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THE CHALLENGES CONFRONTING CHICKEN MEAT PRODUCERS IN GREAT BRITAIN IN RELATION TO LOW PROTEIN DIETS

P.W. GARLAND[1]

Summary

The concept of commercial "low protein diets" used in the UK will be put into context both with typical Australian diets but also within the framework of the commercial environment that has had a significant impact on how chickens are reared. The transition towards lower protein has been a gradual process of evolution rather than an epiphany moment and the history goes back to the early 1990's. This paper will highlight the challenges faced in amino acid supply in commercial broiler diets in the UK currently and the future issues arising from environmental and commercial concerns.

I. INTRODUCTION

The move towards lower protein diets in the UK has been driven by several factors over the last 20 years or so. In the early 1990's the transition was made to formulating using digestible amino acids rather than total; this immediately gave a reduction in protein content and was, in hindsight, a useful cost reduction and performance improving step. By 1996, though, the challenges became apparent with the voluntary removal of meat-and-bone meal, ahead of the total ban implemented by the EU in 2000, which included all ruminant derived products. Overnight, the feeding of broilers was constrained to mainly wheat/soya rations with some fish meal in the early diets.

Quickly, consumer preferences were noted and many retailers required "vegetarian" diets thereby excluding fish meal, tallow and poultry offal meals. In 1999, the industry voluntarily withdrew antibiotic growth promotors from feed (Pritchard, 2015) prior to the EU wide ban in 2006. Whilst this did not necessarily affect protein nutrition, it became evident that nutritional management to improve litter quality would influence our approach to overall crude protein levels. There is now a requirement to cease use of therapeutic antibiotics except where there is a clearly defined clinical need which adds weight to this aspect. Since 2012, antibiotic use has reduced by 71% (BPC, 2017) and one of the primary uses of antibiotic was to treat enteric issues causing wet litter.

The level and source of dietary protein has an effect on intestinal *Campylobacter perfringens* populations with high or excess protein associated with greater incidence of necrotic enteritis or bacterial imbalance leading to enteritis and hence wet litter (Drew et al. 2004). Excess protein increases water consumption; Larbier and Leclercq (1994) estimated that 1% excess protein increases water intake by 3% in broilers. Since wet litter leads to higher incidence of pododermatitis, the avoidance of any increase in water use is regarded as essential. Trial work by Aviagen and reported by Kenny et al. (2010), that increased levels of balanced protein, led to poorer foot pad status confirms practical experience.

Environmental issues have also played a role in shaping our application of protein nutrition. In 1996, the Integrated Pollution Prevention and Control Directive (IPPC) was drafted and came into force in 1999. This requires poultry, and pig, producers to use diets with reducing protein content and the monitoring of ammonia emissions as well as recording the nitrogen content of manures disposed of (IPPC Technical Guidance Note 2010). This has now been superseded by the EPR (Environmental Permitting Regulations 2015 No. 918) and

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requires farmers to demonstrate the use of best available techniques (BAT) to reduce potentially harmful emissions.

Wider global environmental concerns are also beginning to play a role in how we formulate diets. There is a desire in some sectors to reduce our reliance on imported soya bean meal as the main protein source in our feeds and, consequently where possible, the inclusion in feeds of some EU produced vegetable proteins are preferred by major retailers.

II. PRACTICAL DIETS – UK AND AUSTRALIA

It would be tempting to assume that there are differences in broiler diets between the UK and Australia as a result of the European market requirements for animal protein free diets and the aims of the EPR. However, on the basis of a comparison between diets used commercially in both countries, there seems to be relatively little difference in the absolute levels of crude protein and digestible lysine values. Taking the digestible lysine value as an indicator of overall amino acid profile, and relating this to crude protein, does though suggest there are subtle differences. In the example shown in Figures 1 the 2, UK diet regimes have a slightly higher digestible lysine as a percentage of crude protein than the Australian feed programme until about 12 days, thereafter the differences become ambiguous and, in the case of UK A feeds, there is an impact of whole wheat dilution. Both UK feed programmes achieve, at average kill ages of 36 days, liveweight of 2.20 kg and feed conversion ratio (FCR) of 1.60. Clearly there is a different market requirement with the UK not producing the number of heavy birds as is the case in Australia.

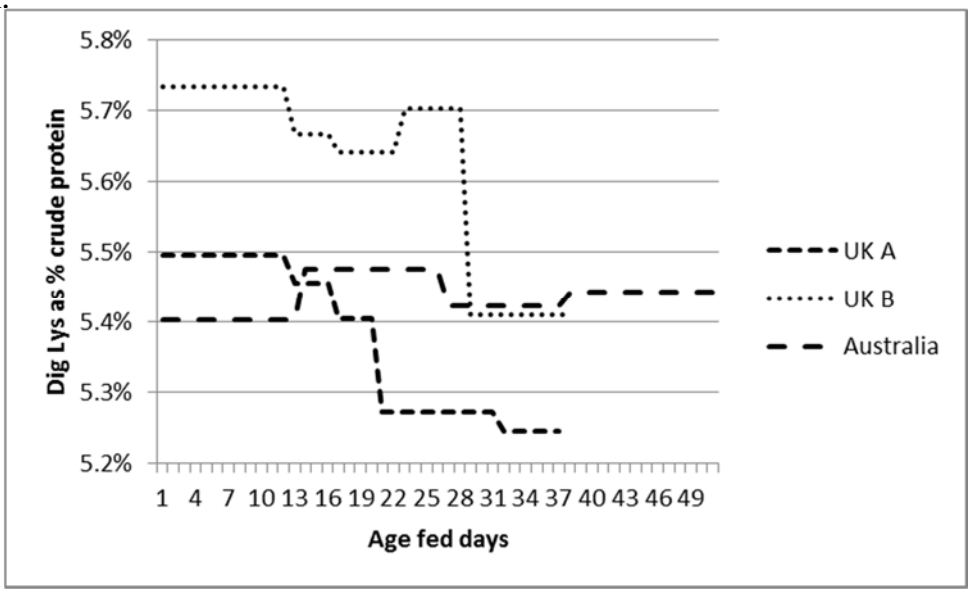

Figure 1 - Comparison between UK and Australian broiler diets in terms of digestible lysine as a percentage of crude protein. (Anonymous sources).

III. IDEAL PROTEIN

The concept of formulating diets to achieve an ideal protein (IP) profile, also referred to as balanced protein, is nothing new, and there are a selection of published profiles to choose from. Some cover the entire life of a commercial broiler whilst others are age specific. Table 2 shows examples of available recommendations and illustrates that, whilst there are some differences in the percentage of individual amino acids in relation to lysine, all broadly follow similar trends. With the availability of feed grade amino acids, achieving the chosen profile should be feasible. However, the economics of feed formulation mean that some of

the target values are aspirational rather than practical, particularly when high digestible lysine diets are used. In particular arginine, valine and isoleucine can be challenging in typical UK diets where, apart from soya bean meal and rapeseed/rapeseed meal, the other proteins available are typically field beans, maize gluten meal and fishmeal where permitted. The use of L-Valine is common practice and the addition of guanidinoacetic acid for its arginine sparing effect is increasing. However, the use of L-isoleucine, L-cystine or combined branched chain amino acids has yet to gain traction due to the current cost of these potential ingredients and lack of approved fermentation production methods to meet EU regulations. The prospect of increased reliance on supplemental amino acids was considered by Selle et al. (2015) and the use of free amino acids as energy sources for the gut mucosa rather than the intended purpose of protein accretion is a potential concern. Commercially, the boundaries are being tested and total supplemental amino acid additions of greater than 10 kg/t seem to give growth and FCR gains.

Table 1 - Ideal digestible amino acid profiles for broiler chickens expressed as percentage of lysine.

Source	Ajinomoto[1]	Evonik[2]		Tillman[3]		Ross[4]		Cobb[5]	
Age period days		1-12	36-48	1-7	29-42	1-10	31-40	1-10	23-42
Lysine	100	100	100	100	100	100	100	100	100
Methionine		40	43	45	47	40	42	38	41
M+C	75	72	78	75	77	74	78	74	78
Threonine	67	63	66	70	68	67	67	65	68
Valine	80	79	81	75	77	75	78	75	77
Arginine	105	102	107	105	107	103	105	105	108
Isoleucine	67	68	72	66	68	67	69		
Histidine	36	33	33						
Leucine	105	107	107			110	110		
P+T	105	116	116						
Tryptophan	17	16	17	16	16	16	16	16	18

[1]Ajinomoto 2015, [2] Evonik 2016, [3]Tillman 2013, [4]Ross 2015, [5]Cobb 2012

With the application of IP and use of added amino acids, it is possible to increase growth rates and lower feed conversion as well as manipulate carcass yield. There is a diminishing return at farm level, as shown by Kemp et al. (2005) who demonstrated that increasing IP to 120% of control diet improved weight gain and FCR, but the economic return did not justify this approach at that time at farm level although processing margins were improved. Perhaps indicating a mismatch between the economics of agriculture and processing.

In a review presented by Ajinomoto (2015), it is proposed that reducing dietary crude protein by 1% gives a 7-10% reduction in N excretion. It is also shown that, using supplemental methionine, lysine, threonine and valine, the crude protein levels in a 1.05% digestible lysine diet can be reduced by 2.9% units in both maize and wheat based diets. Using supplemental amino acids to reduce crude protein has been reported by Dozier and Kidd (2014) in 29 to 40 day broilers where additions of lysine, methionine and threonine were used to maintain an IP profile where digestible lysine was 1.00%. This is not a particularly challenging level but weight gain and FCR were maintained along with carcass and breast yields.

IV. HOW LOW CAN WE GO?

There seems to be considerable scope for reducing crude protein levels in broiler grower and finisher diets and making use of supplemental amino acids. In a trial conducted by van Harn and van Krimpen (2016), broiler grower and finisher diets were reduced in crude protein content by 1, 2 and 3% from control levels of 20.8 and 19.8% protein for the grower and finisher rations respectively. The digestible lysine levels were 1.05% and 0.99% for the control diets, not quite at commercial levels. The IP profile was maintained by the addition of amino acids –L-lysine HCl, DL-methionine, L-threonine, L-arginine, L-valine, L- isoleucine, tryptophan and L-glycine.

The results are encouraging; for 0-35 day performance there was no effect on live weight and significant improvement ($P < 0.05$) in FCR at -2% and -3% crude protein. Water intake was reduced numerically and foot pad scores were significantly ($P < 0.05$) reduced at all protein reductions and litter condition scores were also improved in terms of friability and dryness.

Carcass characteristics showed a slight reduction in breast meat yield at -3% crude protein although carcass weight and yield were unaffected. However, the economic results showed that the cost of adding amino acids gave lower margins per bird of between -€0.024 and -€0.061 per bird. The other aspect reported was that reliance on amino acids has a negative impact on carbon foot print due to energy use in their production. In this trial, the g CO_2-eq/kg is estimated to have increased from 725 to 875 g CO_2-eq/kg live weight.

From a practical or commercial perspective, a number of amino acids that were used are not commercially available or permitted under EU legislation as feed ingredients. It does, though, indicate that there is scope if the materials are available.

As steps are taken to reduce crude protein, it is becoming appropriate to consider the supply of non-essential amino acids that we have taken for granted as being supplied in sufficient quantity with the allegedly surplus protein. The importance of considering glycine and serine in low protein diets was discussed by Siegert and Rodehutscord (2015), where the proteins in the chicken body incorporating both glycine and serine and the metabolic processes requiring adequate supplies of these amino acids are highlighted. In a situation of no antibiotic growth promotors and reduced therapeutic antibiotic use, we need to rethink the importance of individual amino acids and their role in maintaining gut health. In particular, mucin production is of relevance because it aids in protecting the gut mucosa and is dependent upon adequate glycine and serine. Siegert and Rodehutscord (2015) emphasised the importance of interrelationships between essential and non-essential amino acids and the need to consider increasing proportions of, for example, threonine as a precursor for the synthesis of glycine. It is also pointed out that, as commercial nutritionists are tempted to use higher levels of DL-methionine to maintain IP profiles, there is the factor of the role of glycine in the conversion of methionine to cysteine and in diets with low cysteine the requirement for glycine may be increased.

The conclusion drawn is that, with dietary protein concentrations below 20%, glycine and serine will become limiting and that a glycine equivalent value will be required for diet formulation is an indicator of future direction for feed formulation. A glycine equivalent value (Gly_{equi} %) is calculated by glycine % + (0.7143 x serine %) where 0.7143 is the ratio of the molecular weights between glycine and serine, Kidd and Tillman (2016). The same authors note that the determination of digestible glycine and serine is difficult, but fortunately more work is being conducted in this area. In table 2, the Gly_{equi} values for typical raw materials are shown; this illustrates the challenge faced where animal proteins are not available in meeting proposed levels of glycine and serine. The Gly_{equi} requirement varies according to threonine level, and methionine to total sulphur amino acid ratio, according to

Siegert and Rodehutscord (2015), values of 19.0 to 25.0 g/kg dry matter are needed to maximise gain to feed and average daily gain in 7 to 21 day broilers. This is seemingly qualified by Siegert et al. (2015) where a number of trials were used in a meta-analysis and it appears that, if there is adequate cysteine in the diet, then requirement for Gly_{equi} is reduced and crude protein reductions might be feasible. Clearly, with the inability to use meat and bone meal, achievement of lower crude protein diets and maintaining supply of these "non-essential" amino acids will be challenging, unless feed grade sources become available and approved.

V. REDUCING SOYA BEAN USE

Europe relies on imported soya bean meal to provide the majority of protein in monogastric feeds and takes this from the USA and South America. For a number of reasons, there is growing interest in reducing the reliance on this imported protein and increasing the use of locally sourced protein materials. Part of the justification is to improve food security and reduce trade deficits but, amongst retailers, the ability to use soya bean in the food supply chain that is not derived from genetically modified (GM) seed stocks is a key objective. As the acreage of GM derived soya crops increases, it is becoming harder to source non GM meal through a supply chain that handles both GM and non GM material.

Table 2 - Glycine equivalent, crude protein and SID lysine values for raw materials.

Material	Gly_{equi} (g/kg as fed)	Gly_{equi} (g/100g protein)	Crude protein g/kg	Digestible lysine g/kg
Wheat	7.5	7.5	101	2.6
Maize	5.4	7.4	75	2.1
Sorghum	5.7	6.5	89	1.8
Soya bean meal	37.1	7.8	476	25.7
Rapeseed Meal	25.9	8.2	315	15.0
Sunflower Meal	31.0	8.7	355	10.7
Beans (faba)	18.5	7.5	249	12.7
Peas	16.0	7.8	205	13.0
Lupins	25.0	7.5	333	14.5
Wheat bran	12.6	8.1	156	4.5
Wheat DDGS	20.8	7.4	281	2.4
Brewers Yeast	33.2	7.7	429	n/a
Fish meal	63.7	9.4	679	43.0
Meat and bone meal	83.6	17.8	474	15.9
Insect *	68.7	7.7	421	28.0 total lysine

All values from Evonik (2016) except * = Feedipedia

Alternative protein sources available within Europe do not have the amino acid content or digestibility of soya bean meal and therefore any substitution of soya is going to increase crude protein levels if amino acid specifications are to be maintained. The data in Table 2 show that, in terms of protein content, only fishmeal and meat and bone meal are potential direct replacers for soya bean meal and these materials are either not permitted or not favoured by consumers. Much interest has been shown in using legumes, either field beans or peas, with retailer sponsored trials conducted for pig and poultry on commercial units. These have been successful to the point of confirming that they can be used but the substitution of soyabean is limited for a number of reasons. In table 3, the alternative materials available in the UK are compared to soyabean meal in terms of digestible lysine for

ease of comparison and clearly this is not fully reflective of the raw materials since, for example, sunflower and rapeseed meals are good sources of digestible methionine.

This simplistic approach does, though, illustrate that replacing 1% soya bean meal in a ration is going to require considerably more than 1% of an alternative protein meal. This then creates cost in the formulation process through lack of space. The legumes can be used at sensible levels, 12% for beans and 20% for peas would be considered acceptable if they were available in sufficient quantity and of the right quality. The lack of supply means that consistent availability is a problem for feed mills. Field mycotoxin contamination is a concern for beans being harvested late in the season and often subject to rain. Whole rapeseed is used to maximal levels but maintaining pellet quality tends to limit use to about 12%; sunflower is rarely economic. Heat processed blends of whole rapeseed plus beans or peas are widely used and can reach levels of 20% in diets but, again, pellet quality can become an issue and more typical inclusions are 10-15%.

There is much publicity about the use of insect protein for feeds in Europe with pilot plants producing meal from predominately dried Black Soldier Fly larvae. Table 2 shows suggested values but they are not markedly higher than those for soyabean meal. The review by Leek (2016) highlights the potential for insect sources to provide material suitable for aqua-culture and pet feeds, before poultry feeds, purely on a cost basis although the oil extracted from the dried larvae may find value in monogastric feeds. Although approved for use in fish feed, legislation does not yet allow insect proteins to be included in other feeds and it is likely to be some considerable time before it does. Then there are the issues of cost, scale of production and substrate selection for producing insects on. Analysis data shown by Leek (2016) illustrates potential difficulties arising from high calcium content of black soldier fly larvae and the variability of analysis including amino acids and fatty acid profile dependant on the substrate used.

Table 3 - Plant sourced raw material alternatives to soya bean, analysis, inclusion maxima, anti-nutritive factors and amount needed to replace 1% soya bean meal.

Raw Material	Soya - hipro	Field Beans	Peas	Rapeseed meal	Sunflower meal
Protein g/kg	480.0	255.0	205.0	335.0	360.0
Digestible Lysine g/kg	26.2	14.4	11.9	13.6	10.3
Energy MJ/kg	10.30	11.10	11.40	7.00	7.50
Antinutritive factors limiting use	Trypsin inhibitor in under cooked material	Tanins, vicine, convicine, Possible mycotoxin	Possible mycotoxin, trypsin inhibitor	Tanins, sinapine, saponins, phytic acid	Chlorogenic acid.
1% soya requires % inclusion on a digestible lysine basis		1.82	2.20	1.93	2.54

VI. CONCLUSIONS

It is not realistic to suggest that current UK broiler chicken diets are notably lower in protein than those used elsewhere. However, there is pressure from a number of areas to reduce crude protein whilst maintaining diet performance levels. Environmental controls are guiding

farmers towards using lower protein and cost efficiency is clearly a major factor in maximising the efficient use of protein. As these take effect, we need to secure materials which will support attainment of appropriate IP, taking account of the 4th, 5th and 6th limiting amino acids as well as the clearly important role of some non-essential amino acids which are becoming obligatory.

In the face of these challenges, there is the consumer driven need to seek alternative protein sources to soya bean meal which so far has the potential effect of increasing crude protein levels in diets. Perhaps in the future, the reintroduction of correctly processed meat and bone meal will be allowed and fulfil the need of both lower protein and appropriate amino acid supply.

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WHY DO WE NEED LOW PROTEIN MEAT CHICKEN DIETS?

M. HILLIAR[1] and R. SWICK[1]

Summary

The current level of protein used in meat chicken diets is linked to various issues observed in the industry including feed cost and feed efficiency, health and welfare concerns, and negative environmental impacts. This review covers the benefits of low protein diets and how they can be extended into industry with use of feed additives and management. The implementation of low protein diets will contribute to the sustainability and efficiency of the poultry industry.

I. INTRODUCTION

The meat chicken industry is growing rapidly as a result of an increasing population, relatively low production costs and excellent marketability with regards to affordability, sustainability and minimal religious restrictions. Poultry diets with lower crude protein (CP) have generated global interest from the meat chicken industry due to the benefits concluded by published literature. Low protein (LP) diets have been identified to lower feed costs, improve feed utilisation, reduce environmental impacts, and minimise health and welfare concerns. This review will give a comprehensive overview of the benefits of LP diets and methods for their adoption by the poultry industry.

II. THE BENEFITS OF LOW PROTEIN DIETS

a) Feed cost and ingredient concerns

The current CP levels used in the poultry industry contribute to higher feed costs. Poultry feed is not only the most expensive component of chicken production but, with 287 mmt of poultry feed produced globally in 2016 (Alltech, 2017), any unnecessary costs incurred are significant. To satisfy the nutritional requirements of meat chickens and meet production goals, minimum levels of digestible amino acids (AA) must be presented in the feed. In poultry diet formulations, grains can make up half the CP content; the remaining digestible AA requirements are achieved with protein meals and crystalline AA. Protein meals supply a broad range of AA while crystalline AA are used to meet specific AA requirements. Compared to other unprocessed feed ingredients, these products require manufacturing from raw materials, adding to their overall cost. Crystalline AA supplements are becoming more affordable, enabling the practical extension of LP diets in industry.

Protein meals present inconsistent nutrient profiles, are known to contribute to health issues and are linked to negative environmental impacts. Both fish and meat and bone meals vary in amino acid digestibility between batches due to inconsistencies in source and manufacturing (Smith and Scott, 1965; Batterham et al., 1986). Batterham et al. (1986) observed a decrease in lysine availability in meat and bone meal from 93% to 86% and 31% with application of heat (125°C and 150°C, respectively) over four hours. In 2000, use of meat and bone meal in animal feed was banned in the European Union, following transmissible spongiform encephalopathy outbreaks (Ducrot et al., 2013), restricting meat and bone meal use in poultry feed. Animal derived protein meals have also been identified as

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a predisposing factor for necrotic enteritis and are subject to oxidative rancidity, impairing chicken health and efficiency (Drew et al., 2004). Soybean meal (SBM) is the most widely used protein meal in the poultry industry due to its ideal nutrient characteristics. In 2015/2016 Australia imported 775,000 tonnes of SBM compared to local soybean production of only 27,000 tonnes (USDA, 2017). Importing SBM from the US, Argentina and Brazil includes transportation and importation costs and biosecurity and customs regulations not incurred for domestically produced ingredients. SBM produced in Brazil can be associated with tropical deforestation, a result of grazing cattle being pushed into forested areas to enable soybean production in grassland areas; this is of consumer concern and reflects on industries that use this product (Morton et al., 2006). Australian canola meal, a plant based protein meal, is lower in energy compared to SBM and contains anti-nutritive components including glucosinolates, tannins and phytate, which restrains its use in poultry diets (Khajali and Solminski, 2015).

Digestible AA are known as expensive nutrient requirements in meat chicken diets. A review into existing requirements and reducing industry dependence on current protein levels will help reduce environmental impacts and improve bird health, feed cost and feed utilisation.

b) Feed utilisation, health, welfare, and environmental impacts

Excess CP can overload the gastrointestinal tract (GIT) with excessive AA, peptides and undigested protein (Apajalahti and Vienola, 2016). This overload impedes feed efficiency, contributes to health and welfare issues and adds to negative environmental impacts. Excess AA presented in the diet are absorbed and catabolised, producing higher levels of N excretion in the form of uric acid (Wu, 2013). Improved feed utilisation can be achieved by improving N retention. Belloir et al. (2017) investigated the effect of dietary CP on N retention. Birds fed diets containing 150 g/kg CP achieved 70% N retention in comparison to birds on the 190 g/kg CP diet achieving only 60% N retention ($r = 0.86$). Reducing dietary CP can improve N utilisation.

Dietary protein content is correlated with water consumption and excretion (Alleman and Leclercq, 1997; Wheeler and James, 1949), which leads to wet litter at higher dietary CP levels. Higher water consumption may result from the sodium dependent AA transporters drawing water across the lumen with greater AA absorption. Wet litter occurs as a result of increased water excretion and water spillage from more frequent visits to the water lines. Wet litter is known to cause dermatological diseases such as foot pad dermatitis and cellulitis (Harms et al., 1977). Skin infections are the main causes of carcass and chicken paw downgrade, reducing the yield of the meat chicken industry (US Poultry & Egg Export Council, 2009).

Undigested protein that exits the small intestine acts as a substrate for the bacterium *Clostridium perfringens* in the hindgut, a pathogenic bacterium responsible for necrotic enteritis (Drew et al., 2004). The combination of the higher N waste levels (Ferguson et al., 1998), odorants (Sharma, et al., 2017) and wet litter (Wheeler and James, 1949) that are associated with higher CP diets, creates an optimal environment for disease and infection. These have all been reduced with LP diets (Belloir et al., 2017; Sharma et al., 2017; Wheeler and James, 1949), lowering the risk of disease and improving animal welfare. With the removal of antibiotic growth promoters from poultry diets, the risk of necrotic enteritis and other diseases will increase. Any disease preventative measures, including reduction of dietary CP, become increasingly important.

N wastes have become a focus of environmental sustainability due to their impact on waterway pollution and ecosystems (Sims and Wolf, 1994). Reducing dietary CP further

promotes the sustainability and marketability of the poultry industry. Improving industry sustainability with LP diets comes from reduced water intake and N excretion (Wheeler and James, 1949; Belloir et al., 2017).

Decreasing industry dependence on dietary CP improves the health and welfare of meat chickens by improving living conditions as well as feed utilisation. The health benefits of LP diets must also be considered with future regulations on antibiotic growth promoter use. To remain an efficient and sustainable production system, the industry must consider concepts such as LP diets.

III. ACHIEVING LOW PROTEIN DIETS

In order to achieve the widespread use of LP diets in the meat chicken industry, several methods must be investigated. Supplementation of crystalline essential AA such as D,L-methionine, L-lysine HCl and L-threonine to balance the digestible AA in intact protein, reduces dietary CP to the current levels observed in poultry diets.

One method of maintaining performance with LP diets involves using an ideal amino acid ratio to ensure minimum amounts of AA are offered in the required quantities and ratios without overloading the gut with excess protein. A study conducted by Belloir et al. (2017) found that the use of an ideal amino acid ratio described by Mack et al. (1999), with modifications to arginine and threonine, did not negatively affect performance in diets at 190 and 170 g/kg CP.

In conjunction with ideal amino acid ratios, other crystalline amino acid supplements such as nonessential AA have also been investigated. Maintaining performance with diets containing 160 g/kg CP (Dean et al., 2006; Ospina-Rojas et al., 2014) has been achieved with the use of crystalline essential AA and glycine. Many studies consider both glycine and serine (Gly + Ser) levels in LP diets due to their interconversion *in vivo* (Wu et al., 2013). Glycine has been thought to become limiting in LP diets because of its involvement in uric acid synthesis and the prominence of glycine in collagen and other important proteins such as heme (Wu et al., 2013; Shoulders & Raines, 2009). Optimum levels of Gly + Ser are yet to be agreed upon, with Schutte et al., (1997) suggesting 18 g/kg, while Dean et al. (2006) concluded that total levels need to be 23 g/kg and that lower levels of CP require higher levels of Gly + Ser. The requirement level of Gly+Ser must be confirmed for the successful adoption of LP diets in the poultry industry.

Methods of improving nutrient digestibility must also be considered. Protease is an enzyme which increases the digestibility of CP so this additive must be considered in LP diets. Angel et al., (2011) maintained bird performance at 205 g/kg CP with addition of monocomponent protease at a minimum of 200 mg/kg. Use of insoluble fibre (Hetland et al., 2003) and intermittent lighting (Rodrigues et al., 2017) have improved gut health and function by increasing GIT content retention time. These materials and practices may also contribute to maintaining performance under low CP diets, although more work is required to investigate their effects on N digestibility.

The use of ideal amino acid ratios, crystalline amino acid supplements, proteases and gut enhancing materials and practices will contribute to the employment of LP diets in the industry. This adoption of LP diets will have benefits in production and costs while addressing health, welfare and environmental issues.

IV. CONCLUSION

The extension of LP diets in the poultry industry promotes a decrease in feed costs, environmental, health and welfare issues and an increase in N utilisation with proper dietary formulation. These benefits will result in an improvement in environmental sustainability and

marketability of the meat chicken industry. LP diets have the potential to contribute to the successful adoption of antibiotic free animal production, reducing predisposing factors to disease. LP diets can be achieved with crystalline AA, proteases and GIT enhancing practices. However, LP diets require further research to make their extension practical, profitable and worthwhile for the Australian meat chicken industry.

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DIETARY STARCH INFLUENCES PERFORMANCE OF BROILER CHICKENS OFFERED LOW-PROTEIN DIETS

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Summary

A standard diet (219 g/kg protein, 269 g/kg starch) and a low-protein diet (190 g/kg protein, 439 g/kg starch) were offered to eight replicate cages of male Ross 308 chicks from 7 to 28 days post-hatch. Maize grain was decreased and maize starch increased to reduce dietary protein and evaluate the impact of starch in this context. The transition to the low-protein diet significantly increased ileal starch digestibility by 10.8% but decreased digestibilities of essential amino acids and protein (N) by 6.1%. Thus the study suggests that there is interference between the digestion of starch and protein and/or absorption of glucose and amino acids. Paradoxically, the low-protein diet significantly increased free concentrations of lysine, methionine, threonine and valine in plasma taken from the anterior mesenteric vein.

I. INTRODUCTION

Low-protein diets for broiler chickens axiomatically contain high inclusions of supplemental amino acids and have the potential to generate economic, environmental and bird welfare advantages. Low-protein diets are usually formulated by reducing soybean meal inclusions and increasing inclusions of a range of supplemental amino acids. An alternative approach was adopted in the present study where dietary protein levels were reduced by the partial replacement of maize grain with maize starch. The low-protein diet contained relatively high dietary starch levels and was supplemented with amino acids. The rationale for this approach was to evaluate the impact of readily digested maize starch on the digestive dynamics of supplemental and protein-bound amino acids. In the original study (Selle et al., 2018), a standard positive control diet (1A) was compared with five low-protein diets; however, in this presentation 1A is directly compared with low-protein diet 3C as all five low-protein diets generated very similar outcomes.

II. METHODOLOGY

The composition and nutrient specifications of the two experimental diets are shown in Table 1. Maize was decreased (465 to 98 g/kg) and maize starch increased (0 to 483 g/kg) in the transition from diet 1A to 3C, additional supplemental lysine, methionine and threonine were included in diet 3C to which isoleucine, valine and arginine were also added. As a consequence, diet 1A contained 219 g/kg protein and 269 g/kg starch (analysed) while diet 3C contained 190 g/kg protein and 439 g/kg starch. The experimental diets were steam-pelleted at a conditioning temperature of 80°C and crumbled. Both dietary treatments were offered to eight replicate cages (6 birds per cage) of male Ross 308 chicks from 7 to 28 days post-hatch when growth performance was monitored. At 25 days post-hatch, total excreta was collected over a 48 hour period to determine parameters of nutrient utilisation (AME, N retention). At 28 days birds were euthanised to take digesta from the distal ileum to determine digestibility coefficients of starch, protein (N) and essential amino acids. Gizzard pH and

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relative pancreas weights were determined and blood samples taken from the anterior mesenteric vein to determine concentrations of free essential amino acids in the portal circulation.

Table 1 - Composition and nutrient specifications (amino acids expressed as standardised ileal digestibility values) of experimental diets.

Item (g/kg)	Diet 1A	Diet 3C	Item (g/kg)	Diet 1A	Diet 3C
Maize	465	98	Protein	213	178
Soybean meal	370	344	ME (MJ/kg)	12.8	12.8
Maize starch	0	483	Fat	97.6	29.2
Canola oil	68.0	15.8	Fibre	8.8	5.9
Limestone	6.1	5.4	Calcium	7.56	7.50
Dicalcium phosphate	17.2	18.7	Total phosphorus	7.39	6.37
Sodium chloride	2.66	2.27	Available P	3.60	3.60
Sodium bicarbonate	3.89	3.96	Sodium	2.1	2.0
Potassium bicarbonate	0	3.67	Starch	299	545
Choline chloride	1.17	1.19	Lysine	11.00	11.00
Celite	10	10	Methionine	5.46	6.17
Vitamin-mineral px.	2.0	2.0	Methionine + cysteine	8.14	8.14
Sand	5.0	0	Threonine	7.10	7.10
Lysine HCl	0.76	2.64	Arginine	13.10	11.44
Methionine	2.66	4.12	Isoleucine	8.13	7.59
Threonine	0.29	1.63	Valine	8.77	8.75
Isoleucine	0	0.93	*Analysed values*		
Valine	0	1.79	Protein	219	190
Arginine	0	0.37	Starch	269	431

III. RESULTS

Dietary treatment effects on growth performance, nutrient utilisation, ileal digestibility coefficients of starch, protein (N), essential amino acids and their concentrations in plasma from the portal circulation are shown in Table 2. The low–protein diet significantly increased feed intakes by 8.0%, tended to compromise FCR by 5.3% ($P = 0.079$), significantly enhanced AME by 0.68, N retention by 6.4 percentage units and also ME:GE ratios and AMEn (data not shown). The low-protein diet significantly elevated pH, depressed relative pancreas weights, increased ileal starch digestibility by 10.8% (0.967 versus 0.873; $P < 0.001$) but decreased protein digestibility by 6.1% (0.723 versus 0.770; $P < 0.005$). The transition to the low-protein diet significantly compromised the digestibility of all nine amino acids which ranged from reductions of 1.74% (methionine; $P = 0.023$) to 8.34% (leucine; $P < 0.001$). Somewhat paradoxically, the low-protein diet significantly increased free concentrations of lysine by 94% (76.8 versus 39.5 mg/mL; $P = 0.001$), methionine by 69% (21.6 versus 12.8 mg/mL; $P = 0.003$), threonine by 61% (82.0 versus 51.0 mg/mL; $P = 0.003$), and valine by 37% (41.8 versus 30.6 mg/mL; $P = 0.021$) in the portal circulation. Pearson correlations between apparent digestibility coefficients of starch and essential amino acids in three small intestinal segments are shown in Table 3. Nine essential amino acids were assessed; starch digestibility was negatively correlated with the digestibility of seven amino acids in the proximal jejunum, nine in the proximal ileum and eight in the distal ileum to significant extents.

Table 2 - Effects of dietary treatments on growth performance, nutrient utilisation, gizzard pH, pancreas weights, ileal digestibility coefficients of starch, protein (N) and essential amino acids, concentrations of free essential amino acids in plasma from the anterior mesenteric vein from 7 to 28 days post-hatch.

Item	Diet 1A	Diet 3C	SEM	P =
Weight gain (g/bird)	1433	1458	29.36	0.413
Feed intake (g/bird)	1972	2130	39.59	0.013
FCR (g/g)	1.379	1.452	0.0274	0.079
AME (MJ/kg DM)	12.20	12.88	0.0661	< 0.001
N retention (%)	60.23	66.63	0.8325	< 0.001
Gizzard pH	2.57	3.92	0.1517	< 0.001
Relative pancreas weights (g/kg)	2.36	1.79	0.0955	0.001
Apparent ileal digestibility coefficients				
Starch	0.873	0.967	0.0086	< 0.001
Protein (N)	0.770	0.723	0.0118	0.002
Arginine	0.873	0.834	0.0076	0.003
Histidine	0.839	0.794	0.0076	0.001
Isoleucine	0.835	0.775	0.0086	< 0.001
Leucine	0.827	0.758	0.0092	< 0.001
Lysine	0.849	0.822	0.0071	0.017
Methionine	0.921	0.905	0.0045	0.023
Phenylalanine	0.834	0.769	0.0098	< 0.001
Threonine	0.751	0.707	0.0086	0.003
Valine	0.815	0.768	0.0087	0.002
Concentrations in portal circulation (mg/mL)				
Arginine	95.0	86.0	7.75	0.425
Histidine	17.3	13.1	1.95	0.156
Isoleucine	20.1	24.9	2.11	0.148
Leucine	35.6	30.1	2.66	0.166
Lysine	39.5	76.8	6.68	0.001
Methionine	12.8	21.6	1.73	0.003
Phenylalanine	25.5	25.9	1.50	0.862
Threonine	51.0	82.0	6.09	0.003
Tryptophan	10.5	10.1	0.31	0.409
Valine	30.6	41.8	3.03	0.021

IV. DISCUSSION

The dietary transition was accomplished by substituting maize grain with maize starch which modified the dietary compositions considerably. The resultant increased gizzard pH and decreased pancreas weights with the low-protein diets are indicative of depressed gizzard functionality. The large proportion of negative correlations between starch and amino acid digestibility coefficients in the three caudal small intestinal segments observed is consistent with the concept advanced by Vinardell (1990) that there is mutual inhibition between sugars and amino acids for intestinal absorption. There is the possibility that glucose and amino acids effectively compete for intestinal uptakes when co-absorbed with sodium (Na) via their respective Na^+-dependent transport systems. This is predominantly SGLT-1 for glucose; however, several Na^+-dependent (and independent) transport systems are involved with the absorption of amino acids. In the present study, arginine, isoleucine and phenylalanine appeared to be the amino acids most likely to have their digestibility compromised by a large

dietary concentration of readily digestible starch and competition with glucose for intestinal uptakes.

Table 3 - Pearson correlations between apparent digestibility coefficients of starch and essential amino acids in three segments of the small intestine.

Amino acid	Distal jejunum		Proximal ileum		Distal ileum	
	r =	P =	r =	P =	r =	P =
Arginine	-0.436	0.002	-0.502	< 0.001	-0.389	0.007
Histidine	-0.322	0.026	-0.443	0.002	-0.382	0.009
Isoleucine	-0.353	0.014	-0.500	< 0.001	-0.442	0.002
Leucine	-0.268	0.065	-0.432	0.002	-0.412	0.004
Lysine	-0.216	0.140	-0.367	0.010	-0.286	0.054
Methionine	-0.301	0.038	-0.401	0.005	-0.342	0.020
Phenylalanine	-0.317	0.028	-0.507	< 0.001	-0.414	0.004
Threonine	-0.296	0.041	-0.364	0.011	-0.347	0.018
Valine	-0.297	0.041	-0.450	0.001	-0.407	0.005

Intestinal uptakes of nutrients are probably a critical limiting factor on broiler performance (Croom et al., 1999). However, absorbed nutrients must transit across the gut mucosa to enter the portal circulation and it has been demonstrated that glucose and amino acids, especially glutamate and glutamine, are catabolised in avian enterocytes to meet the copious energy requirements of the digestive tract (Watford et al., 1979). A curious outcome was that the transition to the low protein diets significantly decreased ileal digestibilities of isoleucine, lysine, methionine, threonine, and valine but, enigmatically, significantly increased concentrations of lysine, methionine, threonine, and valine in plasma taken from the anterior mesenteric vein and numerically increased isoleucine. It is probably relevant that the five amino acids mentioned were the only ones added to diet 3C as supplemental amino acids.

The proportions supplemental amino acids represented of dietary totals were in the order of 12, 19, 67, 23 and 37% for isoleucine, lysine, methionine, threonine, and valine, respectively. Thus this outcome is at least consistent with the possibility that supplemental amino acids are more likely to gain entry into the portal circulation than their protein-bound counterparts. If so, there are probably two responsible factors. Supplemental amino acids are completely bioavailable (Izquierdo et al., 1998) do not require digestion and rapidly appear in the portal circulation (Wu, 2009). Supplemental amino acids are absorbed in anterior segments of the small intestine where more glucose is present as an alternative (and more efficient) energy substrate for catabolism in the gut mucosa (Fleming et al., 1997). Therefore, supplemental amino acids may be more likely to be spared from catabolism in the gut mucosa than their protein-bound counterparts.

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RAPIDLY DIGESTIBLE PROTEIN INFLUENCES STARCH AND PROTEIN DIGESTIVE DYNAMICS, DIGESTIBILITY AND CONCENTRATIONS OF AMINO ACIDS IN PORTAL CIRCULATION IN BROILER CHICKENS

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Summary

A 'slow protein' foundation diet and a 'rapid protein' summit diet were offered to male Ross 308 chicks from 7 to 28 days. The foundation diet contained protein derived from soybean and canola meals, maize and limited quantities of supplemental lysine, methionine and threonine. The summit diet contained casein and additional supplemental amino acids as partial replacements for soybean meal. The transition from foundation to summit diets accelerated protein (N) disappearance rates in the three posterior small intestinal segments to highly significant ($P < 0.001$) extents. However, this transition significantly increased starch digestibility coefficients in three posterior small intestinal segments by 4.46% (0.844 versus 0.808) in the distal jejunum (DJ), 3.96% (0.946 versus 0.910) in proximal ileum (PI), 3.11% (0.962 versus 0.933) in distal ileum (DI) and accelerated ($P < 0.01–0.001$) starch disappearance rates in all four small intestinal segments by 16.2% (26.78 versus 23.04 g/bird/day) in PJ, 14.4% (32.38 versus 28.30 g/bird/day) in DJ, 14.0% (36.14 versus 31.71 g/bird/day) in PI and by 13.1% (36.72 versus 32.46 g/bird/day) in DI. Rapid protein positively influenced amino acid digestibility coefficients to highly significant extents and the summit diet significantly increased plasma concentrations of methionine (16.5 versus 11.6 μg/ml; $P < 0.005$) and threonine (81.8 versus 58.8 μg/ml; $P < 0.005$) in the portal circulation. Thus the inclusion of rapidly digestible protein as casein and synthetic amino acids increased protein and starch digestibility and disappearance rates and influenced post-enteral availability of amino acids.

I. INTRODUCTION

The digestive dynamics of starch and protein are considered pivotal to broiler performance (Liu and Selle, 2015). Moreover, it was proposed that the rates of starch digestion and glucose absorption exceed the rates of protein digestion and amino acid absorption and this asynchrony in digestive dynamics compromises the performance of broiler chickens. If so, it follows that the provision of rapidly-digestible protein should enhance broiler performance in a reciprocal manner to the slowly digestible starch concept. A rapid protein summit diet was formulated principally by partially replacing soybean meal with casein in the slow protein foundation diet, a blend of both diets constituted an intermediate diet. Thus the objective of this study was to examine the hypothesis that the dietary provision of rapidly digestible protein enhances the performance of broiler chickens. The absorption of nutrients is considered to be a more important rate limiting factor on the growth performance of broiler chickens than their digestion (Croom et al., 1999). However, following their absorption, amino acids may be subject to catabolism in small intestinal enterocytes for energy provision to the gut (Wu, 1998) and this is a partial determinant of their entry into the portal circulation and their post-enteral availability for protein accretion. Therefore, plasma concentrations of

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free amino acids in the portal (anterior mesenteric vein) circulation was determined in birds offered the foundation and summit diets.

II. METHODS AND MATERIALS

A 'slow-protein' foundation and a 'rapid-protein' summit diet were formulated to be iso-energetic (12.97 MJ/kg). Protein (220 g/kg) in the foundation diet was derived from soybean meal (252 g/kg), canola meal, maize and standard quantities of supplemental lysine, methionine and threonine. Protein (209 g/kg) in the foundation diet was derived from casein (50 g/kg), at the expense of soybean meal (82 g/kg), supplemental arginine, isoleucine and tryptophan in addition to the sources already outlined. Digestible amino acid levels in the dietary treatments were comparable. Each dietary treatment was offered to eight replicate cages (6 bids per cage) from 7 to 28 days post-hatch. Birds had unlimited access to feed and water during the experimental feeding period under a '16-hours-on' lighting regime and room temperature was gradually reduced from 32°C at day 1 to 22°C at day 28. Growth performance and parameters of nutrient utilisation (AME, ME:GE ratios, N retention, AMEn) were determined by standard procedures. At day 28, all birds were weighed, euthanised and digesta samples collected from four small intestinal segments. Protein (N) and starch digestibility coefficients and disappearance rates in proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI) and distal ileum (DI) were determined using acid insoluble ash (Celite) as the dietary marker. Disappearance rates (g/bird/day) were calculated from the following equation:

$$\textit{Disappearance rate} = \textit{feed intake}_{(g/bird/day)} \times \textit{dietary nutrient}_{(g/kg)} \times \textit{digestibility coefficient}.$$

Blood samples were taken from the anterior mesenteric vein of birds following euthanasia (intravenous injection of sodium pentobarbitone). Blood samples were centrifuged and the decanted plasma samples were then kept at −80^{o}C prior to analysis. Concentrations of eighteen proteinogenic amino acids in plasma from the portal circulation were determined as fully described in Selle et al. (2016). The feeding study was conducted so as to comply with specific guidelines approved by the Animal Ethics Committee of the University of Sydney.

III. RESULTS

The overall performance of male broiler chicks from 7 to 28 days post-hatch (weight gain of 1576 g/bird, FCR of 1.463) compared very favourably with 2014 Ross 308 performance objectives (weight gain of 1387 g/bird, FCR of 1.479). The effect of rapid versus slow protein diets on protein (N) and starch apparent digestibility coefficients and disappearance rates in four small intestinal segments are shown in Table 1. The transition from foundation to summit diet significantly ($P < 0.001$) increased protein (N) digestibility coefficients in DJ by 10.4% (0.740 versus 0.670), in PI by 8.57% (0.798 versus 0.735), in DI by 6.11% (0.816 versus 0.769) and significantly ($P < 0.005$) accelerated protein (N) disappearance rates in DJ by 9.27% (16.5 versus 15.1 g/bird/day), in PI by 9.09% (18.0 versus 16.5 g/bird/day) and in DI by 6.36% (18.4 versus 17.3 g/bird/day). The transition from foundation to summit diet significantly ($P < 0.05$ - < 0.001) increased starch digestibility coefficients in DJ by 4.46% (0.844 versus 0.808), in PI by 3.96% (0.946 versus 0.910), in DI by 3.11% (0.962 versus 0.933) and significantly ($P < 0.005$) accelerated protein (N) disappearance rates in PJ by 16.5% (26.8 versus 23.0 g/bird/day) DJ by 14.5% (32.4 versus 28.3 g/bird/day), in PI by 13.8% (36.1 versus 31.7 g/bird/day) and in DI by 13.3% (36.7 versus 32.4 g/bird/day).

The treatment effects on distal ileal amino acid digestibility coefficients and concentrations of free essential amino acids in plasma taken from the anterior mesenteric vein are shown in Table 2. The transition from foundation to summit diets increased ($P < 0.001$)

the digestibility of all amino acids; the average apparent digestibility coefficients of nine amino acids were increased by 5.82% (0.854 versus 0.807) in the distal ileum. The dietary transition increased plasma concentrations of methionine by 42.2% (16.5 versus 11.6 μg/ml; $P < 0.005$) and threonine by 39.1% (81.8 versus 58.8 μg/ml; $P < 0.005$) in the portal circulation. Curiously, the balance of amino acids was not influenced by treatment.

Table 1 - Treatment effects on protein (N) and starch apparent digestibility coefficients and disappearance rates (g/bird/day) in proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI) and distal ileum (DI) in chicks at 28 days post-hatch.

	Protein (N) digestibility coefficient				Protein (N) disappearance rate			
Treatment	PJ	DJ	PI	DI	PJ	DJ	PI	DI
Slow protein	0.560	0.670	0.735	0.769	12.5	15.1	16.5	17.3
Rapid protein	0.630	0.740	0.798	0.816	13.8	16.5	18.0	18.4
SEM	0.031	0.004	0.004	0.006	0.719	0.222	0.204	0.228
Significance (P)	0.141	< 0.001	< 0.001	< 0.001	0.258	< 0.001	< 0.001	0.004
Treatment	Starch digestibility coefficient				Starch disappearance rate			
Slow protein	0.664	0.808	0.910	0.933	23.0	28.3	31.7	32.4
Rapid protein	0.703	0.844	0.946	0.962	26.8	32.4	36.1	36.7
SEM	0.019	0.011	0.003	0.006	0.695	0.479	0.449	0.495
Significance (P)	0.184	0.037	< 0.001	0.007	0.002	< 0.001	< 0.001	< 0.001

Table 2 - Treatment effects on distal ileal digestibility coefficients and plasma concentrations (μg/ml) in portal circulation of essential amino acids in chicks at 28 days post-hatch.

	Distal ileal digestibility coefficient				Concentration in portal circulation			
Amino acid	Slow protein	Rapid protein	SEM	P =	Slow protein	Rapid protein	SEM	P =
Arg	0.858	0.901	0.0029	< 0.001	92.7	101.7	5.041	0.234
His	0.799	0.838	0.0036	< 0.001	16.2	14.5	1.066	0.295
Ile	0.786	0.842	0.0056	< 0.001	17.8	20.0	1.150	0.200
Leu	0.803	0.860	0.0053	< 0.001	31.7	33.2	1.742	0.556
Lys	0.806	0.850	0.0048	< 0.001	31.4	29.9	2.676	0.694
Met	0.929	0.963	0.0026	< 0.001	11.6	16.5	0.694	0.001
Phe	0.808	0.857	0.0049	< 0.001	22.3	22.4	1.128	0.927
Thr	0.705	0.762	0.0069	< 0.001	58.8	81.8	3.780	0.002
Trp	-	-	-	-	6.5	6.8	0.295	0.484
Val	0.770	0.817	0.0057	< 0.001	29.9	30.0	1.321	0.982

IV. DISCUSSION

As anticipated, the transition from foundation to summit diet significantly accelerated protein (N) disappearance rates in the three posterior small intestinal segments. Therefore, substitution of soybean meal in the foundation diet with casein and supplemental amino acids in the summit diet had the intended impact of generating a ‘rapid protein’ diet. The importance of biophysical and biochemical starch-protein interactions on energy utilisation in poultry is accepted (Rooney and Pflugfelder, 1986), although these interactions have yet to be described precisely. It may be that more readily and rapidly digestible protein ameliorates these interactions along the digestive tract, thereby increasing starch digestibility and disappearance rates. This positive influence of rapid protein on starch/energy utilisation appeared to be driving significant improvements in AME of 0.35 MJ, ME:GE ratios by 6.81%, N retention by 7.11% and in AMEn of 0.32 MJ (data not shown). Starch and protein

disappearance rate ratios were calculated to exclude the confounding influence of feed intakes. On this basis starch:protein disappearance rate ratios in the distal ileum were positively correlated to parameters of energy utilisation including AME ($r = 0.509$, $P < 0.05$) and ME:GE ratios ($r = 0.573$, $P < 0.025$). These outcomes demonstrate the relevance of starch and protein digestive dynamics to growth performance of broiler chickens. However, there is a precedent for this outcome as unequivocal impacts of starch and protein digestive dynamics were reported by Sydenham et al. (2017). In this study there were significant ($P < 0.001$) quadratic relationships between starch:protein disappearance rate ratios in the proximal jejunum with 15-28 day weight gain ($r^2 = 0.722$) and FCR ($r^2 = 0.702$).

The transition from slow to rapid protein diets significantly increased distal ileal digestibility coefficients of protein (N) by 6.11% (0.816 versus 0.769) and the average of essential amino acids by 5.82% (0.854 versus 0.807). The likelihood is that the magnitude of these improvements is somewhat more pronounced than would be expected to stem from the differences in diet composition. Nevertheless, the dietary transition increased ileal digestibility of methionine by 3.66% and threonine by 8.09% but increased their concentrations in the portal blood-stream by 42.2% and 39.1%, respectively. Supplemental methionine comprised 35% of the dietary total and the corresponding figure for threonine was 14%. The combined portal plasma concentrations of the six amino acids present in the summit diet in both 'free' and protein-bound forms were increased by 18.6% from 156 to 185 μg/ml by the transition to the summit diet. The corresponding increase for the four protein-bound amino acids was a more modest at 6.1% from 147 to 156 μg/ml. While not conclusive, this outcome appears to be consistent with the possibility that supplemental amino acids are less likely to undergo catabolism in the gut mucosa than their protein-bound counterparts. Both amino acids and glucose are catabolised in avian enterocytes (Watford et al., 1979) to meet the substantial energy requirements of the digestive tract. However 'free' or supplemental amino acids do not require digestion and are rapidly absorbed in the upper small intestine where more starch/glucose is available as an alternative energy substrate. So it is possible supplemental amino acids are more likely to be spared from catabolism in the gut mucosa than their protein-bound counterparts because of their more proximal sites of absorption. Considerable research remains to be completed if the mechanisms by which 'rapid protein' influences starch digestive dynamics and energy utilisation and influences the transition of amino acids across the gut mucosa are to be identified. However, it does appear that such research would be advantageous for chicken-meat production.

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AMINO ACID NUTRITION UPDATE TO ENSURE SUCCESSFUL LOW PROTEIN DIETS IN BROILER CHICKENS

W. LAMBERT[1] and E. CORRENT[1]

Summary

Improving protein efficiency is a key driver in feed formulation in order to reduce nitrogen (N) emissions to the environment, improve health and welfare of animals, reduce feed costs and alleviate the dependency on protein-rich feedstuffs. By applying adequate amino acid (AA) recommendations, combined with a better understanding of interactions and possible influencing factors, it is nowadays possible to drastically reduce dietary crude protein (CP) in broiler rations. A more precise nutrition and balanced AA profile is greatly beneficial to the overall sustainability of broiler meat production. Important reduction of N and NH_3 emissions and climate change, acidification or eutrophication impacts can be achieved as well as improved litter quality and meat quality.

I. AMINO ACIDS: TOWARDS MORE PRECISE FORMULATION

Dietary protein reduction in swine or poultry is driven by economic, environmental and societal issues - the three pillars of sustainability. Formulating on the basis of each indispensable amino acid (IAA) instead of a minimum crude protein (CP) level allows a reduction in feed cost, a decreased dependency on imported soybean meal, and a lower pressure on animal health. In addition, the increasing availability of feed grade amino acids (AA; i.e. L-Tryptophan, L-Valine) has made possible the further decrease of dietary CP and changed the way of addressing risk management in feeds for monogastric animals. Indeed, moving from a dietary formulation based on protein to a more precise formulation that gives value to each single AA is a shift from an unpredictable risk approach to controlled risk management. Formulating and relying on the protein criteria (defined as N x 6.25) is only considering the quantity of supply of various nitrogenous components but not their quality. In contrast to N, AA are predictors of performance and the precise control of the AA levels is crucial for performance and profitability. It also gives more opportunity for innovative nutritional choice and ends in the most economical feed solution by a better adjustment of the feed recipe to the AA requirements. Increasing knowledge about AA nutrition and focusing on their metabolic roles is therefore mandatory. New insights on next limiting AA, increased availability of feed-grade AA and new knowledge about the benefits of low CP diets on the environment and the health and welfare of animals is raising the interest for reducing dietary CP in poultry birds.

II. REVIEW OF AMINO ACID REQUIREMENTS

The ideal protein is the most practical tool to express the AA requirements of animals. All IAA are expressed in ratio to Lys, as Lys is almost exclusively used for protein synthesis (Boisen, 2003). By formulating on each IAA, the protein level will be adjusted automatically by least cost formulation. The requirement is defined as the minimal amount of the studied nutrient required to obtain the optimal or the maximal performance, assuming that all the other nutrients are provided in adequate amounts (Hauschild et al., 2010).

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Table 1 - Published ideal amino acid profiles for broilers and piglets (IAA are expressed in ratio to Lys, %). Gloaguen et al. (2013) proposed an ideal profile for 7-25kg piglets.

	Mack et al., 1999	Wu, 2014		Tillman and Dozier, 2014			Rostagno, 2017		AEL, 2015	Gloaguen et al., 2013 (INRA)
Stage	Broiler									Piglet
(days or kg)	21-42d	0-21d	21-42d	1-14d	15-28d	29-42d	0-21d	21-56d	0-42d	7-25kg
Lys	100	100	100	100	100	100	100	100	100	100
Met+Cys	75	72	75	75	76	77	74	74	75	60
Thr	63	67	70	70	69	68	66	66	67	65
Val	81	77	80	76	77	78	77	77	80	70
Ile	71	67	69	66	67	68	67	68	67	52
Leu	-	109	109	-	-	-	107	108	105	101
Arg	112	105	108	105	106	107	107	107	105	-
Trp	19	16	17	16	16	16	18	18	17	22
His	-	35	35	-	-	-	37	37	36	31
Phe+Tyr	-	105	105	-	-	-	115	115	105	-
Gly+Ser	-	245	245	-	-	-	147	134	-	-

Numerous published ideal AA profiles are available and some examples are given in Table 1. In general, they are fairly consistent especially for the first limiting and most studied amino acids. For the next limiting AA (Val, Ile, Arg), there is greater discrepancy between publications and deserves further investigation. Glycine plus serine requirement, dispensable AAs, is only given in two profiles and is highly variable depending on the methodology used. Differences between pigs and broilers can be illustrated by a comparison of the ideal AA profile recommendations. Sulphur AA (SAA) requirement is higher in broilers than in swine and therefore SAA are the first limiting AA in broiler diets while they are only the 4th or the 5th in pigs. Broilers are indeed feathered animals and feathers contain high amounts of Cys. The branched-chain AA (BCAA; i.e. Val, Ile and Leu) requirements are also higher in broilers compared to pigs while Trp requirement is much higher in swine than in broilers. Arginine is not considered as an IAA in pigs and no recommendation is therefore provided.

The use of different strains, sexes, ages, dietary nutrient levels, health conditions, chosen performance criteria, AA digestibility systems or regression models in the dose-response studies has contributed to the discrepancy in the published AA recommendations. The meta-analysis approach can integrate inter and intra variability and help to estimate nutrient responses and requirements less dependent on experimental conditions (Sauvant et al., 2008).

Threonine is the third limiting AA in broiler diets and a recent meta-analysis estimated the SD Thr:Lys requirement for optimal ADG, ADFI and G:F to be 67% SD Thr:Lys (Lambert et al., 2015a). Besides protein synthesis, Thr is crucial for optimal gut health and immune response as it is the first AA in the composition of the mucins and immunoglobulins. Star et al. (2012) suggested a higher Thr requirement in broilers challenged with an infection model (inoculation of *Eimeria maxima* and *Clostridium perfringens* at d 9 and 14 of age, respectively). In case of challenging practical conditions, Thr will be diverted from growth towards the synthesis of proteins involved in the immune response, resulting in poor performance.

Valine requirement was also recently updated by meta-analysis (Corrent et al., 2017). Among 28 dose-response studies to Val, eight experiments were selected for modelling with non-linear regression models. Estimated Val requirements varied between 78.6 and 95.1% standardized digestible (SD) Val:Lys depending on the model used and the growth performance criteria. By evaluating the different requirements and responses, it is concluded

that 80% SD Val:Lys ratio is sufficient to ensure optimal growth and feed efficiency of broilers. Results of the meta-analysis were confirmed by recently released in-house trials (Figure 1). The variability of the requirement and the response to Val can be partly explained by the amount of dietary Leu in experimental broiler diets. In contrast to broilers, in piglet AA nutrition, the interaction between BCAA is well described showing increased catabolism of Val and Ile when dietary Leu is in excess, reducing the availability of Val and Ile for protein deposition and growth (Wiltafsky et al., 2010). Based on Corrent et al. (2017) and a total of 37 new trials, interaction between Val and Leu was confirmed in broiler chickens, demonstrating that requirement for Val was not influenced by dietary Leu, but the response to Val was greatly increased when feeding higher levels of dietary Leu (AEL Internal Research Report). These findings were confirmed by Ospina-Rojas et al. (2017) who indicated that ensuring adequate dietary Val avoids a depression of performance in case of excessive secondary limiting AA provision. In addition, increasing dietary Val linearly improved body weight and breast meat uniformity (AEL Internal Research Report). Valine is also known to potentially improve Ca bone mineralization and the immunomodulation response to Newcastle virus (Farran and Thomas, 1992; Foroudi and Rezamand, 2014).

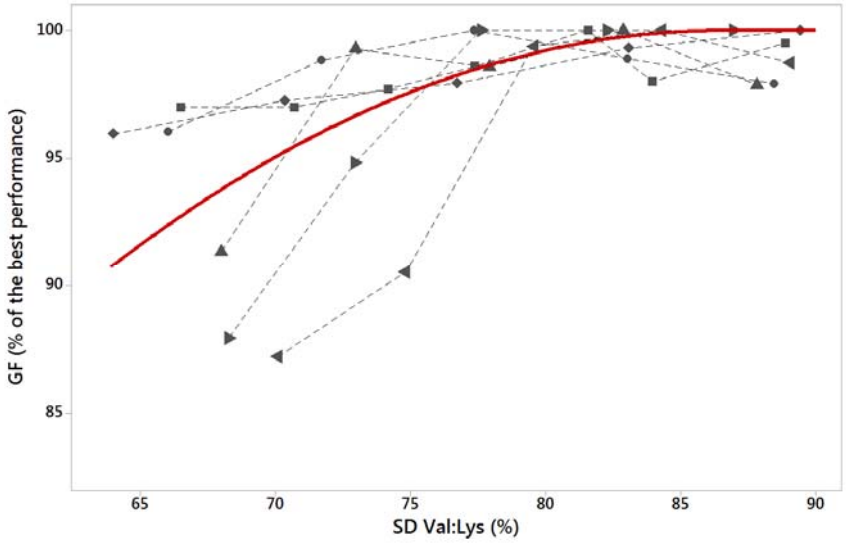

Figure 1 - Gain to feed (GF) according to the dietary standardized digestible (SD) Val: Lys level. Dashed lines = 6 newly released in-house Val trials, solid line = curvilinear-plateau model from Corrent et al. (2017).

Compared to Thr and Val, Ile requirement was much less investigated in recent years. Average of the published recommendation and dose-response trials suggests an Ile requirement of 67% SD Ile:Lys to maximize growth performance. However, this requirement must be fine-tuned in order to formulate efficient low CP diets. Internal desk study on Ile based on 19 trials concludes that the requirement and response to Ile is highly dependent on the experimental diet composition (AEL Internal Research Report). Most of the trials are indeed containing raw materials imbalanced in AA (blood cells, blood meals, corn gluten meal), leading to imbalanced experimental diets. Van Milgen et al. (2012) suggested that, in piglet diets without blood products, the Ile requirement appears to be lower than the currently recommended requirement. These findings also support the idea that Ile requirement is much higher (>67% SD Ile:Lys) for breast meat deposition than for growth performance. The underlying mechanisms still need to be explored and warrants further attention in the future.

In terms of Arg requirement, more than 50 dose-response peer-reviewed papers are available. Contrary to Thr, for instance, it is more difficult to integrate these data into a meta-

analysis as the variability between trials is much greater. Some trials tested extreme levels of Arg, or Arg in interaction with extreme dosage of other nutrients, or Arg under extreme conditions (heat stress, altitude, LPS challenge). General recommendation for Arg is 105% SD Arg:Lys but, depending on the nutrient composition and the farming conditions, Arg requirement can vary substantially. Main factors affecting Arg requirement are the dietary Lys content (Schedle et al., 2015), heat stress (Brake et al., 1998), coccidiosis (Laika and Jahanian, 2017) and altitude (Khajali and Wideman, 2010). Arginine requirement is also highly dependent on age (AEL Internal Research Report).

Although not a strict indispensable AA, Gly is becoming a very studied AA. Glycine requirement is always expressed as the sum of the Gly and Ser requirements as Ser to Gly conversion is equimolar and reversible. Recent published meta-analyses (Akinde, 2014, Lambert et al., 2015b; Siegert, 2016) indicate that it is theoretically possible to estimate Gly+Ser requirement but many dietary factors influence its response and requirement level. The requirement is only valid for young broilers (0-22d) as young broilers are known to have a higher requirement (Coon et al., 1974). Low dietary CP levels were used and convincing evidence suggested a higher Gly requirement at low CP (Dean et al., 2006). Gly is rich in N and increasing dietary levels of Gly logically increased dietary CP levels resulting in diets being not isonitrogenous. Dietary Cys level was low and response to Gly is increased at low dietary Cys (Powell et al., 2011). In contrast to mammals, the majority of the degraded Thr is converted to Gly in broilers and many trials have demonstrated that Thr is capable of sparing dietary Gly in broilers (Ospina-Rojas et al., 2013a,b; Corzo et al., 2009; Siegert, 2015). Evidence from a recent trial confirmed that Gly supplementation does not improve performance of broilers fed a reduced CP diet when Thr is adequately supplied (Lambert et al., 2015c).

III. TACKLING THE CHALLENGE OF DIETARY CRUDE PROTEIN REDUCTION

In Pesti (2009), growth performance of broilers was found to be negatively correlated with dietary CP level, confirming the depressing effect of dietary CP on performance. In piglets, Gloaguen et al. (2014) proved that dietary CP can be drastically reduced down to 14% CP for 1.0% standardised ileal digestible (SID) Lys without any effect on growth performance, indicating that controlling IAA levels allows to reduce dietary CP in piglet diets. Pigs and broilers are monogastric animals and have a comparable digestive system. However, in terms of metabolism, they are completely different animals and they do not share the exact same metabolic pathways. Mammals such as pigs are ureotelic species; the urea cycle is the primary pathway of N excretion. Arginine is the substrate for urea synthesis via the liver arginase and can be *de novo* synthesized via the arginine-ornithine cycle (Morris 2004). In chickens, the urea cycle is inactive in the liver, Arg being not produced via the urea cycle pathway in broilers must be dietary supplied as any IAA. Avian animals are thus uricotelic animals and uric acid is therefore the end product of N excretion. The uric acid pathway is highly demanding on digestible amino acids (DAA - Gln, Gly, Asp) which suggests that some DAA can become limiting for this pathway. More and more knowledge has been pointing at DAA as very important AA in broilers and more research is needed to understand the differences between swine and poultry fed low CP diets.

Several hypotheses were discussed over time to find out why there is a limit in dietary CP reduction in broilers. Aftab et al. (2006) reviewed the possible explanations including dietary electrolyte balance, non-pair feeding regimes, dietary content of DAA, ratio DAA:IAA, inaccurate AA requirement and excess of synthetic AA. From Cirot et al. (2017), based on 367 trials, convincing evidence showed that 97% of published trials where dietary CP was reduced were either not constant in dietary Lys or deficient in one or more IAA. As

previously discussed, among the DAA, glycine seems to be the most pivotal one and its dietary content can be in some cases insufficient to maintain the fast-growing pace of modern broilers fed vegetable diets (Dean et al., 2006). Regarding the impact of free AA supplementation, the general thought is that free AA follow different digestive dynamics compared to protein-bound AA. They do not have to be digested and might be directly absorbed in the most upper part of the small intestine. When replacing AA from protein supply by free AA, it is suspected that great amounts of the first limiting AA will be absorbed earlier in the digestive tract compared to AA coming from protein. The rate of protein synthesis would therefore be impaired by an imbalance in AA in the blood circulation. However, a recent INRA trial indicated that broilers can be fed dietary free AA content up to 45kg/T without any significant adverse effect on performance or carcass characteristics while the average practical inclusion in today's broiler diets is around 6kg/T (Belloir, 2016). In addition, Selle et al. (2015) even indicated that increasing the use of free AA would probably decrease protein-bound AA catabolism in the distal part of the intestine, leading to AA being used primarily for protein synthesis instead of energy supply.

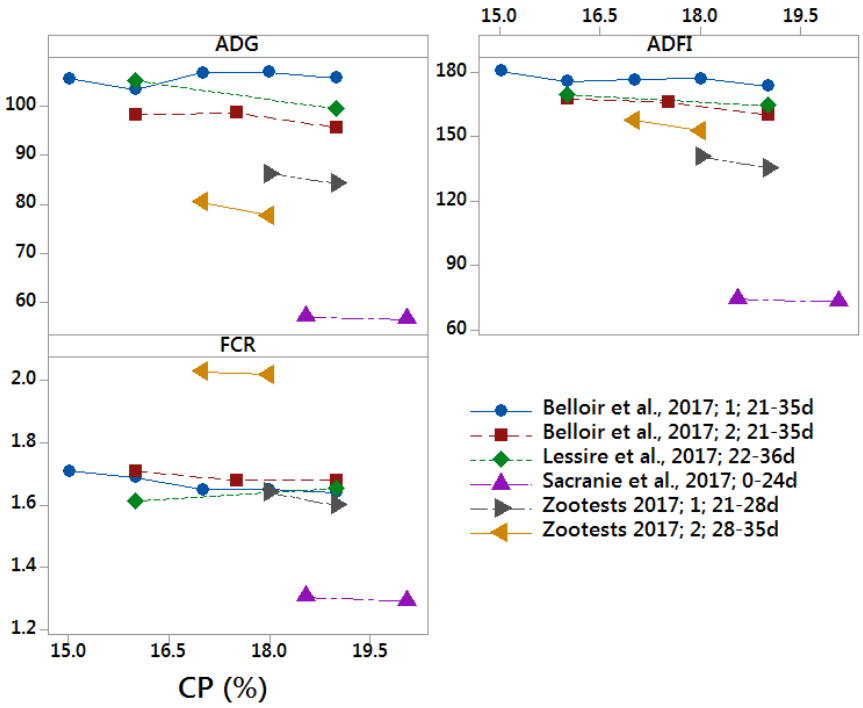

Figure 2 - Average daily gain (ADG), average daily feed intake (ADFI) and gain to feed (GF) according to the dietary Crude Protein (CP) level. References available on request.

In recent trials, low CP could be drastically reduced without affecting growth performance and feed efficiency when dietary Lys was kept constant, dietary Thr was adequately supplied to reduce the need for dietary Gly and main IAA (Met, Val, Ile, Arg) respected the correct requirements (Figure 2). Under these conditions, it could be concluded that no minimum of dietary CP level is needed in formulation (lowest dietary CP reached without compromising performance was 16%), no minimum of dietary SD Gly+Ser was needed in formulation (lowest SD Gly+Ser:Lys was 130%) and no maximum of feed-grade AA supplementation is necessary to be applied in formulation (highest reached was 12kg/T).

IV. BENEFICIAL EFFECTS OF LOWER DIETARY CRUDE PROTEIN CONTENT

Reducing the dietary CP content in broiler diets is an efficient nutritional strategy in order to improve the overall sustainability of the broiler meat chain. In order to quantify the effect of low CP diets, a meta-analysis study in partnership with Laval University (Cirot et al., 2017) and a life-cycle assessment in partnership with INRA (Méda et al., 2017) were conducted. By reducing dietary CP content and controlling dietary content of IAA, SBM is gradually replaced by cereals and feed-grade AA. Nitrogen intake and therefore excretion can be substantially reduced. Cirot et al. (2017), based on a database of 125 trials, showed that N excretion is linearly and significantly reduced by 0.06 g/day or 0.24 g/day per broiler per point of dietary CP reduction for starter or grower-finisher broilers, respectively (Figure 3). This indicates that nutritional intervention by reducing dietary CP is four times more efficient in reducing N emissions to the environment in the last phase of broiler production than in the first phase. The meta-analysis study also shows that there is still potential to increase the N efficiency of modern broilers and that a plateau value seems to be reached at 75%, compared to 55 to 60% for today's broilers.

