae* infection in broiler breeders had no effect on egg shell strength or Haugh units under experimental conditions. These differences in findings might be because the present study was conducted in field conditions. However, controlled experiments would be necessary to study the effects of the Australian strains of M. *synoviae* on egg quality of commercial layers.

ACKNOWLEDGEMENTS

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IDENTIFICATION AND CHARACTERISATION OF FLOCK PERFORMANCE ISSUES IN LAYING HENS USING LOGIC SOFTWARE

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Prototype logic software, capable of identifying issues that affect the performance of laying hen flocks via a user-input questionnaire has been developed. The aim of this prototype was to investigate if it was possible for egg producers, with little veterinary knowledge, to identify sources of performance issues within their flock. The prototype was also built with the aim of teaching egg producers to identify signs of disease, metabolic disorders and other external factors that affect hen welfare and health using basic scientific reasoning. The application consists of three main components; a database with over 100 diseases, conditions, disorders, husbandry malpractices and equipment malfunctions that are known to have an effect on flock performance; a questionnaire consisting of 20 "yes" or "no" questions; and computer software that is able to link the user's answers with the database to provide a diagnosis. The database was compiled using current literature, textbooks and scientific publications, using the following assumption: "each disease, condition or disorder, varies from another by the expression of different signs that can be easily observed by a poultry stockman". These signs were used to categorise each condition and form clusters that could be differentiated by a computer algorithm. Based on the answers to the questionnaire, the algorithm is able to decipher which questions to present to the user and which ones to omit. Unlike other expert systems, the prototype is able to account for certain levels of uncertainty. The algorithm, disease categorisation and the questionnaire were all constructed based on a combination of four scientific reasoning methods first described by Mill (1843) and the theory of reasoning described by Korzybski (1933).Three case studies and more than 60 hypothetical scenarios were performed involving egg producers and industry experts. Results demonstrated that the prototype had an accurate diagnostic power of 0.85. Two main reasons accounted for the prototype failing to reach higher levels of accuracy; uncertainty and user perception. It was found that in certain occasions where too much uncertainty was present, the prototype was unable to eliminate disorders with similar symptoms or clinical signs. When the user did not recognise or identify the signs correctly the prototype was also unable to reach an accurate diagnosis. In addition, when the user misinterpreted the questions an invalid diagnosis was reached. It is concluded that further development and testing is crucial to reach higher levels of accuracy. The prototype's degree of accuracy was completely dependent on the user interaction.

The financial support of the Australian Egg Corporation is gratefully acknowledged.

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MAJOR STRATEGIES AND CHALLENGES WITHIN AECL

J. KELLAWAY[1]

Summary

The Australian egg industry continues to evolve into a large-scale professional business servicing a dynamic market with a range of eggs and egg products by farmers who care about producing the highest quality, safest product; our environment, providing choice; the welfare of our hens; and feeding our growing population. The Australian Egg Corporation Limited (AECL) will continue to assist this growth by developing and driving integrated on-farm, through chain and market services that maximize benefits and revenue for the Australian egg industry and the community while minimizing barriers and costs for Australian egg producers.

I. INTRODUCTION

As part of its continuous planning for the egg industry AECL has recently undertaken its third strategic planning process reflecting good governance and as stipulated in the Statutory Funding Agreement (SFA) between the Australian Government and AECL (Australian Government, 2007). The process has assisted AECL to develop sound strategies based on all key issues or challenges considered by egg producers, our investors, which will be reflected in the final Strategic Plan which will span from 2012 to 2016. These issues or challenges include: flock health & disease management; hen welfare; feed availability & bird nutrition; environmental sustainability; on-farm training, information & technology transfer; supply chain & egg distribution; profitability; food safety; egg advertising & promotion; and egg industry & stakeholder credibility (Kaliber Research, 2011).

But before we can look forward, we need to look back and take account of industry trends and Key Result Areas (KRAs) as defined by egg producers for AECL.

AECL has been in operation now for approximately 9 years since it commenced trading in February 2003. At this time, it subsumed the operations of the Rural Industries Research & Development Corporation (RIRDC) in managing the investment of the Laying Chicken (R&D) levy into an industry-owned company that also commenced receiving and managing the newly instituted Egg Promotion levy. Since inception, the nominal rate of the Egg Promotion levy has not changed but the Laying Chicken (R&D) levy has increased by 88% as approved by egg producers, AECLs investors.

Since commencement, AECL has had a philosophy of establishing the business over the short term, gaining effectiveness of program outcomes over the medium terms and driving efficiencies of operating and program administration over the longer term. AECL KRAs are responsive to the metrics deemed appropriate by investors and as such have and continue to include: egg awareness, demand, sales & consumption; Return-On-Investment (ROI); and industry productivity growth.

II. EGG INDUSTY GROWTH

Prior to the inception of AECL, the egg industry has been steadily growing in line with population trends and industry productivity has been increasing albeit mainly through the investment in new capital infrastructure but also due to the adoption of new technologies and

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research provided through the Laying Chicken (R&D) levy investment over time. However, there has been a decline in egg consumption. As a result, a key focus of AECL to date has been to increase egg consumption and hence growing the egg industry which has resulted in egg production growth (Fig 1.).

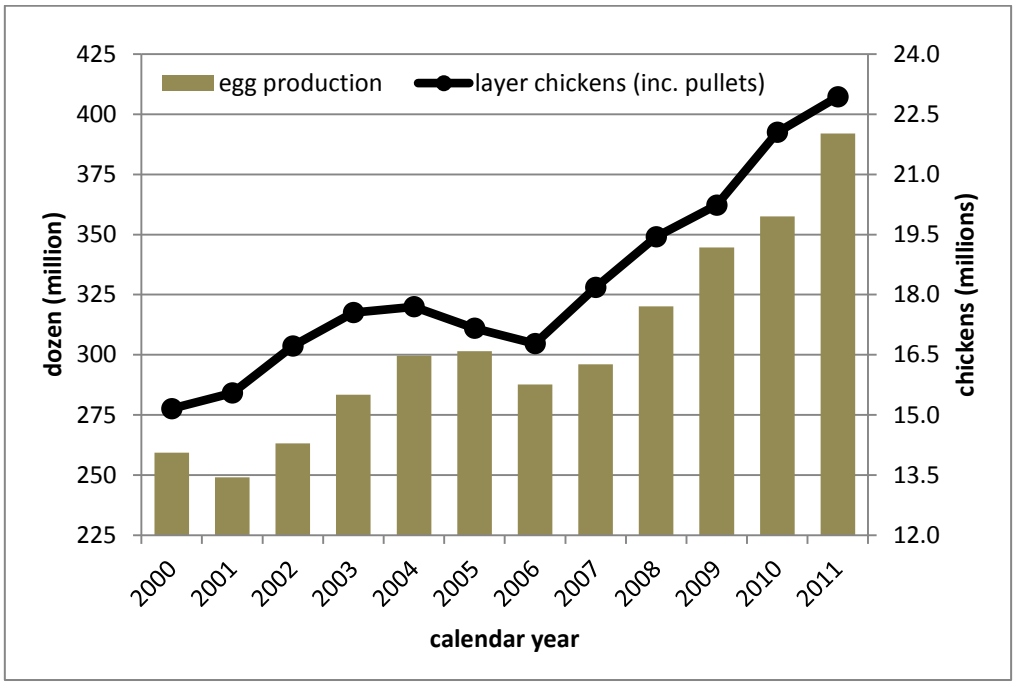

Figure 1: Egg production 2000-2011

Prior to 2003, egg production had a Compound Annual Growth Rate (CAGR) of 1.7%. Since the inception of AECL, the growth rate has increased to a CAGR of 4.6%, well ahead of population growth which has a long term annual growth rate of 1.5% (Australian Bureau of Statistics, 2011). In terms of the number of layer chickens, this has increased at a slightly slower rate depicting increases in hen productivity over time through better genetics, feed formulation and bird husbandry practices. However, this growth trend in productivity is not linear and has started to slow through increased production of eggs in non-cage systems.

The 'push/pull' affect in production created by the egg industry has resulted in per capita increases in egg consumption (Fig. 2). The Australian egg industry has experienced unprecedented growth in the per capita consumption of eggs in recent times with consumption as at December 2011 reaching 213 eggs per person and this increasing trend is not projected to abate over the short term as eggs become cemented as a key part of the weekly meal repertoire in the home, at mainstream dining facilities, restaurants or cafes and among institutional foodservice operators where the use of eggs as a centre-of-the-plate menu item has expanded from solely being a breakfast item.

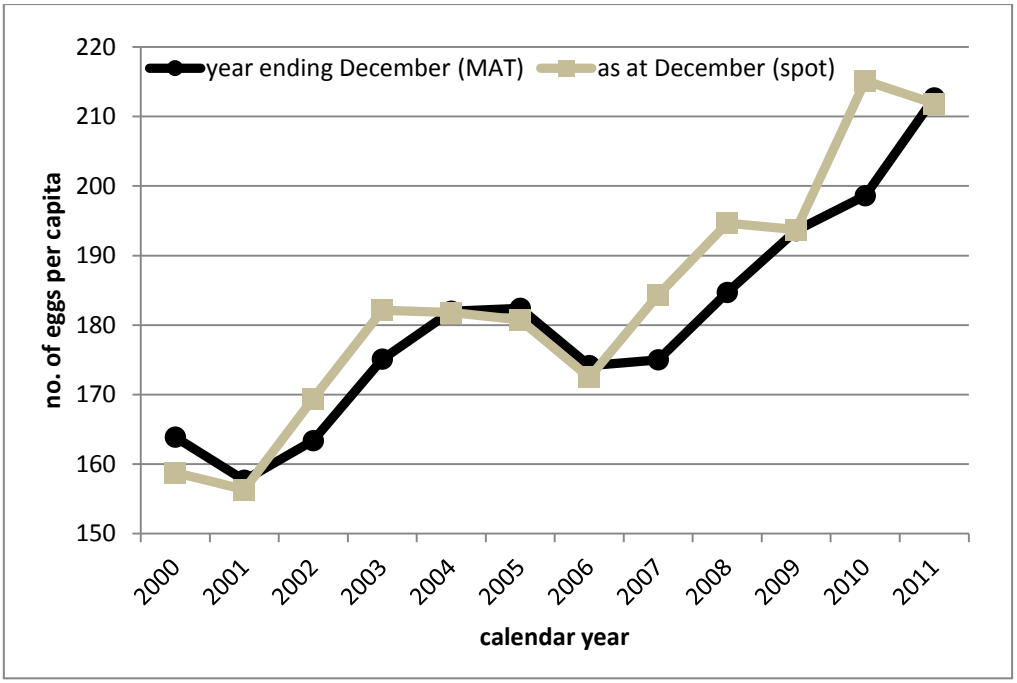

Figure 2. Egg consumption 2000-2011

There are now many varied offerings within the category to suit a highly-varied marketplace ranging from different egg sizes, pack sizes, production systems and brands. While some may suggest this provides confusion, the egg industry strongly believes that this differentiated offering provides revenue growth for the egg industry.

III. EGG INDUSTRY CHALLENGES

The Australian egg industry has a vision of being "a cohesive, profitable and growing Australian egg industry meeting the needs of consumers while operating in a socially and environmentally sustainable fashion." (AECL, 2011). AECL, as the industry's service provider, has a role to play in playing its part to assist the industry reach its vision over the longer term. Through extensive industry consultations, there have been 10 key themes identified that reflect the challenges the industry will face into the future. The challenges identified by egg producers in rank order are: flock health & disease management; hen welfare; feed availability & bird nutrition; environmental sustainability; on-farm training, information & technology transfer; supply chain & egg distribution; profitability; food safety; egg advertising & promotion; and egg industry & stakeholder credibility (Kaliber Research, 2011). It is interesting to note that while 93% of egg producers scored the top-ranked challenge to be important (64% 'very important' and 29% 'important') with a mean score of 1.5 out of 5, a total of 68% of egg producers scored the lowest-ranked challenge to be important (24% 'very important' and 44% 'important') with a mean score of 2.2 out of 5 (Table 1).

The egg industry has experienced incursions of exotic or emergency animal diseases on-farm and the consequences have proven devastating for egg producers through a loss in egg production and a decline in consumer confidence. As a result, minimising disease outbreaks and managing adverse public opinion are both essential to the ongoing sustainability of Australia's egg industry. This includes ensuring effective levels of on-farm biosecurity are maintained, developing industry's understanding of disease characteristics and the development and availability of vaccines on a regular basis. More than 90% of the egg industry consider the containment or control of diseases such as Newcastle Disease (ND),

Infectious Bursal Disease (IBD), Egg Drop Syndrome (EDS) and Avian Influenza (AI), to name a few, as critical challenges into the future. Other key issues included the availability of vaccines; ensuring effective levels of quarantine or biosecurity on-farm; rapid diagnosis of hen health problems; and the availability of science or sufficient disease research as an insurance policy important challenges.

Table 1. Egg industry issues/challenges – 2011

Issues / Challenges	*very important*	*important*	*total*	*mean score (1 to 5)*
Flock health & disease management	64%	29%	93%	1.5
Food safety	56%	31%	87%	1.6
Feed availability & bird nutrition	51%	35%	86%	1.7
Egg advertising & promotion	44%	41%	85%	1.8
Egg industry & stakeholder credibility	43%	37%	80%	1.8
Hen welfare	43%	36%	79%	1.9
Profitability	47%	31%	78%	1.9
Supply chain & egg distribution	43%	35%	78%	1.9
Environmental sustainability	28%	46%	74%	2.2
On-farm training, information & technology transfer	24%	44%	68%	2.2

A number of food borne illness outbreaks have 'implicated' eggs as the source of Salmonella contamination over recent years which remain the community's second most important food safety threat behind Campylobacter. While the egg industry can expect an increased focus by food safety regulators, the industry needs to ensure that any legislative requirements take a whole-of-chain approach as it relates to minimising the risk of food borne illnesses and the subsequent adverse public opinion that is generated. The egg industry believes traceability is an essential component to assist manage and identify the risk, containing any future outbreaks and recognising or rewarding egg producers who do implement appropriate food safety systems as part of their Standard Operating Procedure (SOP).

The security of feed grains at economically viable prices will become more difficult to attain due to increases in the demand for grain world-wide by traditional grain users and new or emerging bio-fuel industries. More effective feed formulations and the introduction or growth of new production technologies such as environmentally controlled shedding will all assist manage feed grain utilisation. In addition, the availability of imported grain at short notice and the emergence of new feed sources will need to be considered to overcome current challenges confronting Australia's egg producers.

Eggs have not had a high awareness or positive association with health, nutrition or convenience in the past relative to other foods that have a strong brand presence and association with consumers. While this is changing, a worrying percentage of consumers and healthcare professionals still view eggs as being bad for heart health and having a confined role in today's diet. This is despite recent research proving that egg consumption is essential as part of a modern and healthy eating plan that has very limited impact on blood cholesterol levels. This long-held cholesterol myth needs to be addressed while promoting the positive and essential health benefits, versatility and convenience of eggs as either an ingredient or a meal solution at any time. This includes expanding the use of eggs and egg products into new markets such as non-human use.

The credibility of eggs and egg producers will be critical if we wish to bring the industry's values to life and reposition the beliefs, views and perceptions of the market which are currently quite negative towards the egg industry. We need to ensure the ongoing support of the community, government agencies and the media in terms of industry practices. Such issues as production system definitions, the use of cages and truth-in-labelling will all be critical to assist improve the industry's image into the future.

While it is regarded by some that egg producers are not welfare-friendly, a large percentage of the community remains unaware of the many advantages and disadvantages pertaining to the egg production systems operating within the egg industry. The availability of rigorous, replicated and peer reviewed science and the communication of key research outcomes as it pertains to best management practice and on-farm animal husbandry will be critical in shaping community attitudes towards the egg industry. Such operational concerns as bird stocking densities, farm raids and public opinion will all depend on the industry's ability to counter biased information 'peddled' by minority groups.

Egg producers are all too familiar with increasing costs and stagnant egg prices. This is a characteristic of limited critical mass, lack of market power and the commoditisation of the egg category. The threat of imports, the nature of retailing in the Australian market and the growth strategy of private-label brands have all assisted to depress price points while significant capital infrastructure and other business inputs have driven increases in business fixed costs. The net result is reduced margins that are being squeezed. These issues will need to be addressed by the industry with long term commitments from egg producers to build appropriate margins back into egg businesses.

Key issues emerging within the supply chain include product quality, egg ullage or wastage, egg labelling, retail category management and differentiating the egg category along meaningful product attributes. This will all assist establish transparent and recognised supply chains that will uniquely position eggs at the Point-Of-Sale (POS) and directly address key consumer requirements as they relate to yolk colour, albumen quality and product performance given its end use.

An increasing focus by government and the community will be on the egg industry's environmental 'footprint' and the capability of industry to create a sustainable future taking account of the natural resources needed to produce eggs. To this extent, understanding and interpreting environmental regulations while managing waste, odour, bird disposal and energy use will all affect the sustainability of Australia's egg production into the future. While the egg industry has one of the lowest carbon footprints among competing protein foods, the community will expect us to continue improving in this part of our business.

Employment turnover and the need to re-train and up-skill current industry employees is essential to maintain the engagement of 'cutting edge' technology and skills as they relate to all facets of egg production, grading, packing, processing and marketing. The availability of on-farm husbandry, business management and marketing skills and information is critical across the industry to assist egg producers plan, invest with confidence and grow business opportunities into the future.

IV. AECL STRATEGIES

While there are a number of issues prevalent among AECLs investors, there are only a small or discrete number of challenges that can be influenced and potentially resolved or addressed by AECL according to egg producers (Table 2). Our investors wish AECL to focus its resources and efforts in assisting address egg promotion & advertising, with the aim of maintaining increases in egg consumption; egg industry & stakeholder credibility, with the aim of raising the integrity and perception of the egg industry; and on-farm training,

information & technology transfer, with the aim of imparting and empowering egg producers to adopt and utilise the latest in research to assist on-farm productivity and business improvement.

Table 2. AECL strategic focus ranked by industry importance

Issues / Challenges	*egg industry importance (total)*	*AECL strategic focus weighting*
Flock health & disease management	93%	19%
Food safety	87%	35%
Feed availability & bird nutrition	86%	8%
Egg advertising & promotion	85%	87%
Egg industry & stakeholder credibility	80%	75%
Hen welfare	79%	17%
Profitability	78%	20%
Supply chain & egg distribution	78%	33%
Environmental sustainability	74%	23%
On-farm training, information & technology transfer	68%	60%

To date, AECL has had a mission "to develop and drive integrated on-farm solutions and through chain market services – with R&D and promotional levy investments – that maximise benefits and revenue for the Australian egg industry and the community while minimising barriers and costs for Australian egg producers." (AECL, 2011). To assist AECL operationalise this mission to date, there have been six key strategies and associated outcomes.

The first strategy is to monitor, analyse, understand and address market attitudes, behaviour, consumption and preferences of eggs and perceptions of the egg industry in the Australian market. The outcome being sought here is to ensure the industry is aware of and addressing consumer attitudes, behaviour and preferences for eggs while the community respects and recognises all egg production systems and minimal food safety risks associated with egg consumption.

The second strategy is to execute integrated and market-responsive product marketing campaigns to assist raise awareness, demand, purchase and consumption of eggs in the Australian market among identified target market segments. The outcome here is to seek continual growth in egg sales providing an effective return on invested funds and positive growth trend in egg sales each year.

The third strategy is to enhance egg distribution channels and the merchandising environment to drive supply efficiencies throughout the value chain with an outcome of generating greater levels of transparency throughout the supply/demand chain with enhanced product merchandising and management of the egg category at the Point-Of-Sale (POS).

The fourth strategy is to identify, measure and address inefficiencies in the Australian egg production and supply chain through benchmarking, research and analysis to ensure on-farm and supply chain inefficiencies are understood, monitored and addressed through consultation with egg producers and resolved or minimised through the application and adoption of research.

The fifth strategy is to initiate the development and uptake of innovations and industry information that will enhance industry competitiveness and sustainability to ensure that industry information and innovation needs are identified, researched and adopted to provide a more competitive and sustainable egg industry.

The last strategy is to build industry resources to deliver stakeholder-responsive programs and information in a timely manner to ensure an adequately resourced egg industry and service provider thereby allowing the provision of technical, market and production-based skills to be engaged on demand.

Moving forward, AECL will wish to consolidate on its core ability to address and assist resolve industry issues or challenges identified and observed by the egg industry. Over the short term, AECL will be working under and making investments in areas outlined in a refined Strategic Plan that has been developed by industry. We will also be complying to a new Statutory Funding Agreement with the Australian Government. 2012 is the Year of the Farmer and AECL intends to be intimately involved with this national initiative promoting the industry's Corporate Social Responsibility (CSR). Additionally, AECL will see the roll-out of the new Egg Standard among all interested egg producers allowing licensees to boast their high quality systems and best practice processors to current and potential customers as well as regulators. The industry's on-farm extension program and training programs will move into 2nd gear up-skilling and adding direct value to egg producers recognizing the training and rewarding farm workers accordingly. Also, our student education program will be expanded given the high levels of success achieved in 2011 in teaching our next generation of egg lovers to assist breach the growing city/country divide.

V. CONCLUSION

AECL will be central to ensuring all key issues or challenges confronted by the egg industry are acknowledged, reviewed, monitored and addressed now and into the future. This will be through communicating messages to increase egg consumption, egg industry credibility and to drive a cohesive egg industry.

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NEW CONCEPTS IN LAYER NUTRITION

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Summary

Laying hen nutrition has advanced dramatically over the past 20 years. In 1992 it was not uncommon to see layer diets containing little more than grain (corn, wheat, sorghum), soybean meal, phosphate, limestone, salt, methionine, and a vitamin and mineral premix. In addition to minimum energy, calcium, available phosporus, and sodium levels the diets were typically formulated to a crude protein minimum and amino acid specifications were limited to total methionine, total methionine plus cysteine and possibly total lysine. However, commercial laying hen diets and nutrition have advanced dramatically over the past 8-12 years. A modern layer diet will typically contain corn, soybean meal, canola meal (where available), distillers dried grains plus solubles, meat and bone meal, bakery by-product meal, wheat middlings or soy hulls, phosphate, limestone, salt, methionine, lysine, threonine, phytase, NSP Enzyme, and a vitamin and mineral premix. The diets will be formulated to a minimum energy, calcium, available phosporus, sodium, chloride, and choline levels. There will be no crude protein minimum and the diets will be formulated to minimum digestible lysine, arginine, methionine, methionine plus cysteine, tryptophan, threonine, isoleucine, and valine levels. This change in nutrition and feeding strategy has been spurred, in part, by research into the impact of enzymes on nutrient availability, research into amino acid digestibility and ratios, increased feed ingredient cost, and improved genetic potential. Age at first egg has decreased by one week every 5 to 7 years, early egg size has improved, and persistency of production has improved. In 1992 a good flock would produce 230 eggs per hen-housed from 20-60 weeks. Today that would be a disastrous flock. A good flock today will produce 260 eggs per hen-housed from 20-60 weeks of age. This improvement has coincided with a significant decrease in body weight and feed intake capacity, all of which increase the importance of nutrition.

In the following paper and presentation I will review some of the modern technologies and feeding methods currently being employed in commercial laying hen nutrition.

I. INTRODUCTION

Most commercial laying hen feeding programs are based on level of consumption, production, egg mass output, and age. This may sound simple but it was not too long ago that all producers had to work with was individual phase 1, phase 2, phase 3, and phase 4 diets allocated by age and fed year round regardless of consumption and production level. A modern feeding program may contain close to 100 diets designed for each stage of production with each diet designed for a specific feed intake level. This enables the nutritionist to target specific nutrient intake targets at each stage of production regardless of intake. All of the primary breeder companies now provide nutrient intake targets in their management guides.

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II. AMINO ACIDS AND PROTEIN

Over the past 20 years, most commercial laying hen nutritionists have transitioned away from diets formulated based on dietary crude protein, total methionine, and total lysine to diets based on digestible amino acids formulated in a specific ratio. Therefore, as the genetic potential of the modern egg layer has improved, commercial layer diet amino acid and crude protein intake levels have been steadily decreasing as nutritionists become more comfortable with formulating layer diets based on amino acids rather than crude protein.

The importance of peaking diet amino acid levels on early egg size and egg production is well established and is, without question, one of the most critical factors involved early layer performance. The tendency of some layer producers to use low amino acid density prelay and pre-peak type diets is a major factor in the failure of many flocks to meet production goals for maturity, early egg size, and peak production. This was illustrated in a study reported by Keshavarz and Nakajima (1995) in which early egg weight and egg production were significantly increased in 18-34 week old Babcock B-300 layers by increasing the dietary protein level from 17 to 21% (Table 1). In a subsequent experiment, Keshavarz (1995) again improved early egg size by increasing dietary protein levels and was also successful in increasing egg size by increasing the dietary methionine level from 0.34 to 0.38%. The effect of methionine in increasing early egg size was consistent across three protein levels (17, 19, and 21 %). Using Hyline W-36 layers from 20 to 36 weeks of age, Brake and Peebles (1992) also showed a clear improvement in egg production, egg weight, and feed conversion with increasing crude protein and amino acid levels (Table 2). In this study, the dietary total sulphur amino acid level was kept at 85% of the lysine level in each treatment. Other researchers have also demonstrated that peaking diet protein and amino acid levels will influence early egg size (Penz and Jensen, 1991; Leeson and Caston, 1996). Increasing dietary methionine levels relative to lysine will increase early egg size in amino acid adequate diets and is a commonly used tool to improve early egg size in commercial egg flocks.

In male broiler chicks, Fernandez et al. (1994) reported that the order of amino acid limitation in a corn-soybean meal diet as the crude protein level decreases is methionine, threonine, lysine, valine, arginine, and tryptophan. Therefore the most recent opportunity to significantly decrease the crude protein level of laying hen diets occurred when synthetic threonine became cost effective in 2002-2003. Yakout et al. (unpublished data) demonstrated this potential in a series of experiments conducted with Hyline W-36 layers from 24 to 67 weeks of age. Four dietary crude protein levels were used in each of three phases with each diet formulated to the same minimum lysine, total sulfur amino acid, threonine, and tryptophan level. In treatment A (the highest protein level) only supplemental synthetic methionine was used, in treatment B synthetic methionine and lysine were used, in treatment C synthetic methionine, lysine, and threonine were used, and in treatment D (lowest protein level) synthetic methionine, lysine, threonine, and tryptophan were used to meet the target dietary amino acid levels. The data from these experiments is presented in Table 3 and show that, when both synthetic methionine and lysine are used, dietary crude protein level can be reduced by 1% without adversely impacting egg production and that a further 1% reduction in dietary crude protein can be achieved with the use of synthetic threonine with minimal impact on performance. The lowest crude protein regime used in this experiment (treatment D) utilizing all four synthetic amino acids failed to maintain adequate performance. Egg weight and egg mass output were reduced in treatment C indicating that one of the other essential amino acids may have become deficient as the dietary crude protein was reduced (Table 3).

When supplementary dietary methionine, lysine, and threonine are used, the next most limiting amino acid in layer diets is most likely isoleucine and field trials have shown that, as isoleucine becomes limiting, egg weight is reduced before egg production is impacted (Table 4). In this study, 60,000 Lohmann LSL Lite layers from housing to 60 weeks of age were utilized and synthetic methionine, lysine, and threonine were made available in all diets. Three different isoleucine ratios were used (72, 69, and 66) and all other amino acid dietary minimums were the same in each treatment. As the isoleucine ratio was reduced from 72 to 66 the dietary crude protein level was reduced by 0.5-1.0% during peak and by as much as 1.5% by 43 weeks of age (Table 5). Egg production and hen-housed eggs were not impacted by the isoleucine level; however egg weight and percent large and above were reduced as the isoleucine ratio was reduced (Table 4). This study clearly demonstrates that it is possible to significantly reduce crude protein level and the cost of production when formulating with synthetic methionine, lysine, and threonine and manipulating dietary isoleucine levels. Because attainment of good early egg weight is economically important it is suggested that a high isoleucine ratio be used early in production and that it can be reduced as the birds age and egg weight goals are attained.

The majority of the published research on the amino acid requirement of layers has been conducted from 20 to 40 weeks of age. Therefore, there is very little published information on the amino acid requirement of layers after peak production and peak egg mass output. All of the primary breeder companies recommend phase feeding programs in which the amino acid intake is gradually decreased with decreasing egg mass output. If implemented carefully, with gradual amino acid density reductions, phase feeding of amino acid intake will reduce feed cost, maintain optimal body weight, and control egg weight without hurting production persistency.

Although many layer nutrition programs continue to be implemented utilizing target levels for only crude protein, total sulphur amino acids (TSAA), and total lysine, there is a gradual trend towards the use of digestible amino acids and an ideal amino acid profile, the use of which can both improve the accuracy of formulation and decrease the cost of production. Several published ideal amino acid profiles are presented in Table 6. The most recent of these studies was reported by Bregendahl et al. (2008) utilizing Hyline W-36 layers from 28 to 34 weeks of age. With the exception of a considerably higher ratio for total sulfur amino acids, the amino acid profile recommended by Bregendahl et al. (2008) is quite similar to that proposed by Coon and Zhang (1998). The higher TSAA value proposed by Bregendahl et al. is a function of the small egg bird used in their studies. Layer producers have long since learned that the egg size profile of a flock can be manipulated by varying the TSAA intake between strains and at different ages. As discussed previously with isoleucine, the ratio of certain amino acids can be varied at different ages to meet production goals. For example, a lower ratio of TSAA, isoleucine, and threonine is used for hard-boiled egg flocks (small eggs) compared to the ratio used for breaker egg flocks (large egg) (Figure 1). The goal of most egg producers is to attain target egg size and mass as soon as possible. However, because excessive egg size is associated with poor shell quality and yield, egg producers also work hard to prevent egg size from becoming excessive as the birds age. We are able to accomplish this without hurting production by reducing the TSAA, threonine, and isoleucine ratios relative to lysine in conjunction with reduced linoleic acid levels as the birds age. There is a school of thought that the ideal ratio should not be changed as the birds age and that we should modify the egg size profile by simply reducing the balanced protein level as a whole. However, this technique tends to reduce production as well as egg weight and that modifying the amino acid profile is most effective. Safaa et al. (2008) has reported that egg size was

reduced with no adverse impact on production by reducing TSAA and linoleic acid levels in late first cycle Hyline and Lohmann brown layers.

In addition to utilizing different amino acid ratio profiles between and within strains to optimize egg size and mass depending on the market, there are also considerable differences in the amino acid requirements of white egg and brown egg layers. Bonekamp et al. (2010) reported that, while egg production in white egg layers was maximized at 600 mg/day digestible lysine, production in brown egg layers was not maximized at the highest level of lysine tested which was 800 mg/day. These findings ilustrated that the amino acid requirement for different performance parameters varies, egg weight and egg mass were increased up to the maximum level tested 800 mg/day lysine, indicating that the maximum lysine level for egg weight and egg mass was not reached in this study. In a study designed to determine the ideal ratio for the key amino acids, Bregendahl et al. (2008) estimated that, in Hyline W-36 layers from 28 to 34 weeks of age, the digestible lysine requirement for egg production, egg weight, egg mass, feed efficiency, and body weight was 482, 649, 538, 693, and 489 mg/day.

III. CALCIUM AND PHOSPHORUS

For optimal skeletal development, pullet diets should contain approximately 1.10% calcium and 0.45-0.50% available phosphorus. To ensure optimal medullary bone development in early maturing birds, a prelay diet prelay diet containing 2.50 to 2.75% calcium and 0.45 to 0.50% available phosphorus should be introduced 7 days before light stimulation and used for no more than 7-14 days after which time the first layer feed should be introduced.

At the onset of production, the diets should be formulated to provide 3.90 to 4.10 g/hen/day of calcium and 430 to 470 mg/hen/day of available phosphorus to birds in production. Failure to achieve target calcium and available phosphorus intake goals at the onset of production will result in cage layer fatigue and poor shell quality for the life of the flock. Because the efficiency of calcium utilization decreases and the phosphorus requirement decreases with age, the daily intake of calcium should gradually be increased and the daily intake of phosphorus decreased as the bird ages (Table 7).

Starting with the prelay diet, one half of all supplementary dietary calcium should be supplied in large particle form (2-3 mm). As the bird ages, the proportion of large particle limestone should be gradually increased to approximately 70% of supplementary limestone. This will ensure that, during the night when the birds are depositing the egg shell, calcium will be present in the gastrointestinal tract and available for absorption and egg shell deposition. This leads to better shell quality and improved skeletal integrity. Small particle limestone travels through the gastrointestinal tract in 2 to 3 hours. If no large particle calcium is present in the digestive tract when the bird is forming the eggshell, the calcium is taken from the skeletal system. Over time, this gradually results in a loss of skeletal integrity, poor shell quality, and an increased rate of cage layer fatigue.

There is considerable debate over the correct phosphorus level to feed to both growing and adult birds. Despite widespread knowledge of the problems associated with improper phosphorus nutrition and numerous papers dealing with the phosphorus requirement of laying hens, phosphorus is still one of the most controversial subjects in poultry nutrition. This is evidenced by wide range of available phosphorus levels recommended by nutritionists (from less than 200 to more than 600 mg available phosphorus/hen/day). This debate is due, in part, to a poor understanding of the availability of phosphorus from plant sources, continuing debate on the impact of exogenous phytase on phosphorus availability, and the relationship of phosphorus to shell quality and feed cost. Because phosphorus is expensive and because some

reports have indicated that reducing dietary phosphorus can occasionally improve shell quality, many nutritionists and producers feed marginal levels of dietary phosphorus. This may lead to the slightly increased mortality, increased skeletal problems, and decreased production observed in some flocks, especially as they approach the end of the laying cycle.