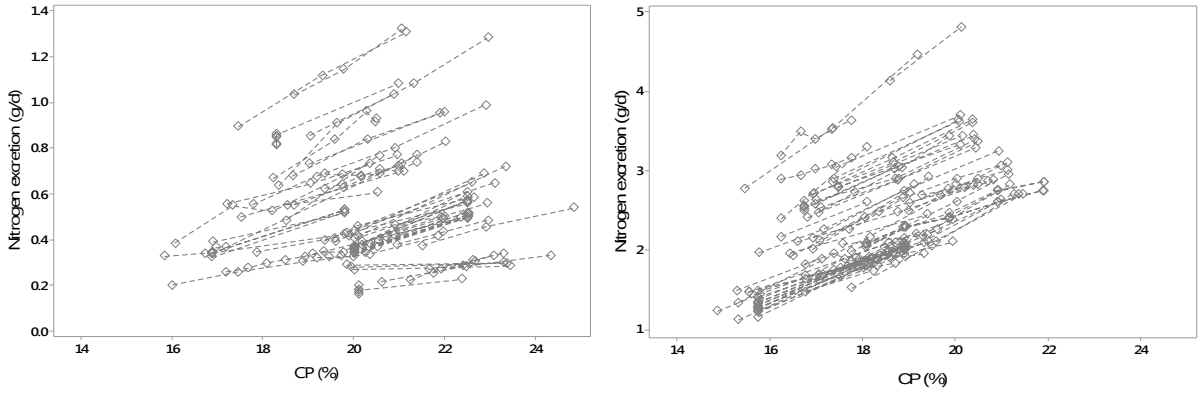

Figure 3 - Nitrogen excretion (gram/day) according to dietary Crude Protein (CP) level for a) 0 to 21 days of age broilers (left, N=41 trials) and for b) 21 to 42 days old broilers (right, N=52 trials).

In Belloir et al. (2017), where dietary CP was reduced from 19 to 15% in 21-35d broilers, combined synergetic effect of lower N excretion (-2g/g BWG per CP percentage point) and lower manure moisture content (-2 points per CP percentage point) resulted in lower N volatilization (e.g. ammonia emissions, -5 points per CP percentage point). Based on published results from Belloir et al. (2017), Méda et al. (2017) conducted a life-cycle assessment to estimate environmental impacts of producing 1 kg of broiler meat fed different dietary CP levels. On average, impacts were reduced by 8%, 7% and 5% for climate change, eutrophication and acidification, respectively, when broilers were fed 16% of dietary CP from 21 to 35 days of age compared to 19%.

When feeding low CP diets to broilers, uric acid synthesis, a high water consuming pathway, is reduced due to a lower amount of AA excesses. On the other hand, SBM content is reduced, gradually replaced by cereals, leading to a lower amount of dietary K. Those two effects combined lead to a reduction of water intake and therefore an improvement of litter humidity. Based on 29 trials, Cirot et al., (2017) showed that water consumption was decreased by 1.4% per CP percentage point, leading to a reduction of 2.2% of litter humidity per CP percentage point (Figure 4). It was also shown in recently published experiments that reducing dietary CP indeed led to lower water:feed ratio, lower litter humidity and lower incidence of foot pad lesions (van Harn et al., 2017; Sacranie et al., 2017; Belloir et al., 2017). In addition, reducing dietary CP is known to have antibacterial effects indicated by

lower pH content (Namroud et al., 2008) and lower E. coli or C. perfringens counts (Dahiya et al., 2006).

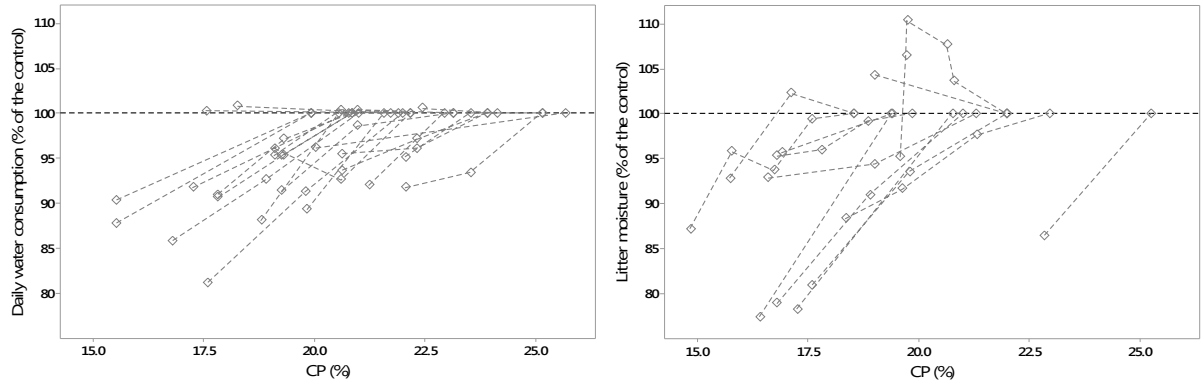

Figure 4 - a) left, Daily water consumption (% of the positive control) and b) right, litter humidity (% of the positive control) according to dietary Crude Protein (CP) level.

Dietary CP reduction also showed improvement in meat quality traits. First of all, breast meat yield (BMY) was improved in recent experiments, showing that reducing excess of dietary AA improved BMY, although a breaking point could be found at 16% CP. In addition, the ultimate pH and drip loss of the breast meat were increased and decreased, respectively, by dietary CP reduction (Belloir et al., 2017). It is speculated that, as dietary CP is reduced, available nutrients for glycogen production are also decreased.

V. CONCLUSION

In conclusion, dietary CP can be reduced in broiler chickens when controlling dietary IAA levels. Many interactions between AA arise in low CP broiler diets, in particular Val x Leu, with growth depression at low Val content exaggerated by an excess of Leu and Thr x Gly, with Thr being able to spare Gly requirement, unique to poultry species. Based on scientific findings, successful practical implementations of low CP diets can be undertaken, enjoying environmental and health benefits.

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LOW PROTEIN DIETS AND SYNTHETIC GLUCOCORTICOIDS ALTER INTESTINAL BARRIER FUNCTION AND PERFORMANCE OF BROILER CHICKENS

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The practice of reducing dietary protein along with supplemented amino acids has the potential to result in impaired performance and unbalanced supply of amino acids. There is little published information on the impact such diets have on intestinal health. An experiment was conducted to study the effect of low protein (LP) diets compared with a standard (SP) diet and a high protein (HP) diet on intestinal permeability and performance. This experiment had two parts. The first part was related to the growth performance study of the birds fed the three experimental diets in each phase of feeding (G/F: grower/finisher) in a completely randomised design. The three experimental diets were: LP (17/15% CP) fortified with essential amino acids, SP (20.2/19%) and HP (22/21%). Diets were formulated to meet the Ross 308 specifications. Low and SP diets contained the same level of essential amino acid concentration, while the HP diets contained 10% above the recommended Ross specifications. Each diet was replicated 6 times (10 male birds per replicate/pen). The second part investigated intestinal permeability and function on a subset of 72 male birds receiving the same three experimental diets. Glucocorticoids have been shown to induce gut leakage (Vicuña et al., 2015). On d 14, a total of 36 birds (12 birds per diet) were injected intramuscularly with dexamethasone (DEX), a synthetic glucocorticoid at 0.5mg/kg body weight. The injections were repeated on d 16 and 20. The remaining 36 birds received sham injections (saline solution). The fluorescein isothiocyanate dextran (FITC-d) method was utilized as the gut permeability test on d 21. All 72 birds were then euthanized for organ weights and tissue collection. Birds fed LP diets had lower body weight gain (BWG) and higher FCR compared to SP and HP diets in both grower and finisher phases of feeding (P<0.001). For the challenge part, birds that received DEX showed higher FCR independent of dietary treatments. Diet and DEX interacted (P<0.001) for BWG, whereby the effect of diet was only evident in sham injected birds. Birds fed LP had a higher (P<0.05) FITC-d concentration indicating a more permeable intestine compared to HP but comparable to SP (Table 1). Independently, administration of DEX resulted in a significant increase in FITC-d concentration in all dietary treatments. The relative weights of lymphoid organs were also found to be lower in birds injected with DEX. Bursal atrophy was evident in LP diets and challenged group of birds (data not shown). The results of this experiment confirmed that intestinal barrier function can be impacted by both DEX and dietary protein level even when amino acids were supplemented at or above the industry recommendations. Further investigation is underway for the role of individual amino acids that have the potential to improve intestinal function and performance of birds fed low protein diets.

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Table 1 - Concentration of FITC-d (µg/ml) in serum samples of broilers at d 21 of age.

Dietary protein	Low		Standard		High			*P* values		
DEX	-	+	-	+	-	+	SEM	Diet	DEX	Diet x DEX
Serum FITC-d	0.664	1.178	0.688	0.928	0.551	0.847	0.0311	0.021	<.0001	0.396

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INVESTIGATING THE EFFECTS OF GLYCINE AND GLYCINE EQUIVALENTS ON MEAT CHICKEN PERFORMANCE UNDER LOW PROTEIN DIETS

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Low protein diets are being investigated to reduce feed cost and address health, welfare and environmental concerns associated with feeding meat chickens excess dietary crude protein (CP). Low CP diets supplemented with only essential amino acids (AA) have failed to improve performance to that observed with standard CP diets (Dean et al., 2006). The non-essential amino acid glycine (Gly) is believed to become conditionally limiting as supplementation of Gly and essential AA in low CP diets has improved performance (Dean et al., 2006). Threonine (Thr) and serine (Ser) *in vivo* degradation both produce Gly as a product (Wu et al., 2013). Therefore, the aim of this study was to investigate the effects of supplementing Gly in comparison to Ser and Thr in low CP diets on meat chicken performance. Male day-old Ross 308 chicks (n=528), were fed a common starter diet containing wheat, sorghum, soybean meal and meat and bone meal from d 0 to d 7. On d 7, chicks were allocated to 48 pens of equal weight, resulting in 6 replicate pens of 11 chicks per pen for each treatment. Feed and water were provided *ad libitum* throughout the trial. Essential AA were supplemented when determined limiting using AMINOChick®2.0 software. The dietary treatments were: a standard CP diet containing meat and bone meal with a CP of 227 g/kg (SP), a LP vegetarian diet with a CP of 191 g/kg (LP) and the LP diet supplemented with Gly, Ser and Thr at two different concentrations, resulting in 8 treatments. The first AA level was to equal the amount of Gly+Ser in SP (16 g/kg), with Gly, Ser and Thr supplemented on an equimolar basis. The second AA level was based on a recommended Gly+Ser level of 18 g/kg suggested by Shutte et al. (1998). Feed intake and weight gain per pen from d 7-21 were recorded and the feed conversion ratio (cFCR) calculated, and corrected for mortalities. The SP diet had significantly lower cFCR compared to LP (1.241 vs 1.351, $P < 0.001$). Supplementation of Thr to the LP with 18 g/kg Gly+Ser significantly ($P < 0.005$) improved cFCR by 3.93% compared to the LP diet (1.298 vs. 1.351), but also resulted in significantly ($P < 0.02$) lower BWG with a difference of 65 g/bird (801 vs 866 g/bird). Supplementation of Gly and Ser at both levels to the LP treatment resulted in no significant improvement in BWG or cFCR. This proposes that the dietary level of Gly+Ser recommended by Shutte et al. (1998) is excessive at this CP level. The data from this study suggests that Thr cannot be supplemented as a precursor of Gly to offset lower BWG associated with low CP diets and that there is no difference between Ser and Gly supplementation.

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THE SELECTION ELEMENT IN WHOLE GRAIN FEEDING REGIMES

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Summary

Seven dietary treatments consisting of a ground grain control and six treatments with pre- and post-pellet whole grain inclusions of 7.5, 15.0 and 30.0% were offered to a total of 252 male Ross 308 broiler chicks from 7 to 28 days post-hatch. With the post-pellet approach, whole grain and pelleted concentrate were offered in separate feed trays to give birds an unhindered opportunity to select between the two ration components. The 30% post-pellet whole grain treatment generated the most efficient feed conversion of 1.260 which represented an improvement of 7.01% (1.260 versus 1.365 $P < 0.01$) in comparison to the 30% pre-pellet counterparts. Also, 30% post-pellet whole wheat generated the greatest responses in AME and ME:GE ratios. Birds offered 30% post-pellet whole grain exhibited the strongest preference for a high protein diet, consuming proportionally more pelleted concentrate and less whole grain than the 7.5 and 15% post-pellet whole grain treatments. Thus, the opportunity of choice feeding and the selection of higher protein dietary intakes contributed to enhanced performance under whole grain feeding regimes.

I. INTRODUCTION

Whole grain feeding (WGF) involves the partial substitution of ground grain with whole grain in boiler diets. Whole grain (WG), usually wheat, may be added either prior to (pre-pellet) or following (post-pellet) steam-pelleting. WGF generates heavier and presumably more functional gizzards which are thought to be the genesis of responses in feed conversion ratios (FCR) and energy utilisation (Liu *et al.*, 2015). However, post-pellet WGF also provides broilers with the opportunity to select between the 'low protein' WG and the 'high protein' pelleted concentrate component of the ration, which have similar energy densities. As WG inclusions increase so does the differential in protein content between the two components. Cumming (1992) championed WGF on the basis that "each fowl can accurately select its own nutritional requirements" as a result of free choice-feeding. To investigate this aspect, 7.5, 15.0 and 30% post-pellet WG rations were offered with whole wheat and pelleted concentrate in two separate feeding trays to provide birds with an unhindered opportunity to select or choice-feed, and to permit the individual feed intake recording of the two components. Therefore, WG inclusions of 7.5, 15 and 30% were applied to both pre-pellet and post-pellet WGF regimes to compare these dietary treatments with a conventional ground-grain control diet.

II. MATERIALS AND METHODS

Seven dietary treatments with six replicates each (6 birds per cage) were offered to a total of 252 male Ross 308 broiler chicks from 7 to 28 days post-hatch, the diets were identical (532.8 g/kg wheat, 310.0 g/kg soybean meal, 218.6 g/kg protein, 336 g/kg starch) except for the presentation of wheat. A wheat-soy steam-pelleted diet, containing ground grain (3.2 hammer-mill screen), which met Ross 308 nutrient specifications served as the control. Pre- and post-pellet whole wheat inclusions were added in substitution for ground wheat and comprised the remaining six treatments with WG inclusion levels of 7.5, 15.0 and 30.0%.

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The post-pellet WG and the corresponding pelleted concentrate were offered separately and feed was added daily into two adjacently positioned feeding trays to permit the monitoring of feed intakes of both components. When appropriate, wheat was ground through a 3.2 mm hammer-mill screen prior to steam-pelleting through a 4.00 mm die at a 75°C conditioning temperature. A non-starch polysaccharide degrading enzyme (Danisco Xylanase, 16,000 BXU/kg) was added across all wheat-based diets. Growth performance, gizzard characteristics and total tract energy utilisation were determined by standard procedures described in Moss *et al.* (2017). Data were analysed using IBM® SPSS® Statistics (IBM Corporation. Somers, NY). A probability level of less than 5% was considered statistically significant. The study complied with guidelines approved by the Animal Ethics Committee of The University of Sydney.

III. RESULTS

Overall, the Ross 308 2014 growth performance objectives were surpassed. Effects of dietary treatments on growth performance, gizzard characteristics and energy utilisation are shown in Table 1. Also, absolute and relative ration components and starch and protein intakes of birds offered separate post-pellet WG diets are tabulated. WGF did not significantly influence weight gain, but significantly influenced feed intake ($P < 0.001$) with 15 and 30% post-pellet WG depressing feed intakes relative to the control. On the basis of a pair-wise comparison, the 30% post-pellet WG treatment improved FCR by 7.69% (1.260 versus 1.365; $P < 0.01$) in comparison to the ground grain control. Pre- and post-pellet WG significantly increased relative gizzard weights ($P < 0.001$), with 30% post-pellet WG generating the largest increase of 37.3% (19.23 versus 14.01 g/kg; $P < 0.001$) by a pairwise comparison. Post-pellet WG inclusions of 15 and 30% and the 30% pre-pellet inclusion significantly increased relative gizzard contents, with the 30% pre-pellet WG treatment generating the largest increase of 51.1% (9.79 versus 6.48 g/kg). Pre-pellet WG inclusions of 7.5 and 15% and post-pellet inclusions of 15 and 30% significantly reduced gizzard pH. All post-pellet WG inclusions significantly increased average metabolisable energy (AME) and metabolisable energy to gross energy (ME:GE) ratios ($P < 0.001$), with the 30% post-pellet inclusion enhancing AME by 1.31 MJ (13.25 versus 11.94 MJ/kg; $P < 0.001$) and ME:GE ratios by 9.88% (0.756 versus 0.688; $P < 0.001$).

Ostensibly, birds offered post-pellet WG treatments should have consumed 7.5, 15.0 and 30.0% whole wheat to maintain a constant dietary protein intake of 226 g/kg. However, the actual consumptions of WG were 8.4, 8.2 and 16.4%, respectively. WG inclusion was linearly correlated to starch to protein ratios ($r = 0.689$; $P < 0.005$), where higher WG inclusions resulted in a significant condensation ($P < 0.001$) of starch to protein intake ratios from 0.83 (7.5% WG) to 0.55 (30.0% WG) as birds elected to increase their relative protein intakes given the unfettered opportunity to select between the two ration components.

IV. DISCUSSION

The growth performance response observed in the current study followed the trends discussed in Liu *et al.* (2015), where WGF maintained weight gain, reduced feed intake and enhanced feed conversion in a number of feeding studies. However, the hallmark response to WGF regimes is increased relative gizzard weights. In the current study, post-pellet WGF generated a robust increase of 37% (19.23 versus 14.01) in gizzard weight, which was correlated with FCR ($r = -0.457$; $P < 0.005$), AME ($r = 0.775$; $P < 0.001$) and ME:GE ($r = 0.759$; $P <0.001$). Thus, the clear implication is that heavier, and presumably more functional, gizzards advantaged feed conversion and nutrient utilisation in broiler chickens.

Nevertheless, heavier gizzard weights may not be the only consequence of WGF regimes which enhance performance. Gous and Swatson (2000) found that broiler chicks will attempt to maximise performance by selecting the best possible combination of protein sources when given the opportunity. Forbes and Covasa (1995) support the concept that choice feeding is contributing to the responses observed in broiler chickens under WGF regimes. Birds offered post-pellet WG had the unhindered opportunity to choice feed by selecting between the pelleted concentrate (high protein) and WG (low protein) components provided in separate feed trays. It is evident that birds offered the 15 and 30% post-pellet WG treatments did not consume the entire WG component allocated, and instead selected a proportionally higher protein to starch intake. While this outcome was not anticipated, Amerah and Ravindran (2008) observed essentially similar findings with birds displaying a preference for the pelleted concentrate over WG. Additionally, across all treatments, starch to protein ratios were correlated with FCR ($r = -0.382$; $P < 0.02$), AME ($r = 0.698$; $P < 0.001$) and ME:GE ($r = 0.683$; $P < 0.001$), such that the higher the starch intake, the poorer the feed conversion and energy utilisation. The 30% WG treatment provided the greatest opportunity for selection as the analysed protein content of the pelleted concentrate increased from 234 to 246 and 273 g/kg with increasing WG inclusions. As a consequence of selection, birds elected to consume dietary intakes with protein contents of 226, 237 and 253 g/kg, respectively.

Instructively, Corzo *et al.* (2005) found that enhanced feed conversion efficiency and white meat yield were realised by diets containing amino acid levels greater than typical industry standards. Accordingly, the current WGF study demonstrates that higher than standard dietary protein concentrations improved feed conversion efficiency; however, this approach would need to be cost-effective to be adopted in practice.

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Table 1 - Effects of dietary treatments on growth performance from 7 to 28 days post-hatch, relative gizzard weights, contents and gizzard pH at 28 days post-hatch, energy utilisation from 25-27 days post-hatch and absolute and relative ration components and starch and protein intakes of birds offered separate post-pellet inclusions from 7 to 28 days post-hatch.

Treatment		Weight gain (g/bird)	Feed intake (g/bird)	Feed conversion ratio (g/g)	Relative gizzard weight (g/kg)	Relative gizzard content (g/kg)	Gizzard pH	AME (MJ/kg DM)	ME:GE ratio
Description	Wholegrain inclusion (%)								
Control	0.0	1651	2252c	1.365b	14.01a	6.48b	2.96c	11.94ab	0.688a
Pre-pellet	7.5	1621	2266c	1.364b	15.17b	4.53a	2.43ab	11.59a	0.670a
	15.0	1644	2240c	1.362b	15.17b	6.50b	2.43ab	12.05ab	0.693a
	30.0	1590	2210bc	1.355b	15.58b	9.79c	2.72bc	12.10b	0.693a
Post-pellet	7.5	1608	2147bc	1.334ab	17.94c	6.99b	2.36a	13.05c	0.744b
	15.0	1532	1959a	1.296ab	19.22d	8.86c	2.15a	13.18c	0.752b
	30.0	1681	2098b	1.260a	19.23d	9.61c	2.31a	13.25c	0.756b
SEM		53.2	47.1	0.0263	0.365	0.544	0.102	0.168	0.0096
Significance (P =)		0.553	< 0.001	0.046	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
LSD (P < 0.05)		-	135.2	0.0755	1.049	1.562	0.292	0.484	0.0277

Treatment		Pelleted concentrate (g/bird)	Whole wheat (g/bird)	Proportion of wheat (%)	Protein intake (g/bird/day)	Starch intake (g/bird/day)	Starch to protein intake ratio
Description	Wholegrain inclusion (%)						
Post-pellet	7.5	1981a	165b	8.39b	23.07b	19.15b	0.83c
	15.0	1779b	147b	8.18b	22.15b	14.58a	0.66b
	30.0	1751b	290a	16.43a	25.32a	13.92a	0.55a
SEM		55.8	40.0	2.188	0.487	1.106	0.043
Significance (P =)		0.021	0.046	0.027	0.001	0.009	0.001
LSD (P < 0.05)		168.1	120.5	6.594	1.469	3.334	0.129

[abcd] Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

EFFECTS OF DIETARY INSOLUBLE AND SOLUBLE NON-STARCH POLYSACCHARIDES ON PERFORMANCE AND ILEAL AND EXCRETA MOISTURE CONTENTS IN BROILERS

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Summary

This study examined the effect of dietary insoluble and soluble non-starch polysaccharides (NSP) in common broiler diets on performance and ileal and excreta moisture contents in broilers. These effects were assessed by feeding 24 diets with varying levels of insoluble and soluble NSP to broiler chickens from d21 to d28; insoluble NSP level ranged from 60.8-220.7 g/kg and soluble NSP ranged from 8-14.6 g/kg. Dietary insoluble and soluble NSP level had a significant impact on feed conversion ($P < 0.001$) and ileal and excreta moisture contents ($P < 0.001$). The feed conversion ratio and feed intake were highest, and digesta and excreta moisture contents were high, in birds fed the diet with the highest insoluble NSP and high soluble NSP contents. Strong relationships were observed between dietary soluble NSP content and ileal and excreta moisture content. The optimum diets contained a combination of soluble NSP ranging from 6.24-6.72 g/kg and insoluble NSP ranging from 176-182g/kg, based on observed low FCR and high digesta and excreta dry matter content. The findings from this study reconfirm the significant role that insoluble and soluble NSP play in dictating feed conversion and digesta and excreta moisture content in broilers fed a variety of diets based on common feed ingredients in Australia.

I. INTRODUCTION

NSP refer to a wide variety of polysaccharide molecules of cell walls with varying degrees of water solubility, size and structure. Their physiological functions are defined by whether they are water soluble or insoluble. The amount of soluble and insoluble NSP varies greatly among different ingredients; for example, corn and sorghum contain very low levels of NSP but wheat, rye and triticale contain high amounts of both soluble and insoluble NSP (Choct et al., 2010). The structure and physiochemical characteristics of the NSP also differ widely among ingredients and even within the same ingredient. The prevalence of soluble and insoluble NSP must be measured to accurately determine the effects of fibre on nutrient and energy digestibility; physiological properties of fibre cannot be predicted from the monomeric composition of the constituents (Bach Knudsen, 2014). It is important to consider both soluble and insoluble NSP as their impacts on digestion differ substantially. Insoluble NSP act as nutrient diluents and a physical barrier to enzymes, such as amylase and protease, thereby reducing efficient digestion of nutrients within the cell wall matrix of grains. Soluble NSP increase digesta viscosity, thereby affecting nutrient digestion and absorption and reducing digesta transit time, and display anti-nutritive properties resulting in reduced ileal digestibility of starch, protein and lipid. Moderate levels of NSP are however advantageous, in that insoluble NSP stimulates the gizzard, improving starch digestibility and increasing digesta passage rate, ensuring fermentation occurs in the large intestine and caeca as opposed to earlier in the gastrointestinal tract, and soluble NSP stimulates the growth of beneficial gut microbes. The aim of this study was to investigate the extent of impact that insoluble and soluble NSP have on broiler performance and ileal and excreta moisture contents in diets based on common feed ingredients in Australia.

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II. MATERIALS & METHODS

Male day-old Ross 308 chicks (n=720) were housed in 144 cages, 5 birds per cage, from d21-28 post-hatch. The birds were fed a standard corn-soybean meal based starter diet from d0-10 and grower diet from d10-21. They were then allocated to one of 24 dietary treatments from d21-28; composed of varying levels of wheat, corn, soybean-meal, sorghum and canola-meal, without any supplemental enzymes. Diet 1 and 6-9 were corn-soybean meal based diets with varying levels of SBM (ranging from 30-50%), Diet 2-5 and 10-13 were wheat, corn and soybean based diets with varying levels of wheat (ranging from 15-60%, with wheat either replacing the energy bearing component of the diet or replacing corn like-for-like), Diets 14-17 were corn, canola meal and soybean meal based with varying levels of canola meal (ranging from 15-30%), Diets 18, 20, 21 and 22 were corn, sorghum, wheat and soybean based with varying levels of sorghum (ranging from 2.5-45%), Diet 19 was wheat, sorghum and soybean meal based, Diet 23 was corn, sorghum and soybean meal based and Diet 24 was just wheat-soybean meal based. All diets were formulated to meet Ross 308 nutrient specifications and feed and water was provided *ad libitum* throughout the trial.

Birds and feed per cage were weighed on d21 and d28, to determine body weight gain (BWG), feed intake (FI) and the feed conversion ratio (FCR). On d28, two birds per cage were euthanised by CO_2 asphyxiation and ileal digesta samples were collected. Excreta was collected daily from each cage from d25-28. These samples were homogenized and moisture content was determined by oven-drying at 105°C until constant weight. Performance and, ileal and excreta dry matter (DM) data were analyzed using IBM SPSS v. 23; after Kolomogorov-Smirnov testing to confirm normality, one-way ANOVA was used to test equality of the means and Tukey's post-hoc tests were conducted to differentiate them. Pearson product-moment correlation coefficient was used to determine the relationship between the dietary soluble and insoluble NSP content and the ratio of soluble: insoluble NSP with performance and ileum and excreta dry matter. Significance was accepted at $P < 0.05$.

The insoluble and soluble NSP content of each diet was analyzed, based on the method of Englyst et al. (1994). Briefly, the diet sample was fat extracted and the free oligosaccharides removed. The starch in the resulting residue was then gelatinised and α-amylase and amyloglucosidase added. The resulting supernatant contained the soluble NSP and residue contained the insoluble NSP. For the soluble NSP analysis, released sugars were removed using ethanol and the residue was hydrolyzed with trifluoroacetic acid. For the insoluble NSP analysis, glucose released from the starch digestion was removed and the cellulosic polymers were hydrolyzed with dilute H_2SO_4. An internal standard was added (allose, 4mg/ml) to each sample. The sugars in all the samples were then reduced and acetylated; $NaBH_4$ was added (excess was decomposed with $C_2H_4O_2$), followed by 1-methylimidazole, $C_4H_6O_3$, water and dichloromethane respectively. The pellet was dried and reconstituted with ethyl acetate and water, and the resulting supernatant was analyzed by gas chromatography.

III. RESULTS

Table 1 illustrates that dietary insoluble and soluble NSP content has a significant impact on feed conversion and ileal and excreta moisture contents in broilers. Birds fed the diet with the highest insoluble NSP level, coupled with a high soluble NSP level, presented the highest FCR and numerically highest feed intake. Birds fed this diet also had high digesta and excreta moisture content. Birds fed the diet with the lowest soluble NSP level had the second highest feed conversion ratio and the numerically lowest BWG. The optimum diets in this study contained a combination of soluble NSP ranging from approximately 6.24-6.72 g/kg and insoluble NSP ranging from approximately 176-182g/kg, based on observed low FCR (1.31)

and high digesta and excreta DM content. There was no significant relationship between the dietary insoluble and soluble NSP content and the performance parameters measured ($P > 0.05$), but there were weak relationships between the soluble: insoluble NSP ratio and feed intake and FCR ($r = -0.162$, $P = 0.043$ and $r = -0.173$, $P = 0.038$). There were strong relationships observed between soluble NSP and, ileal and excreta DM content ($r = 0.822$, $P < 0.001$ and $r = 0.872$, $P < 0.001$, respectively) and between the ratio of soluble: insoluble NSP, ileal and excreta DM content ($r = 0.608$, $P < 0.001$ and $r = 0.602$, $P < 0.001$, respectively).

Table 1 - Effect of diets containing varying levels of insoluble and soluble non-starch polysaccharides (NSP) on individual bird performance and, ileal and excreta dry matter content in broilers from d21-28.

	Diet NSP Content		Performance			DM (%)	
Diet[1]	Insoluble (g/kg)	Soluble (g/kg)	Feed Intake (g)	BWG (g)	FCR	Ileum	Excreta
18	60.8	8.0	965	675	1.43^{efgh}	21.5^{bcde}	23.7^{fgh}
24	61.2	13.2	969	676	1.43^{defg}	20.0^{defg}	21.2^{kl}
20	62.5	8.2	942	661	1.43^{efgh}	21.2^{cdef}	23.1^{efg}
13	64.5	13.4	985	703	1.40^{fghi}	19.7^{efg}	20.7^{kl}
14	64.6	8.5	982	635	1.55^{cd}	20.8^{cdefg}	22.6^{fghi}
23	65.2	7.4	992	652	1.52^{cde}	22.0^{abcde}	24.6^{cd}
21	65.7	8.8	942	648	1.43^{defg}	20.7^{cdefg}	22.6^{fghi}
22	66.1	10.4	904	619	1.46^{defg}	20.3^{defg}	21.3^{ijkl}
19	67.3	14.6	954	666	1.43^{efghi}	18.7^{g}	20.1^{l}
12	68.3	10.8	991	709	1.40^{fghi}	20.2^{defg}	21.2^{jkl}
15	69.4	7.6	1003	640	1.57^{c}	22.0^{abcde}	24.4^{cde}
11	72.4	9.5	989	728	1.36^{ghi}	20.7^{cdefg}	22.2^{ghij}
1	74.1	6.4	978	670	1.42^{fghij}	23.7^{ab}	26.3^{ab}
16	74.8	6.6	992	609	1.63^{c}	22.7^{abc}	24.9^{cd}
10	75.3	7.8	969	704	1.38^{ghi}	21.7^{abcde}	24.1^{cde}
17	76.4	5.3	1030	603	1.71^{b}	22.2^{abcd}	24.9^{cd}
2	94.2	8.2	971	670	1.45^{defg}	21.2^{cdef}	23.7^{def}
6	144.7	6.9	910	673	1.35^{ghij}	22.0^{abcd}	24.6^{cd}
3	146.0	9.7	1014	674	1.50^{cdef}	20.5^{cdefg}	21.9^{ghijk}
4	148.2	10.0	1065	680	1.57^{c}	20.4^{cdefg}	21.5^{hijk}
7	156.9	6.7	884	674	1.31^{ij}	23.9^{a}	26.7^{a}
8	172.6	6.5	897	684	1.31^{j}	23.6^{ab}	25.3^{bc}
9	181.5	6.2	891	678	1.31^{hij}	23.9^{a}	26.5^{ab}
5	220.7	13.5	1082	611	1.77^{a}	19.1^{fg}	20.6^{kl}
SEM			6	7	0.01	0.2	0.1
P-Value			0.084	0.298	<0.001	<0.001	<0.001

[a-k] Means within the same column with no common superscript differ significantly ($P \leq 0.005$)
[1] Data arranged in order of increasing dietary insoluble NSP content

IV. DISCUSSION

The novelty of this study was that the diets fed did not contain very high or extreme levels of NSP, they were common diets and the birds performed to the breed standard, yet significant NSP induced effects were still observed. As predicted, feed conversion was reduced with very high dietary total NSP content and ileal and excreta DM content were lowest in the diets containing high levels of soluble NSP (Choct and Annison, 1992). The extreme differences observed between feeding 181.5 g/kg and 220.7 g/kg insoluble NSP highlight that feeding

diets with an insoluble NSP level higher than approximately 180 g/kg has a significant anti-nutritional impact and resulting detrimental impact on performance. The positive effects on FCR observed with a reasonably high level of insoluble NSP likely reflects the ability of insoluble NSP to stimulate the gizzard, improving digestibility by heightening retention time and increasing hydrochloric acid secretion. These improvements at the beginning of the tract, coupled with presence of the poorly fermentable fraction, possibly resulted in a higher proportion of beneficial micro-organisms in the tract, promoting integrity of the intestinal lining and heighted nutrient digestion. Further investigation is warranted to determine the precise optimum, excessive or insufficient level of dietary NSP. The regression analysis illustrates that soluble NSP strongly influences intestinal water absorption, as soluble NSP has high water holding capacity and the presence of unabsorbed sugars in the tract causes osmosis of water into the gut lumen, resulting in heightened digesta and excreta and resulting poor litter quality. The results from this study emphasize the significance of the ratio between insoluble and soluble NSP in a diet; observing their individual effects is largely inconclusive. Diet NSP content alone does not dictate feed efficiency, a number of factors and combination of dietary influences also contribute towards the differences observed between the diets, but this study highlights the need for enhanced understanding of the form and chemical composition of different NSPs and the impact of NSP in different ingredient combinations. This is required in order to achieve the greatest benefits when using NSP-degrading enzymes to regain lost performance and improve litter quality.

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INFLUENCE OF CARBOHYDRASE SUPPLEMENTATION ON METABOLISABLE ENERGY AND ILEAL NUTRIENT DIGESTIBILITY OF TWO CULTIVARS OF BARLEY FOR BROILERS

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The use of barley in broiler diets is limited because of the presence of β-glucans, which negatively influence nutrient utilisation and bird performance. These negative effects may be partly overcome by exogenous carbohydrases containing β-glucanases. In the current study, the influence of a multi-component carbohydrase (Ronozyme multigrain, DSM Nutritional Products, Singapore) on nitrogen-corrected apparent metabolisable energy (AMEn; assay 1), and, apparent ileal digestibility (AID) of starch and standardised ileal digestibility (SID) of nitrogen (N) and amino acids (AA; assay 2) of two barley cultivars was examined. A 2 × 2 factorial arrangement of treatments was used in both assays with two barley cultivars (normal hulled barley [NB] and waxy hull-less barley [WHB]) and two levels of enzyme supplementation (0 and 200 g/tonne feed). Assay diets contained barley (962 and 917 g/kg in assays 1 and 2, respectively), fortified with mineral and vitamin supplements. Each assay diet was fed to six replicate cages (eight 14-day old male broilers per cage). In assay 1, total excreta collection was carried out from day 17 to 21 post hatch for the determination of AMEn. In assay 2, ileal digesta samples were obtained on day 21 post hatch and apparent digestibility was calculated using the ratio of the inert marker (Titanium dioxide at 5 g/kg) in the diet and digesta. The SID of N and AA were calculated using endogenous losses determined in broilers fed a nitrogen-free diet.

Differences were observed in N and fibre contents of the barley cultivars. The contents of dry matter, N, insoluble and soluble dietary fibre of NB and WHB were: 893 and 907, 101 and 133, 142 and 110, and 29 and 68 respectively. Starch content was higher in NB (610 g/kg) than WHB (554 g/kg), and the composition of starch differed markedly between the cultivars. The amylopectin content of the starch in NB was 343 g/kg, whereas in WHB cultivar the content was 477 g/kg. β-glucan content also differed, with WHB having higher concentration compared to NB (69 and 38 g/kg, respectively). Higher β-glucan content of WHB is in agreement with previous data (Izydorczyk *et al*., 2000). The main effect of cultivar was significant ($P < 0.001$) for AMEn, with higher value (12.17 MJ/kg) observed for NB compared to WHB (9.92 MJ/kg). Added enzyme increased ($P < 0.001$) the AMEn (11.38 vs 10.72 MJ/kg) and AID of starch (0.946 vs 0.911) in both cultivars, but the magnitude of response was greater in WHB resulting in a tendency ($P < 0.07$ - 0.10) for a cultivar x enzyme interaction. The main effect of cultivar was also significant for the SID of N ($P < 0.01$) and most AA ($P < 0.05$- 0.001), with higher values determined for NB. The coefficient of SID of N in NB and WHB were 0.79 and 0.75, respectively. Enzyme addition tended ($P = 0.07$) to improve N digestibility, but had no effect ($P > 0.05$) on AA digestibility. The present data indicate that the responses in starch digestibility and AMEn with enzyme addition were greater in WHB than in NB. The poorer nutrient utilisation indices of WHB, however, highlight the need to consider β-glucan content in the selection of barley cultivars for use in broiler diets.

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PRECISION FEEDING OF BROILER BREEDERS

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Summary

Precision livestock feeding relies on real-time sensor feedback from individual birds to decide whether or not to allocate feed, according to the needs of each individual. A broiler breeder precision feeding system has been developed to feed broiler breeder pullets and hens exactly what they require to achieve targeted growth rates. The system has also been used successfully with other poultry, including broilers, layers, and heritage breeds. The precision feeding system is an excellent tool to discover individual nutritional needs of broiler breeders during growth and reproduction, and to evaluate metabolism in response to feeding. However, the system presents a very different paradigm compared with conventional practices. In current practice, broiler breeders are fed large meals infrequently, usually once every 24 or 48 hours. In contrast, precision fed birds can be fed multiple smaller meals in a 24 hour period. As a result, interesting and sometimes unexpected changes in nutrient partitioning have been observed. One of the surprises from our research was that when we precision fed broiler breeders according to the breeder-recommended target body weight, we observed poor egg production compared with conventionally fed birds. This problem related to a delay in the onset of lay, and was remedied with a higher target BW and photoperiod management. These findings suggest that current recommended broiler breeder target BW curves are too low for optimal reproductive performance in precision fed broiler breeders, and may in fact point to similar and imminent problems in conventionally fed broiler breeders. Precise control of lighting schedules is still required for optimal reproductive performance, although our research with precision feeding has led us to hypothesize that metabolic triggers may at least partially override or otherwise counteract the negative effects of suboptimal photoperiod management.

I. INTRODUCTION

Modern broilers grow 5 times faster on 40% less feed than they did 60 years ago (Zuidhof et al., 2014). Because high body weight (BW) correlates negatively with reproduction and health (Decuypere et al., 2010), the severity of broiler breeder feed restriction increases every year relative to broiler growth potential. This intensifies competition for feed, resulting in unequal distribution of feed and poor flock uniformity. Achieving and maintaining high flock uniformity is one of the biggest management challenges for contemporary hatching egg producers. A precision feeding (PF) system was developed at the Poultry Research Centre at the University of Alberta to solve this issue. The PF system allocates feed to individual floor housed birds after comparing each individual's BW to a recommended target BW. The system automates complex feed allocation decisions, freeing the flock manager to focus on higher level decisions. The PF system can now consistently achieve 1% coefficient of variation (CV) in BW at photostimulation age, so we can now refine our questions to include, "What is the right target BW for broiler breeders?" "How much should we feed broiler breeders, and when?" and "What optimal dietary nutrient concentrations optimize a broiler breeder's entry into lay, and subsequent reproductive performance?" The PF system requires a radical change to the traditional thinking that is second nature for those experienced in

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feeding birds once per day as one big group. Precision feeding has been challenging the way we think about nutrition, reproductive physiology, and behaviour in broiler breeders. The big data collected by the system provides analytical opportunities we have never had before. This has evolved our thinking, and has led to new insights around broiler breeder metabolism, physiology, productivity, and behaviour. Precision feeding has also yielded some unexpected results, which are always an excellent opportunity to develop new hypotheses. The goal of this paper is to explore some of the interesting and important new discoveries the PF system has begun to yield.

II. METHODS

a) Overview of precision feeding studies

The observations reported subsequently in this stem from several completed precision feeding studies. A brief description is provided here to help the reader understand both the experiments that will be discussed, followed by a description of how the precision feeding system worked.

Two studies were conducted as direct comparisons of conventional and precision feeding. In the first of these comparison studies, a commercial broiler breeder was used (Ross 308); in the second, a Cobb grandparent (GP) line was used (L35; male line female). Conventional feeding treatments used skip-a-day or daily feeding, and feed allocations were based on weekly BW measurements.

A third study was conducted to evaluate the impact of metabolizable energy (ME) intake on the onset of lay. In this third study, precision fed Ross 308 broiler breeders were divided into two treatments: 1) a standard commercial diet (11.9 MJ/kg) fed to achieve the breeder-recommended target BW, or a high ME diet (13.2 MJ/kg) provided ad libitum.

Two studies evaluated the interacting effects of BW and light management (rearing day length and photostimulation age) on the reproductive efficiency of Ross 708 pullets. The precision feeding system is a sequential feeding system. Instead of all birds eating simultaneously, birds eat any time of the day, one after another. Therefore, we have been exploring questions related to whether we can provide light to stimulate the retina without stimulating the reproductive axis. This would allow us (and eventually commercial users) to increase the time birds can access the feeding system, and decrease capital costs. In both studies, breeder-recommended target BW profiles were contrasted with a higher target BW designed to achieve the 21 wk BW at 18 wk of age, a 22% increase. The first of these studies evaluated the impact of rearing daylength (8L:16D, 10L:14D, and 12L:12D) on breeder performance. The second study explored whether highly uniform birds on the increased target BW could be photostimulated. Thus, birds on the high and low BW treatments were photostimulated at 18 or 21 wk of age.

b) Precision feeding operation

A more complete description of the feeding system and its operation is disclosed elsewhere (Zuidhof et al., 2016; Zuidhof et al., 2017). Only a brief description is provided here. The PF station is a sequential feeding system. The main functional area of each feeding station is the feeding chamber. The feeding chamber has an entry door, an exit door with an associated ejector mechanism, radio frequency identification (RFID) reader, scales to weigh the birds and feeder, and a feed access door.

Visits to the station are recorded whenever a bird enters the station, whether or not it is provided access to feed. The user can specify the amount of feed to provide in the feeder, and the duration a bird that is allowed to feed retains access to the feed. This time defines the

length of each feeding bout. We typically provide 10 to 25 g of feed, and allow access to the feed for 45 or 60 seconds. Birds are allowed to eat any time of the day or night as long as they meet the criteria for a meal, which normally means their BW was less than the target BW at the time of the visit. If multiple birds entered the station, the target BW is greatly exceeded, and the system ejects the birds from the station without feeding them. Prior to each feeding bout, an auger draws feed from a hopper into the feeder until the desired feed amount is present. At the end of each feeding bout, the weight of the remaining feed is measured and feed intake is calculated. After every visit to the station, the date and time at the start and end of each visit, BW, RFID, and feed intake are recorded as a database record.

Over the last couple of years, we have successfully controlled feed intake according to several criteria, including 1) real-time comparison to a set target BW, 2) maximum daily feed allowances, or 3) pair feeding, where we restricted feed intake proportionally to ad libitum fed birds. We have successfully fed males and females, broiler breeders, broilers, layers, and heritage lines.

III. GROWTH AND DEVELOPMENT

a) Body weight management

With PF, we have been able to consistently achieve a coefficient of variation for pullet BW of 1 to 2% by the time of photostimulation. van der Klein et al. (2017) reported a CV of 0.8 % CV for BW at wk 21. In a direct comparison with conventional feeding, Zuidhof (2017) reported a reduction in BW CV in pullets at the time of photostimulation from 14% to 2% using precision feeding. At 21 wk of age, the precision fed birds in that study weighed 99.0% of the target BW compared to 95.3% in the conventionally fed treatment.

b) Nutrient partitioning and body development

Precision feeding had some unexpected effects on development. Higher feeding frequency with the PF system appears to change the nutrient partitioning priorities of feed restricted broiler breeders. This is likely related to the fact that precision fed birds do not regularly fluctuate between the large positive and negative energy balances that occur daily with conventional feeding practices.

By photostimulation age (23 wk), precision fed Ross 308 breeder pullets were leaner, with more breast muscle (20.1% vs. 19.0% of live BW) and less abdominal fat (1.2% vs. 1.6% of live BW) compared with skip-a-day fed pullets (Carneiro, 2016). In a Cobb GP line, conventionally fed birds had 2.6 g of abdominal fatpad at 16 wk of age, compared with only 1.48 g in birds fed with the PF system ($P < 0.05$), but no difference was observed at photostimulation. This particular GP line was extremely lean. At the end of lay, the precision fed GP hens had a 10% larger breast muscle (124 g increase, $P < 0.05$) compared with conventionally fed hens.

Similar results were also observed in the commercial Ross 308 line. At the end of lay (55 wk of age) in the study evaluating the interaction between BW and rearing daylength, standard BW hens had a significantly higher proportional breast weight compared with high BW hens (27.5% vs 25.8%, respectively; $P = 0.006$), and a lower proportional fatpad weight compared high BW hens (1.5% vs. 2.4%, respectively; $P < 0.001$). This coincided with a lower egg production in the standard BW hens, but it is not clear whether altered body composition caused the drop in egg production or vice versa (van der Klein et al., 2017). In this study, almost one fifth of the standard BW hens never reached sexual maturity. Those hens that did not begin egg production before wk 55 had a 1.15 times the breast muscle of hens that had laid eggs, and 0.63 times the fatpad of hens that had laid eggs. This suggests

that if a threshold body fat content or fat mass is required for the onset of lay, it may not have been achieved by some of these precision fed standard BW birds.

IV. EFFICIENCY

a) Feed conversion

During the rearing period, cumulative FCR of precision fed Cobb GP pullets was 3.2% lower compared with conventionally fed pullets ($P < 0.05$). From 10 to 23 wk of age, cumulative FCR for Ross 308 broiler breeders in the precision fed pullets was 20% lower compared with skip-a-day fed pullets (4.0 vs. 4.8, respectively, $P < 0.05$). Precision feeding provided us with a means of evaluating efficiency in individual free run birds. In the study with standard and high BW broiler breeders, cumulative FCR phenotypes from d 16 to wk 21 averaged 3.95 ± 0.165. Cumulative FCR for hens on the high BW treatment was 0.33 higher compared with hens on the Standard BW treatment (

Figure 1, $P < 0.001$). Improved efficiency in precision fed broiler breeder pullets is presumed to be due to the increased feeding frequency (de Beer et al., 2007). Fed once every 24 or 48 h, the pullets need to store nutrients during the immediate post-prandial period when birds are in a high positive energy balance. Conversely, they must mobilize nutrients after nutrient supply from the gut to the bloodstream is reduced. These processes are not 100% efficient, thus the birds expend energy, which is lost as heat, which manifests as a measurably higher FCR. A similar phenomenon has been observed in daily vs. skip-a-day fed pullets (Zuidhof et al., 2015).

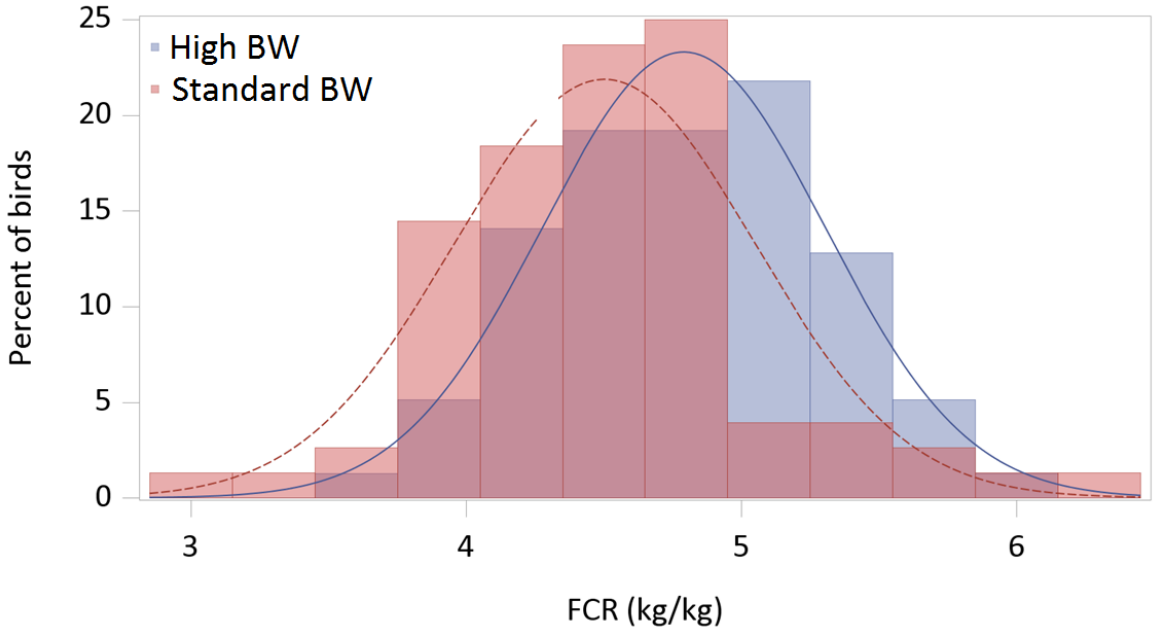

Figure 1 - Distribution of cumulative FCR phenotypes in 180 Ross 708 pullets from 16 d to 21 wk of age grown on a standard breeder recommended target BW, or on a high target BW curve adjusted to reach the 21 wk target BW at 18 wk.

Of course, feed consumed per chick produced is the most important measure of efficiency for broiler breeders. Notably, egg production in the high BW treatment was 57% higher (van der Klein et al., 2017), so the extra cost of feeding to the higher BW would pay back large dividends.

b) Heat production

From 10 to 23 wk of age, total heat production of precision fed Ross 308 pullets was 74.5 kJ/kg $BW^{0.68}$ less than skip-a-day fed pullets (464 vs. 540 kJ/kg $BW^{0.68}$, respectively; $P < 0.0001$). During the early laying phase (23 to 34 wk of age), after the conventional hens were transitioned to daily feeding, this trend continued. Precision fed hens had a lower total heat production (1252 kJ/d) than conventionally fed hens (1307 kJ/d; $P < 0.0001$).

In Ross 708 pullets from 16 d to 21 wk of age, average daily ME allocated towards maintenance (energy which was lost as heat) was 425 ± 20 kJ/$kg^{0.7}$. The ME requirement for BW gain was 10.2 ± 0.24 kJ/g. Hens on the 8L:16D schedule allocated 10.5 kJ/$kg^{0.7}$/d less towards maintenance compared to hens on 12L:12D schedule ($P = 0.003$), and hens on the 10L:14D schedule were intermediate. This was likely due to increased activity levels with longer photoperiods. Hens on the standard BW allocated 21.9 kJ/$kg^{0.7}$/d less toward maintenance ($P < 0.001$) compared to hens on the high target BW (van der Klein and Zuidhof, 2017). Animals are able to adjust feed intake, energy expenditure or both to maintain BW. Feed intake affects energy expenditure and heat production primarily in two ways: diet induced thermogenesis (NRC, 1981), and through homeostatic self-regulation of metabolic rate, which allows birds to maintain their energy balance (Richards and Proszkowiec-Weglarz, 2007). The precision feeding system has allowed us for the first time to model energy expenditure of individual birds in a group housed setting.

V. REPRODUCTION

Reproduction in chickens is under the control of the hypothalamic-pituitary-gonadal (HPG) axis which integrates external (photoperiod, seasons) and internal (age, body status) cues to ensure survival of the species via hatch of healthy chicks. This axis involves the combination of stimulatory and inhibitory neuropeptides and hormones which, upon photostimulation, result in the activation of the ovary (Bedecarrats, 2015). In turn, early ovarian follicles release estradiol which prepares the hen's body for egg production by stimulating the development of the reproductive tract, the synthesis of yolk components by the liver, and by terminating skeletal longitudinal growth in favor of medullary bone for egg shell formation. Thus, optimum reproductive fitness is achieved only if the age, body frame and composition, and photoschedule are coordinated. The process of egg formation is energy demanding for the hen and sufficient reserves need to be achieved. However, it is well documented that broiler breeders, when left unrestricted, tend to become overweight at an early age which results in health problems and poor reproduction (Decuypere et al., 2010; Mench, 2002; Yu et al., 1992). As a result, feed restriction to match target growth curves recommended by primary breeders evolved as standard industry practice to optimize reproductive efficiency. However, our most recent data suggest that continuous increase in broiler growth potential may have increased the optimum weight breeders need to reach at the time of photostimulation and, body composition of breeders is directly linked to their ability to respond to photostimulation. Precision feeding is the only on-farm technology which allows nutritional intervention at the bird level to ensure proper growth and body composition to match breeder hens' metabolic status, age and photoperiod.

a) Ovularche (first ovulation)

Bedecarrats et al. (2016) hypothesized that in addition to the classically considered sexual development thresholds (age, daylength, BW, and carcass fat content), metabolic status also

plays an important role in the onset of lay. Precision fed birds are provided small meals throughout the day, rather than the conventional single large meal. “Just in time” nutrition allows PF birds to access nutrients directly from the gut, and their metabolism appears to shift from fat storage toward preferentially building muscle tissue. This is consistent with the observation that skip-a-day fed pullets retain less breast muscle and more fat compared with conventionally fed pullets fed once per day (Zuidhof et al., 2015). This may explain the unexpected negative impact of PF on egg production when broiler breeders are exactly at the breeder-recommended target BW. Serendipitously, high feeding frequency with PF may have allowed us to see how close current target BW recommendations are to being insufficient for sexual maturation and reproductive success.

In Ross 708 hens, a long (12L:12D) rearing photoschedule caused a delay in the onset of lay. However, this effect and the associated negative impact on total egg production was mitigated by growing birds to a greater BW target (van der Klein et al., 2017). In fact, all hens on the high BW target reached sexual maturity, whereas 38% of standard BW treatment birds on the long rearing photoschedule did not begin laying during the entire trial (to 55 wk of age). This leads us to the hypothesis that a yet-to-be defined metabolic cue may play an important role in the timing of sexual maturation.

When Ross 308 broiler breeders were allowed to consume a lot of energy after photostimulation, they also started to lay earlier compared with the birds on a standard BW trajectory and diet. High ME intake increased the expression of gonadotropin-releasing hormone gene in the hypothalamus and its receptor in the anterior pituitary, which is known to activate the HPG axis leading to sexual maturation and ovularche. By 26 wk of age, only 50% of the standard ME intake birds had ovulated, but 100% of the high ME intake treatment ($P < 0.001$).

b) Egg production

In our first two studies comparing reproductive efficiency in conventional and precision fed hens, we rejected the hypothesis that a highly uniform flock would increase egg production. Egg production in precision fed Ross 308 breeders was only 84% of the conventionally fed birds ($P < 0.0001$). Egg production in precision fed Cobb GP hens was only 80% of the egg production of conventionally fed breeders ($P < 0.001$). However, in another study, it became clear that a higher BW target corrected the problem of reduced egg production by PF hens. Precision fed hens on the high BW treatment produced 138 eggs to 55 wk of age, while PF hens on the standard BW treatment produced only 88 eggs ($P < 0.001$) (van der Klein et al., 2017). These results, together with the decrease in abdominal fatpad, are consistent with the hypothesis that metabolic triggers are also likely involved in sexual maturation. Broiler selection has occurred so rapidly that the primary breeders understandably have not been able to keep up with the development of optimal target BW recommendations for breeder stock. These have changed very little since feed restriction became standard practice (Renema et al., 2007), and may very soon need to be adjusted upward.

c) Fertility

In trials with parent and grandparent stock, overall fertility to 52 weeks of age was 91.8%; significantly higher compared with conventionally fed pullets (89.9%; $P = 0.002$). In Ross 708 breeders, fertility to 55 wk of age was over 95% in both the standard and high target BW treatments, and they were not significantly different from each other. The high levels of fertility may well be attributable to strict control of male BW. Precision fed males are always in a slight positive energy balance because the target BW increases slowly during the laying and mating period. If males are reproductively active, they lose more dietary energy as heat.

However, this metabolic weight loss is replenishable in the PF system. Although these birds would lose weight more quickly, they would also qualify for a meal more quickly, and gain back any lost weight. Conversely, less active males are prevented from becoming overweight because they are weighed every time they attempt to have a meal. In the Ross 708 study, there were several roosters that needed to be replaced even though their BW was very tightly controlled. There were many hatching egg producer concerns about that particular rooster strain as being hard to manage. Notably, in the Cobb GP trial, no spiking was needed in the PF treatment.

VI. CONCLUDING THOUGHTS

Precision feeding is a valuable tool for research in chickens that generates large volumes of information that have allowed our thinking about nutrition, metabolism, and reproduction to evolve. We will use the PF system to investigate additional factors influencing onset of sexual maturity and reproductive fitness in chickens. Our research has raised a warning flag about an impending biological limit to feed restriction, and clearly indicates a need to re-evaluate target BW standards. It also points to the importance of identifying metabolic factors that mediate the function of the HPG axis. Because the precision feeding system allows us to collect large amounts of data and control feed intake to minimize BW variation, it serves as an excellent tool to study the interacting effects of diet composition, feed allocation, and other management factors on subsequent changes to body composition and reproductive success at the individual hen level. Even as these fundamental questions are addressed, precision feeding has excellent potential as a management system for commercial broiler breeders and upstream breeding stock.

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EFFECTS OF DIETARY PROTEIN LEVELS DURING REARING OF BROILER BREEDERS ON EGG PRODUCTION AND FERTILITY DURING PRODUCTION, AND OFFSPRING GROWTH PERFORMANCE

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Summary

A dose-response study into increasing the level of dietary protein to energy content during the rearing phase of broiler breeder production was conducted. Nine hundred and sixty ROSS 308 day-old breeder chicks were assigned to 1 of 6 dietary treatments including -20, -15, -10, -5, 0, and +5% of the recommended CP levels during rearing. Each experimental diet was fed to 4 pens (replicates) with 40 birds in each pen from 2 to 22 weeks. At the end of the rearing phase (22 weeks), 10 birds from each pen were dissected and body composition was determined. From 22 weeks onwards, 27 female broiler breeders and 3 males were located in each pen and egg production performance and fertility were studied until 64 weeks of age. Grow out studies of the offspring were conducted from breeder eggs collected at 28, 43, and 60 weeks of age. Broiler chicks received one of 3 dietary CP levels including standard, -7.5, and -15% during starter and standard, -6, and -12% during grower and finisher. Growth performance parameters were studied to 34 days. Breeders on low CP diets deposited more fat-pad than breeders on standard or high CP diet at a similar target body weight at the end of rearing. Reduced CP groups (-10 and -15% CP) tended to have a higher %lay and slightly higher total number of eggs produced. Offspring showed quadratic tendencies for better growth performance which was in agreement with parent flock egg production performance. However, the growth performance of offspring was impaired by reduced dietary CP, indicating that feeding lower dietary CP to breeders did not make their offspring more efficient when dietary CP level was lowered.

I. INTRODUCTION

Body composition, more specifically breast muscle and the abdominal fat pad, at the end of the rearing period of breeder pullets seems to play an important role in egg production, lay persistency, and fertility in the laying phase. Management of feeding by changing feed allowance or nutrient composition of the breeder pullet's diet during rearing can alter body composition at the onset of lay. Adequate dietary protein is necessary to have optimum ovary development, and a certain fat deposition is required which can be used as an energy source for egg production and persistency. However, the optimum body composition at the end of rearing that will support the best egg production performance and persistency is not defined (Van Emous et al., 2013). In broiler breeder nutrition, major attention has been given to the number of saleable chicks per hen; however, maternal nutrition can also affect the performance of offspring by changing the nutrients deposited in the egg or via transgenerational epigenetics (Lopes and Leeson, 1995a & b). The objective of the current study was to estimate the optimum dietary crude protein (CP) during the rearing phase in relation to egg production, fertility, and lay persistency as well as offspring growth performance. Offspring dietary CP levels were also assessed.

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II. MATERIALS AND METHODS

Nine hundred and sixty ROSS 308 day-old breeder chicks were used in a dose-response trial with 6 × 4 pens (6 treatments, 4 replicates) of 40 birds each and received a standard diet at 0 - 2 weeks of age and 6 experimental diets starting from week 3 to the end of rearing (22 weeks). Experimental diets were formulated to have -20, -15, -10, -5, 0, and +5% of the recommended CP levels during rearing. Other nutrients including energy were provided to fulfil requirements. All birds were reared to the same target body weight at the end of rearing based on supplier guidelines. At the end of 22 weeks, 10 birds from each pen were randomly selected for dissection and carcass, fat pad, and breast weights were measured for each dietary treatment. From 22 weeks onwards, 27 hens and 3 roosters were placed in each pen and received the same standard lay diets to the end of the trial (64 weeks). Eggs were collected and weighed daily. Egg fertility and hatchability were measured 8 times during the lay phase at weeks 28, 32, 38, 43, 53, 57, and 60. Eggs from 28, 43, and 60 week old breeders were incubated and chicks were hatched for the 3 grow out trials. Chicks in each grow out trial received one of 3 experimental diets including standard and 2 reduced CP diets (-7.5 & -15% in starter and -6 & -12% in grower and finisher phases). Feed intake and body weight in each trial were recorded weekly and feed efficiency was calculated for 0 - 34 days.

III. RESULTS AND DISCUSSION

The breeder birds on reduced dietary CP diets were allocated more feed in order to achieve the target body weight at the end of rearing. Protein intake levels, however, were still below recommendations for the -20, -15, -10 and -5% CP levels. Table 1 presents the results for body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) during rearing, and carcass composition at the end of rearing. Birds achieved similar target BW at 22 weeks by feeding management. Reduced dietary CP reduced breast weight ($p < 0.001$) and increased abdominal fat pad linearly ($p < 0.001$).

Table 1 - Growth performance parameters (0-22 weeks) and carcass composition at the end of rearing (22 weeks) of female breeders.

Treatment	CP in rearing diets						SEM	*p*-value	Linear contrast	Quadratic contrast
	-20%	-15%	-10%	-5%	Std	+5%				
BW 22 wk (g)	2683.5	2680.4	2677.2	2663.8	2661.5	2680.0	12.3	0.43	0.27	0.21
ADG 0-22 wk (g)	17.0	16.9	16.9	16.8	16.8	16.9	1.4	0.98	0.57	0.77
ADFI 0-22 wk (g)	72.9	69.3	66	64.1	61.9	60.4	2.6	<0.001	<0.001	0.01
FCR 0-22 wk (g/g)	4.42	4.86	4.20	4.32	3.99	4.01	0.41	0.009	0.003	0.57
BW dissection (g)	2483.8	2428.0	2501.6	2514.0	2494.2	2559.2	0.03	0.025	0.007	0.28
Carcass weight (g)	1958.9	1951.8	2022.0	2037.0	2018.0	2058.9	0.03	0.024	0.002	0.15
Breast weight (g)	525.9	557.8	612.6	629.8	639.8	682.7	0.02	<0.001	<0.001	0.25
Fat pad weight (g)	53.6	44.3	37.4	34.4	25.3	23.8	0.004	<0.001	<0.001	0.32
Mortality	16.12	8.67	8.67	9.29	7.43	14.27	2.9	0.1	0.58	0.02

Table 2 shows egg production, fertility and hatchability parameters during the laying phase (22 - 64 weeks). Larger breast weight was associated with higher feed intake with no differences in production performance, which suggests a higher maintenance requirement for heavier breasts. Reduced CP (-15 and -10%) treatment groups tended to have a higher %lay and total number of eggs, e.g. 4 more eggs in the -10% treatment compared with the standard group.

Table 2 - Egg production, fertility and hatchability parameters during laying phase (22-64 weeks) breeders.

Treatment	CP in rearing diets						SEM	*p*-value	Linear contrast	Quadratic contrast
	-20%	-15%	-10%	-5%	Std	+5%				
ADFI (g)	147.0	151.0	155.4	152.5	155.7	159.1	1.9	0.002	<0.001	0.60
ADG (g)	5.0	5.0	4.9	5.2	5.2	5.0	0.1	0.13	0.13	0.51
FCR (g/g)	3.37	3.33	3.38	3.46	3.47	3.54	0.07	0.18	0.009	0.42
Laying (%)	67.7	70.1	71.0	67.9	69.7	67.7	1.4	0.124	0.649	0.08
Total no of eggs	197.6	204.7	207.3	198.2	203.5	197.6	4.0	0.12	0.65	0.08
Fertility (%)	99.0	99.0	99.0	98.0	98.0	99.0	0.58	0.47	0.60	0.56

The main effects of different breeder rearing dietary CP levels on offspring growth performance are presented in Table 3. The effects of parent rearing nutrition on offspring growth performance were not strong; however there were quadratic tendencies for final body weight (BW 34 d) and average daily gain (ADG 0-34 d). Mortality and feed efficiency were not affected. The reduced dietary CP of the breeder rearing diet did not result in more efficient offspring in terms of CP utilization, as their performance decreased in response to lowered dietary CP. Body weight 34d, ADG (0-34d), ADFI (0-34 d), and gain to feed (G:F 0-34 d) were linearly reduced by reducing offspring dietary CP level as shown in Table 4. The interactions of different breeder rearing diet and offspring reduced CP diets on offspring performance (data not presented) were not significant for the overall trial and only ADG (p = 0.02) and ADFI (p = 0.009) of offspring was affected in the last week (28 – 34 d).

Table 3 - Main effects of breeder rearing diet on offspring final body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), gain to feed (G:F).

Item	CP in breeder rearing diets[1]						SEM	*p*-value	Linear contrast	Quadratic contrast
	-20%	-15%	-10%	-5%	Std	+5%				
BW 34 d (g)	1783	1844	1832	1840	1841	1804	86	0.44	0.62	0.065
ADG 0-34 d (g)	50.6	52.5	52.2	52.3	52.3	51.4	2.0	0.42	0.54	0.068
ADFI 0-34 d (g)	81.0	83.2	82.8	83.2	83.0	81.5	3.3	0.66	0.81	0.104
G:F 0-34 d	0.626	0.630	0.628	0.627	0.628	0.629	0.004	0.599	0.437	0.980
Mortality 0-34 d (%)	2.2	1.9	1.6	2.3	2.1	1.2	1.4	0.51	0.29	0.49

[1] Standard diet (Std) is the recommended dietary crude protein level and the other treatments are 5, 10, 15, and 20 percent lower or 5 percent higher in crude protein content compared to recommendations in breeder rearing diets.

Table 4 - Main effects of reduced crude protein diets on offspring growth performance.

Item	CP in broiler chicken diets[1]			SEM	*p*-value	Linear contrast	Quadratic contrast
	Std	-7.5/-6%	-15/-12%				
BW 34 d (g)	2087	1831	1554	84	<0.001	<0.001	0.506
ADG 0-34 d (g)	59.5	52.1	44.0	2.0	<0.001	<0.001	0.534
ADFI 0-34 d (g)	91.2	82.6	73.6	3.1	<0.001	<0.001	0.762
G:F 0-34 d	0.653	0.631	0.600	0.003	<0.001	<0.001	<0.001
Mortality 0-34 d (%)	1.9	1.9	1.7	1.1	0.805	0.60	0.68

[1] Standard diet (Std) is the recommended dietary crude protein level and the other treatments are -7.5 and -15% (starter and grower diet) and -6 and -12% (finisher diet) lower in crude protein content compared to recommendations.

IV. CONCLUSION

The overall conclusion of the current study is that dietary CP level could be reduced during rearing of broiler breeders resulting in the deposition of a slightly higher abdominal fat pad at the end of rearing, with the -10% CP diet resulting in higher egg production performance and persistency. The broiler chicken offspring of breeders reared on lower CP diets did not become more efficient in CP utilization as their growth performance declined as the dietary CP reduced, which indicates that decreasing breeders rearing dietary CP has less pronounced effects on the growth performance of their offspring.

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VITAMIN K ENRICHED EGGS: BENEFITS FOR THE CONSUMER, THE FARMER AND THE HEN

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Summary

Vitamin K (VK) is essential to the health and well-being of both humans and animals. It has been demonstrated that VK deficiency is involved in human diseases, especially bone and joint disorders. The 'modern' or processed diet of both humans and animals is low in VK. In two separate studies, the effect of Vitamin K_1 (VK_1) supplementation on bone density of laying hens and the effect on VK_1 content of eggs was evaluated. Hens fed 250 µg VK_1/day showed a 3.8% increase in bone density compared to a 5.4% decline in bone density of unsupplemented hens. Eggs from VK_1 supplemented hens contained on average 9.9 µg VK_1/100 g yolk, representing 5-25% of the daily requirement for children. Hens on the VK_1 supplemented diet laid 15.7% more eggs than unsupplemented hens. The number of hens used in these studies was small and the results should be confirmed using larger numbers of hens. These findings do, however, suggest that re-instatement of VK_1 into the ration of laying hens could improve hen health, increased human VK_1 intake and increase egg production.

I. INTRODUCTION

The significance of vitamin K (VK) to human health, well-being and ageing is currently receiving worldwide attention. Beyond its role in coagulation, the importance of VK in bone health is well established. Other roles in cardiovascular disease, inflammatory response, neural and cognitive health are currently under investigation (Card et al., 2014). Vitamin K_1 (phylloquinone, VK_1), occurs naturally in Fresh, Leafy, Green, (FLG) plant material and in the tissues (including eggs and milk) of animals consuming the same plant materials. The 'modern', processed, human diet is low in VK for several reasons:

1. Consumption of VK_1 rich foods, especially FLG vegetables, has declined in many populations (ABS, 2015);
2. Loss of VK_1 occurs due to light exposure in post-harvest storage of vegetables (Biffin et al., 2008);
3. Low fat diets are recommended by some health professionals/organisations. Absorption of dietary VK_1 requires co-consumption of fat, and;
4. FLG material, and hence VK_1, has been removed from the diet of many food production animals.

Many of the VK dependent proteins are conserved through the animal kingdom, and animal models have been widely used to inform the VK requirements of humans; yet the role of VK in animal health has received little attention. For example, osteocalcin is a well-known VK dependent protein. It is the most abundant, non-collagenous protein found in the bone of all vertebrates (Lanham-New, 2008). Its synthesis is dependent upon vitamin D but its ability to bind calcium is dependent upon VK.

In the absence or severe dietary shortage of VK, bone mineralization and bone strength are compromised (Binkley et al., 2002). The VK requirements of domesticated and farmed animals are, however, rarely considered beyond what is required to maintain normal

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blood coagulation. Several large studies of human populations have shown a direct correlation between increased VK intake and decreased risk of hip fracture (Shea and Booth, 2016). We propose that some of the diseases of VK deficiency, especially those involving bone mineralization, may have parallels in the animals we feed. Two separate studies were conducted to determine if addition of VK1 to a standard layer ration could:

1. Improve bone mineralization in the laying hens, and;
2. Increase vitamin K1 content of eggs for the benefit of the consumers.