The use of phytase in commercial pullet and layer diets is widespread. In North America, the industry standard has been to use 300 FTU/kg of a fungal phytase with a release of 0.09% available phosphorus and 0.09% calcium. At 300 FTU/kg, the fungal phytase will decrease phosphorus excretion by 15-18%. Field trials have shown that the use of 300 FTU/kg phytase has no adverse impact on any performance parameter, including shell quality (Table 8). However, recent reports show that when using *E-Coli* derived phytases 500 FTU/kg phytase will release 0.12% available phosphorus and 0.12% calcium plus small levels of key amino acids and energy. Because of the significant cost savings I have moved all of my feeding programs to 500 FTU/kg *E-Coli* derived phytase.

IV. FAT AND LINOLEIC ACID

The beneficial effect of added fat on early egg size and performance is well established. Using isolaloric diets Jensen (1983) demonstrated that the effect of added dietary fat on egg size is independent of dietary energy level. Subsequent research has shown that linoleic acid is the factor in vegetable oils required for optimal egg size. Manipulation of the dietary linoleic acid content, independent of the dietary energy level, is an effective means of maximizing early egg size. Parsons (1993) increased egg size by supplementing a corn-soy based diet with 2, 4, and 6% added corn oil. Almost all of the increase in egg size occurred with the first 2% addition of corn oil. The diets were not isocaloric but daily calorie intake was similar between treatments. The data indicate that the egg size response with corn oil supplementation was due to increased dietary linoleic acid levels. Using isocaloric peaking diets (11.79 and 12.71 MJ/kg (1277 and 1377 kcal/lb.)) and two added animal-vegetable fat blend (20.6% linoleic acid) levels (0 and 4%), Keshavarz and Nakajima (1995) showed a significant effect of added fat on egg size. Increasing dietary energy without added fat and with added fat had no effect on egg size (Table 1). This clearly shows that the effect of added fat in increasing egg weight is independent of dietary energy level. If dietary energy level during peak was a limiting factor for early egg size increasing the energy level from 11.79 to 12.71 MJ/kg (1277 and 1377 kcal/lb.) would certainly have increased egg size.

The use of non-starch polysaccharide (NSP) enzymes in layer diets worldwide is now common practice. Layer producers are using these products because they improve the digestibility of dietary nutrients, improve performance, decrease variability, decrease nutrient excretion, and decrease feed cost. Early field trials in corn-soy based diets conducted in 2001 and 2002 showed that both Avizyme 1502 (Danisco Corp.) and Rovabio (Adisseo) supported production levels equivalent to the controls indicating that both products successfully liberated energy from the corn and soybean meal. However, in both trials, egg weight was reduced in the enzyme supplemented diets resulting in reduced production of large eggs in younger layers. The egg size reduction was due to reduced dietary linoleic acid levels as a consequence of reduced dietary fat coming into the NSP enzyme containing diets. After our initial trials, we started using an NSP enzyme product with our large egg breeds, but before we started using an NSP enzyme in diets formulated for small egg breeds, we conducted a trial in which we fixed the linoleic acid level of the enzyme diet at the same level as that of the control diet. The results from this trial, conducted with Hyline W-36 layers from housing to 46 weeks of age, are presented in Table 9 and clearly show that imposing a dietary

minimum linoleic acid level to match the linoleic acid level of a non NSP enzyme diet will return egg weight to that achieved in the control treatment.

To maximize early egg size in smaller egg breeds, peaking diets should contain a minimum of 2.00% linoleic acid. In larger egg breeds a minimum of 1.50% linoleic acid is sufficient. Good sources of linoleic acid are corn, animal-vegetable fat blend, vegetable oils, extruded soybeans, and high fat rice bran.

V. SHELL QUALITY

With the incidence of undergrades due to deteriorating shell quality ranging from 2 to 10% as the bird ages, managing for optimum shell quality should be a major part of a pullet and layer farm management program. Shell quality is impacted by numerous factors, including genetics, nutrition, feeder management, body weight and age at light stimulation, heat stress, egg gathering, egg belts, de-escalators, processing equipment, disease, and gut health.

As with all production traits, the various commercially available breeds vary in their ability to consistently produce eggs with good quality shells. The breeder companies recommend breed specific body weights that must be attained prior to light stimulation. This is an important part of pullet management. Light stimulation at an earlier than recommended body weight will bring birds into production underweight and before they are physiologically ready. These birds will remain underweight throughout their productive life and will suffer from excess cage layer fatigue and poor shell quality. Conversely, light stimulation should not be delayed. Stimulation at a heavier than recommended body weight will result in a heavier mature body weight and increased difficulty in controlling egg size, which can lead to shell quality problems.

A well-balanced and properly implemented feeding program is a key factor involved in the development of a sound skeleton and the maintenance of good shell quality. Although nutrition for optimal skeletal development starts at hatch, the most critical period starts at approximately 15 weeks of age. During this period, the bird starts to develop the medullary bone, a source of labile calcium for eggshell production. As the bird starts to develop the medullary bone and reproductive tract, the correct implementation of the prelay diet and first layer feeds is very important for future skeletal integrity and shell quality. A mistake at this point can damage the skeleton for the life of the flock. With the prelay diet, the dietary calcium and amino acid levels are increased to provide for optimal medullary bone and reproductive tract development. Starting with this diet, 50% of all dietary calcium should be supplied in large particle form (2-5 mm). As discussed previously, the prelay diet should be introduced 7 days before light stimulation and used for no more than 7-14 days after which time the first layer feed should be introduced. During lay, each laying hen has a specific daily calcium and available phosphorus intake requirement for maintenance and production. Layer feeding programs should be designed to precisely deliver that requirement at each stage of production and at each level of feed intake. This has been discussed previously in this paper.

a) Addressing Shell Quality Problems

The first thing a nutritionist should do when receiving a shell quality complaint is to verify that the correct diet is being ordered and that that diet is being manufactured correctly. Once this is confirmed, nutritional methods to improve shell quality should be implemented. If the decrease in shell quality is associated with increased cage layer fatigue mortality, a cage layer fatigue treatment should be implemented (Week 1: increase the dietary calcium level by 0.60% and the available phosphorus level by 0.14%, Week 2: increase the dietary calcium

level by 0.30% and the available phosphorus level by 0.07%, Week 3: Normal diets or 0.15% increased calcium and 0.035 increased available phosphorus continuously). If the decrease in shell quality not associated with an increase in mortality, increase the dietary calcium by 0.30% and add additional vitamin D_3 in the feed. Additional vitamin D_3 (2500 IU/liter of drinking water) can also be added to the water for 7 consecutive days and 2 separate days per week thereafter.

Researchers have suggested that the high calcium levels in modern layer feeding programs may adversely impact the utilization of inorganic mineral sources such as manganese and zinc. A number of studies have suggested that amino acid complexed zinc and manganese may improve layer performance and shell quality. In a large scale field trial utilizing 135,000 Isa White layers from 18 to 65 weeks of age Fackler et al.(2002) reported that 40 mg/kg supplemental amino acid complexed zinc improved egg production, mortality, and egg quality compared to that achieved by 66 mg/kg zinc in the form of zinc oxide.

In several trials conducted at the Wenger's Feed Mill, Inc. research farms 40 mg/kg supplemental amino acid complexed zinc and manganese has been shown to improve the shell quality of both first and second cycle layers. Data from one such study conducted with single cycled Hyline W-36 layers from 61 to 81 weeks of age is presented in Table 10 and clearly shows that the supplemental amino acid complexed zinc and manganese decreased percent cracks and improved shell strength.

Research at Auburn University has indicated that *Bacillus* based probiotics may improve shell thickness in commercial egg laying birds (Sohail et al., 2002). A study was designed in which the effect of two *Bacillus* based products, Provalen and Calsporin, on the performance of Hyline W-36 layers from 53 to 68 weeks of age was studied. Provalen, developed by Agtech Products, Inc., is composed of *Bacillus subtilis* and *Bacillus licheniformis* fermentation product. The enzymes produced by Provalen's bacteria have been shown to decrease ammonia and other odors associated with poultry manure and field observations have indicated that this product may also improve feed efficiency. Calsporin, developed by QTI of Japan, is composed of *Bacillus subtilis* C-3102 fermentation product. Research at the University of Arkansas has shown that Calsporin will improve broiler performance and reduce the incidence of necrotic enteritis in the absence of antibiotics (Fritts et al., 2000). The results from the study are presented in Table 11 and show that both both products improved shell strength and percent cracked eggs compared to the control. The shell strength improvement compared to the control was greatest in the Calsporin fed birds. QTI has suggested that their *Bacillus* promotes osteoclast activity which could help long term skeletal integrity and shell quality. They also have data showing that *Bacillus* fed birds have a thicker film of lactobacillus on the villus suggesting that their *Bacillus* also promotes a healthier gut environment. Egg production, egg weight, hen-housed eggs, haugh units, and mortality were not impacted by dietary treatment. Feed intake was reduced in the Provalen fed group compared to the control and Calsporin fed birds resulting in slightly improved feed efficiency in the Provalen fed birds compared to the other two treatments. Calsporin had no impact on feed intake or feed efficiency compared to the control. This research suggests that *Bacillus* based probiotics may be useful in improving shell quality in commercial egg laying flocks as they age.

VI. DISTILLERS DRIED GRAINS WITH SOLUBLES

With the exception of phytase and NSP enzymes no ingredient in North America has stimulated more discussion over the past 10 years than distillers dried grains with solubles (DDGS). More than 50% of the layer formulations in the USA now contain DDGS with the

inclusion rates varying from 5-20%. The wide range in usage level is a function of quality and variability concerns, formulation technique (total amino acids or digestible amino acids), and widely varying recommended usage levels. Several researchers have seen no negative response with dietary DDGS levels up to 15% of the diet (Roberson et al., 2005, Lumpkins et al., 2005) while others have suggested that 25% and 32% DDGS can be fed without a negative impact on layer performance (Masa'deh et al., 2011 and Loar *et al.*, 2010 respectively). In their study suggesting that 32% DDGS could be fed to layers without a negative response compared to an un-supplemented control Loar et al. (2010) reported that maximum performance was obtained at 16% DDGS (Table 12). More typically layer performance tends to drop as DDGS levels exceed 12% with the optimal level on a cost of production basis being around 15% DDGS. In a recent field trial utilizing 265,500 Lohmann LSL Lite layers from 18-69 weeks of age housed in an individual layer house with a split feeding system the impact of 15% DDGS from 18-35 weeks followed by 20% DDGS from 36-69 weeks was compared with a program utilizing 12% DDGS from 18-35 weeks followed by 15% DDGS from 36-69 weeks of age. Some of the data from this trial is presented in Table 13 and clearly show that hen-housed eggs and overall egg mass were decreased in the high DDGS group. Feed intake was increased in the high DDGS group suggesting that the energy value of the DDGS may be overestimated. The lower average cost per ton in the high DDGS group was not able to overcome the impacted of reduced egg numbers and elevated feed intake. Clearly there are some anti-nutritional factors in DDGS that we are not fully able to compensate for with formulation. These can be mitigated by formulating on a digestible amino acid basis, making synthetic methionine, lysine, threonine, and tryptophan available, and by utilizing services such as Evonik's Amino Red service on the NIR to evaluate amino acid digestibility. Energy content should be frequently recalculated to adjust for varying protein, fat, and fiber levels.

VII. CONCLUSION

This is an exciting time to be involved with laying hen nutrition. Genetic potential improves with each generation with earlier maturity and improved production persistency, all with smaller body weight and reduced feed intake capacity. The ever increasing cost of feed ingredients has made us more acceptable of alternate feed ingredients and additives. Available to us are various enymes, pro and pre biotics, organic minerals, and an assortment of synthetic amino acids. Universities and suppliers continue to help us advance nutrition knowledge and provide us with tools to properly evaluate ingredients. It is our job as commercial nutritionists to put all of this knowledge together and deliver a nutrition package that best optimizes the cost of production.

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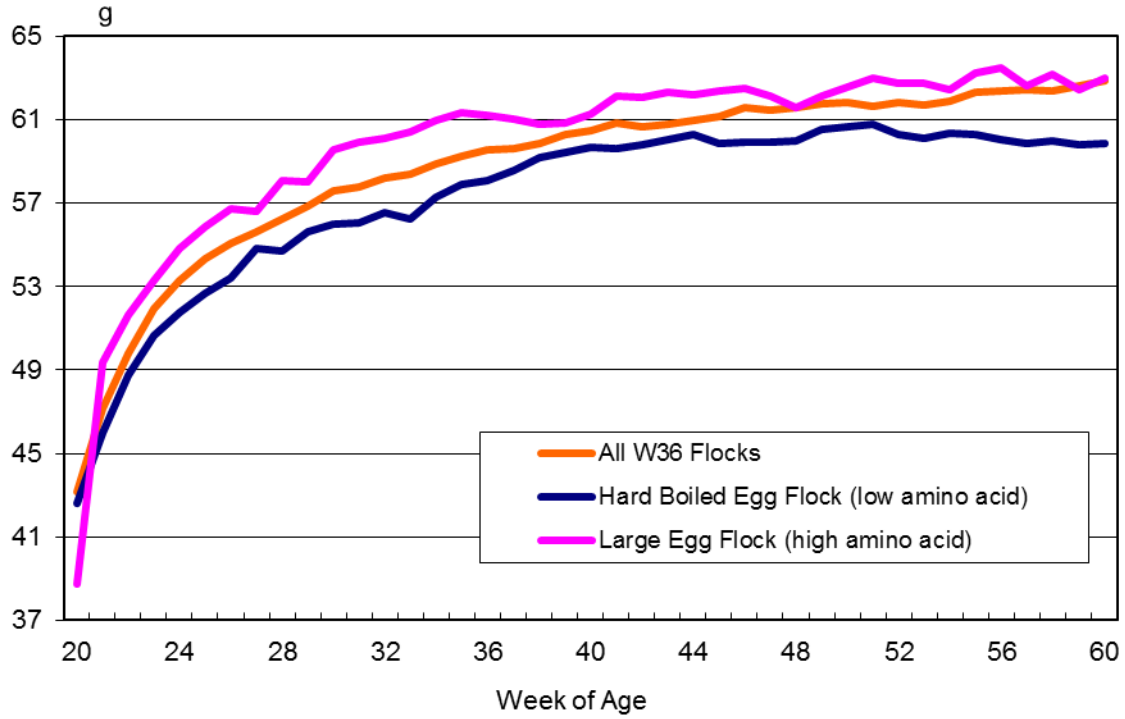

Figure 1. Impact of amino acid level on the egg weight of Hyline W36 layers from 20 to 60 weeks of age

Table 1. Effect of dietary regime on the performance of Babcock B-300 layers from 18 to 34 weeks of age

Treatment	Body weight		Egg production	Egg weight	Large + XL
	18 wk	34 wk			
	g		%	g	%
Energy constant, 2816 kcal/kg					
Protein effect					
17%	1,243	1,661	73.4[b]	50.9[b]	23.2[b]
21%	1,243	1,663	76.1[a]	51.9[a]	29.7[a]
Fat effect					
0%	1,244	1,636	75.6[a]	51.0[b]	24.0[b]
4%	1,241	1,689	73.8[b]	51.8[a]	28.8[a]
Fat constant, 4%					
Protein effect					
17%	1,242	1,682	73.6[b]	51.2[b]	23.9[b]
21%	1,241	1,702	76.2[a]	51.9[a]	29.9[a]
Energy effect					
2816 kcal/kg	1,241	1,689	73.8[b]	51.8	28.8[a]
3036 kcal/kg	1,241	1,695	75.9[a]	51.3	25.0[b]

[a,b]Means within a column not sharing a common superscript differ (P<0.05)
Keshavarz and Nakajima, 1995

Table 2. Effect of dietary crude protein and amino acid content on the performance of Hyline W-36 layers from 21 to 37 weeks of age

Dietary content[1]			Ave Egg Prod.	Egg weight	Large eggs	Ave. feed Intake	Feed conversion
Protein	Lysine	TSAA					
	%		%	g	%	g/day	g:g egg
14.94	0.775	0.660	79.4[a]	56.7[a]	69.5[a]	99.7	2.22[c]
14.12	0.725	0.620	78.9[ab]	56.1[b]	66.3[b]	99.8	2.25[b]
13.44	0.675	0.577	77.8[b]	55.6[c]	62.2[c]	99.7	2.30[a]

[a,c]Means within a column not sharing a common superscript differ (P<0.05)
[1]Basal diet containe 2866 kcal/kg (1300 kcal/lb), 3.90% calcium and 0.39% available phosphorus
Brake and Peebles, 1992

Table 3. Influence of dietary crude protein level and synthetic amino acid supplementation on the performance of Hyline W-36 layers from 24 to 67 weeks of age

Experiment [1]	24 to 50 weeks of age			
Treatment[1]	BW gain g/bird/period	Feed Intake g/day	FCR g/g	Egg Prod. %
A. Met	178.4	89.64	1.760	86.89
B. Met, Lys	141.32	89.30	1.779	87.66
C. Met, Lys, Thr	156.65	88.92	1.797	85.67
D. Met, Lys, Thr, Trp	-34.29	84.68	1.937	79.37
Experiment 2		55 to 67 weeks of age		
Treatment				
A. Met	6.67	98.96	2.21	70.87
B. Met, Lys	30.06	99.54	2.25	71.43
C. Met, Lys, Thr	8.03	99.13	2.25	70.22
D. Met, Lys, Thr, Trp	-13.64	97.86	2.22	67.13

[1]Values are an average of phase 1 and 2. Phase 1 crude protein levels: A=19%, B=17%, C=15%, D=13%. Phase 2 crude protein levels: A=18%, B=16%, C=15%, D=13%
Yakout *et al*, 2007

Table 4. Influence of isoleucine to lysine ratio and dietary protein level on the performance of Lohmann LSL Lite layers from housing to 60 weeks of age

Treatment	Ave Egg Prod.	Ave. Egg Wt	Large & Above	HH Eggs	Ave. Feed Intake	60 wk. BW	Total Mortality
	%	g	%		g/day	g	%
Normal feeding program, Ile=72[1]	90.47	59.48	77.2	256	106.68	1683	2.62
Treatment 1 with Trp=17, Ile=69[2]	90.05	59.28	76.8	255	105.32	1701	2.90
Treatment 1 with Trp=17, Ile=66[3]	90.07	59.22	76.5	255	105.82	1683	2.80

[1]Amino acid to lysine ratios in control diet are: Arg 116, Met 40, M+C 76, Trp 19, Thr 67, Ile 72
[2]Treatment 2 amino acid to lysine ratios are: Arg 116, Met 40, M+C 76, Trp 17, Thr 67, Ile 69
[3]Treatment 3 amino acid to lysine ratios are: Arg 116, Met 40, M+C 76, Trp 17, Thr 67, Ile 66
Research conducted at the Wenger's Feed Mill, Inc. research farm, 2007

Table 5. Isoleucine trial crude protein levels

Diet	Age Fed	Treatment		
		1	2	3
	weeks		---------- % ----------	
118	18-21	18.99	18.50	18.33
119	22-23	19.05	18.56	18.37
120	24-26	18.36	17.90	17.63
121	27	17.76	17.28	17.04
122	28-36	16.89	16.43	16.29
122A	37-42	16.89	16.07	16.07
122A	43-53	17.05	16.09	15.53
123A	54-57	15.94	15.04	14.51
223	58-60	15.75	14.56	14.15

Research conducted at the Wenger's Feed Mill, Inc. research farm, 2007

Table 6. Published Ideal Amino Acid Profiles For Laying Hens

	NRC 1994	CVB 1996	Coon & Zhang 1998	Rostango 2005	Bregendahl *et al.* 2008
Lysine Requirement, mg/day	690	700	676		538
Lysine	100	100	100	100	100
Methionine	43	50	49	50	47
Met+Cys	84	93	81	91	94
Tryptophan	23	19	20	23	22
Threonine	68	66	73	66	77
Isoleucine	94	79	86	83	79
Arginine	101	-	130	100	-
Valine	101	86	102	-	93

Table 7. Sample layer feeding programs

White egg layers					
		Level of production			
Nutrient	Units	Peak & > 90%	90-85%	85-80%	80-75%
Metabolizable Energy	Kcal/day	285	285	285	285
Crude Protein	g/day	17.20	16.60	16.00	15.60
Calcium	g/day	4.00	4.20	4.35	4.45
Avail. Phosphorus	mg/day	450	440	410	380
Methionine, Total	mg/day	415	375	340	304
Meth. + Cystine, Total	mg/day	730	665	625	590
Lysine, Total	mg/day	890	850	830	800
Linoleic Acid	g/day	1.50	1.45	1.40	1.35
Brown egg layers					
		Level of production			
Nutrient	Units	Peak & > 90%	90-85%	85-80%	80-75%
Metabolizable Energy	Kcal/day	315	315	315	315
Crude Protein	g/day	17.70	17.10	16.50	16.10
Calcium	g/day	4.30	4.50	4.60	4.70
Avail. Phosphorus	mg/day	460	430	410	395
Methionine, Total	mg/day	425	400	370	345
Meth. + Cystine, Total	mg/day	750	720	680	660
Lysine, Total	mg/day	925	910	880	860
Linoleic Acid	g/day	1.50	1.45	1.40	1.35

Table 8. Influence of supplementary dietary phytase and phosphorus on the performance of Hyline W-98 layers from 17 to 61 weeks of age[1]

Treatment	Ave Egg Prod.	Ave Egg Wt.	HH Eggs	Feed intake	Total mortality	Feed conv.	Cracked Eggs
	%	g		g/day	%	kg/doz	%
Control[2]	85.1	60.6	247	95.7	2.9	1.33	4.15
Control with no phytase	85.2	60.7	248	96.0	2.5	1.33	4.36
Control plus 50 mg AP/day[3]	85.3	60.7	248	96.7	2.5	1.34	4.70

[1]Performance numbers are from 20 to 61 weeks of age
[2]Normal diet contained 300 FTU/kg phytase from a fungal source
[3]Daily available phosphorus intake increased by 50 mg per day
Research conducted at the Wenger's Feed Mill, Inc. research farm, 2001

Table 9. Influence of Avizyme 1502 on the performance of Hyline W-36 layers from 17 to 46 weeks of age[1]

Treatment	Ave Egg Prod.	Ave Egg Wt.	HH Eggs	Feed intake	Total mortality	Age at 60.5 g egg wt	Cracked Eggs
	%	g		g/day	%	wk	%
Control	91.10	56.4	160	93.6	0.61	38	3.74
Control with AV-1502 + LA Min[2]	90.91	56.4	160	94.2	0.62	38	3.65
Control with AV-1502[3]	91.28	56.3	161	95.8	0.57	41	3.87

[1]Performance numbers are for the test period only
[2]Normal layer diet with Avizyme 1502 and Linoleic Acid level same as treatment 1
[3]Normal layer diet with Avizyme 1502 and no Linoleic Acid minimum
Research conducted at the Wenger's Feed Mill, Inc. research farm, 2005

Table 10. Influence of organic zinc and manganese on the Performance of Hyline W-36 layers from 61 to 81 weeks of age[1]

Treatment	Ave Egg Prod.	HH Eggs	Feed intake	Total mortality	Feed conv.	Cracked eggs	Shell strength
	%		g/day	%	kg/doz	%	g/force
Normal diet	75.3	96	97.1	1.71	1.55	6.96	3,122
Normal diet plus Zn and Mn[2]	75.4	97	95.7	1.91	1.52	6.41	3,219

[1]Performance numbers are for the test period only
[2]Normal diet plus 40 PPM Zinc and Manganese from Availa-Z/M
Research conducted at the Wenger's Feed Mill, Inc. research farm, 2005

Table 11. Influence of bacillus based probiotics on the performance of Hyline W-36 layers from 53 to 68 weeks of age[1]

Treatment	Ave Egg Prod.	HH Eggs	Feed intake	Total mortality	Feed conv.	Cracked eggs	Shell strength
	%		g/day	%	kg/doz	%	g/force
Control	84.11	87	99.7	0.91	1.42	6.58	3,332
Control plus Provalen[2]	83.84	87	98.6	0.67	1.41	6.35	3,417
Control plus Calsporin[3]	84.07	87	99.7	0.76	1.42	6.04	3,405

[1]Performance numbers are for the test period only
[2]Normal program plus 1.0 lb/ton Provalen
[3]Normal program plus 0.5 lb/ton Calsporin
Research conducted at the Wenger's Feed Mill, Inc. research farm, 2005

Table 12. Live production characteristics of Bovans White commercial egg layers fed various levels of distillers dried grains with solubles from 72 to 86 weeks of age

Treatment	Ave Egg Prod.	Feed/doz	Feed intake	Egg weight	Total Mortality
	%	kg/doz	g/day	g	%
DDGS, %					
0	87[b]	1.63[ab]	114	71.4	1.8
8	85[b]	1.72[a]	113	70.4	2.1
16	93[a]	1.55[b]	115	71.4	0.4
24	85[b]	1.72[a]	114	69.2	0.0
32	89[ab]	1.58[b]	113	69.4	2.6
SEM	2	0.04	0.002	0.8	1.2
Anova, P-value					
DDGS	0.04	0.01	0.84	0.22	0.46
Week	0.0001	0.0001	0.0001	0.0001	0.0001
DDGS X week	0.61	0.99	0.005	0.27	0.95

[a,b]Means within a column not sharing a common superscript differ (P<0.05)
Loar *et al.*, 2010

Table 13. Impact of dietary DDGS level on the performance of Lohmann LSL Lite layers from 18 to 69 weeks of age[1]

Treatment	Ave Egg Prod.	Ave. Egg Wt	HH Eggs	Ave. Feed Intake	Feed Efficiency		Egg Mass
	%	g		g/d	kg/doz	kg/kg	kg egg/HH
Control[2]	90.73	59.14	307	100.9	1.34	1.88	18.29
Control + High DDGS	89.07	59.31	301	102.3	1.38	1.92	18.01

[1]Performance numbers are for the test period only
[2]Control diet contained 12.0% DDGS to 35 wks and 15.0% thereafter. Test diet contained 15.0% DDGS to 35 weeks and 20.0% thereafter
Research conducted at Rembrandt Enterprises 2010-2011

CASE REPORT: ADVERSE REACTIONS FOLLOWING PARENTERAL ADMINISTRATION OF LIVE ATTENUATED SALMONELLA VACCINE IN A STRAIN OF LAYER CHICKENS.

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During an experiment using different *Salmonella* vaccines in a Rhode Island layer strain, an unexpected adverse reaction to the administration of a live *Aro-A* deletion mutant *Salmonella* Typhimurium vaccine when given by sub-cutaneous (s/c) injection of 10^8 cfu was observed. All birds were free of *Salmonella* antibody prior to vaccination. All birds (344) that received the live attenuated vaccine by s/c injection diluted in sterile phosphate buffered saline at 6 weeks of age were affected. The current label dose for this vaccine is 1 $\log_{10}$ lower and the use of this vaccine by the s/c route is currently off-label. Within 1½ hours, all birds so treated exhibited depression, somnolence and reluctance to move. Sister birds that remained unvaccinated (130) or received an inactivated *Salmonella* vaccine (130) by intramuscular injection at the same time showed no such reaction. At 4 hours post vaccination, some affected birds were moving and beginning to eat. By about 15 hours, birds were largely improved and by 24 hours they appeared to be completely recovered. No birds died. Titration of the vaccine used showed the birds received approximately 2.5 x 10^8 colony forming units per bird which was near the intended application.

Under the trial protocol, a second vaccination was intended for 12 weeks of age. Following guidance from Birling Animal Ethics Committee which supervised the work, five birds only received this administration (s/c) and were observed closely for 2 hours. Two of the five again exhibited adverse signs, similar to the original administration. This treatment was abandoned in this experiment. Body temperatures were monitored over this time but no febrile reaction was detected in vaccinated or unvaccinated birds.

This dose rate of the same batch and method of administration (s/c) had been used previously in another strain of layer chicken (Groves and Sharpe, 2010). Hence we suspect that there may be a difference in bird strain reaction to live attenuated *Salmonella* vaccine administered by subcutaneous injection. The cause of the reaction is not known but has been suggested to involve bacterial endotoxin release (C. Jackson, pers. comm.). Endotoxins are the lipid part of the lipopolysaccharide complex associated with the cell walls of Gram-negative bacteria (Harper et al., 2011). Endotoxins have been suggested to be involved in elicitation of acute phase responses in chickens (Xie et al., 2000) and clinical responses to endotoxin may include reactions including fever, diarrhoea, prostration, shock or death (Todar, 2011). Birds observed during the second vaccination exhibited neither a rise in body temperature nor diarrhoea. Animals have been noted to vary in their susceptibility to endotoxin. Another possibility for the unexpected occurrence may involve undetected exposure to nonspecific endotoxins in previous studies, as these can be antigenic (Todar, 2011). Any Gram-negative bacteria may release minute amounts of endotoxin during their growth and thus could have exposed previously studied birds to endotoxin and allowed them to develop some degree of immunity. This may have prevented occurrence of the signs previously. Arguing against this possibility however is the re-occurrence of the signs in a substantial proportion of the birds during the second vaccine administration in this experiment. Further work is needed to understand this reaction.

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RECOVERY OF ENTEROBACTERIACEAE FROM SHELL SURFACE AND SHELL IN EARLY MID AND LATE LAY

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Summary

Microbial populations from samples of six pooled eggs were enumerated for Enterobacteriaceae on violet red bile glucose agar (Oxoid, Australia) plates with overlay (purple-red colonies). Presumptive colonies were counted and reported as log cfu/mL of egg rinsate. Translucency tended to be higher in late lay flocks. The Enterobacteriaceae count on the egg shell surface was slightly higher in late lay flocks but was not significantly different from that of the flocks in early lay. *Salmonella* Infantis was isolated from egg shell rinse. Samples were collected throughout the year 2010-2011. This study is on-going so, as the sample size increases, new insights will be obtained.

I. INTRODUCTION

The egg contents are an ideal growth medium for microorganisms which are hazardous to humans. It has been observed that the microflora of the eggshell is dominated by Gram-positive bacteria, whereas Gram-negative bacteria are best equipped to overcome the antimicrobial defences of the egg content (De Reu et al., 2008). The egg industry in Australia is periodically implicated in cases of food poisoning. Cage laying systems are the major source of whole shell eggs for the supermarkets in Australia. It is possible that egg shell translucency may increase the incidence of bacterial penetration (Chousalkar et al., 2010). The Australian poultry industry is considered free from *Salmonella* Enteritidis which is of major concern to the food industry all over the world. *S*. Infantis outbreaks have not been recorded recently in Australia by OzFoodNet during the years 2004–6, (OzFoodNet Working Group, 2005, 2006, 2007). However, *S*. Infantis was consistently a common serotype in human notifications in South Australia between 2005 and 2008; and a common serotype in two other Australian states in 2004 and 2006 (OzFoodNet Working Group, 2005, 2006, 2007, 2009). More recently, *Salmonella* Typhimurium (ST) 108, 9, 44 outbreaks have been reported (OzFoodNet Working Group, 2009) Cox et al. (2002) reported that *Salmonella* Infantis was the predominant *Salmonella* serovar in the Australian egg industry. Studies on microbial contamination of egg shells have been reported (Musgrove et al., 2004; Musgrove et al., 2005). However, small defects in the egg shell may provide means for the predominant bacterial spp. on the egg shell to penetrate and move into the egg contents (De Reu, et al., 2006). Abnormalities in egg shells (thin shells, increased shell pore numbers) can potentiate the entry of food borne pathogens into the eggs (De Reu et al., 2008). It has also been found that bacterial contamination of air cells, shells, and egg contents is more common in eggs from older hens than from younger hens (Jones et al., 2004). In the present study, visibly clean eggs collected from commercial egg farms from hens at various stages of lay were tested for the presence of *Salmonella* spp. The Enterobacteriaceae populations on the egg shell surface, in the egg shell pores and egg internal contents were also monitored.

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II. MATERIALS AND METHODS

Visually clean eggs (n=1200), collected from the cage front from various farms, were processed for total Enterobacteriaceae from egg shell surface and egg shell crush. The farms included in this study had either Hyline or Isa Brown laying hens. Eggs were candled to ensure they were intact eggs without cracks then whole eggs were placed in Whirl-Pak bags with sterile PBS at room temperature. To recover bacteria from egg shell pores, eggs were then washed in 70% alcohol at room temperature to kill bacteria on the egg shell surface and half of the egg shell was crushed in the Whirl-Pak bag containing sterile PBS. Inoculated PBS (egg shell surface + egg shell crush) was pour-plated on violet red bile glucose agar plate and McConkey agar plate for enumeration of Enterobacteriaceae. The inoculated PBS (with egg shell crush + egg shell surface) and egg contents samples were also inoculated into buffered peptone water (1:4) and incubated overnight. 100 µL of this sample was inoculated into Rappaport Vasidalis (RV) broth (Oxoid) which was then incubated at 42°C overnight. The samples were streaked on brillant green agar (Oxoid) and bismuth sulphite agar (Oxoid). The suspected colonies were inoculated on Triple Sugar Iron agar (TSI) slants (Oxoid). The suspected Salmonella cultures were sent to the Salmonella reference centre, Adelaide, SA. All the isolates were stored in glycerol stock. Half of the egg shell was washed, air dried and preserved for later shell ultrastructure work.

III. RESULTS

The Enterobacteriaceae count on the egg shell surface did not vary significantly across early, mid and late laying phases (Table 1). However, it tended to be higher on the egg shells collected from the flocks during late lay. The Enterobacteriaceae count in the egg shell pores did not vary significantly during early, mid or late laying phase (Table 1). However, again, it tended to be higher in the egg shell pores of the eggs collected from flocks during late lay. The *E. coli* count on the egg shell surface did not vary significantly during early, mid or late laying phase. However it tended to be higher on the egg shells collected from the flocks during late lay. The *E. coli* count in the egg shell pores did not vary significantly during early, mid or late laying phase. Eleven *Salmonella* Infantis and two *Salmonella* sub 1, ser 4,12:d isolates were isolated from egg shell rinse of eggs collected from the flocks in early, mid or late lay.

Table 1. The egg total bacterial (TBC) and Enterobacteriaceae count (TEC) recovered from egg shell wash and egg shell pores from eggs obtained at various stages of lay

Phase	Early lay (n=420)	Mid lay (n=240)	Late lay (n=540)	P value
TBC on egg shell (log CFU)	2.9 ± 0.13	3.1 ± 0.12	3.4 ± 0.08	0.002
TBC on egg shell crush/pores (log CFU)	0.40 ± 0.11	0.48 ± 0.14	0.48 ± 0.13	NS
TEC on egg shell (log CFU)	1.6 ± 0.13	1.5 ± 0.18	1.8 ± 0.17	NS
TEC on egg shell crush/pores (log CFU)	0.20 ± 0.07	0.29 ± 0.13	0.24 ±0.01	NS

1. Early lay – Up to 40 weeks of age
2. Mid lay- Up to 55 weeks of age
3. Late lay- More than 55 weeks of age

IV. DISCUSSION

In relation to bacterial contamination of eggs, in Australia and most European countries, there is some debate about the benefits of washing eggs. Previous research suggests that washing removes faecal material and reduces microbial load on the egg shell surface which could ultimately reduce the likelihood of horizontal transmission occurring as well as reducing the potential for cross contamination during food handling/preparation. However, research has also shown that wet washing can damage the cuticle layer (which prevents the entry of bacteria across the egg shell), thereby leaving pores exposed and potentiating bacterial penetration (Sparks and Burges, 1993). Egg washing is widely used in many countries including Australia (Hutchison et al., 2004). The Australian egg industry has often been implicated in *Salmonella* food poisoning outbreaks although minimal formal research has been conducted to study the prevalence of *Salmonella* in table eggs. The findings from the present study differs with the earlier finding by Daughtry et al. (2005) who undertook a microbiological survey of commercial eggs in Australia to determine the prevalence of *Salmonella* contamination. During Daughtry's study, *Salmonella* spp. were not isolated from the internal contents of 20,000 eggs sampled. In the present study, *Salmonella* spp were not detected in any of the egg internal contents. Our finding regarding the presence of *S*. Infantis in layer flocks is in agreement with Cox et al. (2002) however the genetic diversity of these Salmonella isolates needs further investigation. Also further extensive survey work is essential. In the present study, shell rinse and crush methods were used to recover Enterobacteriaceae from commercial shell eggs as described earlier by Musgrove et al. (2004), however these isolates have not been characterised. Further identification and characterization of Enterobacteriaceae is in progress. Our findings regarding no significant differences between the Enterobacteriaceae count on the egg shell surface during early, mid and late lay is in agreement with Protais et al. (2003) and De Reu et al. (2005) who found no effect of age of the hens on bacterial eggshell contamination.

It has been demonstrated that the extent of cuticle deposition can influence the egg shell penetration of *Salmonella* Enteritidis at 20°C (De Reu et al., 2006). However it is not clear whether the extent of cuticle deposition can also influence egg shell penetration by Enterobacteriaceae including *S.* Infantis at various temperatures. Such studies are in progress.

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COMPARISON OF OMEGA-3 LEVELS IN TWO STRAINS OF BROILERS AND LAYERS FED HIGH ALPHA LINOLENIC ACID DIETS

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Summary

Long chain omega-3 (n-3) polyunsaturated fatty acids (n-3 LCPUFA) have numerous health benefits. Previously, we have demonstrated that increasing the levels of alpha-linolenic acid (ALA) while controlling linoleic acid in the diet promotes increased n-3 LCPUFA levels in chicken meat. In the current study, we performed a series of experiments to evaluate the capacity for high ALA diets to increase n-3 LCPUFA in meat and eggs. Importantly, we compared n-3 accumulation in two strains of layers and two strains of broilers.

I. INTRODUCTION

Adequate consumption of long chain omega-3 polyunsaturated fatty acids (n-3 LCPUFA) is important for humans in order to reduce the risk of cardiovascular disease and other inflammatory disorders. Most Australians do not consume sufficient n-3 LCPUFA and as such, additional dietary sources of n-3 LCPUFA are required. As chicken meat is the most consumed meat in Australia, it is an attractive candidate for increasing n-3 LCPUFA consumption.

There are numerous reports of n-3 enrichment of poultry via dietary fish oil (Lopez-Ferrer et al., 2001; Bou et al., 2005), however, these are often associated with reduced sensory properties. Enrichment with vegetable oils that are high in the n-3 fatty acid, α-linolenic acid (ALA), has also been investigated (Rymer and Givens, 2006), as ALA can be converted into n-3 LCPUFA by the chicken. Previously, we have demonstrated significant improvements in n-3 LCPUFA enrichment of chicken meat by use of high ALA/low linoleic (LA) diets (Kartikasari et al., 2009). In further studies, we demonstrated that increasing levels of LA in the diet can reduce conversion of ALA into n-3 LCPUFA (Kartikasari et al., 2010).

In the current study, we continue our research and compare n-3 LCPUFA accumulation in Cobb 500 and Ross 308 birds. We also assessed the impact of high ALA diets on bird performance, and importantly, performed sensory analysis to identify any impact on the quality of the chicken meat. In addition, we performed a preliminary study comparing two strains of layer hens to determine the impact of high ALA diets on egg fatty acid composition, performance and sensory properties of the egg.

II. MATERIALS AND METHODS

a) Broiler study

Two hundred and forty male birds (120 x Cobb 500 and 120 x Ross 308) were allocated to three dietary treatments (n=4 pens per diet x strain). Blended vegetable oils (60 g/kg) were added to a common wheat-based basal diet to produce three experimental diets with

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increasing levels of ALA. The low ALA diet contained 1.2 g/kg ALA and 11.2 g/kg LA, the mid ALA diet contained 14.7 g/kg ALA and 21.2 g/kg LA, and the high ALA diet contained 28.5 g/kg ALA and 21.3 g/kg LA. Birds were fed *ad libitum* from day of hatch until day 40. Body weight and feed intake were recorded weekly and FCR calculated. At the completion of the trial, three birds from each pen were killed and samples of breast collected for fatty acid analysis. A further three birds per pen were processed at a registered abattoir and samples collected for sensory analysis. Performance and fatty acid data were compared by ANOVA.

b) Layer study

Forty eight laying hens (24 Hy-Line white and 24 Hy-Line brown) were allocated to three dietary treatments. Blended vegetable oils (60 g/kg) were added to a common basal diet to produce three experimental diets with increasing levels of ALA. The low ALA diet contained 1.5 g/kg ALA and 11 g/kg LA, the mid ALA diet contained 15.7 g/kg ALA and 21.3 g/kg LA, and the high ALA diet contained 29.9 g/kg ALA and 21.4 g/kg LA. Birds were placed at point of lay and fed *ad libitum* for 12 weeks. Eggs were collected prior to the commencement of the trial, at 4 weeks and at 12 weeks. Birds were weighed at the beginning and end of the trial and feed was recorded weekly. FCR was calculated at the end of the trial. Eggs were collected during the final week of the trial for sensory analysis. Performance and fatty acid data were compared by ANOVA.

c) Sensory analysis

Sensory analysis was performed on breast meat and boiled and scrambled eggs. Panellists assessed a number of parameters based on flavour, aroma, taste and overall acceptance using a validated scale system ranging from 0-15. Data were compared by ANOVA.

III. RESULTS

a) Broiler study

At day 40, Cobb 500 birds were significantly heavier than Ross 308 birds ($P < 0.05$; Table 1). There was a diet x strain interaction that was approaching significance ($P = 0.06$), in which the body weight gain of Ross 308 birds appeared to decrease as ALA levels in the diet increased. FCR was not significantly different between strains, and it was not influenced by diet ($P > 0.05$).

Table 1. Performance measurement in broiler experiment

	Low ALA		Mid ALA		High ALA		SEM	P value		
	Cobb	Ross	Cobb	Ross	Cobb	Ross		D	S	D x S
BWG	2782	2674	2890	2526	2750	2436	52	*	****	0.06
FCR	1.592	1.644	1.683	1.610	1.675	1.667	0.028	NS	NS	NS

Values are least squares means. * P <0.05. **** P <0.0001.

Cobb 500 birds had a higher breast tissue fat level than Ross 308 birds when fed the high ALA diet, resulting in a significant diet x strain interaction (data not shown). The proportions of total n-3 PUFA were increased by high ALA diets (Table 2). There were significant diet x strain interactions for 18:3n-3 and 20:5n-3 where Cobb 500 birds appeared to have higher levels than Ross 308 birds as ALA levels in the diet increased. No strain effects were observed for 22:5n-3 and 22:6n-3.

Table 2. Fatty acid levels (mg/100g of meat) in total lipid fraction of breast meat

	Low ALA		Mid ALA		High ALA		SEM	P value		
	Cobb	Ross	Cobb	Ross	Cobb	Ross		D	S	D x S
Sats	292.97	233.55	345.67	304.47	347.20	204.71	23.86	*	***	NS
Monos	761.41	550.09	683.41	565.65	544.12	243.35	61.20	***	***	NS
18:2n-6	125.12^{c}	90.45^{c}	251.34^{a}	209.44^{ab}	254.59^{a}	129.80^{bc}	19.56	***	***	*
20:4n-6	19.55	18.44	16.57	17.18	9.69	10.18	0.847	***	NS	NS
Total n-6	159.00	121.66	278.29	237.03	272.32	146.46	19.96	***	***	NS
18:3n-3	9.33^{c}	5.76^{c}	141.76^{b}	109.45^{b}	285.22^{a}	113.62^{b}	19.73	***	***	***
20:5n-3	2.37^{d}	2.22^{d}	9.34^{bc}	7.88^{c}	13.59^{a}	9.95^{b}	0.493	***	***	**
22:5n-3	5.71	5.50	23.76	24.17	24.75	25.01	1.305	***	NS	NS
22:6n-3	5.99	5.18	11.81	13.29	9.04	9.90	0.700	***	NS	NS
Total n-3	24.14^{c}	19.26^{c}	189.65^{b}	157.54^{b}	337.85^{a}	162.47^{b}	20.69	***	***	***

Sats, saturated fatty acids. Monos, monounsaturated fatty acids. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Values are least squares means.

For the sensory properties of breast meat, lower ALA diets had a more pleasant aroma; however the high ALA diet was not considered unpleasant. Off flavours were significantly greater in the high ALA diet; but still received relatively low scores out of 15. Strain effects were observed for juiciness and after taste. Strain x diet interactions were observed for metallic taste, although the scores were relatively low in all treatments, and tenderness. Overall, the sensory properties of the meat were comparable to that of a standard commercial bird (data not shown).

b) Layer study

There was no significant effect of diet or strain on performance measurements including body weight gain, egg weight, egg production and FCR ($P > 0.05$; data not shown). The high ALA diet significantly increased the relative proportions of 18:3n-3, 20:5n-3 and 22:5n-3 compared to the mid ALA diet, which in turn was significantly higher than the low ALA diet (data not shown). Interestingly there was a strain effect for 22:5n-3 where Hy-Line brown birds had higher proportions compared to Hy-Line white. There was a significant diet x strain interaction for 22:6n-3 where Hy-Line brown birds fed the mid ALA diet had a larger proportion of 22:6n-3 compared to Hy-Line white birds fed the mid ALA, and either strain fed the high ALA diet.

When scrambled eggs were assessed, Hy-Line brown eggs received higher scores for egg aroma, butter flavour and sulphur flavour (data not shown). The high ALA diet led to higher scores for sulphur aroma and sulphur flavour. For boiled eggs, no diet effects were observed. Strain effects were observed for after taste, with Hy-Line brown birds receiving a higher score, and sulphur flavour, with Hy-Line white birds receiving a higher score. Diet x strain interactions were observed for butter aroma, off odour and aroma preference.

IV. DISCUSSION

The results of the current study demonstrate successful incorporation of n-3 LCPUFA into the breast meat of broiler chickens and eggs of layer hens as a result of feeding high ALA diets. The levels achieved in the breast meat in the current study failed to reach levels achieved by our research group previously (unpublished data) and other studies (Rymer and Givens, 2006), but exceeded some earlier reported levels (Zelenka et al., 2008). A possible

reason for the higher levels achieved by Rymer and Givens (2006), is the higher level of n-3 LCPUFA in the basal diet due to fishmeal inclusion; whilst our earlier studies used lower ratios of LA to ALA and younger birds.

Interestingly, we found that there appeared to be a genotype effect on n-3 LCPUFA incorporation, with Cobb 500 birds being more efficient than Ross 308 for 18:3n-3 and 20:5n-3; however no differences were seen for the longer chain n-3 fatty acids. This finding is partially consistent with Rymer and Givens (2006) who found no significant difference in accumulation between the two genotypes; albeit a trend was observed indicating that Cobb 500 birds may indeed accumulate 22:6n-3 more efficiently. For laying hens, there appeared to be a genotype effect on n-3 LCPUFA incorporation into eggs, with Hy-Line brown birds having greater proportions of 22:5n-3 and 22:6n-3.

Importantly, feeding high ALA diets did not appear to have major consequences on the sensory properties of the meat or eggs. Future research in this area will include a large-scale commercial validation to evaluate any impact of diet formulation on bird performance.

Table 3. Sensory properties of breast meat

	Low ALA		Mid ALA		High ALA		SEM	P value		
	Cobb	Ross	Cobb	Ross	Cobb	Ross		D	S	D x S
Aroma	7.77	8.25	7.58	8.40	7.35	7.07	0.256	**	NS	NS
Aroma intensity	8.24	8.30	8.12	9.17	7.66	7.50	0.283	**	NS	NS
Tenderness	8.85^{abc}	9.89^{a}	9.33^{ab}	8.42^{bc}	8.11^{c}	8.86^{abc}	0.365	NS	NS	*
Chewiness	8.93	8.38	8.55	8.83	8.73	8.77	0.327	NS	NS	NS
Fibrousness	6.21	5.19	5.66	6.09	6.62	6.64	0.330	*	NS	NS
Juiciness	6.42	5.57	6.40	5.78	6.78	6.12	0.353	NS	*	NS
Chicken flavour	8.32	8.51	8.37	8.45	8.19	8.03	0.227	NS	NS	NS
Corn flavour	4.68	5.02	4.54	4.74	4.12	3.98	0.234	**	NS	NS
Broth flavour	6.20	5.70	6.30	6.40	6.03	5.90	0.255	NS	NS	NS
Mushroom flav.	4.45	4.65	4.69	4.54	4.39	4.61	0.231	NS	NS	NS
Off flavour	2.23	2.30	2.65	2.36	3.77	2.98	0.299	**	NS	NS
Savoury taste	7.23	7.62	7.74	7.48	7.03	7.13	0.254	NS	NS	NS
Metallic taste	4.60^{a}	3.84^{ab}	3.54^{b}	4.59^{a}	3.72^{b}	3.88^{ab}	0.280	NS	NS	**
After taste	7.43	8.32	7.92	8.37	7.14	7.74	0.243	*	**	NS

Scores are given on a scale from 0-15. * $P < 0.05$, ** $P < 0.01$.

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EGG QUALITY AND FOOD SAFETY OF TABLE EGGS: EGG QUALITY AND AGE OF FLOCK – A HORIZONTAL STUDY

J.R. ROBERTS[1] and K.K. CHOUSALKAR[2]

Summary

Eggs were collected from commercial caged layer flocks in early, mid, late and very late lay. Eggs were candled and scored for translucency. Cuticle cover was estimated using MST cuticle stain and a Konica Minolta hand-held spectrophotometer. Traditional measures of egg quality were determined using specialised equipment (TSS, U.K.) Shell ultrastructural features were scored following plasma ashing of shell samples and viewing under a benchtop scanning electron microscope. Translucency tended to be higher in late lay flocks and shell quality was lower. Cuticle cover was relatively uniform for young flocks but much more variable in older flocks. The incidence of unfavourable shell ultrastructural features was higher for older flocks. This study is on-going so, as the sample size increases, new insights will be obtained.

I. INTRODUCTION

Although eggs produced in Australia are considered medium to low risk for food borne illness, the egg industry in Australia is periodically implicated in cases of food poisoning. Fortunately, the Australian poultry industry is considered free from *Salmonella* Enteritidis which is of major concern to the food industry all over the world, and as a consequence, many of the microbiological issues in Australia differ from other continents. Most of the issues of food borne illness associated with eggs or egg products in Australia relate to *Salmonella* Typhimurium contamination of eggs post-oviposition.

It is difficult for bacteria to move across an intact good quality egg shell. However, small defects in the egg shell may provide means for the predominant bacterial species on the egg shell to penetrate and move into the egg contents (De Reu et al., 2006). It is possible that egg shell translucency may increase the incidence of bacterial penetration (Chousalkar et al., 2010). In addition, there is evidence that an intact cuticle presents an effective barrier to bacterial ingress into eggs (Leleu et al., 2011).

In the present study, unwashed eggs collected directly from the cage front were scored for translucency and tested for egg quality measurements. Egg shells were stained for cuticle deposition and the ultrastructural characteristics of the mammillary layer of the eggshell were scored.

II. MATERIALS AND METHODS

Eggs were collected from commercial flocks in different stages of lay: early (<25-40 wks), mid (40-55 wks), late (55-65 wks) and very late (>65 wks). A total of 180 eggs was collected directly from the cage fronts for each flock.

Ninety eggs were used for measurement of egg quality. Thirty eggs were scored for translucency and analysed for traditional egg shell quality measurements: shell colour (reflectivity), egg weight, egg shell breaking strength, shell deformation until breaking, shell weight, shell thickness, percentage shell (equipment by Technical Services and Supplies,

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U.K.). Egg internal was also measured in the form of albumen height, Haugh Units and yolk colour.

Thirty eggs were stained with MST cuticle blue stain and the cuticle colour measured using a Konica Minolta hand-held spectrophotometer. The colour of the egg shell cuticle, stained with MST cuticle blue dye was measured using the L*a*b* colour space. L* has a maximum of 100 (white) and a minimum of 0 (black). Green is indicated by -a* and red by +a*. Blue is indicated by -b* and yellow by +b*.

The remaining thirty eggs were scored for the extent of translucency, photographed individually on a candling box, and processed for viewing of the ultrastructure of the mammillary layer. Small pieces of shell (1 mm^2) were cut from the equator of the egg shell, soaked overnight in distilled water and the shell membranes removed. The samples were then dried and placed in a Biorad PT7 150 Plasma Asher for 4 hours to remove any remaining shell membrane (Reid, 1983). Samples were then mounted on aluminium stubs using conductive silver paint, gold sputter coated in a Jeol MP-19020NCTR Neocoater and viewed under a Jeol JCM-5000 Neoscope desktop scanning electron microscope. Each sample was scored for ultrastructural features as described by Solomon (1991) and Brackpool (1995).

III. RESULTS

Translucency score varied significantly among age categories, generally increasing with the age of the flock although the very late flocks, which had been moulted, were similar to the flocks in mid lay (Table 1). As hen age increased, shell reflectivity increased (shells became lighter in colour), egg weight increased, shell breaking strength decreased, shell deformation to breaking point decreased, shell weight increased, percentage shell decreased and shell thickness generally decreased. There were significant negative correlations between translucency score and albumen height, Haugh Units, shell weight, percentage shell and shell thickness.

Table 1. Traditional measures of egg shell quality

Measurement	Early Lay	Mid Lay	Late Lay	Very Late Lay	P Value
Shell Quality					
Translucency score	^{b}2.31	^{ab}2.46	^{a}2.64	^{ab}2.43	0.0212
Shell reflectivity %	^{b}27.8	^{a}31.0	^{a}31.6	^{a}31.5	<0.0001
Egg Wt g	^{d}56.6	^{c}61.0	^{b}62.8	^{a}66.3	<0.0001
Break strength N	^{a}46.7	^{a}45.0	^{b}36.1	^{b}36.6	<0.0001
Deformation μm	^{a}343.7	^{b}308.1	^{c}290.1	^{c}286.6	<0.0001
Shell weight g	^{d}5.50	^{b}5.86	^{c}5.68	^{a}6.11	<0.0001
Percentage Shell %	^{a}9.83	^{a}9.60	^{c}9.05	^{b}9.27	<0.0001
Shell thickness μm	^{b}387.8	^{a}396.4	^{c}372.8	^{ab}392.0	<0.0001
Internal Quality					
Albumen ht mm	^{a}8.62	^{b}7.68	^{b}7.88	^{c}6.91	<0.0001
Haugh Units	^{a}93.1	^{b}86.7	^{b}87.5	^{c}80.2	<0.0001
Yolk colour score	^{a}10.53	^{ab}10.38	^{b}10.19	^{ab}10.30	0.0339

For the cuticle colour, “L” increased significantly with the age of the flock with early lay being significantly lower than all other age categories (Table 2). However, there were no statistically significant differences among flock age categories for “a”. Mean values for “a”

ranged from negative 0.728 to positive 0.302 with the most negative values being for the flocks in late lay. However, there was great variability among eggs from any one flock and this variability generally increased with flock age. For "b", there were significant differences (P=0.046) among age categories. Values ranged from 32.4 to 33.2 and decreased with flock age.

Table 2. Spectrophotometric measurements of stained cuticle

Measurement	Early Lay	Mid Lay	Late Lay	Very Late Lay	P Value
L	^{b}53.31	^{a}55.01	^{a}54.87	^{a}55.43	0.0080
a	0.302	-0.254	-0.728	1.051	0.0978
b	^{a}33.23	^{ab}33.05	^{b}32.53	^{b}32.40	0.0460

For the eggs studied for shell ultrastructure, there were no statistically significant differences among age categories for translucency score. However, there were some statistically significant effects of age category on the ultrastructural scores. The variability of mammillary cap size was lower in early lay than for the other age categories. The incidence of confluence was higher in early and mid lay than for the older age categories. The incidence of early fusion was lowest for late lay and the incidence of late fusion was lowest in early lay. The incidence of alignment was greatest in mid and very late lay and lower for early and late lay. The incidence of Type B bodies and aragonite increased as the age of the flock increased except that the incidence of aragonite was lower in very late lay than in late lay. There were no significant correlations between translucency score and the scores for various ultrastructural features of the mammillary layer.

Table 3. Mammillary ultrastructure scores

Measurement	Early Lay	Mid Lay	Late Lay	Very Late Lay	P Value
Cap size	^{b}2.14	^{a}2.44	^{a}2.45	^{a}2.48	<0.0001
Confluence	^{a}3.05	^{a}2.88	^{b}2.63	^{b}2.63	<0.0001
Cap quality	^{b}3.04	^{b}2.98	^{a}3.23	^{b}3.05	0.0100
Early fusion	^{a}2.32	^{a}2.23	^{b}1.99	^{a}2.18	<0.0001
Late fusion	^{b}3.58	^{a}3.72	^{a}3.80	^{a}3.77	0.0007
Alignment	^{b}2.34	^{ab}2.46	^{b}2.25	^{a}2.54	0.0004
Type A bodies	1.33	1.36	1.43	1.41	0.4643
Type B bodies	^{c}1.76	^{b}2.16	^{b}2.51	^{a}2.56	<0.0001
Aragonite	^{c}1.15	^{bc}1.19	^{a}1.60	^{b}1.36	<0.0001
Cubics	1.17	1.23	1.16	1.24	0.4419
Cubic cones	1.59	1.51	1.58	1.64	0.4730
Cuffing	1.64	1.23	1.55	1.28	<0.0001
Depression	1.01	1.05	1.04	1.03	0.1180
Erosion	1.01	1.04	1.02	1.03	0.3663

IV. DISCUSSION

Results on egg quality are consistent with previous reports in relation to the changes that occur in egg quality as flocks get older (Leary, 1999). However, this is the first report of increases in the incidence of egg shell translucency with flock age. The use of MST cuticle

blue stain for determining the extent of cuticle cover in avian egg shells requires further verification and experiments are underway to correlate the extent of staining with the appearance of the shell cuticle under the scanning electron microscope. Results to date suggest that the MST cuticle blue stain is, in the main part, a reliable indicator of the presence of cuticle on the egg shell surface. The use of shell colour, as measured by a spectrophotometer, is confounded by the underlying colour of the egg shell. Shells which are pale but have intact cuticle end up bright green whereas shells which were dark brown become a khaki colour following cuticle blue staining. "L" was lowest for the early lay flock indicating a lightening of shell colour with age. However, the pattern was less clear for "a" as the most negative group (indicating the most green shells) was the flock in late lay. All flocks were positive for "b" although this value declined with flock age.

The lack of correlations between egg shell translucency scores and the scores obtained from examination of the mammillary layer of the egg shells suggest that the ultrastructure scoring system is not closely correlated with shell translucency. It is still not clear what structural features of the eggs cause translucency although alignment of the mammillae and microcracks appear to produce linear translucent features. Preliminary studies have commenced to examine egg shells using a Phoenix v|tome|x dual X-ray system, basically a CT scanner. It is anticipated that this system will allow the quantification of the amount of "space" within the mammillary layer of the egg shell which is expected to be correlated to the extent of translucency and hence the translucency score.

Once clearer methodologies have been consolidated that objectively define the variation of the cuticle deposition, and shell translucency, microbiological studies will be undertaken to study the rates of bacterial penetration through shells with a broad range of these characteristics.

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EGG QUALITY AND FOOD SAFETY OF TABLE EGGS: EGG QUALITY AND AGE OF FLOCK - A LONGITUDINAL STUDY

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Summary

Eggs were collected from a cage and a free range flock at the ages of 25, 35 and 45 weeks and divided into 4 different groups after candling and scoring for translucency. Thirty eggs were used for the estimation of the amount of cuticle present on the egg shell using a hand held Konica Minolta spectrophotometer before and after MST Cuticle Blue staining. The cage system eggs were visually more uniformly stained than the free range eggs. Significant effects were recorded for production system and age for L*a*b* values, but there was no significant interaction between the two. Under the scanning electron microscope (SEM), unstained patches showed no cuticle whereas stained areas had intact cuticle irrespective of the rearing system. None of the egg shells showed 100% cuticle coverage. Traditional egg quality measurements were carried out using specialized equipment supplied by TSS (UK). A significant effect was recorded for flock age and production system for all the egg quality variables except shell breaking strength, while interaction between the two was significant for translucency score, egg weight, shell weight, shell thickness and yolk score. Shell ultrastructural features of thirty eggs per flock were scored using SEM following plasma etching. Shell ultrastructural variables significantly affected by production system and age were mammillary cap size, early fusion, late fusion and depression, while interaction between production system and flock age was recorded only for confluence, alignment, type A bodies, cuffing, changed membrane, depression and erosion. The remaining 60 eggs per flock were used for microbiological studies, which showed negative results for *Salmonella spp*.

I. INTRODUCTION

A good quality egg shell plays an important role in the resistance of an egg to microbial penetration and alterations in eggshell properties may be related to increased risk of egg contamination (Hincke et al., 2010). Complex morphological variations occur in the mammillary layer of the egg shell as hens age (Solomon, 1992). Ultrastructural features such as high mammillary density, cuffing, mammillary confluence, good cap formation and early fusion of the mammillary layers are factors resulting in the formation of a good quality shell, while late fusion, Type A bodies, Type B bodies, aragonite, pitting, alignment, cubics, changed membrane and low mammillary density decrease the quality of the shell and resistance to microbial penetration (Solomon, 1992).

The Australian egg industry is thought to be relatively safe and free from microbes including *Salmonella* Enteritidis. Cox et al. (2002) found that the dramatic increase in *Salmonella* Enteritidis infections occurring in other countries has not been observed in Australia and the Australian poultry industry is generally considered to be free from this serotype (Chousalkar et al., 2010).

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II. MATERIALS AND METHODS

Eggs (150 per flock) were collected from two different management systems, cage and free range, at flock ages of 25, 35 and 45 weeks. Both flocks received locally formulated feed containing wheat (or wheat plus sorghum), soybean meal, meat meal, vegetable oil, limestone and yolk color pigment as major components. Eggs were collected directly from the cage fronts and nest boxes of farms and brought to the New England University Egg Laboratory, where they were divided into 4 groups after candling and scoring for translucency. Translucency score was between 0, the least translucent and 5 the highest. Thirty eggs were used for traditional egg quality measurements using specialized equipment (Technical Services and Supply, TSS, UK) in which egg shell reflectivity (%), egg weight (g), egg shell breaking strength (N), shell deformation (μm), shell weight (g), percentage shell, shell thickness (μm), albumen height (mm), Haugh Unit and yolk colour score were determined.

For determination of the amount of cuticle present on each of 30 eggs per sample, egg shell reflectivity (%) was measured and shell colour determined using a hand held Konica Minolta spectrophotometer (CM-2600d) before and after staining with MST Cuticle blue dye. Eggs were soaked in the cuticle blue stain at room temperature for 1 minute, then rinsed in distilled water 2 to 3 times to remove excess stain and allowed to dry. Simultaneous measurements were made for Specular Component Included (SCI) and Specular Component Excluded (SCE). In the "L*a*b*" colour space, "L" has a maximum of 100 (white) and minimum of 0 (black). For "a", green is towards the negative end of the scale and red towards the positive end. For "b", blue is towards the negative end and yellow towards the positive end of the scale. For SEM of the cuticle, the egg contents were first removed by making a small hole at the blunt end and eggs were washed thoroughly with tap water, taking care not to wash off any cuticle dye. After drying, a piece from the equator of the shell was cut from each egg using a Dremel High Speed rotary tool 300 series and mounted on an aluminum stub, and gold sputter coated for 5 minutes prior viewing under a scanning electron microscope (Neoscope Benchtop SEM).

For examining the ultrastructure of the egg shell (third group of 30 eggs), cut shell pieces were soaked in tap water overnight to ensure that the shell membrane could be peeled off. After removal of shell membrane, the samples were allowed to dry thoroughly and were plasma ashed for 4 hours in a BioRAD RF Plasma Barrel Etcher PT 7150. Samples were then gold sputter coated, viewed under the SEM and scored for the incidence of ultrastructural features, as described by Solomon (1991).

The fourth group of 60 eggs was sent to Charles Sturt University, Wagga Wagga, N.S.W. for determination of the total bacterial and Enterobacteriaceae count on the shell surface, in the pores of the eggshell and internal contents. Samples were also processed for isolation of *Salmonella spp*.

III. RESULTS

There was a statistically significant effect of both production system and flock age on translucency score, shell reflectivity, egg weight, shell deformation, shell weight, percentage shell and shell thickness and a significant interaction between production system and flock age for translucency score, egg weight, shell weight, percentage shell and shell thickness. For the free range flock, as compared with the conventional cage, translucency score, egg weight, shell weight, percentage shell and shell thickness were lower and translucency score, shell reflectivity and shell deformation were higher. With increasing flock age, shell reflectivity, egg weight, shell weight and shell thickness increased and shell deformation decreased. There was a statistically significant effect of production system and age on albumen height, Haugh Unit and yolk score, and a significant interaction between production

system and flock age for yolk score only. For the free range flock, as compared to the conventional cage system, Haugh Unit and yolk score were lower. Haugh Unit decreased with increasing flock age in both the systems, while albumen height decreased only in the conventional cage system. In free range flocks albumen height first slightly increased and then declined with increased age.

A statistically significant effect was observed for both production system and age on the values of L*a*b* for both SCI and SCE but there was no significant interaction between flock age and production system. The mean values of SCI L*a*b* and SCE L*a*b* were higher for the free range flock than for the conventional cage system and increased with increasing age of the flocks. The mean values of SCI a & b and SCE a & b showed a slight increase and then decreased with flock age in both production systems. Under the scanning electron microscope, the unstained shell specimens either showed no cuticle at all or very little cuticle. Densely stained specimens had good intact cuticle with cracks and fissures while the lightly stained specimens showed thin patchy cuticle. No difference was observed at microscopic level between the two production systems.

Egg shell ultrastructure results showed a statistically significant effect of production system and flock age for mammillary cap size, early fusion, late fusion, type A bodies, changed membrane and depression and a significant interaction between production system and age for confluence, alignment, type A bodies, cuffing, changed membrane, depression and erosion. No significant effect was recorded either for production system or age or interaction between the two for cubic, cubic cone formation and hole. The mammillary cap size and quality of mammillary caps decreased slightly in the 35wk old flocks and then increased with age in both production systems. The incidence of confluence, poor cap quality, late fusion, type B bodies, cubic cone formation and changed membrane was higher in the conventional cage system as compared to the free range flock. Cuffing, cubic cone formation, aragonite, type A bodies and confluence increased with flock age.

The total bacterial count varied significantly with flock age. However, there were no significant differences for total bacterial count in egg shell crush or total Enterobacteriaceae count on egg shell surface or in egg shell crush. Bacteria were not isolated from any of the egg internal contents. *Salmonella spp* were not isolated.

Table 1. The total bacterial and Enterobacteriaceae count from eggs at various stages of lay

Production	Cage			Free range			P value
Age (weeks)	25	35	45	25	35	45	
TBC on egg shell	3.4 ± 0.08	1.5 ± 0.52	3.08 ± 0.35	4.2 ±0.16	3.4 ± 0.08	2.7 ± 0.18	< 0.0001
TBC in egg shell crush	0.35 ± 0.35	0.58 ± 0.39	1.3 ± 0.44	0.89 ± 0.52	1.37 ± 0.56	1.09 ± 0.37	NS
TEC on egg shell	0.5 ± 0.08	0.63 ± 0.31	0.86 ± 0.36	0.34 ± 0.28	0.98 ± 0.41	0.76 ± 0.31	NS
TBC in egg shell crush	0.35 ± 0.35	0.58 ± 0.39	1.3 ± 0.44	0.89 ± 0.52	1.36 ± 0.56	1.09 ± 0.37	NS

TBC- Total bacterial count; TEC- Total Enterobacteriaceae count; all values in Log CFU.