II. MATERIALS AND METHODS

In the first study, a colony of 42 ISA Brown hens at point of lay was housed in 6 separate cages with 7 birds per cage. All hens were fed a standard commercial layer ration providing 250 µg/hen/day of Menadione Nicotinamide Bisulphite (MNB) as the dietary source of VK. After a period of 5 weeks, consistent laying was achieved. At 27 weeks of age the hens were divided into two groups. Group A was fed the same commercial layer ration. Group B was fed a diet that contained VK_1 (as QAQ®[3]) instead of MNB, also at 250µg/hen/day. At this time, the metatarsal bones of all hens were assessed using Radiographic Bone Aluminium Equivalence (RBAE) (Meakim et al., 1981). This assessment was repeated at 36 weeks and changes in bone density were calculated.

In the second study, two groups of 11 Australorp hens were fed diets containing either MNB or VK_1 (as QAQ®, a water-soluble, UV protected Vitamin K1 supplement, which is a registered product of Agricure Pty Ltd) in equimolar concentrations. The number of eggs from each feed group was recorded daily. Every 4 weeks, all eggs were assessed for: egg weight, yolk weight, shell thickness and VK_1 content. VK_1 was measured by HPLC using reverse-phase C_{18} column and post-column reduction according to the method of Jakob and Elmadfa (2000).

III. RESULTS

In the first study, addition of VK_1 to the ration of laying hens resulted in a significant increase ($P < 0.001$) in bone density over a 9 week period. Hens consuming a commercial diet, with no added VK_1, showed a significant decrease ($P < 0.001$) in bone density over the same period of time. In the second study, hens fed a diet supplemented with VK_1 laid eggs containing significantly more ($p < 0.01$) VK_1 than the unsupplemented hens. The presence of VK_1 in the unsupplemented eggs is due to small amounts of VK_1 present in the ingredients of the commercial diet, especially soy meal and canola oil. No significant differences were found in egg mass, yolk mass or shell thickness (Table 1). An unexpected finding was that hens fed the supplemented ration laid more eggs over the duration of the study (48 weeks). The supplemented hens continued to lay at >80% compared to 65% for unsupplemented hens at 90 weeks of age (Data not shown).

Table 1 - Mean concentration of VK1, egg mass, yolk mass and shell thickness for VK1 supplemented and unsupplemented hens.

Diet	VK1 Concentration (µg/100g yolk)	Egg mass (g)	Yolk mass (g)	Shell thickness (mm)	Total eggs laid over 48 weeks (dozen)
Supplemented 650µg/hen/day	9.90 ± 2.1^a	68.1 ± 6.1	20.2 ± 2.5	0.44 ± 0.04	22.61
Unsupplemented	2.57 ± 1.8^b	62.9 ± 5.5	17.3 ± 1.2	0.36 ± 0.03	19.54

[a,b,] Means bearing different superscripts within a column are significantly different ($P < 0.01$) according to Welch's t-test for unequal variances

IV. DISCUSSION

The aim of Study 1 was to determine if addition of VK_1 to the ration of laying hens could improve the bone density of these hens (as assessed by RBAE). At 36 weeks of age, hens fed a VK_1 supplemented diet, showed a 3.8% improvement in bone density compared to the unsupplemented group, which showed a 5.4% decline in bone density from the 27 week baseline. Bone disorders have been a perennial problem in the poultry industry (Knowles et al., 2008) and, despite efforts to identify underlying dietary causes, the condition remains largely unresolved. Given that VK is an essential cofactor in the activation of osteocalcin, it is likely that VK deficiency plays a significant role in bone weakness. Further research is required to confirm these findings and to determine if the bone density of broilers could also be improved with VK_1 supplementation.

The aim of Study 2 was to determine if increased VK_1 in the hen diet resulted in increased VK_1 in the eggs. The eggs from supplemented hens contained up to 14.7 µg/100g yolk (mean 9.9µg/100g yolk). Research suggests that much of the population is VK deficient (Hayes et al., 2016, Shea et al 2008, Thane et al., 2006) yet the addition of VK_1 to human food is not permitted in Australia, with the exception of Infant formula (NHMRC, 2006). Hen eggs enriched with VK_1 represent an ideal delivery system – providing both UV protection and a fat-rich medium for this vitamin. Enrichment of eggs can contribute to the daily intake of VK_1 for consumers, especially those reluctant to eat or unable to access FLG vegetables.

An unexpected finding in Study 2 was the laying of more eggs by the supplemented hens compared to the unsupplemented hens. In a study by Lavelle et al (1993), hens in a VK_1 deplete diet laid more eggs than those given a standard diet containing MNB. The effect of MNB on laying efficiency warrants further investigation. Whilst these results were generated from a relative small number of layers, they do suggest that supplementation of the layer diet with VK_1 may improve bone density in the hen, can enrich eggs for human consumption and may improve or extend layer efficiency. Further study with more replicates is required to consolidate the findings.

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THE EFFECT OF CHOICE FEEDING OF DIETS VARYING IN DIETARY CA AND AVAILABLE-P CONCENTRATIONS AND RATIOS ON INTAKE AND EGG QUALITY OF LAYERS

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Summary

An optimum inclusion rate of dietary calcium (Ca) and available phosphorus (avP) for laying hen diets is difficult to determine due to complex interactions post-ingestion between these two essential minerals and involvement with other digestive processes. Identifying appropriate inclusion rates and ratios for Ca and avP is complicated further depending on the outcome variable of interest, for example; optimum feed utilisation may necessitate a lower dietary total Ca level when compared with that required for optimum eggshell quality. To identify whether hens possess an ability to regulate Ca intake and if this can be extended to P, individually housed ISA Brown hens were offered a choice of diets selected for diverse dietary Ca and avP concentrations and ratios. Egg production and quality, and feed efficiency were assessed over an 8 week period. The results of this study show that, when given a choice, birds will selectively consume diets of high Ca density where Ca intake was similar at 4.68g/day while intake of avP differed significantly between 0.75-0.96g/day over 8 weeks, suggesting that birds ate until they met a certain Ca requirement. No impact on egg FCR or egg quality were observed when birds were offered two diets varying in Ca and avP, with the exception of choice treatment 2 producing significantly inferior albumen height and Haugh unit compared to the other choice treatments. Choice treatment 2 had the highest intake of P and, consequently, the lowest Ca:avP intake. The results of this study showed that layers will consume equal amounts of Ca when presented with varying concentrations of dietary Ca plus P, and thus have the capacity to manipulate their Ca intake.

I. INTRODUCTION

The laying hen has a considerable requirement for dietary calcium (Ca) and phosphorus (P) to support optimum egg production, egg quality and other physiological processes. In particular, a high intake of Ca ranging from 4 to 4.5% of the diet is considered important as the laying cycle progresses from early to late lay to achieve optimum egg number and egg shell quality. A capacity for birds to increase Ca intake from a separate Ca source as dietary Ca levels diminish has been observed (Wilkinson et al., 2011). The ability of the hen to regulate Ca intake may be utilised to support optimum productivity and egg quality. There is markedly less focus on P requirements in layers outside of research concerning phytase. The relatively fewer studies with a strong focus on P likely reflects the greater importance placed on Ca intake for egg and egg shell optimisation, despite close interaction of Ca and P in the mineralisation processes. The requirements for Ca and P by the laying hen must be considered in tandem due to their interaction and the tendency for Ca to complex with phytate which impedes the digestibility and hence availability of Ca and to a greater extent P. Commercially, laying hens are fed a single mixed diet that is formulated to meet all of the nutritional requirements of the bird to support optimum performance. However, a single

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mixed diet enables birds to primarily exercise their appetite for energy (Henuk and Dingle 2002). This can result in some laying hens having to rely on their medullary bone reserves for minerals, leading to a decrease in egg production, egg quality and the development of osteoporosis. The ability to exploit Ca regulated intake of laying hens may permit a lower dietary Ca component in the diet and allow the birds to consume Ca separately to meet their individual requirements. Allowing birds to self-regulate their Ca intake may also aid in increasing the digestion of P and phytate-P. This study investigated the presence of Ca specific appetite of layers and whether this appetite is extended to P. Additionally, the potential for layers to manipulate their Ca and P intake to improve feed efficiency and egg quality was investigated.

II. METHODS AND MATERIALS

All experimental procedures conducted had approval from The University of Sydney Animal Ethics Committee and were in accordance with the Australian Code for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2013). Birds were offered experimental diets based on wheat-soybean meal (11.7 MJ/kg AME) that comprised three nutrient densities of low, medium and high values of total Ca plus avP (30, 40 and 50 g/kg respectively) and within each density, two ratios of dietary Ca:avP (15.7:1 and 6.1:1). ISA Brown hens were housed separately in cages with three adjacent cages forming the replicate unit (six replicates of three birds per replicate) and fed for 8 weeks on a choice of two diets which were selected to provide a choice of high or low dietary Ca:avP and/or dietary Ca and avP concentrations as shown in Table 1. Egg quality, feed intake and feed:egg conversion efficiency were assessed weekly over an 8 week period. Experimental data were analysed as a one-way ANOVA of dietary treatments using the IBM® SPSS® Statistics 20 program (IBM Corporation. Somers, NY USA). Treatment differences were considered significant at $P < 0.05$.

III. RESULTS

The effect of choice feeding of three combinations of two diets, varying in Ca and avP concentrations and ratios, on intake and egg quality are shown in Table 1. Total feed intake averaged 120 g/bird/day over the 8 week trial period and did not differ significantly between treatments. Average daily feed intake of diets within a choice treatment were the same in treatment 1 and treatment 3 while birds in treatment 2 consumed 65% more of high Ca+P/low Ca:P diet (HD/LR) than the low Ca+P/high Ca:P diet (LD/HR; $P < 0.001$). Intakes of Ca and P were significantly different between choice treatment and diets ($P < 0.001$) where Ca intake increased linearly with higher dietary Ca ($r = 0.9912$, $P < 0.001$) and P intake increased linearly with higher dietary P ($r = 0.8274$, $P = 0.0421$). However, the linear relationship between dietary Ca and Ca intake was diminished at the choice treatment level where overall Ca intake was equal across the three choice treatments. Birds offered a high Ca + avP density diet (5% Ca + avP; HDR and HD/LR diet) alongside a low density diet (3% Ca + avP; LDR and LD/HR diet) consumed more Ca and P from the high density diet by a factor of 3.35 and 1.34, respectively, while birds in choice treatment 3 had a similar Ca and P intake of both medium Ca + avP density diets (4% Ca + avP; MD/HR and MD/LR diet). Ultimately, intake of total Ca did not differ between choice treatments where birds consumed a daily average of 4.68g Ca. As a consequence, total P intake and Ca:P intake ratios were dissimilar between treatments ($P < 0.001$). Choice treatment 2 acquired the highest P intake of 53.6 g and the lowest Ca:P intake ratio of 4.9:1. Choice feeding did not have an effect on egg FCR or egg quality, with the exception of choice treatment 2 producing significantly inferior albumen

height and Haugh unit compared to the other choice treatments. This treatment also obtained significantly lower Ca:avP intake.

Table 1 - The effect of choice feeding of two diet combinations varying in dietary Ca and avP concentrations and ratios on average daily feed intake, total feed intake and egg quality at 8 weeks.

Choice combination	1		2		3			
Nutrient composition:	HDR	LDR	HD/LR	LD/HR	MD/HR	MD/LR	SEM	P-value
Ca (%)	4.7	2.6	4.3	2.8	3.8	3.4		
avP (%)	0.30	0.42	0.70	0.18	0.24	0.56		
Ca + avP	5	3	5	3	4	4		
Ca:avP	15.7	6.1	6.1	15.7	15.7	6.1		
Diet effect (g):								
ADFI (g/day)	66.34^{bc}	57.52^{ab}	75.58^{c}	45.87^{a}	67.25^{bc}	64.54^{bc}	3.874	<0.001
Total Ca Intake	213^{f}	40^{a}	183^{e}	81^{b}	151^{d}	117^{c}	8.32	<0.001
Total P Intake	26.3^{c}	16^{ab}	39.2^{d}	14^{a}	19.5^{b}	28^{c}	1.30	<0.001
Choice effect (g):								
Total Ca Intake	254		265		268		9.55	0.55
Total P Intake	41.9^{a}		53.58^{c}		47.5^{b}		1.2	0.001
Ca:tP intake	6.1^{c}		4.9^{a}		5.6^{b}		0.119	<0.001
Feed Intake (8 weeks)	6504		6667		6952		166	0.19
FCR (g feed/g egg mass)	1.92		1.98		2.20		0.103	0.166
Egg weight (g)	63.4		65.9		64.4		1.144	0.312
Albumen height (mm)	9.96^{b}		9.06^{a}		10.5^{b}		0.280	0.008
Dry shell weight (g)	6.41		6.67		6.32		0.241	0.575
Shell thickness (mm)	0.429		0.455		0.416		0.014	0.159
Haugh unit	98.2^{b}		93.0^{a}		100.5^{b}		1.404	0.006

HDR, high Ca and avP density and ratio diet; LDR, low Ca and avP density and ratio diet; HD/LR, high Ca and avP density and low Ca:avP ratio diet; LD/HR, low Ca and avP density and high Ca:avP ratio diet; MD/HR, medium Ca and avP density and high Ca:avP ratio diet; MD/LR, medium Ca and avP density and low Ca:avP ratio diet; ADFI, average daily feed intake.
abcd Means within rows not sharing common suffixes are significantly different at the 5% level of probability

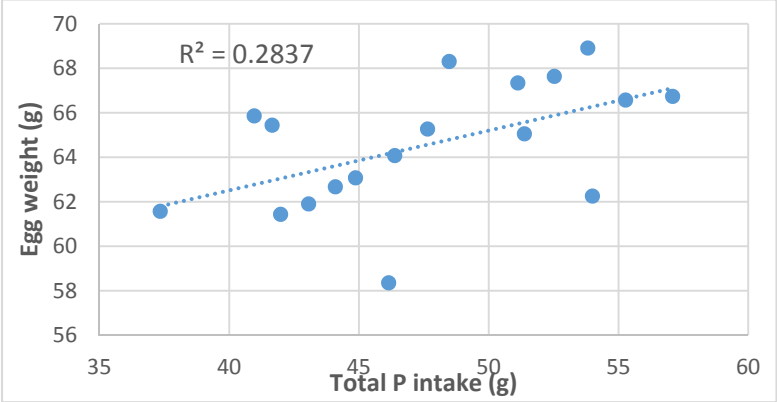

Figure 1 - Linear relationship between egg weight and total P intake in week 8 of experimental period (r = 0.533, P < 0.03).

IV. DISCUSSION

Based on total intakes of Ca and P from the combined consumption of the two diets, it appears birds were defending a Ca target (range 4.5 - 4.8 g/day) and this resulted in a variable P intake (range 0.75 -0.96 g/day) between dietary treatments. Even when given a choice of varying Ca and P content in the diet, birds appeared to prioritise their intake of Ca, as observed by the higher intakes of high Ca dense diets HDR (4.7% Ca) and diet HD/LR (4.3%

Ca) in choice treatments 1 and 2. This intake effect dissipated when the diets offered contained similar dietary levels of Ca as were provided by diets MD/HR (3.8% Ca) and MD/LR 10 (3.4% Ca). Poultry have been shown to possess a specific appetite for Ca as reviewed by Wilkinson et al., 2011 and the results of this study are in agreement. The mechanisms that control the Ca specific appetite remain uncertain. Animals are thought to respond to excess, deficits or imbalances of nutrients. Calcium is the most important driver of egg production and hence may explain the biological necessity for high producing layer hens to seek Ca to meet their high requirements. However, P is an also an important nutrient in the utilisation of Ca for egg production as shown by Figure 1 which depicts the positive linear effect of P intake on egg weight ($r = 0.2837$, $P < 0.03$); meanwhile the relationship between total Ca intake and egg weight was not significant ($P > 0.05$). Phosphorus is involved in many essential physiological functions and is critical to growth performance and bone mineralisation. Similar to Ca, the major use of P in the body relates to the formation and maintenance of bone. Inadequate dietary P may cause demineralisation of the skeleton in the laying hen. Panda et al., (2005) observed a significant reduction in shell thickness, weight and strength when dietary avP was reduced from 2.4 g/kg to 1.2 g/kg. This depletion in egg shell quality was reversed with the addition of phytase. Thus commercial laying hens have a substantial need for dietary Ca and avP to achieve optimum egg production and maintain physiological Ca and P homeostasis. An inadequate supply of these minerals can impact both the quantity and quality of eggs produced, and the longevity of the bird in the flock due to diminished bone mineral reserves leading to a compromised skeleton (Whitehead, 2004). In this study, layers expressed a Ca specific appetite, prioritising their intake of Ca while varying their P intake and in doing so, produced eggs of comparable quality and efficiency.

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PERFORMANCE AND EGG QUALITY OF LAYERS FED DIETS WITH LOW AND HIGH NET ENERGY : METABOLISABLE ENERGY RATIO

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Feed represents 65-75% of total production cost while energy represents approximately 50% of the diet cost. Apparent metabolisable energy corrected to zero nitrogen retention (AMEn) is most commonly used in poultry feed formulation. AMEn, however, does not consider energy lost during digestion as heat increment (HI). AMEn minus HI gives net energy (NE) which is energy available to the bird. An equation to predict net energy for production of ingredients for layers has been developed at UNE. Performance and egg quality were measured in hens fed diets with different levels of NE and NE:AMEn ratio formulated using the equation. The completely randomized study used 62 Hy-Line Brown laying hens of 44 weeks of age for 11 weeks. Two diets were fed to 31 replicates of individual birds, housed in single cages. The NE:AMEn, AMEn (MJ/kg), NE (MJ/kg), crude protein (CP) (g/kg), ether extract (EE)(g/kg) and added canola oil (g/kg) of the diets were, respectively: 1) 0.764,11.55, 8.82, 187, 35, 4.5 and 2) 0.792, 11.63, 9.21, 186, 73, 40.6. Birds fed the high NE:AMEn diet produced larger eggs ($P < 0.01$), with greater egg mass ($P < 0.05$) and had lower feed conversion ratio (FCR) ($P< 0.01$) compared to those fed the low NE:AMEn diet (Table 1). Safaa et al. (2008) showed that increased dietary EE was beneficial to egg weight, egg mass and FCR and according to the prediction equation, higher NE:AMEn is a result of higher dietary EE. Birds fed the high NE:AMEn diet had eggs with higher shell reflectivity ($P< 0.01$), Haugh units ($P < 0.001$) and yolk colour score ($P< 0.001$). Hamilton and Parkhurst (1990) showed a positive relationship between dietary EE and yolk colour score due to enhanced intestinal absorption carotenoid pigments. Paler shell colour in larger eggs may be due to the pigment produced being deposited over a larger surface area (Roland, 1979). The results of the current experiment show that higher dietary NE:AMEn results in beneficial effects in laying hens and the formulation of layer diets based on NE may benefit the egg industry.

Table 1 - Effect of low and high NE:AMEn diet on layer birds performance and egg quality.

	Low NE:AMEn	High NE:AMEn	SE	P > F
FCR, (feed/eggs)	2.124	2.007	0.02	0.004
Hen day production HDP, %	95.9	94.9	0.53	0.323
Egg wt, g	60.5	63.4	0.49	0.002
Egg mass, g/d	58	60.1	0.54	0.050
Shell colour	18.34	19.03	0.12	0.003
Haugh unit	90.2	92.88	0.27	0.001
Yolk colour score	11.37	11.7	0.03	0.001

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COMPARATIVE EVALUATION OF PRODUCTION TRAITS IN COMMERCIAL LAYERS UNDER FREE RANGE, SEMI-INTENSIVE AND INTENSIVE HOUSING SYSTEMS

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Summary

A 14 week study was executed to examine the effects of different rearing systems on production performance, egg geometry and quality traits in two commercial layer strains, Hy-line W-36 white layer and Bovans White. In total, 150 hens (18 weeks of age), comprising 75 from each strain, were randomly assigned to 6 treatment groups in a 2 (strain) × 3 (housing system) factorial arrangement under a randomized complete block design (RCBD). Each treatment had 5 replicates with 5 birds per replicate. Body weight, egg production, egg weight, egg mass and livability parameters of production performance were evaluated. The results indicated higher ($P \leq 0.05$) body weight, egg production, and egg weight in hens under the intensive housing system compared to those under semi-intensive and free range production. Hy-line strain showed greater egg production and egg weight than Bovans. Interaction of treatments showed maximum body weight in Bovans and egg production in Hy-line strain under the intensive housing system, whereas egg weight was found to be minimum in Bovans strain under the free range rearing system. In conclusion, rearing commercial layers in the intensive system had positive effect on production traits.

I. INTRODUCTION

Alternative rearing systems are becoming increasingly important mainly because of growing public concerns about intensive systems. Regulations have been established in some countries to restrict or ban the use of conventional systems. The European Union banned the use of conventional battery cage systems in 2012 and alternatively, new enriched colony cages, free range production systems or barn systems have been introduced as substitutes (Leinonen et al., 2014). As a result, poultry farmers are shifting from conventional to alternative rearing systems like free range, barn and enriched colony cages (Thaxton et al., 2016). These systems provide the birds with an enriched environment designed to improve their behavior and well-being. Furthermore, excreta of free range birds can act as organic fertilizers, enhancing soil fertility and crop yield (Hilimire et al., 2012). Alternative rearing systems could be viable options for better returns to small poultry farmers, helping to break the cycle of poverty. In Pakistan, some changes in the rearing system of laying hens will probably be required to follow the international guidelines for animal welfare, but current literature is sketchy about the use of commercial layer strains in different rearing systems. Therefore, the present study was performed to evaluate the effects of different rearing systems (free range, semi-intensive and confinement) on production performance traits in two strains of commercial layer (Hy-line W-36 white and Bovans white).

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II. MATERIALS AND METHODS

a) Ethical approval and experimental design

The present study was conducted at the Layer Unit, Ravi Campus, University of Veterinary and Animal Sciences (UVAS), Lahore, for 14-week duration (19 to 32 weeks). In total, 150 pullets reared at the Layer Unit, comprising two commercial strains (Hy-line and Bovans), 75 from each, were randomly assigned to 6 treatment groups in a factorial arrangement of 2 (strain) × 3 (housing system) under a RCBD. Each treatment group was replicated 5 times with 5 hens per replicate. Experimental birds were reared and handled under the code and conduct of ethical committee approved by the University of Veterinary and Animal Sciences, Lahore, Pakistan.

b) Rearing systems and management

The experimental birds were maintained in a well-ventilated poultry house in pens on a deep litter floor. Each pen (5 × 5ft) represented a replicate. Feed was offered through manual feeders placed at the front of each pen. A free range area (15 × 30 m) was located adjacent to the house. A photoperiod of 16L:8D was applied during the entire study period. In the intensive system, an average daily temperature of 30°C and Relative Humidity (RH) of 75% were maintained throughout the experimental period. All birds were kept under similar management and hygienic conditions and vaccinated against ND and IB (ND on monthly basis and IB after two months in drinking water) under the supervision of a qualified veterinarian.

Birds in the intensive system were kept 24 h in house (pens) and offered 100% layer ration (0700 h) formulated in accordance with the recommendations of the NRC (1994). The diet was iso-nitrogenous (CP 15.04) and iso-caloric (2682 kcal/kg ME). The birds under the Semi Intensive (SI) housing system, had 'half-day' (07:00-12:00 h) access to free range and subsequently were kept in the house (pens) (12:00-07:00 h) with 50% of the daily feed allowance. A one week adjustment period was provided to the birds under the free range and semi-intensive rearing systems; they were acclimatized by directing them to the free range and then replacing them into the pens to avoid subsequent handling stress. The birds under the free range rearing system had 'whole-day' access to the free range area (07:00-0500 h) where seasonal vegetation, including legumes (beans, lentils, peas and grasses) and non-legumes were grown. Subsequently, they were shepherded to the same house and kept in pens (05:00-07:00 h) with rice husks as bedding material. Fresh water was provided to the birds through a nipple drinking system. On the free range, birds were protected from predators by installing a wire-mesh enclosure (2.44 m high) surrounding the free range premises.

c) Parameters studied

Body weight (BW), egg production (EP), egg weight (EW), egg mass (EM) and livability (LB) [(dead birds/total birds × 100) -100] parameters were studied. Data collected included weekly body weight / bird, percent weekly egg production, fortnightly egg weight, fortnightly and cumulative egg mass, and livability percentage. Egg production was recorded on a hen/day basis. Egg production percentage was calculated as a ratio between total egg production and number of hens multiplied by 100. Egg weight was recorded by using a digital scale with 0.01-g precision, whereas egg mass was calculated as the total number of eggs multiplied by average egg weight. Throughout the study period, mortality if any, was recorded daily, to calculate the livability percentage.

d) Statistical Analysis

The data were analyzed through ANOVA technique under factorial arrangement by using the GLM procedure of SAS 9.1 for windows, Cary Inc. NY. Rearing system and strain were taken as the main effects and their interaction was also tested. Comparison among treatment means was done through Duncan's multiple range (DMR) test) at 5% probability level. Each pen was taken as an experimental unit.

III. RESULTS AND DISCUSSION

a) Body weight (g)

Housing system had a pronounced effect ($P \leq 0.05$) on final body weight, whereas strain alone did not influence ($P > 0.05$) final body weight. Birds under the intensive rearing system showed greater ($P \leq 0.05$) body weight compared to those under the semi-intensive and free range rearing systems (Table 1). Higher body weight in the intensive rearing system may be attributed to the availability of a balanced diet and most importantly to the absence of natural behavior such as foraging, walking, which may result in reduced body weight. Conversely, it is reported that birds under free range rearing system have freedom for the expression of their natural behavior (Chen et al., 2013. Likewise, increased body weight was observed in broiler breeder, quails, cockerels and turkeys (Macek et al., 2004) under an intensive housing system as compared with those having access to the free range.

Table 1 - Production performance of commercial layer under different housing systems.

		BW (g/bird)	EP (%)	EW (g/bird)	EM (g)	LIV (%)
Commercial layer strain						
Hy-line W-36		1438.33±16.00	55.04±0.87^{a}	53.86±0.24^{a}	16152.28±697.13	96.00± 2.13
Bovans White		1465.33±19.56	54.05±0.79^{b}	53.05±0.34^{b}	15011.78±301.45	94.66± 2.36
Housing system						
FR		1410.50±14.10^{b}	50.67±0.43^{c}	52.86±0.41^{b}	15016.98±148.61	92.00± 3.26
SI		1417.50±21.36^{b}	55.20±0.35^{b}	53.64±0.35^{ab}	15430.84±135.18	96.00± 2.66
I		1527.50±19.56^{a}	57.77±0.38^{a}	53.88±0.33^{a}	16298.27±147.79	98.00± 2.00
Commercial layer strain × Housing system						
Hy-line W-36	FR	1396.00±11.66^{c}	51.10±0.82^{d}	53.91±0.39^{a}	16416.24±2223.73	92.00±4.89
	SI	1426.00±31.80^{bc}	55.59±0.52^{bc}	53.98±0.58^{a}	15586.93±148.35	96.00±4.00
	I	1493.00±27.89^{ab}	58.44±0.41^{a}	53.70±0.32^{a}	16453.67±126.53	100.00±0.0
Bovans White	FR	1425.00±25.61^{bc}	50.24±0.29^{d}	51.81±0.28^{b}	13617.72±113.74	92.00± 4.89
	SI	1409.00±29.98^{c}	54.81±0.47^{c}	53.29±0.39^{a}	15274.75±219.18	96.00± 4.00
	I	1562.00±23.94^{a}	57.10±0.52^{ab}	54.06±0.61^{a}	16142.86±264.97	96.00± 4.00

$^{a-d}$Superscripts on means within a column show significant difference ($P \leq 0.05$); BW = Body weight EP = Egg production EW = Egg weight EM = Egg mass LIV = Livability FR = Free range SI = Semi-intensive I = Intensive

b) Egg production (%)

Housing system and strain of bird, separately and in interaction, showed a significantly impact ($P \leq 0.05$) on egg production. Layers of the Hy-line strain had greater egg production than the Bovans strain. Layers under the intensive rearing system had higher egg production ($P \leq 0.05$) compared to those under semi-intensive and free range (Table 1). The difference in egg production between the two strains may be attributed to the difference in their genetic

make-up as discrepancy in egg production among different genetic groups of poultry (Rehman et al., 2017) has already been reported.

c) Egg weight and egg mass (g)

Egg weight was affected ($P \leq 0.05$) by the treatments and their interaction. Layers under the intensive rearing system produced heavier eggs than those under free range. Hy-line strain layers produced eggs with higher weight than those of the Bovans strain. Interaction between housing system and strain of bird resulted in minimum egg weight in layers of the Bovans strain under the free range rearing system (Table 1). Egg weight is a genotype dependent parameter, which varies from breed to breed and strain to strain (El-Fiky et al., 2000). Similarly, variations in egg weight were observed among different strains and breeds of poultry (Yakubu et al., 2008). However, Wall et al. (2010) observed no effect of different poultry breeds or strains on egg weight.

d) Livability (%)

Neither strain, rearing systems, nor their interaction influenced ($P > 0.05$) livability (Table 1). A comparable value for livability percent in free range and intensive systems indicates that birds have genetic propensity to adjust well to free range conditions. However, disease, predation and injuries due to cannibalism are reported to be the major reasons behind low livability in free range rearing system (Elson, 2015). From the present findings, it can be concluded that housing of commercial layers in an intensive housing system had a positive effect on production traits.

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DETECTION OF UNDER AND OVER-PROCESSING OF SOY PRODUCTS BY NIR TECHNOLOGY

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Summary

Protein quality of soy products is related to both the reduction of anti-nutritional factors (ANFs), and the optimization of protein digestibility. Under-processing of soybeans fails to destroy the ANFs whereas over-processing reduces the content of lysine and cysteine and the availability of all amino acids mainly via the Maillard reaction. NIR technology allows soy users to detect and classify all soy products with regard to the degree of processing ranging from raw soybeans to highly over-processed soybean meals (SBM).

I. INTRODUCTION

SBM quality is a function of soybean quality and processing conditions. Currently, the analytical techniques most commonly used to measure SBM quality includes Urease activity (UA), trypsin inhibitor activity (TIA), KOH solubility, protein dispersibility index (PDI) and reactive lysine. However, each of these methodologies has limitations in terms of determining under or over processed SBM. SBM processors and the end users of SBM in the animal feed industry need reliable, rapid and cost-efficient methods to control the quality of their protein meal.

II. MATERIALS AND METHODS

Over 1195 soy product samples of global origin (65 countries) from 2008 till 2016 covering all important suppliers and different production processes were analyzed for TIA (Hamerstrand, 1981), KOH protein solubility (Araba and Dale., 1990), PDI (AOCS, 2011), reactive lysine (RL) and RL to lysine ratio (Fontaine et al., 2007). The quality parameters were then intelligently combined to design a new parameter, the Processing Conditions Indicator (PCI). Individual calibrations for each of the quality parameters were further developed to accurately predict the degree of processing of soy products in a fast and reliable manner. The calibration statistics for the 1195 samples analyzed are presented in Table 1. Around 100, 000 samples from top 5 SBM producing countries globally were analyzed using the newly developed NIR technology (April 2016 to June 2017).

Table 1 - Calibration statistics of soy products from different origin.

Parameter	Data Set					Calibration			
	Mean	CV	SD	Min	Max	SEC	RSQ	SECV	1-VR
TIA (mg/g)	5.34	110.59	5.908	0.2	36.6	1.606	0.93	1.74	0.91
KOH solubility (%)	80.19	12.16	9.752	19.4	99.6	2.617	0.93	2.82	0.92
PDI (%)	15.97	88.31	14.101	4	85.5	2.856	0.96	3.104	0.95
RL (%)	2.31	12.94	0.298	0.823	3.007	0.042	0.98	0.046	0.98
RL/Lysine (%)	88.11	5.07	4.468	55.49	94.38	1.11	0.94	1.203	0.93
PCI	13.8	28.78	3.97	4.73	28.01	1.01	0.94	1.086	0.93

SEC – standard error of calibration, RSQ – coefficient of determination, SECV – standard error of cross validation, 1-VR – variance ratio, coefficient of determination in cross validation

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III. RESULTS AND DISCUSSION

An overview of the SBM samples analyzed (Figure 1) from USA, Brazil (BR), Argentina (AR), China (CN) and India (IN) revealed major contribution coming from BR.

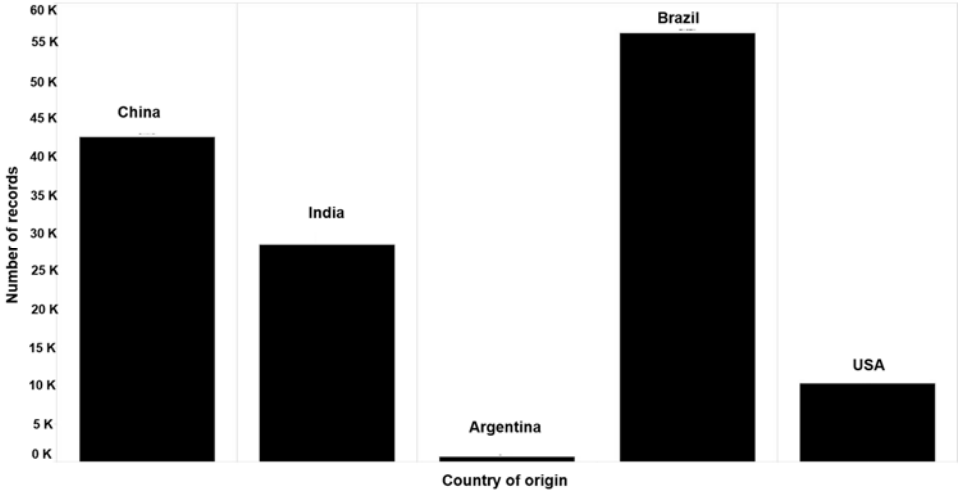

Figure 1 - Overview of SBM samples analyzed from April 2016 - June 2017.

The quality parameters of SBM from different origin (Table 2) indicated quite some variation with TIA values as low as 0.1 to as high as 27. SBM from Argentina (AR) had 95% samples within the desired value of TIA (4mg/g) whereas the same for Brazil (BR), China (CN), India (IN) and USA were 60%, 91%, 85% and 65% respectively. Herkelman et al. (1991) showed TIA to be a more reliable indicator than UA on performance of male broilers fed heat-treated full-fat soybean. Despite the extreme usefulness of TIA to determine whether soy products have been sufficiently processed to deactivate ANFs, it is recognized that the assay is not a good indicator to assess whether the soybean product has undergone an excessive heat treatment. Palic et al. (2011) in their animal feeding studies with raw soybeans showed KOH solubility to be more sensitive to over-processing and the limits were shown to fit well with animal performance. Research comparing protein solubility to other measures of protein quality indicate that KOH solubilities between 73 to 85% are optimal for animal performance (NOPA, 1999).

Table 2 - Quality parameters of SBM from different origin.

Country	TIA (mg/g)	KOH solubility %	PDI	Reactive lys/lys
Argentina	0.2-8	63-91	4-28	81-95
Brazil	0.2-22	45-96	4-57	74-95
China	0.1-26	40-94	4-59	72-95
India	0.1-27	33-98	4-52	75-95
USA	0.1-17	55-93	4-58	80-94

Around 1% of the SBM from AR had KOH solubility below 73% and close to 5% were over the desired levels of KOH solubility. The analyzed values for protein solubility for SBM from BR, CN, IN and USA indicated 94%, 91%, 91% and 89% respectively were within the desired range of KOH solubility. PDI is often considered the simplest, consistent and most sensitive measure of SBM quality (van Eys et al., 2004) with the recommended levels between 15-30%. Despite its simplicity and initial indications that it might be the best indicator of soy processing, Palic et al. (2011) demonstrated in an inter-laboratory study that PDI cannot be recommended as a reliable indicator of protein quality. If we consider PDI as

the quality parameter for SBM (Table 2), all of the origin had less than 50% SBM falling under the recommended levels except for USA (62.5%). Serrano et al. (2013) concluded that KOH solubility values are less influenced than PDI values by the length of storage of the SBM, which might partly explain the huge variation observed above in PDI compared to KOH solubility. The analysis of SBM samples for RL showed values ranging from 2.23-2.84 for AR, 1.94-2.83 for BR, 1.64-2.84 for USA, 1.55-2.81 for CN and 1.85-2.85 for IN (Figure 2). Interpreting protein quality based on the reactive lysine value alone may be misleading rather the ratio of RL to total lysine can be used as a reliable indicator of over-processed soy products.

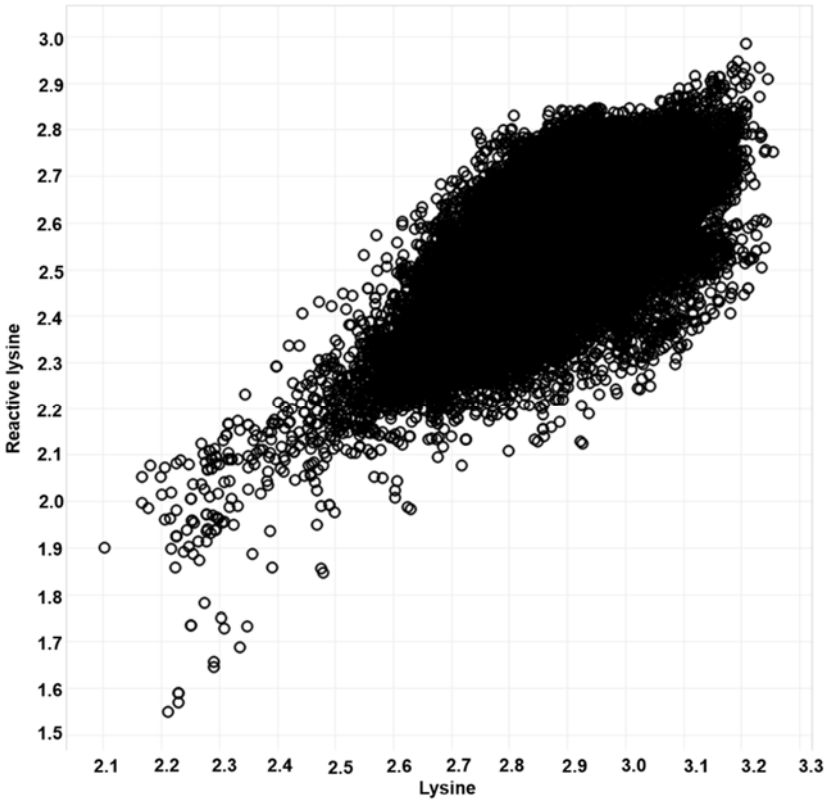

Figure 2 - Reactive lysine of SBM from different origin.

RL to lysine ratio of the analyzed samples revealed AR and USA SBM to be less impacted with lower range (Table 2) compared to SBM from other origin. Different quality parameters resulted in diverse interpretation about SBM quality. The newly established parameter PCI, integrates the various factors of under, adequate and over processing as one numerical index taking into consideration various anti-nutritional factors as well as heat damage effects on amino acid contents and protein solubility. The PCI values of the SBM (Figure 3) indicated 97.5%, 93%, 87%, 79% and 76% of USA, AR, BR, IN and CN to be adequately processed. PCI values for soy quality ranges from 0-30 in the recently developed calibration model and is signified as over processed (0-10), adequately processed (11-20) and under processed if >20. For eg: raw soybeans will have a PCI of about 24 and, with increased processing, the PCI will be reduced. The optimal nutritional value will be reached at a PCI of 12-14. Further processing of soy products will lead to additional reduction of the nutritional value and will be indicated by a drop of the PCI. In conclusion, it is necessary for ingredient quality control programs to understand the appropriate assays to determine if soy products have been subjected to under or over-processing. Deeper understanding of soy products and a precise evaluation of their quality allows a more accurate feed formulation and thus a more

consistent animal performance over time. The newly established calibration model uses NIR technology to measure soy product quality in a fast, reliable and accurate manner.

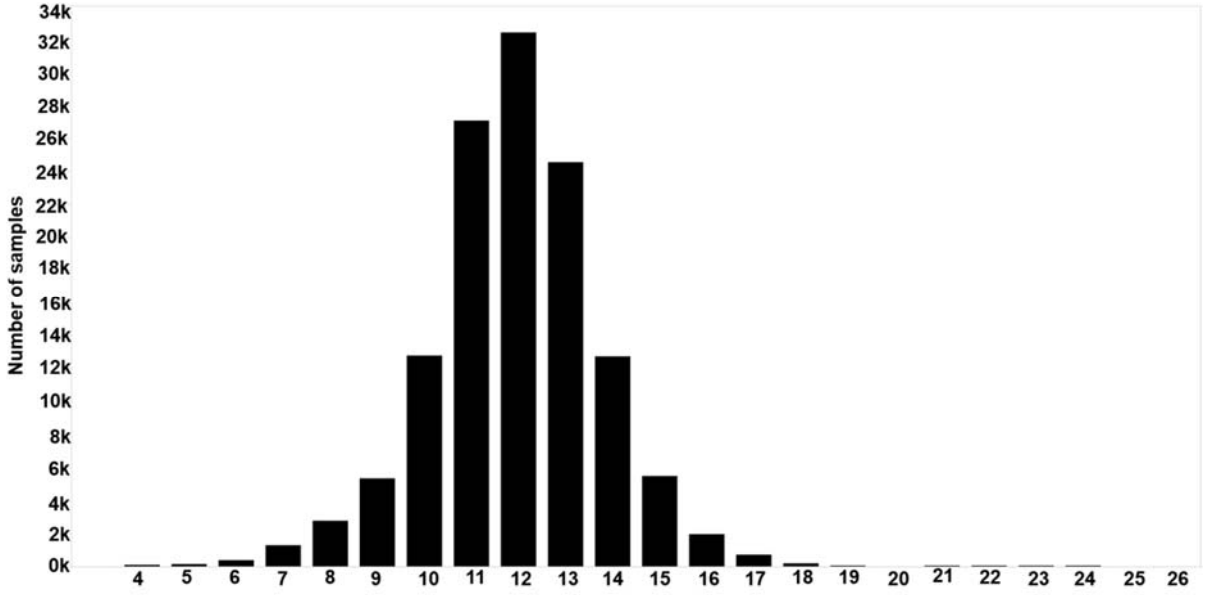

Figure 3 - PCI histogram of SBM from different origin.

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ANIMAL WELFARE STANDARDS & GUIDELINES – THE ROLE OF SCIENCE AND ETHICS IN PUBLIC DEBATES

J. DUNN[1]

Public consultation is due for completion in February on the next animal welfare standards and guidelines for the poultry industry. Practically and importantly, these new standards and guidelines will take the industry from a voluntary specification based model code, to an outcomes-based set of legally enforceable standards. For farmers, this is a paradigm shift. We've traditionally supported voluntary codes whilst making a commitment to the community that we will always improve. At the commencement of this standard setting process, egg farmers indicated their support for the mandating of these standards. This was viewed as an appropriate recognition that community expectations had risen and that part of our compact with the community required support for a firmer regulatory hand. It was also an expression of our view of animal welfare – central to who we are and what we do; we welcome the imposition of legal requirements. If a farmer doesn't care for their animals, then they're in the wrong profession.

This formal support for mandatory standards may be slightly technical, but it is significant, and we continue to engage with the community on its importance. However, increasingly we have found the communication challenge of that engagement to be difficult and frustrating. Incremental reform is cast as insincere; the distinction between voluntary codes and legal standards framed as of no consequence; and what started as a consultation has turned quickly into a fierce campaign. Campaigns aren't the best place for a conversation of nuance or an engagement on the gradual, technical process of improving. Campaigns aren't the place to talk about context, like the joy of achieving improvement while still producing 15 million eggs every day.

The complexity of this operating environment is challenging enough for farmers – how do we explain the multi-faceted process of trading off certain natural behaviours for a better welfare dividend in health? And if we manage to even do that what's our catchy hashtag campaign to make it cut through the noise in 140 characters? Of course, animal welfare isn't unusual in this regard. Campaigning is an important part of cultural expression and identity - farmers aren't alone in their weariness of twitter. Thankfully, the campaign-based nature of public discourse has traditionally been protected by our ability to pivot to science as the independent arbiter of disputes; the bipartisan steward that we can all revert to when the slogans get too much. Right? Wrong.

The greatest casualty of the current consultation process has been the appropriation of science as a campaign tool and, as a result, the integrity of the discipline is now squarely in the frame. As farmers grapple to better engage with new areas of animal welfare science, it is timely to reflect on how our most sacred institution and discipline became a play-thing of a values campaign and what we need to do to restore its respected place as the bastion of evidence and independent thought.

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RANGE ENRICHMENT ON COMMERCIAL FREE RANGE LAYER FARMS

C.T. DE KONING[1], S. KITESSA[1] and K. DRAKE[2]

Hens using the outdoor range display better plumage condition suggesting lower incidence of injurious feather pecking (Chielo et al. 2016; Rodriguez-Aurrekoetxea and Estevez 2016). However, ensuring that the majority of the flock utilizes the range has been difficult. Therefore strategies such as range enrichment are required to induce more birds to use the range and encourage natural behaviour. An uncontrolled observational study was conducted in South Australia on two commercial fixed-range, free range layer farms (Farm 1: 3,000 hens and Farm 2: 10,000 hens, with range stocking density of 1,500 and 10,000 birds/ha, respectively). Hens were Hy-Line Brown and beak treated. A variety of enrichment structures was placed on the ranges of both farms to assess the level of use and attractiveness (e.g. shelters, sand pits, peck toys and hay bales). Farms were visited monthly (April to October 2016), when the enrichment structures were filmed for 1 h at each of 1100 h (AM) and 1500 h (PM) whereby the number of hens visiting the structures were counted continuously. The time of day, AM vs PM, did not influence the number of visits to the structures (Figs 1 & 2). Sand pits (for dust bathing) and overhead cover provided by constructed shelters received most hen visits (Figs 1 & 2). Although hay bales were visited, the number of visits was lower than for shelters. Distance of structures from the shed also influenced the level of hen visits. Visualization of the data (Fig 1) suggests the closest shelter on Farm 1 (Shelter 1 = 20 m from the shed) and hay bale (Hay 1 = 35 m from shed) had more visits than the distant shelter (Shelter 2 = 50 m) and hay bale (Hay 2 = 70 m). In conclusion, hens visited a variety of enrichment structures on the range that were cheap and easy to build.

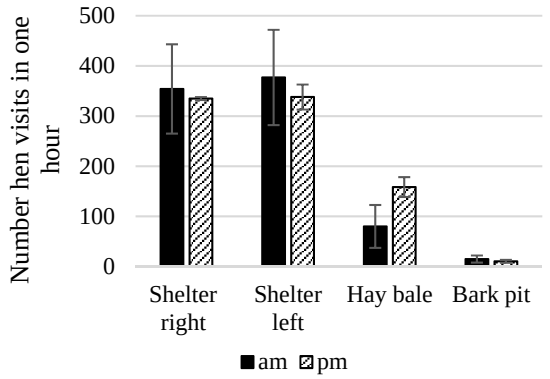

Figure 1 - Average number of hen visits in one hour to various range enrichment structures on Farm 1.

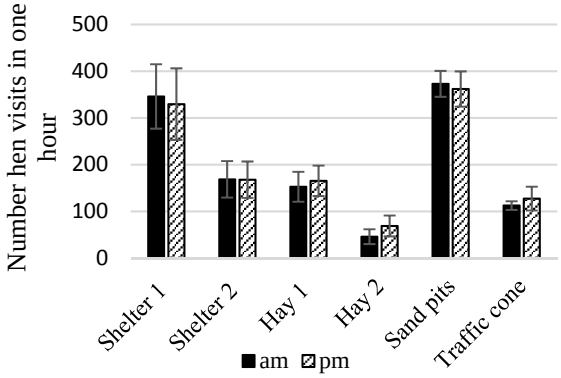

Figure 2 - Average number of hen visits in one hour to various range enrichment structures on Farm 2.

ACKNOWLEDGEMENTS: We thank the Poultry CRC for providing the funds for this project and the collaborating farmers for allowing us to conduct the trial work on their properties.

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A REVIEW OF ENVIRONMENTAL ENRICHMENT FOR LAYING HENS DURING REARING IN RELATION TO THEIR BEHAVIOURAL AND PHYSIOLOGICAL DEVELOPMENT

D.L.M. CAMPBELL[1] and C. LEE[1]

The environment in which a laying hen is reared can have long-term impacts on their behaviour, health and welfare later in life (Janczak and Riber, 2015). Alternative housing systems for layers provide access to larger areas and allow greater expression of behavioural repertoires leading to positive acceptance by consumers. But the complexities of alternative systems can also place greater physical and behavioural demands on the birds leading to increases in skeletal injuries or inter-bird aggression. For optimal welfare and production of hens, it is best to match the rearing environment with the layer environment (Janczak and Riber, 2015), but this is not always possible. Environmental enrichment – defined as a modification in the environment that increases behavioural possibilities leading to improvement in biological function (Newberry, 1995), is a potential method of modifying rearing conditions for improvements during lay. Enrichment can include additional objects, sensory stimuli or structural modifications including alternative rearing systems themselves (e.g. furnished or aviary systems). The effectiveness of enrichments can be determined by quantifying impacts on the physiology and behaviour of the birds. The literature was reviewed to compile the impacts of different types of enrichments during rearing on skeletal development, immune system development, neurophysiological development, visual and auditory development and behavioural development including fear and foraging/pecking.

Briefly, the literature identified that environmental enrichment has variable impacts on pullets depending on the type of enrichment provided. Alternative rearing systems that encourage jumping and flying or rearing systems that include perches can improve bone strength, skeletal symmetry, and reduce the incidence of keel fractures during lay. Furnishings within cages can enhance immune responses and increased environmental complexity can reduce fear responses. Access to litter reduces the development of abnormal feather pecking behaviour. Modifications made within the first few weeks of life can have long-lasting effects.

The majority of research on enrichment during rearing has been conducted overseas with a lack of understanding of impacts on commercial farms within Australia. In particular, birds destined for free-range systems may benefit from enrichment during rearing to better prepare them for outdoor access. New suggestions for enrichments, timelines for provision of enrichments and knowledge gaps are identified.

ACKNOWLEDGEMENTS: This research was supported by PoultryHub Australia.

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ESTIMATION OF OPTIMAL METHIONINE, GLYCINE, AND TRYPTOPHAN LEVELS TO IMPROVE PLUMAGE CONDITION IN ISA BROWN LAYING HENS

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Summary

Optimal dietary levels of digestible methionine, glycine, and tryptophan were estimated using a Box Behnken design to improve plumage condition in laying hens. In the present study, 156 ISA Brown hens were housed in group cages (three replicates of four birds each) and supplemented with varying combinations of digestible methionine (dMet), glycine (dGly), and tryptophan (dTrp) to determine the effect of amino acid supplementation on the plumage condition. Significant effects of dMet and dTrp inclusion were observed on the overall plumage condition of birds. The maximal likelihood of observing perfect overall plumage condition (61.31%) was predicted when dMet, dGly, and dTrp inclusion rates were 0.56%, 0.88%, and 0.26%, respectively, as determined by the proportional odds linear regression model. Plumage condition of the breast region was weakly affected by dGly inclusion rates ($P=0.06$), with optimal dGly level predicted at 0.88%. Similarly, plumage condition of the back region was affected by dTrp inclusion rates ($P<0.001$), with optimal plumage condition predicted at 0.26%.

I. INTRODUCTION

Supplementation of amino acids to reduce feather pecking behaviour and improve plumage condition has been previously studied and found to be successful (Savory, 1998, van Hierden et al., 2004). Feather eating behaviour performed by feather pecking birds suggests that feathers (predominately composed of keratin protein) may be consumed as an alternative source of amino acids, if digested.

Methionine is an essential amino acid required for egg production and feather development/growth. Although feathers contain low amounts of methionine, it may be required for feather synthesis and regeneration after feather pecking damage. Glycine is a non-essential amino acid and has a substantial presence in feathers, but requires dietary inclusion due to a high excretion rate and insufficient *in vivo* synthesis (Kleyn, 2013). Added Tryptophan has been reported to reduce gentle (van Hierden et al., 2004) and severe (Savory, 1998) feather pecking behaviour; however its use in combination with additional amino acids is unknown.

The aim of this trial was to determine an optimal combination of dMet, dGly, and dTrp levels that could effectively improve plumage condition. The trial used a Box Behnken experimental design which is based on a second order three-level incomplete factorial design and allows for the use of response surfaces and multivariate optimisation (Ferreira et al., 2007).

II. MATERIALS AND METHODS

A total of 156 ISA Brown hens (68 weeks old) was used in this trial. Birds were selected from a previous trial based on observed feather pecking behaviour directed at artificially presented feathers. Birds were housed in group cages containing two feather pecking and two non-feather pecking hens and given one week to acclimatise to new housing. Each group cage was randomly allocated to one of 13 dietary treatments set according to a three-factor,

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three-level Box Behnken Design with three replicates per diet. The centre point levels of dMet, dGly, and dTrp inclusion as analysed were 0.49, 0.76, and 0.23%, supplemented levels were 0.56, 0.88, and 0.26% and reduced levels were 0.43, 0.65, and 0.20%, respectively. Requirement levels of dMet, dGly, and dTrp were 0.51, 0.59 and 0.21%, respectively. Feed was provided *ad libitum* to each group cage. Amino acid analysis of both the excreta and diets was conducted by Evonik AMINOLab, Singapore.

Feather pecking was measured directly via behavioural observations of hens in the group cages, and indirectly via plumage condition scoring at the beginning and completion of the trial. Each hen was scored using a feather scoring system adapted from Tauson et al. (2005). Plumage condition was assessed on six body regions (neck, breast, back, wings, tail, and vent) with each region assigned an ordinal score from 1-4 with "four" being perfect plumage condition and "one" completely denuded. Plumage condition of each region was perfect at the beginning of the trial in all regions except the neck. The neck region was excluded from analysis due to plumage damage caused by rubbing of the neck feathers on the cage during feeding rather than feather pecking. Feather score data were analysed using proportional odds linear regression MASS and RESHAPE2 packages in R (R Foundation for Statistical Computing, Austria). Feather pecking behaviour observations were conducted twice daily on each cage from days 14-28. Observations were conducted for two minutes per cage, and all instances of feather pecking behaviour and severity of pecking bout were recorded. Feather pecking bouts not directed towards the aforementioned body regions such as the comb, wattle, head, or feet were categorised as "other" and excluded from the analysis due to difficulty in condition assessment.

III. RESULTS

A total of 254 feather pecking incidents was recorded over the course of the trial. The proportion of 'gentle' and 'severe' feather pecking bouts, out of all feather-pecking bouts directed at each body region, is displayed below (Table 1). As shown in Table 1, about two-thirds of bouts were classed as 'gentle', and overall about one-half of observed bouts were directed at the neck region of hens.

Table 1 - Percentage of gentle and severe feather pecking bouts out of all feather pecking bouts directed toward each body region and the minimum feather score (FS) observed in each region.

	Neck	Back	Breast	Wing	Tail	Vent	Other	Total
Gentle	0.33	0.13	0.02	0.01	0.02	0.01	0.11	0.63
Severe	0.20	0.03	0.01	0.00	0.00	0.00	0.13	0.37
Total	0.53	0.16	0.03	0.02	0.02	0.01	0.24	1
Min. FS	2	2	2	3	3	4	-	

A proportional odds linear regression model was fitted to the sum of feather scores per bird at the end of the trial. Regression analyses indicated linear effects of dMet ($P < 0.001$) and dTrp ($P = 0.03$) inclusion but no quadratic, or interactive effects of dMet, dGly, or dTrp inclusion on the sum of feather scores. Using the RESHAPE2 package in R, the probability of a bird obtaining each overall feather score (ranging from 16-20) for every level of dMet, dGly, and dTrp could be predicted (Figure 1).

A generalised linear regression on the predicted probability of observing perfect plumage condition based on dMet, dGly, and dTrp was conducted. All predictors were found to be significant ($P < 0.001$, $P < 0.001$, and $P < 0.001$ respectively), with the probability of observing plumage damage decreasing as dMet, dGly, and dTrp inclusion rates increased. The minimum dMet, dGly, and dTrp inclusion rates required to ensure the majority of birds

maintained perfect plumage condition were predicted at 0.46, 0.71, and 0.22%, respectively. The maximal likelihood of observing perfect plumage condition (61.31%) was predicted when dMet, dGly, and dTrp inclusion rates were 0.56, 0.88, and 0.26%, respectively.

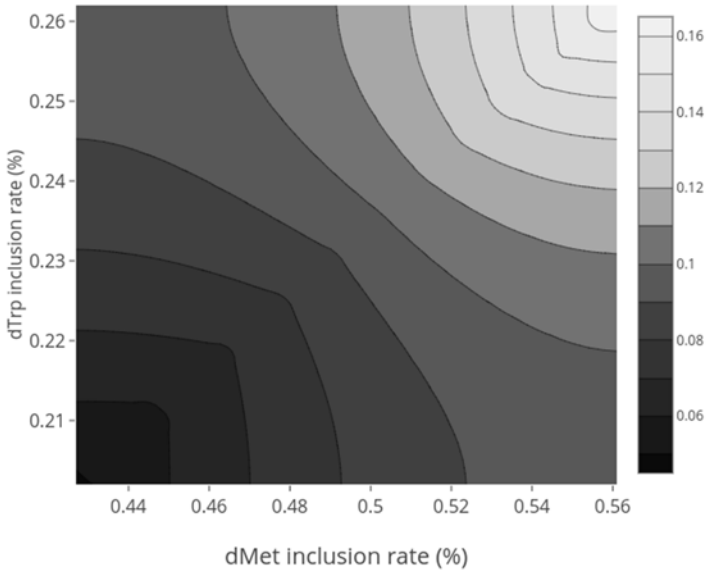

Figure 1 - Predicted probability of observing perfect plumage condition (feather score 4) as influenced by dMet (%) and dTrp (%) inclusion rates.

Proportional odds linear regression analysis on the plumage condition of individual body regions indicated a weak effect (P = 0.06) of dGly inclusion on breast region feather score, and a strong effect of dTrp inclusion on back region feather score (P < 0.001). The predicted probability of observing each plumage condition score for the back region are displayed in Figure 2. The maximal likelihood of obtaining perfect breast (99.95%) and back (99.68%) plumage feather scores was predicted when dMet, dGly, and dTrp inclusion rates were 0.56, 0.88, and 0.26%, respectively.

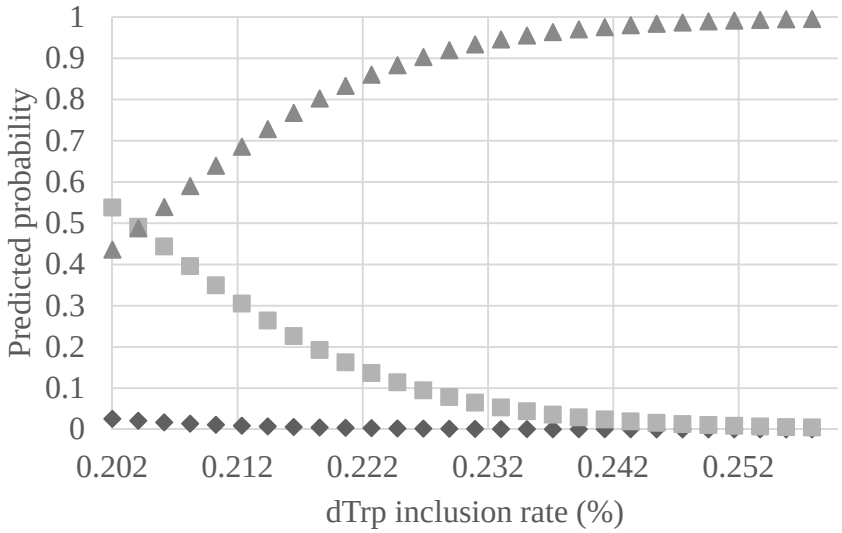

Figure 2 - Predicted probability of observing back plumage condition scores of 4 (triangle), 3 (square) and 2 (diamond) as influenced by dTrp inclusion rate (%).

IV. DISCUSSION

The significant effect of methionine supplementation on overall plumage condition in this trial contradicts the findings of Kjaer & Sorensen (2002), who observed no improvement in integument condition when methionine + cysteine content was supplemented up to 0.82% inclusion.

To date, no other studies have reported on the effect of glycine inclusion levels on feather pecking behaviour. However, results of this trial suggest only a slight effect of dGly inclusion on plumage condition on the breast region.

Tryptophan supplementation of up to 2% has been previously found to reduce gentle (van Hierden et al., 2004) and severe (Savory, 1998) feather pecking behaviour. Since dTrp supplementation in this trial resulted in a significant reduction in back region plumage damage and increase in overall plumage condition, this study supports the notion that feather pecking behaviour is positively influenced by tryptophan inclusion.

The results of this study indicate that reduction of dMet, dGly, and dTrp inclusion levels below recommended requirement (0.51, 0.59 and 0.21%, respectively) tends to increase the likelihood of plumage damage occurring. Similarly, increasing inclusion rates above recommended requirements tended to the increase the likelihood of perfect plumage condition scores.

Supplementation above recommended dietary requirement of dMet, and dTrp significantly affected the overall plumage condition of birds across five body regions (breast, back, wings, tail, vent). The use of a Box Behnken design allowed for prediction of optimal levels of dMet, dGly, and dTrp for perfect plumage condition at 0.56, 0.88, and 0.26%. Moreover, the presence of plumage damage in the back region becomes more prevalent than no damage when dTrp levels are reduced below 0.20%.

Further study investigating the effect of amino acid supplementation in larger groups, with additional replicates, and over a longer time frame is recommended to overcome the limitations of this study which may have resulted in the low level of feather pecking observed.

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INSIGHTS INTO ASSESSMENT OF THE WELFARE OF LAYING HENS IN AUSTRALIA

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Summary

This paper discusses the need for research to develop reliable practical means to assess the welfare of hens. The need for an assessment tool, or index, of hen welfare is vital in order to be able to improve the welfare of hens and to demonstrate this improvement. Both objectives are paramount in order for the Australian egg industry to thrive. These objectives are in line with other livestock industries nationally and internationally that are striving to continuously improve the welfare of their animals. This is a direct result of increased public awareness and concern over animal welfare. We propose that a similar research focus to that being undertaken in mammalian livestock should be applied to laying hens. This is a multidisciplinary approach to develop the means to understand both the biological functioning (physiology and behaviour) and affective (emotional) states of animals throughout the supply chain. Finally, this research effort should include engagement with the entire value chain, which includes the public, consumers, processors, retailors, producers and accreditors. The ultimate objective is to provide a suite of measures that will provide a robust and repeatable means to assess the welfare of hens in practice.

I. INTRODUCTION

In Australia, the National Animal Welfare Research Development and Extension (RD&E) Strategy is a cross-sectoral strategy under the National Agricultural RD&E Framework. Each of the livestock industries in Australia, including the egg industry, is represented by this National Strategy, which is active in identifying the key areas of importance for the welfare of livestock. Over the last four to five years, in particular, this National Strategy has consistently identified assessment of animal welfare as a critical area of research pursuit. This includes laying hens. Therefore, the focus of this review will be the development of the means to assess the welfare of laying hens.

II. THE IMPORTANCE OF ASSESSING ANIMAL WELFARE

A robust and practical means of assessing animal welfare is paramount in order to develop the means to improve welfare and to demonstrate this improvement. These are now accepted objectives of the livestock industries globally which are striving to continuously improve the welfare of their animals. This is a direct result of increased public awareness and concern over animal welfare. The livelihoods of producers and processors can be dramatically and catastrophically altered by rapid social media-induced changes in consumer behaviour. The significance of this risk is very real. Indeed, a recent economic appraisal of the Australian red meat industries showed that there is no greater risk currently facing the red meat industries in Australia than that of not engaging in animal welfare research and development (MISP, 2015). A similar economic appraisal has not been conducted for the poultry industries; nonetheless, it is clear that the need to demonstrate acceptable levels of welfare, at least, is necessary for community acceptance of egg and chicken meat production.

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In addition to the demand of society to improve welfare, there is often a general acceptance that improving animal welfare will improve productivity from animals but there is remarkably little quantitative evidence to demonstrate a causal relationship between welfare and level and quality of productivity. There are many examples in the literature where there are relationships between various measures of welfare and productivity but the causal element is frequently inconclusive or absent and the benefits of improving welfare in economic terms have rarely been addressed. Nevertheless, in a recent comprehensive review it was clearly articulated that there are financial benefits of good animal welfare (Dawkins, 2016). Furthermore, we demonstrated that optimizing floor space and the design features of pens for sows improves welfare with likely improvement in productivity (Hemsworth et al., 2013; Hemsworth et al., 2015).

Certainly, if welfare is poor, productivity can be impacted and this may well be reason alone to ensure that there is appropriate investment to improve animal welfare. A pertinent example of this is feather pecking in laying hens, which is a major negative welfare situation (Glatz, 2000; Ru and Glatz, 2000; Glatz, 2001; Glatz, 2005; Cronin et al., 2014). Feather pecking can result in pain and threats to pecked birds and can result in decreased feed conversion leading to poor feathering (Glatz, 2001). This can result in an increase of 7-12% in the cost of egg production (Glatz, 2001). Furthermore, if the feather pecking develops into cannibalism it can lead to mortalities as high a 25-30% of the flock (Glatz 2001; Cronin et al. 2014). The application of beak trimming can reduce this with savings of up to $AUD240,000 for a flock of 100,000 birds (Glatz and Hinch, 2008) but beak trimming itself presents as a controversial welfare issue (Glatz, 2000; Ru and Glatz, 2000; Glatz, 2005; Cronin et al., 2014). This is clearly an area requiring further investigation.

Irrespective of the relationships or otherwise between improved welfare and productivity, it is clear that the Australian egg industry, like other livestock industries in Australia, must strive to improve the welfare of their hens to ensure protection of the market and a social licence to operate. This requires the robust and practical means to assess the welfare of hens.

III. THE NEED TO DEFINE ANIMAL WELFARE

A universally accepted definition of animal welfare is essential in order to develop methods to assess the welfare of animals. The acceptance of such a definition has proved a major challenge in animal welfare science. Various approaches have been presented to define the welfare of animals including, although not limited to, the “five domains” model (Mellor and Reid, 1994; Fraser et al., 2013; Beausoleil and Mellor, 2015; Beausoleil and Mellor, 2015; Mellor, 2016), the “two question” approach to defining good welfare (Dawkins, 2008, 2016), the Welfare Quality Project® (European Union; http://www.animalwelfareplatform.eu/Welfare-Quality-project.php), the biological functioning, affective states and natural living frameworks of animal welfare and the concept that animals have “lives worth living” (Green and Mellor, 2011; Mellor, 2016). Of the definitions developed, we have contended that the most useful in terms of understanding, and thereby assessing, animal welfare are the biological functioning and affective states frameworks (Hemsworth et al., 2015; Tilbrook and Ralph, 2017a,b). The biological functioning framework refers to biological activity that is required to allow an animal to cope with a challenge and adapt to its environment. The biological activity can be extensive, involving many physiological systems and behavioural responses. The affective states framework considers the capacity of an animal to have emotional experiences and how these influence its welfare.

The biological functioning and affective states frameworks each have limitations (for details see Hemsworth et al., 2015) especially if considered in isolation of each other. Consequently, we have suggested that the biological functioning and affective states frameworks should be integrated (Hemsworth et al., 2015) and we have discussed the value of integrating these frameworks in understanding the welfare of group-housed sows (Hemsworth et al., 2015). There is now opportunity to extend this approach to assessment of the welfare of hens. This will require determining how these frameworks are related and where they intersect with respect to the welfare of hens.

IV. THE CONTINUUM OF ANIMAL WELFARE

Since the assessment of animal welfare cannot afford to deal with the biological functioning and affective states in isolation, the development of practical measures of physiological, behavioural and emotional functioning is essential. Importantly, there is a continuum of levels of welfare of animals that ranges from negative to positive (Mellor, 2012; Fraser et al., 2013; Hemsworth et al., 2015; Mellor, 2015, 2016; Tilbrook and Ralph, 2017a) so methods of assessment of animal welfare must be able to provide critical information about the welfare of animals throughout this continuum of welfare. It is now recognised that the continuous improvement of animal welfare requires the ability to move animals from the negative to the positive regions of the welfare continuum. In other words, the ultimate goal is to provide animals with positive states of welfare. This includes laying hens.

V. ASSESSING HEN WELFARE

The continuous improvement of the welfare of hens is dependent on the ability to rigorously assess their welfare in the field. This includes all housing systems in which hens are kept, including cages, barns and free-range systems. As indicated, an assessment tool, or index of welfare, will necessarily be comprised of a range of physiological, behavioural and affective parameters. Presently, no welfare assessment tool exists for laying hens and there is a need to identify the relevant parameters, or biomarkers, of welfare. This is required before it will be possible to implement strategies to improve the welfare of laying hens in the field. This is an area requiring a substantial research effort.

We have recently extensively reviewed the assessment of animal welfare with a focus on mammalian livestock species (Hemsworth et al., 2015; Ralph and Tilbrook, 2016; Tilbrook and Ralph, 2017a,b). Development of the methods of assessment of animal welfare requires the use of multiple indicators from multiple disciplines (Hemsworth et al., 2015; Ralph and Tilbrook, 2016; Tilbrook and Ralph, 2017a,b). Consequently, our approach in mammals is to take a multidisciplinary approach to develop the means to understand both the biological functioning (physiology and behaviour) and affective (emotional) states of animals throughout the supply chain (Tilbrook and Ralph, 2017a). In addition to a scientific approach, we believe that a critical feature to achieving improved animal welfare is to engage with the entire value chain, which includes the public, consumers, processors, retailors, producers and accreditors (Tilbrook and Ralph, 2017a). Our research with cattle, sheep and pigs utilizes novel scientific and technological approaches to provide the practical means to assess physiological, behavioural and emotional functioning under extensive and intensive conditions. The ultimate objective is to develop a suite of measures that will provide a robust and repeatable means to assess animal welfare in practice (Tilbrook and Ralph, 2017a). We believe that a similar approach is required for laying hens. This approach must include practical measures of both biological functioning and affective states as well as an integration of these measures. Below, we briefly outline some of the measures being considered in mammals and challenge that a similar research pursuit be undertaken in laying hens.

a) Biological functioning

Assessment of biological functioning involves quantifying biological activity. Many measures have been undertaken to understand biological functioning, such as measures of the physiological systems that are activated to allow an animal to adapt to, and cope with, its environment. These include body repair systems, immunological defences and physiological stress responses, as well as a variety of behavioural responses (Tilbrook and Ralph, 2017a,b). A large range of behavioural measures have been used in many species, including laying hens, that include stereotypies, redirected behaviours, fearfulness, aggression and displacement activities, amongst others (Hemsworth et al., 2015). One of the most common approaches to assessing biological functioning is quantification of front-line physiological stress systems, such as the hypothalamo-pituitary adrenal (HPA) axis. Recently, we have critically assessed a range of measures used to assess biological functioning, including the HPA axis, in terms of their value in assessing animal welfare (Ralph and Tilbrook, 2016; Tilbrook and Ralph, 2017a,b). It is clear that the physiological responses to challenges are complex and varied, which can make interpretations of the impact of these responses on animal welfare difficult (Ralph and Tilbrook, 2016; Tilbrook and Ralph, 2017a,b).

With respect to stress responses, it is appreciated that there is not always a clear relationship between acute stress and welfare (Ralph and Tilbrook, 2016; Tilbrook and Ralph, 2017). Nevertheless, the reality is that chronic stress, or repeated ongoing stress, almost always has detrimental effects on the normal functioning of animals (Turner et al., 2011; Ralph and Tilbrook, 2016; Tilbrook and Ralph, 2017b) thereby impacting their welfare. Consequently, the development of indexes of welfare must include quantification of stress responses in addition to a range of other physiological, behavioural and affective (see below) measures. Importantly, for these measures to be useful in the assessment of welfare they need to reflect sustained changes over time because chronic activation of stress systems, such as the HPA axis, is when welfare is most likely to be impacted. In mammals, measurement of stress hormones, such as the glucocorticoid cortisol, in hair and wool provide a chronic date stamp of adrenal activity (Burnard et al., 2017) which can be useful in developing an index of welfare when combined with other measures of biological functioning and affective state. In hens, an obvious medium to consider to obtain chronic measures of HPA axis activity is the feather. Indeed, measurement of the principal glucocorticoid in avian species, corticosterone, has been shown to provide a long-term integrated measure of stress physiology in red-legged partridges (Bortolotti et al., 2008), Clark's nutrackers (Fairhurst et al., 2011) and broilers (Carbajal et al., 2014). Moreover, administration of corticosterone to European starlings resulted in increased concentrations in feathers (Lattin et al., 2011). Studies such as these are lacking in the laying hen, although recently we have been investigating the value of measuring corticosterone in secretions of the uropygial gland (C. de Koning, K. Drake, R. Barekatain, C. Ralph and R Hughes, unpublished) and excreta (C. de Koning, E. Narayan, R. Barekatain, R Hughes and C Ralph, unpublished) from chickens.

It is important to re-emphasise that chronic measures of stress need to be put into the appropriate context when attempting to assess the welfare of hens. On their own, they may be of little value and it is crucial that they are integrated with other physiological (as mentioned above) and behavioural measures, as well as with measures of affective states.

b) Affective states

In comparison to biological functioning, it was long considered that assessing emotions was virtually impossible but this belief is now uncommon and a variety of approaches has been undertaken to quantify affective states. Most of these have been behavioural approaches, with one of the most common being research that investigates the choices that animals make for a

chosen environmental option or motivation to perform a type of behaviour (for review see Hemsworth et al., 2015). The premise underlying this approach is that animals will make choices that are in their best interest (Fraser and Nicol, 2011). Other approaches to assessing affective states include measures of behaviour and cognitive bias (Boissy et al., 2007; Mendl et al., 2009) and Qualitative Behavioural Assessment, which uses the intuitive perception of human observers (Wemelsfelder and Mullan, 2014). As intimated above, many of these measures are limited by not taking account of biological functioning. Furthermore, most approaches to measure emotions have focussed on assessing negative affective states and there is a need to be able to measure positive affective states, consistent with the desire to not simply mitigate against poor animal welfare, but to strive for positive animal welfare. In other words, to move animals along the welfare continuum.

Recently, we emphasised the importance of the brain in controlling affective states and proposed that an understanding can be gained from biomedical science to help understand affective function in animals (Tilbrook and Ralph, 2017a). It has been shown, primarily with rodents, sheep and primates, that there are measurable changes in defined regions of the brain that indicate the affective state of an animal (Bergholm et al., 1984). While there have been various studies on regions of the brain and neurophysiological systems in various avian species (e.g. O'Connell and Hofmann, 2011, 2012; Riters et al., 2014; Cordes et al., 2015) there have not been specific attempts to identify these in laying hens with respect to affective states that may impact welfare. This is an area requiring research.

Our recent review detailed the regions of the brain, and the key neural pathways and neurotransmitters that regulate positive and negative affective states (Tilbrook and Ralph, 2017a). The knowledge on this in mammals is extensive. The research effort that has been undertaken in mammalian livestock is now required in laying hens in order to understand affective states to the point where they can be manipulated to induce positive affective states. A primary objective must be to develop procedures to assess the welfare of hens by identifying the neural pathways that generate key emotions, such as reward, fear and pain, and then by developing the means to determine the activity of these pathways.

We have put forward the thesis that candidates for biomarkers of central neuronal activity, among others, may be specific micro RNA’s (miRNAs) that are expressed in response to the activation of various neuronal systems (Tilbrook and Ralph, 2017a). In various mammalian species, these miRNA’s have been measured as signals (biomarkers) of the activation of regions of the brain and neurophysiological systems associated with various affective states (Tilbrook and Ralph 2017a). We are currently undertaking research in this in pigs (L. Marsh, A. Tilbrook, S. Hiendleder and C Ralph, unpublished). It is unknown if miRNAs may also be a biomarker of affective states in hens, but the possibility that they may be surely makes this an enticing area to pursue.

VI. PRACTICAL ASSESSMENT OF HEN WELFARE

Fundamental research is required to develop a suite of measures that can be used to develop a comprehensive assessment of the welfare of hens in practice, both on farm and when spent. Currently, there is insufficient knowledge to develop such an index of the welfare of hens. Biomarkers of biological functioning and affective states are required that can be easily applied in the field. There are clearly some promising candidates for assessing biological functioning, such as targeted measures from feathers (see above). Candidates to assess affective states in laying hens are less obvious and this underscores the need for research in this area. While miRNA’s appear promising candidates in this domain in mammals, the opportunities in laying hens are unexplored. Ultimately, there is requirement for the

identification and development of biomarkers of affective state in laying hens that can be applied in the field. This is will be a challenging but important area of research.

VII. CONCLUSION

There is a need to develop reliable practical means to assess the welfare of animals, including hens. This is crucial in order to improve welfare and to demonstrate this improvement, a need that has been largely driven by increased public awareness and concern over animal welfare. While addressing this concern should be a priority of the Australian egg industry, improving animal welfare will likely result in increased consumer recognition of the industry, increased ethical production of high quality safe eggs, market protection and, in some cases, improved production.

Research is necessary to develop a suite of measures that will provide robust and repeatable measures of the welfare of hens. The approach must be multidisciplinary and multifaceted and must encompass measures of physiology, neurophysiology, behaviour and emotions (affect). The assessment of the welfare of hens must extend throughout the supply chain and the research effort should include engagement with the entire value chain, which includes the public, consumers, processors, retailors, producers and accreditors.

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NATURALLY-INSPIRED INTERMITTENT LIGHTING SCHEDULES TO IMPROVE BEHAVIOURAL SYNCHRONISATION IN LAYER CHICKS

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Naturally brooded domestic chicks spend a large proportion of time resting under and gaining warmth from their mother, spending multiple short bouts in relative darkness (Shimmura et al., 2010). In contrast, during rearing, commercial chicks experience one continuous light period each day. In this situation, behaviours become unsynchronised, with the potential for active chicks to disturb and direct feather pecks towards resting conspecifics (Riber et al., 2007; Gilani et al., 2012). Intermittent lighting (IT) schedules have the potential to mimic natural brooding and synchronise chick behaviour. Previous IT studies have used sucessive alternating periods of around 40 min L:40 min D (Malleau et al., 2007), based on field observations of wild populations (Wood-Gush et al., 1978). Chicks on this simulated brooding cycle (SBC) rested more than control chicks, with no compromise in weight or feed conversion. However SBC chicks still performed some resting during the light period, suggesting that they could benefit from a shorter light period. In the current study, we used video recordings of 27 broody hens and their chicks up to two weeks of age to determine suitable mean natural brooding patterns. We then developed an IT schedule based on these natural brooding patterns.

Eighty layer chicks (Lohmann classic) were obtained at one day old and were reared in 16 groups of five chicks for 2 weeks under either continuous ("C" 14h L:10h D, n=40) or intermittent ("IT" 8 min L:8 min D for 22h with an additional 2h D from 00:00h to 02:00h, n=40) lighting schedules. Behavioural synchronisation (% of observations in which behaviour was synchronised) was assessed on days 2, 7 and 14 and weight and feed consumption were measured throughout. At the end of the two week period, welfare outcome measures were taken; these included tonic immobility, human approach and novel obejct tests, and eye temperature response to handling.

IT chicks showed significantly higher behavioural synchronisation on days 2 ($p = 0.003$) and 7 ($p = 0.02$) than C chicks, but there was no significant difference on day 14. IT chicks also showed significantly higher weight gain (days 1-14 C: 6.26g/day, IT: 7.23g/day, $p = 0.001$) and a lower feed conversion ratio (days 1-14 C: 6.85g/g, IT:4.70g/g, $p < 0.001$) than C chicks. There were no effects of lighting schedule on welfare outcome measures. Naturally-inpsired intermittent lighting schedules are a promising tool to improve behavioural synchronisation in early rearing, with no detrimental welfare effects. However, further research is needed to determine the longer-term effects of naturally-inspired intermittent lighting schedules, including those on commercial laying hen farms.

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THE EFFECTS OF LIGHT LEVEL FROM 1 TO 7 WEEKS OF AGE, AND STRESS AT 16 WEEKS, ON PLUMAGE DAMAGE AND INJURIOUS PECKING IN ISA BROWN PULLETS REARED FOR FREE-RANGE EGG PRODUCTION

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Summary

Non-beak-trimmed ISA Brown chicks were reared from day-old to 41 weeks, to study the development of plumage damage (PD) due to severe feather pecking (SFP), in an experimental free-range layer flock. Factors investigated were Light level from 1-7 weeks of age (Low: <5 vs. Normal: 40 lux), and stress from Transport, Relocation and Mixing at 16 weeks (TRM vs. not TRM: NT). At 16 weeks there were 16 pens of 50 hens. An SFP outbreak occurred after 18 weeks (in mid-winter), but the imposed treatments had only a minor influence on PD ($0.05 < P < 0.1$). However, there was an effect ($P = 0.017$) of the experimental blocking factor, side of the shed, on PD. Injurious pecking (IP) contributed to an overall reduction in flock size of ~20% due to either cannibalism or removal from the study of wounded hens. IP losses (mortalities plus removals) were about six times greater if the pen was located on the south than north side of the shed. These findings, whilst unexpected, may contribute to our developing knowledge of this complex problem, and may assist direct future research to identify underlying causes of the behaviour and thus improve prevention and intervention strategies for egg producers.

I. INTRODUCTION

Severe feather pecking (SFP) is an abnormal behaviour which reduces both hen welfare and the efficiency of egg production. While the specific causes are unknown, it is acknowledged to be multifactorial, complex and unpredictable (Rodenburg et al., 2013; Hartcher et al., 2016). The problem is compounded because the behaviour seems to spread rapidly via social learning (Zeltner et al., 2000) and is therefore difficult to control in large groups of hens.

A reliable method to reduce pecking problems in indoor housing systems is to maintain low light intensity. However, there are practical difficulties in free-range systems due to light entering the shed via the pop holes. The effect of low light level during rearing on the development of SFP was investigated by Kjaer and Vestergaard (1999), who reared chicks to 15 weeks in 30 vs. 3 lux. At 10 weeks, pullets reared in the brighter light performed three times more SFP than the 3 lux treatment. Cannibalism also tended to occur more in the laying period in the 30 lux treatment. This and other research (Jensen et al., 2006; Gilani et al., 2012) suggested the existence of a sensitive period in early life for the development of IP in chicks. The present experiment therefore, investigated whether maintaining chicks under low lux in early life would provide long-term benefits by reducing SFP in free-range hens.

A second proposed factor in the development of SFP is elevated stress. In industry, the transfer of pullets from the rear to the layer farm occurs at about 14-16 weeks of age, and this process combines a number of 'stressors' - transport, re-housing and re-grouping. El-Lethey et al. (2000, 2001) showed evidence for the involvement of stress and elevated corticosterone concentrations in the expression of SFP, while Bestman and Wagenaar (2003) reported that bringing hens onto the farm at about 16 weeks was associated with higher levels of SFP, compared to rearing them on the egg farm or relocation to the new farm by about 10 weeks. Thus, stress around relocation at 16 weeks may also be an underlying cause of SFP.

This experiment tested two hypotheses: (1) chicks housed under low light in early rearing would have better plumage condition and lower mortality from pecking injuries as adults, and (2)

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the combined stressors of transport, relocation and mixing at 16 weeks would result in poorer plumage condition and higher mortality from pecking injuries as adults.

II. MATERIALS AND METHODS

A total of 880 day-old, non-beak-trimmed chicks (ISA Brown) were purchased from a hatchery in Tamworth, NSW, Australia, and transported to the research facility. Chicks were randomly distributed among 16 floor pens (2.0 m × 3.2 m) bedded with wood shavings. Shed temperature, photoperiod and illumination were managed according to the ISA Brown Management Guide (2010), and feed and water were available *ad libitum*. At regular intervals from day 6, all birds were individually weighed and inspected for plumage damage (PD) on seven body areas (head, neck, back, rump, tail, side and vent). PD was recorded as either absent or present for each area. In addition, at the completion of the experiment (41 weeks) the level of PD at each area was scored as 0 = nil damage; 1 = some feathers missing; 2 = about half of the feathers missing; and 3 = most feathers missing (Tauson et al., 2005). Hens that died were necropsied to identify cause of death, and injured hens were assessed and treated as necessary.

The experiment had a 2×2 factorial arrangement with replication. Factor 1 compared light intensity between 1 and 7 weeks of age (<5 lux: Low vs. min. 40 lux: Normal). At 9 weeks, the 16 pens of pullets were carefully relocated to another shed, whilst remaining in their original pen-groups. In the new shed, groups were allocated at random to pens within blocks, based on shed aspect (north vs. south). All pens measured 1.83 m × 3.25 m, had wood shavings on the floor and contained a feeder, drinker, perch unit and nest-box unit (closed). At 14 weeks, the number of pullets was reduced to 50 per pen and photoperiod was increased weekly to stimulate egg production by providing an additional 30-min light per day, until 15hL:9hD was reached in week 19. Nest boxes were opened at 15 weeks, and at 16 weeks. Factor 2: Transport, Relocation and Mixing (TRM) was imposed on eight pens of pullets. Pullets were placed in poultry crates (max. 8 per crate) and transported by motor vehicle for 35-40 minutes. Upon return to the shed, crates were unloaded and pullets placed in a new pen, in which they were mixed 50/50 with both familiar and unfamiliar pullets. The other 8 pens were not treated (NT), that is, did not experience TRM. In week 20, the pop-holes to the outdoor ranges were opened.

Differences in mean bird weight per pen were analysed using ANOVA (Genstat) at each assessment date, after blocking on side of the shed. Number of PD sites per hen at each assessment date, and the highest (worst) feather score recorded across the 7 areas per hen at 41 weeks, were analysed using GLMM with binomial distribution and a logit link function in Genstat. Light and TRM were fixed effects and block/pen were random effects. Due to apparent differences in PD of hens in pens on the north compared to south side of the shed, analyses were repeated with shed block as a fixed effect, and pen as a random effect. Differences in the number of mortalities (including hens removed with pecking injuries) per pen were analysed using survival analysis with censored data based on week (Genstat).

III. RESULTS

While mean bird weight per pen did not differ due to the main effects at 6 days and 8 weeks of age (pooled means 68 and 683 g, respectively), at 14 weeks Low light pullets tended to weigh less than Normal pullets (1.296 vs 1.338 kg, respectively; sed 0.021, $P = 0.074$). However at 18 weeks, two weeks after imposing the TRM treatment, TRM pullets were heavier ($P = 0.026$) than NT pullets (1.623 vs 1.570 kg, respectively; sed 0.021). By 24 weeks the difference was a weak tendency (1.865 vs 1.816 kg, respectively; sed 0.026, $P = 0.08$) and thereafter there were no differences due to the main effects or interactions on average hen weight per pen.

PD was not apparent at, or prior to, 18 weeks. However, an outbreak of SFP/PD occurred soon after 18 weeks and, at the next assessment (24 weeks), ~40% of hens had at least some PD. Figure 1 shows the proportion of the flock that displayed PD from 18 weeks. Behaviour observations (not reported here) showed SFP progressed to IP, and in some pens hen death from

cannibalism or permanent removal of pecked hens were recorded. IP occurred especially around the rump/uropygial gland area. In two pens, regular wounding/cannibalism occurred and both pens were removed from the study on ethical grounds in week 30 and the remaining hens re-homed. Overall, by 41 weeks flock size had been reduced by over 20%.

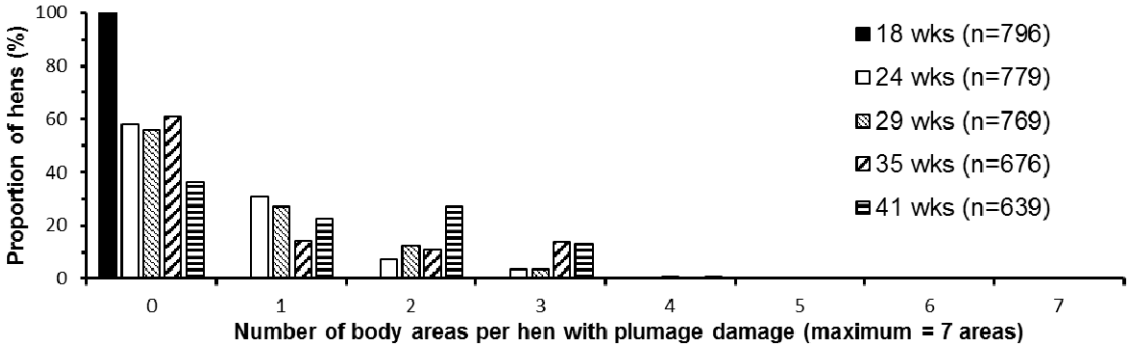

Figure 1 - The proportion of hens in the flock that had nil, or some areas of the body that had plumage damage, when assessed between 18 and 41 weeks of age. The data in the figure are pooled across treatments and pens. The number of hens alive at each assessment point is indicated in the legend.

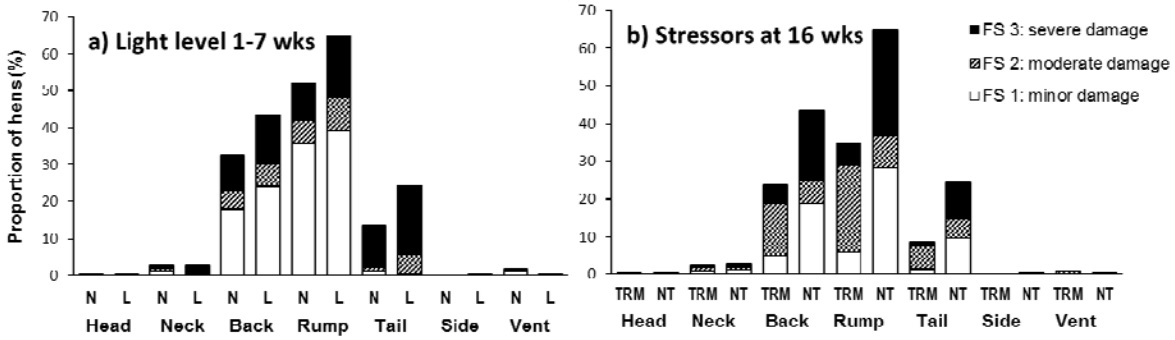

Figure 2 - The effect of different light levels applied between 1 and 7 weeks of age (N: normal lux vs L: low lux; figure a), and combined stressors in the form of transport, relocation and mixing applied at 16 weeks (TRM) compared to no imposed stressors (NT; figure b), on the proportion of hens with different levels of plumage damage (FS: feather-score) on the 7 body areas, when scored at 41 weeks of age.

There were no differences due to main effects or interactions on the number of hens with PD, or the number of PD sites per hen. In week 41 there was a weak difference ($P = 0.087$) due to the Light main effect on feather-score, with the Low light treatment having 'worse' PD than Normal treatment (back-transformed means {btm} of max. feather-score: 1.8 vs 0.9, respectively). There was no effect of TRM, and no Light × TRM interactions. Figure 2 shows the proportion of hens at 41 weeks with different levels of severity of PD at the 7 body areas, for the two main effects. However, at 41 weeks there was an effect ($P = 0.017$) of side of the shed on PD, with hens in pens with south- compared to north-aspect having worse max. feather-score (btm = 2.1 vs 0.7, respectively). Similarly, side of the shed affected the number of PD areas per hen (in weeks 24, 29, 34 and 41; $P < 0.01$), with hens in south-aspect pens having more PD areas than north-aspect pens (e.g., btm for week 41 = 2.0 vs 0.4 areas, respectively).

Analysis of hen mortality plus removals identified a weak effect of the Light main effect ($P = 0.062$), with a tendency for a higher loss of hens reared in the Low than Normal lux (22.7 vs. 16.7% of hens, respectively), and no difference due to the TRM main effect. However, there was a strong ($P < 0.001$) effect of side of the shed on mortality plus removals, with losses of 33.7% of hens from south- compared to 5.8% from north-aspect pens to week 41.

IV. DISCUSSION

An important finding from this study was that, when an SFP outbreak occurred in the experimental flock, differences due to the Light and TRM main effects were relatively minor compared to the differences between pens on the different sides of the shed. Hence our hypotheses were not proven. Clearly, other (unknown) factors had greater influence on the initiation of SFP than the imposed rearing treatments. Resultant PD, which was mainly evident on the rump, back and tail areas of hens, appeared to predispose hens to IP and cannibalism. Flock size consequently declined by ~20% of hens between weeks 16 and 41, with most dead or wounded hens receiving pecking injuries to the uropygial glands. In attempting to identify factors associated with the SFP/IP outbreak, there was a major rain event (133.6 mL) over 12 days during winter (late June to early July) that preceded the onset. Further investigation to identify differences in stimuli (and associated stressors) present between pens on the (colder/damper) south compared to (warmer/drier) north sides of the shed, could inform future research to identify the underlying cause(s) of SFP.

In general, the imposed experimental rearing treatments did not affect hen growth. While some differences in body weight were found (e.g., at 14 weeks due to Light; at 18 weeks due to TRM), these were relatively transient and by the next weigh-day; differences were non-significant suggesting hens had adapted to the environmental challenges which influenced body weight. In contrast, the findings regarding differences in SFP/PD and IP/cannibalism due to side of the shed were unexpected. However, the findings could help contribute to our developing knowledge of this very complex behavioural and welfare problem.

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THE IMPACT OF RANGE USE ON FLOCK UNIFORMITY IN COMMERCIAL FREE-RANGE LAYING HENS

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Flock uniformity is crucial to increase the performance and profitability of commercial egg production (Corzo et.al, 2004). Obtaining flock uniformity in a free-range system may be challenging due to the variation in feeding and ranging behaviour of individual birds.

The current study investigated the effect of body weight on initial range utilization and flock uniformity in two free-range laying flocks. A total of 6,250 Lohmann Brown hens were weighed and assigned to 2 identical commercial free-range sheds (Flock A; Flock B) at 16 weeks of age. Each of the sheds was sub-divided into 5 pens holding 625 hens/pen, allowing for 5 replicates/flock. Hens had access to the range daily from 9am to 8pm at 17 to 21 weeks of age with individual time on the range monitored using radio frequency identification (RFID) leg bands. Individual body weight was obtained at 16 and 21 weeks of age, and assigned to the individual RFID number of the hens. Hens in Flock A had the initial standard average body weight of 1.36kg while Flock B was below the standard weight (1.27kg) at 16 weeks of age. Flock uniformity was calculated as the percentage of hens within plus or minus 10% of the average body weight. Average time on the range, number of events at range, body weight, flock uniformity, change in flock uniformity data were analysed by one-way ANOVA, using SPSS Statistics v.24.

The results (Table 1) indicate that hens of Flock B with lower initial body weight spent significantly less time on the range and gained more weight ($P < 0.001$) to 21 weeks of age compared to hens of Flock A. Flock uniformity of Flock B increased by 7% while the uniformity of hens in Flock A (hens that spent significantly more time ranging) decreased by 2.8% ($P = 0.003$).

Table 1 - The effect of range use on flock uniformity and body weight[†].

Parameters	Time point (Week)	Flock A	Flock B	P-Value
Body weight (kg)	16	1.36 ± 0.003	1.27 ± 0.003	< 0.001
	21	1.75 ± 0.005	1.74 ± 0.005	0.035
Body weight gain (kg)	16-21	0.38 ± 0.007	0.47 ± 0.003	< 0.001
Flock uniformity (%)	16	88.4 ± 0.535	81.3 ± 1.675	0.004
	21	85.6 ± 1.595	88.3 ± 0.683	0.161
Δ Flock uniformity (%)	16-21	2.81 ± 1.925	7.01 ± 1.369	0.003
Time on range/ day (h)	16-21	1.51 ± 0.009	0.91 ± 0.017	< 0.001
Maximum ranging time/ day (h)	16-21	10.9 ± 0.321	10.8 ± 0.321	0.811
Hens that accessed the range (%)	16-21	85.9 ± 0.789	74.2 ± 5.718	0.077
Number of visits/ hen	16-21	27.5 ± 0.382	26.0 ± 0.571	< 0.001
Number of days on the range/ hen	16-21	8.45 ± 0.087	6.35 ± 0.088	< 0.001

[†]All values in the table are represented as Mean ± SEM.

In conclusion, hens with lower body weight at placement spent significantly less time on the range, gained more weight and had increased change in flock uniformity compared to hens with high body weight at placement.

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CONTROL AND MONITORING OF *SALMONELLA* IN EGG-LAYING CHICKENS

R.K. GAST[1]

Summary

Contaminated eggs have been internationally significant sources for the transmission of *Salmonella* infection to humans for several decades. Both the public and private sectors have invested substantial resources in comprehensive risk reduction and monitoring programs for *Salmonella* in commercial egg-laying flocks. The most effective overall strategy for controlling *Salmonella* in layers has been the application of multiple interventions throughout the egg production cycle. Although a large proportion of egg-transmitted illness is attributed to *Salmonella* serovar Enteritidis, other serovars (notably *S.* Heidelberg and *S.* Typhimurium) are also implicated. Contamination of the edible interior contents of eggs with *S.* Enteritidis results mainly from colonization of the reproductive tissues of systemically infected laying hens, although salmonellae can also penetrate through eggshells after contamination of the exterior surface. Managing storage temperatures is vital for limiting the growth of *Salmonella* growth inside eggs. Managing environmental and housing conditions for laying flocks is critical for reducing opportunities for the introduction, transmission, and persistence of *Salmonella*. Different hen housing systems for commercial egg production influence these environmental parameters, and unique risk factors and management challenges are characteristic of each system.

I. INTRODUCTION

Egg-associated *Salmonella* infection is a significant international public health problem. Internal contamination of eggs with *S.* Enteritidis has been the principal concern in North America and Europe, whereas external contamination with *S.* Typhimurium has been the predominant issue in Australia. Some strategies for controlling egg-borne *Salmonella* are designed to act with precision against epidemiologically important serovars, but others are applicable against all serovars. The incorporation of particular risk reduction practices into control programs is guided by their efficacy and cost-effectiveness under local conditions. This paper discusses the example of *S.* Enteritidis control in the United States to illustrate the utilization of both serovar-specific and serovar-independent strategies.

Over a period of nearly three decades, a high prevalence of *S.* Enteritidis infections in many regions of the world has been attributed to the consumption of contaminated eggs (Pires et al., 2014). Both retrospective epidemiological analysis and active disease surveillance have associated the incidence of human illness with the prevalence of *S.* Enteritidis in commercial egg-laying flocks (Arnold et al., 2014a). In a multi-national European survey (De Knegt et al., 2015), laying hens were found to be the most prominent reservoir for human salmonellosis, accounting for 42% of all cases (96% of which involved *S.* Enteritidis). Implementation of federal egg safety regulations in the USA was estimated to potentially reduce annual human health costs (due to 79,000 egg-related illnesses) by USD $1.4 billion (US Food and Drug Administration, 2009).

II. *S.* ENTERITIDIS INFECTION AND EGG CONTAMINATION

Young chicks are highly susceptible to colonization of the intestinal tract by *S.* Enteritidis,

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and infection introduced shortly after hatching can sometimes persist until maturity (Van Immerseel et al., 2004). Older hens are less susceptible and gut colonization usually declines steadily after exposure to the pathogen (Gast et al., 2011), although extended persistence is sometimes observed. Sustained intestinal carriage in even a small proportion of the hens in a flock could prolong opportunities for horizontal transmission and subsequent egg contamination. Fecal shedding of *Salmonella* by colonized birds is a leading source for continuing contamination of the poultry housing environment (Trampel *et al.*, 2014). Mature hens infected with large oral doses of *S.* Enteritidis can sometimes shed this organism in their feces for several months (Gast et al., 2013b). In commercial flocks, the prevalence of *Salmonella* shedding in feces typically declines gradually after peaking just before egg laying begins (Gole et al., 2014), although the overall prevalence of fecal shedding in flocks may fluctuate considerably over time. Highly persistent fecal shedding of *S.* Enteritidis in a flock is not always indicative of the likelihood of systemic infection or egg contamination.

S. Enteritidis can invade past the intestinal tract to colonize livers and spleens of laying hens within just a few hours after they are orally infected (He et al., 2010). Colonization of internal organs declines steadily during the first few weeks after mature birds are exposed to *S.* Enteritidis, although infection may persist in some individuals (Gast et al., 2007b). Deposition of *S.* Enteritidis inside the edible interior contents of eggs results principally from colonization of reproductive organs (Gantois et al., 2009). However, a high frequency of reproductive organ invasion by *Salmonella* does not inevitably lead to egg contamination at a correspondingly high incidence (Gast et al., 2004). *S.* Enteritidis can invade both the ovary (the site of yolk maturation) and oviduct (the site of albumen secretion around descending yolks), and can thus be deposited in either of these major edible egg fractions (Gast et al., 2007b).

In experimentally infected hens, the likelihood of *S.* Enteritidis contamination inside eggs is directly related to the orally administered bacterial dose (Gast et al., 2013a). Exposure by horizontal contact has predictably led to the production of very few contaminated eggs. Nevertheless, the deposition of *S.* Enteritidis usually occurs at low frequencies and involves small initial populations of contaminants, even after inoculation with very large doses (Gast and Holt, 2000a). In commercial flocks, naturally occurring *S.* Enteritidis infections are often acquired via exposure to low pathogen doses from environmental sources or horizontal contact, and are accordingly associated with highly infrequent egg contamination (DeWinter et al., 2011). The location of *Salmonella* deposition within eggs is pivotal for determining whether the pathogen has an opportunity to multiply during storage. Egg yolk offers abundant nutrients that support rapid and prolific bacterial growth at warm temperatures (Gurtler and Conner, 2009), but albumen proteins can limit iron availability and disrupt bacterial membranes (Baron et al., 2016). *S.* Enteritidis is most often deposited in the albumen or on the exterior surface of the vitelline (yolk) membrane of contaminated eggs (Gast and Holt, 2001), and it can rapidly migrate across this membrane to access yolk nutrients at warm temperatures (Gast et al., 2010). However, prompt transfer of eggs to refrigeration temperatures can significantly diminish *Salmonella* penetration into and multiplication inside egg yolks (Gast et al. 2006).

III. EGG CONTAMINATION BY *SALMONELLA* STRAINS AND SEROVARS

Different *Salmonella* strains or serovars can vary significantly from each other in their characteristic consequences for infected laying hens. *S.* Enteritidis strains have sometimes differed very substantially in the frequencies at which they cause reproductive organ invasion and egg contamination after experimental infection of hens (Gast and Holt, 2000a). Strains which efficiently cause egg contamination have been differentiated from other environmental

salmonellae by the expression of traits such as adherence to reproductive tract mucosa and survival in forming eggs. Among the responsible genes for these abilities are those found in the major pathogenicity islands, involved in cell wall or lipopolysaccharide structure, or related to stress responses (Raspoet et al., 2014). Deposition inside developing eggs may be the ultimate consequence of sequential expression of multiple phenotypic properties which are each necessary at specific stages of the overall infection process (Guard et al., 2010). Even between strains of a single *S.* Enteritidis phage type, the accumulation of small genetic changes may lead to divergence in abilities for invasion of reproductive tissues and eggs (Guard et al., 2011). However, individual strains have not been reported to have affinities for particular reproductive tract sites or locations inside eggs (Gast et al., 2007b). Although phage typing of *S.* Enteritidis isolates has been valuable for establishing epidemiological relationships, phage types have not been linked to any consistent pattern of abilities to cause egg contamination (Gantois et al., 2009).

The poultry housing environment may be a reservoir from which strains able to cause systemic infection and egg contamination may periodically emerge. Environmental *S.* Enteritidis isolates typically include a greater diversity of phage types than are recovered from contaminated eggs. The expression of diverse putative *Salmonella* virulence factors (including flagella, fimbria, outer membrane proteins, and iron uptake systems) can be influenced by environmental conditions such as pH and temperature. Impaired resistance to environmental stressors has been reported to reduce the pathogenicity of *S.* Enteritidis isolates for chickens (Shah, 2014). Additional pressure to select for expression of virulence properties is also exerted in the tissues of infected hens. Highly expressed genes in *S.* Enteritidis isolates from oviducts of infected hens were similarly highly expressed in isolates from eggs (Gantois et al., 2008). Repeated passage of *S.* Enteritidis strains through the internal organs of infected hens increased their association with egg contamination (Gast et al., 2003).

Although differences between individual *Salmonella* strains have been found regarding multiplication in egg yolks (and penetration through yolk membranes (Gast et al., 2007a), these properties do not seem to be associated with phage type. The frequency of *S.* Enteritidis migration into yolks can vary among eggs from genetically different commercial lines of laying hens (Gast et al., 2010). *S.* Enteritidis strains have been reported to survive better in egg albumen than isolates of other serovars (De Vylder et al., 2012). Reduced survival and growth in egg albumen were observed among *S.* Enteritidis strains which were sensitive to both acidic and oxidative stress (Shah et al., 2012).

Although a uniquely strong epidemiological association has been established between *S.* Enteritidis and egg-borne disease, some strains of other *Salmonella* serovars can invade reproductive organs of laying hens and cause egg contamination (Gast et al., 2011). In North America, egg contamination by *S.* Heidelberg (a common serovar in laying housing environments) has been implicated in occasional reports of human illness (Chittick et al., 2006). Likewise, *S.* Typhimurium has caused sporadic egg-transmitted disease in Australia (Moffatt et al., 2016). Other serovars which are prevalent in the housing environment of commercial laying flocks, such as *S.* Kentucky in the USA, are rarely linked to egg contamination. Experimental infection with serovars Heidelberg and Typhimurium has sometimes resulted in colonization of reproductive organs at relatively high frequencies (Gantois et al., 2008), but egg contamination has been observed far less often than for *S.* Enteritidis (Gast et al., 2011). This may be because *S.* Enteritidis adheres more strongly to reproductive tract mucosa, or because other serovars elicit more intense protective immune responses. Egg-associated illness in Australia appears to be primarily the consequence of external contamination of eggshells by *S.* Typhimurium (Moffatt et al., 2016).

IV. ENVIRONMENTAL INFLUENCES ON *SALMONELLA* IN LAYING FLOCKS

Environmental conditions in egg production facilities have a powerful influence on the opportunities for introduction and dissemination of pathogens in laying flocks (Trampel et al., 2014). Persistent environmental contamination is sometimes responsible for the transmission of infection into successive laying flocks over extended periods of time (Dewaele et al., 2012). Contaminated dust and feces can distribute *Salmonella* contamination widely throughout laying houses (Im et al., 2015). Severe rodent or insect infestations can amplify *Salmonella* levels sufficiently to threaten the efficacy of standard protocols for cleaning and disinfection of poultry facilities (Wallner-Pendleton et al., 2014).

Once introduced into laying flocks, rapid and extensive horizontal dissemination of salmonellae can be facilitated by direct contact between hens, ingestion of contaminated feed or feces, movement of personnel and equipment, and airborne circulation of contaminated dust and aerosols. Environmental stressors, such as excessive heat and deprivation of feed or water, can heighten the susceptibility of hens to horizontal transmission of infection (Okamura et al., 2010). Risk assessment for *Salmonella* in poultry is made difficult by the environmental complexity of commercial egg production facilities and management practices. Even within individual flocks, the prevalence of *Salmonella* infection often varies considerably over time (Wales et al., 2007). Among the risk factors most often associated with higher *Salmonella* prevalence are larger flock size, greater flock age, housing in older facilities, and multiple-age stocking (Denagamage et al., 2015).

V. HOUSING SYSTEMS AND *SALMONELLA* IN LAYING FLOCKS

The relative merits of different production housing systems for commercial laying hens have been actively debated in recent years in regard to their implications for issues as diverse as animal welfare, economic viability, and public health. The principal management system options include conventional laying cages (housing small groups of hens at relatively high stocking densities), enriched colony cages (providing lower stocking densities for larger hen groups plus environmental enhancements such as perches, nesting areas, and scratching pads), aviaries (allowing birds to move freely among multiple open levels of enriched cage and floor areas within houses), and free-range housing (offering greater opportunities for freedom of movement via varying degrees of access to outdoor forage or pasture areas). Each of these systems is associated with intrinsic facility design features and management practices which can affect the persistence and transmission of pathogens (Jones et al., 2015).

Although numerous studies have assessed the food safety consequences of different types of housing for egg-laying chickens, no overall consensus has emerged to suggest the superiority of any one system. Experiments comparing the prevalence of *Salmonella* environmental contamination, infection, or egg contamination attributable to housing flocks in various cage-based or cage-free systems have yielded a wide range of results. Some studies have reported higher frequencies of *Salmonella* infection from cage-based housing systems (Denagamage et al., 2015), other studies have associated cage-free housing systems with a higher incidence of egg and environmental *Salmonella* isolation *(*Jones and Anderson, 2013), and a third group of studies have not found differences in *Salmonella* prevalence between housing systems (Van Hoorebeke et al., 2011). A large field study conducted under commercial egg production conditions (Jones et al., 2015) identified no major differences between housing system in the presence of pathogens in the environment or on eggs, although each system had specific risk factors which posed unique management challenges (such as contaminated scratch pads in enriched cages and contaminated floor eggs in aviaries).

In experimentally infected hens, *S.* Enteritidis was isolated from internal organs at

significantly higher overall frequencies from hens in conventional cages than in enriched colony cages (Gast et al., 2013b). This suggested that stress associated with some intrinsic characteristic of conventional cage housing (such as bird density or behavioral restriction) might compromise immunity, resulting in increased susceptibility to the systemic dissemination of *S.* Enteritidis infection. Chickens housed at high stocking densities have exhibited suppression of both humoral and cellular immunity and increased *S.* Enteritidis invasion of internal organs (Gomes et al., 2014). However, no difference was evident between conventional and enriched colony cage systems in the production of internally contaminated eggs by experimentally infected hens (Gast et al., 2014). Although systemic dissemination and reproductive organ invasion are necessary precursors to the deposition of *S.* Enteritidis inside eggs, the frequency at which they occur does not consistently not consistently predict the likelihood of subsequent egg contamination (Gast et al., 2011).

VI. *SALMONELLA* MONITORING IN LAYING FLOCKS AND EGGS

Monitoring for the presence of *S.* Enteritidis in hens, their environment, and eggs is a central component of most programs for controlling this pathogen. Testing serves both to identify flocks which potentially threaten public health and verifies the cost-effectiveness of investments made in risk reduction practices. Laying flock testing programs often focus exclusively on *S.* Enteritidis as the epidemiologically preeminent serovar associated with eggs. This approach may arguably represent a cost-effective use of limited resources for protecting public health, but monitoring for the emergence of previously infrequent or inconsequential serovars can also have important proactive value. The efficacy of testing for eradicating *S.* Enteritidis in poultry and eggs is limited by the continuing opportunities for re-introduction of the pathogen into flocks from diverse environmental sources of infection. Moreover, making decisions about the fate of flocks or eggs on the basis of testing results can be complicated by fluctuations over time in the prevalence of *S.* Enteritidis in the environment, hens, and eggs.

The presence of *S.* Enteritidis in the housing environment of laying flocks has been shown to correlate strongly with the likelihood that they will produce contaminated eggs. Because contaminated eggs are typically produced at very low frequencies by infected flocks, testing environmental samples for *S.* Enteritidis is often employed as a screening method to identify potentially infected flocks for further attention. Voided feces from infected hens are leading sources of laying house environmental contamination with *Salmonella*, although testing for fecal shedding does not always predict overall environmental sampling results (Wales et al., 2006). Dust samples have sometimes provided a higher frequency of *S.* Enteritidis isolation than fecal samples (Arnold et al., 2014b); testing both dust and feces may be superior to either sample individually. An assortment of environmental sampling methods have been used effectively in poultry facilities, including drag swabs, boot swabs, and the collection of litter material or dust from locations such as egg belts, fan blades, or nest boxes. *Salmonella* isolation and identification from environmental samples usually involves traditional selective enrichment culturing methods, followed by biochemical and serological confirmation, although rapid assays based on the recognition of specific genetic sequences or antigenic molecules are increasingly employed to identify particular strains with a high degree of precision.

The production of specific antibodies by infected chickens provides another option for detecting *S.* Enteritidis in laying flocks. Both serum and egg yolk antibodies can be detected, sometimes at high titers, for long periods of time after hens are exposed to *S.* Enteritidis (Gast et al., 2002). Testing for egg yolk antibodies to *S.* Enteritidis has been demonstrated to achieve a similar sensitivity for detecting infected flocks as did culturing environmental

samples (Klinkenberg et al., 2011). Serological methods can be valuable as screening methods for flock infection because of their high detection sensitivity, but they have been infrequently applied in recent years because of concerns about antigenic cross-reactivity with other serovars and the persistence of a detectable antibody response long after active infection has been cleared.

The most definitive documentation that a laying flock poses a threat to public health risk is the isolation of *Salmonella* from the edible internal contents of eggs. Accordingly, egg culturing is a pivotal component in most *S.* Enteritidis monitoring protocols for laying flocks. However, even in flocks known to be infected with *S.* Enteritidis, egg contamination is infrequent, sporadic, and transient, thus limiting the diagnostic sensitivity of testing eggs for this pathogen (Gantois et al., 2009). Because salmonellae are generally found inside eggs at low frequencies and in very small cell concentrations, pools of the entire liquid contents (yolk plus albumen) of up to 20 eggs are often employed to keep sample numbers within logistically feasible limits. These egg contents pools may be pre-incubated or supplemented with an iron source to encourage expansion of initially small *S.* Enteritidis populations to more consistently detectable levels before continuing with traditional enrichment culturing methods (Gast and Holt, 2003). Eggshells are also sometimes cultured to detect external surface contaminants.

VII. *SALMONELLA* RISK REDUCTION IN EGG-LAYING FLOCKS

Controlling the prevalence of *S.* Enteritidis infection in commercial laying flocks is essential to reducing the risk of egg-transmitted illness for humans. The most promising overall strategy for achieving this objective involves the implementation of multiple interventions distributed throughout the egg production cycle (Trampel et al., 2014). Many risk reduction practices to control *S.* Enteritidis are similarly applicable against salmonellae of other serovars. Preventing *Salmonella* introduction into poultry facilities requires the enforcement of strict biosecurity measures, stocking with replacement pullets which are demonstrably uninfected, controlling populations of rodent and insect pests, and securing farms against access by wildlife. Some of these practices are also important for reducing persistence and horizontal dissemination of salmonellae within flocks. Indirect horizontal transmission of *Salmonella* to subsequent flocks via contaminated environmental sources can be minimized by thorough cleaning and disinfection of laying houses after depopulation.

Vaccination is a risk reduction practice that often has a serovar-specific target. Immunization of laying hens against *S.* Enteritidis seeks to reduce the susceptibility of individual birds to infection, vertical and horizontal transmission between birds, the overall pathogen load in poultry house environments, and the frequency of egg contamination. Both inactivated (killed) and attenuated (live) *Salmonella* vaccine products are commercially available, eliciting varying degrees of mucosal and systemic immunity. In experimental challenge studies, administration of either type of *S.* Enteritidis vaccine preparation to pullets or hens has typically reduced - but seldom altogether prevented - fecal shedding, organ invasion, and egg contamination (Trampel et al., 2014). Additionally, *Salmonella* vaccines sometimes fail to provide protection against infection when confronted with high challenge doses of the pathogen or when the immune responses of hens are reduced by stressors such as feed deprivation, water deprivation, or environmental heat (Barrow, 2007). Nevertheless, significantly lower frequencies of egg contamination and human *S.* Enteritidis infections have been attributed to the inclusion of vaccination as a component in risk reduction programs for commercial egg production (Cogan and Humphrey, 2003).

Egg refrigeration has often been identified as an important risk mitigation strategy to protect consumers against egg-borne transmission of *S.* Enteritidis infection to consumers.

Although inadequate management of egg storage temperatures is uncommon in modern commercial egg production, one study concluded that nearly half of egg-associated illnesses were associated with such problems (DeWinter et al., 2011). Accordingly, many risk reduction plans include specifications for temperature control during egg storage. Because initial *Salmonella* populations inside eggs are usually very low, prompt refrigeration as soon as possible after egg are laid can prevent multiplication to higher levels more likely to cause human illness (Gast and Holt, 2000b). The effectiveness of egg refrigeration depends on the initial location and cell numbers of contaminants, the migration of bacteria or nutrients within eggs during storage, and the rate of achieving growth-limiting temperatures.

The currently applicable food safety regulations for shell production in the USA are provided by the rule for Prevention of Salmonella Enteritidis in Shell Eggs during Production, Storage, and Transportation (US Food and Drug Administration, 2009). This program mandates several specific risk reduction practices and a two-tiered monitoring program for egg-laying flocks. Commercial egg producers must develop a written *S.* Enteritidis prevention plan, purchase all replacement chicks from breeder flocks certified as uninfected, enforce comprehensive facility biosecurity, and stringently control rodents and insects. All eggs must be stored and transported under refrigeration at 7.2° C within 36 hours after laying (although equilibration to room temperature is allowed before processing to prevent egg sweating). Monitoring for *S.* Enteritidis in flocks is performed during both pullet rearing and egg laying. Samples are collected from the poultry housing environment and cultured to detect *S.* Enteritidis at 14-16 weeks of age from pullet flocks, at 40-45 weeks of age from laying flocks, and again at 4-6 weeks after induced molting. When *S.* Enteritidis is found during environmental monitoring of a flock, several lots of 1,000 eggs each are then also collected and cultured. Isolation of *S.* Enteritidis from any egg samples requires all eggs from that flock to be diverted for pasteurization until repeated negative egg testing results are achieved. Contaminated poultry houses must be thoroughly cleaned and disinfected between flocks. Regulations in the USA do not currently require the vaccination of egg-producing flocks against *S.* Enteritidis. This contrasts with the UK Lion Code of Practice for Shell Egg Producers, which mandates *Salmonella* immunization of egg-producing flocks in addition to risk reduction and monitoring (O'Brien et al., 2013).

VII. CONCLUSIONS

No single disease-control strategy appears to offer a completely effective solution to the complex problem of *Salmonella* in poultry flocks and eggs. In the United States, where *S.* Enteritidis has been the greatest concern, comprehensive efforts to assure the microbial safety of eggs have been built on a foundation of risk reduction practices: biosecurity, sanitation, pest control, and egg refrigeration. Vaccination has often used to augment these measures by inducing an additional protective barrier against infection. Flock and egg testing have been employed to identify situations requiring further attention and to evaluate the efficacy of risk reduction efforts. Problems with other serovars, such as *S.* Typhimurium in Australia, can present unique challenges to public health authorities and the egg industry. Nevertheless, many of the general underlying principles for *Salmonella* control in poultry flocks and eggs are internationally applicable.

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EFFECT OF FEED ACIDIFICATION AND CONDITIONING TEMPERATURE ON FEED HYGIENE AND SALMONELLA RECOVERY FROM MASH AND PELLETED BROILER FEED

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Summary

The present study investigated the interaction between feed acidification and conditioning temperatures as they both pertain to feed hygiene based on recovery of *Salmonella* over time. Wheat-based broiler diets were formulated to contain 4 levels of a feed acidifier containing formic acid and sodium formate (Amasil® NA) at 0, 4, 7, and 10g/kg. *Salmonella enterica* subsp. *enterica* serovars Enteriditis (ATCC 13076) was inoculated into the 4 feeds at 4 $\log_{10}$ cfu/g of feed. Inoculated feed samples were then steam-conditioned at temperatures of 60, 75 and 90°C and pelleted. A portion of the pelleted feed was then reinoculated with *S. enterica* at 7 $\log_{10}$ cfu/g of feed. Feed samples were collected pre- and post-pelleting, and evaluated for growth of *Salmonella* on days 0, 1, 2, 4, 7, and 14 post-inoculation or post-pelleting as appropriate. Pelleting, regardless of conditioning temperature, was sufficient to eliminate all *Salmonella* from the feed in the present study. Acidification of the feed reduced the recovery of *Salmonella* from both unconditioned mash feed, as well as from pellets that were reinoculated with *Salmonella* post-pelleting.

I. INTRODUCTION

Poultry feed is routinely pelleted for many reasons such as increased feed intake, feed efficiency, and feed hygiene (Abdollahi et al., 2013). However, pelleting is not without its drawbacks, particularly when using higher pelleting temperatures (Abdollahi et al., 2010). The industry has been trending towards ever higher conditioning temperatures due, at least in part, to efforts to improve feed hygiene and thus decrease reliance on in-feed antibiotics for prevention and treatment of disease. To our knowledge, the present study is the first designed to understand the interaction between feed acidification and conditioning temperature on survival of *Salmonella* on feed specifically.

II. MATERIALS AND METHODS

Four 100 kg lots of a wheat/SBM-based broiler diet (Table 1) were treated with Amasil NA at 0, 4, 7, and 10 g/kg. The next day, feed samples were inoculated with previously prepared stock solutions of *Salmonella* at 4 $\log_{10}$ cfu/g of feed.

Preparation of inoculum: Previously prepared stock culture of *Salmonella enterica* subsp. *enterica* serovar Enteritidis (ATCC 13076) maintained at -80°C was transferred to fresh trypticase soy broth (TSB), incubated at 37°C, with subsequent transfers to fresh TSB for a total culture volume of 1L for each feed sample to be inoculated. The culture was then directly applied to the feed using a spray applicator with thorough mixing.

Sampling pre-pelleting: Following inoculation and mixing, 1 sample of each contaminated batch of feed (0, 4, 7, and 10 g/kg of Amasil NA) was collected. These samples were taken back to the lab, stored at 20°C, and used to enumerate *Salmonella* in the mash feed samples at d 0, 1, 2, 7, and 14 post-inoculation.

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Sampling post-pelleting: The remainder of the inoculated feed in each lot was then placed in the hopper of the pellet mill (California Pellet Mill CL5 Laboratory Pellet Mill) and conditioning temperature increased to a target of 60, 75, or 90°C for 45 seconds. Once the target temperature was achieved, hot pellets were collected aseptically and immediately placed on ice. This first pelleted sample was later reinoculated with *Salmonella* (ATCC 13076) to simulate post-pelleting recontamination of feed within the mill. These samples were used to enumerate *Salmonella* on d 0, 1, 2, 7 and 14 post-re-inoculation. A second sample of hot pellets was collected and cooled for 10 minutes using a pilot-scale room-air pellet-cooler. Following cooling, this second pelleted sample was also placed on ice and used to determine the impact of conditioning temperature on *Salmonella* enumeration without being reinoculated. These later samples were used to enumerate *Salmonella* on d 0, 1, 2, 7 and 14 post-pelleting.

Salmonella enumeration: Enumeration was conducted according to methods previously described in Huss et al. (2017). In brief, a subsample of the previously collected feed samples was collected aseptically, serially diluted with buffered peptone water, and the appropriate dilutions spread plated in duplicate on xylose lysine deoxycholate agar (XLD; Becton, Dickinson and Company, Franklin Lakes, NJ). All XLD plates were incubated at 37°C for 24 hr. After incubation, all plates were enumerated by counting black colonies, typical for *Salmonella* and serotyped by the National Veterinary Services Laboratory (Des Moines, IA). The number of observed colonies was then multiplied by the dilution factor to determine the total count in cfu/g for the sample. For all plates with no growth, overnight-enriched samples were serial diluted and spread plated to XLD and incubated. Growth on enriched plates were noted as positive or negative for *Salmonella* but not counted.

Table 1 - Ingredient composition of the experimental broiler diets.

	Amasil NA concentration			
Ingredient	0g/kg	4g/kg	7g/kg	10g/kg
Soybean meal	297.70	298.41	298.94	299.47
Wheat	637.19	632.28	628.59	624.91
L-Lysine HCl	3.46	3.45	3.45	3.44
DL-Methionine	2.87	2.88	2.89	2.90
L-Threonine	1.13	1.13	1.13	1.14
Choline chloride	0.25	0.26	0.26	0.27
Vitamin-mineral premix[1]	3.00	3.00	3.00	3.00
Limestone	11.93	11.92	11.91	11.89
Salt	1.42	1.42	1.43	1.43
DCP	9.84	9.87	9.89	9.91
$NaHCO_3$	2.86	1.88	1.15	0.42
Amasil NA[2]	0.00	4.00	7.00	10.00
Natugrain TS[3]	0.10	0.10	0.10	0.10
Natuphos E 10000 G[4]	0.10	0.10	0.10	0.10
Soy oil	28.15	29.30	30.16	31.02
Total	1000.00	1000.00	1000.00	1000.00

[1] Contains per kg of premix a minimum of 40 g Mn, 40 g Zn, 20 g Fe, 4.5 g Cu, 0.6 g I, 60 ppm Se, 3080000 IU vitamin A, 1100000 ICU vitamin D3, 6600 IU vitamin E, 4.4 mg B12, 330 mg of menadione, 2.64 g riboflavin, 0.44 g thiamine, 2.64 g D-pantothenic acid, 11 g niacin, 0.55 g vitamin B6, 275 mg folic acid, 154 g choline, 13.2 mg biotin.
[2] Sodium buffered formic acid containing 61% formic acid and 20.5% sodium formate.
[3] NSP enzyme providing thermostable xylanase (min. 5600 TXU/g) and thermostable glucanase (min. 2500 TGU/g) activity.
[4] Hybrid-6-phytase providing min. 10000 FTU/g.

Statistical analysis: Calculated cfu/g data was converted to $\log_{10}$ and analyzed using the GLIMMIX procedure of SAS with fixed effects of treatment (0, 4, 7, and 10 g/kg of Amasil NA), day (0, 1, 2, 7, and 14), and form (mash vs pellet). Contrasts for mash vs. pellet

were also evaluated. Data from the two sets of pelleted samples (with or without re-inoculation) were analyzed separately.

III. RESULTS AND DISCUSSION

Inoculation of the acidified mash feed resulted in between 3.39 and 3.88 log_{10} cfu/g on d 0 post-inoculation, with numerically lower recoveries in diets containing higher concentrations of Amasil NA (Table 2). The main effect of time decreased *Salmonella* recovery from mash feed (3.74, 2.54, 2.17, 1.48, 0.80 log10 cfu/g; $P < 0.01$). The main effect of 7 and 10 g/kg of Amasil NA decreased *Salmonella* relative to the control (2.92 vs 1.74 and 1.14 log10 cfu/g, respectively; $P < 0.05$). The 7 and 10 g/kg inclusion levels resulted in no detectable *Salmonella* growth with 14 and 7 days of inoculation, respectively. These results clearly show that acidification of mash broiler feed can greatly improve feed hygiene through direct elimination of *Salmonella*.

Table 2 - *Salmonella* detection after pre-pelleting inoculation.

Treatment	Day 0	Day 1	Day 2	Day 7	Day 14
0 g/kg Mash	3.88	3.46	2.92	2.66	1.70
0 g/kg @ 60°C	+	+	-	n/a	-
0 g/kg @ 75°C	-	-	-	n/a	-
0 g/kg @ 90°C	-	-	-	n/a	-
4 g/kg Mash	3.85	3.30	2.71	2.54	1.48
4 g/kg @ 60°C	+	+	-	n/a	-
4 g/kg @ 75°C	-	-	-	n/a	-
4 g/kg @ 90°C	-	-	-	n/a	-
7 g/kg Mash	3.84	2.11	2.04	0.70	-
7 g/kg @ 60°C	+	+	-	n/a	-
7 g/kg @ 75°C	-	-	-	n/a	-
7 g/kg @ 90°C	-	-	-	n/a	-
10 g/kg Mash	3.39	1.30	1.00	-	-
10 g/kg @ 60°C	-	-	-	n/a	-
10 g/kg @ 75°C	-	-	-	n/a	-
10 g/kg @ 90°C	-	-	-	n/a	-

Salmonella counts are provided for pre-enrichment enumeration (log_{10} cfu/g); + indicates positive for growth of *Salmonella* (black colonies) on enrichment media; - indicates negative for growth of *Salmonella* (no black colonies) on enrichment media; n/a indicates enrichment was not performed either because *Salmonella* was detected in the original enumeration or the previous enrichment resulted in no *Salmonella* growth. Enrichment was performed on d 14 for all samples to confirm that they are still negative.

In the present study, pelleting at 60, 75, or 90°C resulted in feed from which no *Salmonella* could be enumerated at any time point (data not shown). Enrichment of pelleted samples revealed that, for feed containing 0, 4, and 7 g/kg of Amasil NA, the 60°C pellets were still *Salmonella* positive for the first 24-hr or so, despite not being culturable by direct plating on XLD. Starting on day 2, no *Salmonella* could be cultured from any of the pelleted samples. It has previously been shown that thermal or pH stress can push *Salmonella* in to a non-reproductive phase temporarily (Carrique-Mas et al., 2007). The additional enrichment step enables detection of these populations. However, pelleting at 75 or 90°C for 45 seconds, regardless of level of acidification, resulted in no detectable *Salmonella* even after enrichment. Interestingly, the combination of 10 g/kg of Amasil NA plus pelleting at 60°C also resulted in no detectable *Salmonella* after enrichment. This would indicate that acidification and pelleting temperature are potentially additive.

Re-inoculation of acidified broiler pellets with *Salmonella* resulted in approximately 6 to 7 log_{10} cfu/g on day 0 (Figure 1). *Salmonella* recovery from the re-inoculated samples naturally decreased over time, even in the unacidified mash/pellets, but reduced more rapidly

the higher the inclusion of Amasil NA. On day 0, the feed samples containing 4, 7 and 10 g/kg Amasil NA were an average of 0.14, 0.26 and 0.72 log_{10} cfu/g lower (SE = 0.23), respectively, relative to the unacidified samples. This indicates that, at higher concentrations, feed acidification offers some level of immediate protection against recontamination of pellets and mash feed with *Salmonella*. As highlighted by Davies and Wray (1997), the pellet cooler can itself serve as a reservoir of *Salmonella* that can potentially undo the work of thermal treatment on feed hygiene. In feed originally inoculated with roughly 7 log_{10} cfu/g of *Salmonella*, acidification with 10 g/kg of Amasil NA reduced the level of quantifiable *Salmonella* to 0 cfu/g in less than 7 days, although it could still be detected by overnight enrichment, and to undetectable even with enrichment within 2 weeks.

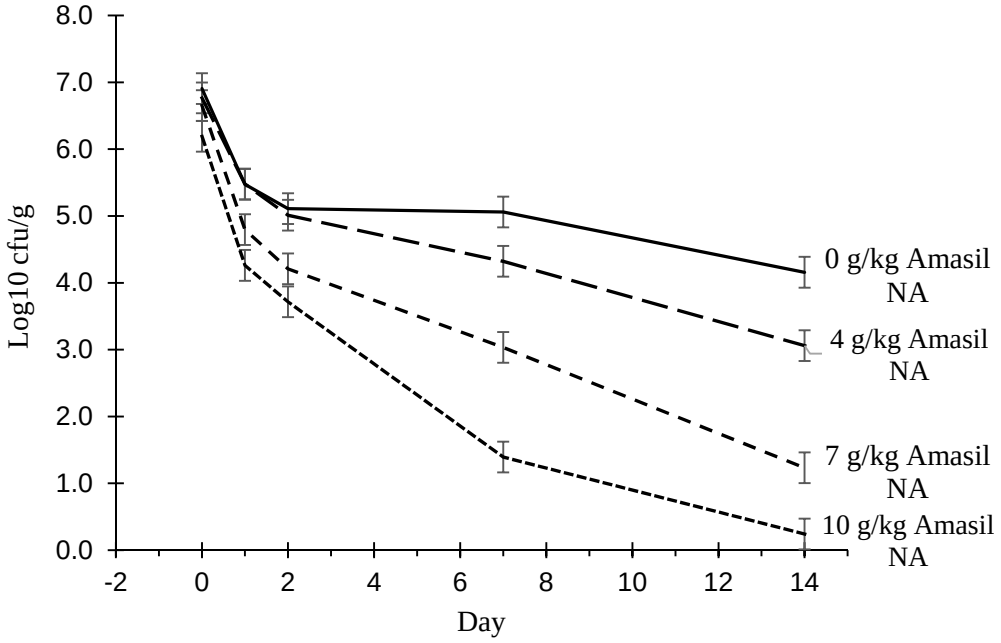

Figure 1 - Salmonella enumeration after second inoculation to simulate a post-pelleting challenge to broiler feed containing 0, 4, 7, and 10 g/kg of Amasil NA (pooled across mash and pellets).

IV. CONCLUSIONS

Both pelleting and acidification with Amasil NA were shown to be effective at increasing the hygiene of broiler feed. Pelleting at 75 and 90°C for 45 seconds were equally effective regarding the pre-pelleting inoculation, but offered only minimal protection against post-pelleting recontamination. Acidification was effective regardless of feed form or conditioning temperature of pellets. Lower pelleting temperatures plus feed acidification with Amasil NA are shown to be complementary tools for promoting feed hygiene.

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COMPATIBILITY OF A NEW MULTISTRAIN PROBIOTIC WITH A LIVE-ATTENUATED SALMONELLA VACCINE IN CHICKENS

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Summary

Vaccination of chickens and probiotic supplementation in feed are nowadays regarded as relevant measures to increase the resistance of birds against *Salmonella* exposure and decrease shedding. A pilot study was undertaken to assess the compatibility of a new multistrain probiotic with a live-attenuated *Salmonella* Typhimurium (ST) vaccine. Experimental units were individual birds. There were three experimental treatments: control group without ST-vaccine administration or probiotic in the diet (T1), ST vaccinated group without probiotic (T2) and ST vaccinated group with probiotic in the diet (T3). On day 3 of test chicks were challenged with 10^7 CFU/ml *Salmonella* Heidelberg by oral gavage. Prior to challenge, four chicks from each group were randomly selected and the vaccine was able to be re-isolated from the spleens from treatments receiving the vaccine, indicating the probiotic did not affect the vaccine's initial colonization. At study termination (day 39) caeca were sampled for *Salmonella* prevalence and number. There was no significant difference in *Salmonella* prevalence among experimental treatments. However, the vaccine alone or with probiotic showed numerically lower MPN $\log_{10}$ of *Salmonella* than the challenge control.

I. INTRODUCTION

The basis for effective control of *Salmonella* infections in poultry production is good farm managment and hygienic practices as well as testing and elimination of positive flocks in some countries. While many different measures have been recommended in meat chicken farms, vaccination with live-attenuated vaccines is likely to have a central role in the reduction of *Salmonella* in commercial operations by increasing the passive immunity of birds and blocking the horizontal transmission of *Salmonella*. The advantage of live-attenuated vaccines is that attenuated *Salmonella* bacteria replicate, colonize, and invade intestinal and visceral organs of inoculated chickens, producing long-lasting protective immunity (Dórea et al., 2010). Furthermore, as part of measures against *Salmonella* in poultry, it has been demonstrated that the addition of *Bacillus*-based probiotics in poultry feed may result in significant reductions of *Salmonella* load in the intestinal tract of chickens as well as in the surrounding environment, thereby potentially reducing the risk of infection between birds and decreasing the amount of *Salmonella* entering the slaughterhouse, thus potentially improving food safety (Knap et al., 2011). The objective of this pilot study was to evaluate the effect of a *Bacillus*-based probiotic on the colonization of a live *Salmonella* Typhimurium (ST) vaccine and its subsequent ability to protect against a *Salmonella* Heidelberg challenge in broiler chickens.

II. MATERIAL AND METHODS

One hundred and twenty (120) day-of-hatch Ross x Ross non-sexed broiler chicks were obtained from a commercial broiler company (Georgia, US). At the experimental facility (Southern Poultry Research Group, Inc. -SPRG-, Georgia, US), birds were randomly distributed in three isolated room divisions (40 birds per division), with each room division

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assigned to one of the following experimental treatments: control group without ST-vaccine administration or probiotic in the diet (T1), ST vaccinated group without probiotic (T2) and ST vaccinated group with probiotic in the diet (T3). All birds were vaccinated with an approved broiler coccidiosis vaccine, 30 minutes before administrating live ST-vaccine to T2 and T3 birds. The ST-vaccine was coarse sprayed, as per manufacturer's recommendation, at one day of age at one dose per bird in a volume of 0.25 ml per chick.

The birds were raised under thermostatically controlled gas heaters and ambient humidity and were provided a lighting program as per the primary breeder recommendations. At placement, each isolated division contained approximately 10 cm of fresh pine shavings. Litter was not replaced during the course of the study. Each isolated division contained one tube feeder and one bell drinker resulting in a forty bird/feeder and drinker ratio. Feed and water were administered *ad libitum.* Since performance assessment was not an aim of the study, only two feeding phases were established: starter phase from day zero to day 21, grower-finisher phase from 22 to day 39. Diets were fed as crumbles (starter phase) or pellets (grower-finisher phase). Feed formulations for this study consisted of un-medicated commercial-type broiler starter and grower diets compounded with commonly used United States feedstuffs representative of local formulations, calculated analyses to meet or exceed NRC standards. No antibiotics were added to any feed. Treatment feeds were prepared from a basal starter feed with quantities of all basal feed to prepare treatment batches documented. Treatment feeds were mixed at the SPRG feed mill and pelleted in a California Pellet mill at 80 °C. Starter feed and grower-finisher feed for T3 birds was supplemented with a multi-strain probiotic composed of the following probiotic strains: *Bacillus subtilis* DSM32324 (8 x 10^5 CFU / g of feed), *Bacillus subtilis* DSM32325 (5 x 10^5 CFU / g of feed) and *Bacillus amyloliquafaciens* DSM25840 (3 x 10^5 CFU / g of feed). Thus, the total content of supplemented *Bacillus* strains in T3 feed was 1.6 x 10^6 CFU / g of feed.

On the third day of life, all birds were orally gavaged with a 3 x 10^7 CFU nalidixic acid-resistant *Salmonella* Heidelberg. Prior to challenge, four ceca and four spleens from each treatment were weighed and collected to confirm vaccine colonization. The samples were analyzed by decimal dilutions in buffered saline with gelatine. The diluted samples were plated in drops on Salmonella-Shigella agar and incubated at 37°C for 24 h (permitting detection of 10^2 CFU of salmonellae per g of sample). The presence or absence of salmonellae at concentrations below 10^2 CFU/g in the sample was tested by ability or inability to detect salmonellae in samples incubated in selenite cysteine broth at 37°C for 48 h, subcultured on Salmonella-Shigella agar, and incubated for 24 h at 37°C. Samples positive by selective enrichment in selenite cysteine broth were recorded as 10 CFU, and negative samples were recorded as 0 CFU (Hassan and Curtiss, 1994).

On day 39, 10 birds per treatment were taken from each isolated division, euthanised (by cervical dislocation), and caeca aseptically removed. After removal, each caecal sample was placed in one sterile plastic sample bag (Fisher Scientific), labeled and stored on ice and transferred to the onsite laboratory for *Salmonella* analysis. The caeca samples were weighed, sterile saline was added to them, and they were stomached. An aliquot of 1 ml was removed for Most Probable Number (MPN) procedure.

For all 10 caeca, a sample of 1 ml of stomached peptone broth was transferred to three adjacent wells in the first row of a 96-well 2 ml deep block. An aliquot of 0.1 ml of sample was transferred to 0.9 ml of tetrathionate broth in the second row. This process was repeated for the remaining rows in order to produce five ten-fold dilutions. Blocks were incubated (24 hours at 42°C). and 1 µl of each well transferred onto XLT-4 agar (containing nalidixic acid) with a pin-tool replicator. Plates were incubated at 37 °C for 24 hours, the final dilution of each sample being recorded and entered in MPN calculator (to determine sample MPN).

Suspect *Salmonella* isolates were confirmed by Poly-O *Salmonella* Specific Antiserum (MiraVista, Indianapolis, IN), according to Berghaus et al. (2013) and Alali et al. (2013).

Salmonella prevalence was compared between treatment groups using Fisher's exact test. *Salmonella* MPNs in culture-positive samples were compared between treatment groups using one-way analysis of variance. MPN values were log-transformed prior to statistical analysis. Post-hoc pairwise comparisons were performed using the Bonferroni procedure to limit the type I error probability to 5% over all comparisons. All tests assumed a two-sided alternative hypothesis, and $P < 0.05$ was considered statistically significant. Analyses were performed using commercially available statistical software (Stata 15.0, StataCorp LLP, College Station, TX). Although statistical comparisons were performed for informational purposes, they are not valid under the pilot study design because birds within the same room division (pen) are not statistically independent, and there was no pen-level replication of treatments.

III. RESULTS AND DISCUSSION

Liver and spleen samples were cultured from four birds per treatment group on day 3. *Salmonella* prevalence is summarized in Table 1. There was a significant overall treatment effect with respect to *Salmonella* prevalence ($P = 0.002$). Prevalence in T1 was zero, whereas in T2 and T3 was 75% and 100%, respectively, indicating that probiotic supplementation in the diet did not interfere with the response to the vaccine. Due to the small sample sizes, none of the individual pairwise comparisons between treatments were significant when using the Bonferroni procedure to limit the type I error probability to 5% over all comparisons. All of the *Salmonella* isolates obtained on day 3 were identified as belonging to serogroup B, which was consistent with the vaccine strain (*Salmonella* Typhimurium).

Table 1 - *Salmonella* prevalence in liver/spleen samples collected from four birds in each of three treatment groups on day 3.