IV. DISCUSSION

Results from the first three collections at 10 week intervals of this on-going study showed significant differences between production systems and flock age for some of the traditional quality variables. Free range eggs were higher than cage system eggs for translucency score, shell reflectivity and shell deformation. Solomon (1986) reported that translucency score increased with increased egg weight but, in the present study, translucency score was not clearly related to egg weight. Production system and flock age affected all shell quality variables except shell breaking strength. The cage eggs were higher for egg weight, shell weight, percentage shell and shell thickness than the free range eggs. As flocks aged, shell

reflectivity, egg weight, shell weight and shell thickness increased and shell deformation decreased. The increase in shell thickness was due to the flock peak production stage. Percentage shell increased with hen age for the cage eggs but not in the free range eggs. Similar changes in egg shell quality with hen age have been reported previously (Leary, 1999). The egg internal quality factors; yolk score, albumen height and Haugh Unit were higher in the cage flocks and decreased with flock age. Differences in feed might contribute to egg quality as the two production systems were on locally formulated diets which were not identical. The overall L*a*b* higher values for the free range flock indicate that less colour pigment is being deposited in the egg shell and therefore there may be less cuticle, as most of the pigment is deposited in the cuticle. A linear increase in the values of SCI L and SCE L indicates that, with increase in flock age, less pigment is deposited as L* value is inversely proportional to pigment deposition in the cuticle and shell. The values for "a", where a more negative value is indicative of greener colour, are more difficult to interpret. The cage flock had negative values at 25 and 45 weeks of age whereas the free range flock had a negative value only at 45 weeks of age. The underlying shell colour complicates the interpretation of these results and investigations are currently underway to measure the L*a*b* colour both before and after staining with MST cuticle dye, in order to allow for the existing shell colour.

Age has an important effect on egg shell quality and, with increased flock age, the incidence of ultrastructural variables such as late fusion, confluence, type A bodies, type B bodies, aragonite, depression, erosion increases which, in turn, may reduce the quality. However, in this study, the overall effect of these variables is negligible as the flocks are still relatively young. With increasing flock age, egg ultrastructural quality factors like confluence, early fusion, late fusion and depression increased irrespective of the production system, suggesting that flock age affects shell quality more than production system. Knape et al. (2002) reported that the average bacterial shell contamination is 6.33 CFU, the average bacterial contamination in this study was lower than reported earlier. The findings regarding total Enterobacteriaceae count is in agreement with De Reu et al. (2008), who reported low Enterobacteriaceae load on eggs from furnished and non cage production systems. It has also been reported that bacterial contamination of eggs is greater in eggs from older hens than from younger hens (Jones et al., 2004).

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EFFECTS OF RETAILER PRESSURE ON THE EFFICIENCY OF AGRICULTURAL INDUSTRIES

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Summary

Historical gains in reducing starvation have been dramatic. These gains have been achieved through increased use of arable land and adoption of new technologies. Future gains will depend much more on increased adoption of new technologies, but will need to be even more rapidly achieved than in the past to meet the increase in demand for food. Intensive industries, such as poultry are under pressure from those engaged with a naturalistic fallacy. Technologies such as antibiotics in chickens, or hormonal growth promotants (HGPs) in beef that are safe for people, reduce environmental impacts of production, increase profits for producers and improve animal well-being will be needed to achieve these gains.

The precedent set in the EU in banning HGPs can be understood as a response to the illegal abuse of diethyl stilboestrol in the EU and as a non-tariff trade barrier to reduce the importation of beef from more efficient producers. The banning of antibiotics in the EU reflects the unwise application of a 'precautionary principle' through which risks are not soundly assessed. The unilateral ban established by Coles on HGPs in Australia, however, represents a more dangerous development in which marketing ploys have been accorded a higher value than the care of animals, environment or profit for producers. Decisions such as these have reduced the viability of animal production in the UK and pose a threat to sustainable agricultural production in Australia.

I. HISTORICAL ACHIEVEMENTS OF AGRICULTURE

Perhaps the most remarkable technological achievement of the last century was the change resulting from the adoption of new technologies in agriculture that resulted in many more people on the planet being fed with fewer starving in year 2000 than in 1900. Associated with this change and advances in medical science, human longevity increased markedly in many countries (Fogel, 2004). Despite this achievement, today there are approximately 1 billion people starving and another 1 billion suffering from malnutrition (Anon, 2002).

Much of the success in reducing starvation was achieved through the 'green revolution' based on genetic selection of crops and use of nitrogenous fertilisers that resulted in much higher crop yields (Borlaug, 2000). In animal agriculture, production of poultry and pigs radically changed over the last century through adoption of more intensive housing and feeding strategies and genetic selection. In the USA, poultry and egg production increased by a compound 3.55% over the period 1948 to 1994 (Ahearn et al., 1998), resulting in an increase in efficiency of around 500% over that period. Similar, though less dramatic changes were achieved in dairy and beef production. Animal products are important in human nutrition, as these are nutrient dense and provide approximately one sixth of human energy intake and one third of protein intakes (CAST, 1999). The density and availability of proteins, biological value of these proteins, and bio-availabilities of macro-minerals and micronutrients (calcium phosphorus, iron, zinc, magnesium and manganese, B vitamins, niacin) in animal products is superior to that in vegetable sources (CAST, 1999).

Despite some misconceptions that animal welfare suffered with intensification, the combination of application of technologies and pressure to improve production associated with responses to lower economic margins for goods sold, resulted in marked improvements

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in animal health and well-being. This improvement is evident in the elimination of many animal diseases from substantial populations, development of anti-parasitic treatments, better therapeutics and vaccines. Better nutrition has greatly reduced animal morbidity and mortality. Housing conditions are being constantly improved to reduce morbidity and increase weight gains. Simply put, mistreating animals is at odds with efficient production and completely unacceptable to the vast majority of farmers. Within that context, however, there is a constant need to improve the housing and husbandry of animals as new understandings of housing and well-being are identified.

II. CHALLENGES FOR FUTURE FOOD SUPPLY

Despite the achievements of the previous century, there remain challenges in terms of food production. Perhaps the most substantial challenge facing the planet is the need to feed the approximately 9 to 9.6 billion people who will be on the planet by 2050 (Anon, 2002, Beddington 2011). Increasing demands for animal proteins in emerging nations and increasing affluence in Asia suggest that we will require 100% more food than produced currently by 2050. A more sanguine assessment by DEFRA in the UK (DEFRA, 2010) suggests that there will need to be a 70% increase, based on gains in post-harvest efficiency and changing some assumptions of the Food Agriculture Organisation of the United Nations (FAO). A number of workers have noted the potential for geopolitical instability to flow from food shortages. Notwithstanding, the differences in estimated needs for food, we can be confident that the increases in production will not be made from increased areas of land. The Beddington report (2011) notes that land will be lost to urban encroachment, salinity, desertification and sea level rise. The highly fertile San Joaquin Valley of California, for example, has lost approximately 21% of the arable land to housing since peak land availability in 1986 (Anon, 2011a). There are challenges also in regard to water, energy supply, phosphorus and potassium supplies for food production. The increased production of food will largely need to be driven by increased adoption of new technology.

The challenge under these conditions will be to provide a sustainable integration of practices to provide the food, fibre and fuel required from agriculture. Perhaps the most critical challenge is firstly to define sustainable. A simple definition is ‘the capacity of the planet to meet current production needs for humans, without impairing, or preferably enhancing, the capacity to meet future needs, while maintaining biodiversity of populations of organisms on the planet.’ It can be argued that the latter function is superfluous to the former, but from it could equally be argued, that we far from understand fully the potential for our ecosystem to benefit humanity.

Beddington (2011) consider that ‘More food must be produced sustainably through the spread and implementation of existing knowledge, technology and best practice, and by investment in new science and innovation and the social infrastructure that enables food producers to benefit from all of these.’ In this context, it is worth examining the effects of societal, governmental and retailer pressure on two technologies; specifically use of antibiotics in chickens and hormonal growth promotants (HGP) in cattle. The effect of the EU ban on certain antimicrobials is a matter of considerable controversy, while in Australia the unilateral ban on HGP by one of Australian supermarkets resulted in a strong response by Australian scientists who criticised the ban.

III. SOCIAL ACTIVISM, THE NATURALISTIC FALLACY AND FOOD PRODUCTION

There is a small but vocal group in society who fear, or wish to disrupt, the adoption of new technologies. Slovic (2000) published a landmark series of studies that explored societal responses to risk from new technologies. Perceptions of risk vary markedly among individuals and are influenced by the level of knowledge of the risk and fear of that risk. It can be hypothesised that agriculture will face increased risks of activism as fewer people have a connection with the land and have less knowledge of the technologies involved. Notable activist groups include the Luddite movement in the UK in the early 1800's who fought against the adoption of new methods of textile production and industrialisation in general. Other examples include the organic movement originating in Europe. Many of the adherents of such movements are engaged in a 'naturalistic fallacy', described and addressed by the philosophers Hume and Moore (Greene, 2003; Tanner, 2006). The position of those opposed to modern animal production systems is exemplified by a response of Mr Brian Sherman, a former financier and animal activist, in the Australian Newspaper (Sherman, 2011) in which he argued against positions presented by approximately 40 of Australia's leading animal and veterinary scientists (Anon, 2011b) regarding the unilateral ban by Coles supermarkets of hormonal growth promotants (HGP) in Australia. Mr Sherman in doing so, invoked an argument in regard to management of pigs, that we must 'know and feel instinctively' natural is better. It is not difficult to challenge the naturalistic fallacy. If we relied on such 'natural systems', humans would live to an average of around 35 years, frequently die in child birth, and suffer high rates of infant mortality. Natural or organic systems could not feed the current human population, nor the increasing human population (Trewavas, 2001; Beddington, 2011). Moreover, the extended period of co-evolution between humans and domestic animals over the last 10 to 14,000 years redefines concepts of 'natural' in regard to domestic animals. The latter observation is particularly pertinent to poultry production. The naturalistic fallacy has also been invoked by spokesmen for Coles Supermarket that placed a unilateral ban on HGPs in cattle. Recently, Mr Durkan of Coles said 'consumers were looking for "natural food grown under ethical production".' No data have been provided by Coles to support their contentions that consumers desire this (Herald and Weekly Times, 2011). Without quantitative data and clear evidence of the structure of survey questions, it is difficult to assess the strength of the assertions from Coles.

IV. ANTIBIOTICS AND POULTRY PRODUCTION IN THE EU

The EU, in 1999, decided to ban a number of antimicrobial agents used in agriculture, particularly poultry production. In 2006, this ban was extended. These decisions were made despite the advice of the EU's own Scientific Committee on Animal Nutrition (SCAN, 1996; SCAN, 1998a; SCAN, 1998b) and review by the EU courts that concluded that there were serious logic flaws in the ban. The ban reflected the adoption of the 'precautionary principle' invoked by EU regulatory authorities on the basis that there was a risk to human health from the use of antibiotics in poultry production (Castanon, 2007; Phillips, 2007). In the UK, retailer pressure was a factor in promoting these bans (Anon, 1999).

Fleming raised concerns about the possibility of antibiotic resistance as early as the 1940's. Subsequently, the problems of transmissible plasmids, genetic material that can be passed from bacteria to bacteria passing on antibiotic resistance, and potential for antibiotic resistance spread were examined in the Swann report of 1969. Outcomes from that report led to the development of prudent use criteria for antibiotics and the removal of some antibiotics from veterinary use. Veterinary curricula included detailed information on the potential for the inappropriate use of antibiotics to increase the prevalence of resistant bacterial

populations in animals and in man from the early 1970's. The risk to man from antibiotic use in animals has been reviewed by a number of authoritative bodies including the report published by the Heidelberg Appeal Netherlands Foundation in (1999), NRC (1999); SCAN (1996); SCAN (1998a); SCAN (1998b); OIE (1999). All of these reviews found that there was little risk of in-feed antimicrobial treatment increasing treatment failure in humans. Critically, to date, after more than 60 years of use of antibiotics in agriculture there are few, if any, documented cases of in feed antibiotic treatments of animals causing treatment failure in man.

However, there continues to be a very active media campaign implying or stating that the risk of human treatment failure is high, because of antibiotic treatment of animals. In the last 10-15 years since many of the reports cited above were released, many millions of dollars have been spent looking for links between animal treatment and infection or treatment failure in man. No longer does a lack of cases of treatment failure or infection reflect a lack of surveillance, because there has been extremely active surveillance in many countries using sophisticated gene probe methods. Increasingly also, evidence based on risk assessment approaches indicate that while the potential for such a hazard exists, the risk is very low (Cox and Popken 2003). This is unsurprising, as there are steps that intervene between the use of antibiotic and treatment failure in man. Casewell et al. (2003) suggest that indicators of human health relevant to risks associated with antimicrobial use in animals may have deteriorated in association, or even as a result, of the ban.

Recent risk assessments estimate the upper end (maximum) preventable deaths from an immediate ban in use of virginiamycin in all species in the USA (population ~ 250 million) would be less than 1 person in the next 15 years. To put this in context, the death rates due to bee sting are 1 per 6 million people per year and those due to dog bite 1 per 18 million per year. The view of the epidemiologists (Casewell et al., 2003; Phillips et al., 2004; Doores et al., 2003) contrasts greatly from microbiologists, as evident in the consistent support of these for bans based on precautionary principles (Turnidge, 2004). The impact of the bans on animal health and productivity is not clear, as many of the studies are of poor design. However, a number note declines in poultry health and productivity, whereas others note a decrease in tonnes of antibiotics fed following the ban.

There are three key aspects in understanding the impacts of these bans i) the risk of banning in feed antibiotics on human health is bi-directional, in other words, removal of preventive medicines can increase use of therapeutic antibiotics, which also impose risks to man ii) in industries such as poultry, small changes in efficiency of production are critical to profitability; and iii) a mix of fear, and activism can result in poor policy. A thoughtful article highlights the need for more sophisticated approaches to making policy decisions in agriculture based on understanding ignorance in decision making (Rivera-Ferre and Ortega-Cerda, 2011). The article argues for a more integrated approach to making agricultural decision including the use of risk assessment, but engaging also with understandings of uncertainty, ambiguity and ignorance.

IV. HORMONAL GROWTH PROMOTANTS IN BEEF

Another recent example of retailer pressure is the unilateral ban of Coles supermarkets on HGPs. The decision replicates bans on such products in the EU. The HGPs were first used in the USA in the late 1940's and since the late 1970's in Australia. Early growth hormone research was a response to the food deprivations associated with the Second World War and initial investigations were designed to mimic the steroid and peptide growth hormones naturally produced by livestock. In some countries and in early trials, diethyl stilboestrol (DES) was used. DES has the potential to create residues and be hormonally active in those

eating the meat. Further, concerns were raised in regard to the carcinogenic potential of DES. These impacts lead to some concerns about safety of HGPs. Consequently, subsequent products have been extremely rigorously assessed for potential risks to consumers.

There are several different agents registered for use in Australia and approximately 40 other countries. These include naturally occurring steroids (oestradiol 17β, oestradiol benzoate, progesterone, testosterone), a synthetic steroid (trembolone acetate) and zeranol – a synthetic, non-steroidal oestrogen, originally derived from a naturally occurring fungus.

In Australia, the HGPs that are registered have been evaluated for safety and efficacy in animals by the Australian Pesticides & Veterinary Medicines Authority and the Therapeutic Goods Administration, and in the USA by the Food and Drug Administration (FDA, 2011). Further opinions and reviews were provided by the most widely recognized authority on the safety of therapeutics in food, the Codex Alimentarius Commission, the Codex Committee on Residues of Veterinary Drugs in Foods, and the Joint Expert Committee on Food Additives (JECFA). All these organisations and other national registration authorities have concluded that these products are safe for human use, based on understandings of the biology of the products and the very low residues. A further independent and authoritative independent review, the Lamming report (1982), commissioned by the European Union (EU) concluded that these products were safe to humans. This report was suppressed by EU authorities, but was published by the committee in 1987 (Lamming et al., 1987). The conclusions of the Lamming Committee were confirmed by a joint World Health / FAO committee (Anon, JECFA 1988). Despite these consistent findings from regulatory authorities, the EU banned the use of HGPs from 1985 and banned imported beef from countries including the USA and Canada from 1989. A full review of safety of these products in humans is available from the APVMA (2011).

The genesis of the EU political antagonism to HGPs can be understood in the context of several major influences. Extraordinarily, there had been abuse of DES in some member states, especially Italy, in the early 1980's. This abuse had justifiably raised consumer concerns about food safety, in regard to hormone treatments. Further, policies in the EU were designed to reduce over-production of subsidised food, including beef. The Common Agricultural Policy of the EU involved minimum pricing for products, trade barriers, tariffs and market controls that were directed towards providing protection for EU internal markets on the basis that many of the member states wished to protect their farmers and maintain viable rural populations. Consequently, means of reducing access to products from more efficiently producing countries were devised, i.e non-tariff trade barriers. The combined effects of these lead to an invocation of the 'precautionary principle', that result in a decision by EU to ban the HGPs, despite the overwhelming conclusion by those who examined the human safety aspects of the HGPs that the products were safe. A consequence of the ban on the HGP was that in an action under the World Trade Organisation the USA and Canada gained reparations from the EU to a value of $US93 million per annum on the basis that the ban was not based on grounds based on science or a proper risk assessment (APVMA, 2011).

V. SAFETY AND EFFICACY OF HGP IN CATTLE

The use of HGP has been the subject of substantive, quantitative literature reviews (Preston, 1999; Wagner et al., 2007; Hunter 2010). Responses of cattle to HGP are positive and consistent in cattle on adequate diets. The growth rate of steers and heifers is increased by 10–30%, feed conversion efficiency by 5–15% and fat content of carcasses reduced by 5 to 8% (Hunter, 2010; Preston, 1999). A review of studies from the north of Australia, obtained

from Hunter (2010) and other sources shows that cattle treated before the wet season have the potential to hold gains achieved in that period into the dry over control cattle. This is important for production, efficiency of production, meeting markets of higher value and animal well-being. Table 1 outlines the positive and negative aspects of using HGPs on cattle performance, the environment, profitability of production and meat quality. The HGP have substantial and positive effects on the environment, with up to 40% of Australian cattle being treated. The negative impacts on cattle health are outlined in Table 1, arise infrequently, and were not of sufficient concern to prevent registration of the products. The positive value of having additional body tissue reserves, especially in adverse nutritional environments, can be substantial. Claims that there are impacts on the capacity of cattle to thermo-regulate are based on a single flawed (temporally pseudoreplicated) study (Gaughan et al., 2005). These products are, on balance, positive for cattle health, especially in northern Australia.

Table 1. Positive and negative aspects of using HGPs on cattle performance, the environment, profitability of production and meat quality.

Area of Impact	**Positive Effects**	**Negative Effects**
Animal Health	Weight gains retained into dry period	Buller steers, behavioural changes, rare abscess formation at implant sites
Animal Productivity	Increased production by 10 to 30% and a 10 to 15% reduction in intake, Overall efficiency of production increased by 30%	Nil
Sustainable Management	Reduced use of land and water, reduced greenhouse gas production	Nil
Profit of Production	Value in 2008, $210 million	Nil
Meat Quality	High Quality markets met by use in Northern Australia, reduced fat in meat (Hunter 2010)	Reduced meat quality, overcome by aging the carcase or tenderstretch methods (Hunter 2010)

Gains in profit of $210 million per annum estimated by the NSW Department of Agriculture for producers, needs to be viewed in the context that many beef properties do not make a profit (ABARE 2009). As for manipulations in poultry, even modest improvements in returns have a large effect on profitability. Products that allow producers to meet high value markets, reduce environmental impacts and improve animal well-being have an especially valuable place in cattle production. The effect of HGPs on the eating qualities of meat has been used by Coles to support their position in banning HGPs. A meta-analysis (Watson, 2008) on the effects of HGPs on beef palatability provides evidence of negative effects on tenderness and palatability. Very few individual studies reviewed by Watson (2008) showed negative effects on tenderness or palatability, but overall effects were significant and consistent in the meta-analysis. However, there is a difference between significance and importance of effect, and these effects on quality are modest. Hunter (2010) notes that tenderstretch methods or aging remove these negative effects. Further, a reduction in lipid associated with treatment can be viewed as a positive nutritional effect in a leaner beef that, however, has reduced flavour. These effects on meat quality, positive and negative, are incorporated into Meat Standards Australia grades that account for all the variables that have

an influence of meat quality. Consequently, the claims of Coles in regard to the effects of HGPs on meat quality lack a sustainable, rational basis and have been refuted by Meat and Livestock Australia (Gorman, 2011) and the CSIRO (Bell, 2011), the organisations whose work was cited by Coles in support of their ban.

It is difficult to assess the impact of retailer activism on food buying habits. Recent surveys in Australia suggest that beef sales for retail butchers have been maintained and Woolworths have increased more than those of Coles. Data supplied by the Beef Central publication on 31st July (2011) indicates that Coles "Hormone-free beef" campaign has had little impact on their sales of beef. Market share of independent retailers was maintained at 29.4%; market share for Woolworths increased from 27.5 to 28.9% over the same period, whereas a smaller increase from 20.8 to 21.6% was recorded by Coles. More recent findings, published by Beef Central and based on independent survey data for the next quarter of the year suggest that Coles are losing market share in this sector (Condon, 2011).

VI. SOCIETAL RISKS OF ARBITARY BANS ON ANIMAL HEALTH AND PERFORMANCE TECHNOLOGIES

There is no agreed ethical basis by which marketing is conducted, rather the ethics need to be determined by each organisation in marketing. However, it is disingenuous to claim that 'it is just marketing' to ban a product. A decision to reject legal, regulatory agent evaluated, industry accepted technologies imply a decision that such technologies are somehow flawed. This assertion resonates with the statements cited above that Coles are seeking to produce food on a 'more ethical basis'.

The effects of arbitrarily banning technologies can be serious. The ethics are complex and the science of applying any new technologies must contain some uncertainties. It has been argued that is important for scientists with knowledge to engage with policy makers to explore the complexity of application of new technologies from a rational and risk-based perspective using qualitative and quantitative assessment methods (Stirling, 2010).

Perhaps that most dramatic effect on human health has resulted from the ban initiated by EU activists on genetically modified (GM) 'golden rice'. This GM rice that includes a gene to produce beta-carotene was developed to address the needs of much of the population in the Indian subcontinent for beta-carotene to prevent blindness. It has been argued that around a 9% reduction in the national disabilities, blindness and deaths associated with vitamin A deficiencies of around 1.4 million healthy life years in India per annum could have been saved more cost-effectively than by other means using the rice (Stein et al., 2006). Despite previous positions in regard to the adoption of technology driven by consumer activism and agro-political positions the EU, have responded to the impending challenges of food security and over-turned the previous positions on GM crops. Beddington (2011) argue strongly for the use of GM crops to allow sufficient and efficient production to be achieved.

Coles' decision to ban HGPs, could have had flow on effects through other retailers resulting in a loss of $210 million to beef producers. The arbitrary ban on technologies will have, however, other profound effects. Such actions create uncertainty among those wishing to improve animal well-being and production through use of new technologies. Any new technology will have some adverse effects, whether these be minor adverse reactions in animals, such as for HGPs, or more substantial effects on production systems, such as for housing animals.

If retailers are to simply use any difference as a point of differentiation in the market through a ban, this raises grave doubts over the investments made in new technology by universities, other research organisations or commercial entities with vested interests, such as feed or pharmaceutical companies. Further, such bans make production less viable within a

country and make producers less profitable and efficient, thereby increasing the environmental footprint of production and producing a vulnerability to imports. The effects of policies in the UK largely lead by Tesco, similar to those prosecuted by Coles, on the UK farming community have been devastating. From a position of being a net food exporter, the UK now imports food including beef and poultry meat (AgriStats, 2011).

VII. CONCLUSIONS

It is very clear, that we will need more food in the future. The increase in production will need to substantially come through the adoption of new technologies. The rejection of technologies, such as antibiotics in chickens or HGPs in beef that increase producer profit, improve animal well-being, provide safe, highly nutritious food at reasonable cost and that reduce the environmental impact of production should be regarded as unethical. The additional risks that may increase the costs and barriers to adoption of new technologies needed to feed the future population should be regarded as extremely worrying. The ban on HGPs by Coles stimulated an unprecedented response by senior and leading agricultural and veterinary scientists for these reasons.

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EVALUATION OF NEW HIGH YIELDING TRITICALE CULTIVARS FOR INCREASED BROILER PRODUCTION

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Summary

Five high yielding triticale cultivars, bred by the University of Sydney, were grown in a number of different environments and evaluated with and without a blend of xylanase and phytase enzymes in a broiler energy metabolism study. Significant interactions between cultivar and growing environment were observed for apparent metabolisable energy (AME) and feed conversion ratio (FCR). Within a cultivar, AME differences ($P < 0.01$) of up to 0.76 MJ/kg dry matter were observed between growing environments, and similarly within an environment AME differences between cultivars, up to 0.72 MJ/kg dry matter, were recorded. For FCR, the largest difference ($P < 0.01$) between environments of 1.60 to 1.80 was found for the variety Tobruk. Significantly different ($P < 0.01$) FCR values of 1.64 and 1.75 were also found between cultivars grown at Gerogery in 2008. Significant AME responses to the addition of enzymes between 0.39 and 0.45 MJ/kg of dry matter were found for four of the five triticale cultivars. Enzyme treatments did not affect cultivar FCR.

I. INTRODUCTION

Triticale (*X Triticosecale*) is a unique cereal crop resulting from a synthetic cross between wheat and rye, incorporating both the high yielding capacity of wheat with the tolerance to poor growing environments of rye. Triticale is used in animal feeds and in recent years breeding has improved both its yield and nutritive value for poultry diets (Elangovan et al., 2011). While triticale has shown good yield characteristics under a range of environments, there is limited research into how different growing conditions impact on its nutritional value. The purpose of this study was to determine apparent metabolisable energy (AME) content and broiler performance when several high yielding triticale cultivars sourced from a range of environments were fed to broilers in diets with and without enzyme supplementation.

II. MATERIALS AND METHODS

Five triticale cultivars, including the registered varieties Tobruk and Berkshire and University of Sydney breeding lines JRCT55, JRCT101 and JRCT117 were used in the study. All cultivars were grown in Narrabri in 2008 and 2009. Berkshire was also grown in Corowa, Gerogery and Young in 2009 and Tobruk was grown in Henty, Dunedoo and Young in 2008 and Young and Gerogery in 2009. Moisture availability in each growing environments is shown in Table 1 below.

Table 1. Rainfall (mm) available within each environment (location x year) at planting (April – June) and during the main growing period (July – Nov). (*pre-plant irrigation)

	Corowa 2009	Dunedoo 2008	Gerogery 2009	Henty 2008	Narrabri 2008	Narrabri 2009	Young 2008	Young 2009
April - June	139	77	156	62	46 (50*)	138	88	158
July - Nov	123	297	312	233	317	109	226	203

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The study consisted of two conventional energy balance experiments, which were statistically analysed together and accounted for between experimental variation through the use of common grains included in each experiment. Experiment one contained the eight 2008 grown triticale samples and nine other samples not reported in these results, but which were included in the experiment to allow 'experimental connectivity' to previous experiments and contribute to the AusScan NIR database (Hughes et al., 2012; in these Proceedings). Experiment two contained the 10 triticale samples grown in 2009 plus five 2008 triticale samples in common with experiment one to allow 'experimental connectivity' between the two experiments in the study. All triticale samples were fed with and without enzyme supplementation consisting of a blend of commercial xylanase and phytase enzymes. Responses were measured for grain apparent metabolisable energy (AME) content and feed conversion ratio (FCR) with regards to triticale variety, growing environment and enzymes treatment.

Triticale AME values were determined by measuring feed intake and excreta output and the gross energy values of both the feed and excreta using bomb calorimetry (Mollah et al., 1983). Both experiments in the study used two batches of broiler chickens. Each batch of day-old chickens was raised on a commercial broiler diet in two single-sex floor pens. At 22 days of age birds were transferred in groups of five to single-sex metabolism cages in a controlled temperature room maintained between 22 and 25^{o}C. Experimental diets contained 80% grain, 15.2% casein, 1.1% dicalcium phosphate, 1.3% limestone, 0.7% DL-methionine, 0.2% vitamin premix, 0.3% sodium chloride, 0.2% choline chloride (60%). All diets were cold-press pelleted and fed with and without a blend of commercial xylanase (Porzyme 93010 at 50g/tonne) and phytase (Phyzyme at 50g/tonne) enzymes to two cages of male and two cages of female birds (four dietary replicates). The diets were fed for seven days and bird weight was measured at the beginning and end of the seven day period. During the first three days, birds adapted to diets, then for the final four days excreta was quantitatively collected and oven dried at 80^{o}C to a constant dry weight. Feed intake was measured in both the adaptation and collection phases. The dry matter content of the grain, casein and pelleted diets was measured and the gross energy (GE) of the dried excreta and pelleted diets were determined with a Parr isoperibol bomb calorimeter. AME grain (MJ/kg dry matter) and FCR (g feed : g gain) values and significant effects were determined using a statistical model (ASReml software) with average start weight fitted as a covariate and included terms which accounted for between and within experimental variation.

III. RESULTS

Triticale cultivar and growth environment (location x year) had a significant effect on both grain AME and FCR. Figure 1 shows the AME values for the five triticale cultivars grown in different environments. Large differences ($P < 0.01$) in grain AME due to environment, possibly related to moisture availability, were found for four of the five cultivars, with the largest AME difference of 0.76 MJ/kg dry matter observed in Tobruk between the Narrabri 2008 and 2009 growing environments. Significant differences ($P < 0.01$) in AME for JRCT55 (0.66 MJ/kg dry matter) and JRCT101 (0.55 MJ/kg dry matter) were also found between Narrabri 2008 and 2009, though AME values were similar for the JRCT101 and Berkshire cultivars grown in these environments.

Within three of the four common growing environments; Gerogery 2009 and Narrabri 2008 and 2009, significant interactions were observed between the triticale cultivars. For example, at Gerogery 2009, Tobruk had a significantly higher ($P < 0.01$) AME value than Berkshire (15.04 vs 14.32 MJ/kg dry matter), but the situation was reversed in the Narrabri 2009 environment where Berkshire had a better ($P < 0.05$) AME value than Tobruk (14.80 vs 14.46 MJ/kg dry matter). However, no significant difference was observed between these cultivars at Narrabri in 2008.

Cultivar and environment also had an effect on FCR as shown in Figure 2. JRCT101 had a significantly ($P < 0.01$) better FCR of 1.60 in Narrabri 2008 compared to 1.72 recorded in Narrabri 2009, though no significant differences were found in FCR among the other cultivars in these two environments. Significant differences in the FCR of Tobruk were observed between a

number of growing environments. For example, the FCR for Tobruk grown in Young 2009 (1.60) was significantly better ($P < 0.05$) than Henty 2008 (1.80), Gerogery 2009 (1.75), Young 2008 (1.72), and Dunedoo (1.69). Conversely, the FCR of Berkshire was not significantly different across all five environments in which it was grown. Within a growing environment, however, Berkshire had a better ($P < 0.05$) FCR than JRCT101 when grown at Narrabri 2009 (1.63 vs 1.72) and a better ($P < 0.01$) FCR than Tobruk grown at Gerogery 2009 (1.64 vs 1.75).

Figure 1. The apparent metabolisable energy of the five triticale cultivars grown in different environments (location x year). Standard error bars are shown and the least significant difference at the 5% level is 0.34.