Treatment	N	No. positive (%)	†P
T1 (untreated group)	4	0 (0.0)[a]	0.002
T2 (*Salmonella* vaccine alone)	4	3 (75.0)[a]	
T3 (*Salmonella* vaccine + probiotic)	4	4 (100)[a]	

† Fisher's exact test. Percentages with a superscript in common do not differ with a level of significance of 5% over all comparisons.

Salmonella prevalence in ceca samples at day 39, based on MPN data, is summarized in Table 2. There was no significant difference between treatments ($P = 0.125$). All of the *Salmonella* isolates obtained from caeca samples were identified as belonging to serogroup B. As nalidixic acid was used in the MPN procedure, all isolates obtained were presumed to be the challenge strain. Although there was not a significant reduction, there was a consistent numerical reduction in prevalence of the challenge strain in both treatments.

Table 2 - *Salmonella* prevalence in caeca samples collected from 10 birds in each treatment group on day 39.

Treatment	n	No. positive (%)	†P
T1 (untreated group)	10	7 (70.0)	0.125
T2 (*Salmonella* vaccine alone)	10	5 (50.0)	
T3 (*Salmonella* vaccine + probiotic)	10	6 (60.0)	

†Fisher's exact test.

Salmonella MPNs for the culture-positive caeca samples are summarized in Table 3. There was no significant difference between treatments with respect to the mean $\log_{10}$

Salmonella MPN / g in culture-positive caeca samples on day 39 ($P = 0.135$), although the application of the live-attenuated vaccine alone or with probiotic resulted in lower values than the untreated group.

Table 3 - Log_{10} *Salmonella* MPN/g for culture-positive ceca samples on day 39.

Treatment	N	Mean log_{10} MPN/g (SE)	†***P***
T1 (untreated group)	7	0.85^a (0.29)	0.135
T2 (*Salmonella* vaccine alone)	5	0.05^a (0.35)	
T3 (*Salmonella* vaccine + probiotic)	6	-0.01^a (0.32)	

†One-way analysis of variance. Means with a superscript in common do not differ with a level of significance of 5% over all comparisons.

This study was primarily designed to demonstrate that the tested probiotic would have no negative effect on the ability of the live *Salmonella* vaccine to replicate and induce protection. This study clearly demonstrated that this *Bacillus*-multistrain probiotic does not have any detrimental effect. In addition, the study indicated there may be an additive effect to having both the vaccine and probiotic.

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EFFICACY OF COMMERCIAL DISINFECTANTS AGAINST BIOFILMS FORMED BY *SALMONELLA* TYPHIMURIUM

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Summary

In this study, the susceptibility of *Salmonella* Typhimurium biofilms to three different commercial disinfectants (Products A, B and C) that are used in the poultry industry was investigated. Biofilms were formed for 24 and 48 hour (h) using the MBEC™ system at 22 ºC and treated with commercial disinfectants at 0.5 or 1.0% concentrations of products A and C and 0.4 and 0.8% of product B for 30 s. All three disinfectants produced a significant reduction in viable biofilm cells at 24 and 48 h in a dose dependent manner. The age of the biofilm was associated with resistance towards all disinfectants at both concentrations. In conclusion, commercial disinfectants tested in this study were able to reduce the viable cells in biofilms. However, complete eradication of biofilm cells was not achieved. These findings highlight the need to consider the optimisation of application of disinfectants to control biofilms formed by *S.* Typhimurium.

I. INTRODUCTION

Contamination of eggshells by *Salmonella* Typhimurium is a major public health and food safety issue to the Australian poultry industry. In Australia, the majority of the egg and egg related foodborne outbreaks are caused by *S.* Typhimurium (The OzFoodNet Working group 2015).

Biofilm formation is one of the survival mechanisms utilised by *Salmonella* spp. against physical and chemical stress factors in the environment (Steenackers *et al.* 2012). A biofilm is a community of cells attached to biotic and abiotic surfaces in a self-produced extracellular matrix component (Donlan and Costerton 2002). Cells in a biofilm exhibit a greater resistance to antimicrobials and environmental stressors than their planktonic counterparts (Steenackers *et al.* 2012). Hence, the eradication of biofilms in both domestic and industrial settings is difficult. Previous studies have demonstrated that susceptibility of biofilm cells to commonly used disinfectants was associated with the age of the biofilm (Shen *et al.* 2011).

Biofilm formation contributes to the persistence of *Salmonella* in the food processing environment and detachment of biofilm cells from surface could lead to cross contamination of other food products, compromising the food quality (Møretrø *et al.* 2012). The objectives of this study were to test the susceptibility of 24 and 48 h old biofilms formed on a plastic surface to different concentrations of commercial disinfectants following 30 s exposure. All commercial disinfectants tested in this study are currently being used in the Australian poultry industry. In this study, each product was tested at the user recommended concentration or twice the recommended concentration against *S.* Typhimurium biofilms.

II. MATERIALS AND METHODS

Twelve isolates of *S.* Typhimurium isolated during epidemiological studies were used for biofilm formation. The stock culture of *S.* Typhimurium was streaked onto XLD agar plates and incubated at 37 °C for 24 h for the preparation of biofilm inoculum (Pande *et al.* 2016). The MBEC™ Assay system (Innovotech Inc. Edmonton, Canada) was used to form *S.*

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Typhimurium biofilms. Biofilms were formed by adding 200 µL of *S.* Typhimurium inoculum in each well of MBEC plate and plates were incubated at 22 ± 2°C for 24 or 48 h on a rocking platform shaker (ROCKit, Select Bio-Products, NJ, USA). Three commercial disinfectants (Table 1) commonly used in the Australian poultry industry were tested in this study against *S.* Typhimurium biofilms according to the manufacturer procedural manual version 1.1 (Innovotech Inc. Edmonton, Canada), at 1x or 2x the recommended dose. Products A & C were tested at 0.5 or 1.0%, and product B was tested at 0.4 or 0.8%. Freshly prepared working solutions of each commercial disinfectant were prepared from stock solution and used immediately on the challenge day. Positive and negative controls were also used in the experiment. After biofilm establishment, peg lids were aseptically removed at 24 or 48 h and rinsed with 0.9% normal saline solution (NSS) for 1 min in 96 well flat bottom microtiter plates (Nunc™, Thermo Scientific, Australia). For each disinfectant studied, the peg lids were placed in the disinfectant plate and exposed for 30 s. Sterile NSS was used as a negative control and *S.* Typhimurium biofilm without disinfectant was used as a positive control in the disinfectant challenge plate.

After 30 s exposure, the peg lids were rinsed two times (one min each rinse) in NSS, and then immersed in a neutralising broth (Difco™ Neutralizing Broth) for one min. The peg lids were then aseptically transferred to a 96 well plate containing LB broth. The plates were sonicated at high speed for 5 min (Model 160TD, Soniclean Pty Ltd, Australia) and number of viable cells was enumerated by plate count method. In this study, two biological replicates of each *S.* Typhimurium isolate were used to test the activity of each disinfectant against biofilms. Statistical analysis was performed using GraphPad Prism version 6 Software, Inc. CA, USA. Mean recovery of viable cells from control and disinfectant treated groups, at different concentrations and days of biofilm formation, were analysed by two-way ANOVA followed by Tukey's multiple comparisons test. Data were expressed as mean log CFU peg^{-1} ± standard error of mean (SEM). P values <0.05 were considered statistically significant.

III. RESULTS

In comparison to the positive control (7.05 log CFU peg^{-1}), product A significantly reduced the viable cell count to 4.38 and 4.43 log CFU peg^{-1} in the 24 h biofilms at 0.5 and 1% concentrations, respectively. Similarly, in comparison with the positive control (7.02 ± 0.09 log CFU peg^{-1}), product A significantly reduced the viable cell count to 2.72 and 3.38 log CFU peg^{-1} in 48 h biofilms at 0.5 and 1% concentration, respectively. However, no significant difference was observed between the two concentrations in the recovery of viable biofilm cells after 24 and 48 h of biofilm formation. The sensitivity of 48 h biofilm cells to product A at 0.5% was significantly reduced (P=0.0002) and more cells were recovered from 48 h biofilms (4.29±0.19 log CFU peg^{-1}) than the 24 h biofilm (2.67±0.34 log CFU peg^{-1}). There was a trend (P=0.053) towards decrease in efficacy of product A between 24 (2.63±0.29 log CFU peg^{-1}) and 48 h (3.65±0.26 log CFU peg^{-1}) biofilms at the 1% concentration.

Compared to the positive control (7.05 log CFU peg^{-1}), product B tended to reduce the viable cell counts to 4.35 and 4.57 log CFU peg^{-1} in the 24 h biofilms at 0.4% and 0.8% concentrations, respectively. In comparison with the positive control (7.02 ± 0.09 log CFU peg^{-1}), product B significantly reduced the viable cell count to 1.86 and 2.79 log CFU peg^{-1} in 48 h biofilms at 0.4 and 0.8% concentrations, respectively. However, no significant difference was observed between the two concentrations in the recovery of viable biofilm cells after 48 h of biofilm formation. The sensitivity of 48 h biofilm cells to product B at 0.4 % was significantly reduced (P=0.0001) and more cells were recovered from 48 h biofilms (5.16±0.22 log CFU peg^{-1}) than the 24 h biofilm (2.70±0.41 log CFU peg^{-1}). Similarly, more

cells were recovered from 48 h biofilms (4.23±0.22 log CFU peg^{-1}) than the 24 h biofilms (2.48±0.38 log CFU peg^{-1}) when treated with the 0.8% concentration of product B.

In comparison with the positive control (7.05 log CFU peg^{-1}), product C at 0.5% and 1% concentrations significantly reduced the viable count to 4.95 and 4.92 log CFU peg^{-1} for the 24 h biofilms, respectively. However, no significant difference in the recovery of viable biofilms cells after 24 h was observed between 0.5 and 1% concentrations. In comparison with the positive control (7.02 log CFU peg^{-1}), product C significantly reduced the viable cell count to 1.91 and 2.24 log CFU peg^{-1} in 48 h biofilms at 0.5 and 1% concentrations, respectively. However, no significant difference was observed between the two concentrations in the recovery of viable biofilm cells after 48 h of biofilm formation.

There was significant difference in the resistance to product C at all concentrations between biofilm ages. The sensitivity of 48 h biofilm cells to product C at 0.5% was significantly reduced (P=0.0001) and more cells were recovered from 48 h biofilms (5.11±0.11 log CFU peg^{-1}) than the 24 h biofilms (2.10±0.40 log CFU peg^{-1}). Similarly, more cells were recovered from 48 h biofilms (4.78±0.09 log CFU peg^{-1}) than the 24 h biofilms (2.13±0.34 log CFU peg^{-1}) when treated with a 1% concentration of product C.

Table 1 - Details of commercial disinfectants used in the study.

Product Name	Composition	Intended use and dose
A	Chlorinated liquid (potassium hydroxide + sodium hypochlorite)	Egg shell sanitizer; 1:10, 1:100
B	Quaternary Ammonium compound (QAC)	Egg shell sanitizer, used as sanitizer in poultry sheds, animal pens, farm equipment; 1:50, 1:100
C	Twin-chain QAC (didecyldimethylammonium chloride + ethanol + alcohols, C12-14, ethoxylated)	Sanitizer in food processing plants; 1:10

IV. DISCUSSION

The three commercial disinfectants (Products A, B and C) tested in this experiment significantly reduced viable biofilm cells. However, none of the products completely eliminated the biofilm cells. The effects of disinfectants are highly concentration-dependent (Russell and McDonnell 2000), and it has been shown that high concentrations of disinfectants were able to reduce more viable cells from biofilms, or even demonstrated 100% reduction in viable cells (Møretrø *et al.* 2009). In contrast, in this study, even at twice the recommended user concentration, complete elimination of biofilm cells was not achieved by any of the products. Product A, a sodium hypochlorite based compound, has already been tested with variable effects as a disinfectant against biofilms (Wong *et al.* 2010). Product A was effective in reducing viable biofilm cells at both 0.5 and 1% concentrations; however it was not able to eliminate all viable cells. However, previous studies have shown that sodium hypochlorite, when used at a concentration of 5% (m/v) for 1 and 5 min, was able to completely remove viable cells from 5 or 7 day (d) old *Salmonella* biofilms (Wong *et al.* 2010). The differences in concentration, exposure time (30 s) and age of biofilm (24 and 48 h) could have contributed to the discrepancies in the results. Product B contained quaternary ammonium compounds (QAC), and product C was a twin-chain QAC. QAC have powerful disinfectant activity (Hegstad *et al.* 2010). Exposure to QACs leads to disruption of the lipid bilayer of the cytoplasmic membrane and outer membrane of gram-negative bacteria leading to leakage of cytoplasmic components and eventually cell lysis (Quinn *et al.* 2011). In our

study, both products B and C were able to reduce the number of viable biofilm cells of two different ages at both tested concentrations. The decrease in viable cell numbers was greater in Product C which is a fourth-generation twin chain QAC containing didecyl dimethyl ammonium chloride with greater germicidal performance than conventional QACs. These twin chain QACs have superior germicidal activity, are low foaming and have a high tolerance to protein loads and hard water.

It has been reported that increase in the concentration and contact time of disinfectants can increase their effectiveness against *Salmonella* (Wong *et al.* 2010). In contrast, while in our experiment all three products reduced the number of viable biofilm cells, increasing their concentration did not alter the results significantly. The difference in concentrations, biofilm (24 and 48 h age) and short disinfectant exposure time (30 s) may be one of the reasons for this effect. Also, effective cleaning procedure prior to application of sanitisers is important.

The results of this study showed that older biofilm age was associated with increased resistance to disinfectant treatments. None of the products in this study was able to remove biofilm cells completely (100%), even at the double recommended user concentration. Products A and B are used in egg washing. Given that *S.* Typhimurium is able to form biofilms on egg shells (Pande *et al*. 2016), further studies are required to investigate the efficiency of these products in the removal of biofilms from the egg shell surface. In this study, each product was tested at the user recommended concentration or twice recommended concentration against *S.* Typhimurium biofilms. This was a research study with no intention to support the use of commercial products at concentrations other than those recommended and registered as such. This study does not make any recommendations for tested commercial products to be used at concentrations other than those recommended on the label.

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THE IMPACT OF RANGE USE ON CAECAL MICROBIOTA COMPOSITION IN FREE-RANGE LAYING HENS

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The composition of gastrointestinal microbiota has been demonstrated to affect behaviour and vice versa (Neufeld et al., 2011; Berthoud, 2008). In free-range laying hens, the individual preference to range can lead to characteristic sub-populations of the flock (Hinch & Lee, 2011). The purpose of this study was to investigate the impact of ranging behaviour on caecal microbiota in laying hens.

A flock of sixty ISA Brown laying hens was housed in the UNE research facilities with daily range access from 21 weeks of age. Radio-frequency identification (RFID) tracking technology was employed to evaluate daily range usage of the individual hens. The experiment started when hens were 43 weeks of age. The RFID data of ranging behaviour across 39 consecutive days were used to select hens; Group 1 (n=10) spent the least while Group 2 (n=10) spent the most, time on the range (Table 1). Caecal content was collected from hens at 49 weeks of age, DNA extracted (Kheravii et al., 2017) and microbial profiling done at the Australian Genome Research Facility. Statistical analysis was performed using univariate non-parametric Mann-Whitney tests with adjustments for multiple comparisons using the Benjamini-Hochberg adjustments (significance set at $P < 0.05$).

Table 1 - Range usage of hens that were sampled for caecal microbiota characterisation (Mean±SEM).

	Group 1	Group 2	P-value
Average time on the range/hen/day (h)	4.64 ±0.34	7.49±0.20	<0.000
Δ Ranging time	-25.7±5.40	19.9±3.16	<0.000
Average visits to the range/hen/day	22.4±5.57	23.8±1.24	0.786
Total days accessed the range/hen	37.9±0.67	39.0±0.00	0.075
Total visits to the range/hen	860±220	883±48.2	0.911
Total time spent on the range/hen (h)	177±14.36	280±13.3	<0.000

At the phylum level, only *Tenericutes* differed significantly between the two groups (P=0.05), whereas no significant difference was observed for *Actinobacteria*, *Bacteroidetes*, *Deferribacteres*, *Elusimicrobia Firmicutes*, *Fusobacteria*, *Lentisphaerae*, *Proteobacteria*, *Spirochaetes*, *Synergistetes* and *Verrucomicrobia*. At the family level, significant differences were observed for unclassified RF39 (Mollicutes), *Porphyromonadaceae* and unclassified RF32 (Alphaproteobacteria) between the two groups ($P < 0.05$). In conclusion, the ranging activity of free-range laying hens at 49 weeks of age had a minor impact on caecal microbiota composition.

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CALCIUM PIDOLATE IMPROVES EGG QUALITY WHEN IT IS FED TO COMMERCIAL LAYERS FROM 50 WEEKS OF AGE

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Summary

Calcium pidolate is claimed to have a positive impact on bone quality (in rearing) and to improve egg quality (in lay). Supporting data, however, are quite limited and often relate to studies carried out on small numbers of birds. In this study, we compared egg quality and bone strength in eight commercial free range flocks which received either a standard diet supplemented with 300ppm of calcium pidolate (Treatment n=4) or just the standard diet (Controls n=4) from 50 weeks of age. The results show that there were 0.89% fewer eggs graded as seconds ($P < 0.001$) in the treatment group and 1.4% increase in eggs graded as large ($P < 0.001$). A small but significant increase in eggshell breaking strength ($P = 0.004$), shell weight ($p = 0.38$) and shell colour ($P < 0.001$) was also observed. No evidence was found that bone quality at 70 weeks of age was better in the treatment group (tibia or humerus breaking strength, keel bone radiographic density and keel bone deformity scores). It can be concluded from the study that supplementing a layer diet with 300ppm calcium pidolate from 50 weeks of age could be a cost-effective way of maintaining egg quality in longer laying cycles.

I. INTRODUCTION

With the current genetic focus being on longer laying cycles, there is a need to look for new ways of improving calcium metabolism in laying birds which are prone to osteoporosis in the latter stages of lay (Bain et al, 2016). Calcium pidolate (a highly soluble, absorbable salt with excellent gastrointestinal tolerance) has been around for 10-15 years in the commercial sector of the egg industry. Existing evidence suggests that this supplement has beneficial effects on egg and bone quality (Agblo and Duclos, 2011; Valderrama and Roulleau, 2013). Additional independently derived data from commercial flocks fed this supplement are needed to help farmers make a decision about using calcium pidolate since it adds a significant cost to production.

The aim of this study was to provide reliable data to support the hypothesis that providing laying hens with a 300ppm of calcium pidolate supplementation from 50 weeks of age improves egg quality and bone health. A unique design feature of this study was the access to eight commercial free range (FR) flocks that were located across four different study sites. Each site offered two identical sheds with flocks of the same stocking density (12,000-16000), genotype and age. By providing one flock at each site with the calcium pidolate supplement (treatment) and the other the control diet, the four study sites served as replicates in our statistical models.

II. MATERIALS AND METHODS

Pre-trial baseline data (egg grading, egg quality and bone quality) were collected for all eight study flocks between 45 and 50 weeks of age. From 50-70 weeks of age, one flock per site

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was given a calcium pidolate supplemented diet (300ppm), the other flock received the same standard layer diet without supplementation (control).

Weekly egg grading data (% extra large (XL), % large (L), % medium (M), % small (S), % extra small (XS), % Seconds, First quality (Doz), Seconds (Doz), Total Eggs (Doz) and Average Egg Weight (g) were provided for each flock pre and post intervention from 45 to 70 weeks of age.

For egg quality and bone quality parameters, power calculations were used to determine the optimal sample sizes (total eggs and bones for analysis per flock) using published data. Egg quality was assessed on 120 eggs per flock every five weeks from 45 to 70 weeks of age. Egg quality was assessed in terms of egg weight (g), dynamic stiffness (Kdyn) (N/mm), breaking strength (N), shell colour (% reflectance @ 640nm), shell thickness (mm) and shell weight (g). 31 birds from each flock were culled at 45 (pretrial data) and at 70 to assess their bone health status. The right wing, right distal limb, and keel (whole with muscle still attached) were carefully excised, bagged, labelled and deep frozen prior to testing. Bone breaking strength (tibia and humerus) was determined by a three-point destructive bending test, Keel bone radiographic density measurements were made by taking a radiograph of each keel bone and analysing each image using Image J1.32 as described by Fleming et al. (2004). Keel bone damage was visually assessed by scoring each keel on a scale of 0-3, 0 being no damage and 3 being severely deformed involving evidence of bone fracture.

Multivariable linear regression models were built for each outcome variable within each of the egg grading, egg quality and bone quality data sets. Age (or sample number as a proxy of age) was included in all models, because of known associations between age and many of our measured outcomes. ‘Site’ (n=4) was included as a random effect variable in all models to account for between ‘site’ variations. Thus, variation in the measured outcomes that was associated with age and any unmeasured variables or differences that existed between sites was accounted for in the analyses.

For the bone data the primary variable of interest, representing the control or treatment group, was forced into each model. For both the egg grading and egg quality datasets, two sets of models were developed for each of the measured outcomes. The first model included the primary variable of interest which compared the control group samples and the treatment group samples with the pre-trial samples. The second model included only the data post intervention and so compared the treatment group using the control group as the reference for each of the measured outcomes.

III. RESULTS AND DISCUSSION

Model 1 compared the post intervention treatment group and the control group with the pre-trial data. For the egg grading data set, the only significant effect we observed was a small reduction in the average egg weight (-0.8g; $P = 0.025$) in the Control group. For the egg quality data set, a significant post intervention effect was observed for breaking strength and shell colour in both the Treatment and Control groups: respectively a reduction in breaking strength of 5.5N, ($P < 0.001$) and 6.2 N ($P < 0.001$) and a reduction in shell colour of 10.73%, ($P < 0.001$) and 11.48 %, ($P < 0.001$). The fact that both post intervention treatments were different to the pre-trial data suggests that there could have been errors associated with using ‘bird age at sampling’ as a proxy for age in the egg quality data set model. For the bone quality data set, no significant post intervention effects were observed.

Our second model compared the post intervention Control and Treatment data sets with each other. This revealed that there was a significant increase ($P < 0.001$) of 1.4% in

eggs graded as Large and a significant reduction (P < 0.001) of 0.89% in Seconds from the Treatment group (Figure 1).

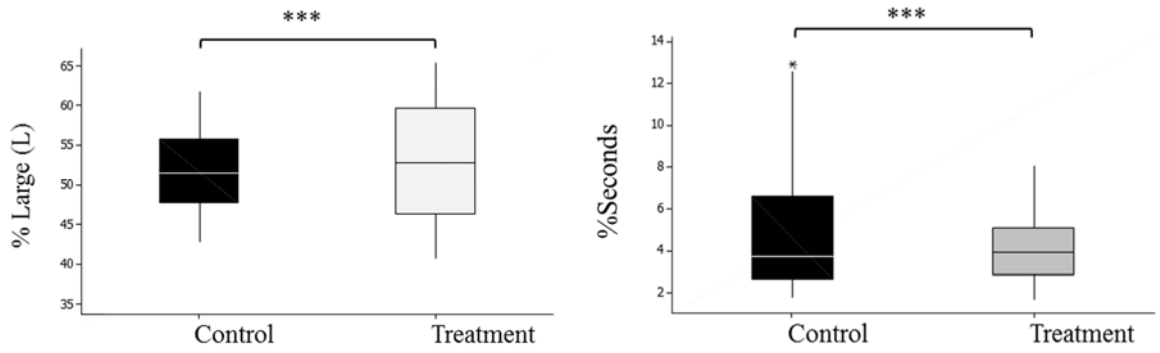

Figure 1 - Comparison of %Large and %Seconds for Treatment (Calcium Pidolate) and Control groups. The combined weekly data from 51-70 weeks of age are presented as medians with 25-75 percentile range as the box and the whisker as 10-90 percentiles (***P < 0.001).

Eggshell breaking strength (+0.7N; P = 0.004), shell weight (+0.48g; P = 0.014) and shell colour (-0.75% delta% ref; P < 0.001) were also significantly improved in the Treatment group (Table 1), although in absolute terms the differences were quite small. Perhaps a more marked difference between the control and treatment groups might be evident if the trial had been continued for longer.

Table 1 - Egg quality data for Treatment and Control groups: Mean and standard deviations for all data collected from the 4 flocks on treatment and the 4 flocks on the control diet (NB: For shell colour, a lower delta %ref corresponds to a browner egg).

	Control (n=4)	Treatment (n=4)	P value	Coefficient of Variation
Breaking strength (N)	41.3 +/- 7.6	42.0 +/- 7.7	0.004	(0.7)
Egg weight (g)	65.2 +/- 5.1	65.4 +/- 5.2	ns	
Kdyn (N/mm)	15457 +/- 2084	15496 +/- 2176	ns	
Shell weight (g)	6.395 +/- 0.619	6.443 +/- 0.597	0.014	(0.05)
Shell thickness (mm)	0.372 +/- 0.282	0.372 +/- 0.281	ns	
Shell colour (delta% ref)*	69.1 +/-6.4	68.4 +/- 6.5	0.001	(-0.75)

As for the previous model, there was no significant difference in any of our bone quality measurements (Table 2).

Table 2 - Bone Quality. Mean and standard deviations for all data collected from the 4 flocks on treatment and the 4 flocks on the control diet.

	Control	Treatment	P value
Humerus BS (N)	225.76 +/- 50.12	224.62 +/- 50.96	ns
Tibia BS (N)	280.75 +/- 78.18	278.21 +/- 59.90	ns
Keel RD	0.6323 +/- 0.1195	0.6155 +/- 0.0989	ns
Keel Score	0.23 +/- 0.63	0.20 +/- 0.61	ns

IV. CONCLUSIONS

This study provides evidence that providing commercial free range layer hens with a diet supplemented with calcium pidolate from 50 weeks of age can improve egg quality: the % seconds decreased and eggshell strength and shell colour were also improved. For the

producer, this means more ‘saleable’ eggs, which probably justifies the additional cost when laying flocks are to be kept for longer. Confirming the previous experiences of the supplier, no improvement in bone strength or quality was seen in this study with treatment at end of lay, which can be attributed to the fact that the birds were already too old for any significant improvement to occur. It can, therefore, be suggested that to improve bone health, the intervention should be directed towards the rearing period when the medullary bone reserves are first forming.

ACKNOWLEDGMENTS: This trial was carried out as an independent study by the University of Glasgow commissioned by Lakes Free Range Company (UK). Both parties would like to thank Dietaxion for supplying the calcium pidolate and Benjamin Pollet for his helpful contributions to this manuscript. Prof Tim Parkins is also acknowledged for his statistical advice and all the staff at the Lakes Free Range Company for their help with sample collection. This study was funded through a Sainsbury's ‘Big Data’ Agriculture Research and Development Grant (2014-15).

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SPOTTY LIVER DISEASE

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Summary

Spotty Liver Disease has become common in the Australian layer industry. *Campylobacter hepaticus*, the bacterial pathogen that causes the disease, has recently been identified and characterised. Following on from the initial discovery of the causative pathogen, whole genome sequencing of a collection of isolates has been carried out and molecular assays have been developed. The molecular assays allow culture free detection and quantification of *C. hepaticus* in microbially complex samples such as gastrointestinal tract samples. In addition, a multiplex PCR assay has been developed to that is capable of simultaneous detection of *C. hepaticus*, *C. jejuni*, and *C. coli*.

I. INTRODUCTION

Spotty Liver Disease (SLD) is characterized by the occurrence of multiple grey/white spots in the liver. It causes mortalities and reduction in egg output and is prevalent within the layer industry in Australia, especially within the free range sector of the industry (Grimes & Reece, 2011). The disease is less commonly found in barn and cage birds and parent stock (Scott, 2016). The recent identification of *Campylobacter hepaticus*, as the causative agent (Van *et al.*, 2016), and the development of an experimental disease induction method (Van *et al.*, 2017a), provide the tools to facilitate the study of disease pathogenesis and the evaluation of experimental vaccines.

The development of specific and sensitive PCR detection methods allows the detection of *C. hepaticus* in the gut of diseased birds. *C. hepaticus* occurs throughout the gut, increasing in abundance down the gut. To date we have only detected *C. hepaticus* in the gut of birds from sheds that have clinical signs of disease. *C. hepaticus* could not be detected in the gut of birds from other sheds, on the same farms, that have not had a history of clinical disease. Currently, we do not have a highly selective culture method for *C. hepaticus* so the organism can only be isolated from samples such as liver and bile, that are not infected with other bacteria, as *C. hepaticus* is slow growing and hence is rapidly overgrown by any other contaminating bacteria.

II. IDENTIFICATION OF THE CAUSATIVE AGENT

Disease cases with similar clinical presentations as modern day SLD were reported in the USA in the 1950's (Tudor, 1954; Delaplane *et al.*, 1955). Bacteria described as "vibrios" were cultured from diseased birds, initially by passage in chicken embryos and subsequently cultured on rich agar media of various compositions. In one case, cultured bacteria were fairly comprehensively characterised for fermentation and enzymatic activities; however the bacterial genus was not identified and no subsequent study of the isolates has been reported (Peckham, 1958). Other researchers have suggested the possible involvement of a number of bacterial species, including *Campylobacter jejuni*, *Campylobacter coli*, *Clostridium sordellii*, and *Helicobacter pullorum* (Burnens *et al.*, 1996; Forsyth *et al.*, 2005; Jennings *et al.*, 2011). The *Campylobacter* and *Helicobacter* species would be consistent with the previous findings

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of “vibrio” like bacteria, but in no cases could the disease be experimentally reproduced with the candidate cultured bacteria.

Crawshaw *et al.* recovered a number of bacterial isolates from SLD affected hens from UK flocks and identified them as campylobacters (Crawshaw *et al.*, 2015). Van *et al.* isolated similar bacteria from Australian cases of SLD and went on to fully characterise the organism and identified it as a new species that they named *Campylobacter hepaticus* (Van *et al.*, 2016). The role of *C. hepaticus* as the etiological agent of SLD was confirmed by its ability to induce lesions, typical of clinical cases of disease, in experimentally infected birds (Van *et al.*, 2017a).

III. ISOLATION OF *C. HEPATICUS* FROM CLINICAL SAMPLES

C. hepaticus was first isolated from the livers of layer birds with typical indications of SLD. The two groups who have reported successful isolation of the organism have used slightly different culturing methods (Crawshaw *et al.*, 2015; Van *et al.*, 2016). In both cases aseptically collected internal fragments of liver were macerated in Preston broth and incubated under microaerophilic conditions at 37^{o}C; the UK group for 7 days and the Australian group for 2 days. Samples from the pre-enrichment step were plated onto 5% sheep blood agar (SBA) (UK group) or Brucella agar with 5% horse blood (BAB) (Australian group) and again incubated microaerophilically for several days. The Australian isolates produced clearly visible colonies within 3-5 days whereas the UK group reported that some isolates required up to 7 days before growth was obvious. An easier route to isolation of *C. hepaticus* from diseased birds, taken by both groups, is the direct plating of bile onto either SBA or BAB and incubation under microaerophilic conditions at 37^{o}C for several days.

There is currently no highly selective media available for *C. hepaticus* isolation and so strains have only been recovered from tissue samples that only carry the target organism. When other bacteria are present any potential *C. hepaticus* colonies are rapidly overgrown by more rapidly multiplying bacteria.

IV. CULTURING AND CHARACTERISATION OF *C. HEPATICUS*

Following primary isolation, *C. hepaticus* can be reliably grown on BAB but grows poorly in liquid culture without blood supplementation. It grows at 37^{o}C and 42^{o}C but not at 25^{o}C and does not grow under aerobic conditions. Electron microscopy (EM) showed that *C. hepaticus* has typical *Campylobacter* morphology. Cultures consist mainly of S-shaped cells and longer helical cells, but some cocci forms are also present (Figure 1). Some cells have bipolar unsheathed flagella while many appear to have single polar flagella or no flagella; the variation observed under EM may be due to the sensitivity of the flagella to mechanical breakage as the scanning EM appears to show a lot of broken flagella fragments. Whole genome sequencing and comparison to the genomes of other *Campylobacter* species indicated that *C. hepaticus* is most closely relate to *Campylobacter jejuni* and *Campylobacter coli.*

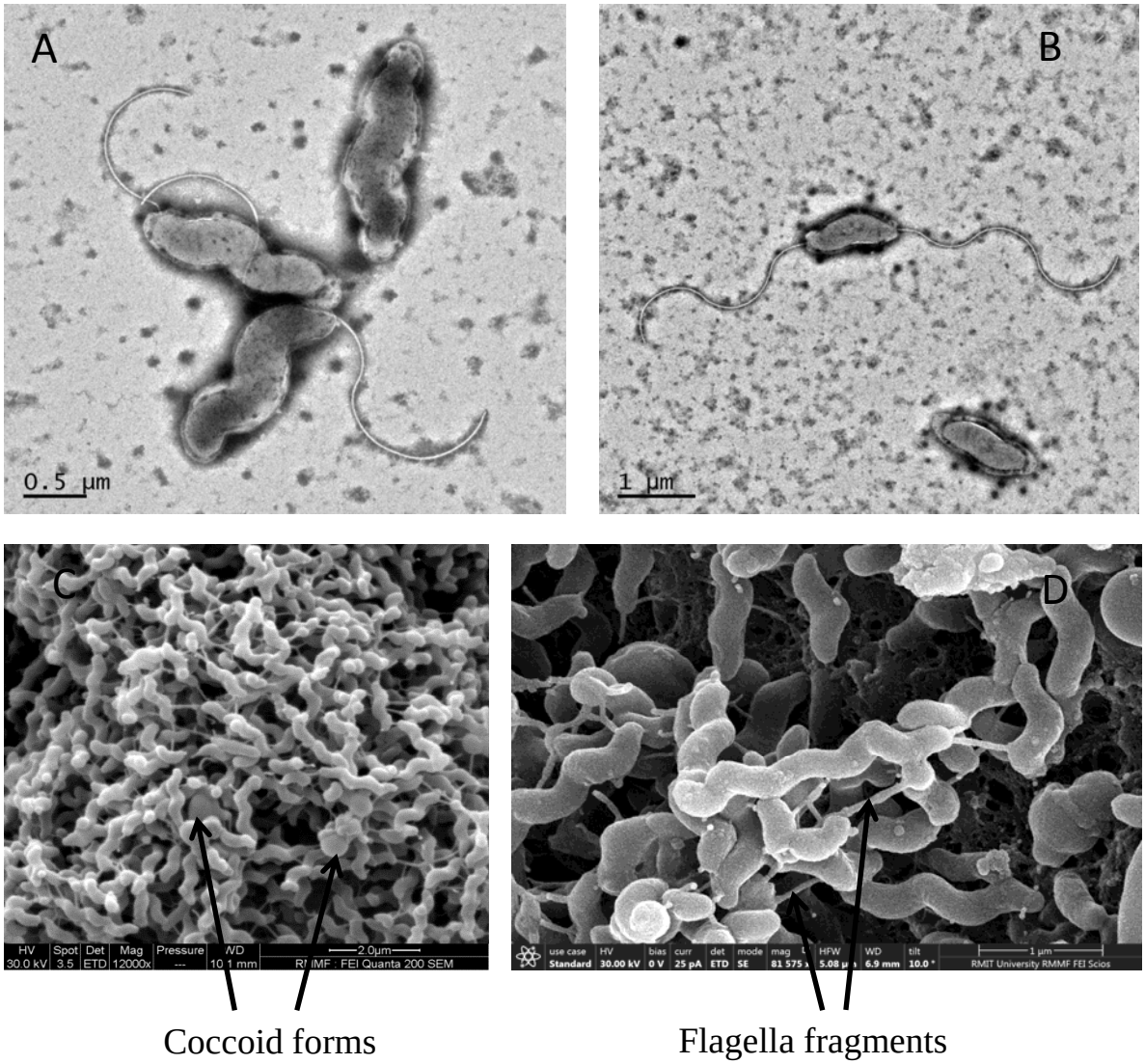

Figure 1 - Panels A and B: transmission electron micrographs of isolated *C. hepa*ticus cells. Note the long bipolar flagella shown in Panel B. Panels C and D: scanning electron micrographs of surface of a colony of *C. hepaticus* cells. Note in panel D the variation in cell length, ranging from the S-shaped cell in the top centre of the panel to the long helical cell in the centre of the panel.

V. EXPERIMENTAL REPRODUCTION OF DISEASE

Early attempts to induce pathology used some of the embryo passaged or cultured bacteria isolated from US cases in the 1950's. The "vibrio" bacteria caused death and lesions in challenged chicken embryos and, in some cases, signs of clinical disease were reproduced in inoculated adult bird (Hofstad *et al.*, 1958; Peckham, 1958; Sevoian *et al.*, 1958). More contemporary attempts to reproduce clinical disease, using the recent UK isolates in specific pathogen free chicks, resulted in microscopically visible lesions in the liver of challenged birds (Crawshaw *et al.*, 2015).

It is only with the use of the Australian *C. hepaticus* isolates in birds coming into lay that full-blown disease typical of field cases of SLD has been successfully reproduced following experimental infections (Van *et al.*, 2017a). Those studies fulfilled Koch's postulates (Grimes, 2006) to unequivocally demonstrate that *C. hepaticus* causes SLD (Van *et al.*, 2017a). Disease induction was achieved by inoculating birds from flocks with no

history of SLD with 10^9 to 10^{10} CFU of *C. hepaticus* HV10^T via direct oral gavage. The severity of disease in the 24 challenged birds varied from no macroscopically obvious disease in one bird to severe disease covering the entire surface of all lobes of the liver in a few birds. Most of the birds had moderate numbers of macroscopically obvious lesions on the liver surface. No long term trials to investigate the effect of experimental disease challenge on egg output have yet been reported. From both the success of the oral gavage in the disease induction experiments and the findings of SLD in cage facilities (relatively rare compared with free-range operations) where it is usually birds on the lower layers that suffer disease, it is concluded that natural SLD infection probably occurs via the faecal-oral route.

VI. *C. HEPATICUS* GENOME

Whole genome sequencing has shown that the genomes of 14 Australian isolates range in size from 1.48 to 1.53 Mb (unpublished data). Sequencing of 10 British isolates showed a wider range of genome sizes from 1.50 to 1.80 Mb (Petrovska *et al.*, 2017). The type strain, HV10^T (=NCTC 13823^T; =CIP 111092^T), has a genome of 1,520,669 nucleotides and is predicted to encode 1494 protein coding sequences and 52 RNA coding genes (unpublished results). Overall whole genome comparison, on a single nucleotide polymorphism gene-by-gene basis of the core genome, showed that the Australian type-strain isolate differed from the three sub-clades of the British isolates. The Australian isolate had a lower GC content; 27.9% compared with an average of 28.4% for the British isolates (Petrovska *et al.*, 2017). The *C. hepaticus* isolates have smaller genomes than typically found for the closely related species, *C. jejuni* and *C. coli*, with approximately 140 fewer genes encoded, including a notable reduction in the number of genes encoding products predicted to be involved in iron acquisition and general metabolism. There were also fewer putative virulence, disease, and defence subsystem genes predicted in the genomes of *C. hepaticus*. *C. hepaticus* genomes encoded more genes involved in carbohydrate, fatty acid, lipid, and isoprenoid metabolism than typically found in *C. jejuni* genomes (Petrovska *et al.*, 2017). Petrovska et al. (2107) have suggested that the reduced genome of *C. hepaticus* may result from the more specialised lifestyle that *C. hepaticus* has compared to *C. jejuni*, in particular, the reduction in iron acquisition may result from specialised niche adaptation to the iron rich environment within the liver.

It is of particular interest to interrogate the genome of *C. hepaticus* for potential toxins that may be important in disease pathogenesis, in particular the pathology observed in the liver. To date no obvious toxin encoding genes have been identified although it should be noted that, like all genomes, the *C. hepaticus* genome contains many genes for which a function could not be predicted. There are only a few genes in other *Campylobacter* species that have been identified as encoding toxins that may play some role in disease pathogenesis. In *C. jejuni* cytolethal distending toxin may play a role in disease pathogenesis but all the genes involved in its synthesis are absent from *C. hepaticus* (Petrovska *et al.*, 2017).

VII. MOLECULAR ASSAYS

Whole genome sequencing of *C. hepaticus* supplied the DNA sequence information required to develop polymerase chain reaction (PCR) methods to specifically and sensitively detect *C. hepaticus* (Van *et al.*, 2017b). Such methods are particularly useful for *C. hepaticus* given its slow growing and fastidious nature and the current inability to selectively culture the organism. It is only with the use of PCR detection methods that the bacterium can be identified and quantified in microbially complex samples such as samples from the gut. Application of the PCR assay (Figure 2A) to gut samples showed that *C. hepaticus* was present within the gut and increased in abundance along the length of the small intestine (Van

et al., 2017b). The detection of sheading of *C. hepaticus* in the faeces supports the contention that SLD is likely to be transmitted via the faecal-oral route. In the future the PCR assay could be used to investigate the epidemiology of bacterial acquisition and spread within a flock and the presence of the bacterium within the environment. It will important to identify the source of infection so that preventative measures can be taken to reduce the incidence of disease in the Australian flock. A multiplex PCR assay that can detect and differentiate three *Campylobacter* species commonly found in chickens has also been developed (Figure 2B), further facilitating the epidemiological investigation of *Campylobacter* carriage in chickens.

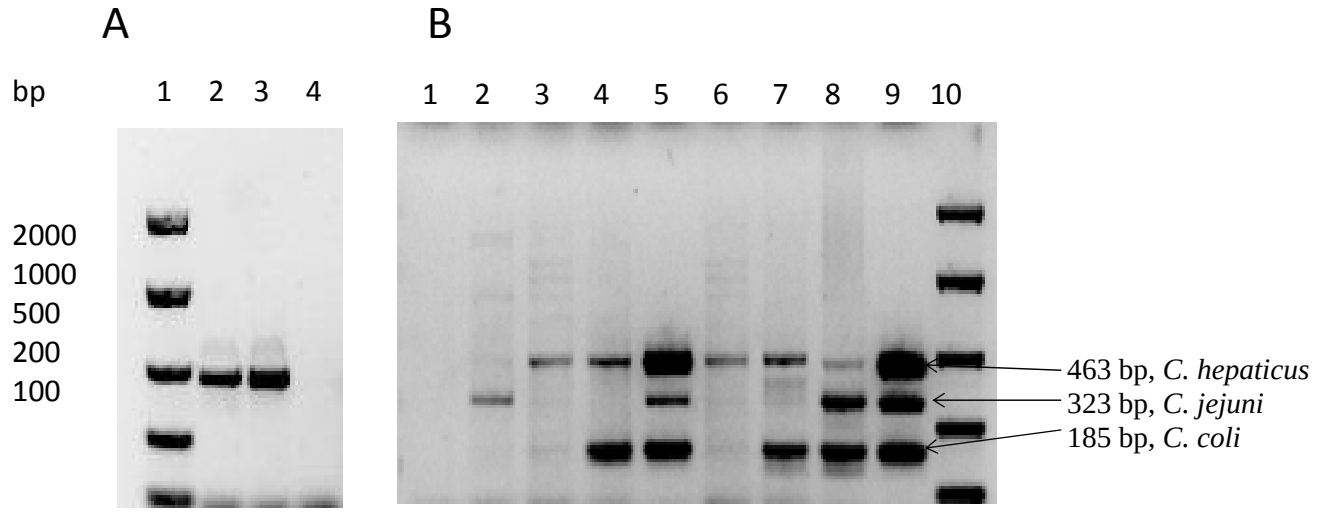

Figure 2 - Panel A: PCR identification of *C. hepaticus* in microbially complex samples. Lane 1: Molecular size standards, EasyLadder (Bioline); Lanes 2 and 3: DNA extracts from chicken caecum samples from birds with clear clinical indications of SLD; Lane 4: Negative control – chicken caecal DNA from a bird from a flock with no history of SLD. Panel B: Multiplex identification of *C. hepaticus*, *C. jejuni* and *C. coli* in caecum samples from chickens (lanes 2-8); lane 1, no DNA negative control; lane 9, spiked DNA positive control; lane 10, Molecular size standards, EasyLadder (Bioline).

VIII. CONCLUSIONS

Recent research has identified *C. hepaticus* as the etiological agent of SLD. Further, characterisation of *C. hepaticus* and the development of an experimental disease induction model have provided the basic tools that can now be used to investigate and develop treatment options for SLD. The disease model can be used to test the efficacy of experimental vaccine formulations under controlled conditions and the model can also be used to test the effectiveness of other potential interventions such as prebiotics, fatty acids, phytobioics, and probiotics. Development of treatment options would be also be advanced by elucidating basic mechanisms of pathogenesis and understanding how *C. hepaticus* traffics from the gut to the liver. In the future, epidemiological studies, facilitated by the application of the developed molecular assays, may inform management practices that could be modified to reduce disease incidence.

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PREVALENCE OF PLUMAGE DAMAGE AND INVESTIGATION OF ASSOCIATED NUTRITIONAL FACTORS FOR FREE RANGE LAYING HENS IN AUSTRALIA

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Summary

Feather pecking (FP) is a detrimental behaviour observed in layer hens in all housing systems, presenting challenges to animal welfare and significant economic consequences. Epidemiological studies on prevalence of plumage damage (PD) attributed to feather pecking have been conducted in numerous countries; however not enough data are available for Australian farms. Fifteen free range farms across Australia, with hens ranging between 34 to 80 weeks of age, were visited to assess the prevalence and severity of PD in a cross-sectional study. Husbandry practices were investigated and feed analysed for nutrient levels. PD attributed to FP was prevalent in all but one of the farms investigated. On average, 33% of hens in a flock displayed PD. Of the different body locations assessed, tail had the highest PD at 70%, while wing had the lowest at 12%. Hens fed pellets showed a higher PD score than in those fed mash ($P = 0.02$). Gross energy, crude protein, calcium, and phosphorus levels in feed showed a significant association ($P < 0.05$) with average PD scores; however further studies are required to investigate and confirm this.

I. INTRODUCTION

It has been shown that FP occurs in all housing systems, from conventional cages, to barn and free range systems (Green et al., 2000). However, there is a lack of data available on the true prevalence of FP in commercial free-range layer farms in Australia. In a survey in the UK, 65% of flocks showed FP during lay as reported by free-range farmers but, when the same flocks were assessed by researchers, prevalence reached 89% and 69% at 25 weeks, and 73% and 86% at 40 weeks for gentle and severe FP, respectively (Lambton et al., 2010). The aetiology of FP is multifactorial and not completely understood, despite extensive research. FP has been proposed to be due to a complex relationship involving genetic and environmental factors (Hartcher et al., 2016; Rodenburg et al., 2013). Included in environmental factors are nutrients in feed and feeding management. FP has been theorised to be redirected foraging and feeding behaviour (Hartcher et al., 2016) and is exacerbated by nutrient deficiencies (Ambrosen and Petersen, 1997; Kjaer and Bessei, 2013). The objective of the study was to determine the prevalence and severity of PD attributed to FP in free range layer farms in Australia and to identify the nutritional factors associated with its occurrence.

II. MATERIALS AND METHODS

A cross-sectional study was conducted on 15 free range layer farms selected from a list generated by an online survey. A sampling frame was created to include farms that had consented to further contact by researchers. Farms were selected based on their location (South Australia (6), Queensland (2), New South Wales (5), and Victoria (2)); number of birds on farm (7 small (200-7,000 hens), 4 medium (14,000-37,000 hens) and 4 large farms (50,000-300,000 hens); age of birds (34-80 wks); and housing styles (fixed sheds/mobile caravan style sheds) to capture the most diverse practices and demographics. On-farm visits involved generating data on husbandry practices using a questionnaire. Feed samples were collected from silos for analysis to determine dry matter percentage, gross energy, crude

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protein percentage and levels of calcium, phosphorous and sodium. Excreta samples were used to calculate apparent metabolisable energy.

Two researchers simultaneously recorded PD on all farms, each selecting five areas randomly in the same shed and visually assessing plumage damage on a group of about 50 birds before they were let out on the range for the day. Scores were based on the severity of damage (with a value of 4 for severe, 3 for moderate, 2 for slight, and 1 for no damage) and were scored for six body locations individually (neck, breast, back, wing, tail and cloaca) (Lambton et al., 2010). It was assumed that all PD was caused by FP except that on neck which can also be due to mechanical damage (Assurewel, 2014). A visual assessment was used to estimate the percentage of flock affected. Data from the two researchers was collated and PD scores across each body location were averaged to obtain final estimates. PD scores for this study were calculated by multiplying the percentage of plumage damage in the body location by the above mentioned factor of severity (nil, slight, moderate and severe). The values were summated to come to a total with the highest possible value as 400, and lowest as 100. Overall prevalence of PD was calculated by dividing the number of farms affected by PD by the total number of farms. The percentage of flock displaying PD to each body location scored was also calculated.

Data were subjected to least square analysis using JMP Pro v11.0 (SAS Institute, Cary, NC, USA) to determine the effect of different nutritional practices and feed nutrients on average and individual body location PD scores. Mean values were reported along with the SEM and compared using Tukey's HSD (considered significantly different at $P<0.05$).

III. RESULTS AND DISCUSSION

Plumage damage (PD) attributed to feather pecking was observed in all but one farm. An average of 33% of hens in a flock were affected by PD (Figure 1).

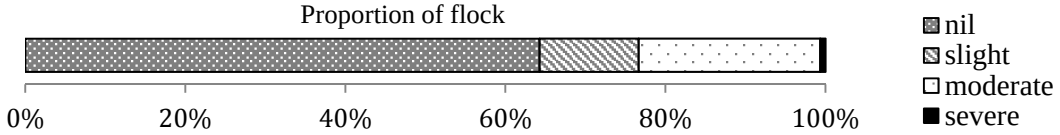

Figure 1 - Average proportion of hens in a flock showing plumage damage at different severity levels as observed on fifteen free range farms.

Of the different body locations assessed, tail had the highest plumage damage at 70% followed by 36%, 34%, 27%, 20% and 12% for back, neck, cloaca, breast and wing, respectively (Figure 2). The highest mean feather pecking score was seen for the tail (213 ± 19) and the lowest for wing (115 ± 8). A similar finding was reported by Petek et al. (2015) where feather score of the tail region was significantly greater than in the other body locations. Since PD on the head and neck indicate either aggressive pecking to maintain the dominance hierarchy or mechanical damage (Assurewel, 2014), the observed plumage damage on the tail, back and cloaca in this study is more likely a result of feather pecking than abrasive and mechanical causes. The significance of cloacal feather loss relates to its association with vent pecking and cannibalism (Lambton, 2015; McAdie et al., 2005). Also, due to the increased risk of wound contamination from faecal matter and the environment, feather loss and damage to the integrity of the skin in the cloacal region can result in septicaemia and death (Lambton et al., 2015).

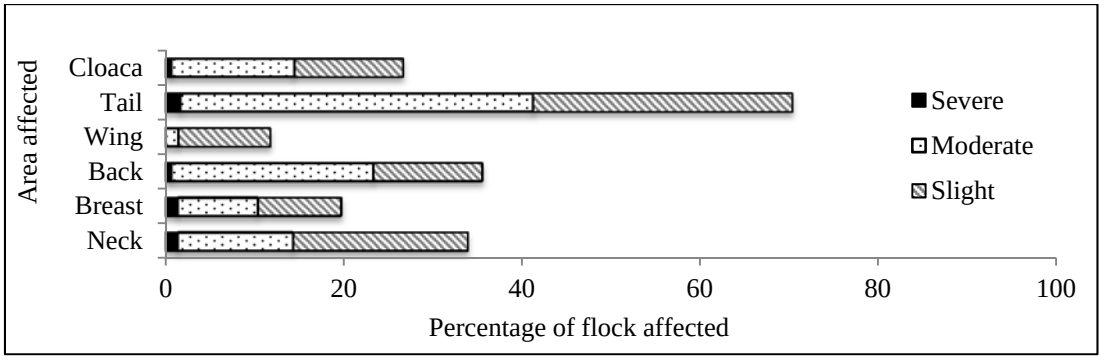

Figure 2 - Prevalence and severity of plumage damage (PD) for six different body locations as observed on fifteen free range farms.

Pelleted feed was used by 40% of farms and consistently showed higher average PD scores (169 ± 11) compared to mash feed (132 ± 8) (P = 0.02), which is in agreement with previous studies (Lambton, 2010; Lindberg and Nicol, 1994).

In the present study, the lowest PD scores were observed on farms that fed crude protein levels between 15-16% (108 ± 13) (P = 0.01). Both very low and high CP levels, as compared to the recommended levels, showed increase in the incidence of PD. Earlier studies have shown that low protein diets can have detrimental effects on feather pecking behaviour (Kjaer and Bessie, 2013, Gerum and Kirchgessner, 1978). While Ambrosen and Petersen (1997) showed that no further significant improvement in plumage condition could be obtained beyond 15.2% CP in feed, the increased PD scores with increasing CP levels seen in this study could be related to the age of the bird since it was farms with older flocks that showed this trend. Moisture content of feed ranged from 6.9% to 11.2% and did not show any significant association to PD.

Gross energy of feed had a significant effect (P=0.01) on PD scores with higher levels associated with higher PD scores. The recommended level for ME is between 11.7-12.0 MJ/kg (Leeson and Summer, 2005). Metabolisable energy (ME) ranged between 10.4 and 12.7 MJ/kg on farms. A significant association of ME levels and PD could not be demonstrated; however, farms with feed that had ME ranging between 11-12 MJ/kg consistently had the highest PD scores, with a mean score of 168±10. Gerum and Kirchgessner (1978) have also reported that broilers fed diets with increasing energy levels of ME showed an increase in feather eating.

Table 1 - Effect of nutritional factors on plumage damage (PD) in laying hens.

	Feed Structure	Crude Protein (CP) %	Gross Energy (GE) (MJ/Kg)	Calcium (Ca)%	Phosphorous (P)%
P-value	*	*	*	**	*
SEM	13.73	16.19	11.56	11.94	12.72
R^2	0.23	0.83	0.62	0.68	0.48
Mean PD (X)	151.20	139.07	141.75	147.48	143.72

Nutrients were calculated on the basis of DM of feed fed on farm; significant differences in means are represented by P-value *(P<0.05); **(P<0.01); SEM = standard error of mean; R^2 = regression coefficient.

No farms had calcium levels within the recommended range of 4.2-4.6% (Leeson and Summers, 2005), with 13 farms below and two farms above this range (between 0.4 - 6.7%). The recommended level of sodium and phosphorous is 0.15-0.17% and 0.3-0.5%, respectively (Leeson and Summer, 2005) and feed analysis identified a range of 0.02-0.22% and 0.2-0.7% respectively. Higher FP scores were recorded for both deficient and above recommended levels of Ca (P=0.01), Na (P=0.41) and P (P=0.02). Hughes and Woodgush

(1973) reported calcium deficiency increased locomotor and general pecking activity termed 'exploratory' behaviour in chickens. Similar effects were reported for sodium deficiency in laying hens (Bessei, 1978). Thus it could be concluded that nutritional deficiencies had a stimulating effect on activity and exploratory behaviour leading to FP. However, it was also observed that levels above the recommended range for these nutrients resulted in the higher feather pecking scores. This effect could be attributed to the fact that, when the feed contains adequate levels of all nutrients and a surplus of certain specific nutrients, the time required for feed intake is reduced. The mismatch between the spontaneous activity for feed-related behaviours (pecking, scratching, locomotion), and the actual time required for feeding is considered a cause of feather pecking (Baum, 1995).

In conclusion, 33% of hens in a flock showed PD, with the tail showing the highest and the wing the lowest of all body locations scored. Providing mash rather than pelleted feed provides an easy implementable strategy which has been shown in the present and previous studies to have an effect on plumage damage. Feed analysis indicated great variability in the levels of nutritional components in the feed. Comparison to recommended levels revealed that none of the feeds adhered to suggested levels for calcium; however feather pecking scores increased with increasing calcium levels. Feather pecking scores were the lowest when diets were within the recommended range for CP%, sodium and phosphorus. This epidemiological study helped to identify dietary nutrient and nutritional management factors that may be implemented on free range laying farms to reduce the prevalence of plumage damage in hens. Other studies looking at a bigger sample size and including practices of pullet rearing and early lay will be useful in developing mitigation strategies to reduce PD in hens.

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PERCEPTIONS, PERFORMANCE AND PERSONALITY TRAITS OF AUSTRALIAN COMMERCIAL CHICKEN FARMERS WITH REGARDS TO BIOSECURITY PRACTICES

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Summary

It is prudent in today's age that farmers uphold high level compliance of biosecurity practices on Australian commercial chicken farms. This is especially so with the large, recent expansion of free-range farms due to consumer demand, which has raised concerns amongst industry experts of the potential increase in disease introduction and spread from more interactions between wildlife and chickens. Farmer compliance with biosecurity practices is dependent on a number of psychological factors, including perceived importance of biosecurity practices and personality traits. An on-farm survey was conducted which interviewed 25 free range layer farms, nine cage layer farms, nine barn layer farms, 15 free range meat chicken farms, and 15 barn meat chicken farms. This survey involved on-farm interviews which asked questions on farmer perceived importance of biosecurity practices and actual performance on farm. Univariable logistic regression analyses were used to estimate the association between farmer perceived importance of these practices and actual performance. It was found that there were significant statistical associations ($P<0.05$) between reported compliance and the perceived importance of all biosecurity practices except for disinfection of equipment between sheds ($P=0.71$). In addition, all significant associations were positive ($OR>1$), with the exception of rodent control and wild bird proofing sheds. A literature review also identified that personality traits were found to influence job compliance. The Work Approach and Behaviour Test is a personality test based on the five factors; emotional stability, extraversion, open-mindedness, agreeableness and conscientiousness. Racicot, Venne, Durivage, and Vaillancourt (2012) found that personality traits grouped under conscientiousness and emotional stability were found to be significantly associated with positive famer biosecurity practice compliance in commercial poultry farms in Canada. Further research on personality traits influencing farmer biosecurity practice compliance, especially in the context of Australian commercial farms, is needed due to the limited area of this research.

I. INTRODUCTION

The Australian commercial chicken industry is experiencing major changes; a significant proportion of both meat and layer farm types are becoming free range and this is driven by consumer demand (ACMF 2011; AECL 2015). Amongst industry experts, there is concern the risk of disease introduction and spread in Australian commercial chicken farms will be raised from the increased potential of interactions between wildlife and commercial chickens on free range farms (Scott et al., 2009). It is therefore prudent on today's farms that farmers uphold strict biosecurity on their farms to reduce the potential for disease introduction and spread. There are various factors influencing farmer compliance of biosecurity practices on farms; including farmer-perceived importance of biosecurity practices which is influenced

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from farmer education and training, and farmer personality traits. This study details results obtained from an on-farm survey conducted on Australian commercial chicken farms of farmer perceived importance of biosecurity practices and correlation with actual on-farm performance of those biosecurity practices. This study also evaluated personality traits that strongly influence job performance for a number of workplaces, as well as biosecurity practice compliance on commercial chicken farms through a literature review (Morrison et al., 2008; Racicot et al., 2012).

II. METHODS

A research project was commenced in 2015 to quantify the likelihood of avian influenza (AI) virus introduction and spread between different types of commercial chicken farms in Australia and to evaluate the reduction in likelihood of alternate on-farm mitigation actions. This project involved an on-farm survey which was conducted on commercial chicken farms in the Sydney basin and South-East Queensland regions of Australia from June 2015 to February 2016. In total, 25 free range layer farms, nine cage layer farms, nine barn layer farms, 15 free range meat chicken farms and 15 barn meat chicken farms were visited. Commercial layer farms were defined as those having more than 1,000 hens, and commercial meat chicken farms were defined as those having more than 25,000 birds. The survey involved conducting on-farm interviews on each of these farms where a range of questions was asked which captured data related to general farm information, water source and use, poultry health, range information, farmer observations of wild birds and other wild animals, and biosecurity. This study focusses on answers from the biosecurity section, where farmers were asked what biosecurity practices were performed on-farm and their perceived importance of these biosecurity practices in terms of preventing the introduction and spread of diseases. For the latter, farmers rated the importance of biosecurity practices using a scale from one to five; one being ‘not at all important’ and five being ‘extremely important’. Univariable logistic regression analyses were used to estimate the association between actual performance of biosecurity practices and farmer perceived importance of these practices. The statistical program JMP® was used (© 2012 SAS Institute Inc., Cary, USA). A literature review was also performed to review other factors influencing biosecurity compliance on poultry farms, with specific focus on farmer personality traits.

III. RESULTS AND DISCUSSION

Results from the on-farm survey revealed that most biosecurity practices were rated on average as ‘very important' by farmers across the farm types. Some practices gained an average rating of ‘extremely important’ but only by meat chicken farms. There was a relatively low rating of importance for disinfection of equipment between sheds by barn meat chicken, cage layer and free range layer farm types. Visitor recording and turnaround times in sheds were also rated relatively low by cage layer farms.

A univariable logistic regression analysis to determine the association between reported compliance with a biosecurity practice on farm and farmer-perceived importance of the biosecurity practice was unstable when performed for the biosecurity practice of hand washing, and therefore the results were omitted. There were significant statistical associations ($P<0.05$) between reported compliance and the perceived importance of all biosecurity practices listed except for the practice of disinfecting equipment between sheds ($P=0.71$). Except for rodent control and wild bird proofing sheds, all significant associations were positive ($OR>1$), meaning that, when the practice was present, the importance rating was higher. Farmer-perceived importance of rodent control may be clouded by failure to reduce rodent numbers despite implementing rodent control, due to the ideal conditions for rodent

presence and breeding on farms in general and the possible resistance to rodent baits. Similarly, farmer-perceived importance of wild bird control may be due to general unawareness of the significance of wild bird presence inside sheds in terms of pathogen transfer. Improved education and training of farmers of the impact and importance of biosecurity practices is likely to heavily influence farmer compliance of biosecurity practices.

Personality tests can be used to assess personality traits in the workplace; a common one used is The Work Approach and Behaviour Test which is based on the five factors; emotional stability, extraversion, open-mindedness, agreeableness and conscientiousness (Morrison et al., 2008). Specific types of personality traits are grouped under these five. One study on poultry farms in Canada found that two personality traits grouped under conscientiousness; responsibility and complexity, and one personality trait grouped under emotional stability; action-oriented, were significantly associated with positive biosecurity compliance in poultry farms (Racicot et al., 2012). Tett and Jackson (1991) found that in other workplaces such as police, sales and professionals; agreeableness was the best predictor of good job performance. Personality tests could potentially be used during the selection process of job candidates on farms and also as a tool to understand poor farmer compliance of biosecurity practices with follow-up education and training to improve compliance.

IV. IMPLICATIONS AND CONCLUSIONS

Studies relating to farmer compliance of biosecurity practices on commercial chicken farms are limited in general. This is especially the case for Australia, and so further research is required. Understanding farmer perceived importance of biosecurity practices and personality traits of farmers helps with understanding the level of farmer compliance of biosecurity practices on farm. Improved education and training of farmers to highlight the significance of biosecurity practices can improve farmer perceived importance of biosecurity practices which will increase the likelihood of compliance. Similarly, personality tests can be used when assessing job candidates on farms but these should be used in combination with other selection processes as the understanding of personality traits influencing biosecurity compliance in commercial chicken farms is limited.

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HAPPY CHICKENS LAY TASTIER EGGS: MOTIVATIONS FOR BUYING FREE-RANGE EGGS IN AUSTRALIA

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Summary

This paper summarizes recent work within the Food Values Research group that has been previously published in full (Bray and Ankeny, 2017) and which used qualitative research approaches to explore consumers' motivations for buying free-range (or cage-free) eggs. This work was motivated by public interest in so-called "ethical" food production such as attention to animal welfare, and increasing sales of 'free-range' eggs. Although previous studies have examined consumers' willingness-to-pay for free-range eggs, and attitudes to animal welfare, there has been little work that unpacks the assumption that free-range egg purchases are linked to consumers' concerns about the welfare of caged hens. Qualitative, thematic analysis of focus groups and interviews involving 73 participants revealed that free-range and cage-free eggs were perceived as better quality, more nutritious, and safer, and having superior sensory characteristics than caged eggs. In response to open-ended questions, free-range and cage-free eggs were described as easy to identify and affordable, compared with other animal products with humane production claims. Although caged-egg production was described by many participants as cruel, the desire to purchase free-range eggs was more often described in connection to efforts to avoid "industrialized" food than in relation to taking a stance on the issue of caged-hen welfare.

I. INTRODUCTION

Australia is highly urbanized, with 80% of people living in the major cities (Australian Government Department of Infrastructure and Regional Development, 2015) and, although Australians believe that farmers do a good job of looking after their animals (Cockfield & Botterill, 2012), there are low levels of agricultural knowledge among the general public (Worsley et al., 2015). Egg production has become increasingly prominent in public discussions of farm animal welfare in Australia, for example the Animals Australia "No way to treat a lady" campaign, targeting caged-egg production (http://www.animalsaustralia.org/no-way-to-treat-a-lady). Until March 2016 (Han, 2016), there was no legally enforceable standard for eggs sold as "free-range" and at the time that this research was performed, labels such as "free-range" could be used to describe a range of production systems (Parker et al., 2013).

Celebrity chefs such as Jamie Oliver, popular books including Michael Pollan's *The Omnivore's Dilemma* (2006), and films such as *Food, Inc.* (2008) have stimulated public interest in "ethical" food production and consumption, including avoidance of food produced from intensively farmed animals. Retailers also have had major roles in bringing products with ethical claims more into the mainstream (Dixon, 2003). Ethical food consumerism (Ankeny, 2012) describes a set of voluntary food choices directed toward a "moral other" because of values and beliefs, and may involve avoiding foods that can be morally problematic, or choosing certain foods over others because of a percieved ethical superiority. For example, a consumer who purchases free-range eggs because he or she believes it is wrong to keep hens in cages is participating in an act of ethical consumerism. Ethical consumerism can be thought of as a conscious or political act, where consumers "vote with

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their dollar" (Shaw et al., 2006; Willis & Schor, 2012) or "vote with their forks" (Parker, 2013), for example, purchasing free-range eggs with the ultimate aim of eliminating caged-egg production through market forces. However, the idea that people can simultaneously act as citizens and consumers has been challenged by some scholars, such as Johnston (2008) and Guthman and Brown (2016; see also Ankeny, 2016 for the contrast between food citizens and consumers).

Research on public perceptions of farm animal welfare has tended to focus on what people know about how animals are treated on farms (Coleman, 2010), what people think about farm animal welfare (Boogaard et al., 2006; Boogaard et al., 2011; Coleman et al., 2016; Prickett et al., 2010; Vanhonacker et al., 2010; Vanhonacker et al., 2012), or whether consumers are willing to pay premiums for products with ethical claims (Chang et al., 2010; Elbakidze and Nayga, 2012; Taylor and Signal, 2009). However, the assumption that such decisions are acts of ethical consumerism or directly related to concerns about animal welfare has not been tested previously. The findings presented in this paper were part of a larger project that aimed to examine Australians' understandings of "ethical" food choices; however we found that conversations about animal production were dominated by discussions about eggs. Hence, we specifically focus in this paper on motivations to purchase certain types of egg products and in what ways they were considered preferable to "conventionally" produced counterparts. We were particularly interested in whether participants spontaneously explained their purchasing decisions in terms of ethical consumption or whether there were other factors associated with purchasing choices. We also explored other factors such as knowledge of and trust in egg production systems, and whether there were barriers to consumers making purchasing decisions that aligned with their values, for example price.

II. METHODS

This research was approved by the University of Adelaide's Human Research Ethics Committee (H-2012-054). The research took place in 2014 in Adelaide, the capital city of the state of South Australia (population of approximately 1.2 million), with a large urban area surrounded by a number of agricultural regions. Consistent with qualitative approaches (Denzin and Lincoln, 1994), we used a combination of focus groups and interviews with semi-scripted, open-ended prompts that allowed participants to address the questions posed, explore the reasoning underlying their responses, and connect these understandings to other food practices, as well as broader social and ethical issues and concerns. Participants were asked to reflect on their regular food purchases and to identify anything that they thought of as being locally produced, organic, free from genetic modification, or produced in a way that promoted good animal welfare. They then were asked to explain why they purchased the particular items which they identified. In addition, participants were asked whether there was anything they avoided purchasing for ethical reasons. In this paper, we only report discussions directly related to animal welfare and eggs.

A total of 31 people participated in four focus groups and were recruited through community announcements, newsletters, social media announcements, and flyers distributed at public events. In addition, we held 42 interviews at two suburban shopping centers ("mall intercepts"; Bush and Hair, 1985) in areas frequented by those of lower socio-economic status (based on postcodes and diverse ethnicities), to ensure that we were able to capture a range of views. The focus group and interview discussions were recorded digitally, transcribed, and anonymized, and checked for accuracy against hand-recorded notes taken by one of the researchers. The transcripts were treated as rich, narrative texts, and analysis was performed by one researcher coding the transcripts for major themes emerging from the data, similar to the "open coding" method described by Corbin and Strauss (1990), using a general

inductive approach. Validity was checked by the second researcher by comparing these themes to those identified independently by her in the transcripts, and coding for consistency across the themes.

Of the 73 total participants in the research, 70% were women. Age was distributed evenly between 18 and 24 years and 65+ age groups, with the lowest represented group being 35–44 (n = 8) and the highest represented group being 55–64 (n = 16). Fifty-five per cent were married or in a *de facto* relationship, 68% had children, and 54% were not currently working, which was also reflected in the high proportion (51%) of low income earners (indicating that they had a household income of less than AUD50,000 per year[1]). Seventy-five per cent lived in inner metropolitan areas based on residential postcodes and the Australian Standard Geographical Classification system. The educational profile of the participants was mixed: 29% had completed high school only, 22% had a vocational qualification, 22% had completed a university degree and 16% had postgraduate qualifications, and 23% were currently studying either full- or part-time.

III. RESULTS AND DISCUSSION

a) Motivations for Purchasing/Eating Free-range and Cage-free Eggs

Participants associated free-range or cage-free eggs with superior quality in comparison with eggs from caged hens. Quality was mentioned much more readily as a motivating factor for purchase rather than concerns for hen welfare, suggesting that the behavior is directed more toward the consumer, rather than the hens. Participants talked about the superior sensory characteristics of free-range eggs, in particular their taste and yolk color, and free-range eggs also were said to provide greater nutritional benefits than their conventionally produced counterparts. Leaving aside the possibility that these products in fact may have superior attributes over caged-eggs (Hammershøj and Steenfeldt, 2015), labelling may be influencing the association between egg production system and quality. Participants may be using the labels as proxies for “good” or “bad” (Eden, 2011); however, the emphasis placed on superior sensory characteristics suggests that our participants are making an implicit association between free-range and a better, healthier product, and this tendency likely is a result of a “halo effect,” where the evaluation of one attribute strongly influences another (Lee et al., 2013). The label itself may also influence perceptions of taste; it has been shown that people rate animal products labeled with “humane” as tastier than those with other labels (Anderson and Barrett, 2016).