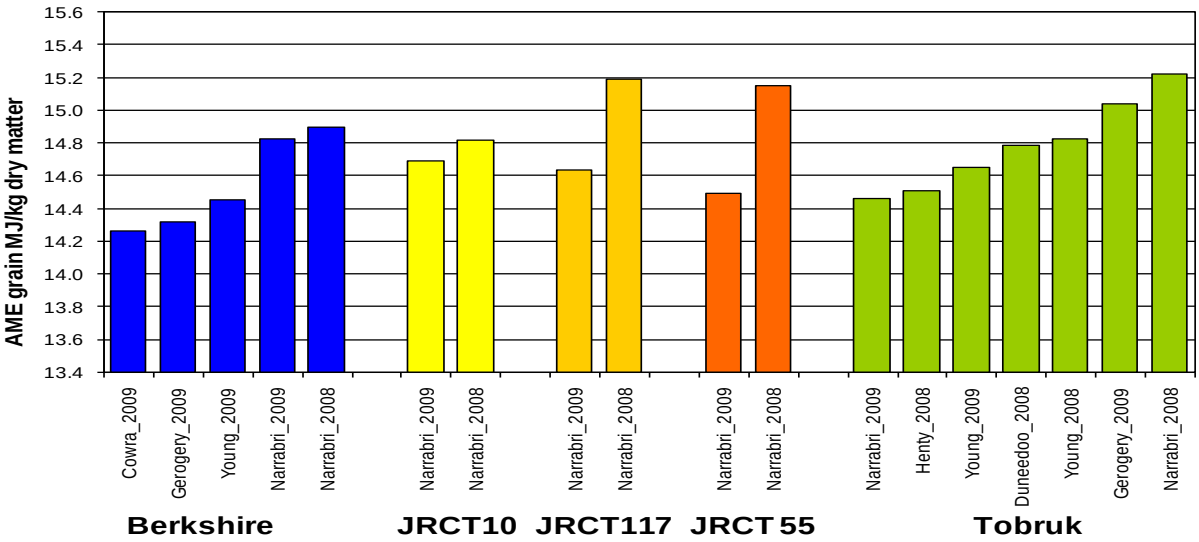
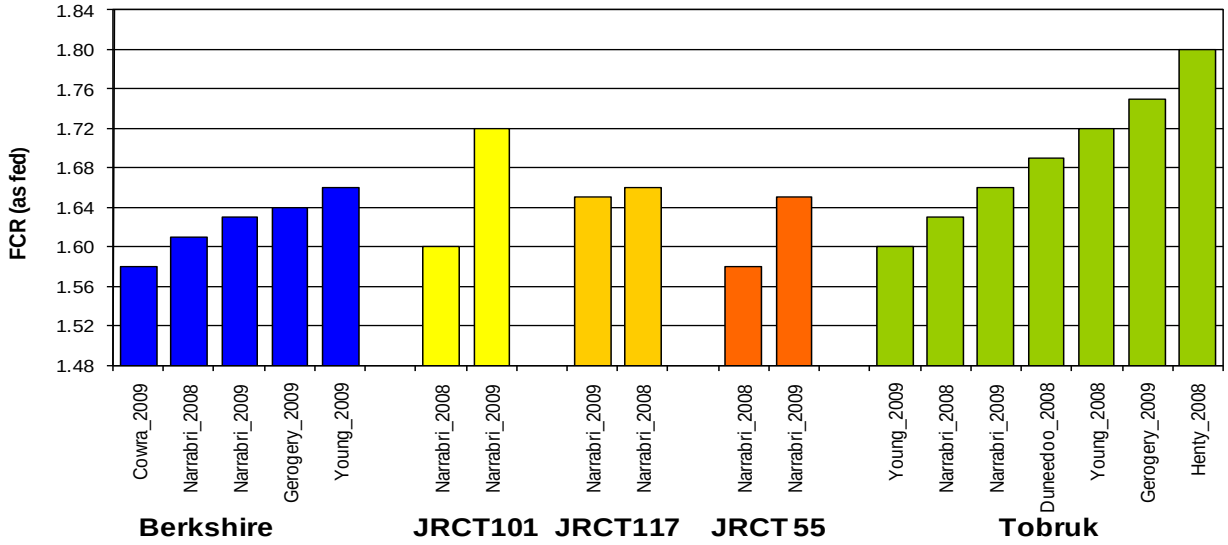
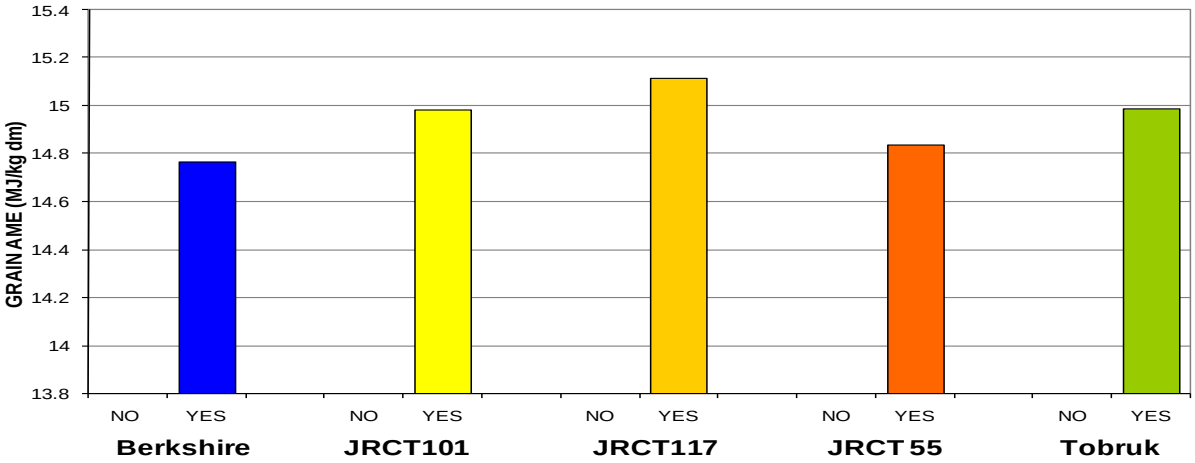


Figure 2. Feed conversion ratio for the five triticale cultivars grown in different environments (location x year). Standard error bars are shown and the least significant difference at the 5% level is 0.08.



No interaction between growing environment and response to enzymes was observed. However, a significant cultivar x enzyme interaction was observed where four of the five triticale cultivars had significantly higher ($P < 0.01$) AME values as a result of supplementation with xylanase and phytase enzymes. As shown in Figure 3, JRCT101 had the largest response to the addition of enzymes with an increase in AME of 0.45 MJ/kg dry matter. No significant effect of enzymes was observed between triticale cultivars for FCR.

Figure 3. The effect of adding xylanase and phytase enzymes (no or yes) to the AME of five triticale cultivars. Standard error bars are shown and the least significant difference at the 5% level is 0.37.



IV. DISCUSSION & CONCLUSION

The results indicate that triticale cultivars suitable for feeding to broilers can be developed with superior FCR and higher AME values. However, the significant effect of environment, most probably related to moisture availability and the existence of cultivar x environment interactions will complicate the process. Despite the effect of environment on AME, there was no significant interaction between growing environment and enzyme treatments. Most of the triticale cultivars tested had a significant AME response to enzyme supplementation, suggesting that enzymes may be effectively used to improve AME in triticale based broiler diets. Further research is required however, to understand the cultivar x enzyme interaction for AME, so that breeding might enhance the AME response to enzymes.

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THE ROLE OF GASTROINTESTINAL TRACT MICROBIOTA IN CHICKEN PRODUCTIVITY

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Summary

A comparison of the microbiota present in the gut of individual chickens indicates that the composition can vary considerably, even when reared at the same time under identical conditions. By studying these microbial changes and correlating them with other changes in the bird's biology we aim to define important host-microbe interactions. Using the latest high throughput DNA sequencing methods to characterize microbial populations we have found that the differential abundance of some bacterial species correlates with differences seen in the feed conversion ratios of individual birds, indicating that there are microbes present that may positively or adversely affect bird productivity. We are now investigating these interesting bacterial species to see if manipulation of their numbers can affect productivity outcomes.

I. INTRODUCTION

In the poultry industry, uniformity and level of flock performance are of special interest as they have a profound effect on profitability and competitiveness. The performance of a chicken flock can be measured by a range of parameters such as body weight gain (BWG), feed conversion ratio (FCR), apparent metabolizable energy (AME) and the time to achieve market weight. In a flock of the same breed, fed an identical diet, some birds are able to convert food to body weight or to extract the available nutrients very efficiently, while others lose a greater proportion of energy via the excreta (Hughes 2003, 2004). The mechanisms underlying this variation are yet to be elucidated. In the study presented here we have investigated whether there are specific bacterial species in the gastrointestinal tract (GIT) which are correlated with improved bird performance. We have used high throughput sequencing of 16S rRNA amplicons to characterize microbial populations from birds at the extremes of both AME and FCR performance.

II. MATERIALS AND METHODS

The present experiment was previously described in detail (Moore et al., 2011). Briefly 96 male Cobb 500 broiler chickens were housed in individual cages and a number of performance indicators calculated, including AME and FCR. Caecal content samples were taken for microbiota analysis and processed and sequenced as previously described (Moore et al., 2011). A total of 24 samples, 12 for high and 12 for low FCR birds were selected for sequencing and separately another 24 for the AME investigation. There were 16 birds that were represented in both AME and FCR groups resulting in the sequencing of 32 samples. One sample was removed from the analysis due to the low number of sequences generated. The amplified 16S rRNA gene fragments were sequenced using a Roche/454 FLX Genome Sequencer. Output sff files were split into fasta and qual files using PyroBayes (Quinlan et al., 2008), and data were analysed using the most recent release of Qiime software (v1.3.0) as per the Qiime defaults, unless stated otherwise. Sequences were assigned to Operational

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Taxonomic Units (OTU) with 97% within group similarity, a level which is approximately equivalent to a species designation.

III. RESULTS

a) Animal trial

One-way ANOVA was used to test for statistically significant differences in feed intake, growth and feed conversion between birds. There was no significant difference in feed intake in the birds selected for analysis while differences in AME, FCR and BWG were statistically significant $P < 0.0001$.

b) FCR related differences in microbiota

ANOVA was used to investigate and compare the relative abundance of each specific OTU across sample groups between 12 high and 12 low FCR birds. There were 30 OTUs differentially abundant between the two groups ($P < 0.05$), only three were more abundant in low FCR birds (Table 1), while the 27 remaining OTUs were overrepresented in high FCR birds (data not presented). The three potentially beneficial OTUs identified were uncultured Lachnospiraceae (OTU 661), *Lactobacillus* sp. (OTU 423) and uncultured Clostridiales (OTU 308). Of the 27 OTUs more abundant in high FCR birds, 12 could only be classified down to phylum level as unclassified Firmicutes, while others included unknown species from genera *Roseburia*, *Acetivibrio*, and family Ruminococcaceae.

c) AME related differences in microbiota

Euclidean distance between samples, presented as a 3D PCoA plot in Figure 1, shows some grouping of low AME birds indicating possible presence of gut bacteria that may impact ability of bird to utilize energy from food. ANOVA analysis detected 12 OTUs of differential abundance, with four OTUs present only in high AME birds and another four more than 3 times more abundant in high AME birds compared to low AME birds. Table 1 shows OTUs more abundant in high AME birds.

IV. DISCUSSION

A growing body of published information indicates that performance may be linked to gut microbiota. Most previous studies in chickens have used electrophoresis based methods to characterize microbial populations and select OTUs for further investigation. The current study reported here provides in-depth information on broiler caecal microbiota with over 600,000 quality sequences produced from 32 samples.

A significant proportion (11%) of the generated sequences could only be classified as “unknown phylum”. It has been previously suggested that only 10% of gastrointestinal bacteria are known culturable species (Apajalahti et al., 2004). We have also identified significant microbiota variation between animals with some comprised exclusively of Firmicutes and others of Bacteroidetes with remaining birds covering the range between the two extremes. This may contribute to large variations in chicken performance between birds grown under the same conditions. It is known that the ratio of Firmicutes and Bacteroidetes (F/B ratio) plays a role in human weight management with F/B ratio significantly higher in obese individuals and significantly reduced during weight loss (Ley et al., 2006). In the present study F/B ratio did not appear to significantly influence performance, but whether F/B ratios in chickens can be manipulated to enhance performance remains to be further investigated.

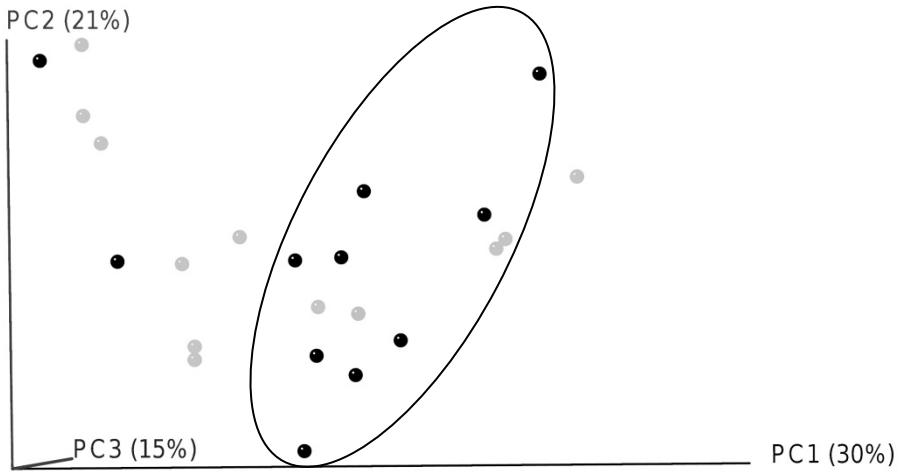

Figure 1. 3D plot of caecal bacterial OTU from birds divergent for AME. Highest AME ranked birds (grey circle; n=12) and lowest AME birds (black circle; n=11). PCoA plot was based on Euclidean distances.

Table 1. OTUs found potentially beneficial for bird performance using FCR (first 3 rows) and AME extreme bird comparisons. Fold represents fold of higher abundance in birds showing good perfortmance, namely in high AME and low FCR. Inf value indicates total absence of OTU in poorly performing birds.

OTU	p-value	Fold	RDP classification
661	0.04097	+inf.	Lachnospiraceae
423	0.04203	+inf.	*Lactobacillus*
308	0.04765	+inf.	unclassified Clostridiales
418	0.01628	+inf	unclassified Ruminococcaceae
706	0.01750	+inf	unclassified Ruminococcaceae
245	0.02305	6.5	unclassified Lachnospiraceae
83	0.03311	6.5	*Syntrophococcus*
229	0.03832	3.3	*Bacteroides*
99	0.03967	4.3	*Coprococcus*
1284	0.04097	+inf	*Lactobacillus*
673	0.04203	+inf	unclassified Lachnospiraceae

There were 11 OTUs identified as being associated with birds exhibiting superior performance: three more abundant in low FCR birds and eight more abundant in high AME birds. The FCR selected OTUs include Lachnospiraceae, *Lactobacillus* and unclassified Clostridiales while AME selected OTUs also include two OTUs selected by FCR comparisons, namely Lachnospiraceae and *Lactobacillus*, and additionally Ruminococcaceae, *Syntrophococcus*, *Coprococcus* and *Bacteroides* classified OTUs. The PCoA analysis shows no grouping of energy efficient birds and a slight grouping of low AME birds. This may indicate that low AME birds may share an OTU that promotes bad performance.

A number of the selected OTUs are related to bacteria that have well documented beneficial effects. For example some *Lactobacillus* isolates are widely used in humans and

animals and claimed to have health giving benefits and Lachnospiraceae are known as beneficial bacteria in the human intestine, participating in fermenting carbohydrates into short-chain fatty acids (SCFA) (Duncan et al., 2007). Members of the *Bacteroides* genus are known as one of the genera with the highest hydrolytic activity, being very effective degraders of indigestible carbohydrates, including resistant starch and cellulose (Al-Sheikhly and Al-Saieg 1980) and also SCFA producers (Collier et al., 2008). Most of the known Ruminococcaceae have been isolated from rumen ecosystems where they play a role as major cellulose and starch degraders (Duncan et al., 2007). Torok et al., (2011) investigated bird performance as measured by FCR using different diet compositions, broiler breed and bird age, and they also reported Lachnospiraceae, *Lactobacillus* and Ruminococcaceae related OTUs. *Coprocccus* have been reported to produce butyrate (Duncan et al., 2007) while the Clostridiales are known SCFA producers. All of these have the potential to contribute to increased performance. The key to further progress is to culture and directly test whether any of these candidate performance enhancing bacteria can be used to manipulate the GIT microbiota and thus deliver performance improvements for poultry producers.

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RESPONSIVENESS OF SORGHUM AND TRITICALE GRAINS TO SEPARATE BLENDS OF XYLANASE AND PHYTASE ENZYME PRODUCTS

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Summary

Twenty five sorghum grains donated by commercial nutritionists were evaluated along with five triticale and eight sorghum "connectivity" grains used in previous energy metabolism experiments which contributed to the AusScan NIR calibration database. All grains were fed with and without a blend of xylanase and phytase enzyme products. Separate blends were used for sorghum and triticale. The inclusion of "connectivity" grains enabled data to be analysed statistically for valid comparisons across many previous experiments conducted in the period 1998 to 2011.

The mean apparent metabolisable energy (AME) content for all sorghum samples with and without enzymes was 16.5 MJ/kg dry matter. The results show that 31 of 33 sorghum samples were unresponsive to the enzyme blend, with one sample showing a significant increase in AME (0.4 MJ/kg dry matter) and the other a significant decrease in AME (0.5 MJ/kg dry matter). The mean AME content value for all triticale samples fed with enzymes was 15.2 MJ/kg dry matter. Four of the five triticales showed significant uplifts in AME ranging from 0.5 to 0.8 MJ/kg dry matter when fed to male chickens, but there were no responses in females. Growth, feed intake and feed conversion were unaffected by the enzyme blend, but were highly significantly different between male and female chickens with males having larger weight gain and feed intake, and poorer feed efficiency.

I. INTRODUCTION

Calibrations based on near infrared (NIR) spectroscopy for estimating the AME content and AME Intake Index of cereal grains for broiler chickens have been developed in Australia (Black et al., 2009). The calibrations were developed from results obtained in the Premium Grains for Livestock Program. Updated NIR calibrations were reported by Black et al. (2010). These new calibrations included results from an extra 55 grains, comprising 30 high screenings grains and 25 grains donated by industry. This greatly improved the ability to predict values for unknown grains and the accuracy of prediction. Further improvements are anticipated when results from an additional 25 industry grains are included in the database. Nevertheless, inspection of the measured versus predicted AME values indicates that the database is sparsely populated with sorghum, particularly with samples having lower than average AME values, and triticale in general. The latter grains are discussed by Tredrea et al. (2012) in these Proceedings.

This paper describes a series of four AME experiments to evaluate a total of 25 sorghum samples donated by industry nutritionists. A number of these samples were chosen on the basis of having lower than average AME determined by NIR measurement. In addition, five triticale and eight sorghum grains fed previously were also assessed in order to provide statistical "connectivity" with all other experiments contributing to the AusScan NIR

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calibrations. All grains were fed with and without a blend of xylanase and phytase enzyme products. This paper describes the responses in grain AME to feed enzymes.

II. MATERIALS AND METHODS

The AME values of grains were determined across a series of conventional energy balance studies involving measurements of feed intake and excreta output as described by Mollah et al. (1983) with minor modifications, and subsequent measurement of gross energy values of feed and excreta by bomb calorimetry.

The experiment was conducted with four separate batches of day-old feather-sexed broiler chickens raised in floor pens on a commercial broiler diet to 22 days of age and then transferred in single-sex groups of five to metabolism cages in controlled temperature rooms. Air temperature was maintained at 26^{o}C at the start of the 7-day experiment and lowered daily until it was 23^{o}C at the end. Experimental diets contained grain, casein, dicalcium phosphate, limestone, DL-methionine, mineral and vitamin premix, salt, and choline chloride. All grains were fed with and without a blend of xylanase (Porzyme 93010 at 50 g/tonne for triticale and Rovabio Excel at 200 g/tonne for sorghum) and phytase (Phyzyme TPT at 50 g/tonne) enzyme products. Dietary treatments were replicated four times (two cages of males and two cages of females). Cold-pressed diets were fed for seven days. The first three days enabled the chickens to adapt to the feeds. During the following four days, all excreta were collected and dried at 85^{o}C. Feed intake was measured during the adaptation and collection phases of the study. Birds were weighed at the start and end of the 7-day period. Dry matter contents of samples of pelleted and milled feeds were measured. Gross energy values of dried excreta and milled feeds were measured with a Parr isoperibol bomb calorimeter. AME of the grain was calculated by subtracting from the total energy intake the energy contribution of casein, which was assumed to be 20.1 MJ/kg dry matter (Annison et al., 1994).

III. RESULTS

The xylanase/phytase enzyme blend had no effect on feed intake, weight gain, feed conversion ratio or grain AME intake during the 7-day energy balance study. There were highly significant differences ($P < 0.01$) between males and females in live weight at the start (934 vs 839 g/bird), weight gain (60.8 vs 58.6 g/bird/day), feed intake (116.7 vs 108.1 g/bird/day), feed conversion (1.96 vs 1.87 g feed to g gain), and grain AME intake (1.54 vs 1.45 MJ/day).

The AME values for 33 sorghum samples are shown in Figure 1. Samples 11 and 14 were the only sorghums affected by enzymes. Sample 11 responded positively to enzymes (16.1 vs 16.5 MJ/kg dry matter), whereas sample 14 responded negatively (16.7 vs 16.2 MJ.kg dry matter). Figure 1 also indicates that eight industry samples were significantly lower than average, and 13 were significantly above average in AME.

The effects of sex and enzyme blend on apparent metabolisable energy values for triticale are shown in figure 2. Four of the five triticales showed significant uplifts in AME ranging from 0.5 to 0.8 MJ/kg dry matter when fed to male chickens, but there were no responses in females.

IV. DISCUSSION

Recent Australian studies indicate that sorghums are, or should be, responsive to exogenous feed enzyme exogenous blends having sufficient xylanase, protease and phytase activities (Selle et al., 2010; Gidley et al., 2011). This contrasts with the general lack of response to the

particular enzyme blend used in the current study. Together with the positive effect of enzymes on sorghum sample 11 and the negative effect on sorghum sample 14, it is clear that further study of the comparative effects of various xylanase products, particularly when used in combination with phytase, is warranted. The recent work of Faizah et al. (2011) suggests that hindgut microflora are not implicated.

In a previous study which contributed to the AusScan data base, we observed numerical but non-significant increases in AME in response to enzymes in both males and females for all five triticale samples used in the present study. Inconsistencies between experiments may reflect differences in experimental sensitivity or may be associated with as yet to be determined differences between males and females in digestive physiology, as speculated by Hughes et al. (2001; 2011). Sex-related responses to exogenous enzymes pose significant problems for nutritionists formulating diets for broilers.

In conclusion, the results show that the particular blend of xylanase and phytase enzymes had no effect on AME values for 31 of out 33 sorghums, but a separate blend of enzymes improved AME values for 4 out of 5 triticale grains fed to male chickens.

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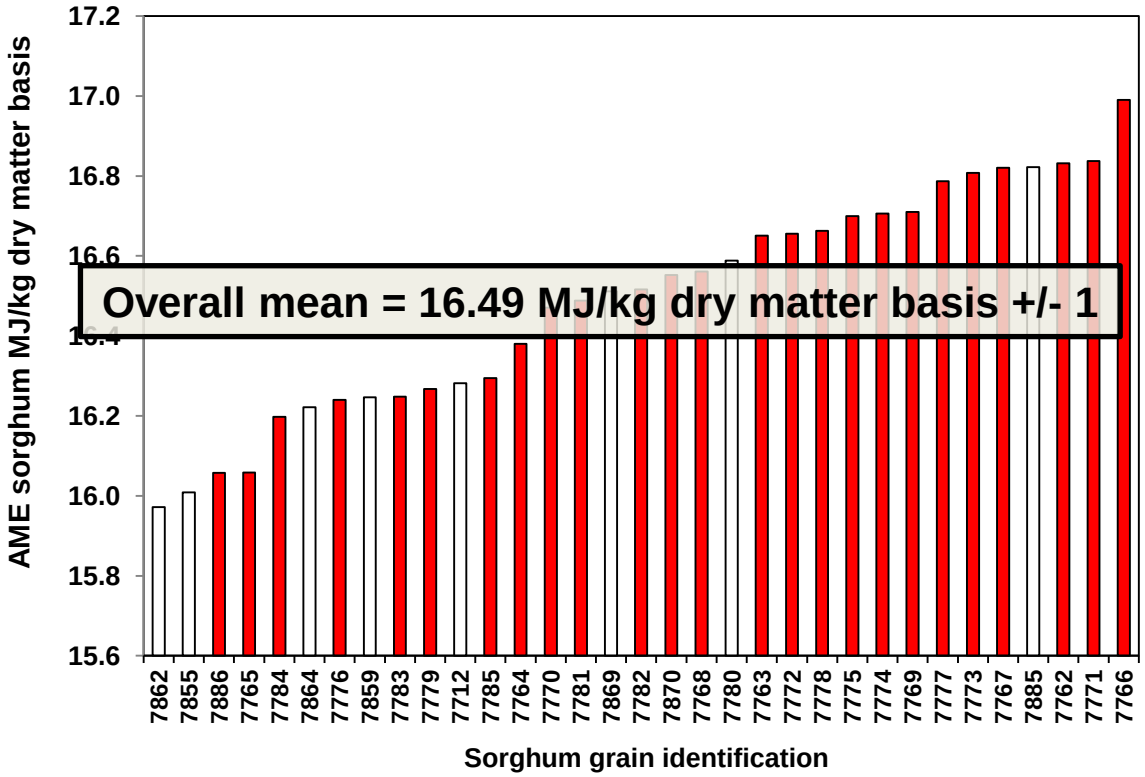

Figure 1. The apparent metabolisable energy values for individual sorghum samples. Samples represented by filled bars were donated by industry nutritionists.

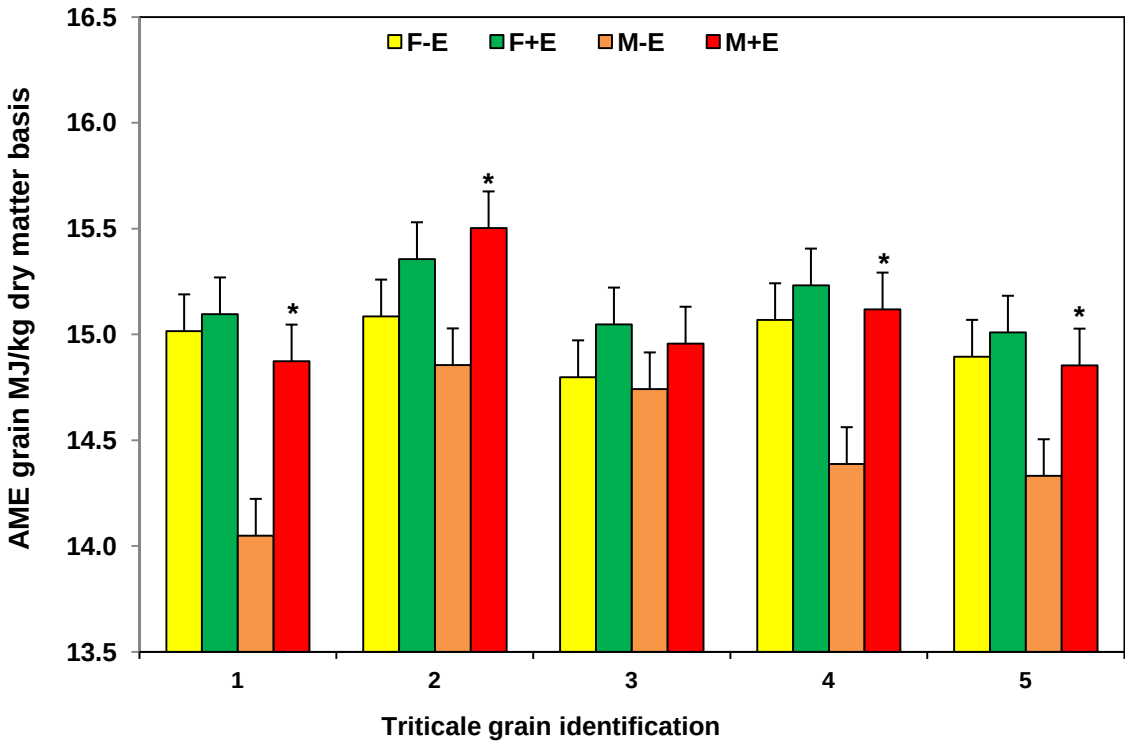

Figure 2. The effects of sex and enzyme blend on apparent metabolisable energy values for triticale. The error bars are standard deviations. An asterisk represents a significant effect ($P < 0.05$) of enzyme within sex.

AUSTRALIAN WHEATS: DEGRADABILITY AND ENZYME RESPONSE

Y.G. LIU[1], C. GADY[2] and P. DALIBARD[2]

The study reported here assessed composition of proximate nutrients, amino acids and the impact of a non-starch polysaccharide (NSP) degrading enzyme on the *in vitro* dry matter degradability of six wheat samples harvested in Australia in the year 2011. The analyses carried out in Carat Lab, France showed that the proximate composition of the six wheat samples was in the normal range, in %: dry matter 87.1 - 88.1; crude protein 11.1 - 13.4; fat 2.1 - 2.6; NDF 9.9 - 11.5; ADF 2.5 - 3.0; lysine 0.30-0.33; methionine 0.17-0.20; cystine 0.27-0.30; threonine 0.35-0.41; tryptophan 0.15-0.19.

The determination of dry matter degradability followed the *in vitro* method of McNiven *et al.*, (2002). Each treatment used 8 replicate nylon bags (Linen Bultex 50µm, 14x12), with 1 g of wheat in each bag. The bags were placed into a flask containing 67 ml buffer solution (pH 5.2), incubated in water bath for 40°C with agitation for 2 h, washed, drained and dried in an oven at 60°C overnight. The enzyme used was Rovabio Excel (xylanase, β-glucanase plus various side activities) and was included at 1 ml for each 8 replicates.

Table 1. *In vitro* degradability of dry matter with and without enzymes (Mean and SD)

Wheat sample	**Dry Matter %**	**Control %**	**+ Enzyme %**	**Increase %**
Tli19449	90.9	45.3 ± 2.0	85.6 ± 3.2	89.2
Tli079503	90.7	54.7 ± 4.0	86.9 ± 1.5	58.8
Tli079428	91.3	41.6 ± 4.0	82.6 ± 7.2	98.3
NTH NSW	91.2	41.4 ± 2.4	80.1 ± 7.2	93.4
STH QLD	91.1	38.1 ± 2.2	85.7 ± 2.6	124.8
Mill Tare	90.6	47.4 ± 1.2	84.4 ± 1.3	78.0
Mean	91.0	44.8 ± 2.6	84.2 ± 3.8	90.4

The results showed that the *in vitro* dry matter degradability of the 6 wheat samples varied from 38.1 to 54.7%, average of 44.8%. Enzyme addition increased the degradability of all samples, from 78.0 to 124.8%.

Early studies on various wheat samples from Europe revealed a strong negative correlation (R^2=0.90) between dry matter degradability and the enzyme responses (Preynat *et al.*, 2009). Results of this study showed the same trend. The highest enzyme response (124.8%) was obtained from the sample STH QLD that had the lowest degradability (38.1%); whereas sample Tli79503 had high degradability (54.7%) but with the lowest enzyme response (58.8%). The results suggest that the enzyme can significantly enhance dry matter degradability of all wheats especially the ones of low degradability. The authors also investigated correlations between the dry matter degradability and *in vivo* digestibility or AME values of these wheat samples. Our initial study with broiler birds did not find a strong correlation, as anticipated. Further *in vivo* studies (DAA, AME) on these 6 samples are in progress.

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MICROBIAL PHYTASE INFLUENCES KINETICS OF STARCH-PROTEIN DIGESTION IN BROILER CHICKENS

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While it is clear that microbial phytase improves phosphorus and calcium retention, the effects on protein and energy utilisation are equivocal. The aim of this study was to investigate the effects of phytase on the kinetics of starch and protein digestion.

In a 21-day deep litter trial, day-old male broilers (n = 840) were offered diets based on wheat and soybean meal (7.5 g/kg Ca, 4.0 g/kg nonphytate-P) and four phytase inclusion levels (0, 500, 1000, 2000 FTU/kg). The diets were steam-pelleted at ~ 85°C and each dietary treatment was offered to 6 pens (35 birds/pen). Body weights, feed intake and mortalities were recorded. On day 21, five birds from each pen were euthanized and digesta collected from the proximal jejunum, proximal and distal ileum to determine apparent starch and nitrogen digestibility. Since the rate of nutrients absorption potentially limits growth performance in hatchlings and growing birds, nutrient uptake rates (daily nutrient intakes × apparent digestibility coefficients) at the three sites were introduced to evaluate phytase influences. Starch and protein uptake rates are shown in the Table.

Table 1. Phytase inclusion effects on uptake rates of starch and protein

	Starch uptake rate (g/day)			Protein uptake rate (g/day)			uptake ratio (Starch/protein)		
Phytase (FTU/kg)	Proximal jejunum	Proximal ileum	Distal ileum	Proximal jejunum	Proximal ileum	Distal ileum	Proximal jejunum	Proximal ileum	Distal ileum
0	16.58^{a}	20.98^{a}	21.98^{a}	7.10^{a}	8.04^{a}	8.38^{a}	2.34^{ab}	2.61^{b}	2.62^{b}
500	18.27^{ab}	24.21^{ab}	25.11^{b}	6.98^{a}	8.67^{a}	8.83^{a}	2.73^{b}	2.77^{c}	2.84^{c}
1000	20.19^{b}	23.31^{ab}	24.92^{b}	7.88^{ab}	8.35^{a}	8.67^{a}	2.58^{ab}	2.79^{c}	2.88^{c}
2000	19.54^{ab}	23.64^{b}	24.10^{b}	9.41^{b}	9.95^{b}	10.06^{b}	2.10^{a}	2.38^{a}	2.42^{a}
SEM	0.8610	0.7800	0.6446	0.4941	0.2733	0.2335	0.1323	0.0428	0.0258
Significance (P=)	0.037	0.041	0.009	0.012	0.000	0.000	0.016	0.000	0.000
Linear effect(P=)	0.027	0.118	0.103	0.001	0.000	0.000	0.103	0.008	0.017

Means with different superscripts are significantly different (P<0.05)

Increasing phytase inclusion from 0 to 2000 FTU/kg improved starch uptake rates at all the three sites in small intestine (17.8%, 12.7% and 9.6% respectively). Protein uptake rate increased significantly until the phytase supplementation reached 2000 FTU/kg. At this level of supplementation, phytase drove up protein uptake rates at each site by 32.5%, 23.8% and 20.0% respectively. This dramatic improvement tended to reduce the starch and protein uptake rates ratio as well, which was not found in 500 and 1000 FTU/kg phytase. High phytase inclusions were associated with improved feed conversion ratios and superior weight gain (data not shown). These beneficial effects may be explained by more synchronous absorption of glucose and amino acids from the small intestine in addition to enhanced protein digestion. It appears that high dietary phytase inclusion may synchronize the absorption of glucose and amino acids from the small intestine and this better balance in nutrient uptake rates may enhance energy and protein utilisation.

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MAXIMISING THE ENERGY VALUE OF CASSAVA PRODUCTS IN DIETS FOR BROILER CHICKENS

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Summary

The objective of this study was to investigate the utilization of energy as well as performance of broiler chickens on diets based on cassava chips and pellets with or without enzyme supplements. Feed intake to day 21 was lower ($P < 0.01$) on the diet containing cassava chips than on diets with maize or cassava pellets, in spite of enzyme supplementation and enzyme supplements improved ($P < 0.01$) feed intake on all diets. Live weight at day 21 was also significantly reduced ($P < 0.01$) on the diet based on cassava chips but improved ($P < 0.01$) by the enzyme supplements. Metabolizable energy intake was reduced ($P < 0.01$) by both cassava chips and pellets but was improved ($P < 0.01$) on all diets by enzyme supplementation. A similar trend was observed for net energy of production (NEp), generally being higher ($P < 0.01$) on the maize-based diets than on diets containing cassava. Enzyme supplementation improved ($P < 0.01$) NEp. Heat production was highest ($P < 0.01$) on diets containing cassava pellets. In general, it may be possible to use cassava pellets in diets for broiler chickens at close to 50 % of the diet to reduce cost, and the nutritive value of such diets can be improved through supplementation with appropriate microbial enzymes.

I. INTRODUCTION

Cassava chips and pellets are two alternatives to cereal grains in cassava-producing areas for example, Asia or other regions that import these products. Both products are not currently used by the poultry industry in Australia but their use may increase as the pressure on grain supplies intensifies. In Asia and Africa cassava products are used in animal feeding to replace some or all of the cereal grain in diets for poultry. A study by Brum *et al.* (1990) showed that up to 66.7 % of maize in broiler diets can be replaced by cassava meal without adversely compromising growth performance. Other researchers have reported variable response on diets containing cassava products such as chips and pellets in broiler diets (Obikaonu and Udedibie, 2006). These inconsistencies may be due to differences in cultivars or product processing prior to feeding. In general, the utilization of energy in cassava products by poultry has not been extensively studied, particularly under controlled environments. This is an area that needs to be investigated, in order to position cassava as an alternative source of energy in poultry diets. The current research focused on improving carbohydrate digestion and energy value of cassava products in diets for broiler chickens.

II. MATERIALS AND METHODS

The two products, cassava chips and pellets, were received from Thailand and analysed for nutrient composition prior to feed formulation. The cassava chips and pellets contained (g/kg), CP 22, 21; crude fat 12, 15, and starch 750, 680. Lysine and methionine were (g/kg protein) 77, 75, and 17, 20 (g/kg protein) while calculated ME was 12.63 and 11.65 MJ/kg, respectively. The ME content of cassava chips and pellets was calculated according to the

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equation [ME (Kcal/kg) = 53+38 × (% CP + 2.25 × % EE + 1.1 × % Starch + % Sugar)] developed by Carpenter and Clegg (1956). In this trial, 384 one-day-old male Cobb broiler chicks were used in a 3 x 2 factorial design to compare the two cassava products (chips and pellets) with maize and with or without microbial enzyme (carbohydrase and phytase) supplementation. Each diet was replicated 8 times, with 8 chicks per replicate. The chicks were reared in cages in an environmentally controlled house. Semi-purified diets were formulated to contain the cassava products (50%) or maize (58%) as sole energy source in the diet with or without enzyme supplementation. All diets were iso-caloric and iso-nitrogenous. The ingredients were ground through a hammer mill, mixed to obtain a complete diet and then pelleted using a cold pelleter. The AME was measured through marker (TiO_2)-based faecal collection between 18 and 21 days of age. The representative faecal sample was collected from each cage over three days, pooled, freeze-dried and analysed. At 21 days, 2 chicks per replicate were randomly selected and slaughtered through cervical dislocation. The carcasses were minced and a sub-sample was freeze-dried. The meat samples were analysed for energy, protein and fat contents. The diets and excreta samples were also analysed for these nutrients and TiO_2. The data were used to calculate nutrient retention, net energy of production (NEp), heat production and efficiencies of utilization of metabolizable energy for protein, fat and energy retention as described by Olukosi *et al* (2008). Data were analysed in accordance with the general linear model of SPSS, version 18 (SPSS Inc, 2010).

III. RESULTS AND DISCUSSION

Feed intake up to d 21 was lower ($P < 0.01$) on the diet containing cassava chips than on diets with maize or cassava pellets, regardless of enzyme supplementation (Table 1). The enzyme supplements improved ($P < 0.01$) feed intake on all diets. Live weight at day 21 was also significantly reduced ($P < 0.01$) on the diet based on cassava chips but improved ($P < 0.01$) by the enzyme supplements. There was a significant increase ($P < 0.01$) in FCR in groups with the cassava products but this tended ($P<0.08$) to be improved by enzyme supplementation. The decrease in feed intake of the cassava chips-based diet may be due to the viscous nature of the cassava products, especially at high temperature. In solution, the material may create a gut-filling effect, thereby reducing appetite. However, the enzyme supplements improve feed intake in all diets, which is in agreement with the findings of Kumar and Dingle (1996) in research on cassava supplemented with a yeast product. There was an increase in live weight with the inclusion of enzymes, similar to reports by Akinfala *et al*., (2009) who observed a beneficial effect of feed additives in cassava-based diets fed to broiler chickens.

Metabolizable energy intake was reduced ($P < 0.01$) by both cassava chips and pellets but was improved ($P < 0.01$) on all diets by enzyme supplementation (Table 2). A similar trend was observed for NEp, generally being higher ($P < 0.01$) on the maize-based diets than on diets containing cassava. Enzyme supplementation improved ($P < 0.01$) NEp. Heat production was highest ($P < 0.01$) on diets containing cassava pellets and further increased ($P < 0.01$) by enzyme supplementation on all diets. This agrees with Iji *et al.,* (2011) who reported that NEp and heat production were reduced by cassava pulp but were improved by enzyme supplements similar to those used in the present study. There may be a heat increment associated with further digestion of the product, even though that also led to an improvement in NEp. More ($P < 0.01$) energy was retained as protein and fat in the maize-based diets than on diets containing cassava and this was increased ($P < 0.01$) on all diets as a result of enzyme supplementation. The efficiencies of utilization of ME for energy, lipid, and protein retention were reduced ($P < 0.01$) by the cassava products but these were unaffected by enzyme supplementation.

In conclusion, it is possible to use cassava pellets in diets for broiler chickens at around 50 % of the diet. The nutritive value as well as efficiencies of utilization of nutrients of such diets can be further improved by use of appropriate microbial enzymes supplements.

Table 1. Feed intake, body weight and FCR of broiler chickens at 21 days of age on diets based on cassava products with or without microbial enzymes

	Enzyme	FI (g/bird)	BW (g/bird)	FCR (g:g)
Maize (control)	–	948.5^{bc}	696.1^{b}	1.46^{d}
	+	1199.7^{a}	881.7^{a}	1.44^{d}
Cassava Chips	–	754.9^{d}	453.5^{e}	1.86^{a}
	+	940.1^{c}	574.6^{d}	1.79^{ab}
Cassava Pellets	–	1006.4^{b}	637.1^{c}	1.71^{bc}
	+	1162.5^{a}	749.4^{b}	1.67^{c}
Pooled SEM		*23.136*	*21.100*	*0.026*
Model P		*<0.001*	*<0.001*	*<0.001*
Source of variation				
Energy base		*<0.01*	*<0.01*	*<0.01*
Enzyme		*<0.01*	*<0.01*	*0.08*
Energy base x enzymes		*0.08*	*NS*	*NS*

$^{a, b, c, d, e}$ Values with unlike superscripts within each column are significantly different at the levels indicated ; NS = Not significant (P > 0.05); SEM = Standard error of mean.

Table 2. Metabolizable energy (ME) intake, net energy of production (NEp), heat production (HP), energy retained and efficiencies of ME use for energy, lipid and protein retention in broiler on the different diets with or without enzyme supplementation to 21 days

Treatment			Energy utilization (kj/day)		Energy retained (kj/day) as		Efficiencies of ME use for energy, lipid and protein retention		
	Enzyme	ME intake (kj/d)	NEp	HP	Protein	Fat	Energy	Lipid	Protein
Maize (control)	–	557.9^{c}	253.6^{b}	304.3^{d}	96.0^{ab}	130.5^{b}	0.45^{a}	0.23^{a}	0.17^{a}
	+	704.8^{a}	326.6^{a}	378.2^{ab}	108.3^{a}	166.6^{a}	0.46^{a}	0.24^{a}	0.15^{ab}
Cassava Chips	–	465.4^{d}	157.1^{d}	308.3^{d}	52.4^{d}	75.3^{e}	0.34^{c}	0.16^{c}	0.11^{c}
	+	549.3^{c}	197.0^{c}	352.3^{c}	68.3^{c}	100.2^{d}	0.36^{bc}	0.18^{bc}	0.12^{c}
Cassava pellets	–	587.9^{c}	216.1^{c}	371.8^{bc}	87.9^{b}	107.5^{cd}	0.37^{bc}	0.18^{bc}	0.15^{ab}
	+	649.6^{b}	256.9^{b}	396.8^{a}	111.2^{a}	126.0^{bc}	0.39^{b}	0.19^{b}	0.17^{a}
Pooled SEM		*13.92*	*9.93*	*6.55*	*4.07*	*5.58*	*0.01*	*0.01*	*0.01*
Model P		*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*
Source of variation									
Energy base		*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*
Enzyme		*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*0.08*	*NS*	*NS*
Energy base × enzymes		*<0.01*	*NS*	*<0.01*	*NS*	*NS*	*NS*	*NS*	*<0.02*

$^{a, b, c, d, e}$ Values with unlike superscripts within each column are significantly different at the levels indicated ; NS = Not significant (P > 0.05); SEM = Standard error of mean.

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ENERGY UTILIZATION AND GROWTH RESPONSES OF BROILER CHICKENS ON VEGETABLE PROTEIN PROTEIN DIETS

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Summary

This study was undertaken to investigate the gross response and energy utilization of broiler chicks fed on vegetable protein or conventional diets. Two hundred and fifty-two day-old Cobb-500 male broiler chicks were randomly assigned to five experimental groups and raised on a control diet (containing tallow) or diets containing fish meal (SBM50 and Can50) or diets with no animal products (SBM75; Can75) (predominantly soybean or canola meal). Birds were reared mainly on litter under similar environmental and management conditions from 1 to 21 days on starter diets. Feed intake was highest ($P<0.001$) on the SBM50 and Can50 diets, and lowest on the SBM75 diet. Birds in the SBM50 and Can50 diet groups were heavier ($P<0.001$) than those in other groups, with SBM75 and Can75 diet groups being the lightest. Birds on SBM75 and Can50 achieved superior feed conversion ratio (FCR), while birds on Can75 diet were the poorest. The dietary apparent metabolisable energy (AME) contents were similar, but ME intake on the SBM50 and Can50 diets was higher ($P<0.001$) than in other groups. Heat production (HP) was similar, but net energy of production (NEp) was improved ($P<0.05$) in the birds on SBM50 and Can50. Birds on SBM50, Can50 and Control diet groups retained higher ($P<0.05$) energy as fat (RE_f), while energy retention as protein (REp) was highest ($P<0.05$) in the SBM50 and Can50 diet groups. The efficiencies of utilization of ME for energy (K_{RE}), protein (K_{REp}) and fat (K_{REf}) retentions were unaffected. The results demonstrated that birds on the conventional diets (SBM50; Can50) utilized energy better, and grew faster than the birds on vegetable protein (SBM75; Can75) and Control diets.

I. INTRODUCTION

Feed costs represent a major cost in poultry production (about 70%), with energy sources occupying the greatest portion (70 to 75% of the diets) (Van der Klis et al., 2010). Birds are prone to feed mainly to satisfy their energy requirement and, once this is met, they will not consume any more feed even if the requirements for other nutrients like protein, vitamins or minerals have not been met (Sing and Panda, 1992). For this reason, the energy content of the diet represents a central theme in diet formulation for poultry. The performance of birds is closely associated with feed nutrient and energy utilization, which is primarily related to availability of more nutrients and energy from the feed ingredients (Olukosi et al., 2008).

Most of the dietary energy comes from plant sources in the form of carbohydrates from cereal grains. Around the world, soybean is the premier vegetable source of protein while canola seed meal is also gaining in popularity. Animal protein sources are usually more balanced in amino acids than vegetable protein sources but there is a wide variation in the material that is used around the world. Processed meals from abattoirs, such as meat meal, meat/bone meal, blood meal, feather meal, etc. are common in countries with a well developed animal processing industry. In major fishing areas in Asia, South America and Africa, fishmeal is also an important animal protein source. The main advantage that plant protein sources hold is their safety from zoonotic diseases. In a previous study, we showed

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that diets containing fishmeal supported better growth than diets based entirely on vegetable protein sources (Hossain et al., 2011). In the current study, we examined the utilization of energy and efficiency of utilization of this energy in protein and fat in broiler chickens raised on diets containing fishmeal or vegetable protein.

II. MATERIALS AND METHODS

A total of 252 day-old Cobb-500 male broiler chicks were assigned to five dietary treatments, each with six replicates, and eight chicks per replicate in a completely randomized design. Two diets (SBM75 and Can75) contained only vegetable proteins, with soybean and canola meals combined in a 3:1 ratio (Table 1). The other two diets (SBM50 and Can50) were conventional, formulated with soybean meal and canola meal at a ratio of 2:1, along with fish meal. A fifth diet, used as control, was a commercial type diet containing canola, soybean, mung beans and tallow mixer in addition to the basal cereal grains. All diets were iso-caloric and iso-nitrogenous, supplemented with carbohydrase and phytase enzymes (Danisco Animal Nutrition, UK), and supplied in pelleted form. All diets were formulated to meet or exceed the NRC (1994) nutrient requirements for broiler chickens between hatch and 21 days.

Table 1. Composition of diets fed during the trial (1-21days)

	Diets				
	SBM75	Can75	SBM50	Can50	Control
Ingredient composition (g/kg)					
Maize	405.9	377.5	412.7	617.1	0.0
Wheat	210.0	187.5	203.8	0.0	260.0
Sorghum	0.0	0.0	0.0	0.0	320
Vegetable oil	0.0	20.3	0.0	0.0	0.0
Tallow mixer	0.0	0.0	0.0	0.0	10.0
Soybean meal	246.9	93.8	154.9	84.0	200.0
Canola meal	82.3	281.25	77.45	167.9	52.5
Fishmeal	0.0	0.0	77.5	84.0	0.0
Mung bean	0.0	0.0	0.0	0.0	100.0
Key nutrient composition (g/kg)					
ME (MJ/kg)	12.38	12.38	12.39	12.38	12.37
Crude protein	210.0	211.1	211.1	211.5	210.8
Ether extract	24.0	28.4	29.3	32.2	24.0
Calcium	11.5	10.5	12.8	12.4	12.3
Available P	5.2	5.3	6.4	6.2	6.0
Lysine	12.8	12.1	13.7	13.4	13.0
Methionine	5.5	5.2	6.4	6.3	5.1
Threonine	8.2	8.4	8.4	8.5	6.8
Sodium	1.8	1.8	2.2	2.1	1.9
Chlorine	2.5	2.7	2.7	2.5	2.9

Diets also contained Limestone, dicalcium phosphate, DL-methionine, lysine, sodium chloride, vitamin-mineral premix, choline chloride, microbial enzymes, Zinc bacitracin and marker (TiO_2).

A total of 30 cages were set up on litter in climate-controlled rooms to accommodate the birds for 21 days. On day 18, four birds from each cage were transferred to metabolic cages in order to collect excreta samples for the last three days of the trial period. These samples were used to determine AME. The birds were brooded at 33°C for the first two days, after which the temperature was gradually reduced to 24°C at 19 days of age, and maintained

until the end of the trial period. Sixteen hours of lighting per day were provided throughout the trial period. Feed and water were provided *ad libitum*. Starter diets (ME 12.37 MJ/kg; CP 210g/kg) were used throughout the trial period.

The gross response of birds in terms of feed intake, body weight and FCR was assessed weekly. The AME contents of the diets were determined and the comparative slaughter technique was used to assess energy utilization according to the methods described by Olukosi et al. (2008). These data were used to derive values of net energy of production (NEp), heat production (HP), and efficiencies of utilization of ME for energy (K_{RE}), protein (K_{REp}) and fat (K_{REf}) retention. All data were subjected to statistical analysis, using Minitab software (Minitab, 2010).

III. RESULTS

Birds on the SBM50 and Can50 diet groups ate more (P<0.001) and were heavier (P<0.001) than the birds in the other dietary groups. Birds of the Can75 diet group were the poorest (P<0.001), while the birds of SBM50 dietary group were the best in terms of feed conversion. Birds on the SBM75 diet were similar to the birds of the Control group in FCR throughout the trial period.

Table 2. Metabolizable energy content of diets, metabolizable energy intake (MEI), protein intake, heat production (HP) and net energy of production (NEp) of different diets fed to broilers

	SBM75	Can75	SBM50	Can50	Control	Pooled SEM
ME (MJ/kg)	12.3	12.1	12.3	12.3	12.1	0.05
MEintake (KJ/d/bird)	567.9^{c}	592.0^{c}	686.6^{a}	672.2^{a}	634.2^{b}	4.00***
Protein intake (g/b)	48.0	44.7	49.9	49.1	47.6	1.17
HP (KJ/d)	304.7	325.7	358.7	346.0	346.3	6.91
NEp (KJ/d)	263.2^{b}	266.3^{b}	327.9^{a}	326.2^{a}	287.9^{ab}	7.38*

Data (calculated on DM basis) denote mean values of six replicate cages with four broilers per replicate cage at 21 days; ^{a,b,c}Means bearing uncommon superscripts within a row are significantly different (*P<0.05 and ***P<0.001); SEM= Pooled standard error of mean.

The dietary AME content, ME intake, protein intake, HP and NEp of different treatment groups are shown in Table 2. The dietary AME content varied from 12.1 to 12.3 MJ/kg and was unaffected by treatment. The ME intake but not protein intake was increased (P<0.001) in the SBM50 and Can50 dietary groups. Heat production of broiler chickens was found to be similar (P>0.05) between the different dietary groups, while NEp was significantly increased (P<0.05) in SBM50 and Can50 dietary groups.

Birds on SBM50, Can50 and Control dietary groups retained similar but higher (P<0.05) amounts of energy as fat than the birds in the other diet groups (SBM75 and Can75) (Table 3). Energy retention as protein was highest (P<0.05) for the SBM50 and Can50 diets, while birds on Can75, SBM75 and Control diets retained the least energy as protein. The $K_{RE,}$ K_{REp} and K_{Ref} ranged between 0.45 to 0.49, 0.22 to 0.26 and 0.21 to 0.24, respectively, and were generally not affected by dietary treatment.

IV. DISCUSSION

Broiler chickens raised on conventional diets demonstrated significantly improved live weight through increased feed consumption and superior FCR, as compared with the other treatment groups. The poor growth on the vegetable protein diets may be due to a reduction in ME intake on those diets. This reduction in ME intake may be due to high fibre contents and presence of anti-nutritive factors contained in these diets, as has been observed by Barteczko et al. (2008).

Table 3**.** Carcass energy retention as fat (REf) and as protein (REp) and efficiencies of ME used for energy, fat and protein deposition in broilers receiving different diets

	SBM75	Can75	SBM50	Can50	Control	Pooled SEM
Energy retention						
REf(KJ/d)	131.8^{bc}	123.0^{c}	161.3^{a}	159.6^{a}	150.3^{ab}	3.54
REp(KJ/d)	142.5^{b}	130.1^{b}	176.9^{a}	169.5^{a}	154.1^{b}	3.87
Efficiencies of energy utilization						
K_{RE}	0.46	0.45	0.47	0.49	0.45	0.011
K_{Rep}	0.25	0.22	0.26	0.25	0.24	0.006
K_{Ref}	0.23	0.21	0.23	0.24	0.24	0.005

Data denote mean values of six replicate cages with four broilers per replicate cage at 21 days. ^{a,b,c}Means bearing uncommon superscripts within a row are significantly different ($P<0.05$). SEM = Pooled standard error of mean.

Broilers on the conventional diets also used energy better as shown by the higher NEp, without significant differences in HP. More energy was retained as protein than as fat, especially on the conventional diets. This also represents a more efficient use of energy and the carcass would be less fatty as a consequence. In this study, the birds were still in the active growing phase of production, and had not reached the stage at which fat accumulation would be significant.

V.CONCLUSION

It can be concluded that birds on the conventional dietary groups had superior feed intake, higher body weight and better FCR than birds on the alternative diets, partly as a result of better energy utilization.

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COMPARING A THERMOTOLERANT XYLANASE WITH A MULTI-ENZYME BLEND IN WHEAT-BASED BROILER DIETS

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Summary

A total of 1920 Ross 308 day-old male broilers were assigned to 3 diets with 8 replicates of 20 birds per diet. A starter crumbled pelleted diet was fed from 0-21 days and a pelleted grower diet from 21-42 days. Diets were based on wheat and soybean meal, were pelleted at 70°C, and were either unsupplemented or supplemented with a new thermotolerant xylanase (XT) or a xylanase multi- enzyme blend (WP). Diets were formulated with a standard wheat ME value or with an ME value of the wheat increased by 6%, 8% or 10% this being equivalent to a reduction in dietary ME of 0.48, 0.65 and 0.84 MJ/kg, respectively. Day 42 liveweights were over 3 kg at an average FCR of less than 1.70. Increasing the matrix energy value of wheat tended to increase feed intake and thus FCR ($P < 0.05$). Xylanase supplementation improved performance at 42 days, with average live weight-corrected FCR being 9 points lower than control ($P < 0.05$), and with no interaction ($P > 0.05$) between dietary ME and enzyme addition. Enzyme addition compensated for the 10% increase in the matrix ME of the wheat by restoring performance to at least the level seen in the standard control diet. The birds fed the new xylanase tended to outperform those fed diets supplemented with the xylanase-based multi-enzyme blend.

I. INTRODUCTION

Wheat based diets are routinely supplemented with xylanases as a means to increase digestibility and reduce the incidence of wet litter (Bedford 2000). Traditionally these enzymes have been used commercially to increase the ME of wheat by approximately 6%. In this way the energy value assigned to the enzyme is intrinsically associated with the inclusion level of the substrate. Most commercial xylanases have been in use for over 15 years and have well established matrices. The introduction of a new product necessitates comparison with the incumbents so that the suitability of a similar matrix can be confirmed. In particular, new, monocomponent xylanases may display different values compared to more multi-enzyme products, particularly if the ancillary activities of the multi-enzymes are essential for efficacy. As a result, the objective of the current experiment was to compare performances of a new, highly thermostable monocomponent xylanase, with that of a well established multi-enzyme blend containing xylanase in a wheat based diet where wheat energy levels assigned to the wheat varied by 6 to 10%. Although one of the objectives of developing the new xylanase was that it is thermotolerant, testing this characteristic was not the purpose of this trial.

II. MATERIALS AND METHODS

1920 Ross x Ross 308 male broilers were fed wheat-soy test diets from 1 to 42 days of age. Eight replicate cages of 20 birds per cage (7 animals/m^2) were allocated to one of 12 treatments in which wheat was formulated into a starter and grower ration at standard, +6, +8 or +10% ME which amounted to 12.87, 13.64, 13.90 or 14.16 MJ/kg of wheat

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respectively. Into each of these 4 basal diets was included a standard dose of Econase XT (XT, new thermotolerant xylanase), Wheat Plus (WP, a traditional *Trichoderma* xylanase) or no enzyme at all for a 4 x 3 factorial design. Feed (pelleted at 70°C) and water were offered. A starter formulation was fed from 1-21d of age and a grower from 21-42d of age *ad libitum*. Diets were formulated to meet all nutrient requirements with the exception of energy in those diets where the energy matrix of wheat was increased. Birds were weighed and feed consumption determined at day 21 and 42, and FCR calculated for the same periods. At 21 d of age, 2 birds per pen of average cage weight were sacrificed and the small intestine from the end of the duodenal loop to Meckels diverticulum excised and the contents removed by gently squeezing between fingertips into a small beaker. This was carefully mixed and viscosity determined as per the method of Bedford and Classen (Bedford and Classen 1992).
The trial was designed as a completely randomised design and analysed using the model Y= initial weight, AME uplift %, Enzyme and AME uplift %* Enzyme. If initial weight was not significant it was removed and the model re-run. Cage was the observational unit.

III. RESULTS AND DISCUSSION

The results are shown in Tables 1 and 2. Overall performance was excellent with the birds being well ahead of the breeder gain target of 2.8kg and 1.70 FCR at 42d. Nevertheless there were still depressions in performance with energy being taken out of the diet (i.e. by increasing AME content of wheat).

a) 0-21d of age

No interactions were noted at 21 d of age but significant effects of enzyme on gain, FCR and viscosity were noted along with significant effects of AME uplift on FCR (and a very strong tendency in gain, P = 0.0532). In all cases where the enzyme effect was significant, addition of either enzyme enhanced gain, FCR and reduced viscosity compared with the control treatment with no differences between enzymes being apparent.

b) 0-42d of age

No interactions were noted at 42d of age with the significant main effects of enzyme being on intake and FCR plus an effect of AME on FCR. Feed intake was reduced with the addition of either WP or XT which is expected in low viscosity diets with the effect tending to be more evident in XT versus WP supplemented rations (contrast P = 0.20).
The FCR improved with addition of either enzyme and deteriorated with increased wheat energy uplift. On average, XT improved FCR by 1.6 points more than WP (P = 0.076) suggesting that there is a difference in performance between these two enzymes in wheat based diets. The differences between these enzymes only became apparent at 42d of age.

The wheat AME uplift resulted in reduced performance in the absence of enzymes. Enzyme addition resulted in restoration of performance, particularly with XT. Both enzymes were able to compensate for a 10% uplift in wheat AME. The new xylanase performed similarly to the multi-enzyme blend in WP which may suggest that the ancillary enzymes present in WP were not essential for efficacy in these wheat based diets.

Table 1 The effect of enzyme and wheat energy value uplift on 0-21d performance

AME uplift	Enzyme	Intake	Gain	FCR	Viscosity
0		1217	902	1.349	2.81
6		1213	884	1.374	2.79
8		1224	896	1.366	2.94
10		1225	883	1.388	3.01
	None	1232	881	1.400	3.75
	WP	1214	897	1.354	2.55
	XT	1213	896	1.355	2.38
Initial weight		**0.0007**	0.0995	**0.0394**	
AME uplift		0.6976	0.0532	**0.0148**	0.7444
Enzyme		0.1201	**0.0426**	**0.0000**	**0.0000**
AME uplift * Enzyme		0.3831	0.0607	0.2782	0.3165

Table 2 The effect of enzyme and wheat energy value uplift on 0-42d performance

AME uplift	Enzyme	Intake	Gain	FCR	Mortalilty
0		5053	3058	1.674	3.1
6		5048	3013	1.695	1.9
8		5086	3023	1.703	1.7
10		5174	3032	1.725	1.7
	None	5176	3001	1.743	1.7
	WP	5075	3052	1.686	2.3
	XT	5019	3041	1.670	2.2
		ANOVA			
Initial weight		**0.0221**	**0.0016**		
AME uplift		0.0513	0.4257	**0.0001**	0.3404
Enzyme		**0.0020**	0.1009	**0.0000**	0.7234
AME uplift * Enzyme		0.7604	0.6462	0.4441	0.9546

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OVIPOSITION FEEDING: EFFECT OF REDUCED ENERGY AND PROTEIN LEVELS ON PERFORMANCE OF LAYING HENS

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Summary

The following trial was conducted as part of an on-going project investigating the effect of oviposition determined feeding system on hen performance. One hundred and forty, individually caged, ISA Brown laying hens, were divided into five groups of 28 replicates to investigate the effects of feeding two diets a day with different AME and crude protein levels from 65 to 80 weeks of age. Treatment 1 consisted of a feed with an AME level of 2750 kcal/kg and a crude protein level of 170 g/kg available the whole day. This feed was also provided in treatments 2, 3 and 4 during the morning. During the afternoon, the feed contained 2650 kcal/kg AME and 155 g/kg crude protein in treatment 2, 2650 kcal/kg AME and 140 g/kg crude protein in treatment 3 and 2750 kcal/kg AME and 140 g/kg crude protein in treatment 4. In treatment 5, a feed with an AME level of 2750 kcal/kg and a crude protein level of 155 g/kg was provided during the whole day. The results of the experiment indicate that there is a possibility of reducing the AME content of the afternoon feed from 2750 to 2650 kcal/kg in the period from 65 to 80 weeks without negatively affecting performance. The crude protein content can be reduced from 170 to 155 g/kg in both the morning and afternoon feed from 65 to 80 weeks of age. Both measures translate into feed cost savings.

I. INTRODUCTION

In a previous experiment, presented also at this meeting (de los Mozos et al., 2012), the hypothesis of providing a high energy, high protein and low calcium feed during the morning and a low energy, low protein and high calcium feed during the afternoon was tested. In this previous experiment, performance was maintained fairly well at a significantly lower daily energy and protein intake with this feeding programme compared to the control group in which a normal feed was provided. It was concluded that the utilization of nutrients was improved by feeding two different feeds during the day. Since in that experiment, the moment of oviposition was determined for each hen individually and the hen was fed according to this, it was also concluded that oviposition feeding should be tested on the basis of a group mean for the moment of oviposition during a longer period of time. Therefore, this second experiment was conducted.

According to Etches (1996) and Keshavarz (1998a; 1998b), varying dietary crude protein levels during the day may not improve egg weight and protein efficiency. A reduction in dietary energy levels might contribute to a reduction in feed costs, and therefore, this experiment was focussed on a reduction in crude protein and energy levels. The calcium level was not increased in the feed that was provided in the afternoon, since Novo et al. (1997), Keshavarz (1998b), Waldroup and Hellwig (2000) and Goto et al. (2002) already had demonstrated that providing extra calcium during the afternoon improved eggshell strength significantly.

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II. MATHERIALS AND METHODS

One hundred and forty ISA Brown Classic laying hens, individually caged, at 65 weeks were housed in one facility of the Nutreco Poultry and Rabbit Research Centre. The experiment was performed for a period of 15 weeks. Water and feed were available ad libitum, and hens were exposed to 16 hours of continuous light per day. The experiment included 5 treatments (Table 1) with 28 replicates per treatment in the experiment. Treatment 1 consisted of a feed with an AME level of 2750 kcal/kg and a crude protein level of 170 g/kg available for whole day. This feed was also provided in treatments 2, 3 and 4 during the morning hours. During the afternoon, the feed contained 2650 kcal/kg AME and 155 g/kg crude protein in treatment 2, 2650 kcal/kg AME and 140 g/kg crude protein in treatment 3 and 2750 kcal/kg AME and 140 g/kg crude protein in treatment 4. In treatment 5, a feed with an AME level of 2750 kcal/kg and a crude protein level of 155 g/kg was provided during the whole day. Total calcium and retainable phosphorous were kept constant in all the feeds (40 and 2.7 g/kg respectively). Morning feeds were provided from 07.30 to 14.30 hours and afternoon feeds from 14.30 to 07.30 hours. The aim was to achieve a feed intake distribution of 40 % of the total feed intake during the morning and of 60 % during the afternoon. Morning and afternoon feed intake, egg production and egg weight were recorded weekly. Eggs were collected every morning for the duration of the trial. Once a week, all the eggs from the previous seven days were weighed. Live weight was measured at the beginning and the end of the trial. Whole body composition was analysed for moisture, ash, crude protein and crude fat at the start of the experiment and at the end of the experiment (8 samples per treatment).

Data were analysed by using the general linear models (GLM) procedure of SAS® to determine the effect of treatments on all the studied parameters. Means were separated by using the LS means Test.

III. RESULTS

The effects of providing different feeds during the day on laying hen performance in the period from week 65 to 80 are summarized in Table 1. Lowering the AME content from 2750 to 2650 kcal/kg and the crude protein content from 170 to 155 g/kg in the afternoon (treatment 2) did not result in significant changes in feed intake, laying percentage, egg weight, egg mass, feed conversion ratio, live weight and live weight gain in comparison with the control group (treatment 1).

Table 1. Effect of different dietary AME (kcal/kg) and crude protein (CP, g/kg) levels during the day on performance of ISA-Brown laying hens in the period from week 65-80

treatment	1	2	3	4	5	
AME-CP morning	2750-170	2750-170	2750-170	2750-170	2750-155	P-value
AME-CP afternoon	2750-170	2650-155	2650-140	2750-140	2750-155	
feed intake, g/day	121.9	119.9	121.6	120.9	121.0	0.9308
egg production, %	92.37	92.85	89.65	91.67	93.42	0.3385
egg weight, g	66.51	67.12	66.11	66.48	67.29	0.8681
egg mass, g/day	62.00	62.33	59.37	61.22	62.83	0.1245
f.c.r.	2.000^{ab}	1.976^{b}	2.101^{a}	2.067^{ab}	1.963^{b}	0.0473
weight, week 65, g	2159	2079	2138	2118	2157	0.5564
weight, week 80, g	2160	2084	2193	2142	2175	0.4054
weight gain, g	1.1	5.7	31.7	24.1	18.4	0.8240

When the crude protein content in the afternoon feed was further reduced to 140 g/kg (treatment 3), laying percentage and egg mass decreased numerically and feed conversion ratio increased significantly compared to treatments 2 and 5.

The differences for egg mass and feed conversion ratio were significant between treatment 2 and 3 and laying percentage tended to be significantly different (P=0.0978). Compared to treatment 1, egg mass and feed conversion ratio tended to be impaired (P=0.0677 and 0.0649). Live weight gain was not reduced in treatment 3 as compared with treatments 1 and 2.

When only the crude protein content was reduced from 170 to 140 g/kg in the afternoon feed (treatment 4), laying percentage, egg mass and feed conversion ratio were impaired compared to treatment 1, but differences were not statistically significant. A reduction of the crude protein content in both the morning and afternoon feed (treatment 5), which provided the same daily crude protein intake as in treatment 4, resulted in a numerically higher laying percentage and egg mass and in a lower feed conversion ratio than in treatment 4. The difference in feed conversion ratio was almost significant (P=0.0555). During the experimental period, no mortality occurred.