The hen’s diet was very important to our participants, and was used to explain both the superior quality as well as how caged egg production was “not natural” more readily than freedom to roam or other behaviors. By their accounts, birds in free-range systems had more natural or better diets, mostly because of what they were thought *not* to be eating, specifically “chemicals” such as hormones and antibiotics. In addition, participants described hens in cages as being fed unknown substances that hens would not choose themselves in comparison with feed available in free-range systems. This concern about hen diets is a novel finding with respect to preferences for non-caged eggs. Confinement was seen to restrict natural behaviors, but in particular it is seen as preventing the hens from consuming a “natural” diet. A general preference for “natural” foods (relating to process of production more than content; Rozin, 2005), has been well documented, particularly in relation to genetically-modified (GM) foods (Rozin et al., 2012; Mielby et al., 2013). We suggest that it is the perceived role of “additives” (Rozin, 2005; Rozin et al., 2009) in the hen’s diet that is the main driver in our participants’ descriptions of non-caged and free-range eggs as “natural.” In addition, based on our participants’ responses we suggest that disgust, which influences food purity attitudes (Clifford and Wendell, 2016), also is closely aligned with preferences for non-caged eggs.

Some participants made broader links between animal wellbeing and their own health. The idea that "what is better for the animal is better for me", and that non-caged eggs were better for people to eat was thought to be obvious by our participants, though this conclusion was typically based on limited and subjective evidence. These associations between animal diet and wellbeing, and egg quality, and the obviousness attributed to them, suggest that the participants felt that these factors are linked to health in a "you are what you eat" manner, and could be interpreted as "magical thinking" (Rozin et al., 1986), for example the 'laws of contagion' and transmission of 'stress' from caged-hens as mentioned by one of our participants. Magical thinking has been explored in relation to GM and organic foods (Saher et al., 2006) and warrants further examination in relation to animal products.

b) Eggs Compared with Other Animal Products

There are four key factors that help to explain the dominance of discussion of free-range eggs over other products with animal welfare claims, despite asking generic questions about ethics in relation to animal products. First, there were high levels of awareness about the use of cages in egg production, which participants thought was undesirable. Participants mentioned recent advertisements by activist groups as well as documentaries and the activities of celebrity chefs as sources of information. Second, participants compared free-range eggs with other products such as free-range chicken meat in their explanations, typically mentioning clearer labelling and prominent positioning within the supermarket as contributing to purchases. Third, for many respondents, the price difference between caged eggs and other products was perceived to be small enough that even those from lower socio-economic groups could purchase free-range despite the higher price, whereas meat was already an expensive item and thus free-range meat was considered too expensive. Finally, participants described obtaining eggs from their own hens or sourcing them from friends or family, and described these as "free-range".

c) Information and Trust

Although increasing public concern about animal welfare in Australia is often linked with the so-called "urban-rural divide," (Meyer et al., 2012), eggs provide an interesting counterexample, given increasing numbers of small urban flocks which allow urban dwellers more direct contact with poultry. Participants talked about backyard egg production as a way to control "unknowns," particularly about what hens were being fed, and as such reduce the risks to which they and their families were exposed. Having 'backyard chooks' was also spoken about as a way of knowing about good farm animal welfare. Participants who had poultry in the past, or who currently kept small numbers of hens for household egg production, used their personal knowledge to justify their claims that intensive production was cruel and "disgusting". Backyard hens may be more appropriately considered as pets than production animals (Elkhoraibi et al., 2014), and so may be supporting a range of different values and associations than would be typical for food production animals. This topic warrants further investigation particularly given the rapid increase in the numbers of people keeping such animals in Australian cities.

The relationship between trust and risk reduction is characteristic of many contemporary consumer interactions, particularly in highly risk-adverse environments such as ours (Lupton, 1999). Trust was unsurprisingly extended to family and friends, but butchers also were seen as important sources of information on provenance and as such were a preferred place to buy both meat and eggs. However, it seems that for most participants, basic labels provided enough information to enable them to choose one product over another (free range/cage-free over caged eggs) at the point of purchase and there was infrequent discussion

of additional information on labels such as stocking densities or voluntary certification. Despite this, many participants were skeptical of the labels and some had even attempted to verify the claims made by checking the companies' websites for details about conditions.

IV. CONCLUSIONS

Our findings show that there is a strong link between free-range (or cage-free) eggs and perceptions of quality motivating people to purchase these products and appears to be playing much greater roles among these consumers than considerations about animal welfare. There were high levels of awareness of caged-egg production and strongly-held perceptions that caged-egg production is "wrong," unnatural, and even disgusting, with diet and confinement being key (negative) aspects mentioned by our participants. However, these were only secondary reasons why participants were buying free-range eggs, as their main focus was on quality. Although emotional states of nonhuman animals are increasingly becoming an important area of animal welfare science, in the marketing of animal products happiness is presented as being a factor contributing to better-tasting food (Miele 2011) and our participants largely viewed the happiness of the chicken as "good" because of its influence on the eggs produced by them, rather than as "good for the chicken" as such. The idea that free-range or cage-free production systems are better for hens was not questioned or critiqued by the participants in our study, despite the presence of factors that can affect animal welfare in these systems, as it was not seen by them as central to their purchasing decisions.

These findings present challenges for those interested in improving animal welfare as well as advocates for ethical or political consumerism. It could be argued that, if perceptions of quality drive consumption of products that ultimately generate better welfare for laying hens, then a lack of engagement with ethical issues in egg production on the part of consumers may not matter. However, as highlighted by Parker and Costa (2016) and Miele (2011), ethical and animal welfare issues are not absent in free-range systems. In addition, if increasing consumption of free-range and cage-free eggs (along with other products with animal welfare claims) is being viewed by industry and government as an indicator of community concern for farm animal welfare, then estimates about the levels of concern, and resulting shifts in policy and/or production methods, may be based on false assumptions. Hence, for those interested in promoting animal welfare, it is critical to note that purchasing preferences alone may not indicate increasing support for humane production processes. Instead, it is critical to engage with consumers around the values underlying their preferences in order to better comprehend evolving understandings of various ethical food categories.

Overall, we contend that purchasing free-range or cage-free eggs was not considered to be an act of political consumerism with respect to farm animal welfare by most of our participants. However, the perception that caged-egg production was in various senses "bad" suggests that, as citizens, the participants in our study are not supportive of caged-egg production. More research is needed to understand and unpack community sentiments and explore whether policy changes, either with regard to production methods or labelling would be supported. This study also reveals that even within the "ethical consumption" domain, purchasing decisions are complex and include a range of factors that operate outside what most would strictly consider to be "ethical" considerations.

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ENHANCING RESEARCH COMMUNICATION THROUGH INFORMATION DESIGN AND VISUAL STORYTELLING: REFLECTIONS ON 10 YEARS OF APSS PROCEEDINGS FIGURES

M. KRZYWINSKI[1]

Summary

Figures have the potential to illustrate complex concepts and patterns that would otherwise be difficult to express concisely in words or notice quickly from a table. Figures that are clear, concise and attractive help to form a strong connection with the audience, communicate with immediacy and accelerate understanding and progress. This can be achieved by employing principles of graphic design, which are based on our understanding of how we perceive, interpret and organize visual information. This article presents practical guidelines for visually communicating data and experimental protocols to assist poultry researchers in presenting their research to colleagues and those outside of the field. Several redesign examples using figures taken from past APSS Proceedings are presented to concretely illustrate the application of information design concepts such as data encoding, use of color, visual conventions and metaphors. The examples demonstrate how to more clearly show essential aspects of the data, combine different modalities and correlate patterns across observations and treatments. To strengthen reach to industry, the public and policy makers, an unadorned and field-agnostic visual style is used to improve consistency and clarity of how information is presented.

I. INTRODUCTION

All of us are schooled in 'written design' (grammar) and most have had some experience with 'verbal design' (public speaking). However, relatively few have had training in 'visual design' (information design and visualization). Thus, when we need to present complex information visually, we may find ourselves at a 'loss for words', graphically speaking.

Just as text must be grammatically and semantically sound, figures should embody equivalent visual qualities. These qualities can be realized by using principles of graphic and information design that are based on our understanding of how we perceive, interpret and organize visual information. This information is underpinned by conclusions from studies in visual perception and awareness (Cleveland and McGill, 1984, 1985; Yantis, 2005; Fecteau and Munoz, 2006). In addition to informed choice of data encoding (e.g. bar, line, scatter plot, etc.), the use of color, placement of labels and annotation, visual weight of navigation elements and flow of information across panels all influence how well the figure can communicate concepts, proportions and patterns. These elements must be in balance to match salience to relevance - drawing the eye to important patterns and subtly discouraging irrelevant or misleading interpretations that can arise when it is not clear where to look (Wong, 2011).

To make the design advice presented here practical, I have applied it to examples that are representative of the current poultry research literature. I have reviewed and tabulated all figures in the past 10 years (2008–2017) of the Proceedings of the Annual Australian Poultry Science Symposium (APSS), categorizing them by type, use of color, use of 3-D and texture (Figure 1). I counted 481 distinct figure panels, of which the majority (366, 76%) grouped into three basic types: 162 (34%) bar charts, 118 (25%) line charts and 86 (18%) scatter plots (Figure 1A). More than 50% of all figures were black-and-white and 34% of the top three

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types used one or more colors (Figure 1B). 23% of bar charts used textures and 51% of line plots used dotted, dashed, or otherwise stippled lines to distinguish traces (Figure 1C).

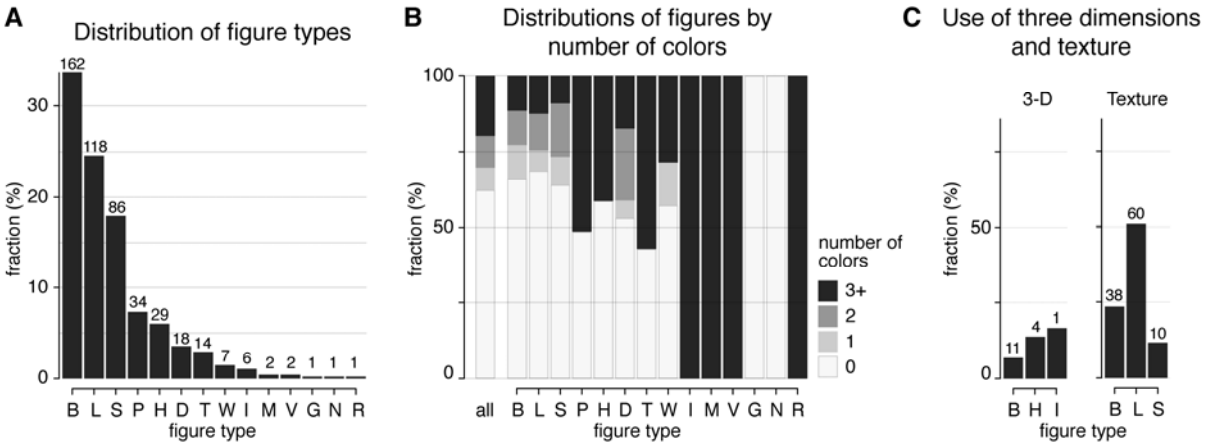

Figure 1 - The distribution of plot types and format properties of 481 figures that appeared in 10 years of Proceedings of the APSS during 2008–2017. Values above bars show the absolute count. (A) Fraction of each figure type. (B) Breakdown of all figures (first column) and figures by type (remaining columns) by the number of colors used (0 indicates black-and-white or greyscale). (C) The fraction of figures that appeared in three-dimensions and employed the use of textured patterns. Texture for line and scatter plots indicates use of dashed, dotted, or otherwise stippled lines. Plot type codes are: B (bar), L (line), S (scatter), P (photo), H (heat map), D (diagram), T (ternary), W (box plot), I (pie chart), M (geographical map), V (Venn diagram), G (gel), N (network layout), R (rose plot).

II. BAR CHARTS

The bar chart is the most common figure type in APSS Proceedings. This example will focus on presenting raw data together with output of statistical testing, selecting grey levels and managing labels in the plot and in the legend.

The original figure (Figure 2A) (Hughes, et al., 2016) shows the effect of a feed enzyme additive on the apparent metabolisable energy (AME) of wheat. In crowded charts (this one has 58 bars), white bars can be difficult to distinguish from the space between bars—an effect described by the figure-ground Gestalt principle (Wong, 2010a, b). Bars with a solid fill stand apart from the background more effectively (Figure 2B). Angled axis labels make it difficult to include labels for all 29 wheats in the original figure - only 15/29 labels are shown and only for 5/10 wheats with a significant effect. Angled labels also match poorly with their bars because they extend beyond neighbouring bars. If labels are long (e.g. complex experimental conditions) but need to be easily read, a horizontally formatted figure is better. When labels are short sample IDs, as here, a vertical label orientation is a good compromise between legibility and compactness. The axis tick marks appear between bar groups in Figure 2A, which is inconsistent with how ticks work in other data encodings. The role of the tick mark is as a callout line to its label and not a group separator - groups can be separated by space.

To visually assist hypothesis testing, figures should include the output of statistical tests, such as significance. The observation that 7/10 wheats with untreated AME < 13 showed an enzyme effect would be made more salient if the '*' significance symbol were placed closer to the untreated AME bar. However, this is difficult to achieve when bars are so close together. The significance stars are more optimally placed near the wheat labels (Figure 2B). This has the effect of easily connecting the treatment effect to the label as well as facilitating counting the stars (because they are now aligned) for a given AME cutoff.

Although reporting statistical significance using a *P* value cutoff (so-called bright line testing) is widely used, the *P* value alone does not tell the full story (Krzywinski and Altman, 2014b; Altman and Krzywinski, 2016, 2017). Ideally, both the effect size and 95% confidence interval (CI) should be reported (Krzywinski and Altman, 2013a) and both have been added for each wheat in Figure 2B. The effect size relates to the magnitude of the change that is observed, which may be statistically significant but not necessarily biologically relevant. The CI tells us the range of AME changes that are statistically compatible (do not test as significant) with the observed change. Thus, when the 95% CI includes zero, we conclude that no effect is present. Showing CIs enhances our interpretation of significance - we can now tell how close we are to the bright line cutoff (impossible from a statement like '$P < 0.05$') and whether increasing sample size might make the observation significant. Note the two wheats in Figure 2B that are annotated with an arrow - the left shows an effect (just barely) and the right does not (again, just barely). If the experiment was repeated, we would have good reason to expect that the significance status of these wheats changes. Always keep in mind that, because samples are variable (Krzywinski and Altman, 2013b), so are their 95% CIs.

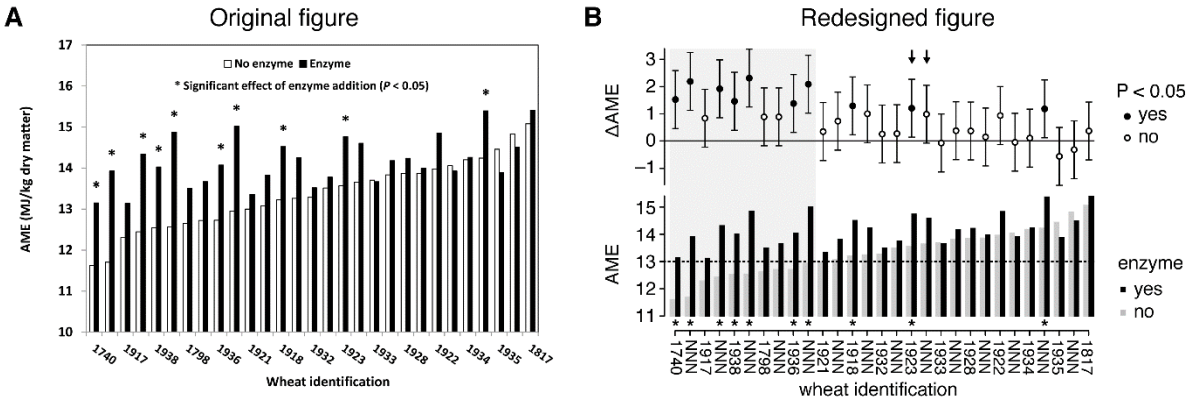

Figure 2 - A grouped bar chart can show the effect of a treatment but a large number of observations can reduce legibility of values and labels. (A) Original figure from (Hughes, *et al.*, 2016). (B) The redesigned figure. Statistical significance is communicated both with the conventional '*', conveniently next to the wheat label, as well as a scatter plot of AME change and 95% confidence interval (CI) (arrows point to one pair of near-identical cases of which only the left is significant, barely). Focus on wheats with AME < 13 is achieved with a horizontal dashed line and grey highlight. The label 'NNNN' is used for wheat whose identifier was not available in (A). The 95% CIs drawn are visual placeholders for actual intervals that would be drawn—ones shown are estimated and made all identical.

The panels in Figure 2B share the horizontal axis and both vertical axes for AME and ΔAME have the same scale to facilitate comparison of vertical distances between panels. Vertical axes are clipped to the range of the data to allocate more of the figure's space to where data are changing. In the original figure the AME axis range of 10–17 is too generous - there are no values for AME < 11 or AME > 16. Where possible, the legend should not encroach on space that would be better used by data and should be set as a table with as little duplication in text as possible. There is also usually no need for a top and right axis, which unnecessarily contain the plot and add clutter.

From the scatter plot of ΔAME in Figure 2B, we can glean that all the significant observations have ΔAME > 1, a reflection of the power of the experiment. Power should always be reported - when it is low, only large effects can be detected, and negative results (such as the sample called out with the right arrow in Figure 2B) cannot be reliably interpreted (Krzywinski and Altman, 2013c). Since a pair of bars showing untreated (AME–) and treated (AME+) AME can just as easily be shown as a scatter plot, I've adopted this approach in Figure 3A. I have also adopted the notation in which untreated and treated

conditions are denoted as a suffix (– vs +). Note that the treatment suffix is not a dash '-' but an n-dash '–', which is longer and visually more compatible with '+'. The n-dash is the appropriate character for intervals (e.g., 10–17) and negative quantities (e.g., $x = –2$).

The scatter plot of ΔAME vs AME– clearly shows that all differences ΔAME > 1 are significant and makes any outliers evident. It is more useful than the plot of AME+ vs AME– , since the experiment is more cogently explained in terms of AME– and ΔAME because it is the change in AME, not its final value, that is more relevant.

One essential observation from the experiment is the extent to which untreated AME affects the efficacy of enzyme treatment. The point that 7/10 wheats with AME < 13 showed an effect (Figure 2B) has already been made anecdotally. By fitting the fraction of wheat in a range of AME values (e.g. 11–12, 12–13, and so on) as a function of AME– we can quickly see that the higher the AME–, the less likely we are to observe an enzyme effect (Figure 3B). Furthermore, from the slope of the fitted line, we can say that each unit of AME– increase in the range 11–16 leads to an absolute decrease in the fraction of wheat with an effect by 25%.

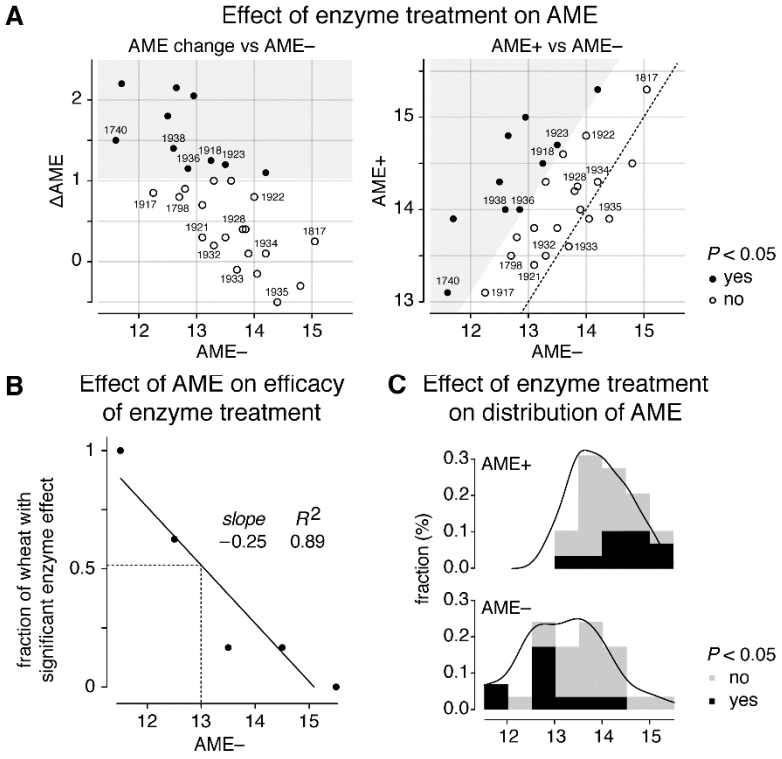

Figure 3 - Alternative ways to a bar plot for displaying a 2-level single factor experiment. (A) The change in AME in the presence of enzyme treatment (ΔAME, left) and treated AME (AME+, right) shown as a function of untreated AME (AME–). The first clearly shows the ΔAME > 1 significance cutoff, which is harder to identify quantitatively in the second panel because it is a vertical distance between two oblique lines. Point labels are wheat identifiers. (B) A fit to the fundamental relationship addressed by the experiment: the impact of enzyme efficacy on AME increase as a function of AME–. Only the slope and R^2 are reported for the line, since the intercept has no meaningful interpretation. (C) Distribution of AME– and AME+ is useful to understand the bounds of the measurement.

Finally, let's use a frequency plot to explore the distribution of untreated and treated AME values (Figure 3C). Although these distributions do not distinguish how AME changed for any specific wheat, they clearly indicate that AME reaches a maximum of about 15, regardless of treatment, and that treated AME has a smaller spread than untreated AME, presumably because the enzyme treatment is more effective for smaller AME values.

Frequency plots and box plots (Krzywinski and Altman, 2014a) powerfully communicate features of populations and samples. Unfortunately, both are underrepresented in the APSS Proceedings. Although they may be at first hand unfamiliar, they are simple to grasp and have great utility. Figure 1 in (Greenhalgh, et al., 2017) shows the variation in feed conversion ratio (FCR) of 140 birds using jittered points (Figure 4A), making it difficult to judge the spread and extent of outliers. These data are better shown as a histogram of bird count for each FCR interval (e.g. 0.1), which makes it is easier to see both the spread and the top and bottom 15% of birds, which were monitored in the experiment (Figure 4B). Importantly, the histogram visually justifies the 15% cutoff for classification as low- and high-efficiency, something that can't be identified from the original in Figure 4A.

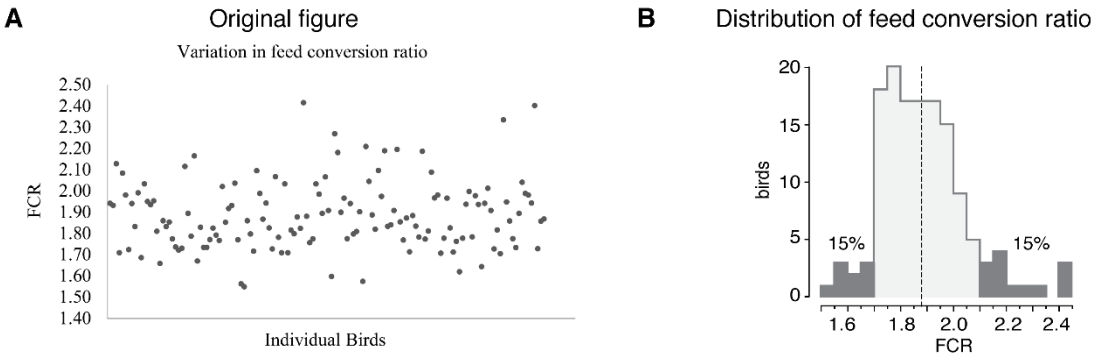

Figure 4 - A histogram clearly communicates the location and spread of a distribution. (A) Original figure from (Greenhalgh, *et al.*, 2017). (B) The same data shown as a histogram with bins of 0.1. The distribution average is shown as a vertical dashed line (1.88) and the bottom and top 15% percentile highlighted.

III. LINE PLOTS

This example is of an experiment studying the effect of 4 levels (1, 2, 3 and 4%) of Ca supplement in the diet. The original figure (Figure 5A) is taken from (Bradbury, et al., 2014) and shows the supplement's effect on daily feed and weekly separate source Ca intake.

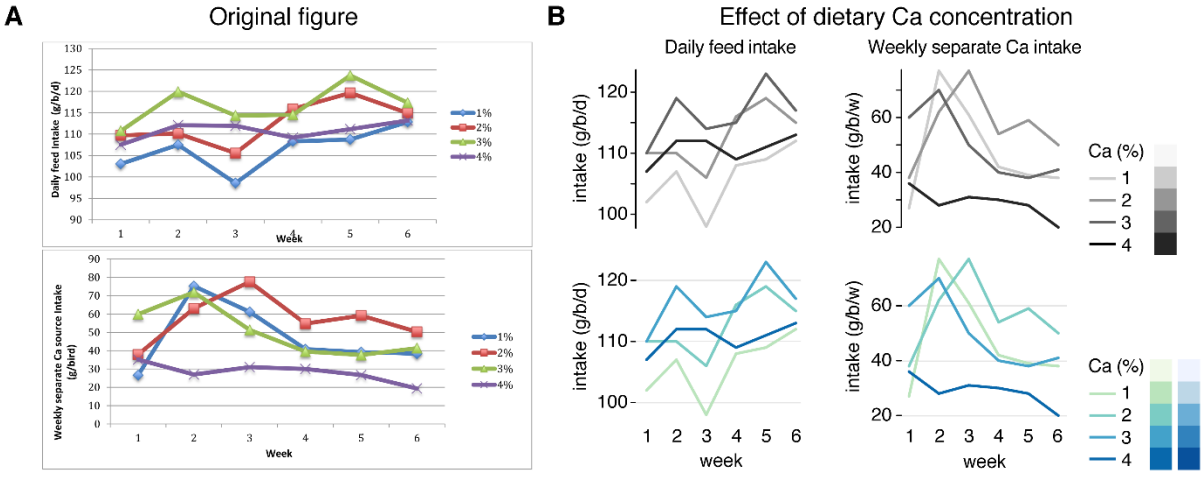

Figure 5 - Effect of Ca supplement level (1–4%) on daily feed intake and weekly separate Ca intake. (A) Original figure from (Bradbury, *et al.*, 2014). (B) Data shown in black-and-white (top row) or color (bottom row) with simplified formatting to emphasize trends in the line profiles. Colors 2–5 from the 5-color grey and blue-green Brewer palettes are used for the lines. Also shown is the 5-color blues Brewer palette (bottom row) as a perceptually quantitative option with constant hue.

The line plot - the second most common APSS Proceedings figure type - is a good choice here. The line emphasizes trends, especially when the number of observations is low

(6 points per trace). The choice of color (blue, red, green, purple), however, is inappropriate for the factor - these colors are more suitable for a categorical variable (one for which there is no inherent order). The Ca level is a quantitative variable and the colors chosen do not reflect this. Purple does not communicate that its level is 4× the level encoded by blue.

The figure can be reformatted to black-and-white (Figure 5B, top panels) without loss of clarity. The grey scales for each trace can be selected from the grey sequential Brewer palette (Harrower and Brewer, 2003), which has steps of grey that are approximately perceptually uniform (the difference in perceived brightness between adjacent tones is similar). Alternatively, a colored sequential Brewer palette (e.g. blue-green) can be used (Figure 5B, bottom). Using color makes comparing the plots easier—hue visually groups objects more powerfully than grey tone by the Gestalt principle of grouping (Wong, 2010a, b).

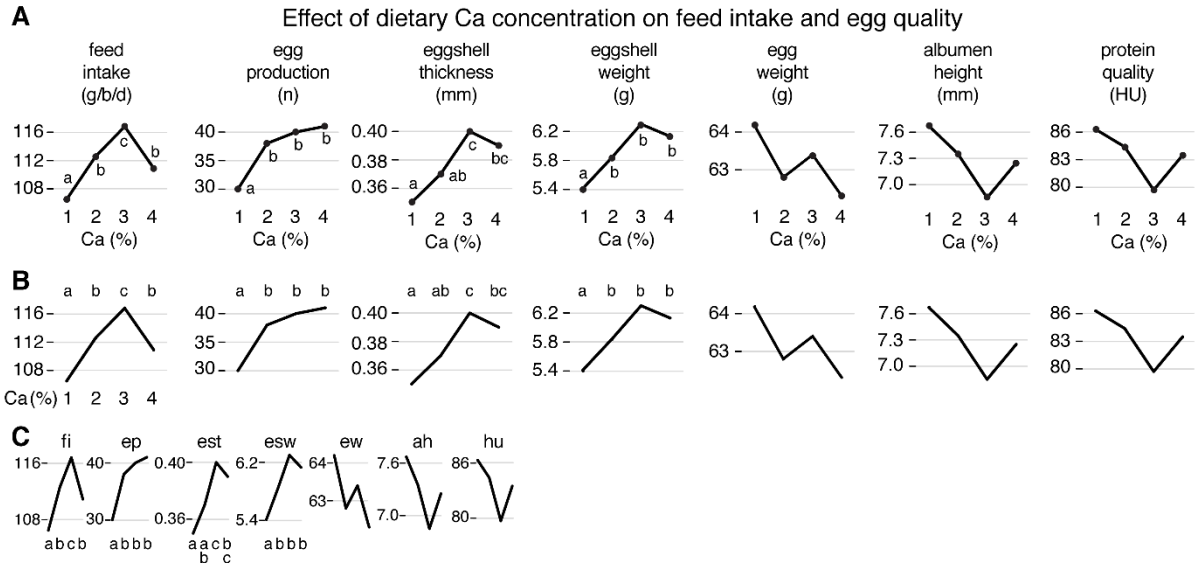

Figure 6 - Change in feed intake and egg quality as a function of Ca supplement level, shown at various level of design detail. (A) Line plots shown with data point glyphs and post-hoc pair-wise significance labels next to the points. The horizontal axis is labeled for each plot. (B) Data point glyphs have been removed and only the first plot has a horizontal axis label. Significance labels aligned on top of the panels to maintain emphasis on data. (C) A tight formatting of the plots, with fewer grid lines and plot titles shown as variable acronyms—a practice that can save space but requires explanation.

Not every plot element in Figure 5B is needed. The horizontal axis for the week number is not necessary—axis lines for categorical, ordinal or otherwise discrete variables can mislead the reader that intermediate values are possible. For example, in Figure 2B the *x*-axis line can be removed—the bottoms of the bars provide a sufficient visual anchor. In Figure 5B the vertical axis can be removed—now any line with a vertical component represents data.

Figures with subtle formatting are ideal when there are a lot of data or insufficient space (or both). Navigational elements (axes, grids, ticks) should have less visual weight than data to keep the data-to-ink ratio high (Krzywinski, 2013a). For example, a 20% opacity is recommended for grid lines (Stone and Bartram, 2009). Sometimes removing elements from a plot altogether helps add salience to data patterns. If you're not familiar with this practice, you may be initially uncomfortable with a plot missing elements that are traditionally present. However, many of these elements are actually superfluous (e.g. plot or legend border, frequent ticks or grids) and should be included only if they enhance your ability to understand the data, not because they are the default settings in your plotting.

Figure 5B shows only part of the picture. The experiment explored the effect of Ca supplement on 7 variables, all of which are tabulated (Table 1 in (Hughes, *et al.*, 2016)). Although tables are excellent for looking up precise values, they do not quickly reveal trends. If we plot the change in the 7 variables as function of Ca supplement level (Figure 6A), we can see that trends in the variables fall into groups. For example, feed intake, eggshell thickness and eggshell weight all from 1-3% then decrease. Similarly, albumen height and protein quality have almost the same profile shape, not surprising since the former informs the latter.

Not all trends in Figure 6A, however, are significant. Including letter subscripts to indicate significant pair-wise comparison (post-hoc Tukey test) helps focus on potentially meaningful changes. The letters should be carefully placed and aligned to avoid disorder and the inevitable confusion that follows. Letters chosen are arbitrary, though traditionally from the beginning of the alphabet in order of appearance. This is not optimal however, since some letters have similar shapes, such as 'a', 'c' and 'e'. It is better to select letters that have different shapes, such as 'h', 'q', 'x' (Krzywinski and Wong, 2013), especially for large plots in which many letters are used, such as Figure 4 from (Dersjant-Li and Kwakernaak, 2017) that uses 8 letters a–h with both positioning and color inconsistently applied.

When dealing with a large number of variable profiles, simplifying the figure by removing elements adds focus on the data (Figure 6B, C). For example, moving the pair-wise significance letters away from data points helps with comparison (because the letters are now aligned) and relieves clutter in the data panel. Other strategies to avoid crowding and emphasizing data and not formatting (Krzywinski, 2013b) include cropping the vertical scale to allow as much room for data as possible (McInerny and Krzywinski, 2015) and limiting the use of large glyphs, drop shadows or gradients (Figure 4A has all three).

IV. EXPERIMENTAL PROTOCOLS

The final example visualizes both the experimental protocol and data simultaneously. The experiment depicted in the original table and figure (Figure 7) explores the effect of two differently changing lighting schedules on egg laying times and rate (Cronin, et al., 2010).

A Original table

Table 1. Clock times (h) when the room lights were switched on and off each day over progressive weeks of the experiment, and the median times of egg laying per week for birds in the Gradual and Abrupt treatments.

	Gradual treatment				Abrupt treatment				Median egg laying times		
Hen age (wks)	On	Off	On	Off	On	Off	On	Off	Gradual	Abrupt	SED
17	0600	1900			0600	1900			0740	0740	0.553
18†	0600	1900	0230	0300	0600	1930			0620	0730	0.601
19	0600	1900	0200	0300	0600	2000			0545 [a]	0740 [b]	0.473
20	0600	1900	0130	0300	0600	2030			0630 [a]	0730 [b]	0.316
21	0600	1900	0100	0300	0600	2100			0540 [a]	0720 [b]	0.298
22	0600	1900	0030	0300	0600	2130			0650 [a]	0830 [b]	0.511
23‡	0600	1900	0000	0300	0600	1900	0300	0600	0620 [a]	0805 [b]	0.620
24	0600	1900	0000	0300	0600	1900	0300	0600	0650	0725	0.659
25	0600	1900	0000	0300	0600	1900	0300	0600	0720	0715	0.554
26	0600	1900	0000	0300	0600	1900	0300	0600	0730	0735	0.625

Within hen age pairs, values with different superscripts differ a,b: P<0.05
SED: standard error of difference between the means
Start of Gradual and Abrupt Light Introduction Treatments indicated by † and ‡, respectively

B Original figure

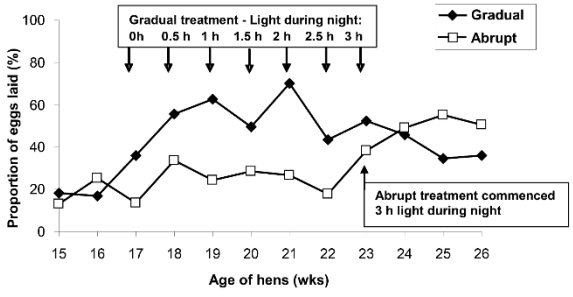

Figure 7 - The effect of two light-dark protocols on egg laying times and rate. From (Cronin, *et al.*, 2010) (A) The light schedule and median egg laying times for gradual (interrupted night) and abrupt (longer day) treatments. (B) The effect of the treatments in (A) on egg laying rate.

The light schedule table (Figure 7A) is easy to understand but requires time to parse. Many values are actually repeated and these would be better shown as a dot to indicate repetition of value in the row above. For example, it is not immediately clear that the amount of additional light each week is the same (30 minutes) for both protocols. This fact is more easily understood from the text and, at that point, the table is no longer required. Similarly, trends in the egg laying times are also difficult to spot.

It is reasonable to try to use a clock to explain the two lighting schedules (Figure 8). This format is familiar and it is easy to spot the difference between the schedules: interrupted night (gradual) and longer day (abrupt). However, it takes longer to identify the weekly constant 30 minute light increase.

For all its familiarity, the real trouble with Figure 8 is that it is very difficult to overlay egg laying times and rates in a way that is simple and quantitative—there is no single axis on which egg laying time can fall. Placing these times on the clocks would make identifying trends across the clocks difficult. Using the clock metaphor has helped one aspect of communication but hindered another. Although we could draw the observations in a separate figure, as done in the original publication (Figure 7B), let's see how both the schedule and observations can be combined the same panel—I present two options.

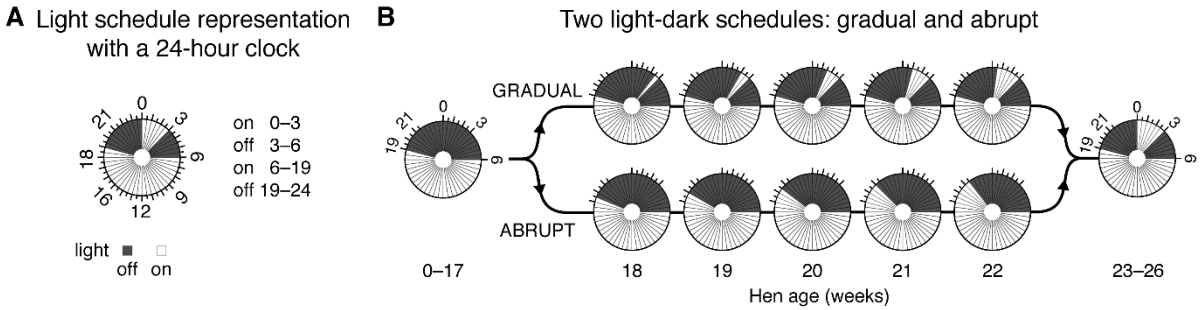

Figure 8 - Representing light schedules using a 24-clock. (A) The face of a 24-hour clock is colored based on light (white) or dark (black) conditions. Here light is applied between midnight–3am (0–3) and between 6am–7pm (6–19). (B) Two light-dark schedules for transitioning hens from a full night of darkness to 3 hours of light. The gradual schedule acclimatizes the hens to 3 hours of night light by subjecting them to increasingly more light each week. The abrupt schedule extends light during the day by 30 minutes each week but does not change night light conditions. For simplicity, only times at which light schedule is varying are labeled on the clock.

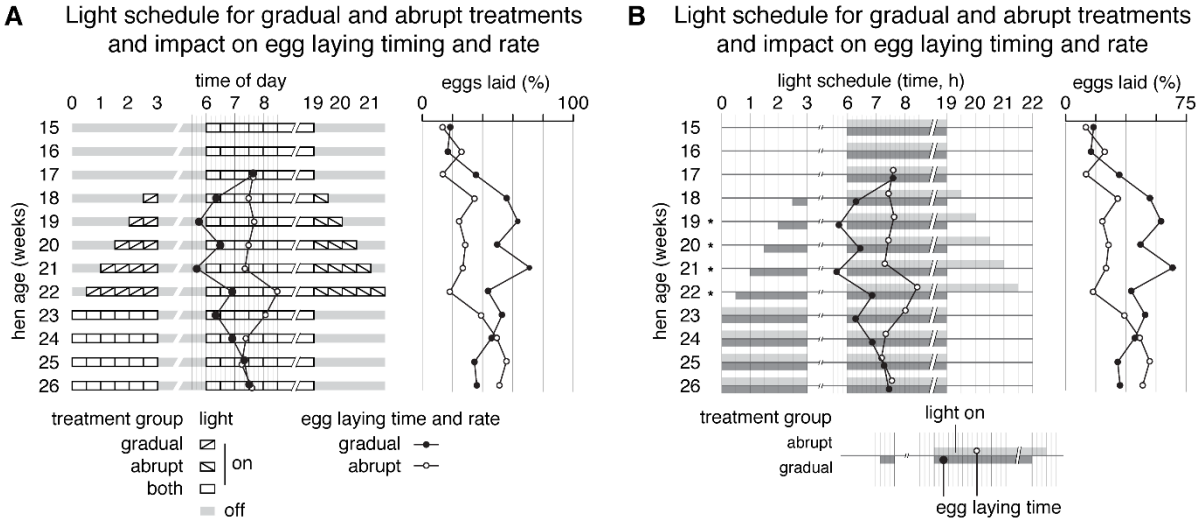

Figure 9 - Two alternatives for combining light-dark schedules and experiment results in a single panel. (A) Light/dark metaphor informs the color of boxes that indicate absence and presence of light. (B) Ink is used to show when light is on and darkness is inferred from where bars that indicate light are absent.

In the first option (Figure 9A) adopts the dark/light metaphor whereby white boxes are used to indicate light condition and grey strip as dark condition. Light during the gradual and abrupt treatments is distinguished by the direction of the line through the box. The day line acts as a natural time axis for the time laying times. By overlaying experimental

observations on top of light schedule, we can correlate patterns better. For example, we can quickly see something that is very hard to spot from the table in Figure 7A: there are two weeks at which hens are laying eggs in the dark (week 19 and 21 in the gradual treatment). We can also see that the highest egg laying rate happens during precisely these two weeks.

In the alternative presentation (Figure 9B), the light/dark metaphor is not used. Instead, horizontal bars show the duration of light for each group. Using ink in the figure to encode when light is present and no ink for when it's absent connects more closely to the theme of the experiment. Note that the legend of Figure 9B is formatted similarly to the figure itself—a practice that helps explain the position and identity of all the elements in a complex display.

V. PARTING THOUGHTS

An experiment that took months (or years) to complete deserves a figure that took several hours (or days) to prepare. Begin designing a figure by identifying the core message, which should be salient so that it can be grasped quickly. Avoid distracting and unnecessary elements to ensure that the visual communication is immediate. Default software settings often pollute output with unnecessary garnish—once done with a figure, check for elements that may be removed without impacting the message. Follow best practices (Tufte, 1992) and resist the urge to depart from effective visual paradigms unless your data set absolutely requires it.

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EFFECTS OF FEED ACIDIFICATION AND CONDITIONING TEMPERATURE ON NUTRIENT DIGESTIBILITY AND PERFORMANCE OF BROILER STARTERS FED WHEAT-BASED PELLETED DIETS

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Summary

The present experiment was designed to examine the influence of acidifier inclusion and conditioning temperature in wheat-based pelleted diets on the performance and nutrient utilisation of broiler starters. The experimental design was a 3 × 2 factorial arrangement of treatments, which included three inclusion levels of an acidifier (Amasil® NA; 0, 7.0 and 10.0 g/kg) and two conditioning temperatures (60 and 90 °C). The acidifier increased ($P < 0.05$) the apparent ileal digestibility (CAID) of dry matter (DM), nitrogen (N), fat and phosphorus (P) at both inclusion levels, and of starch at 10 g/kg inclusion. Increasing conditioning temperature from 60 to 90 °C reduced the CAID of DM, starch, fat and Ca. Neither the main effects nor the interaction between acidifier and conditioning temperature was significant ($P > 0.05$) for weight gain and feed per gain. Current findings demonstrate that feed acidification, through inclusion of organic acids, is beneficial to nutrient digestibility in broilers fed pelleted diets and also confirm the previously reported detrimental effects of application of high conditioning temperatures for poultry feed manufacture.

I. INTRODUCTION

Currently, the majority of feed used in the production of broilers is fed in pelleted or crumbled form. One of the major issues in the manufacture of pellets is the application of high conditioning temperatures. The need to reduce potential levels of feed-borne pathogens such as salmonella and campylobacter for feed safety and to achieve high pellet quality has led to the application of relatively high (between 80 and 90 ºC) conditioning temperatures during conventional pelleting processes, a practice which may not favour optimal nutrient availability. However, the true impact of conditioning temperature on nutrient availability of pelleted diets has not been clearly delineated due to the confounding effects of conditioning temperature and feed form or has been ignored due to a focus on physical pellet quality and feed safety. Abdollahi et al. (2011), by differentiating the effects of conditioning temperature from feed form, showed that application of high conditioning temperatures *per se* adversely influenced nutrient digestibility and energy utilisation in wheat-based diets. In a recent study with maize-soybean meal diet, Loar II et al. (2014) found that, as conditioning temperature increased from 74 to 85 and 96 ºC, digestibility of some amino acids decreased by 3 to 5%, and feed per gain was impaired by 3 points (1.96 vs 1.99) and 8 points (1.96 vs 2.04), respectively.

There are several strategies which can be employed to improve the physical quality of the pellets, instead of applying high conditioning temperatures. Pre-conditioning moisture addition in the form of water and, to a lesser extent, pellet binder addition, as well as using small diameter die holes and longer pellet lengths can create high quality pellets under low conditioning temperature (Abdollahi et al., 2013). The only remaining concern is the need to eliminate salmonella in feed, which is thought to require high-temperature heat treatment.

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Heat treatment is currently thought to be the most practical method to achieve satisfactory feed safety. But, considering the rising cost of feed ingredients and the negative impact of high conditioning temperature on nutrient availability and feed efficiency, there is a need to find new approaches to improving feed hygiene which are not detrimental to feed nutrients. The objective of the present study was to elucidate the influence of feed acidification and conditioning temperature on broiler growth performance and ileal nutrient digestibility.

II. MATERIALS AND METHODS

The experimental design was a 3 × 2 factorial arrangement of treatments evaluating three inclusion levels of a feed acidifier containing formic acid and sodium formate (Amasil® NA; 0, 7.0 and 10.0 g/kg) and two conditioning temperatures (60 and 90 °C). Three basal wheat-soybean meal-based diets with the three different inclusions of acidifier were formulated to contain similar levels of apparent metabolisable energy (AME), amino acids and other nutrients. All the diets had a background of 100g BASF Natuphos E 10,000 G (hybrid 6-phytase) and 100g BASF Natugrain TS (endo-xylanase and β-glucanase). Following mixing, each diet was divided into two equal batches. One batch was steam-conditioned at 60 ºC and the other at 90 ºC, and then pelleted using a pellet mill capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3-mm holes and 35-mm thickness. Conditioning time of the mash was 30 seconds and the conditioning temperature was measured at the outlet of the conditioner. All diets contained titanium dioxide as an indigestible marker. Each of the six dietary treatments was offered ad libitum to six replicate cages (eight birds per cage). Body weights and feed intake were recorded at weekly intervals throughout the 21-day trial. On d 21, ileal digesta were collected for determination of apparent ileal digestibility (CAID) of dry matter (DM), nitrogen (N), starch, fat, calcium (Ca) and phosphorus (P).

III. RESULTS AND DISCUSSION

The influence of acidifier inclusion and conditioning temperature on pellet durability index (PDI), growth performance and CAID of nutrients in broilers is shown in Table 1. Neither the main effects nor the interaction between acidifier addition and conditioning temperature was significant for weight gain and feed per gain ($P > 0.05$). There was a tendency ($P = 0.066$) for the acidifier to increase feed intake at 10.0 g/kg compared to diets with no acidifier. Whilst acidifier inclusion and increasing conditioning temperature both increased PDI, a significant ($P < 0.001$) interaction between acidifier addition and conditioning temperature also existed. While increasing conditioning temperature from 60 to 90 ºC improved the PDI, regardless of the acidifier inclusion, its effect was attenuated in diets containing acidifier.

Significant main effect of acidifier was observed for DM ($P < 0.001$), N, starch and P ($P < 0.05$), and fat ($P < 0.01$) digestibility. The acidifier increased the CAID of DM, N, fat and P at both inclusion levels, and of starch at 10 g/kg inclusion. There was a significant ($P < 0.05$) effect of conditioning temperature on the CAID of DM, starch, fat and Ca, with diets conditioned at 90 ºC having lower digestibility coefficients.

The use of acidifiers in poultry diets has increased over the years and their benefits on gut health (Adil et al., 2011), nutrient digestibility (Ao et al., 2009) and growth performance (Palamidi et al., 2017) have been documented. The current work also confirms the benefits in terms of enhanced nutrient digestibility, with acidification increasing the CAID of DM, N, starch, fat and P by an average of 6.6, 3.0, 1.1, 2.7 and 14.5%, respectively. It has been suggested that pH reduction in the digestive tract as a consequence of organic acid inclusion may increase the digestibility of protein, by enhancing pepsin activity and mineral absorption (Lückstädt and Mellor, 2011). It may also be speculated that, due to the shorter digesta

retention time and an elevated gizzard pH, because of an under-developed gizzard in pellet-fed birds (Abdollahi et al., 2013), the beneficial effects of feed acidification might be more pronounced in pellets than mash.

Feeding diets conditioned at 90 ºC was associated with digestibility reduction by 3.6, 1.3, 1.9 and 36.5% for DM, starch, fat and Ca, respectively, compared to those conditioned at 60 ºC. Several studies have shown the negative impacts of high conditioning temperatures on nutrient digestibility and bird performance (Raastad and Skrede, 2003; Abdollahi et al, 2010; Loar II et al., 2014). Abdollahi et al. (2011) reported decreases in starch digestibility from 0.98 in wheat-based diets conditioned at 60 ºC to 0.94 and 0.91 in diets conditioned at 75 and 90 ºC, respectively. Increasing conditioning temperatures above 60 ºC also reduced the AME of diets from 14.2 MJ/kg in diets conditioned at 60 ºC to 13.9 MJ/kg in those conditioned at 75 and 90 ºC. Overall, the current findings demonstrate that feed acidification, through inclusion of organic acids, is beneficial to nutrient digestibility in broilers fed pelleted diets, and also confirms the previously reported detrimental effects of application of high conditioning temperatures for poultry feed manufacture.

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Table 1 - Influence of acidifier inclusion and conditioning temperature on pellet durability index (PDI, %), growth performance, coefficient of apparent ileal digestibility (CAID) of dry matter (DM), nitrogen (N), starch, fat, calcium (Ca) and phosphorus (P) in broilers fed pelleted diets[1].

Item			Growth performance			CAID					
Acidifier (g/kg)	C. temp. (°C)	PDI	Weight gain	Feed intake	Feed per gain	DM	N	Starch	Fat	Ca	P
None	60	75.7e	1022	1351	1.333	0.612	0.783	0.940	0.885	0.215	0.494
	90	88.7b	1029	1364	1.330	0.593	0.766	0.936	0.875	0.121	0.474
7.0	60	82.7d	1061	1389	1.310	0.663	0.807	0.955	0.920	0.292	0.578
	90	88.2b	1033	1370	1.333	0.629	0.789	0.929	0.897	0.168	0.545
10.0	60	84.2c	1049	1394	1.337	0.647	0.794	0.957	0.909	0.249	0.561
	90	90.3a	1043	1401	1.344	0.632	0.800	0.953	0.890	0.189	0.531
Pooled SEM		0.36	13.3	16.4	0.0111	0.010	0.009	0.006	0.008	0.041	0.026
Main effects											
Acidifier (g/kg)											
None		82.2	1025	1358	1.331	0.603	0.774	0.938	0.880	0.168	0.484
7.0		85.4	1047	1380	1.321	0.646	0.798	0.942	0.909	0.230	0.562
10.0		87.2	1046	1398	1.340	0.639	0.797	0.955	0.899	0.219	0.546
C. temp., °C											
	60	80.8	1044	1378	1.326	0.641	0.794	0.951	0.905	0.252	0.544
	90	89.1	1035	1378	1.336	0.618	0.785	0.939	0.888	0.160	0.517
Probabilities, P ≤											
Acidifier		0.001	0.207	0.066	0.246	0.001	0.034	0.025	0.005	0.296	0.015
C. temp., °C		0.001	0.422	0.984	0.321	0.013	0.260	0.029	0.015	0.011	0.208
Acidifier x C. temp.		0.001	0.417	0.598	0.531	0.641	0.373	0.131	0.729	0.744	0.968

Acidifier, a mixture of formic acid and sodium formate (Amasil® NA); C. temp, conditioning temperature.
Means in a column not sharing a common letter (a,b) are significantly different (P < 0.05).
[1] Each value represents the mean of six replicates (eight birds per replicate).

CAN XYLANASE INHIBITORS AFFECT THE EFFICACY OF XYLANASES IN POULTRY DIETS?

H. GRAHAM[1] and R. JONES[2]

Summary

Xylanase inhibitors are reported to be widely present in cereals, and can influence the assay of supplemental xylanases in feeds. However, little is known about the presence of these inhibitors in animal feeds or how they might affect the efficacy of supplemental xylanases. In the present study, a method was developed for the indirect quantification of xylanase inhibitors. This method demonstrated that commercial feed xylanases differ considerably in susceptibility to xylanase inhibitors, and that many of the cereals and all feeds tested contained varying levels of inhibitors. These inhibitors were largely unaffected by processing of the feed up to 90ºC, and partly survived passage through the broiler gut as far as the ileum. Thus, the variations in the level of xylanase inhibitors and in the susceptibility of xylanases could help explain some of the differences in efficacy between commercial products in animal trials.

I. INTRODUCTION

Xylans and cellulose are the predominant fibre-polysaccharides in animal feeds, and xylanases, together with phytases, are the most widely used supplemental microbial enzymes in feeds. Proteinaceous xylanase inhibitors (XI), primarily attributed to three types, *Triticum aestivum* xylanase inhibitor (TAXI), xylanase inhibiting protein (XIP) and thaumatin-like xylanase inhibitors (TLXI), are found in a wide range of plant feedstuffs, particularly cereals grains such as wheat, barley and maize (Goesaert et al, 2004). These proteins inhibit the activity of family 10 and 11 xylanases from both bacterial and fungal sources. As such they interfere with the analysis of xylanases in-feed, introducing uncertainty and reducing accuracy. However, it is not known if these inhibitors directly reduce the effectiveness of xylanases in the animal digestive tract, and thus could result in differences in efficacy between xylanases and responses in animal trials (Verhoeven et al, 2005). The current study adapted a quick method for the indirect determination of XI in feeds. This method was used to determine the susceptibility of a number of commercial xylanases to XI, and investigated whether feed processing or passage through the broiler digestive tract influences XI activity.

II. METHODS AND MATERIALS

A variation of the standard Megazyme Xylazyme AX xylanase in-feed assay method (McCleary and Monaghan, 1999) was used (pH 5.0 in acetate buffer, 50ºC, 60 min, OD at 590 nm). The test cereal or feed was extracted (10 g + 80 ml) into pH 5.0 acetate buffer, centrifuged (2000g, 10 minutes) and the supernatant titrated into the assay mixture to give an effective final feed concentration of 0-125 mg/ml. The concentration of feed giving 50% xylanase inhibition was calculated.

A number of cereals, including wheat, barley, maize and sorghum, were analysed for xylanase inhibition, and several commercial xylanases were tested for susceptibility to XI. A typical xylanase-free wheat-soyabean meal (SBM) broiler grower diet was conditioned for 30 sec at 70-90ºC, followed by pelleting in a semi-commercial pilot plant. The extracts from

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these feeds were analysed for xylanase inhibition Another xylanase-free wheat-SBM broiler grower diet was fed to chicks and jejunal and ileal digesta were collected at 21 days of age. After freeze-drying, these digesta samples were analysed for xylanase inhibition.

III. RESULTS

Analysis of a number of feedstuffs, including wheat, rye, barley, maize and sorghum, and broiler feeds established that most cereals and all feeds tested contained XI as demonstrated by xylanase inhibition. However, the commercial xylanases tested varied considerably in susceptibility. Some xylanases, such as Xylanase E, were not inhibited by any of the extracts tested, while others such as Xylanase W were inhibited by wheat, maize and feed extracts (Table 1).

Table 1 - Level of sample (mg/ml) need to reduce xylanase activity by 50%.

Sample	Xylanase E	Xylanase R	Xylanase W
Wheat-based broiler feed 1	>50	5.6	1.8
Wheat-based broiler feed 2	>50	5.2	2.5
Wheat	>50	3.9	1.4
Maize	>50	>50	31
Micronized maize	>50	>50	>50

Conditioning at 70-90ºC followed by pelleting through a pilot feed plant had no influence on the ability of the extract to inhibit wheat XI-susceptible Xylanase W, with feed at 12.5 mg/ml resulting in ~70% inhibition, irrespective of processing conditions (Figure 1).

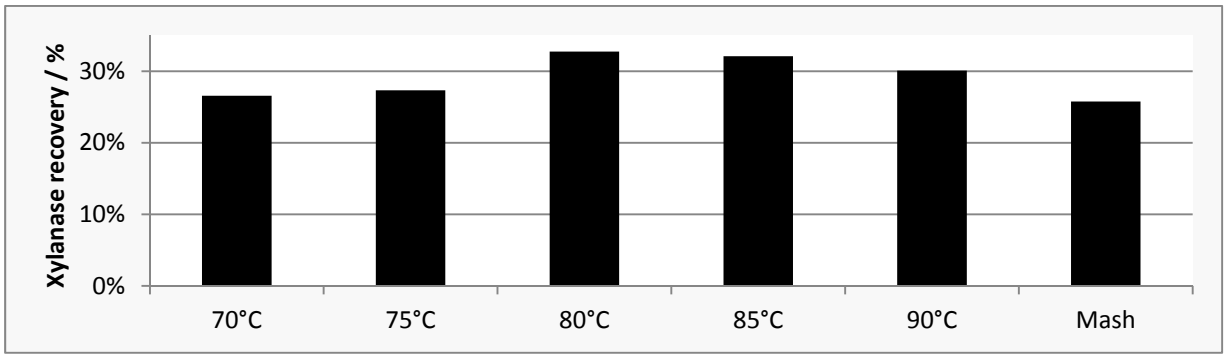

Figure 1 - Influence on the activity of xylanase W (% of expected) of wheat based broiler feed conditioning temperature (30 sec) when included at 12.5 mg/ml.

Extracts of a wheat-based feed fed to broilers and sampled at the jejunum and ileum confirmed that the XI survived sufficiently through the gut to inhibit xylanases R and W at both digesta sites (Table 2). There appeared to be a small inhibition of xylanase E at the jejunum, but this could be due to the presence of proteases which may have partly degraded this xylanase.

Table 2 - Level of sample (mg/ml) need to reduce the xylanase activity by 50%.

Sample	Xylanase E	Xylanase R	Xylanase W
Feed	>50	5.2	2.5
Jejunal digesta	16	9.6	10
Ileal digesta	>50	25	5.8

IV. DISCUSSION

The method developed for indirect XI analysis proved robust, allowing the rapid detection of xylanase inhibition activity in feedstuffs and feeds. The commercial enzymes tested varied considerably in susceptibility, with some not inhibited, some inhibited by both wheat and maize extracts and others inhibited only by wheat extracts. All the feedstuffs and feeds tested were shown to have XI, with activity varying both within and between feedstuffs.

Micronizing and autoclaving eliminated xylanase inhibition, as did heating the extract at 90^{o}C *in vitro* for 30 minutes, as might be expected for a proteinaceous inhibitor. However, conditioning feed at 70-90^{o}C for 30 sec followed by pelleting had little effect on the XI activity in the feed tested, showing that xylanase inhibitors are very likely to be present in the majority of xylanase-supplemented feeds as fed to animals. The survival of a significant fraction of these inhibitors through the gut as far as the ileum would further suggest that they could influence the activity of a number of supplemental feed xylanases *in vivo*. This may account for the differences in efficacy seen between xylanases, and the variation in animal response seen in xylanase trials.

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BIG DATA FOR POULTRY – WHAT IS POSSIBLE?

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Summary

The term big data may at first appear incongruous to animal agriculture. However, by capturing, analysing, reporting and sharing of through-production chain data with decision makers, organisations are better equipped to make informed decisions. Pivotal to this process is the type of data, its relevance, accuracy, and integrity. The importance of capturing relevant, accurate data that is reported in a meaningful time and space cannot be underestimated. However, significant challenges exist with big data for poultry production, not least with the very basics of capturing data, storage, security, analysing as well as effecting meaningful change based on the data. This paper will review current technologies available or in development for the poultry industry and highlight opportunities for their application.

I. INTRODUCTION

Agricultural industries are on the cusp of a digital revolution. Rising demand for higher yields, combined with constraints on finite resources such as land and water, has placed increased pressure on the input side of agriculture. The increased demand on agricultural outputs from an increasing global population and socioeconomic growth has intensified the pressure on the agricultural sector to produce more with less. Current projections for population growth estimate that the world population will reach 9 billion people by 2050 and, in order to feed this number of people, overall food production will need to increase by approximately 70% between 2007 and 2050 (FAO, 2009). Traditionally, to meet this increase in demand, the agricultural sector would more often than not apply the 'bigger is better' principle and expand production by clearing more land or increasing the intensity of production. However, this strategy is becoming increasingly difficult from an environmental perspective and is often in conflict with spreading population centres that prioritise arable land for urban development. Adding to this dilemma is the estimate from the Food and Agriculture Organisation that between 20-40 % of annual global crop production is lost to pests and diseases. To counter this inefficiency, a simplistic approach would be to apply more fertiliser and/or insecticides yet paradoxically (from a production volume view) consumers and governments are demanding fewer chemicals be applied. This somewhat parallels the current direction of poultry production. Traditional technologies such as antibiotic growth promoters are facing increased scrutiny and pressure globally to be reduced or removed entirely. While poultry production can expand by adding more sheds (within limits) to meet rising demand, the volume of poultry products that can be produced from each unit on a square metre basis has also faced downward pressure on account of reduced stocking densities. These scenarios have the potential to impair the growth in production volume and lead to shortages in food production at the very moment when more is required. It is also evident that the poultry industry cannot rely on past expansion strategies alone to meet this increased demand. To help meet this challenge, a proposed key to facilitate increased food production in a time of tightening inputs lies with Agriculture 4.0 and big data technologies.

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a) Smart Farming

The development and application of smart farming began in the late 1990's with the introduction of precision farming whereby technology was applied to the production of agricultural commodities for the first time. However, precision farming focussed largely on farm machinery used in the production of crops with assistive technologies such as global positioning systems to reduce overlap when turning at the ends of the field and therefore improving sowing, harvesting and fuel efficiencies. The next iteration of smart farming is termed Agriculture 4.0 which is a continuation of precision farming and is hailed as the new era in modern agriculture. The foundations of Agriculture 4.0 rely on the increased use of mechanised processes (from paddock to plate) that are supported by the Internet of Things (IoT), big data, wireless/mobile communications and cloud computing. Agriculture 4.0 monitors each step of the food production chain from the first input to the last output.

The Internet of Things and big data are terms used to describe technologies that are embedded in everyday objects and are interconnected via the internet and ultimately produce large data sets. For poultry production, this will result in more sensors and data inputs at each step of the value-chain. However, a consequence of this will be that the data sets produced will be so big and vast that traditional data processing software is insufficient to handle these data sets. Importantly, big data also refers to the use of predictive analysis that moves beyond the basics of reporting data and analyses data for correlations and patterns from which businesses may then extract value.

b) Data acquisition

Data acquisition is perhaps one of the easiest components of big data for poultry production. Currently, there are numerous sources of data acquisition ranging from the production statistics on the breeder farm right through the value chain to consumer preferences at the retail level. However, not all of these data are collated and able to be analysed in depth, with some sources of data analysed (at best) or sitting unanalysed in isolation (at worst). Yet to achieve improved efficiency, it is important that all of these data are captured and analysed in a holistic manner.

It is often described that organisations build a data lake which is akin to constructing a man-made water reservoir (Figure 1). First the dam is created, is then filled with water (data) and once the lake begins to fill, the water (data) is then used for other value adding purposes. A data lake provides a platform for rapid data accumulation and, potentially, its application. While this represents a significant advancement, the transformation analysis and application of the data is more complex and represents a major challenge to organisations. After a data lake is created, the propensity to measure and capture data increases significantly and may lead to an overload of data. Measuring something for the sake of measuring it should be avoided for "sometimes what counts, can't be counted and what can be counted, doesn't count (Cameron, 1963). For each new data stream, an analysis of the proposed benefits should be applied prior to its creation, and a review after it is active, to evaluate the value of the data. The value of data streams may be under or overestimated and it is the analysis and interpretation of these data where expertise is required in order to maximise the value and application of big data.

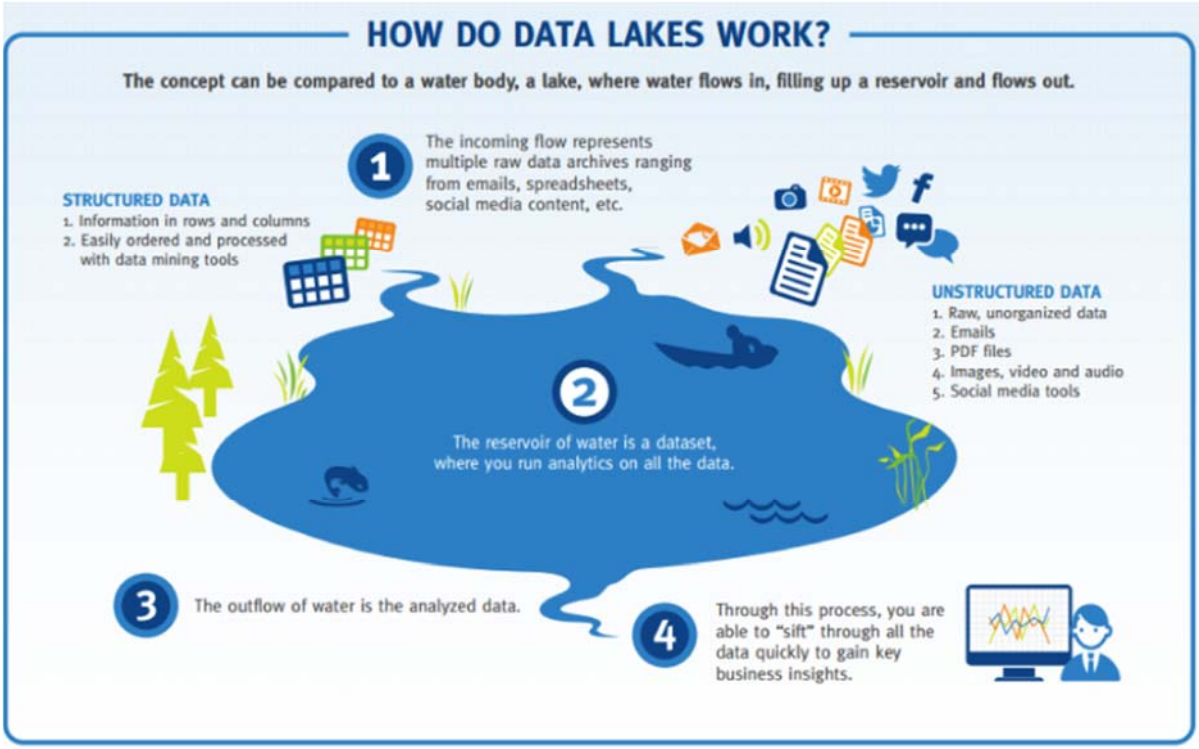

Figure 1 - A Data Lake: how does it work? (Source: Realworldanalystics.com)

c) When good info goes bad: the cost of data errors

Efficient poultry production is reliant on accurate data. Performance targets currently exist for each step of the production chain from the breeder farms, through to the hatchery, for on farm growth, feed efficiency as well as processing. For most integrators, these values may be summarised as cents/kg of poultry meat products or cents/dozen eggs for table egg producers. If we accept the average benchmark of a 1% error rate in manual data entry and multiply this by the instances of manual data entry, the consequences of these missteps can be profound. The human ability to catch or avoid errors is inherently flawed and if data needs to be entered multiple times, this only exacerbates the problem. A common business concept is the 1-10-100 rule which illustrates the importance of correcting data entry mistakes at the source. According to the 1-10-100 rule, it costs $1 to verify the accuracy of the data at the point of entry, $10 to correct or clean up the data in batch form, and $100 (or more) per record if no corrective action is performed. While the absolute value of individual and cumulative data errors to companies may differ, the principle remains the same. Reliable and timely data are essential. Using the underlying technologies of Agriculture 4.0 to capture and report this data automatically using connected sensors and online platforms will lead to increased accuracy and facilitate timely decision making.

The following categories of data described in this paper represent some suggested data streams for big data in poultry production with a focus on streams that have the potential to be transformational.

II. ON FARM DATA

a) Environment

With the advent of tunnel ventilation for poultry housing over three decades ago, the ability to control and monitor environmental conditions such as temperature, relative humidity,

ventilation, lighting, air quality and heat index/bird comfort has advanced significantly. Since these technologies currently exist and are widely used, the focus of this review will concentrate on emerging technologies and identify opportunities for development. However, it is worthwhile noting that, although regulation and monitoring of environmental conditions has increasingly become more automated, the reporting and dissemination of these records beyond the farm is often fragmented and remains an area for improvement.

b) Water

Water meters are more common on farms than feed measuring devices; however, not every farm or shed has these. It is perhaps stating the obvious that water consumption is a crucial indicator as to the health of birds and, by extrapolation, may give some indication of feed intake. Yet water data is often over looked on farm or not recorded and reported in a manner so that it best supports the optimal management of flocks. Recording hourly, if not daily water intake data would assist in identifying trends in consumption, particularly decreased intake which may precede a health problem and provide an opportunity for early investigation and intervention.

c) Feed

When it comes to poultry production, key drivers of efficiency and profitably are centred around feed. While feed is but one of the many components of poultry production, its contribution to production efficiency warrants particularly close attention. Feed costs reportedly account for 60-70% of production costs and, therefore, its importance to the economics of a poultry company cannot be over-emphasised.

However, somewhat paradoxically, this metric is perhaps the least well reported. The volume of feed consumed for each batch or production cycle is approximated on-farm using varying combinations of feed mill weigh bridge receipts and subjective estimates which range from somewhat technologically advanced to throwing rocks at silos. Of the more technologically advanced methods for feed usage estimation, some are prone to error and require considerably more maintenance than others. Whichever method is used, although it might be considered a step forward in monitoring feed, the accuracy of the data may be questionable and therefore potentially misleading. The need (and desire) to accurately measure and report feed intake in real-time is significant and the benefits of these data should not be underestimated.

Another factor which contributes to the imprecision of feed reporting is that, despite best intentions, more often than not feed usage is reported after the batch has finished and with incomplete data. The accuracy of this also relies on the estimate of feed left in the silo at the end of the batch. Given the above methods that are routinely used to estimate silo inventory, application of more accurate feed volume monitoring is key to providing meaningful data daily and even hourly, and in real time. Emphasising the potential of this data stream to improve poultry production decision making results in the ability to benchmark flocks, sheds, farms and changes in management/nutrition. Currently unless research farms are available, the ability to accurately quantify effects on performance/efficiency of birds in response to changes in feed formulation, feed additives and feed manufacture is limited. The potential to monitor feed delivery into sheds, and therefore calculate daily feed consumption, is perhaps one of the most significant challenges on-farm, yet the opportunities here are enormous.

d) Live body weight and uniformity

Live body weights and flock uniformity are important to assess growth, feed efficiency and underlying health or welfare issues (Vranken *et al*., 2005). Currently, the average body weight and uniformity of birds is obtained by manual weighing of a subset of the flock or, less commonly, automatic weighing platforms. Manual weighing of birds is laborious and limits the number of birds sampled, potentially misrepresenting the flock. Automatic weigh platforms are subject to the vagaries of bird behaviour and body weight. Heavier birds are less likely to step onto the weigh platforms leading to an underestimation of flock body weight by as much as 30% (Chedad *et al.,* 2003). Concordantly, this scenario is most evident towards the end of the production cycle in broilers when broiler body weights are crucial to scheduling pick up times. Image analysis techniques that estimate bird body weight based on the surface area of the bird in conjunction with weigh platforms are in development with results showing improved accuracy (less than 5% error) when compared to manual weighing (Vranken *et al.,* 2005).

e) Biosensors

An emerging area in livestock farming is the use of advanced biosensor technologies such as microfluidics, sound analysers and image detection algorithms (Neethirajan, 2017). Sound analysers have been reported to be effective at predicting 'stress' levels in laying hens (Lee *et al*., 2015) thermal comfort of chicks during the brooding stage (Moura *et al.,* 2008), growth performance of broilers (Fontana *et al*, 2015) and chicks pre-hatch (Exadaktylos *et al.*, 2011). The monitoring of the spatial distribution of birds may provide indicators of bird behaviour, environmental conditions and bird activity. It is envisaged that these sensors will be incorporated into poultry production units and feed data (information) to poultry livestock managers to enable appropriate decision-making relating to the management of the birds. Currently, the adoption of these technologies is low; however, these technologies represent the direction for which poultry production is heading and will further contribute to filling the data lake.

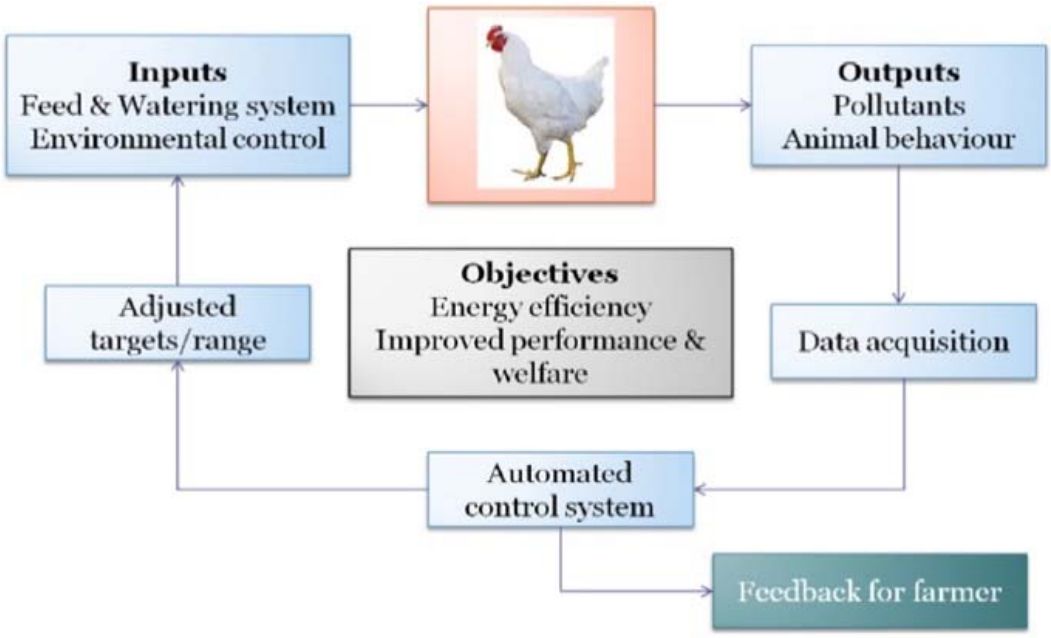

Figure 2 - Schematic of how biosensors may be used on poultry farms to improve production. (Adapted from Corkery *et al.*, 2013).

III. LIMITATIONS AND BARRIERS TO ADOPTION

Digital technology is a key enabler across the food chain; however, despite clear trends in other countries, Australia lags significantly in using digital information and software platforms. Impediments to the adoption of digital technology are multifactorial but can largely be attributed to the capital constraints required to implement such systems and inadequate telecommunications coverage, especially on farms located in remote areas. Efforts to improve connectivity to the internet in remote areas are making progress, yet internet access still remains inconsistent, unreliable and slow in many areas. Work around solutions to this are expensive and, given the fragmented location and ownership of farms, it is unlikely a single poultry farm could justify the capital required.

Another consideration in adopting digital monitoring and reporting technologies is that of data ownership and security. In fully integrated companies where farms are owned or managed by the company, the issues around data ownership and transparent reporting are perhaps less controversial than in situations where contract growers are engaged. Similarly, larger organisations are more likely to have dedicated IT departments and security protocols in place than smaller operators. In situations where contract growers are employed, sensitivities pertaining data sharing and security may occur with concerns raised as to how the data will be stored, shared and interpreted by poultry companies. The latter may impact contract negotiations or payments and would need clarification at the onset of a data project. Whichever the case, the information generated would benefit both parties provided clear undertakings as to how the data will be used were provided.

IV. CONCLUSIONS

The adoption of technology for monitoring and management should be based on some fundamentals, otherwise there is the risk of being overwhelmed with erroneous or meaningless data. An ideal technology in poultry production should be able to explain an underlying biological process, translate this information into a meaningful action, be cost effective, robust, reliable and precise as well as solution focussed. A caveat to the use of big data for poultry production is that the combination of people and data is critical to success. Skilled people will be required to interpret the data as well as managing flocks in the field; big data is not a replacement for skilled people rather a tool to enable decision making. To maximise the value of big data in poultry production, a whole value-chain approach will need to be employed and will also require adjustments in how data are currently shared. An overarching objective of using big data in poultry production should be to provide the right data to the right person at the right time. Achieving meaningful data sets and analysis thereof will facilitate increased data driven decisions and improve production efficiency.

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THE EFFECTS OF A MULTI-ENZYME AND *BACILLUS* PROBIOTIC COMBINATIONS ON CALCIUM AND PHOSPHORUS DIGESTIBILITY AND BROILER PERFORMANCE

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Summary

This study evaluated the effect of a multi-enzyme combination and varying doses of a 3-strain *Bacillus* probiotic on broiler digestibility and bird performance. 450 day-old male Ross 308 chicks were randomly allocated to 3 dietary treatments with 6 replicate pens per treatment and 25 birds per pen. Diets were supplemented with 500 FTU commercial phytase and a xylanase and beta-glucanase mixture (XB; to provide 1220 U xylanase and 152 U beta-glucanase/kg feed) alone or in combination with 75,000 CFU/g feed or 150,000 CFU/g feed of a 3 strain *Bacillus* spp. combination. Supplementation of the *Bacillus* probiotic numerically and significantly improved bodyweight corrected feed conversion ratios (FCRc) in diets containing XB. Digestible calcium and phosphorus were also increased when *Bacillus* were supplemented on top of diets containing XB.

I. INTRODUCTION

Bacillus-based probiotics have gained increased attention as alternatives to antibiotic growth promoters (AGP) to support broiler performance in systems aiming to reduce the use of antibiotics and maintain gut health and function. Combatting poor gut health and disease susceptibility in an era without antibiotics has been made increasingly difficult due to the increasing cost of commercial feed ingredients. This has forced some producers to use cheaper dietary alternatives including by-products (such as corn distiller's dried grains with solubles (corn DDGS) and wheat middlings) which often contain high levels of anti-nutritional factors such as non-starch polysaccharides (NSP). Consequently, feed enzymes, such as carbohydrases and phytases, have been increasingly used in animal feed to increase the nutrient availability and utilisation of ingredients and confer beneficial changes to intestinal microbial populations and support gut health through described prebiotic modes of action.

With no "silver bullet" available to replace antibiotic use, it is important to understand how different alternatives can support gut health and bird performance.

This study aimed to evaluate the effect of multiple enzymes in combination with *Bacillus* probiotics on nutrient digestibility and broiler performance when fed typical Australian diets.

II. MATERIALS AND METHODS

The study was conducted at the University of Sydney and protocols were approved by the university's ethical committee.

450 day-old male Ross 308 chicks were randomly allocated to 3 dietary treatments with 6 replicate pens per treatment (25 birds/pen). Prior to the trial, the birds were vaccinated against infectious bronchitis, Marek's disease and Newcastle disease. The trial was conducted in a deep litter broiler shed and ran for 42 days. All diets were supplemented with 500 FTU commercial phytase and a xylanase and beta-glucanase mixture (XB; to provide 1220 U xylanase and 152 U beta-glucanase/kg feed; manufactured by DuPont Animal Nutrition) alone or in combination with 75,000 CFU/g feed or 150,000 CFU/g feed of a 3 strain *Bacillus*

[1] Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, UK.
[2] Feedworks Pty Limited, Romsey, VIC, Australia.

spp. combination. Diets were pelleted (80°C, 100 kPa pressure) and fed *ad libitum*; starter diet from day 1 to 15, grower diet from day 16 to 28 and finisher diet from 29 to 42. At the end of each phase, feed intake and bodyweight were measured to calculate bodyweight gains and feed conversion ratios. Additionally, on day 28, 5 birds from each pen were euthanised and the ileal contents were collected and pooled per pen for nutrient digestibility calculations according to the methods of Ravindran et al (2005).

Table 1 - Dietary composition and nutrient specifications of starter, grower and finisher diets.

Composition (g/kg)	Starter	Grower	Finisher
Wheat	566	551	607
Canola meal	75.0	120	150
Soybean meal	298	230	157
Meat meal	15.0	10.0	0.00
Tallow	15.0	35.0	40.0
Vegetable fat blend	5.00	16.5	22.0
DL-methionine	2.80	2.20	1.60
Celite	0.05	15.1	0.05
Sodium bicarbonate	2.20	2.40	2.80
Vitamin and mineral premix	2.00	2.00	2.00
Lysine HCL	2.00	1.70	1.90
Threonine	0.60	0.50	0.50
Salt	1.70	1.60	1.60
Limestone	8.30	7.50	8.40
Dicalcium phosphate	6.00	4.50	5.00
Axtra Phy 10,000 TPT	0.0001	0.0001	0.0001
Axtra XB 201 TPT	0.0001	0.0001	0.0001
Nutrient specifications (g/kg)			
ME (MJ/kg)	11.9	12.3	12.8
Crude protein (g/kg)	247	225	201
Ca (g/kg)	9.06	8.70	8.10
P (g/kg)	4.80	4.35	4.05
Lysine (g/kg)	14.0	13.0	11.0
Digestible lysine (g/kg)	12.0	11.0	10.0
Methionine (%)	6.00	6.00	5.00
Digestible methionine (g/kg)	6.00	5.00	4.00
Methionine and cysteine (g/kg)	11.0	10.0	9.00
Digestible methionine and cysteine (g/kg)	10.0	9.00	8.00

III. RESULTS

Overall (1-42 days), *Bacillus* probiotic supplementation at 75,000 and 150,000 CFU/g feed, combined with XB, significantly ($P < 0.05$) improved FCRc by 7 points (4.5%) and 8 points (5.2%), respectively, compared to enzyme supplementation alone. Furthermore, there was no significant difference in FCRc in diets containing XB when the *Bacillus* probiotic was supplemented at either 75,000 CFU/g or 150,000 CFU/g of feed. No significance differences in either feed intake or bodyweight gain were detected with probiotic supplementation in diets containing XB.

Digestible calcium and phosphorus were significantly ($P < 0.05$) increased by 68.4% and 17.5%, respectively, with the inclusion of the *Bacillus* probiotic at 150,000 CFU/g feed in combination with XB, compared to XB alone.

IV. DISCUSSION

Supplementation of the 3-strain *Bacillus* probiotic in diets containing XB improved broiler performance. *Bacillus* probiotics are well documented to support gut development, in turn allowing nutrients from feed enzymes to be absorbed more efficiently. Interestingly, in diets containing XB at higher dose of the *Bacillus* probiotic did not show further significant improvements in bird performance. This could support the inclusion of a lower probiotic *Bacillus* dose, potentially making it more economically viable. Such feed enzyme and *Bacillus* probiotic combinations have been shown to be efficacious in situations of gut health challenge including coccidiosis and necrotic enteritis (Dersjant-Li *et al.*, 2016). Gut health related issues are costly to the global market. Complex diets and the inclusion of plant by-products negatively impact gut health by increasing the viscosity of the digesta, slowing intestinal transit and allowing the proliferation of non-beneficial microbial populations. An excess of undigested crude protein in the lower gut is associated with necrotic enteritis. It is therefore understandable how feed enzymes can be a key component of any gut health strategy. Improving the digestibility of the diet and reducing the undigested fraction reaching the distal gut is one mechanism for improving gut health. *Bacillus* based probiotics have well documented benefits in supporting the colonisation of a beneficial microbiota and thus supporting optimal gut development and functionality both under unchallenged and challenged conditions. In turn, this has been shown to improve gut function and ultimately bird performance. In conclusion, feed enzymes in combination with *Bacillus* based probiotics can be used to improve nutrient digestibility and bird performance.

Table 2 - Effects of a 3-strain *Bacillus* and XB on bird performance.

	XB 100 g/tonne	XB + 3 strain *Bacillus* 75,000 CFU/g	XB + 3 strain *Bacillus* 150,000 CFU/g
Starter (1-15 days)			
Bodyweight gain (g/d)	33.5^{a}	32.5^{ab}	31.5^{b}
Feed intake (g/d)	37.5$^{(a)}$	37.0$^{(ab)}$	36.1$^{(b)}$
FCR	1.12^{b}	1.14^{ab}	1.15^{a}
Grower (16-28 days)			
Bodyweight gain (g/d)	88.2	91.6	92.1
Feed intake (g/d)	136	132	129
FCR	1.54^{a}	1.44^{b}	1.40^{b}
Finisher (29-42 days)			
Bodyweight gain (g/d)	111	112	112
Feed intake (g/d)	208	205	201
FCR	1.88	1.83	1.81
Overall (1-42 days)			
Bodyweight gain (g/d)	77.5	78.9	78.4
Feed intake (g/d)	120	118	116
FCR	1.55^{a}	1.50^{ab}	1.48^{b}
FCRc	1.55^{a}	1.48^{b}	1.47^{b}

^{ab} Means within a row with different superscripts differ ($P < 0.05$).
$^{(ab)}$ Means within a row with different superscripts differ ($P < 0.10$).
FCRc is bodyweight corrected FCR, 3 points in FCR for every 100g difference versus control.

Table 3 - Effects of a 3-strain *Bacillus and* XB inclusion on ileal digestible calcium and phosphorus at 28 days.

	XB 100 g/tonne	XB + 3 strain *Bacillus* 75,000 CFU/g	XB + 3 strain *Bacillus* 150,000 CFU/g
Digestible calcium (%)	0.171[b]	0.213[b]	0.288[a]
Digestible phosphorus (%)	0.154[b]	0.153[b]	0.181[a]

[ab] Means within a row with different superscripts differ ($P < 0.05$).

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EFFECTS OF DIETARY CALCIUM LEVELS ON THE PERFORMANCE AND BONE QUALITY OF YOUNG BROILERS

C. TORRES[1], A.I. GARCIA[1], A.P. BONILLA[1] and M.A. DIJKSLAG[2]

Summary

Five broiler pre-starter diets (0 to 4 days of age), based on corn and soybean meal, were formulated to contain 3, 4, 6, 8 and 10 g/kg calcium (Ca). All diets were equivalent with respect to digestible phosphorus (dP) content (4.6 g/kg). The influence of dietary treatments on the growth performance and tibia mineralization of a total of 1200 one-day-old male Ross 308 broilers was evaluated to 4 days of age. The birds were allocated to 40 experimental units and randomly assigned to the five dietary treatments.

Feeding broilers with a pre-starter diet containing 10 g/kg calcium resulted in inferior body weight gain and feed intake in the first two days compared to those fed 4 g/kg Ca. Tibia ash content of chicks fed 8 and 10 g/kg Ca increased from day 2 onwards whereas in those given the 3 and 4 g/kg diets, there was no increase with age. Mortality was minimal and no incidence of skeletal anomalies was observed across dietary treatments. The present study suggests that dietary Ca inclusion at 10 g/kg limits performance in the first days after hatch despite improved mineral deposition.

I. INTRODUCTION

Mineralization of the chicken embryo requires calcium (Ca) and phosphorus (P)' macro minerals supplied to the embryo by eggshell + yolk and yolk, respectively. Towards the end of incubation and after hatch, phosphorus in the residual egg yolk is minimal (Li et al., 2014). Yolk calcium reserves at hatch, however, are similar (Yair and Uni 2011) or even higher (Richards and Packard 1996) compared to eggs at setting, as a result of eggshell calcium being transferred to the yolk throughout incubation (Johnston and Comar 1955). In this case, yolk calcium reserves as hatching approaches would likely be sufficient to support neonatal skeletal requirements, but bone growth may be limited by the low egg phosphorus content. These findings, in addition to observations that embryonic bone growth reaches a plateau after 19 days of incubation (Li et al., 2014), suggest a nutritional limitation to embryonic bone growth late in the neonatal period and therefore the need for completion of skeletal growth by day 19 of embryonic development. Under current dietary Ca levels in a typical broiler starter diet (9-10.5 g/kg), the P absorption might be compromised because neonatal mineral metabolic condition might be down regulating intestinal Ca absorption. Excess Ca in the lumen could in turn react with dietary inorganic P to form insoluble Ca orthophosphate and thus reduce P absorption (Selle et al., 2009). In this study, we tested the hypothesis that a significant decrease in the level of Ca at a constant dP of 4.6 g/kg in diet for the first 4 days of life may improve early growth and bone mineralization of newly hatched chicks.

II. MATERIALS AND METHODS

One thousand two hundred male Ross 308 broiler chicks, averaging 45 grams at arrival at the Research Centre, were randomly allocated to 40 floor pens, 30 birds per pen, giving 5 treatments with 8 replicates. Broiler starter diets, based on corn and soybean meal, were

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[2] ForFarmers N.V. Kwinkweerd 12, Lochem, Netherlands.

formulated to be equivalent with respect to energy (2800 AME kcal/kg), amino acid (11.5 g/kg dLys) and digestible phosphorus (4.6 g/kg dp) (Dutch Feeding Tables, 2007) but not calcium. Prior to formulating the diets, main ingredients were analyzed for Ca and P contents and data were used to formulate the experimental diets. Five diets were formulated to contain 3, 4, 6, 8 and 10 g/kg Ca. The lowest (3 g/kg) and highest (10 g/kg) Ca diets were formulated separately and intermediate level diets were produced by mixing these two diets in proportion to meet desired Ca levels. Experimental treatments did not contain phytase in order to prevent confounding effects on P availability as the efficiency of this enzyme to release P from phytate improves when diets contain reduced dietary Ca. Birds had *ad libitum* access to feed and water immediately after placement in the experimental unit and for the duration of the study. Birds and feed were weighed daily and one animal / replicate, with a body weight (BW) within 5 g/kg of the average pen BW were selected. These chicks were blood sampled for Ca and P serum analyses and euthanised. The right tibia was collected, cleaned, weighed and used for tibia ash determination. Performance data was analyzed as one-way ANOVA using the general linear models procedure of SAS in a completely randomized block design. Bone ash and serum were analyzed as repeated measures. Significant differences between means were detected by Tukey test when the model was significant at P<0.05.

III. RESULTS

Table 1 - Influence of dietary calcium concentration on early growth of broilers fed pre-starter diets from placement to 4 days of age.

Variable	Dietary calcium level, g/kg					SEM	P-value
	3	4	6	8	10	(n=8)	
Body weight, g							
0 day	46.08	45.99	45.78	45.94	45.97	0.18	NS
1 day	*56.71*	*56.54*	*56.14*	*56.02*	*55.74*	0.25	*0.07*
2 day	68.75^{ab}	69.42^{a}	68.49^{ab}	67.94^{ab}	67.10^{b}	0.43	**
3 day	85.20^{ab}	86.39^{a}	85.60^{ab}	84.75^{ab}	83.52^{b}	0.62	*
4 day	$*103.5^{ab}$	$*105.9^{a}$	$*104.3^{ab}$	$*103.0^{ab}$	$*102.1^{b}$	0.91	*0.07*
Daily weight gain, g							
0-1	10.63^{a}	10.73^{a}	10.36^{ab}	9.78^{b}	9.77^{b}	0.17	***
1-2	12.27^{ab}	12.88^{a}	12.35^{ab}	12.55^{ab}	11.36^{b}	0.28	*
2-3	16.46	16.97	17.11	16.40	16.43	0.41	NS
3-4	18.28	19.51	18.70	17.90	18.62	0.53	NS
Daily feed intake, g							
0-1	9.04^{a}	8.40^{b}	8.41^{b}	8.16^{b}	8.56^{b}	0.11	***
1-2	11.42^{a}	11.31^{ab}	10.81^{abc}	10.67^{bc}	10.15^{c}	0.17	***
2-3	17.89	18.06	18.04	17.23	17.93	0.53	NS
3-4	24.28	24.86	26.12	24.72	26.61	1.02	NS
FCR, g/g							
0-1	0.83^{ab}	0.78^{b}	0.82^{ab}	0.83^{a}	0.87^{a}	0.012	***
1-2	0.91	0.88	0.88	0.85	0.90	0.015	0.08
2-3	1.073	1.064	1.062	1.097	1.102	0.034	NS
3-4	1.310	1.281	1.407	1.389	1.435	0.047	NS

[a-c] Means within a row with unlike superscripts differ significantly at P <0.05. *= P<0.05, **= P<0.01, ***= P<0.001, NS= not significant.

Table 1 shows the effect of the diets on growth to 4 days of age. Daily weight gain was slightly under Ross 308 performance objectives likely because of stress of chicks being handled daily. Increasing the dietary Ca level had detrimental effects on early growth. Body

weight of the chicks fed 10 g/kg Ca was inferior to those fed diet with 4 g/kg Ca at day 2 and 3 and tended to be lower at day 4 (P=0.07). Despite improved daily weight gain, the feed intake of birds fed 4 g/kg Ca was improved only on the second day relative to broilers fed 10 g/kg Ca. Feed conversion figures were not markably affected by dietary treatment.

Bone growth in the first 2 days of age was minimal among all treatments (Figure 1). Minimal bone growth occurred during experimental period in birds fed the lowest Ca levels despite improved body weight. Mineralization increased from days 3 and 4 when chicks were fed pre-starter with 6, 8 or 10 g/kg Ca. Despite reduced bone mineralization, no incidence of bone lameness or skeletal abnormalities were observed in this study. Likewise, mortality was negligible and not related to any treatment.

Overall, increasing dietary Ca level from 3 to 10 g/kg was followed by increased Ca serum in the blood; regardless of diet the blood concentration of this mineral decreased in the first 3 days followed by an increase at day 4 (data not shown). With respect to P, dietary treatment did not influence P level in the serum; however, its concentration varied in the first 4 days of life. Serum P level increased up to the second day of life after which it appeared to plateau (Figure 2).

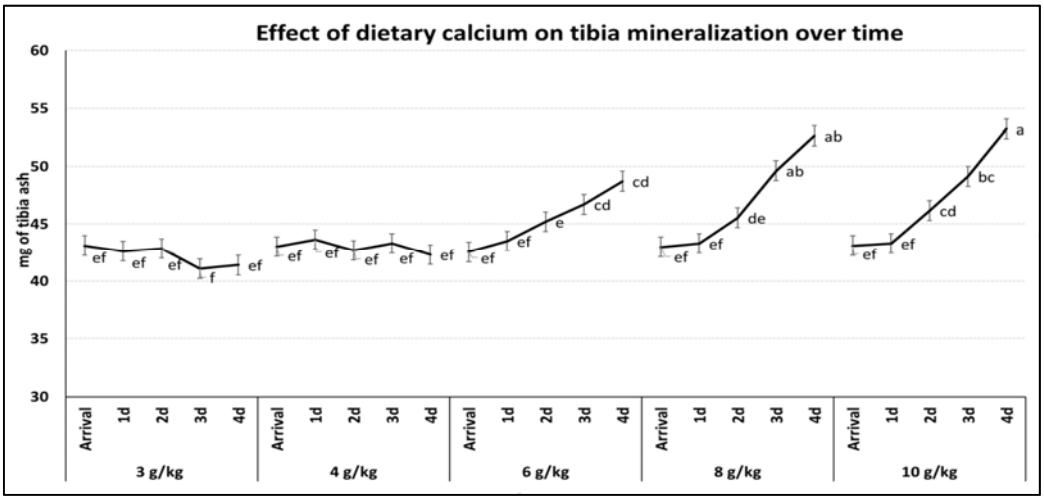

Figure 1 - Influence of dietary Ca on tibia ash (mg) deposition of broilers fed pre-starter diets from placement to 4 days of age. [a-f] Means between chart bars with unlike superscripts differ significantly at P <0.05.

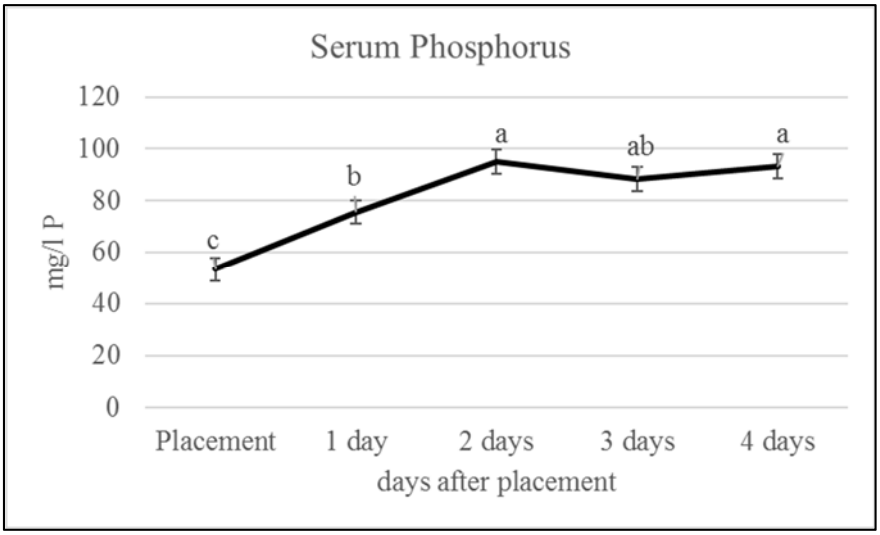

Figure 2 - Time effect on P concentration in the serum of juvenile chickens. . [a -c] Means between chart bars with unlike superscripts differ significantly at P <0.05.

IV. DISCUSSION

In the present study, increasing dietary Ca concentration decreased weight gain in diets without phytase. Abdollahi et al (2016) reported that increasing dietary Ca concentration in the starter diet (1-21d) also suppressed growth, both without and with added phytase. Despite increased body weight, the bone ash deposition was minimal when chicks were fed diets with a low calcium level; yet no incidence of clinical skeletal disorder or mortality was observed. In contrast, 10 g/kg dietary Ca limited performance in the first days after hatch despite improved mineral deposition.

The mechanisms leading to the paradoxical effect of decreasing dietary Ca improving performance but reducing bone ash deposition is intriguing. Phosphorus is the major intracellular anion and it is a vital component of metabolic pathways involving the production of ATP. It is possible that, when birds are fed a diet with more bioavailable P (by reduced Ca inclusion), this mineral is delivered to sustain such metabolic routes, instead of bone mineralization. This would explain the physiological mechanism leading to increased body weight when newly hatched chicks are fed low calcium in the first days after hatch. The results from this study indicate that, despite reduced bone mineralization, 4 g/kg dietary calcium in pre-starter diets of broilers improves body weight gain and feed intake in the first two days compared to those fed 10 g/kg Ca. Further work is warranted to determine if providing pre-starter diet with reduced calcium level for newly hatched chicks improves broiler productivity during grow-out and whether it has any carry over effect on skeletal quality.

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A NOVEL CARBOHYDRASE RESTORED NUTRIENT AVAILABILITY IN A 3% SILICA-DILUTED BROILER DIET

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Summary

The current study investigated the effect of a novel carbohydrase (Enz; Rovabio® Advance), a multi-enzyme complex containing high levels of endo-xylanase (Xyl) and arabinofuranosidase (Abf) activities, on energy and ileal amino acid (AA) digestibility of a wheat/soybean-based diet in broilers. A standard wheat/soybean-based diet was compared with a 3% nutrient-diluted version using silica as an inert diluent. The effect of adding exogenous Enz to these diets was studied using 120 broilers in a 2 x 2 factorial arrangement of four treatments in a complete block design. Enzyme improved energy utilisation (+2.8%; 73.3 vs. 75.4%; $P < 0.001$), leading to a significant increase of AME content of 402 and 410 kJ/kg dry matter (DM) in the diluted and standard diet, respectively. Apparent metabolisable energy content of the diluted diet with Enz was similar to that of the standard diet without Enz ($P = 0.98$) demonstrating the ability of Enz to alleviate the negative effect elicited by 3% nutrient dilution. At ileal level, addition of Enz increased AA digestibility by an average of 4.4% ($P < 0.001$).

I. INTRODUCTION

Wheat is an important cereal grain in broiler diets due to its richness in starch and protein, but there is also considerable evidence that the cell wall non-starch polysaccharides (NSP) in wheat act as anti-nutritional factors and have been shown to impair poultry performance (Choct and Annison, 1992). NSP-degrading enzymes are commonly used in poultry feeds for better hydrolysis of the undigested fraction, resulting in higher energy and nutrient availability. While endo-xylanases can break down the polymers by hydrolysing the xylose (X) backbone in an endo-acting manner, the efficiency of these enzymes is often hampered by multiple arabinose (A) residue substitutions along the backbone (Lagaert et al., 2014). Therefore, it is of importance to understand the A/X ratio, which indicates how ramified the arabinoxylan (AX) fraction is. This ratio varies depending on the cereal (0.74 for corn, 0.62 for wheat; Knudsen, 2014). Arabinofuranosidases can cleave arabinose from the xylose backbone and therefore provide access to endo-xylanase activity. An *in vitro* study showed that a combination of arabinofuranosidase (Abf; GH51) and xylanase (Xyl; GH11) improves DM digestibility of corn and wheat compared to Xyl alone (Cozannet et al., 2016). When tested in broilers, an enzyme complex enriched in Xyl and Abf activities (Rovabio® Advance; Enz) improved energy utilisation and increased the rate of protein, fat and starch digestibility in poultry feed (Cozannet et al., 2017). The current study aimed at evaluating the effects of Enz on ileal digestibility of amino acids and energy availability in standard and 3% nutrient-diluted wheat/soybean-based broiler diets.

II. MATERIALS & METHODS

This experiment was carried out at the Centre for Expertise and Research in Nutrition, Adisseo, France S.A.S. (Malicorne, France). In total, 120 male Ross PM3 broiler chicks were

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used in this study, and it employed a 2 x 2 factorial arrangement of four treatments to study the effect of adding Enz to a standard and a nutrient-diluted feed. The experimental period was divided into three phases: a 7-day adaptation phase (13 - 19 d of age), a 3-day excreta collection phase (20 - 22 d of age) and a 4-day preparation phase prior to ileal digesta collection (23 - 26 d of age). Birds had *ad libitum* access to feed and water. Diets were produced using wheat and soybean meal and formulated to meet or exceed the bird's nutrient specifications for the respective rearing phases, following Rhodimet® Nutrition Guide recommendations (2013). A diluted form of the standard diet was achieved using 3% silica as an inert diluent to give a diet containing an estimated 97% of the nutrients of the standard diet. Titanium dioxide (TiO_2) was added to both experimental diets at 0.5% to serve as an indigestible marker. Dietary treatments included: T1, standard diet; T2, as for T1 with the addition of Enz; T3, diluted diet; T4, as for T3 with the addition of Enz. A liquid form of the multi-enzyme (Rovabio® Advance, Adisseo France S.A.S., Antony, France; inclusion rate: 200 mL/MT) was sprayed on the surface of pelleted feed to provide a minimum of 1,250 visco-units of endo-β-1,4-xylanase, 9,250 visco-units of α-L-arabinofuranosidase and 860 visco-β-glucanase units of endo-1,3(4)-β-glucanase per kg of feed. During the excreta collection phase, feed refusals and spillages were collected daily and analysed for DM content. Excreta were collected daily from trays under each cage, pooled and stored at -18 °C for AME analysis. At the end of the four d preparation phase for digesta collection, broilers were sacrificed using carbon dioxide exposure for ileal sampling. Ileal digesta were gently flushed out with deionized water, pooled in groups of five on a treatment basis, and immediately frozen at -18 °C prior to amino acid analysis. Data (n = 120) were subjected to ANOVA with block (n = 10), diet composition (n = 2) and enzyme (n = 2) as main effects. The ANOVA also considered the complete factorial interactions between enzyme addition, diet composition and nutrient density using the General Linear Models (GLM) procedure of SAS/STAT (SAS Institute Inc., Cary, USA). The means were compared using Tukey's protected least significant difference test ($P \leq 0.05$) only if the overall F test was significant ($P \leq 0.05$).

III. RESULTS

As shown in Table 1, energy utilisation (AME:GE) was similar in both standard and diluted diets at around 73.3% ($P = 0.99$); however, AME was 3% higher in the standard versus diluted diet (14.4 and 14.0 MJ/kg DM respectively; $P < 0.0001$). Apparent metabolisable energy was significantly increased in both diets by the addition of Enz, by 410 and 402 kJ/kg DM in the standard and diluted diet, respectively. Nitrogen corrected AME (AME_N) was significantly lower in the diluted diet relative to standard diet ($P < 0.001$). Addition of Enz significantly improved AME_N ($P = 0.003$), rendering the energy release of the diluted diet comparable with that of the control standard diet.

At the ileal level, no interaction among diet and enzyme effect was observed on AA digestibility except for Thr ($P = 0.03$). The addition of Enz significantly increased ($P < 0.05$) ileal digestibility of Thr in the diluted diet while no difference was observed in the standard diet. Amino acid digestibility was similar for diluted and standard diets, averaging around 75% except for Met (Table 2; $P = 0.02$). Indeed, nutrient dilution of the diet was associated with lower Met digestibility relative to standard diet. In treatments fed standard diets, the most pronounced improvement with Enz was seen on Cys (8.19%), Val (2.88%) and Ile (2.87%); while in those fed diluted diets, the supplementation of Enz had greatest effect on Thr (7.33%), Cys (7.07%) and Val (6.52%). On average, digestibility of all AA was increased by 4.5% in the presence of Enz ($P < 0.001$).

Table 1 - Effect of diet and enzyme (Enz) supplementation on energy digestibility in broilers from 20 to 22 days.

		Digestibility coefficients		Energy values (MJ/kg DM)	
		DM	AME:GE	AME[3]	AME_N[4]
Standard diet	Control	0.687[b]	0.734[b]	14.4[b]	13.7[bc]
	Enz[2]	0.708[a]	0.754[a]	14.9[a]	14.1[a]
Diluted diet	Control	0.665[c]	0.733[b]	14.0[c]	13.5[c]
	Enz[2]	0.683[b]	0.754[a]	14.4[b]	13.7[bc]
Statistics[1]	Block	0.636	0.597	0.591	0.546
	Diet	< 0.0001	0.99	< 0.0001	< 0.0001
	Enzyme	< 0.0001	< 0.0001	< 0.0001	0.003
	Interaction	0.705	0.973	0.976	0.447
R^2		0.51	0.35	0.45	0.41

Values are means of n = 30 values per dietary treatment

[a,b and c]Values with different superscripts are significantly (P < 0.05) different from each other within each column.

[1] From ANOVA on 120 results with block (n = 30), enzyme (n = 2), diet (n = 2) and interaction (n = 4) as main effects.

[2] Enz, Rovabio® Advance.

[3] AME, apparent metabolisable energy.

[4] AME_N, AME adjusted for zero nitrogen balance.

Table 2 - Effect of diet and enzyme (Enz) supplementation on ileal digestibility of AA at 26d.

	Standard diet		Diluted diet		Statistics[1]			R^2
	Control	Enz[2]	Control	Enz[2]	Diet	Enzyme	Interaction	
Amino acid								
Cys	0.684[b]	0.740[a]	0.679[b]	0.727[a]	0.200	< 0.0001	0.126	0.236
His	0.776[ab]	0.798[a]	0.756[b]	0.800[a]	0.192	< 0.0001	0.111	0.198
Ile	0.766[ab]	0.788[a]	0.747[b]	0.793[a]	0.404	< 0.0001	0.148	0.153
Leu	0.767[ab]	0.785[a]	0.747[b]	0.790[a]	0.320	0.000	0.123	0.142
Lys	0.816[bc]	0.833[ab]	0.802[c]	0.837[a]	0.349	< 0.0001	0.118	0.181
Met	0.790[a]	0.805[a]	0.757[b]	0.800[a]	0.020	0.001	0.105	0.153
Phe	0.788[ab]	0.807[a]	0.770[b]	0.808[a]	0.231	0.000	0.199	0.141
Thr	0.727[a]	0.742[a]	0.696[b]	0.747[a]	0.117	< 0.0001	0.029	0.174
Val	0.729[ab]	0.750[a]	0.705[b]	0.751[a]	0.186	0.000	0.143	0.142

Values are means of n = 30 values per dietary treatment

[a,b and c] Values with different superscripts are significantly (P < 0.05) different from each other within each row.

[1] From ANOVA on 120 results with block (n = 30), enzyme (n = 2), diet (n = 2) and interaction (n = 4) as main effects.

[2] Enz, Rovabio® Advance.

IV. DISCUSSION

Previously, it has been shown that Rovabio® Advance (Enz), a multi-enzyme complex containing Xyl and Abf, improves overall digestibility of organic matter by 3% and enhances digestibility of fat, starch and protein in poultry feed (Cozannet et al., 2017). The current study confirms these previous observations using a different approach whereby the effect of Enz on a nutrient-diluted diet was investigated, with specific focus on how it influences AA digestibility.

Dilution of the diet with silica decreased DM digestibility and this is in connection with its indigestible properties. Addition of silica to the diet did not affect GE digestibility but had, as expected, a diluting effect on AME content. The restoration of energy utilisation (AME_N) in the diluted diet, with the exogenous Enz, to a level comparable with that of the standard diet indicates that Enz improved digestibility of dietary raw materials by around 3%. With respect to mechanism of action, previous *in vitro* work showed that Abf activities

present in the current enzymatic solution improved the potential degradation of corn arabinoxylan (AX) by providing better access to the AX backbone for Xyl activity (Cozannet et al., 2016) . This collaborative activity between Xyl and Abf contained within Enz helps to explain the associated improved energy utilisation in the current study. The β-glucanase in the Enz may also play a role as it is known that wheat contains about 1% β-glucan (Knudsen, 2014). In addition, Xyl has been shown to reduce intestinal viscosity caused by soluble NSP (Adeola and Bedford, 2004), which might have contributed to the increased energy utilisation.

In the silica-diluted diet, the greatest absolute effect of Enz on AA digestibility was observed for Thr (+ 5.1% units) and the smallest effect was for Lys (+ 3.5% units). These differences were mainly due to differences in the inherent digestibility of each AA because the inherent digestibility of individual AA has great impact on the magnitude of impact due to enzyme supplementation, and Thr has been reported to have one of the lowest inherent digestibility among all AA (Cowieson, 2010). In the present study, no interaction between diet and enzyme on AA digestibility was found except for Thr. Threonine is strongly implicated in the mucin excretion. Mucin plays an important role in the protection of the intestinal villi against endogenous enzymes and the abrasive effects of digesta (Sigolo et al., 2017). The latter is increased by the addition of silica which could explain the interaction between diet and enzyme on Thr digestibility. In the current study, when Enz-induced improvement in individual AA digestibility in the undigested fraction of the diluted diet was calculated, these ranged from around 15 to 18%. Average improvement (16.5%) is in agreement with Cowieson, (2010) who concludes, in a review of 19 published studies, a consistent 16% improvement in digestibility of the undigested protein fraction in the presence of Xyl based enzyme preparations.

In conclusion, this study has shown that Enz, a multi-enzyme complex containing Xyl and Abf, can restore nutrient availability when the nutrient content of a diet is diluted by 3%. The exact mechanism responsible for this is unclear, although it is apparent that an increase in AA digestibility and a potential effect on endogenous AA flow are, in part, responsible. The study also highlights the importance of considering all the nutrients when diets are reformulated to include enzymes and offers further understanding of AA digestion characteristics in poultry feed.

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COMPARISON OF DIFFERENT METHODS TO DETERMINE THE GASTROINTESTINAL PASSAGE RATE IN POULTRY

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Gastrointestinal (GI) passage rate of feed influences nutrient digestibility and absorption, as it dictates the amount of time the digesta are exposed to digestive enzymes, the intestinal villi and microbial fermentation (Vergara et al. 1989; Svihus 2010). The titanium dioxide (TO_2) marker technique is currently widely practiced for evaluation of GI passage rate. However, this method requires euthanising the bird to obtain digesta samples, which has negative implications from an animal welfare perspective, is labor intensive and eliminates the possibility of follow up studies with the same individual. The aim of this study was to evaluate alternative methods for evaluating GI passage rate, by comparing the TiO_2 marker method to radiographic evaluation of Barium Impregnated Polyethylene Spheres (BIPS) and barium sulphate suspension ($BaSO_4$).

Mature laying hens (80 weeks of age) were assigned to three treatment groups; group 1 (n = 5) and group 2 (n = 5) were orally administered with 30 BIPS of 1.5 mm-diameter and 5 ml $BaSO_4$ (125%) mixed with 5g mash feed, respectively. Whole body radiographs of each individual hen were taken at the following time points: 0, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 24, 36 and 48 hours (h) post-inoculation. Hens in group 3 (n = 36) were orally inoculated with 0.15g TiO_2 mixed with 5g mash feed. Three hens per time point were sacrificed using CO_2 asphyxiation and whole GI organ segments were collected and analysed for TiO_2 content, based on the method of Short et al. (1996). The level of BIPS and TiO_2 in each of the GI segments was quantified, and the presence or absence of $BaSO_4$ in the GI segment was evaluated. Mean percentage level of BIPS and TiO_2 in each GI segment at each time point was assessed using a one-way ANOVA on SPSS statistics v.24.

There was a significantly higher percentage of BIPS present in the crop at 0, 0.5 and 2 h (P = 0.004, 0.001, 0.038 respectively) post-inoculation and in the gizzard at 48 h (P = 0.004) post-inoculation compared to the amount of TiO_2 observed, indicating a comparatively slower transit time of the BIPS. Furthermore, a higher percentage of TiO_2 was present in the proventriculus (P = 0.003) and small intestine (P = 0.031) at 36 h post-inoculation, indicating a longer retention time of TiO_2 compared to BIPS. The percentage of TiO_2 measured at 1.5, 3, 12 and 24h post-inoculation was significantly higher in small intestine (P = 0.006, 0.002, 0.000, 0.002, respectively) and large intestine (P = 0.004, 0.026, 0.011, 0.000, respectively) compared to the BIPS observed. $BaSO_4$ was found to be present in all the organs in 80% of the hens at 3 h post inoculation and, by 36 h it had passed through the small intestine of all hens, with 20% of the hens displaying its presence in the large intestine until 48 h post-inoculation.

In conclusion, the evaluation of the GI transit time is feasible using BIPS, TiO_2, and $BaSO_4$. BIPS particles were retained longer in the GI tract mimicking solid feed particles, while $BaSO_4$ and TiO_2 showed a faster passage rate mimicking the GI transit time of liquids. In order to investigate physiologic digesta transit time, solid and liquid transit time need to be evaluated using different methods.

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INFLUENCE OF HATCH TIME ON MEAT CHICKEN LEG STRENGTH

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Summary

Across three trials, male meat chickens aged 5 weeks were subjected to a Latency to Lie test, and evaluated for signs of leg weakness. It was found that birds which hatched after 498 hours of incubation stood for longer and had fewer signs of leg weakness than those which hatched before 498 hours of incubation. Across four trials, tibial bone mineralisation was seen to decrease when birds spent 24 hours unfed after hatching. Providing earlier access to feed may improve leg strength in meat chickens, if sufficient mineral and nutrient uptake can be stimulated.

I. INTRODUCTION

Production improvements in commercial meat chickens via genetic selection and improved husbandry have resulted in rapid growing birds. These birds have endemically reduced locomotor ability; a conservative estimate is that 30% of birds in a flock are likely to have poor mobility (Knowles et al, 2008). Leg weakness has a direct impact on production due to lower bird quality, which may result in culls or condemnation of carcasses. While not well understood, early rapid growth can result in various nutritional imbalances, bone under-mineralisation, and associated metabolic disease, such as rickets and tibial dyschondroplasia (Angel 2007). These in turn predispose birds to bacterial penetration of bone growth plates and femoral head necrosis. The issue has attracted consumer and media attention and is considered an important welfare concern. Any improvement in bird leg strength is highly desirable.

II. MATERIALS AND METHODS

Four trials were conducted in which Cobb 500 eggs were obtained from hatcheries in NSW, from a total of 5 different breeder flocks of varying ages. The basic procedure of each trial was as follows:

a) Incubation

Eggs were set amongst six Multiquip E3 incubators and incubated at 37.8°C for 17.5 days. At that point, eggs were removed from the incubators and transferred into hatching trays which were divided into 60 individual hatching cells per tray. This allowed individual identification of chicks when they hatched. The hatcher trays were placed in a randomized fashion into one Aussieset™ incubator (Bellsouth Pty Limited, Victoria, Australia) at an initial temperature of 37.2°C and 60% relative humidity. Temperature was dropped to 36.0°C by 21 days of total incubation and relative humidity raised to 65%.

b) Hatch and Sampling

The time of hatch for each egg was observed and recorded from 468 hours of total incubation (HOI) (Embryonic Day (ED) 19.5) at 6 hour intervals, until 516 HOI (ED 21.5, designated Take off, TO). Chicks were only considered hatched once they had cleared the shell and their down was dry. In total, 2411 chicks were hatched. At 492 HOI (ED 20.5, designated Hatcher

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sampling, H) a selection of hatched chicks was removed from the incubator. Birds were euthanised and the right leg removed for tibia bone ash assay to determine bone mineralisation.

At TO, remaining birds were removed from the hatcher, identified, wing tagged and weighed. Chicks were selected again and sampled as described above. The remaining chicks were removed from the hatcher and allocated to small cages, at 4-5 birds per cage.

Three days from placement (designated Day 3, D3), chicks were randomly selected from the cages (approximately 2/cage). These birds were sampled in the same manner as above. In the final experiment, all hatched birds were sampled by this point in time.

Table 1 - Sample times.

Sample point	Hatcher (H)	Take off (TO)	Day 3 (D3)
Hours of incubation	492	516	588
Embryonic day	20.5	21.5	24.5

c) Grow-out Period

Remaining chicks were moved into a tunnel ventilated floor pen shed, combining cages without mixing treatment groups. Individual bird weight was recorded each week. After two and four weeks from TO, all birds were placed on a standard grower and finisher diet, respectively. On day 35 (Week 5), all visibly male birds, as determined by comb and wattle development and size, were selected from pens to be subjected to a modified latency-to-lie (LTL) test, where they were made to stand in a tub with approximately 3cm of tepid water (30-33°C) and timed until they sat down, up to a maximum of 5 minutes, at which point the birds were considered censored. After completing this test, birds were identified, weighed and euthanised. They were then assessed for foot pad dermatitis (FPD) and hock burn (HB). Sex was confirmed at post mortem. Remaining (female) birds were weighed and sent for commercial slaughter on day 35.

d) Analysis

Birds were grouped based on time of hatch – birds hatching before 486 HOI were classified as Early, between 486 and 498 HOI as Mid, and after 498 HOI as Late. Feed intake and FCR could not be calculated as birds were not necessarily grouped per the current hatch group classification scheme in each trial. Birds standing for 5 minutes in the LTL test, the incidence of FPD and HB, and bird weight on D35 were compared via ANOVA with hatch group as the treatment factor. LTL times were compared using Cox's F-Test. Bone ash results of Early and Mid birds were treated as one group and compared using ANOVA with sample time as the treatment. Late hatching bird bone ash results were subjected to a Student's T-test comparing TO and D3 results.

III. RESULTS

Chick hatch times are shown in Figure 1. Approximately 80% of birds hatched before 498 HOI.

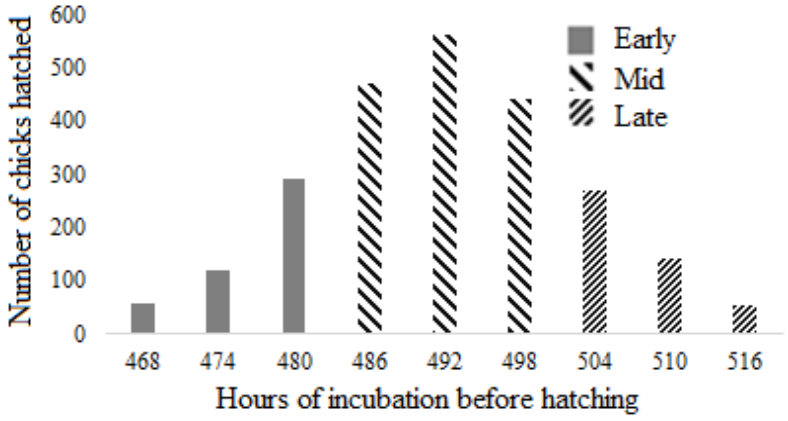

Figure 1 - Chick hatch times.

Survival curves for LTL time based on hatch groups are shown in Figure 2. Late and Mid hatching birds stood for significantly longer times than Early hatching birds (p = 0.004, 0.03). Late birds tended to stand for a longer time than Mid hatching birds (p = 0.055).

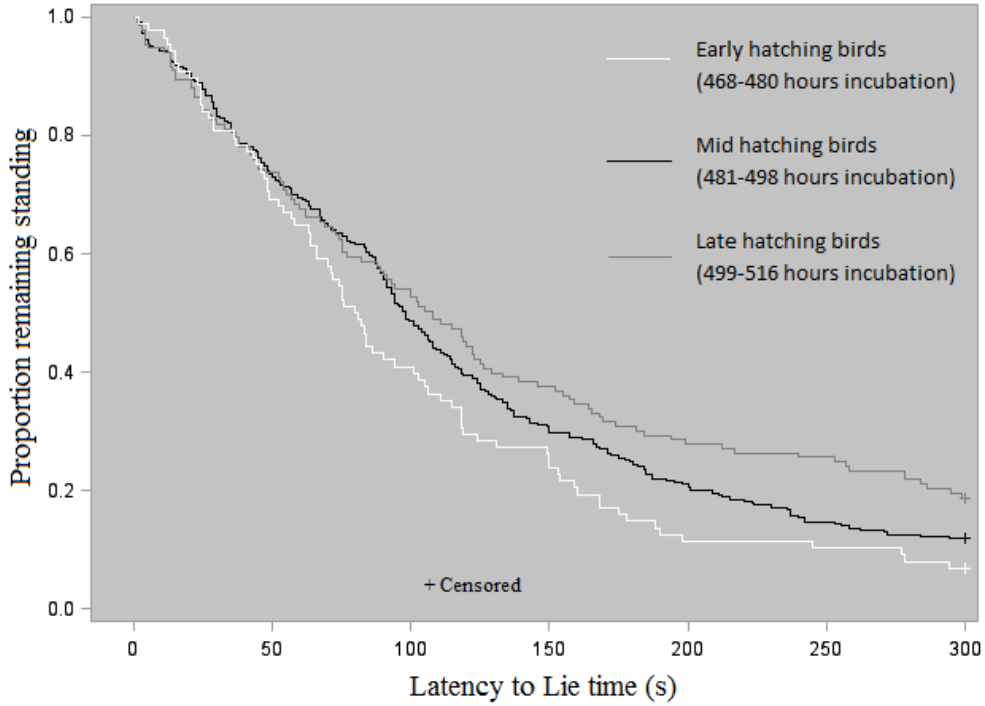

Figure 2 - Survival probability curves of LTL time based on hatch groups.

Table 2 - Descriptive statistics of LTL time based on hatch groups.

	Median	Mean	Standard deviation	Sat before 5 minutes	Stood for 5 minutes	Total number
Early	80.5	104.3	82.85	82	6	88
Mid	98	122.0	91.86	252	34	286
Late	108	136.3	105.61	108	25	133
Total	97	122.7	94.61	442	65	507

Seven percent of Early hatching birds stood for 5 minutes in the LTL test, compared to 12 percent of Mid and 19 percent of Late hatching birds ($p = 0.026$). Early hatching birds had higher incidences of FPD and HB, with Mid intermediate and Late the lowest ($p < 0.0001$). At time of LTL (5 weeks), Early hatching birds were the lightest, weighing 2480g compared to 2600g for Mid and 2654g for Late ($p < 0.001$).

For Early and Mid hatching birds, bone mineralisation decreased from 25.8% at H to 24.7% at TO, then rose to 32.4% at D3. ($p < 0.001$). Late hatching birds went from 25.0% bone mineralisation at TO to 32.9% at D3 ($p < 0.001$).

IV. DISCUSSION

Across trials, later hatching birds performed better in the LTL test, being most likely to remain standing for 5 minutes, and had fewer incidents of FPD and HB. This is a strong indication that leg strength and mobility was higher in these birds (Groves and Muir, 2016). Interestingly, they were heavier than Early hatching birds. This is important as it shows the impact leg strength has on production values. Lower weight in conjunction with less standing ability may indicate Early hatching birds are less able or inclined to stand and eat. This effect is not as prominent in the Mid hatching birds, however it is justifiable to conclude that hatching at a later time has had a positive effect on bird leg strength.

The advantage of a later hatching time may be due to Late hatching birds spending under 24 hours without feed compared to 24-48 hours without feed for Early and Mid hatching birds. It is possible to delay chick hatching time by reducing initial incubation temperature, which improves bone mineralisation at TO, as well as hatchability (Muir and Groves 2017). If fed between H and TO, bone mineralisation in Early and Mid hatching chicks can increase during this period (Muir and Groves 2017), rather than decrease as seen in unfed birds. Twenty-four hours without feed after hatching also impairs muscle growth (Powell et al 2016).

Strategies to provide early feed access include in-ovo feeding, provision of a pre-starter ration inside the hatcher incubator, feeding during transportation, and hatching/brooding systems such as Patio, Hatchbrood and Hatchcare. Identifying hatch time as a factor relating to leg weakness is a key step in improving meat chicken production. Implementing early feed access across the industry is an important reform that will greatly benefit poultry producers, consumers and bird welfare.

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IMPACT OF GRAIN TYPE ON PERFORMANCE AND GUT MICROBIOTA COMPOSITION IN BROILERS UNDER NECROTIC ENTERITIS CHALLENGE

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Summary

The aim of this study was to investigate the effect of grain type on performance and gut microbiota composition in broiler chickens under a necrotic enteritis (NE) challenge. Broilers were fed 3 different diets based on wheat, barley or rye, and were orally administered *Eimeria* and *Clostridium perfringens* to induce subclinical NE. The NE challenge significantly decreased weight gain, feed intake and counts of *Bacillus* and *Ruminococcus* bacteria in the caecal contents. Birds fed the rye based diets showed significantly reduced weight gain and feed conversion compared to the other diets. Rye fed birds also had the highest fluorescein isothiocyanate-dextran (FITC-d) marker in their serum and the lowest concentration of *Ruminococcus* bacteria and acetic acid in caecal contents. These findings suggest that grain type can directly influence the susceptibility and severity of NE challenge in broilers, largely through the impact of the non-starch polysaccharides (NSP) content of grain on nutrient availability, leaky gut, gut microbiota and short chain fatty acid composition.

I. INTRODUCTION

Necrotic enteritis (NE) is of great concern to the poultry industry due to its deteriorating impact on production and increasing mortality, resulting in a US$6 billion global economic loss (Wade and Keyburn, 2015). The causative agent of NE is *Clostridium perfringens*, a gram-positive spore-forming anaerobic bacterium. The subclinical form of NE is financially more devastating than the clinical form. This is due to a lack of obvious symptoms resulting in a delayed instigation of an effective treatment, and consequently a substantial loss in flock performance and reduced feed efficiency. Feed composition strongly influences the gut environment and hence may impact the severity of NE. Cereal grains such as wheat, barley and rye contain high amounts of non-starch polysaccharides (NSP). Soluble NSP increase digesta viscosity and reduce digesta transit time. Insoluble NSP act as a nutrient diluent and create a physical barrier to enzymes, thus reducing starch, protein and lipid absorption and digestion. The purpose of the present study was to compare the impact of high NSP containing grains (wheat, barley and rye) on susceptibility of birds to subclinical necrotic enteritis. Performance, leaky gut, gut microbiota and short chain fatty acid composition of NE challenged broilers were evaluated in the different diet groups.

II. MATERIALS AND METHODS

A total of 468 one-day-old male Ross 308 chicks were randomly assigned to 36 floor pens using a 2 × 3 factorial arrangement. The factors assessed were NE challenge (no or yes) and grain type (wheat, barley or rye based diets). Total NSP (soluble and insoluble) content of wheat (9.29 g/kg), barley (10.73 g/kg) and rye (11.28 g/kg) was evaluated. To induce the NE challenge, birds were orally administered 1 mL *Eimeria* (5,000 oocysts) on d 9, followed by 1 mL *C. perfringens* (10^8 CFU) on d 14 and d 15 (Rodgers et al., 2015). Birds and feed per pen

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were weighed on d 0 and d 24 to determine body weight gain (WG), feed intake (FI) and feed conversion ratio (FCR).

On d 16, two birds per pen were orally inoculated with FITC-d (4.17 mg/kg body weight) and serum samples were obtained 2.5 hours after inoculation, for leaky gut evaluation. The amount of FITC-d was measured by using a microplate reader (Synergy HT, Multi-mode microplate reader, BioTek Instruments, Inc., VT, USA). Caecal digesta samples were taken for bacteria enumeration by quantitative PCR (Shannon et al. (2007) and the method described by Jensen et al. (1995) was used for evaluating SCFA composition.

III. RESULTS

The NE challenge significantly reduced FI (P = 0.001), and WG (P = 0.001) from d 0-24, irrespective of dietary treatment, but FCR was not affected (P = 0.318) by the challenge (Table 1). Chickens fed with rye based diets showed a higher FCR (P = 0.001) and lower WG (P = 0.001). This group also had higher concentrations of FITC-d marker (P = 0.001) in their serum compared to those fed wheat or barley based diets. The NE challenge increased the FITC-d concentration (P < 0.001).There was no significant effect of the challenge or the grain types on mortality rates. Additionally, no grain type by NE challenge interactions for performance or FITC-d was observed (Table 1).

Table 1 - Effect of necrotic enteritis challenge and grain type on broiler performance.

Factor		Day 0-24				Day 16
		WG	FI	FCR	Mortality (%)	Serum FITC-d (mg/mL)
Grain type	Wheat	1326 [a]	2241	1.612 [b]	2.56	0.79 [b]
	Barley	1349 [a]	2156	1.575 [b]	3.21	0.84 [b]
	Rye	1193[b]	2133	1.727[a]	3.21	1.02 [a]
NE Challenge	No	1346 [a]	2251[a]	1.626	2.56	0.76 [b]
	Yes	1233[b]	2102[b]	1.650	3.42	0.99 [a]
P-Value	Grain type	0.001	0.094	0.001	0.935	0.001
	NE challenge	0.001	0.001	0.318	0.607	0.001
	Grain type x challenge	0.065	0.064	0.932	0.935	0.218

Data are expressed as means. Values within a row that do not share a common letter are significantly different (P ≤ 0.05).

Table 2 - Effect of necrotic enteritis challenge and grain type on bacteria count and short chain fatty acid production in the caecum.

Factor		Caecal Bacteria (log^{10} CFU)			Caecal SCFA (μmol/gm)	
		Bacillus	Lactobacillus	Ruminococcus	Acetic	Lactic
Grain	Wheat	9.06	9.17	9.83[a]	47.73	11.65
	Barley	8.78	9.03	9.78[a]	41.68	15.63
	Rye	8.80	9.26	9.28[b]	35.85	19.53
Challenge	No	9.20[a]	9.05[b]	9.83[a]	46.68[a]	11.86[b]
	Yes	8.56[b]	9.25[a]	9.42[b]	36.82[b]	19.35[a]
P- value	Grain type	0.607	0.065	0.001	0.117	0.060
	Challenge	0.017	0.013	0.001	0.046	0.011
	Grain type × challenge	0.942	0.632	0.563	0.267	0.565

Data are expressed as means. Values within a row that do not share a common letter are significantly different (P ≤ 0.05).

Table 2 shows that caecal *Bacillus* and *Ruminococcus* concentrations and acetate concentrations were decreased by the NE challenge (P < 0.05, P = 0.001 and P < 0.05, respectively). However, caecal *Lactobacillus* and lactic acid concentrations increased in

challenged groups ($P < 0.05$ and $P < 0.05$, respectively). Rye diets decreased *Ruminococcus* population in the caeca, compared to wheat and barley diets ($P = 0.001$). There were no grain type by NE challenge interactions for any of bacteria or SCFAs investigated.

IV. DISCUSSION

High NSP contents can damage the gut through either thickening the digesta or causing dysbacteriosis in the lumen (Teirlynck et al., 2011). Higher FITC-d levels observed in the rye diet fed chickens in the current study suggests that these diets had a more acute effect on the intestine tight junctions, causing a more severe leaky gut problem in the chickens. We also observed lower level of *Ruminococcus* bacteria in the caeca of birds fed with rye diets. The aforementioned bacteria are responsible for cellulose digestion, suggesting that lower performance observed in these birds could partially be due to lower polysaccharide fermentation in caeca. In addition, the lower level of *Bacillus* spp. and *Ruminococcus* observed in the NE challenged birds, could affect the acetate concentration due to lower degradation of starch and NSPs, which are the main sources for acetate production in the caecum. Fujiwara et al., (2009) reported that *Bacillus* spp. levels are associated with acetate concentrations in the caecum. Additionally, elevated lactic acid amounts in NE challenged birds have also been observed in other studies (M'Sadeq et al., 2015). It can be concluded that grains containing high amounts of NSP can negatively impact the bacterial population and bacterial enzyme production, consequently reducing the SCFA concentrations in the gut. Rye diets have been shown to have a major negative impact on intestinal tight junctions and also on the bacterial population of the gut. These factors could all lead to lower digestion and utilization of feed nutrients, hence resulting in the lower performance observed in chickens fed on rye diets.

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CONSISTENCY OF EFFICACY OF *BACILLUS* BASED PROBIOTICS ON VARIOUS DIET COMPOSITIONS

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Summary

Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. The efficacy of probiotics may depend on the type of diet and on the type of cereals which represent a major component of a broiler diet. The objective of this study was to evaluate the effect of *Bacillus subtilis* 29784 on growth performance of broilers fed either a corn and DDGS-based diet, or a corn/wheat and DDGS-based diet. This *Bacillus* strain was compared to a commercial *Bacillus subtilis (B. subtilis* X). At 22 d, *B. subtilis* 29784 increased BWG (+2.3%; P = 0.046) and decreased FCR (-2.5%; P = 0.015) compared to control birds, whereas *B. subtilis* X did not improve animal performance. At 42 days, regardless of the diet, the animals receiving *B. subtilis* X had the same performance as the control group, while animals supplemented with *B. subtilis* 29784 showed significantly higher performance (improvement of 2.6% in FCR, P = 0.009). This consolidates the consistency of the effect of strain *B. subtilis* 29784 on broiler performance.

I. INTRODUCTION

Since the ban on using antibiotics (AGP) as growth promoters in the EU in 2006, gut health has become a growing concern. As described by Conway in 1994, the gut health concept is based on three components: the diet, the microbiota, and the gut mucosa. All three components interact to maintain a dynamic equilibrium that ensures proper functioning of the digestive system without pathology. However, this equilibrium is fragile and can be affected by factors such as how the flock is managed (Tsiouris et al., 2015), the presence of pathogens (Moore et al., 2016), or the composition of the feed (Choct, 2009). Probiotics can represent a nutritional solution to reduce the need to use antimicrobial agents in animal husbandry (Sarangi et al., 2016; Rhayat et al., 2017). Probiotics contribute to improving gut health status through different modes of action, including maintaining a normal intestinal microbiota by competitive exclusion and antagonism of harmful microorganisms (Callaway et al., 2008). However, they can be sensitive to the substrates available for their development in the gut. Thus, the source of cereals in the feed may affect the effectiveness of the probiotic (Choct, 2009).

The recently isolated probiotic *Bacillus subtilis* 29784 has been shown to improve animal performance in three different trials, in which broilers were fed on corn- and soybean-based diets, under different rearing conditions. Our *in vitro* results also showed no differences between corn and wheat diets in terms of its germination speed and outgrowth rate. The present study investigated this probiotic and another commercially available, *B. subtilis* X, in 2 types of diets, based either on corn or a mix of wheat and corn. In addition, DDGS was systematically added to the diets as it also offers an opportunity to reduce the cost of nutritionally optimal diets for poultry.

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II. MATERIALS AND METHODS

A total of 3,120 one day-old male broiler chicken, Cobb 500, were randomly allocated according to a 2 x 3 factorial design, including six treatments, with 13 replicates of 40 birds per treatments. Birds were reared until 42 days in floor pens, using wood shavings as bedding. The experimental treatments were: T1, Control 1, corn-based diet; T2, T1 + *B. subtilis* 29784 at 1 x 10^8 CFU/kg feed; T3, T1 + *B. subtilis* X (1 x 10^8 CFU/kg feed); T4, Control 2, corn/wheat-based diet; T5, T4 + *B. subtilis* 29784 at 1 x 10^8 CFU/kg feed; T6, T4 + *B. subtilis* X (1 x 10^8 CFU/kg feed).

The experimental diets (Table 1) contained no coccidiostat and no AGP. Feed intake (FI), weight gain (BWG) and mortality were measured at 22 and 42 days of age, and feed conversion ratios (FCR) were calculated.

Performance parameters were subjected to ANOVA, with complete randomized design using the ANOVA procedure of XLSTAT (Addinsoft 1995-2014) to establish differences among treatments. The model included probiotic and diet as main factors. Pen was considered as the experimental unit. Results are reported as least square means. LS means were assumed to be different at $P < 0.05$. Mortality data were arcsin-transformed for ANOVA analysis.

Table 1 - Dietary composition and nutrient specifications of the experimental diets.

Dietary composition	Corn and DDGS-based diet			Corn/wheat/DDGS-based diet		
	Starter 0-10d	Grower 11-22d	Finisher 23-42d	Starter 0-10d	Grower 11-22d	Finisher 23-42d
Wheat	-	-	-	27.60	29.80	28.00
Corn	54.10	58.80	57.80	27.50	30.30	31.10
Soybean meal (46%)	25.00	21.50	23.50	23.00	19.00	21.00
DDGS	10.00	10.00	10.00	10.00	10.00	10.00
Corn gluten (60%)	5.00	3.00	-	5.00	3.00	-
Sunflower oil	1.00	2.00	4.00	2.00	3.00	5.00
L-lysine	0.40	0.40	0.40	0.40	0.40	0.40
DL-Methionine	0.30	0.30	0.30	0.30	0.30	0.30
L-Threonine	0.20	0.20	0.20	0.20	0.20	0.20
Monocalcium Phosphate	1.20	1.00	1.00	1.00	1.00	1.00
Premix	3.00	3.00	3.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient specifications						
ME, MJ	12.37	12.76	13.13	12.27	12.61	13.00
Crude protein, g/kg	22.10	19.7	18.7	22.2	19.7	18.7
Crude fat, g/kg	4.70	5.8	7.6	5.1	6.1	8.0
Crude fiber, g/kg	3.80	3.6	3.7	3.8	3.6	3.7
Ash, g/kg	5.80	5.5	5.6	5.6	5.4	5.4
dig. Lysine, g/kg	1.18	1.10	1.06	1.21	1.12	1.06
dig. Methionine, g/kg	0.55	0.52	0.50	0.53	0.50	0.47
dig. TSAA, g/kg	0.96	0.87	0.82	0.94	0.86	0.81
dig. Threonine, g/kg	0.84	0.76	0.73	0.85	0.77	0.73
dig. Tryptophan, g/kg	0.19	0.18	0.17	0.20	0.19	0.18
Calcium, g/kg	0.92	0.92	0.92	0.92	0.92	0.92
Total phosphorus, g/kg	0.77	0.75	0.74	0.77	0.75	0.74
Av. phosphorus, g/kg	0.45	0.45	0.45	0.45	0.45	0.45

Table 2 - Growth performance of broiler fed either a corn and DDGS-based diet, or a corn/wheat and DDGS-based diet (0-22 d period).

Treatments	Probiotics	Diets	Performance parameters			
			FI[1] (g)	BWG (g)	FCR	Mortality (%)
T1	No	Corn + DDGS	1215	954	1.27	4.8
T2	*B.* 29784	Corn + DDGS	1232	993	1.24	4.4
T3	*B.* X	Corn + DDGS	1213	964	1.26	4.2
T4	No	Corn/wheat + DDGS	1222	960	1.27	5.0
T5	*B.* 29784	Corn/wheat + DDGS	1199	967	1.24	4.0
T6	*B.* X	Corn/wheat + DDGS	1204	973	1.24	2.9
S.E.M.			10.084	9.904	0.012	1.403
Probiotic effect						
No			1219	957[a]	1.27[a]	4.7
B. 29784			1216	980[b]	1.24[b]	4.2
B. X			1209	969[ab]	1.25[ab]	3.4
Diet effect						
Corn + DDGS			1220	970	1.25	4.4
Corn/Wheat + DDGS			1209	967	1.26	3.8
Significance (P =)						
Probiotics			0.580	0.046	0.015	0.262
Diets			0.158	0.612	0.458	0.220
Probiotics x diets			0.123	0.091	0.548	0.222

[1]FI: feed intake; BWG: body weight gain; FCR: feed conversion ratio
[abc]Values with different letters are significantly (P < 0.05) different from each other.

Table 3 - Growth performance of broiler fed either a corn and DDGS-based diet, or a corn/wheat and DDGS-based diet (0-42 d period).