In Table 2, the body composition of the birds at the start and end of the experiment is presented. At 80 weeks of age, no significant differences in body composition were observed between treatments, although high variation was reported. No indications of a lower protein or fat deposition were found when the AME or the crude protein levels of the afternoon feed was reduced. Interestingly, the ash percentage increased when the AME content of the afternoon feed was reduced, while this percentage decreased in the other treatments as compared with the start of the experiment. However, these differences were not significant. In comparison with the start of the experiment, moisture and crude fat contents remained constant during the experiment, while the ash and crude protein content of the body decreased.

Table 2. Effect of feeding different dietary AME (kcal/kg) and crude protein (CP, g/kg) levels during the day on body composition of ISA-Brown laying hens at the end of the experimental period (80 weeks of age)

treatment	AME-CP morning	AME-CP afternoon	body composition, g/kg			
			moisture	ash	crude protein	crude fat
1-5[1)]			533.0	40.0	184.0	240.1
1	2750-170	2750-170	520.5	34.8	176.0	236.7
2	2750-170	2650-155	533.5	41.0	177.7	229.5
3	2750-170	2650-140	526.0	41.0	175.2	255.7
4	2750-170	2750-140	537.5	37.0	180.0	230.6
5	2750-155	2750-155	528.2	36.7	176.3	250.4
1-5[2)]			529.1	38.1	177.0	240.6
P-value			0.8203	0.4291	0.8598	0.5132

[1)] the data in this row show the results of a pooled sample of birds of all treatments at the start of the experiment (week 65)

[2)] the data in this row show the average results of all treatments at the end of the experiment (week 80)

The results of the present experiment indicate that it is possible to reduce the AME content of the afternoon feed from 2750 to 2650 kcal/kg during the last 15 weeks of the laying period without losing performance. The crude protein content can be reduced from 170 to 155 g/kg at the same time without negative effects on performance, but this does not seem to be related to the fact that protein requirements might be lower in the afternoon as compared with the morning. A reduction in the crude protein content in the afternoon feed to 140 g/kg (treatments 3 and 4) had a negative effect on production, especially when this was combined with a decreased AME content. Treatment 5 provided about the same daily crude protein intake as in treatments 3 and 4 (the difference was 1.5-2 % between these treatments, which normally does not affect egg size and egg mass notably). Therefore, the distribution of protein during the day appears to affect performance and a reduction of the crude protein content during the afternoon has a negative effect on performance. These effects are in line with the first experiments of Keshavarz (1998a; 1998b). On the basis of the results of the current experiment, it is possible to reduce the crude protein level in both the morning and afternoon feed to 155 g/kg without impairing performance.

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EFFECT OF MULTI-NSP ENZYMES AND PHYTASE COMBINATION ON GROWTH PERFORMANCE OF BROILER CHICKENS FED WITH DIFFERENT QUALITY GRADES OF INGREDIENTS

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Summary

Growth performance responses from broiler chickens to two quality grades of corn (high *vs.* low) and soybean meal (SBM, dehulled *vs.* non-dehulled) and reduced nutrient specification diets with a phytase plus multi non-starch polysaccharide (NSP) degrading enzymes combination were determined. The reformulated diets were lower in metabolizable energy (ME, -0.36 MJ/kg), digestible amino acids (-2%), available phosphorus (-0.15%) and calcium (-0.12%). Birds on all treatments reached 2,900g live weight at 40 days of age, with a feed conversion ration (FCR) of around 1.70. Birds grew well on diets containing an actual available phosphorus level of 0.30% for the starter diet and 0.21% for the grower diet when supplemented with Rovabio® Max LC, without negative effects on tibia ash content.

I. INTRODUCTION

Nutritionists aim at formulating diets as accurately as possible, using feedstuffs of heterogeneous origin, to meet requirements of a specific set of nutrients, in an attempt to ensure adequate performance and maximum return of a given category of poultry raised under specific conditions. The ubiquitous and substantial variations of nutritive quality of feed ingredients are the main contributors to less than optimal management of feed ingredients, inaccurate formulation and inconsistent animal performance.

Corn quality depends on a complex interaction between both intrinsic and extrinsic characteristics of the grain. From pervious surveys used to assess the variation of feed ingredients used in Asia, hundreds of corn and soybean meal samples were collected from various feed companies and analysed for contents of moisture, crude protein, fats, crude fibre, ash, calcium and phosphorus (Liu and Liu, 2008). The results clearly demonstrated that nutrient contents are variable in corn and SBM. Corn samples (n>200) had an average content of crude protein of 7.95% with coefficient of variation (CV) of 6.16%, and moisture of 13.19% with a CV of 6.22%. For soybean meal (SBM, n>300), protein content averaged 45.06% (CV 3.2%) and moisture 12.21% (CV 5.81%). The contents of crude fat and crude fibre in SBM had a CV of more than 20% for both corn and SBM, indicating a high degree of variation. Variation of protein solubility was relatively low for both ingredients. The level of xanthophylls in corn exhibited a CV of 32.5% which prompted the use of added pigments to deal with this variation. The AME values were 3,468 kcal/kg for corn and (CV 3.4%) and 2,271 kcal/kg for soybean meal (CV 8.2%) which are indicate a considerable range in AME value as high as 598 kcal/kg for corn and 910 kcal/kg for soybean meal (Liu *et al*., 2011).

The objective of the present study was to continue research in understanding the variable qualities of feed stuffs used in Asia and new opportunities to use NSP degrading enzymes and phytase combinations to save costs in modern poultry diets. The key to successfully using selected enzyme products to influence diet investment will depend on the contribution to energy values and nutrient bioavailability, resulting in better growth performance and the synergistic relationship between NSPase and phytase enzymes.

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II. EXPERIMENTAL DESIGN AND TREATMENTS

This trial was conducted at the Bangkok Animal Research Center, Thailand. A total of six hundreds, one-day-old Abor Acre male broilers were randomly allotted into 4 treatment groups (T1, T2, T3 and T4) that each group consisted of 6 replicates of 25 birds.

The dietary treatments were shown as Table 1. T1 and T3 (control treatments formulated to meet requirements of all nutrients as stated by the NRC) formulated with high (or low) quality corn and de-hulled (or non-dehulled) soybean meal; T2 and T4 (with enzyme treatments; enzyme composed of xylanase, β-glucanase and phytase obtained from two fermentation broths with *Penicillium funiculosum* and *Schizosaccharmyces pombe*) formulated diet with lower specifications for AME (-0.36 MJ/kg), digestible amino acids (-2%) and available P (-0.15%) and calcium (-0.12%).

The diets consisted of corn and soybean meal of two quality grades: 1) Grade A (Corn H) corn is good quality with the lowest content of corn cob and moldy grains, crude protein of 7.47%, purchasing price of Thai Baht (TB$) 11.0 per kg; Grade AAA (Corn L) is poor quality, containing a high level of corn cob, more broken and moldy grains, crude protein of 7.35%, price of TB$ 10.4/kg; 2) two types of soybean meal, as de-hulled (DH, crude protein of 49.8%, price of TB$ 14.1/kg) and non-dehulled (NDH, crude protein of 46.5%, price of TB$ 13.1/kg). Broilers were fed as starter diet from 1 to 20 days, and then a finisher diet to 40 days. Feeds were provided *ad libitum* as crumbles to 12 days and as pellets thereafter.

Mortality was recorded daily, including the probable cause of death. At the end of the trial period, two birds from each replicate per treatment were sacrificed to determine the phosphorus utilization by tibia ash determination. The measurements of performance data were collected at the end of each diet phases.

The experiment was analysed as a randomized block design using ANOVA to compare the treatment means.

III. RESULTS AND DISCUSSION

The results are shown in Table 2. The final body weight on all treatments reached over 2,900g after 40 days with feed intake (FI) around 4,900 g/bird and a corrected FCR of around 1.70. Feeding low quality ingredients caused a higher FI with poorer FCR. Reformulating with enzymes caused a significant increase of 2.8 FCR points with high quality ingredients and a significant decrease in feed intake with low quality ingredients. No other differences were observed between the control treatments and the reformulated treatments.

The potential for specific NSP enzymes to improve growth performance of broiler chickens has been known for over fifty years. Xylanases and β-glucanase are known to degrade plant cell walls leading to the release of nutrients from grain endosperm and aleurone layer cells and thus improving the feed energy value.

Table 1. Main ingredients and chemical composition of experimental diets

	Starter (1-20 d)				Finisher (21-40 d)			
	T1	T2	T3	T4	T1	T2	T3	T4
Ingredients								
Corn H (7.1%)	60.80	60.88	-	-	62.53	63.88	-	-
Corn L (7.35%)	-	-	52.96	58.96	-	-	54.82	60.72
SBM DH (49.8%)	32.19	35.34	-	-	31.11	33.17	-	-
SBM NDH (46.5%)	-	-	36.66	34.71	-	-	35.47	33.59
Palm oil	2.13	-	5.69	2.41	2.39	-	5.92	2.66
Limestone (39%)	1.24	1.27	1.22	1.27	0.99	1.03	0.97	1.02
M-DCP	1.89	1.01	1.72	0.89	1.39	0.53	1.23	0.40
Nutrient value								
Dry matter	87.62	87.24	87.94	87.35	87.54	87.11	87.84	87.25
Crude protein (%)	20.97	22.30	21.05	20.69	20.56	21.51	20.65	20.31
ME (MJ/kg)	12.77	12.41	12.77	12.41	12.98	12.62	12.98	12.62
Dig. lysine	1.15	1.13	1.15	1.13	1.10	1.08	1.10	1.08
Dig. methionine (%)	0.55	0.52	0.57	0.56	0.55	0.52	0.58	0.56
Dig. met. + cys (%)	0.83	0.81	0.83	0.81	0.83	0.81	0.83	0.81
Calcium (%)	0.90	0.78	0.90	0.78	0.72	0.60	0.72	0.60
Total phosphorus (%)	0.75	0.59	0.72	0.56	0.65	0.49	0.62	0.46
Av. phosphorus (%)	0.45	0.30	0.45	0.30	0.36	0.21	0.36	0.21
Ro Max LC (ml/mt)	-	200	-	200	-	200	-	200
Feed cost (TB$/kg)	13.90	13.15	14.43	13.39	13.90	13.10	14.42	13.39

Table 2. Pperformance on high *vs.* low quality ingredients with or without enzymes

Treatments	T1 (H/DH)	T2 (H/DH/Enz)	T3 (L/NDH)	T4 (L/NDH/Enz)
Final live weight (g)	2,938	2,938	2,966	2,909
Feed intake, FI (g)	4,856^{b}	4,940^{ab}	5,030^{a}	4,909^{b}
FCR	1.677^{a}	1.705^{b}	1.720^{b}	1.712^{b}
Livability (%)	98.00	98.67	97.33	98.00
Tibia ash (% dry)	48.31	47.11	47.30	47.76
Feed cost TB$/kg gain	22.98^{c}	21.55^{a}	24.70^{d}	22.04^{b}

a,b Means within the same row bearing different superscripts differ (P<0.05)

The reformulation of starter diets removed 21.3 kg palm oil and 8.8 kg Mono-Dicalcium phosphate (MDCP) for the high quality diet resulting in a cost reduction of TB$725 per tonne of feed. Values for the low quality diet were removal of 32.8 kg palm oil and 8.3 kg MDCP and a cost reduction of TB$800 per tonne of feed. Values for the high quality finisher diet were removal of 23.9 kg palm oil and 8.6 kg MDCP resulting in cost reduction of TB$1040 per tonne of feed, and for the lower quality diet, 32.6 kg palm oil and 8.3 kg MDCP removed with cost reduction of TB$1030 per tonne of feed. Birds fed on the low quality ingredients cost TB$1.04 more than those fed the high quality ingredients for the whole experimental period. Overall, the use of combination of NSPase and phytase saved TB$1.42 (5.9%) per kg weight gain.

Data in Table 3 shows a comparison of ingredients and the effect of the enzyme product. Birds fed on low quality ingredients reached the same live weight with a higher FCR, and cost TB$ 1.04 (4.6%) more per kg weight gain than those on high quality ingredients. This suggests that buying high quality ingredients can achieve better growth performance and an economic return compare with lower quality ingredients. The addition of enzymes showed

benefits for both quality ingredients with more pronounced responses observed from diets with poorer quality ingredients.

Table 3. Effect of ingredient quality and Rovabio® Max LC on growth performance and diet cost

Treatment	Quality of ingrediets		Rovabio® Max LC	
	High	Low	Without	With
Weight gain (g)	2,897	2,897	2,911	2,883
Feed intake (FI) (g)	4,898	4,969	4,943	4,924
FCR	1.691[a]	1.716[b]	1.698	1.709
Performance Index	427	418	425	421
Tibia ash (% dry)	47.71	47.53	47.81	47.21
Feed cost TB$/kg gain	22.85	23.89	24.08	22.66

a,b Means within the same row bearing different superscripts differ (P<0.05)

IV. CONCLUSION

High quality feed ingredients can generate good performance with better economic returns comparing with poorer quality of ingredients at cheaper prices in this experiment. Overall, no significant differences were observed between control treatments and reformulation with Rovabio® Max LC treatments on growth performance during the 40 days of the trial period. The results suggest that enzymes are more effective on the lower quality feedstuffs, as they saved TB$1.42 per kg weight gain.

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EFFECT OF DIFFERENT CONCENTRATIONS OF LYSINE AND METHIONINE ON PERFORMANCE OF WHITE LEGHORN LAYERS FED DIETS WITH SUB-OPTIMAL CONCENTRATIONS OF PROTEIN

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Summary

An experiment was conducted with WL layers to study the effect of two concentrations (7.54 and 8.16 g/kg) of lysine (Lys) and four (2.83, 3.14, 3.45 and 3.77 g/kg) of methionine (Met), in a factorial manner, in diets containing 157 g/kg crude protein (CP). Each diet was fed *ad libitum* to eight replicates (88 birds per replicate) housed in an open sided poultry house. Egg production (EP), feed required to produce kg egg mass (FE), and egg weight (EW) were recorded from 25 to 40 (Phase I) and 41 to 56 weeks of age (Phase II). Egg mass (EM) was calculated using the EP and average EW. Interaction between the concentrations of Lys and Met or concentration of Lys in the diet did not influence ($P > 0.05$) EP, feed intake (FI), FE, EW, EM and body weight at 40 and 56 weeks of age. Increasing dietary concentrations of Met up to 3.45 g/kg significantly increased FE, EW and body weight, while EP and EM increased up to 3.77 g/kg diet during phase I, but not during phase II. Based on the results, it is concluded that 7.54 g Lys/kg diet (729 and 814 mg/b/d, during Phases I and II, respectively) was adequate for layers during Phase I, while the requirement of Met was higher (3.77 g/kg diet) during Phase I compared to that required (2.83 g/kg diet) during Phase II (365 and 305 mg/b/d, respectively, in Phases I and II) in diets containing 157 g protein/kg.

I. INTRODUCTION

In recent years, research has focused on optimizing the nutrient allowances, particularly CP, to minimize the cost of feeding and excretion of nitrogenous substances without affecting bird performance. Limitation of lysine (Lys) and methionine (Met) concentrations in diets containing sub-optimal concentrations of protein may hamper the birds' performance. Increased efficiency of protein utilization in chickens by supplementation of Lys (Uzu and Larbier, 1985) or Met (Schutte et al., 1994) has been reported in the literature. Therefore, it would be ideal to reduce the dietary levels of CP by optimizing the levels of Lys and Met in diet. A better understanding of Lys and Met requirements of layers fed sub-optimal concentrations of CP may help in reducing excretion of nitrogen and reduce the cost of production from intensive poultry farming without affecting laying performance.

II. MATERIALS AND METHODS

A total of 5632 WL (White Bovans) pullets (25 wks of age) were distributed equally among 64 replicates (22 colony cages with four birds in each – 88 birds per replicate). The cages were on an elevated platform in an open-sided poultry house (ambient temperature range 23-37°C). A basal diet (BD) containing 157g CP, 7.54 g Lys and 2.83 g Met/kg was prepared. Crystalline Lys (L-lysine HCl) and Met (DL-methionine) were added to the BD to achieve two levels of Lys (7.54 and 8.16 g/kg) and four levels of Met (2.83, 3.14, 3.45 and 3.77 g/kg) in a 2 x 4 factorial design. The analysed composition of CP, Lys and Met (Llames and

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Fontaine, 1994) was used to achieve the required levels of these nutrients in experimental diets. Each diet was offered *ad libitum* to eight replicates from 25 - 56 weeks of age in two phases: Phase I (25-40 weeks) and Phase II (41-56 weeks).

Eggs were collected twice daily to record daily egg production (EP). Quantity of feed consumed to produce 1 kg egg mass (feed efficiency – FE) was compiled at 28 d intervals from 25 to 56 wks (8 periods). Average egg weight (EW) was recorded during the last 3 days of each period. Egg mass (EM) was calculated by multiplying the average EW by the number of eggs produced in each replicate. Body weight (BW) of the birds was recorded at 40 and 56 weeks of age. The data were subjected to 2 x 4 factor analysis (Snedecor and Cochran, 1980) considering levels of Lys and Met as the independent factors. The treatment means were compared using the Duncan multiple range test (Duncan, 1955) at $P < 0.05$.

III. RESULTS AND DISCUSSION

Most of the parameters studied were influenced ($P<0.05$) by the diet during Phase I but not during Phase II and therefore the data were analyzed accordingly (Table 1). There was no statistically significant effect of the concentration of Lys or interaction between the Lys and Met for any parameter studied during both phases. During Phase I, EP and EM increased progressively with increase in dietary Met concentration up to 3.77 g/kg, at which level a maximum response in these parameters was observed compared to lower Met levels. Neither the variation in concentration of Lys and Met, nor their interaction significantly ($P>0.05$) influenced feed intake (FI) during either Phase I or Phase II. Feed efficiency (FE) and EW during Phase I and body weight during Phase II increased with increase in dietary Met up to 3.45 g/kg but no further increase in these parameters was observed at 3.77 g/kg diet.

The EP of layers during Phase I was slightly lower (0.94 vs 0.88 eggs/day) than the levels recommended by the breeding company (Flock Production Record, Pioneer Bovans 2011), whereas during Phase II the EP was similar to the recommendations. Similarly, the BW of layers at 40 and 56 wks of age was lower than the breed standard by 80 and 66.5g, respectively. None of the production parameters were affected by increasing dietary Lys from 7.54 to 8.16 g/kg during both phases, thus a level of 7.54 g/kg appears to be adequate for WL layers for optimum performance. The breed guidelines suggested 8.30 and 7.80 g Lys/kg diet during Phases I and II, respectively. The calculated average daily intake of Lys during Phases I and II was 729 and 814 mg/b, respectively, which is lower than the levels recommended (850 to 900 mg/b/d) by Schutte and Smink (1998) and Novak et al. (2004). The variation may be due to differences in age of birds and egg size. Schutte and Smink (1998) and Novak et al. (2006) recommended higher levels of Lys (900 and 860 mg/b/d, respectively) during the initial production phase (20-43 wks) compared with 715 mg/b/d during post peak (44-60 wks) production. The higher BW (1600 g) and EW (58-60 g) observed in their studies may be responsible for higher Lys requirements reported. Novak et al. (2004) tested 860 mg/b/d Lys as a minimum level in their experiment and reported the same concentration as the requirement. Similarly, higher concentrations of Lys (850 and 920 mg/b/d, respectively) were suggested by some authors (Pilbrow and Morris, 1974) for layers fed diets based on wheat, which has low amino acid digestibility compared to maize (Ravindran et al., 2005). Egg weight and EM were not affected by increasing the concentration of Lys from 7.54 to 8.16 g/kg in the diet. These observations are contrary to the findings of several reports (Brake and Peebles, 1992; Novak et al., 2004), which found progressive increase in EW with increasing Lys intake (500 to 1613 mg/b/d) by layers. Body weight was not affected by increasing concentration of Lys in diet, which is contrary to our unpublished data (Rama Rao et al., 2011). The minimum level of Lys used in the present study (7.54 g/kg diet) is higher than the optimum levels of Lys observed in our previous study (7 g/kg diet).

Table 1. Effect of dietary concentrations (g per kg diet) of lysine (Lys) and methionine (Met) on performance of WL layers (25-56 wk of age)

AA. g/kg	EP, per day		FI, g/b/d		FE, kg/kg egg		EW, g		EM, g/b		BW, g	
Age, wks	25-40	41-56	25-40	41-56	25-40	41-56	25-40	41-56	25-40	41-56	40	56
Lys												
7.54	0.879	0.901	96.7	108.0	2.06	2.04	53.56	58.97	1319	1487	1362	1476
8.16	0.880	0.906	96.6	107.7	2.05	2.02	53.57	59.09	1321	1498	1359	1471
N	32	32	32	32	32	32	32	32	32	32	32	32
Met												
2.83	0.856[d]	0.903	96.5	107.8	2.14[a]	2.04	52.81[c]	58.75	1267[d]	1485	1345[b]	1452[b]
3.14	0.873[c]	0.906	97.0	108.8	2.08[b]	2.04	53.38[b]	58.98	1306[c]	1495	1356[ab]	1467[ab]
3.45	0.888[b]	0.908	96.4	107.6	2.02[c]	2.01	53.86[a]	59.23	1340[b]	1505	1372[a]	1490[a]
3.77	0.902[a]	0.898	96.9	107.3	1.98[c]	2.03	54.20[a]	59.15	1369[a]	1487	1370[a]	1484[a]
N	16	16	16	16	16	16	16	16	16	16	16	16
P values												
Lys	0.77	0.46	0.88	0.79	0.74	0.38	0.90	0.42	0.74	0.32	0.68	0.47
Met	0.001	0.75	0.90	0.77	0.001	0.78	0.001	0.13	0.001	0.56	0.009	0.005
Lys*Met	0.22	0.93	0.60	0.45	0.08	0.41	0.43	0.40	0.12	0.70	0.58	0.87
SEM	0.0017	0.0032	0.35	0.50	0.008	0.013	0.076	0.077	3.23	5.52	3.09	3.98

AA is amino acid

[a, b, c] Means within a column having no common superscript differ significantly (P<0.05)

Increasing dietary concentrations of Met up to 3.77 g/kg significantly increased EP, FE, EP and EM, while increase in EW and FE was observed up to 3.45 g/kg during Phase I but not during Phase II, during which the dietary Met concentration did not influence these production parameters. Considering the EP and EM, a level of 3.77 g Met/kg diet may be considered as the optimum level for WL layer during Phase I. As the performance (EP, FE, EW and EM) in layers fed 2.83 g/kg diet was similar to those fed the highest concentration of Met (3.77g/kg), the lowest level of Met may be considered as the requirement during Phase II. The optimum value obtained in the current experiment was much lower than the breed recommendations of 4.20 and 4.00 g/kg diet during Phases I and II, respectively. The calculated intake of Met in groups fed 3.77 g Met/kg diet during Phase I is 365 mg/b/d; similarly intake of the amino acid in groups fed 2.83 g/kg diet during Phase II is 305 mg/b/d. A significant increase in EP was observed by Calderon and Jensen (1990) with increasing supplemental crystalline Met in the diet (0, 0.025, 0.05, 0.075, 0.1, or 0.125% of DL-methionine). Similar to the data of the present experiment, Harms and Russell (2003) and Safaa et al. (2008) did not observe any reduction in EP and EM in WL layers during the post peak egg production (45-53 and 55-76 weeks of age, respectively) when birds were fed 3 and 3.1 g Met /kg diet, respectively. The EW and EM increased progressively with increases in dietary Met concentration in the WL layer diet. Similarly, several authors (Schutte et al., 1994; Sohail et al., 2002; Keshavarz, 2003) have reported increased EW with increased intake of Met / TSAA.

Based on the results, it is concluded that 7.54 g Lys/kg diet (729 and 814 mg/b/d, during phase I and phase II, respectively) was adequate for layers, while the requirement of Met was higher (3.77 g/kg diet) during the initial laying period (25-40 weeks) compared to that required (2.83 g/kg diet) during 41-56 weeks of age (365 and 305 mg/b/d, respectively during 25 to 40 and 41 to 56 wks) in diets containing 157 g protein/kg.

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MULTISUBSTRATE ENZYME IMPROVES THE APPARENT METABOLIZABLE ENERGY (AME) OF BROILER DIETS

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With the dramatic rise in maize and soya prices worldwide, alternate feed ingredients are of interest as partial substitutes for traditional ingredients in poultry diets. Although alternate feed ingredients may provide good sources of nutrients, their full potential is generally not exploited by animals as many contain significant quantities of non-starch polysaccharides (NSP), which decrease nutrient digestibility resulting in a reduction in the apparent metabolisable energy (AME) of diets. Supplementation of appropriate NSP enzymes improves feed quality by enhancing carbohydrate digestibility and reducing gut viscosity (Almirall *et al*., 1995). The use of amylases and proteases may also improve nutrient and energy digestibility of poultry feed. The present study evaluated the energy sparing efficacy of a multisubstrate enzyme developed to enhance the metabolizable energy of broiler diets formulated with alternate feed raw materials.

Treatments used for the trial were (1) Positive control diet (corn-soybean diet) (2) Negative control diet with energy reduced by 0.42MJ/kg formulated by replacing corn and soybean meal with alternate feed raw materials (DDGS (4.1%), canola meal (4.1%), wheat middlings (3%) (3) Negative control diet with multisubstrate enzyme (0.5kg/t). Each diet was fed in mash form to six replicates per cage (28-day-old birds) for 7 days. During the last 4 days, feed intake and excreta output were measured quantitatively per cage for the determination of AME.

Table 1. The effect of multisubstrate enzyme on the AME of broiler diets

Diet type	Enzyme	AME (MJ/kg) DM basis
Positive control	None	14.30[a]
Negative control	None	13.96[b]
Negative control	Multisubstrate Enzyme (0.5kg/t)	14.19[a]

[a, b,] Means with different superscripts are significantly different ($P < 0.01$)

Our results show that the addition of a multisubstrate, multienzyme complex resulted in significant improvement of 0.23MJ/kg ($P < 0.01$) in AME of low energy diets formulated with alternate feed ingredients. This energy uplift may be due to the improvement in the digestibility of feed nutrients by the optimum combination of NSPases, amylases and proteases.

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IMPROVED BROILER PERFORMANCE FOLLOWING THE EARLY ADMINISTRATION OF PROTEIN FRACTIONS EXTRACTED FROM MEAT AND BONE MEAL

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Meat and bone meal (MBM), the rendered products of mammalian offal, is a by-product of meat production that is an important source of protein, calcium and phosphorus (Hamilton, 2002). MBM is used as fertiliser or added to pet or stock feeds, including poultry. Its inclusion in stock feeds, however, has been restricted due to between batch variation in its quality (Bryden *et al*, 2009), the potentially negative impact of biogenic amines on animal performance (den Brinker *et al* 2003) and the presence of bovine spongiform encephalopathy in Europe and the North America. Therefore alternative uses for MBM are under investigation. MBM contains up to 50% protein. This supplies essential amino acids and nutrients but additionally the protein component may also possess bioactivity (Meisel 2007), which can affect physiological function including growth and performance. Therefore an exploratory study was designed to assess the presence of bioactivity in MBM-derived protein when administered to hatchlings.

MBM was obtained from a commercial rendering facility. Protein fractions were extracted by size exclusion fractionation. Protein size ranges (and treatment groups) <3kDa, 3-30kDa, 30-100kDa, 3-100kDa and >100kDa, and vehicle only (control) were administered four times via gavage to Ross chicks during their first week post-hatch. The total amount of protein received was <5mg/chick in the three former and 1.3mg/chick in the latter two treatment groups. Chicks were grown to 4 weeks of age, with weekly determination of body and breast weight and intestinal length on10 chicks/treatment.

Treatments with protein fractions 3-100kDa and >100kDa significantly ($P<0.05$) increased the length of the jejunum when birds were 4 weeks old compared to the control chicks. Concurrently the ileum was also significantly ($P=0.015$) longer in both of these treatment groups, as well as in chicks receiving the 3-30kDa treatment. Treatments did not significantly increase chick weights, however the 3-30kDa and 3-100kDa treatments did induce a 7% and 9% increase respectively in mean weights compared to the control chicks at 3 weeks of age. Similarly mean breast weight was not significantly affected by protein treatments. However, the 3-30kDa treatment increased mean breast weight by 9% at 3 weeks of age, and the 3-100kDa treatment increased mean breast weight by 16% at 4 weeks of age, compared to the control chicks. In all of the above observations at least 70% of chicks of each of the treatment groups had body or breast weights numerically greater than the mean of the control chicks.

The administration of MBM-derived protein treatments 3-30kDa and 3-100kDa generated improved body and breast weights and increased intestinal length. These findings support proof of the principle that MBM contains agents with growth-promoting bioactivity.

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EVALUATING THE EFFICIENCY OF A PREBIOTIC (ASPERGILLUS MEAL) ON BROILER PERFORMANCE

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Current trends in animal production point to reduced use of antibiotic growth promoters (Ferket, 2004) and increased use of non-antibiotic feed additives. Fermentation product of *Aspergillus orizae* referred to as Aspergillus meal (AM), has no live cells or spores (Torres-Rodriguez et al., 2005) and has been shown to enhance the digestive efficiency of the gut (Harms and Miles, 1988). Reports that inclusion of Aspergillus meal in nutrient-limited culture medium selectively increased the growth of Lactobacillus spp. suggest that AM may be best described as a prebiotic (Torres-Rodriguez et al., 2005). Prebiotics have been reported to provide optimum conditions for useful gut micro flora to produce and excrete digestive enzymes (Torres-Rodriguez et al., 2005). Also they may change the intestinal microflora and enhance the synthesis of lactic acid leading to lowering of intestinal pH and reduced ammonia synthesis (Chegeni et al., 2011).

An experiment was conducted to evaluate the effects of AM on the performance of broiler chickens and carried out with two experimental treatments consisting of a control diet formulated without antibiotic growth promoters and coccidiostats, and a diet supplemented with the AM prebiotic. Prebiotic was added at 1.8 and 1.0 g/kg (based on inclusion rate recommended by manufacturer) to barley, wheat, corn, soy-based diets in starter (1-21d) and grower (22-42d) periods, respectively. Feed and water were provided *ad-libitum*. The experimental design was completely randomised with 2 dietary treatments each replicated 18 times. Twenty-two broilers (half male and half female housed together) formed the experimental unit. Replicates (pens) were allocated to the treatments to achieve a homogeneous distribution of treatments within the house. The birds and feed consumed were weighed on days 21 and 42 to allow the calculation of feed intake, average daily gain and feed conversion ratio. Data were analysed using the GLM procedures of SAS. Differences between means were determined by Duncan's multiple range test at a significance level of ($P < 0.05$).

Broilers fed prebiotic-supplemented diets had greater body weight gain (36.3 *vs*. 37.8 g/day; P= 0.001) and ate 2 g/day more feed (53.3 *vs.* 55.3 g/day; P= 0.013) during the starter period (from 1 to 21 days). Although feed conversion ratio was improved slightly by supplementing the diet with prebiotic at the end of starter period (1.46 *vs.* 1.47), the improvement was not statistically significant. For the experimental period as a whole (1-42 days of age), feed intake was improved significantly (2.6 g/day) by prebiotic supplementation, but no significant differences were observed for body weight gain and feed conversion ratio.

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THE EFFECT OF A SYNBIOTIC – A COCKTAIL OF PRE AND PROBIOTIC– ON BROILER PERFORMANCE AND LITTER QUALITY

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Summary

A trial was conducted to investigate the effect of fermentation product of *Aspergillus orizae*, as a prebiotic and a lactobacillus-based probiotic, individually and in combination as a synbiotic, on broiler performance and litter quality. Prebiotic was added as 1.8, 1.0 and 1.0 g/kg and probiotic as 0.9, 0.5 and 0.25 g/kg to a corn, soy-based diet in starter, grower and finisher periods, respectively. Dietary treatments were: control diet, prebiotic-supplemented diet, probiotic-supplemented diet, and synbiotic-supplemented diet. Ross day-old broilers (408 male and 408 female) were randomly allocated to 48 pens. Each sex and each dietary strategy was tested with 6 replicate pens. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) parameters were analyzed statistically using ANOVA (Genstat) with sex and diet as factors to evaluate broiler performance. The prebiotic improved BWG and FCR after 36 days significantly ($P<0.05$) by 1 and nearly 2% respectively, whereas the probiotic had no significant effect on BWG and FCR. The synbiotic improved BWG and FCR significantly ($P<0.05$) by 3.0 and 2.1% respectively. Positive effects became significant after 14 days and no interaction between sex and treatments was found. Pre and probiotics had not any significant effect on litter pH and moisture (%), but the synbiotic decreased these amounts slightly at the end of the finisher period. It was concluded that the addition of the prebiotic to the diets was more effective in stimulating broiler performance than the addition of the probiotic. Amalgamating a prebiotic with a probiotic can enhance the useful effects of a prebiotic on broiler performance.

I. INTRODUCTION

Since the importance of a well-balanced gut microflora for adequate health and high performance has been recognized, feeding strategies have been directed to control the microbial gastrointestinal environment by nutritional means. One key strategy is to feed directly the microorganisms which have been shown to exert beneficial effects in the gut. Probiotics are live microorganisms which are supplemented to the feed in order to establish a beneficial gut microflora (Fuller, 1989). Thus, probiotics have the potential to beneficially affect gut health by modification of the gut microflora, especially in young animals, in which a stable gut microflora is not yet established. Prebiotics are defined as "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (Gibson and Roberfroid, 1995). Combinations of prebiotics and probiotics are known as synbiotics.

If litter moisture is not kept at an appropriate level, very high bacterial loads and unsanitary growing conditions may produce ammonia and other gases which in turn cause odors, insect problems, soiled feathers, footpad lesions and breast bruises or blisters (Read, 1986). Maintaining the litter pH is one of the key regulatory aspects of ammonia control, because ammonia release increases above pH 7 with the highest release rate at pH 8 (Reece et al., 1979). This study was conducted to determine the effect of a fermentation product of *Aspergillus orizae* as a prebiotic, and a lactobacillus-based probiotic as dietary supplements on broiler performance and litter pH and litter moisture (%) from 1 to 36 days of age.

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II. MATERIALS AND METHODS

a) Formulating the diets

The trial was conducted with eight treatments, based on four dietary treatments examined in male and female broilers. Dietary treatments were based on the addition of either prebiotic (Fermacto) or probiotic (Primalac) or a cocktail of both to an unsupplemented basal diet in the starter, grower and finisher phases.

One-day-old broilers were randomly allocated to 48 pens with sawdust as bedding material. Lighting schedule throughout the experimental period comprised 23 hours light and 1 hour darkness. The house temperature was gradually decreased from 32^{o}C on arrival according to a standard temperature schedule. At the hatchery, the birds were vaccinated against IB and no other vaccination program administrated during the experimental period. Starter, grower and finisher diets were formulated based on approximately 60% corn and 25% soybean meal (AMEn 12.24, 12.56, 12.56 MJ/kg; lysine 10.7, 10.1, 10.0 g/kg). The nutrient, mineral and vitamin compositions of all diets were nutritionally adequate according to the NRC (1994) requirements for broilers. Diets were supplemented with chemical coccidiostats during the starter and grower phases. The diets did not contain any antimicrobial feed additive and were presented as mash. The birds were provided with feed and water *ad-libitum*. Dietary treatments were tested with 6 replicate pens in two groups (female and male). Body weight gain and FI were measured from 1-14, 14-28 and 28-36 days of age. Body weight gain, FI and FCR were statistically analyzed using ANOVA (Genstat) with sex and diet as treatment factors.

Table 1.[1] Dietary Treatments

Diets	Starter(1-14days) g/kg	Grower(14-28days) g/kg	Finisher(28-36days) g/kg
1.Control	-	-	-
2.Control+Prebiotic	1.8	1.0	1.0
3.Control+Probiotic	0.9	0.5	0.25
4.Control+Synbiotic	1.8+0.9	1+0.5	1+0.25

[1]Inclusion rates of prebiotic and probiotic used in this experiment are based on inclusion rates recommended by the manufacturers.

b) Measuring litter pH and moisture

For measuring the amount of pH and moisture of the litter, 200-g litter samples were collected from various places in each pen when the birds reached 14, 28 and 36 days of age. pH was determined using the method reported by Huff (1984): a 1:10 dilutions in distilled water of duplicate samples were mixed on a vortex for 3-5 min. To measure the H^{+} concentration of the resulting solution, the pH meter was calibrated (pH=10) prior to using and after every 16 samples. Litter moisture was determined gravimetrically by drying the samples at 70^{o}C until the weight of samples reached a constant level. The litter moisture was measured on days 14, 28 and 36 and the litter pH was measured on days 28 and 36.

III. RESULTS AND DISCUSSION

a) Broiler performance

Healthy one-day-old broilers arrived at the institute with an average body weight of 40.5 and 40.3 grams for the males and females, respectively. Bird mortality after 36 days was low (2.6%) and not affected by the experimental treatments. Broiler performance per dietary treatment is presented in Table 2. Differences between male and females were significant as expected. As the study was concentrated on the effect of prebiotic and lactobacillus-based probiotic on production performance and no "sex × additive" interaction effects were observed, no further attention was paid to the sex effect. During the starter period, feed intake was improved (P=0.06) in birds fed the diets containing the prebiotic. Although this effect disappeared in older birds, a positive response was shown on BWG and FCR from 1 to 28 days of age and from 1 to 36 days of age. Up to 28 days of age, dietary supplementation with prebiotic and probiotic resulted in a 2.1% and 1.4% improvement of BWG, respectively.

Table 2. Results for body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of the broilers

	FI	BWG	FCR
	g	g	g/g
1-14d			
Control	493	374	1.327
prebiotic	502	377	1.329
Probiotic	492	372	1.321
Synbiotic	500	380	1.317
P /Lsd(additive)	*0.06*	*0.22*	*0.62*
1-28d			
Control	1989	1328 [a]	1.499 [b]
prebiotic	1992	1355 [bc]	1.471 [a]
Probiotic	1985	1347 [ab]	1.475 [a]
Synbiotic	2013	1371 [c]	1.469 [a]
P /Lsd(additive)	*0.21*	*0.008*	*<0.001*
1-36d			
Control	3290	2061 [a]	1.598 [b]
prebiotic	3290	2101 [bc]	1.568 [a]
Probiotic	3280	2073 [ab]	1.584 [b]
Synbiotic	3321	2124 [c]	1.565 [a]
P /Lsd(additive)	*0.24*	*0.001*	*<0.001*

[a,b,c] Mean values without a common superscript letter within a column differ significantly between diets per age period.

The mixture of both products gave a 3.2% improvement and effects of the synbiotic therefore seemed to be additive. The positive effect of the feed additives after 36 days on BWG was slightly less than shown during the 28 day period, but improvement (in g) was larger (on average 40 and 63 g for prebiotic and synbiotic respectively). Compared to the 28-day period, the positive effect on feed conversion ratio was similar for prebiotic (-1.9%)

and synbiotic (-2.1%) supplementation, but was no longer significant for the probiotic alone (-0.9%).

Average body weights of the male and female broilers from the control group were higher than the Ross standards: approximately +6% and 8%, respectively, whereas the FCR were similar to the Ross standards. After the first 14 day period, however, BWG of the males and females were approximately 6 and 1% lower than the standard values. These results of the control group show that broilers fed mash diets with a high inclusion level of corn can result in good production performance, even without specific feed additives. As these feed additives are assumed to be the most effective under less than optimal gastro-intestinal or management conditions, it is hypothesized that the magnitude of the effects shown in this study will be larger under commercial conditions. In the finisher phase (days 28-36), no effect of probiotic was shown, which was most probably due to the decreased amount of probiotic in the finisher diets. From this trial, it was concluded that dietary supplementation with 1.8, 1.0, 1.0 g prebiotic alone or in combination with 0.9, 0.5, 0.25 g/kg probiotic (lactobacillus-based probiotic) in starter, grower and finisher diets, respectively, improved body weight gain and feed conversion ratio of broilers during a complete production period. Sex had no effect on the response to supplementation with the pre and probiotics.

b) Litter pH and moisture

Prebiotics have been reported to provide optimum conditions for useful gut micro flora to produce and excrete digestive enzymes; also they may change the intestinal microflora and enhance the synthesis of lactic acid leading to lowering of the intestinal pH, and prevention of ammonia synthesis. Also, it is assumed that probiotics can stimulate the growth of beneficial bacteria; therefore bacterial digestive enzymes may enhance absorption and utilization of nutrients and reduce water consumption and excretion by birds, resulting in decreased litter pH and moisture. In this experiment, adding pre and probiotics to diets did not have any significant effect on litter pH and moisture, but the levels of these factors in broilers fed diets containing synbiotic, decreased slightly at the end of the finisher period. Litter pH was 8.11, 7.99, 7.94 and 7.82 for control, prebiotic-supplemented, probiotic-supplemented and synbiotic-supplemented groups, respectively, and the amount of litter moisture (%) was 38.83, 36.12, 37.13 and 35.87 for control, prebiotic-supplemented, probiotic-supplemented and synbiotic-supplemented group, respectively at the end of the finisher period.

The results obtained suggest benefits in using prebiotics in combination with probiotics, synbiotics, to increase performance and improve litter quality.

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BETAINE AS AN OSMOLYTE

A. PHILLIP[1]

There are two commercial forms of betaine (trimethyl glycine) used in poultry feeds, anhydrous betaine and betaine HCl. A claim is sometimes made that anhydrous betaine has osmolyte properties while betaine HCl does not. This paper provides an argument, from knowledge of physical chemistry, as to the relative osmolyte properties of both betaine forms. From a biochemical standpoint, betaine's function as an osmolyte is based on the fact that it is a Zwitterion, i.e. carrying both a positive and a negative charge on the same molecule at the same time (Zheng et al., 2005). The Zwitterion state of betaine is dependent on the pH of the molecule in its solvated state. One can think of betaine as a weak conjugate base A^- and betaine HCl can be thought of as its protonated acid form HA. Since the pKa of betaine is 1.84 and pH = pKa + log [A-]/[HA] , this means that the concentration of the conjugate base (A-) and the acid (HA) are equal when the pH = pKa. The laws of physical chemistry say that this will happen regardless of whether the molecule has started out as a betaine anhydrous or a betaine HCl (Po and Senozan, 2001).

As far as the animal is concerned, a betaine HCl molecule and a betaine molecule are identical. Any difference will be determined not by the betaine, but by the pH in the intestinal tract. Since there will not be enough betaine HCl in the recommended formulation to materially change the pH in the intestinal tract, there will be no difference.

Betaine Hydrochloride has the structure $[(CH_3)_3\ N - CH_2 - COOH]^+\ Cl^-$. This means that the carboxyl group is now protonated and the ammonium nitrogen carries a positive charge as before $[HA]^+\ Cl^-$. To balance the charge on the protonated acid, there is a negative charge on the chloride anion. This reaction is reversible and the equilibrium between the two forms (amphoteric form and acidic form) is determined by the pH of the solution.

In the gut, above pH 3 essentially all added betaine HCl will be present as anhydrous betaine and below pH 3 there will be an increasing amount of betaine HCl and a proportional drop in betaine anhydrous. Then there really is no difference between the two forms whatsoever i.e. whichever form is added, when in the GIT the forms will interchange depending on pH.

Depending on the pH of the solution (concentration of H^+), the relative proportions of these two "species" (Betaine Hydrochloride and Betaine Anhydrous) will vary in accordance with the acid dissociation constant (pKa = 1.84).

Note:

- At pH = 1.0: 87% of the betaine will be present as the hydrochloride and 13% as betaine anhydrous
- At pH = 1.8: 50% will be betaine hydrochloride and 50% will be betaine anhydrous
- At pH = 2.0: 40% will be betaine hydrochloride and 60% as betaine anhydrous
- At pH = 3.0: 7% will be betaine hydrochloride and 93% as betaine anhydrous

These above calculations were obtained using the Henderson-Hasselbach equation for calculating the equilibrium pH in acid-base reactions. It is therefore incorrect to claim that one form is superior to the other as an osmolyte, since both forms will be inter-converted in solution, depending on the exact pH of the solution.

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ENHANCING EGG PRODUCTION PERFORMANCE IN COMMERCIAL PHEASANTS USING PHOTOPERIOD

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Summary

Egg production of commercial pheasants (*Phasianus colchicus*) is highly seasonal, begins in spring and terminates in late summer, limiting the number of eggs and chicks that can be produced. Over two breeding seasons, we studied the response of the adult pheasants to manipulated photoperiod. In the first year of the study, the treated pheasants were exposed to a short photoperiod (8L/16D) for 6 weeks starting early July and ending in mid August, and then to a long photoperiod (16L/8D) for the reminder of the breeding season (mid August to early February). In the second year, the same pheasants were again exposed to the short photoperiod for 6 weeks (early July to mid August), then to the long photoperiod (16L/8D) for a further 6 weeks until mid September, followed by exposure to the natural daylength (12L/12D) in mid September. The control group in both years was maintained under natural daylength all year round. In the first year, egg production under artificial photoperiod was advanced by 4 weeks when compared to controls. In the second year, egg production was advanced by less than 3 weeks and delayed the onset of photorefractoriness. This confirms that, in this species, light manipulation can be used to advance the onset of egg production and increase the peak production.

I. INTRODUCTION

Pheasant production has a small share of the game bird meat market mainly due to seasonality of production and limited demand. Commercial production of pheasants, whether in the Northern or the Southern hemisphere is constrained by seasonality of breeding (Deeming and Wadland 2002; Malecki and Martin 2007). In Australia, pheasants under natural photoperiod begin breeding early October with a photoperiod of approximately 13 hours light, reach a peak egg production in November, then gradually decline in December and terminate in early February under a photoperiod of approximately 13-14 hours light (Malecki and Martin 2007). Such a pattern is typical of the long-day breeding species in which the breeding is stimulated by increasing daylength and is subsequently terminated by development of photorefractoriness to the stimulatory effects of long days (Sharp 1996). Photorefractoriness can be dissipated by dark or dark/light (D/L) treatments, which can reinstate sensitivity to long days. Pheasant producers in Australia take advantage of this phenomenon by manipulating the duration and timing of pheasant breeding with artificial photoperiod (Chaseling 1990). There is, however, little documented evidence on the nature of response to the light treatments and potential benefit of manipulated photoperiod. The aim of this preliminary study was to describe the response of the pheasant breeder birds to stimulation by increasing day length and development of photorefractoriness.

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II. MATERIALS AND METHODS

The study was located at the Olson Game Birds (Swan Hill, Victoria). Normally, the breeding flock is managed under natural photoperiod fluctuating from 9.30 to 14.40 hours. The total flock consisted of 620 breeders aged 1 - 2 years, with a male to female ratio maintained at 1 to 6. For the study, the control treatment (natural photoperiod) consisted of 500 breeders, whereas the photoperiod treatment group consisted of the remaining 120 breeders. The flock managed under natural daylength was housed from early September in the breeder house consisting of 12 pens with large windows allowing natural light penetration. The photoperiod treatment group was housed in a separate shed that did not have windows. All birds were receiving the commercial pheasant breeder ration offered *ad libitum*. The light treatments were as follows: Year 1 – early July (8L/16D for 6 weeks till mid August), then 16L/8D for the rest of the season; Year 2 – early July (8L/16D for 6 weeks till mid August), then 16L/8D for another 6 weeks (mid August to late September) followed by natural daylength. A switch to natural daylength was accomplished by opening doors and allowing access to outdoor yards. Changes in day length were instituted rapidly from one day to the next and there was no attempt to transition changes in day length. Over two breeding seasons, we tested the response to light treatment by measuring the rate of egg production.

II. RESULTS

In the first year, the egg production of the light-treated group was advanced by 4 weeks (28-30 days) (Fig. 1a). The maximum egg output was reached in early October, 50 days from the commencement of long day treatment. The same level of production in the control group was reached in early November. The rate of egg production in the treatment group started then declining, but mimicked the controls and terminated in early January, whereas the controls terminated at the end of January.

In the second year, the light-treated group commenced egg production 3 weeks ahead of the natural daylength group (Fig. 1 b) and reached peak production by mid October. After the peak, egg production gradually declined and became similar to that of birds under the natural daylength. Egg production terminated at the same time as in the controls.

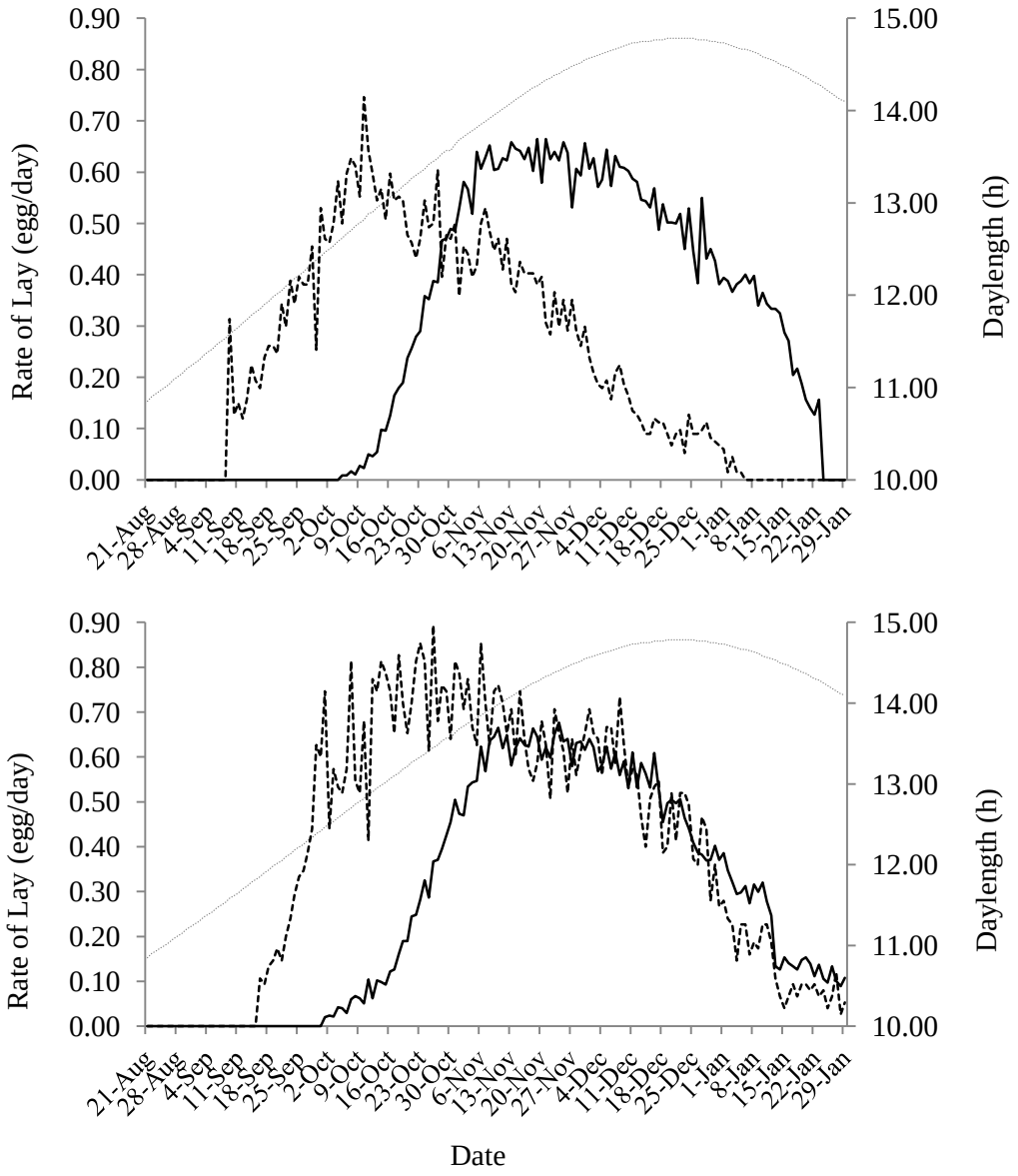

Figure 1. Egg production performance of Ring-necked pheasants (*Phasianus colchicus*) subjected to the photoperiod treatment and natural daily photoperiod.

Figure 1a. Upper graph: Year 1 – response to natural (solid line ——) and to photoperiod treatment (broken line – – –) 16L/8D; (Hairline ·····) superimposed natural daylength (h); Day 1 of the season is when 16L/8D commenced.

Figure 1 b. Lower graph: Year 2 - response to natural (solid line———) and photoperiod treatment (broken line – – –) 16L/8D and then natural daylength; (Hairline ·····) superimposed natural daylength (h); Day 1 of the season is when 16L/8D commenced.

III. DISCUSSION

The commercial Ring Necked pheasants in Australia are responsive to photoperiodic information. They can be stimulated to commence egg production by exposure to increasing daylength and/or long days and can be later inhibited by the same day length, possibly due to development of photorefractoriness (Sharp 1996). The abrupt switching to the natural daylength of 13 h in early October from 16 h of light per day, followed by the normal seasonal increase in daylength from September to October to November appeared to stimulate additional peak production (65% to 80%), and inhibited the steep decline in egg production or abrupt loss of persistency of production. These experiments have illustrated the opportunities for photoperiod manipulation to influence the duration of the egg production season and the peak production, but additional research is required to study the loss persistency of production and to establish a clearer understanding of the impacts of photorefractoriness in these production changes.

V. CONCLUSIONS

Australian Ring-necked pheasants respond to daylength in a pattern similar to that observed in long-day breeding birds. An appropriate light regime can extend the egg production period by both, advancing the onset of laying and delaying the onset of photorefractoriness but the optimal light program remains to be determined.

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NUTRITIONAL STRATEGIES TO ALLEVIATE POOR GROWTH PERFORMANCE OF COMMERCIAL DUCKS UNDER HIGH TEMPERATURE

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Under Australian conditions ducks take an extra 4-6 days to reach market weight in summer than in winter. The objective of the current study was to investigate nutritional strategies that might improve duck performance during periods of high ambient temperature.

Cherry Valley Peking ducks, were raised on deep litter in a tunnel ventilated shed following commercial practices. As day olds, equal numbers (n=15) of males and females were randomly allocated to 24 floor pens (n=4 per treatment; 5.3 birds/m^2). Birds were fed *ad libitum* a starter diet (days 1-14) formulated to supply 12.45 MJ/kg ME and 22.2% protein, and a grower diet (days 15-41) formulated to provide 12.54 MJ/kg ME and 18.8% protein. Each pen had its own water supply with 4 nipple drinkers. Birds were wing tagged and individually weighed on days 14, 28, 35 and 41 of age. The following treatments were applied from days 29-41 of age; control alone water with 250 mg/kg vitamin C, water with betaine at 500 mg /L, betaine in the diet at 1000 mg/kg, feed withdrawal during the high temperature period. A further treatment was supplied with water on days 29-35 and then water with electrolyte salts (2g NaCl, 2.175g NaHCO3 and 1.125g KCl/L) and betaine (Feedworks, Romney, Vic, Australia) 500mg/L on days 36-41 of age. The birds were exposed to high temperature (30-32^{o}C) from 08:30-16:30h and to 20^{o}C for the remainder of each day, over days 36-41 of age. Data were analysed using the REML linear mixed model function of Genstat®. Significance testing of fixed effects was conducted using Wald chi-square tests ($P<0.05$) and where significance was obtained, pair-wise comparisons were made by the least significant difference (LSD) procedure.

Table 1. Liveweight gain of Peking ducks under high temperature (30-32^{o}C)

Strain	Week	Control	Vitamin C	Betaine in water	Betaine in feed	Feed withdrawal	Electrolytes	SEM
Gain (g)	5	353^b	376^a	381^a	365^{ab}	381^a	355^b	18
	6	359^c	333^d	382^b	367^c	355^c	442^a	18

Within rows values with different superscripts are different ($P<0.05$).

The treatment x week interaction was significant ($P<0.001$). In week 5, compared to betaine in feed and control treatments, performance was improved by using vitamin C, betaine in water and feed withdrawal ($P<0.05$). In week 6, the improved gain was only sustained by using the betaine supplementation in water, however, further improvement was made when electrolyte salts were added with the betaine ($P<0.05$). Continued supplementation with vitamin C significantly depressed liveweight gain in week 6 ($P<0.05$). The electrolytes are likely to act to improve acid base balance and water retention. The use of electrolytes and betaine as supplements in water might provide producers with an opportunity to improve duck performance during periods of high temperature although on-farm trials are required to support this recommendation.

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EFFECT OF A PROTECTED COMBINATION OF SODIUM BUTYRATE AND ESSENTIAL OILS (NATESSE) ON BROILER PERFORMANCE AND JEJUNUM EPITHELIUM DEVELOPMENT

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Summary

The protected combination of sodium butyrate and essential oils has been demonstrated to be effective in preventing necrotic enteritis (NE) in broiler chickens. Therefore, it is of interest to evaluate the effect of this concept on healthy animals. A total of 192 one-day-old Cobb broilers were divided equally among 16 pens. The trial compared the biological parameters and the mid jejunum epithelium development of 2 treatments (8 replicates per treatment): a control treatment (C) (normal fattening program) and an experimental treatment (E) (control treatment feed + 1 kg/ton of feed of the protected combination of sodium butyrate and essential oils). The feeding program consisted of a starter diet from day 0 to 21 (330 g/kg corn, 200 g/kg barley, 360 g/kg soy, 66 g/kg lard; 12.56 MJ/kg AME, 210 g/kg CP, 87 g/kg EE and 11 g/kg Dig Lys) and a finisher diet from day 21 to 42 (319 g/kg corn, 233 g/kg barley, 335 g/kg soy, 73 g/kg lard; 12.77 MJ/kg AME, 201 g/kg CP, 94 g/kg EE and 10.3 g/kg Dig Lys); diets were presented as mash. The average body weight of the animals was similar for both treatments at the beginning of the trial (43.41 g for C and 43.18 for E). However, at the end of the trial, the animals in the E treatment tended to weigh more than the C animals (2478 g for C and 2604 g for E; P = 0.0837); this difference is explained by the higher average daily gain (55.3 g/day for C, 58.2 g/day for E; P = 0.0837). The feed intake of the E group was numerically higher (89.3 g/day for C, 91.4 g/day for E; P = 0.53) and the feed conversion ratio was numerically better for the E treatment as well (1.615 for C and 1.569 for E; P = 0.32). At day 42, one animal per pen was euthanized and sampled for mid jejunum morphometric evaluation. There were no statistically significant differences between treatments: villus length was numerically higher for the E treatment (1750 microns for C, 1886 microns for E; P = 0.1690), the animals in the control group presented more crypts/diametric mm in the jejunum (17.53 for C, 15.83 for E; P = 0.2210) and the animals in the E treatment had more villi/diametric mm (5.34 for C, 5.38 for E; P = 0.92). It was concluded that the protected combination of sodium butyrate and essential oils improves growth in healthy animals.

I. INTRODUCTION

Increasing concern about antibiotic resistance led the European Union to ban sub-therapeutic antibiotic usage, starting a trend that affects animal production across the entire world. There is, then, an increasing interest in finding alternatives to antibiotics in poultry production.

Short-chain fatty acids could be an interesting alternative due to different properties. Butyrate is considered to be important for normal development of epithelial cells (Pryde et al., 2002, Mallo et al., 2010), having a direct effect on intestinal epithelium development and, hence, on animal performance: increasing body weight gain and improving feed conversion

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ratio (Hu and Guo, 2007; Mallo et al., 2010). Similar to other organic acids, short-chain fatty acids can be used for the treatment of several intestinal bacterial infections, including salmonellosis (van Immerseel et al., 2005; Fernández-Rubio et al., 2009).

Essential oils have many beneficial effects. They are able to control both gram- and gram+ bacteria, as well as protozoa such as coccidia (Barbosa, 2009; Oviedo-Rondón et al., 2006; Mallo et al., 2010) and can stimulate secretion of digestive enzymes (Williams and Losa, 2001) or have a positive effect on intestinal epithelium growth and regeneration (Jerzsele et al., 2011).

The combination of sodium butyrate with essential oils has a strong effect against necrotic enteritis (NE) (Jerzsele et al., 2011) at a dosage of 1.5 g/kg of feed. With the sodium butyrate, the intestinal epithelium is able to recover faster from the desquamation produced by coccidia and other NE predisposal factors. Sodium butyrate, together with the essential oils, is able to control the population of the gram+ bacteria *Clostridium perfringens*, which induces the most common NE (Gholamiandehkordi et al., 2007).

Bolton and Dewar (1965) indicated that free butyrate quickly disappeared in the upper digestive tract and, whereas almost 60% of the feed source was intact in the crop, less than 1% was recovered from the upper small intestine. The efficacy of butyrate, and hence, of the essential oils, will likely be improved if it is protected from immediate absorption in the upper tract. It is, however, important to leave a part of the product active in the first part of the gastrointestinal tract in order to protect the animal from bacterial infections at the level of the crop (Fernández-Rubio et al., 2009).

This study evaluated the effect of a vegetable fat protected combination of sodium butyrate with 1% essential oils on healthy broiler biological performance at a dosage of 1 g/kg feed, similar to that found effective for protected butyrate (Mallo et al., 2010).

II. MATERIAL AND METHODS

One hundred and ninety-two one-day-old Cobb broilers were divided equally among 16 pens. The trial consisted of 2 treatments, with 8 replicates each, a Control treatment (C), and an experimental treatment (E) (Control diets + 1 kg of Natesse/ton of feed). The composition of Natesse is 700 g/kg of sodium butyrate, 10 g/kg of a blend of essential oils, and 290 g/kg of vegetable fat.

Animals were fed following a two phase mash feed scheme, with a starter diet (day 0 to 21) and a finisher diet (day 21 to 42); both diets were formulated to cover the nutritional needs published by the NRC. The composition of the control diets can be found in Table 1.

In order to compare the biological response of the 2 treatments, animals were weighed at the same time of day on days 0, 21 and 42, and feed intake was recorded every 3 weeks.

To compare the intestinal epithelium development, at day 42, one animal per pen was euthanized and sampled for mid jejunum morphometric evaluation. The samples of jejunum were open longitudinally, and evaluated transversally (relating the measurements, hence, to diametric mm), for villus length, and number of villi and crypts. Figure 1 shows a scheme of the intestinal epithelium evaluation.

III. RESULTS AND DISCUSSION

Table 2 shows the weight of the animals at days 0, 21 and 42. At the end of the trial, the animals in the E treatment tended to weigh more than the C animals (2478 g for C and 2604 g for E; $P = 0.0837$); this difference is explained by the higher average daily gain (Table 3) (55.3 g/day for C, 58.2 g/day for E; $P = 0.0837$); there were no statistically significant differences for feed intake or FCR. These results are in concordance with the reported results

for the protected combination of sodium butyrate and essential oils (Jerzsele et al., 2011, Mallo et al., 2012) and for protected sodium butyrate (Mallo et al., 2010).

Table 4 presents jejunum villus length for the two treatments. The villus length was numerically higher for the E treatment, the animals in the control group had more crypts/mm in the jejunum and the animals in the E treatment had more villi/mm of circumference. Again, these results concur with those observed with protected sodium butyrate (Mallo et al., 2010).

It was concluded that the protected combination of sodium butyrate and essential oils improves growth in healthy animals. It is necessary to confirm the differences observed in the development of the intestinal epithelium with a larger number of animals.

Table 1. Control diets Starter and Finisher

Ingredients g/kg	Starter 0-21 d	Finisher 21-42 d	Nutrients [2]	Starter 0-21 d	Finisher 21-42 d
Corn	334.0	319.0	AME, MJ/Kg	12.56	12.77
Barley	200.0	233.0	Crude Protein g/kg	210.0	201.0
Soy 44%	360.0	335.0	Crude Fibre g/kg	37.0	37.0
Lard	66.0	73.0	Crude Fat g/kg	87.0	94.0
L-Lys HCL (78%)	1.1	0.9	Linoleic acid g/kg	16.5	17.1
DL-Met 99	2.4	2.2	Lys g/kg	12.7	11.9
L- Thre	1.0	0.8	Dig lys g/kg	11.0	10.3
Calcium carbonate	10.0	11.5	Met g/kg	5.5	5.1
Dicalphos	17.1	16.1	Dig Met g/kg	5.1	4.7
Salt	4.0	4.0	Met + Cys g/kg	9.0	8.5
Vit-min premix [1]	4.0	4.0	Dig Met + Cys g/kg	7.9	7.4
			Thre g/kg	8.9	8.4
			Dig Thre g/kg	7.5	7.0
			Trp g/kg	2.6	2.5
			Ca g/kg	9.2	9.5
			Total P g/kg	6.9	6.6
			Av P g/kg	4.2	4.0
			Na g/kg	1.7	1.7

[1]Broiler vit-min premix; [2]Based on FEDNA tables values (1999)

Table 2. Weight of the animals at days 0, 21 and 42

	Body Weight d 0 (g)	Body Weight d 21 (g)	Body Weight d 42 (g)
Control	43.4	706	*2478*
Natesse	43.2	723	*2604*
SEM	-	9.7	46.0
P	-	0.2383	*0.0837*

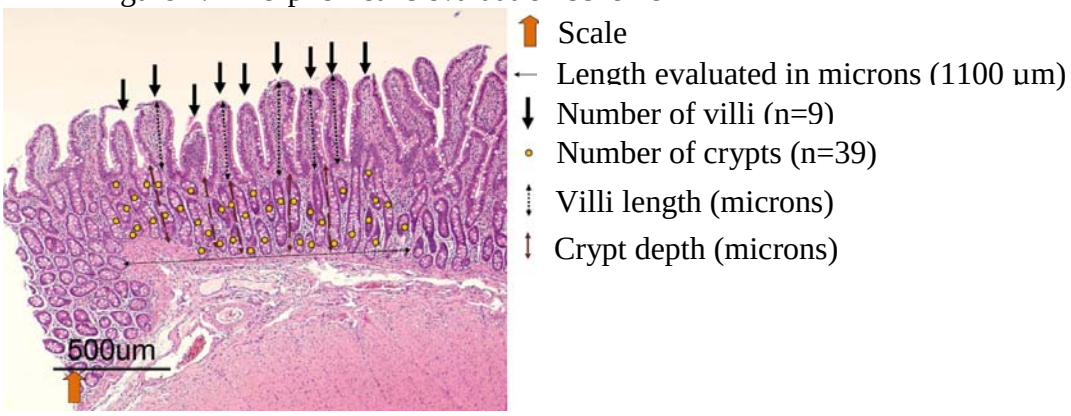
Table 3. Biological response 0 to 42 days

	Average Daily Gain (g)	Average Daily Feed Intake (g)	Feed Conversion Ratio
Control	*55.3*	89.3	1.610
Natesse	*58.2*	91.4	1.570
SEM (n = 8)	1.05	2.19	0.02
P	*0.0837*	0.5376	0.3184

Table 4. Jejunum epithelium development at day 42

	Villi length (microns)	Crypts/mm of circunference	Villi/mm of circunference
Control	1750	17.53	5.34
Natesse	1886	15.83	5.38
SEM (n = 8)	215.4	0.961	0.290
P	0.1690	0.2210	0.9160

Figure 1. Morphometric evaluation scheme



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