Treatments	Probiotics	Diets	Performance parameters			
			FI[1] (g)	BWG (g)	FCR	Mortality (%)
T1	No	Corn + DDGS	4796	2761	1.74	5.0
T2	*B.* 29784	Corn + DDGS	4726	2782	1.70	4.4
T3	*B.* X	Corn + DDGS	4785	2763	1.73	4.4
T4	No	Corn/wheat + DDGS	4831	2737	1.77	5.2
T5	*B.* 29784	Corn/wheat + DDGS	4742	2771	1.71	4.2
T6	*B.* X	Corn/wheat + DDGS	4798	2774	1.73	2.9
S.E.M.			33.675	21.646	0.014	1.271
Probiotic effect						
No			4814	2749	1.75[a]	5.1
B. 29784			4734	2776	1.71[b]	4.3
B. X			4791	2768	1.73[ab]	3.7
Diet effect						
Corn + DDGS			4791	2761	1.74	4.6
Corn/Wheat + DDGS			4769	2769	1.72	4.1
Significance (P =)						
Probiotics			0.057	0.438	0.009	0.132
Diets			0.436	0.656	0.261	0.213
Probiotics x diets			0.941	0.719	0.580	0.159

[1]FI: feed intake; BWG: body weight gain; FCR: feed conversion ratio
[abc]Values with different letters are significantly (P < 0.05) different from each other.

III. RESULTS AND DISCUSSION

At 22 days, a significant effect of probiotics was detected, with no effect of diet, and no interaction between probiotics and diets. Thus, performance of broilers was significantly improved with *B. subtilis* 29784, with +2.3% in BWG (P = 0.046) and -2.5% in FCR (P = 0.015), whereas there was only a tendency towards improvement with *B. subtilis* X (+1.25% in BWG and -2.0% in FCR compared to control birds). There was no difference in mortality between groups during this period.

At 42 days, although there was no effect of probiotics on BWG; FCR was improved without interaction with diets. The performance of broilers was increased with the incorporation of *B. subtilis* 29784 whatever diet type, with an improvement of 2.6% in FCR (P = 0.009). FCR of *B. subtilis* X group did not significant differ from the control group. Also, mortality was not different between treatments, and was 4.2% in average.

In conclusion, these results suggest consistency of the efficacy of *B. subtilis* 29784 on broiler performance when corn or corn/wheat diets were used together with DDGS.

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EFFECT ON PERFORMANCE PARAMETERS OF DIFFERENT DOSES OF A NOVEL BACILLUS CHCC15076 PROBIOTIC FEED STRAIN FOR THE CONTROL OF NECROTIC ENTERITIS IN BROILERS

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The use of *Bacillus* species as probiotic supplements is expanding rapidly and demonstrates immune stimulation, antimicrobial activities, enzyme production and competitive exclusion as the most prevalent modes of action. The objective of this study was to investigate the dosage influence on performance parameters of the novel probiotic strain CHCC15076 in birds recovering from necrotic enteritis (NE) caused by *Clostridium perfringens*.

The experiment consisted of 1920 day of hatch Cobb 500 male chicks distributed in 48 pens starting with 40 chicks per pen. The treatments were replicated in eight blocks, randomized within blocks of six pens each. Feed and watering were performed *ad libitum*.

Four different doses (5 x10^4 CFU/g feed to 3 x10^6 CFU/g feed) were applied to the feed throughout the trial. Mean weight gain (WG), feed intake, feed conversion ratio (FCR), NE lesion scores and % NE mortality were calculated. On days 19, 20, and 21 all pens except for treatment 1 pens were challenged with a field isolate of *C. perfringens* known to cause NE. Each pen received the same amount of *C. perfringens* administrated in the feed. On day 21, three birds from each pen were sacrificed and examined for the degree of presence of NE lesions. The NE lesion scoring was as follows: 0 for normal intestines up to 3 for extreme NE.

Data were analysed with R vs. 3.2.5. Body weight and NE lesion scores were analysed using a regression model (procedure lm from the core package) with treatment group as fixed effect. A logistic regression models (procedure glm of the MASS package) with treatment group as fixed effect was fit to assess the odds of being positive for NE lesions. Residual plots were checked to evaluate model fit. Statistical significance was assessed at $P \leq 0.05$. Results for lesion scores at day 21 were significantly improved for the CHCC15076 treated groups at the dose 1x10^5CFU/g of feed or higher. Likewise, the NE associated % mortality was improved significantly for all birds receiving CHCC15076.

Day 42 performance results	C. perfringens infection	Feed intake	FCR	Avg. Wt. gain	Day 21 lesion score	% NE Mortality
Non-medicated	-	138.63^{ab}	1.627^{c}	2.272a	0.05d	0.0b
Non-medicated	+	136.70^{abc}	1.731a	2.155b	0.58a	4.3a
CHCC15076 5 x 10^4 CFU/g	+	135.64^{bc}	1.669b	2.151b	0.40ab	0.7b
CHCC15076 1 x 10^5 CFU/g	+	131.31^{c}	1.645bc	2.175b	0.28bc	1.4b
CHCC15076 5 x 10^5 CFU/g	+	141.73^{a}	1.645bc	2.303a	0.18cd	0.7b
CHCC15076 3 x 10^6 CFU/g	+	139.47^{ab}	1.629c	2.277a	0.18cd	0.4b

Numbers contain the same letter subscribed were not significant distinguishable ($P \leq 0.05$).

Conclusions for this trial; Inclusion of the novel *Bacillus* CHCC15076 probiotic strain decreases the degree of NE lesion scores and mortality, and several of the doses improve the FCR and final slaughter weight. The experiment illustrates the importance of the correct dosage of probiotic for the performance and efficacy of the birds. The mode of action for this novel probiotic involves antimicrobial activities and competitive exclusion against pathogens.

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PERFORMANCE AND INTESTINAL HEALTH OF BROILER CHICKENS SUPPLEMENTED WITH A PROTEASE AND FED A STANDARD DIET OR A LOW-DENSITY DIET

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Summary

The effect of a protease on performance and intestinal health of broiler chickens was evaluated when supplemented on top of a standard diet or in a low-density diet. Male Cobb chicks (392; 1-42d) were reared in floor pens and allocated in a completely randomized design in a 2x2 factorial arrangement with 7 replicates. There were 2 feed formulations: a standard diet (STD) and a low-density diet (LDD), with 6% reduction in crude protein and main digestible amino acids. The 2 diets were either supplemented (+P) or not (-P) with a protease (Jefo Protease at 125 g/t). The performance was evaluated by feeding period (1-7, 7-21, 21-35 and 35-42d). At day 28, samples of ileum of two bird/replicate were analyzed by a morphometric index for histological alterations. There was no interaction between factors and no differences between the treatments were observed for the first 7 days of age. In general, for all the other periods, birds fed the LDD-P were lighter and/or had poorer feed conversion ratio (FCR) when compared to all other treatments ($P < 0.05$). The supplementation of the LDD with protease increased body weight gain (BWG) and decreased FCR ($P < 0.05$), promoting a performance similar to birds fed the STD-P. At 42d, the birds on the STD+P were the heaviest (124g difference to STD-P, $P = 0.1$) and presented the same FCR as the STD-P and LDD+P groups while being 13 points lower ($P < 0.001$) than the LDD-P group. Regarding the gut health analysis, birds receiving the protease presented the best morphological index ($P \leq 0.06$) mainly as a result of fewer alterations of lamina propria and epithelial thickness and enterocyte proliferation. In conclusion, the protease improved performance and intestinal health indicators of broiler chickens when added on top of a standard diet or with a low-density diet.

I. INTRODUCTION

Protease enzymes can improve the dietary protein utilization. Therefore, it is possible to decrease the level of dietary protein to save on feed cost while maintaining performance, reduce nitrogen excretion in the environment, and minimize the risk of enteric infections. The objective of this study was to evaluate the effect of a protease on performance and intestinal health of broiler chickens fed a standard diet or a low-density diet.

II. MATERIALS AND METHODS

A total of 392 day-old male broiler chickens (Cobb 500) was distributed in 4 treatments with 7 replicates over 42 days. The birds were reared on wood shavings in floor pens (14 birds/pen) and fed *ad libitum*. Each pen represented an experimental unit. Two diets were used: 1) a standard diet based on nutritional recommendations of the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011) and 2) a low density diet, with 6% reduction in crude protein and digestible lysine (the ratio of the other essential amino acids to lysine was

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kept the same as in the Standard Diet). The two diets were either supplemented or not with protease (Jefo Protease, Jefo Nutrition Inc.; Table 1). A four-phase feeding program during the rearing period was used (1-7, 7-21, 21-35, and 35-42 days; Table 2).

Table 1 - Treatments distribution.

	Feed Formulation	Enzyme addition
T1	Standard Diet[1]	-
T2	Standard Diet	Protease[3] (125 g/t)
T3	Low-Density Diet[2]	-
T4	Low-Density Diet	Protease (125 g/t)

[1]Based on the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011); [2]6% reduction in crude protein and amino acids. The ratio of the other essential amino acids to lysine was kept the same as in the Standard Diet; [3]Jefo Protease, Jefo Nutrition Inc.

Table 2 - Composition of the experimental diets (as fed basis).

	1-7 d		7-21 d		21-35 d		35-42 d	
	Standard[1]	-6%[2]	Standard	-6%	Standard	-6%	Standard	-6%
Ingredients (%)								
Soybean meal	39,66	36,49	36,53	32,97	33,07	29,72	29,54	26,63
Corn	53,38	57,21	56,16	60,42	59,15	63,13	63,06	66,53
Soybean oil	2,70	2,06	3,47	2,75	4,39	3,72	4,36	3,78
L-Lysine	0,27	0,26	0,30	0,31	0,22	0,23	0,24	0,24
DL-Methionine	0,22	0,20	0,19	0,18	0,18	0,17	0,17	0,16
L-Threonine	0,10	0,09	0,07	0,07	0,06	0,06	0,06	0,06
NaCl	0,51	0,51	0,48	0,48	0,46	0,46	0,44	0,44
Limestone	0,55	0,54	0,68	0,67	0,68	0,68	0,65	0,65
Phosphate	2,46	2,49	1,97	2,00	1,64	1,68	1,33	1,36
Choline	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05
Vit-Min Premix	0,10	0,10	0,10	0,10	0,10	0,10	0,10	0,10
Nutritional Composition, %								
CP	22,40	21,25	21,20	19,93	19,80	18,61	18,52	17,48
ME, kcal/kg	2960	2960	3050	3050	3150	3150	3200	3200
Dig Lys	1,32	1,25	1,28	1,20	1,13	1,06	1,06	1,00
Dig Met	0,52	0,49	0,48	0,45	0,45	0,43	0,42	0,40
Dig Met+Cys	0,90	0,85	0,84	0,80	0,80	0,76	0,76	0,73
Dig Thr	0,86	0,81	0,79	0,74	0,74	0,69	0,69	0,65
Dig Trp	0,26	0,21	0,24	0,22	0,22	0,20	0,20	0,19
Dig Arg	1,43	1,34	1,34	1,24	1,24	1,15	1,15	1,07
Dig Val	0,98	0,94	0,93	0,88	0,88	0,83	0,82	0,78

[1]Based on the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011); [2]6% reduction in crude protein and amino acids. The ratio of the other essential amino acids to lysine was kept the same as in the Standard Diet.

At d 28, a sample of ileum was collected from two birds per replicate and analyzed by the I See Inside Scoring System Methodology (ISI) according to Kraieski et al. (2017). In this methodology, an impact factor (IF) ranging from 1 to 3 is defined for each alteration in microscopic analysis according to the reduction of the organ's functional capacity, where 3 is the most impactful. In addition, the intensity and extension of each alteration is evaluated and a score from 0 to 3 is assigned, with 0 being the absence of lesion. To reach the final value of the ISI® index, the IF of each alteration is multiplied by its respective score and the results of each individual alteration are summed. Therefore, a lower number of ISI index represents better result. Body weight (BW), feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were evaluated by feeding periods. Data were analyzed by ANOVA

and Fischer LSD using the General Linear Model (GLM) procedure (SAS Inst. Inc., Cary, NC) in a completely randomized design with four treatments. A $P \leq 0.05$ was used to indicate statistical significance and a $P \leq 0.1$ was used to indicate tendency.

IV. RESULTS AND DISCUSSION

a) Intestinal health

Birds supplemented with the protease presented the best total ISI® morphological index ($P \leq 0.06$) mainly as a result of the lower ($P < 0.05$) number of alterations regarding lamina propria, epithelial thickness and enterocytes proliferation (Table 3).

Table 3 - Histology analysis of the ileum / ISI® methodology.

	Standard Diet[1]		Low-density Diet[2]		*P*-value
	No Protease (T1)	Protease[3] (T2)	No protease (T3)	Protease (T4)	
Lamina propria thickness	0.96[b]	0.76[b]	0.82[b]	0.37[a]	0.02
Epithelial thickness	0.07[a]	0.04[a]	0.21[b]	0.07[a]	0.02
Proliferation of enterocytes	0.13[ab]	0.06[a]	0.23[b]	0.10[a]	0.04
Epithelial plasma infiltration	0.64	0.66	0.82	0.76	0.16
Mixed inflammatory infiltration[4]	1.91	1.44	1.62	1.83	0.39
Increase of goblet cells	2.00	1.58	1.74	1.93	0.26
Congestion	0.58	0.42	0.46	0.17	0.15
Necrosis / apical karyolysis	0.09	0.17	0.05	0.05	0.60
Presence of oocysts	0.00	0.00	0.00	0.00	-
Total	6.38[b]	5.13[a]	5.95[ab]	5.29[a]	0.06

[ab]Means followed by different letters differ ($P \leq 0.06$); [1]Based on the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011); [2]6% reduction in crude protein and amino acids. The ratio of the other essential amino acids to lysine was kept the same as in the Standard Diet; [3]Jefo Protease, Jefo Nutrition Inc; [4]in lamina propria.

b. Performance

Comparing both treatments without protease supplementation, birds fed the Standard Diet (T1) presented higher BW and better ($P < 0.05$) FCR at 21 ($P < 0.05$) and 42d ($P < 0.10$) compared to birds fed the low-density diet (T3), showing that the nutritional reduction applied was sufficient to reduce performance (Table 4). Protease supplementation on top of the standard diet (T2) tended to improve ($P = 0.1$) BW, in which treatment birds were 124 g heavier at 42 days than the ones fed the same diet formulation with no protease supplementation (T1); feed intake and FCR were not affected. Similar results were obtained by Lahaye et al. (2016) and Yu et al. (2007). Supplementation of the low-density diet (T4) with protease allowed the recovery of performance losses due to poor BWG and FCR, promoting an overall performance similar to the control treatment (T1). Because FI was not affected, these observations may be explained by a better efficiency of dietary protein use as previously shown by Wang et al. (2008), who demonstrated that protease supplementation could improve nutrient digestibility and FCR of broiler chickens.

Table 4 - Overall performance.

		Standard Diet[1]		Low-density Diet[2]		*P*-value
		No Protease (T1)	Protease[3] (T2)	No Protease (T3)	Protease (T4)	
1-7d	BW 7d, g	170	169	168	169	0.90
	FI, g	166	162	172	165	0.80
	BWG, g	120	120	118	120	0.88
	FCR	1.37	1.35	1.45	1.38	0.55
7-21d	BW 21d, g	876^{a}	848^{ab}	802^{b}	842^{ab}	0.05
	FI, g	1,014	1,027	1,006	1,03	0.79
	BWG, g	706^{a}	679^{ab}	635^{b}	673^{ab}	0.04
	FCR	1.44^{a}	1.51^{b}	1.58^{b}	1.53^{b}	0.004
21-35d	BW 35d, g	2,144	2,146	2,04	2,1	0.23
	FI, g	2,095	2,094	2,222	2,067	0.20
	BWG, g	1,268	1,298	1,237	1,258	0.68
	FCR	1.65^{a}	1.61^{a}	1.80^{b}	1.64^{a}	<.0001
35-42d	BW 42d, g	$2,730^{xy}$	$2,854^{x}$	$2,664^{y}$	$2,769^{xy}$	0.10
	FI, g	1,01	1,194	1,121	1,1	0.27
	WG, g	586^{y}	709^{x}	624^{xy}	669^{xy}	0.07
	FCR	1.72	1.69	1.79	1.64	0.20
1-42d	BW 42d, g	$2,730^{xy}$	$2,854^{x}$	$2,664^{y}$	$2,769^{xy}$	0.10
	FI, g	4,284	4,477	4,521	4,361	0.26
	BWG, g	$2,680^{xy}$	$2,805^{x}$	$2,615^{y}$	$2,719^{xy}$	0.10
	FCR	1.60^{a}	1.60^{a}	1.73^{b}	1.60^{a}	<.0001
	FRC (adj.)	1.61^{a}	1.57^{a}	1.76^{b}	1.60^{a}	<.0001

[1]Based on the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011); [2]6% reduction in crude protein and amino acids. The ratio of the other essential amino acids to lysine was kept the same as in the Standard Diet; [3]Jefo Protease, Jefo Nutrition Inc; [4]Adjusted to 2.754 kg (average BW at 42d). Considers that there is a 0.3 increase in feed conversion for every 1 kg of body weight gain over 2.754 kg; BW, body weight; FI, feed intake; BWG, body weight gain; FCR, feed conversion ratio; Means followed by different letters differ ($^{abc}P < 0.05$, $^{xy}P < 0.1$).

V. CONCLUSION

The protease studied can be used on top of a broiler standard diet to improve bird performance or with a low-density diet as a strategy to reduce feed cost while avoiding loss of performance. In both cases, the protease positively impacted intestinal health indicators.

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DOES MATERNAL FLOCK AGE AND PRE-STARTER DIET COMPOSITION ALTER LEG STRENGTH IN COBB 500 CHICKS?

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While the breeding programs of commercial meat chickens select birds with robust legs (Davis, 2015), poor leg strength and reduced locomotion are frequently observed in late stages of growth. We have previously reported that lower egg shell temperatures during early incubation, which delays chick hatch time, increases bone ash (BA) (Muir and Groves, 2017). Observations from our research and the literature (e.g. Ulmer-Franco et al., 2010) suggest that maternal breeder flock age may influence the amount of some nutrients and minerals within the yolk, which could impact chick bone strength. Additionally, we have proposed that pre-starter diets with varying levels of Ca and P may improve the leg strength of commercial meat chickens (Muir and Groves, 2017). The current experiment evaluated the effect of maternal breeder flock age and pre-starter ration Ca and P levels on chick BA, bodyweight, serum Ca and P levels and, 5 week latency to lie (LTL) in Cobb 500 chickens.

Fertile eggs were sourced from a 34 and a 50 week old Cobb breeder flock and incubated at 37.8^{0}C. At 18 days incubation each egg was transferred into an individual hatching cell in a hatching tray. Chick hatch was observed every 6 hours from 19.5 until 21.5 days of incubation, and was used as a covariate in analyses. At take-off chicks from each breeder flock were randomly allocated to one of three pre-starter diets – standard, low Ca and high P, which they were fed for three days. From 4 days of age all birds were fed a standard ration for the remainder of the 35 day experiment. Bone ash was measured at take-off and day 3. Serum Ca and P was determined at day 3 and, at 5 weeks of age all visibly male birds were assessed for their ability to stand in a LTL test. Bird weights were also measured.

Maternal flock age did not significantly alter BA, day 3 serum Ca and P nor LTL in 5 week old male birds. Chicks arising from the older breeder flock were significantly heavier when 1 and 3 days old ($P<0.05$). At 3 days of age chicks fed the high P diet had significantly higher BA than the standard or low Ca fed chicks. Concurrently serum Ca was significantly lower in chicks fed the low Ca and high P diets, while serum P was lowest in chicks fed the standard ration and, highest in chickens fed high P diet ($P<0.05$). No significant effect of diet was observed on 5 week LTL, but, birds fed the high P ration gave the longest standing times. Hatch time did not affect BA at take-off, but, at day 3 BA was significantly higher in later hatching (>20.75 days) chicks. Similarly late hatch birds had a longer LTL time ($P=0.108$) than earlier hatching birds.

Maternal flock age had no significant effect on the leg strength of their offspring, but, older breeder hens generated heavier chicks at hatch. While the high P diet favoured improved leg strength, chick hatch time had the greatest effects on leg strength, as we have previously reported (Groves and Muir, 2014; Muir and Groves, 2017). A later hatching time increases bone ash in young chicks and, generates longer standing times in 5 week old birds.

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INTERMITTENT LIGHTING IMPROVES RESILIENCE OF BROILERS DURING THE PEAK PHASE OF SUB-CLINICAL NECROTIC ENTERITIS INFECTION

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Reviews made on the impact of intermittent lighting (IL) on broiler performance (Buyse et al., 1996; Rodrigues and Choct, 2017) attest to the consistency of such lighting programs in achieving better feed conversion rates. So far, no research has been conducted to assess the impact of IL on the susceptibility to and recovery from infectious diseases. We hypothesized that an IL program would increase broiler resilience to necrotic enteritis (NE). In order to test this hypothesis, a 2 x 2 experiment was conducted. There were two factors, namely, lighting schedule (continuous lighting (CL, 10-12 lux), 18L:6D vs. IL (IL, 10-12 lux), 1L:3D:1L:3D:1L:3D:1L:3D:2L:6D) and sub-clinical NE challenge (challenge vs. no challenge).

A total of 390 Cobb 500 mixed sex, day-old chicks (initial weight, 35.8 ± 5.0 g) were raised until d 7 under 23 h of light (L, 20 lux) and 1 h of dark (D) (23L:1D). At day 7, 12 birds were selected at random (d 7 weight, 154.8 ± 1.4 g) and allocated to one of 4 treatments in single floor pens (0.7 m x 0.70 m). Sub-clinical NE challenge was performed in half of the birds as per Wu et al. (2014). Birds and feeders were weighed weekly to ascertain the impact of both IL and NE on animal performance (Table 1).

Table 1 - Performance of broilers in the peak phase of CP Type A infection.

Lighting Program (LP)		NE Challenge (NE)	d 14 to d 20		
			Feed Intake (g/bird)	Body Weight Gain (g/bird)	FCR†
CL		No	581	429	1.356[c]
CL		Yes	549	259	2.117[a]
IL		No	547	413	1.327[c]
IL		Yes	422	258	1.647[b]
Main effects	LP	CL	565[a]	344	1.737
		IL	484[b]	335	1.487
	NE	No	564[a]	421[a]	1.342
		Yes	485[b]	258[b]	1.882
P values		LP	0.007	0.566	0.000
		NE	0.008	0.000	0.000
		LP x NE	0.100	0.624	0.002

Data were analysed using 2-way ANOVA (SPSS Statistics, ver. 24). Means were compared using the Tukey multiple range test.
[a-c] Means within a column with different superscripts differ significantly (P < 0.05). †Mortality corrected feed conversion rate'

In the week following CP inoculation, NE negatively impacted FI and BWG of birds (P < 0.05). FI of birds submitted to IL was also lower than CL-fed birds (P < 0.05). FCRc was better for unchallenged animals (P < 0.005); however, the negative impact of the disease on efficiency was attenuated by IL (P < 0.05). In the overall GIT, lesion frequency was higher for challenged birds than for unchallenged birds (P < 0.05), but no interaction was observed between factors (P > 0.05). Throughout the trial, mortality remained low (< 1%) and unrelated to tested factors (P > 0.05). These data and those reported by Rodrigues et al. (2017) show that IL may have potential to minimize the negative impact of sub-clinical NE, especially during the peak phase of infection.

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β-GLUCAN ENRICHED YEAST CELL WALL ADJUVANTS STIMULATED HUMORAL IMMUNE RESPONSE AND PROTECTED BROILERS AGAINST VIRAL CHALLENGE

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Yeast β-glucans consist of a β(1,3)-linked glucose backbone, with β(1,6)- linked side chains at different interval and sizes along the backbone. They are recognized by several receptors present on the surface of innate immune cells, such as the Dectin-1 receptor. Previous research done with crude yeast cell wall (YCW) extracts, have led to the description of β-glucans as immune-stimulant molecules resulting in a strong activation of macrophages that secrete high levels of inflammatory cytokines (Goodridge et al., 2009). However, recent research has shown that this macrophage activation is most likely the result of a combined activation of the Dectin-1 receptor with other pattern recognition receptors (PRRs), including TLR-receptors that can interact with other Pathogen Associated Molecular Patterns (PAMPs) in the crude YCW. When YCW are enriched in β-glucan content, the PAMPs are partially or totally removed, while Dectin-1 signaling remains intact. In vitro assays performed with β-glucan-enriched YCW show only poor macrophage activation (Walachowski et al., 2016). Nevertheless, Walachowski et al. (2017) demonstrated that pretreatment of macrophages with these β-glucan-enriched YCWs, showed an exacerbated cytokine production in response to a secondary stimulation with TLR ligands, a concept also known as immune training. The objective of this study was, therefore, to evaluate the effect of β-glucan enriched YCW adjuvants on broiler immunity during a vaccination and challenge trial.

β-glucan enriched YCW were prepared as 2% aqueous solutions, containing killed Newcastle Disease (ND) vaccine and administered by eye-drop to 21-day old specific pathogen free (SPF) broiler chickens. Two control groups received either the normal vaccine control or physiological solution. Fourteen days after vaccination, blood samples were taken to assess antibody titers. Additionally on day 14 after vaccination, animals were challenged with a virulent ND strain after which survival rates were evaluated. Blood analyses clearly showed that β-glucan enriched YCW adjuvant vaccines were able to stimulate the production of ND specific antibodies to a significantly higher level than the normal vaccine control. In challenged birds, vaccination with β-glucan enriched YCW was able to significantly decrease ($P < 0.01$) the viral loads found in target organs. Finally, a 100% protection against the lethal ND challenge as compared to 80% protection with normal vaccine control or 0% in the negative control birds was observed when using β-glucan enriched YCW as adjuvant.

In conclusion, it was demonstrated that adjuvants based on β-glucan enriched YCW can induce a strong humoral immune response, which we think has greatly contributed to the effective protecting against a virulent ND challenge. Even though, it is imperative to investigate the role of the cell mediated immunity, we believe that it is plausible to hypothesize here that innate immune training achieved with β-glucan enriched YCWs might influence the development of adjuvant strategies as it could affect the antibody titer and the overall protective response.

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EFFECT OF VITAMIN E SUPPLEMENTATION ON PRODUCTION AND HEAT SHOCK PROTEIN 70 IN BROILER CHICKENS DURING HOT-HUMID SUMMER

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Summary

Total of 120 broiler chicks were reared in cages on a standard broiler starter diet up to 14 days of age and thereafter up to 42 days on test diets with or without Vitamin E in a hot-humid environment. There were 5 replicate groups of 8 birds for each of three treatments, viz. T1 (control diet), T2 (vitamin E @ 150mg/kg) and T3 (vitamin E @ 250mg/kg) Body weights and food intake were measured weekly and production parameters were calculated. Production indices (body weight gain and feed efficiency) improved ($P<0.01$) significantly upon supplementation from 21 days onwards. Supplementation of Vitamin E at both levels caused significant ($P<0.05$) down regulation of relative expression of HSP70 in jejunum tissues during 28th and 42nd day. Based on this study it was concluded that supplementation of Vitamin E at 150mg/kg in broiler diets significantly improved their performance and welfare under heat stress conditions.

I. INTRODUCTION

Chicken meat and egg production has been on the increase in all continents with the highest increases in Asia and South America (Mandal, 2010). High temperatures coupled with high humidity impose severe stress on birds and can cause huge economic loss. Such loss is due to reduced performance, immune suppression, and an increase in respiratory disease and mortality (Mandal, 2010). Exposure of chickens to high temperature also increases the induction of heat shock protein 70 (HSP70) in different tissues including the brain, liver and lungs (Sahin *et al.,* 2009). Considerable attention has been paid to the role of nutritional manipulation in minimizing the effects of heat stress. To reduce heat stress losses antioxidant, vitamins and minerals are commonly enriched in the diets of birds reared under conditions likely to result in heat stress (Sahin and Kucuk, 2003). Antioxidant nutrient supplementation, especially Vitamins E inclusion in diet is effective in heat stressed broilers (Felipe *et al*., 2015). Since poultry cannot synthesize Vitamin E and its concentration is reduced under heat stress conditions, Vitamin E requirements must be met from dietary sources. The present study was designed to evaluate Vitamin E as anti-heat-stressor under high temperature and humidity conditions.

II. MATERIALS AND METHODS

120 broiler chicks were reared in cages on a standard broiler starter diet (214.4 g/kg CP and 2839 kcal ME/kg) up to 14 days of age and thereafter finisher diet (197.5g/kg CP and 2891 kcal ME/kg) up to 42 days on test diets with or without vitamin E. Dietary treatment groups were three in number *viz*., T1 (control diet), T2 (vitamin E @ 150mg/kg) and T3 (vitamin E @ 250mg/kg). Each treatment comprised of five replicate of eight birds each. Experiment was carried out during the hot-humid summer (August-September, 26.0±0.12 to 34.2±0.37°C, RH %: 77.0±0.90-86.1±0.61).

Birds and food issues and returns were recorded weekly to 42 days of age and weight gains, food intakes and feed efficiency were calculated over relevant intervals. Mortalities were recorded as they occurred. To assess the HSP70 expression, jejunal samples were

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collected at d 28 and 42. Total cellular RNA was isolated using RNAgents® Total RNA Isolation System (Promega, Madison, USA), purity and quantity were assessed by measuring the optical density of each sample at 260 versus 280 nm in nanodrop. Total RNA sample (2 µg) was reverse transcribed using the RevertAid First Strand cDNA Synthesis Kit (MBI Fermentas, Hanover, USA) according to the manufacturer's instructions. The qPCR assays were evaluated by the generation of a standard curve. Calibration curves for each gene were done on an iQ5 cycler (Bio-Rad Laboratories, Hercules, USA) with five 10-fold serial dilutions (in triplicates) and were calculated by the Bio-Rad Optical System Software (Version 2.1, 2010) with the analysis mode "PCR base line substracted". Expression of HSP70 was studied in real time PCR (SYBR® Green Qiagen GmbH, Hilden, Germany) using the designed primers (Table 1). PCR cycling conditions included initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 30s, annealing for 30s and extension at 72°C for 45s. The mRNA expression levels (expression ratio) of HSP70 gene were analyzed by REST 2009 software.

Table 1 - Oligonucleotide sequence of HSP70 gene primers.

S. No	Gene	Primer	Annealing Temp.	Length (bp)	Reference / Acc. number
1	HSP- 70	F- GGCACCATCACTGGGCTT R- TCCAAGCCATAGGCAATAGCA	56°C	74	HM587997
2	GAPDH	F-CCGTCCTCTCTGGCAAAGTCC R-AGCCCCAGCCTTCTCCATG	57.5°C	266	NM_204305

III. RESULT AND DISCUSSION

Weekly BWG was significantly higher in the vitamin E supplemented treatments between 3 and 4 weeks of age than in the unsupplemented controls; subsequently, there were no significant differences between treatments. The feed intake during different weekly periods except 21st and 35th day period remained comparable. Vitamin E supplementation at both 150 and 250 mg/kg improved feed efficiency each week from 21 to 42 days of age. In agreement with our findings, Sahin *et al.* (2001) concluded that supplemental vitamin E in broilers reared under heat stress conditions, significantly increased feed intake, body weight gain and helped to improve the overall performance of birds. Supplemental Vitamin E resulted in increased feed and water consumption.

Moreover, vitamin E is involved in reducing free radical formation and thus providing welfare besides less secretion of corticosterone, a catabolic hormone. Figures 1 and 2 indicate the relative fold expressions of HSP70, at 28 and 42 days, respectively, of the two vitamin E treatments compared with the control. Data were calculated according to Pfffal *et al.* (2002) and showed significant decrease in expression of the HSP70 gene in the vitamin E treatments. At 28th day post-hatch Vitamin E supplementation at 150 mg/kg as well as 250 mg/kg level bring significant ($P<0.01$) down regulation in HSP70 gene. Significant ($P<0.05$) down regulation in HSP 70 gene is also observed at 42nd day of age. Kapkin *et al.* (2013) concluded that heat stress reduces the secretion of HSP-70 in testes of broilers, when Vitamin E is administered. Similar to present study, Ushakova *et al.* (1996) showed that dietary supplements of Vitamin E can modify gene expression induced by heat shock in vivo and have a protective role against oxidative stress by enhancing the level of endogenous antioxidants and down regulating HSP70 gene expressions in mice. Vitamin C and vitamin E are primary antioxidants in biological systems and break the chain of lipid Peroxidation in cell membranes. Several studies have investigated the relationship between HSP expression and vitamin C (Mahmoud and Edens, 2003).

Table 2 - Effect of supplemental vitamin E on weekly production performance during hot-humid summer.

Treatment	0-14 d	14-21 d	22-28 d	29-35 d	36-42 d
Body weight gain (g)					
T1	206.4	171.6^{a}	338.1	398.7	361.0
T2	206.9	246.4^{b}	368.1	368.2	380.8
T3	207.6	247.1^{b}	367.5	391.5	387.1
SEM	2.20	11.46	7.39	5.68	8.50
P-value	NS	P<0.001	NS	NS	NS
Feed intake (g)					
T1	338.4	287.6^{a}	629.7	794.3^{b}	787.0
T2	338.8	405.4^{b}	681.9	720.8^{a}	803.4
T3	340.9	402.8^{b}	673.7	762.3^{ab}	804.4
SEM	3.95	18.03	13.58	12.12	17.17
P-value	NS	P<0.001	NS	P<0.05	NS
Feed efficiency					
T1	1.64	1.68^{c}	1.86^{b}	1.99^{b}	2.18^{c}
T2	1.64	1.65^{b}	1.85^{b}	1.96^{a}	2.11^{b}
T3	1.64	1.63^{a}	1.83^{a}	1.95^{a}	2.08^{a}
SEM	0.003	0.006	0.004	0.006	0.014
P-value	NS	P<0.001	P<0.01	P<0.001	P<0.001

Means bearing different superscript differed significantly (P<0.05); NS= Non-significant

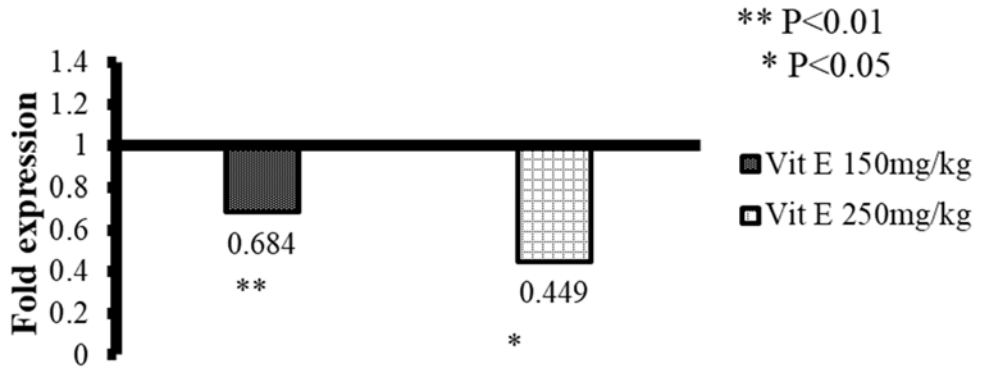

Figure 1 - Effect of vitamin E supplementation on HSP70 expression during hot humid summer on 28th day.

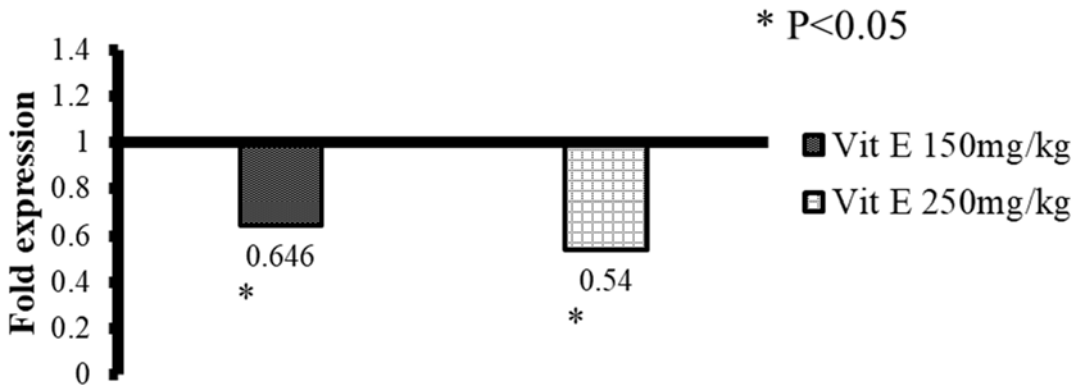

Figure 2 - Effect of vitamin E supplementation on HSP70 expression during hot humid summer on 42nd day.

IV. CONCLUSION

It was concluded that supplementation of Vitamin E at 150mg/kg under hear stress conditions will reduce stress through impact on heat shock proteins leading to improvements in both growth performance and welfare of birds

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EFFECTS OF MEAT AND BONE MEAL, PHYTASE AND ANTIBIOTICS ON GROWTH PERFORMANCE IN BROILER CHICKENS DURING NECROTIC ENTERITIS CHALLENGE

H.K. ZANU[1], N.K. MORGAN[1], M. TOGHYANI[1], S.B. WU[1] and R.A. SWICK[1]

Meat and bone meal (MBM) is a valuable source of protein, calcium and available phosphorus for broiler diets (Anwar et al., 2016). The use of MBM in broiler diets at levels above 50 g/kg minimizes the need for inclusion of inorganic P, thus reducing feed cost. Potential indigestibility of protein in MBM (Kim et al., 2012) may act as a predisposing factor for necrotic enteritis (NE). Increased production of nitrogenous bacterial metabolites including amines and ammonia in the hindgut may increase pH and favour proliferation of *Clostridium perfringens*. A study was designed to investigate the hypothesis that superdosing with phytase in meat and bone meal free diets might enhance growth performance during the challenge period with necrotic enteritis compared with diets containing MBM. The experiment used 672 Ross 308 male broilers in a completely randomized design with a factorial arrangement of treatments. Factors were meat and bone meal (no or yes), antibiotic (zinc bacitracin, 100 mg/kg in starter (S), grower (G) and 50 mg/kg in finisher (F) with salinomycin, 60 mg/kg in S, G, F), phytase (500 using 500 matrix or 1500 using 500 FTU/kg matrix) (Quantum Blue^{TM}, AB Vista, Malborough, UK) and MBM (none or 60, 50 and 50 g/kg in S, G, F). The birds were fed wheat-SBM-canola meal based diets in which MBM partially replaced soybean meal and completely replaced limestone throughout the experiment, but completely replaced mono and di-calcium phosphates only during the starter and grower phases of the study. There were 14 birds per pen and 6 replicates per treatment. All birds were challenged with 5000 unattenuated sporulated oocysts each of *Eimeria acervulina*, *E. brunetti* and *E. maxima* (Bioproperties Pty Ltd) on d 9, and 10^8 CFU of *C. perfringens* Strain EHE-NE18 (known to express NetB toxin (CSIRO) on d 14 and again on d 15. Inclusion of MBM in the diets improved body weight gain on d 7 (pre-challenge; $P < 0.05$). However on d 21 (post-challenge) and d 28, MBM inclusion decreased BW relative to those fed MBM-free diets ($P < 0.05$). No MBM effect was detected on BW on d 35 or d 42 ($P > 0.05$). Interactions between the phytase level and the presence of antibiotics were detected for BW on every weigh day ($P < 0.001$), indicating a positive effect of phytase superdose on BW in the presence of antibiotic (lower incidence of NE after challenge). Similarly, phytase by antibiotic interactions were detected for FCR on d 21, d 28, d 35 and d 42 ($P < 0.01$) indicating a greater effect of antibiotics in decreasing FCR in the presence of superdose phytase. On d 42, (across MBM levels), FCR of birds fed no antibiotics was 1.528 with 500 FTU phytase and 1.623 with 1500 FTU phytase whereas with antibiotics FCR was 1.519 with 500 FTU phytase and 1.553 with 1500 FTU phytase. No MBM by phytase interactions were detected for BW or FCR at any time during the experiment ($P > 0.05$). The results suggest that superdosing with phytase may result in the release of additional nutrients, such as calcium that may influence bacterial growth in the hindgut through elevation of pH. In the presence of antibiotics, birds were protected from NE. The greatest weight gain and lowest FCR was with 1500 FTU phytase, MBM with antibiotic. If NE challenge is expected, antibiotics and full matrix value for the dose of phytase should be used to minimize excess calcium in the hindgut. In conclusion, superdosing phytase improves growth performance of broiler chickens growing under optimum conditions.

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A *BACILLUS SUBTILIS* PROBIOTIC IMPROVES BROILER PERFORMANCE AND FOOTPAD CONDITION

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Footpad dermatitis (FPD) is considered an animal welfare issue. Wet and sticky litter are major causes of FPD (Taira et al., 2014). Our previous study showed that a well selected probiotic strain *Bacillus subtilis* 29784 can improve broiler performance by modulating intestinal microbiota and intestinal inflammatory status (Ghane et al., 2017; Jacquier et al., 2016). We hypothesize that the previous demonstrated benefits of *B. subtilis* 29784 on gut health will improve litter quality and may decrease FPD. In the present study, we evaluated *B. subtilis* 29784 for its effects on performance, litter quality, occurrence and severity of FPD of broiler.

The experiment was carried out at the Center for Expertise and Research in Nutrition (Adisseo, Commentry, France) and was conducted according to the European Union Guidelines of Animal Care and legislation governing the ethical treatment of animals. Three hundred and twenty day old male chicks Ross PM3 were randomly distributed into 2 treatments with 8 pen replicates of 20 birds per replicate (density: 13.3 birds/m²). All birds were housed in the same environmentally controlled house, with a wood shaving litter, and fed on corn-soybean meal based diets. The two treatments were: 1) Control, and 2) Control + *B. subtilis* 29784 (1 x 10^8 CFU/kg of feed, from 0 to the end of experiment). The experiment was conducted until 35 days and broilers were euthanised with inhaled carbon dioxide gas. At 35 days, performance parameters (weight gain, feed intake and feed conversion ratio) were measured. Litter quality and FPD were scored, following the Welfare Quality® (2009) method, on all birds. Mortality was assessed daily. Data were analysed using ANOVA, and Tukey's test for significance.

At 35 days, mortality rate was not influenced by dietary treatments and was 4% on average. Probiotic supplementation improved WG by +4.8% (P = 0.01) and FCR by -2.9% (P = 0.003). 60% of pens in the Control group had highly degraded litter (Score 4), whereas this condition was absent in the pens of the probiotic group (P = 0.031). The probiotic addition significantly improved FPD scores, with 21% decrease of pens scored as "4, highly necrotic" (P = 0.008) and 11% decrease of pens with combined scores "3+4, highly and visibly necrotic" (P = 0.014). As expected, the improvement of litter score resulted in a decrease of FPD. Better litter condition could be at least partially explained by the positive effect that *B. subtilis* 29784 has on gut health parameters, such as microbiota changes and inflammation reduction (Jacquier et al., 2016; Ghane et al., 2017).

In conclusion, the incorporation of *B. subtilis* 29784 into feed resulted in a concomitant improvement in both performance and well-being, as reflected by improvement of litter quality and reduction of severe FPD. These effects are probably a direct consequence of an improvement of gut health parameters, as shown in previous studies.

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ADSORPTION ASSAYS ON *CLOSTRIDIUM PERFRINGENS* ALPHA-TOXIN: *IN VITRO* AND *IN VIVO* APPROACHES

S. RAHMAN[1], N. VAN SAN[1], M. CABALLERO[2], I. HEINZL[2]

Summary

One *in vitro* and two *in vivo* experiments were conducted to test the effects of an adsorbent (ADS), consisting of a combination of clay minerals (natural mixture of illite, montmorillonite and kaolinite), yeast cell walls (from *Saccharomyces cerevisiae*) and plant extracts, on a solution of *C. perfringens* α-toxin after one hour of incubation, and on the performance of broilers orally challenged with a broth culture of *Clostridium perfringens* ($1x10^6$ CFU/mL). ADS demonstrated a dose dependent *in vitro* adsorption on *C. perfringens* α-toxin and had a positive effect on the growth and performance of the animals.

I. INTRODUCTION

Clay minerals and yeast fractions are regarded as effective tools for the prevention of the negative effects of toxic compounds in animal feed and production (Kubena et al., 1990; Lillehoj et al., 2016; M'Sadeq et al., 2015; Phillips et al., 1988). Clays and yeast fractions, when added to animal feeds, can adsorb certain toxic materials in the gastrointestinal tract of the animals, reducing their bioavailability. Dietary supplementation with clays has been shown to improve weight gain and feed conversion in pigs and poultry (Almeida, 2015).

Different modes of action have been proposed with regard to these materials (Almeida, 2015), adsorption being among them (Phillips et al., 1988; Yiannikouris et al., 2004). Clays and yeast fractions can bind to mycotoxins, heavy metals and bacterial toxins, preventing the increase in intestinal permeability and cellular damage exerted by these toxins, thus improving animal health and performance (Kubena et al., 1990; Lillehoj et al., 2016; M'Sadeq et al., 2015; Phillips et al., 1988).

Damage to the intestinal mucosa caused by toxins from *Clostridium perfringens* - among them α-toxin - leads to decreased digestion of feeds and absorption of nutrients and, as a consequence in broilers, weight gain is reduced and feed conversion increased (Elwinger et al., 1992).

The aim of the study was to evaluate the ability of an adsorbent (ADS) composed of clay mineral, yeast cell walls and plant extracts, to bind to *C. perfringens* α-toxin and enhance performance of *C. perfringens* challenged broilers.

II. MATERIALS AND METHODS

a) *In-vitro* experiment: efficacy of ADS on *C. perfringens* α-toxin

Four buffer solutions were prepared with equal α-toxin concentrations and increasing concentrations of ADS (0, 10, 50 and 100 mg/mL). The solutions were incubated at 27°C for one hour. The mixtures were centrifuged and the supernatant of each solution was taken for measurement of toxin concentration using an antigenic sandwich ELISA kit for the detection of *C. perfringens* α-toxin (BioX diagnostics).

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b) *In-vivo* experiments: effect of ADS against *C. perfringens* toxins in a broiler model

Experiment 1

30 birds (Ross 308, males) were individually orally challenged with 1 mL of a broth culture (10^8 CFU of *C. perfringens*) on the 3rd and 4th days of life. Of the 30 challenged birds, 15 received a diet including ADS (1 kg/ton), and 15 were used as a challenged control (CC). 15 additional birds were left unchallenged (NCC). Feed and water were provided *ad libitum*. The feeding programme included pre-starter and starter diets. The birds were weighed on days 1, 8 and 14 of life. The performance of the CC and ADS birds was compared with the NCC group.

Experiment 2

30 broilers (Ross 308, males) were challenged in the same way as in Experiment 1. The birds were divided into two groups, CC and ADS, which were fed and managed identically, except for the feed additive (ADS) that was supplied to the ADS birds.

The feeding program included a pre-starter and starter diets. The birds were weighed on days 1, 8, 14 and 21 of life. To further evaluate the *C. perfringens* challenge, blood serum was collected on day 21, and analyzed for anti-α-toxin antibodies (ELISA).

III. RESULTS AND DISCUSSION

a) *In-vitro* experiment: efficacy of ADS on *C. perfringens* α-toxin binding

The *in vitro* experiment measured the dose response effect of increasing ADS doses (0, 10, 50 and 100 mg/ml) in solutions of 1 mg/mL *C. perfringens* α-toxin on adsorption of α-toxin. The dose response effect of ADS on the toxin was demonstrated with the percentage of adsorption ranging from 0% when no ADS was added to 95% at the highest dose (Table 1).

Table 1 – *In-vitro* adsorption of increasing levels of α-toxin by an adsorbing agent (ADS).

Dose of toxin/adsorbent	ELISA OD_{450}	Adsorption (%)
Toxin (1 mg/ml) + dilution buffer	0.454	0
Toxin (1 mg/ml) + 1% ADS (10 mg/mL)	0.367	20
Toxin (1 mg/ml) + 5% ADS (50 mg/mL)	0.191	60
Toxin (1 mg/ml) + 10% ADS (100 mg/mL)	0.023	95

b) Effect of ADS against *C. perfringens* toxins in a broiler chicken model

In vivo Experiment 1:

The experimental design allowed for assessment of the effect of challenge on performance (CC versus NCC), and for assessment of the treatment effect in challenged birds (ADS versus CC). In this experiment, a significantly higher body weight was obtained in the ADS and NCC groups compared with CC at all measured times, and NCC and ADS had similar results (Table 2). The higher body weight in the challenged birds may have allowed them to allocate more nutrients into growth and production to achieve the performance of the NCC group. No differences in other performance parameters (mortality and FCR) were found among the three groups (data not shown).

Table 2 - Weekly Body Weight ± SD of broilers challenged with α-toxin with and without the supplementation of an adsorbent (ADS) compared with a group of non-challenged control birds (NCC).

	CC	NCC	P value NCC*	ADS	P value ADS*
1 days	38.6 ± 2.7	39.5 ± 2.6	NS	39.0 ± 3.0	NS
7 days	141.8 ± 13.1	158.8 ± 10.2	0.0002	152.5 ± 12.5	0.015
14days	425.9 ± 44.9	469.3 ± 20.0	0.0001	455.0 ± 32.3	0.025

* compared to CC, t-test. NS: non-significant

In vivo Experiment 2

The experimental design allowed only for assessment of the treatment effect in challenged birds (ADS versus CC), as a non-challenged group was not included. Body weight of the ADS group was significantly higher from day 8 onwards (Table 3).

Table 3 - Weekly Body Weight ± SD of broilers challenged with α-toxin with and without ADS.

Age	CC	ADS	P value
1 day	44.9 ± 2.6	44.3 ± 3.2	NS
8 days	212.1 ± 21.8	223.3 ± 13.5	0.05
14days	501.0 ± 35.8	525.4 ± 42.0	0.05
21days	890.6 ± 62.6	949.1 ± 65.3	0.009

NS: non-significant

Anti α-toxin antibody titers were measured in the serum at 21 days of age. The ADS group had significantly lower anti α-toxin antibody titers in comparison with the control group (Figure 1), indicating that the birds may have been less affected by the challenge. Similar results were obtained by Lillehoj et al. (2016) when using a montmorillonite based dietary supplement. They found low α-toxin antibody levels in the birds consuming the supplement compared with challenged birds (co-infection with *Eimeria maxima* and *C. perfringe*ns).

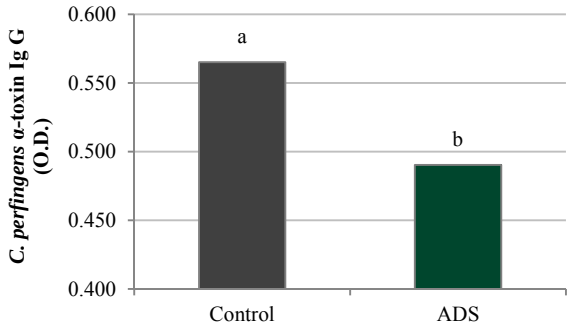

Figure 1 - Anti α-toxin antibody (IgG) titers in serum of broilers at 21 days of age challenged with *C. perfringens* and fed a diet with and without ADS (different superscripts: statistical significance P<0.05).

IV. CONCLUSIONS

Although the adsorption of bacterial toxins by clay minerals and yeast cell walls is still a poorly understood field, the ADS showed binding activity *in vitro* and a positive influence on the growth performance of *C. perfringens* challenged broilers. More research should be conducted in order to understand the effects of ADS under *C. perfringens* challenges and its mechanisms of action in broilers.

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A REVIEW OF THE APPLICATION OF POLYPHENOLS IN POULTRY

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Summary

Polyphenols are a class of compounds that recently have gained increasing interest as feed additives, especially for use in poultry. The growing interest has been strongly driven by the ban of most antibiotic feed additives within the European Union in 1999, with a complete ban enforced in 2006 with ongoing discussion to restrict their use outside of the European Union. The removal of in-feed growth promoters has led to animal performance problems and a rise in poultry disease, such as subclinical necrotic enteritis, making the need to find alternatives to in-feed antibiotics paramount. Polyphenols or phytogenic feed additives are a relatively new class of feed additives which show potential due to their antimicrobial and antioxidant properties.

I. INTRODUCTION

In poultry meat production birds face several challenges, all of which disturb the normal functioning of the organism, with the gastrointestinal tract being the most affected. This results in impaired absorption of nutrients, leading to reduced performance and increased mortality. Previously general practice was to feed antibiotics at sub-therapeutic levels to enable birds to cope with the challenges during growth (Wati et al. 2015). With the European Union passing legislation to ban the use of in-feed antibiotics in 2006, many other countries are pursuing this path, Australia included. This has been the main driving force to seek alternative 'natural growth promoters'.

One of the main challenges Australian antibiotic free producers will face surrounds intestinal health, specifically prevention and control of coccidiosis and necrotic enteritis (Cervantes, 2015). Removing ionophore anticoccidials and antibiotic feed additives is certain to cause problems in controlling coccidial parasites and bacterial organisms, particularly *Clostridium perfringens* (Van der Sluis, 2000). Ideally, an alternative to in-feed antibiotics should have the same beneficial effects when included in the diets; it is generally accepted that in-feed antibiotics and growth promotors elicit some antibacterial actions and thereby reduce the incidence and severity of subclinical infections (Wati et al. 2015). Polyphenols are almost ubiquitous in plants, with certain polyphenols such as quercetin common among all plants, whereas other polyphenols are specific to particular food plants (Manach et al. 2004). Antimicrobial activity and immune enhancement are two major properties possessed by polyphenolic compounds that are essential for the health and wellbeing of poultry and which make polyphenols an ideal candidate as an alternative to antibiotics and ionophores.

The objective of the present paper is to present an overview of polyphenols, their potential mode of action and the role that they can play in replacing in-feed growth promoters in broiler nutrition.

II. OVERVIEW OF POLYPHENOLS

Polyphenols (phenolic compounds or phytogenic feed additives) constitute one of the most extensive groups of chemicals in the plant kingdom, with more than 8,000 compounds being isolated and described (Surai, 2014). Polyphenols are products of secondary metabolism in plants, and they arise biogenetically from two main synthetic pathways; the shikimate pathway and the acetate pathway (Harborne, 1989). Natural polyphenols can range from

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simple molecules such as phenolic acid to highly polymerised compounds such as tannins. They occur predominately in conjugated form, with one or more sugar residues linked to hydroxyl groups, although direct linkages of the sugar molecule to an aromatic carbon atom also exist (Manach et al. 2004; Bravo, 1998). The associated sugars can be present as monosaccharides, disaccharides or even as oligosaccharides, with glucose being the most common (Bravo, 1998).

Polyphenols are classified into different groups as a function of the number of phenol rings they contain, and on the basis of structural elements that bind these rings to one another. The main classes include; flavonoids, phenolic acids, tannins, stilbenes and lignans. Of these groups, flavonoids are the most widely abundant, with more than 4,000 varieties identified. Yang et al. (2009) further classified polyphenols based on their biological origin, formulation, chemical description and purity, and devised four main categories:

1. Herbs – products from flowering, non-woody and non-persistent plants
2. Botanical – entire or processed parts of a plant, e.g. roots, leaves, bark
3. Essential oils – hydro-distilled extracts of volatile plant compounds
4. Oleoresins – extracts based on non-aqueous solvents

There are numerous factors that affect the phenolic content of plants, including but not limited to; part of the plant used, ripeness, time of harvest, environmental factors, geographical origin, processing and storage (Huyghebaert et al. 2011; Windisch et al. 2008). Polyphenolic content in cereal grains is usually less than 1% of the dry matter, with the exception of sorghum (*Sorghum bicolor*) cultivars, which have as much as 10% phenolic content. Isoflavones are the main phenolic compound found in legumes, with the darker legumes such as red kidney beans and black beans tending to have higher polyphenolic content (Hermann, 1988).

III. POLYPHENOLS AND POULTRY FEED

a) Application of polyphenols and coccidiosis infection

Coccidiosis infection caused by *Eimeria spp*. is a common disease challenge facing poultry producers. Annually, this disease causes a global loss of 2.4 billion US dollars (Muthamilselvan et al. 2016). Anticoccidial chemicals, coccidiocides, coccidiostats and ionophores have long been used as mainstream strategies to control coccidiosis (Chapman et al. 2010). Despite the effectiveness of these products, there is a push towards banning and/or limiting their use; therefore, natural products are emerging as a potential way to combat coccidiosis. Currently, there are at least four commercially available plant-based products on the market, many of which contain phenolic compounds. In the literature, there are numerous studies demonstrating the positive effects of phenolic compounds on the prevention of coccidiosis infection. Jang et al. (2007) demonstrated that green tea polyphenols significantly inhibited the sporulation process of coccidian oocycts. This was further supported by Molan and Faraj (2015) who observed that the selenium and polyphenolic compounds in green tea extract were necessary for inactivation of the enzymes responsible for coccidian sporulation. Molan et al. (2009) investigated the used of pine bark extracts (*Pinus radiata*), a natural rich source of condensed tannins, on *Eimeria spp.* oocyst development. They reported a significant reduction of infectious oocysts in the environment, due to the ability of the condensed tannins to penetrate the oocyst and interfere with the endogenous enzymes responsible for sporocyst formation. Naidoo et al. (2008) compared the use of 4 plant phenolic compounds (*Combretum woodii*, *Vitis vinifera*, *L. Artemisia afra* and *Tulbaghia violacea*) with Toltrazuril, a veterinary grade anticoccidial used as a positive control. Dietary inclusion of *Combretum woodii* at 160 mg/kg was shown to be highly toxic to the birds, whereas dietary treatments containing *Tulbaghia violacea* (35 mg/kg), *Vitis vinifera* (75

mg/kg) and *Artemisia afra* (150 mg/kg) produced FCRs comparable to the anticoccidial drug. Birds that were fed *Tulbaghia violacea* showed a decreased in oocysts per gram of faeces compared to infected birds receiving no treatment. In addition to decreased shedding of oocysts, the lower levels were maintained for the duration of time birds were fed *Tulbaghia violacea* and were then seen to increase after treatment ceased. The promising results of polyphenols warrant further research into the use of these plant extracts as therapeutic or prophylactic anticoccidial agents.

b) Polyphenols and gut health

Gut health has recently become a subject of interest in poultry research. The gastrointestinal tract is the pivotal organ which mediates nutrient uptake and use by the bird. The gut is also the main site of potential exposure to pathogens (Yengi and Korver, 2008). When gut function is impaired, digestion and absorption are affected, which in turn compromises health and performance of poultry.

A wide range of spices, herbs and their extracts with phenolic content are known to exert beneficial effects within the gastrointestinal tract (Chrubasik et al. 2005). Stimulation of digestive secretion, bile and mucus as well as enhanced enzyme activity is proposed to be one of the core modes of action of polyphenolic compounds (Platel and Srinivasan, 2004). As polyphenols come from a wide variety of plants, which vary in composition and content of active ingredient, it makes it difficult to understand the mode of action of all phenolic compounds, with some modes of action potentially only being possible when a defined combination of ingredients are available (Grashorn, 2010). However, there has been a lot of speculation as to the possible mechanisms through which polyphenols exert their beneficial effects on the gut (Windisch and Kroismayr, 2007). These include:

1. Modulation of the cellular membrane of microbes leading to membrane disruption of the pathogens.
2. Increasing the hydrophobicity of the microbial species which may influence the surface characteristics of microbial cells and thereby affect the virulence properties of the microbes.
3. Stimulating the growth of favourable bacteria such as lactobacilli and bifidobacteria in the gut.
4. Acting as an immunostimulatory substance.
5. Protecting the intestinal tissue from microbial attack.

Studies have shown that essential oils containing phenolic compounds enhance the activity of trypsin and amylase in broilers. Jang et al. (2007) tested the use of a commercially available blend of essential oils (29% active ingredients including thymol) at different inclusion rates against antibiotics. They observed that, when the fed was supplemented with essential oils and lactic acid, there was a significant increase in trypsin activity compared to the diet supplemented with antibiotics. Total and specific amylase activities were also observed to be significantly increased compared to the antibiotic dietary treatment. However, the dietary treatment group using only essential oils did not stimulate digestive enzyme activity, which is consistent with the findings of Lee (2002).

Saponin is a broad classification term used for amphipathic glycosides. In order for glycosides to be absorbed they must be hydrolysed into their corresponding aglycone, commonly taking place in the large intestine by caecal microflora (Manch et al. 2004). Saponins have been proposed to reduce intestinal ammonia formation, and thus pollution of the housing and environment (Francis et al. 2002). Studies by Killeen et al. (1998) and Duffy (2001) both observed that active phenolic compounds in *Yucca schidigera* were able to lower intestinal urease activity and enzymes involved in the metabolic urea cycle in rats, and Nazeer et al. (2002) further confirmed that Yucca extracts reduced intestinal and faecal

urease activity when fed to broilers. Further research is warranted to clarify the potential use of saponins as feed additives in poultry feed.

IV. CONCLUSIONS

Despite the varying results reported in the literature, the use of polyphenols for their antimicrobial and antioxidant properties in animal health is promising, with a wide application across multiple disease and health challenges. Future research into the antimicrobial and gut health properties of polyphenols in production animals is needed. As metabolic activity differs widely among polyphenols, safety needs to be assessed separately for individual polyphenol products. Studies on the interactive effect between polyphenols and enzymes are limited and further investigation is warranted if polyphenols are to use be as an alternative to in-feed antibiotics.

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APPARENT METABOLIZABLE ENERGY, ILEAL DIGESTIBILITY AND BONE QUALITY OF BROILER CHICKENS FED WHEAT-BASED DIETS SUPPLEMENTED WITH CARBOHYDRASES

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Summary

The objective of this study was to assess apparent metabolizable energy (AME), ileal digestibility and bone quality of broiler chickens offered wheat-based diets supplemented with carbohydrases. Birds were raised in cages in climate-controlled rooms. Excreta samples were collected from trays underneath each replicate cage between 20 and 23 days for estimation of AME. Samples of ileal digesta were collected from three randomly selected birds per cage at 24d for estimation of digestibility of amino acids, crude protein, gross energy and starch. At day 35 two birds per cage were randomly selected; the right drumstick was collected for assessment of bone breaking strength and concentrations of minerals in the tibia.

I. INTRODUCTION

Microbial enzymes are now routinely used in broiler chicken diets to reduce the effects of antinutritive factors (ANF) in feed and/or improve the digestion of nutrients (Bedford, 2011). There is a suite of enzymes that target ANF such as non-starch polysaccharides (NSP) and phytic acid as well as products which improve the digestion of nutrients such as protein and minerals. The major cereals used in poultry diets are wheat, maize, sorghum and barley. The objective of this study was to investigate the effectiveness of two NSPases and a phytase in wheat-based diets fed to broiler chickens. The impact of the products was assessed with respect to digestibility of energy and nutrients such as starch and protein (amino acids) and parameters related to bone quality.

II. MATERIALS AND METHODS

A total of 648 male Ross 308 broiler chickens was randomly assigned to a 3 (none, low (30 mg/kg) and superdose (300 mg/kg) phytase levels) × 2 (none and optimum (100 mg/kg) xylanase levels) × 2 (none and optimum (100 mg/kg) β-glucanase levels) full factorial study in a completely randomized design. Each of the 12 treatments was replicated 6 times, with 9 birds per replicate. The diets were fed *ad libitum* from 0 to 35 days in 3 phases – starter (1-10 d), grower (11-24 d) and finisher (25-35 d). AME was determined using an indigestible marker (TiO_2) and collection of excreta samples from trays underneath each replicate between 20 and 23 days of age. AME was then calculated as: AME = GEi - [GEo× (Ti/To)], where GEi is gross energy (MJ/kg) in feed; GEo is the gross energy (MJ/kg) in excreta, Ti is the concentration of titanium in the diets and To is the concentration of titanium in the excreta. At 35 days, two birds per pen were randomly selected, slaughtered by cervical dislocation; the right drumstick was collected and frozen for later assessment of bone breaking strength and concentrations of minerals in the tibia. Following defrosting of the tibias, adherent muscle, tissue, cartilage caps and fibula were removed manually. Breaking strength is the force required to break the bone and was measured in the range of 0 to 500 N

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using a Lloyd Tensile Testing machine. The entire bones were then dried and ashed, and these samples then analysed for mineral contents. A general linear model procedure was used to analyze the collected data (Minitab, 2013).

III. RESULTS AND DISCUSSION

Interactions ($P < 0.01$) between phytase, xylanase and β-glucanase with regards to gross energy (GE) and apparent metabolizable energy contents are shown in (Table 1).

Table 1 - Apparent metabolizable energy and ileal digestibility of protein, gross energy and starch of birds fed diets supplemented with different levels of Phytase, Xylanase and β-glucanase at 24 d of age.[1]

Phytase levels	Xylanase levels	β-glucanase levels	AME[2]	Protein	GE[3]	Starch
Main effects:						
None			13.80^{c}	0.81^{c}	0.78^{c}	0.95^{b}
Low			14.23^{b}	0.85^{b}	0.81^{b}	0.96^{b}
Superdosing			15.11^{a}	0.88^{a}	0.84^{a}	0.97^{a}
	None		14.30^{b}	0.84	0.80^{b}	0.96
	Optimum		14.46^{a}	0.85	0.82^{a}	0.96
		None	14.25^{b}	0.84^{b}	0.79^{b}	0.96^{b}
		Optimum	14.51^{a}	0.85^{a}	0.82^{a}	0.96^{a}
Source of variation						
Phytase			0.001	0.001	0.001	0.001
Xylanase			0.01	0.58	0.001	0.86
β-glucanase			0.001	0.003	0.001	0.01
Phytase × Xylanase			0.70	0.29	0.85	0.87
Phytase × β-glucanase			0.58	0.36	0.23	0.01
Xylanase × β-glucanase			0.57	0.09	0.13	0.89
Phytase × Xylanase × β-glucanase			0.33	0.28	0.001	0.10

[1] Values are means of 6 replicates (8 birds each cage); [2]AME = Apparent metabolizable energy; [3]GE=Gross Energy
[a,b,c,d]Mean values with different superscripts within the columns are different ($p < 0.05$). SEM = Standard error of means.

Ileal amino acid digestibility was improved ($P < 0.01$) markedly and in a dose-dependent manner by phytase and by β-glucanase to a much lesser extent (Table 2).

Table 2 - Amino acid digestibility of birds on diets supplemented with different levels of Phytase, Xylanase and β-glucanase at 24 d of age.[1]

Phytase	Xylanase	β-glucanase	Ala	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Main effects:												
None			0.78^{c}	0.84^{c}	0.85^{c}	0.82^{c}	0.83^{c}	0.90^{c}	0.94^{b}	0.84^{c}	0.79^{c}	0.80^{c}
Low			0.82^{b}	0.88^{b}	0.87^{b}	0.85^{b}	0.86^{b}	0.91^{b}	0.95^{b}	0.87^{b}	0.83^{b}	0.84^{b}
Superdose			0.86^{a}	0.90^{a}	0.89^{a}	0.88^{a}	0.88^{a}	0.93^{a}	0.96^{a}	0.89^{a}	0.86^{a}	0.87^{a}
	None		0.82	0.88	0.87	0.85	0.86	0.91	0.95	0.87	0.83	0.84
	Optimum		0.83	0.88	0.87	0.85	0.85	0.91	0.95	0.87	0.83	0.84
		None	0.81^{b}	0.87	0.86^{b}	0.85^{b}	0.85^{b}	0.91^{b}	0.95^{b}	0.86^{b}	0.82^{b}	0.83^{b}
		Optimum	0.83^{a}	0.88	0.87^{a}	0.86^{a}	0.86^{a}	0.92^{a}	0.96^{a}	0.87^{a}	0.84^{a}	0.84^{a}
Source of variance												
Phytase			0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Xylanase			0.21	0.77	0.52	0.61	0.40	0.36	0.58	0.60	0.83	0.67
β-glucanase			0.001	0.53	0.01	0.004	0.02	0.01	0.003	0.03	0.001	0.004
Phytase × Xylanase			0.25	0.70	0.61	0.87	0.70	0.50	0.49	0.92	0.38	0.80
Phytase × β-glucanase			0.72	0.59	0.47	0.07	0.10	0.12	0.13	0.06	0.10	0.11
Xylanase × β-glucanase			0.50	0.94	0.14	0.09	0.04	0.02	0.55	0.10	0.09	0.09
Phytase × Xylanase × β-glucanase			0.68	0.02	0.12	0.87	0.90	0.71	0.70	0.87	0.04	0.51

[a,b,c]Mean values with different superscripts within the columns are different ($p < 0.05$).
[1]Values are means of 6 replicates (8 birds each); SEM = Standard error of means.

Bone breaking strength for tibia increased (P < 0.001) with the low dose of supplemental phytase but there was no further increase with the higher dose (Table 3). Supplementation with β-glucanase also improved (P < 0.04) bone breaking strength.

Table 3 - Breaking strength and mineral contents (%) of tibia bone of chicks at 35d of age when fed wheat-based diets supplemented with Phytase, Xylanase and β-glucanase[1].

Phytase levels	Xylanase levels	β-glucanase levels	Br. strength (kg/mm^2)^2	DM	Ash	Ca	P	Mg	K	S
Main effects										
None			360.9^b	69.3^a	49.6	37.3	16.5	0.81	0.59	0.24
Low			398.4^a	66.9^a	49.6	37.2	16.5	0.82	0.58	0.25
Superdose			405.6^a	66.8^a	49.8	37.0	16.4	0.82	0.60	0.26
	None		379.6	68.4	49.6	37.1	16.5	0.82	0.58	0.25
	Optimum		397.0	67.0	49.7	37.2	16.5	0.82	0.59	0.25
		None	378.9^b	67.8	49.6	37.3	16.5	0.82	0.58	0.25
		Optimum	397.7^a	67.5	49.7	37.0	16.4	0.81	0.59	0.25
Source of variance										
Phytase			0.001	0.04	0.57	0.54	0.72	0.41	0.58	0.41
Xylanase			0.06	0.13	0.44	0.94	0.98	0.91	0.43	0.48
β-glucanase			0.04	0.70	0.48	0.30	0.73	0.09	0.74	0.89
Phytase × Xylanase			0.56	0.89	0.51	0.93	0.99	0.43	0.27	0.52
Phytase × β-glucanase			0.80	0.80	0.24	0.71	0.41	0.15	0.63	0.32
Xylanase × β-glucanase			0.64	0.71	0.98	0.21	0.21	0.49	0.59	0.22
Phytase × Xylanase × β-glucanase			0.87	0.93	0.35	0.83	0.42	0.76	0.88	0.99

[a,b]Mean values with different superscripts within the columns are different (p <0.05); [1]Values are means of 6 replicates (2 birds each);
[2]Breaking strength for right tibia of birds; SEM = Standard error of means.

IV. CONCLUSIONS

The phytase and β-glucanase used in the study improved the apparent metabolizable energy, ileal amino acid digestibility and bone breaking strength.

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IMPACT OF FEEDING CONSTANT LEVELS OF COTTONSEED MEAL WITH ENZYMES ON BROILER MEAT YIELD, VISCERAL ORGAN DEVELOPMENT, ENDOGENOUS ENZYME ACTIVITY AND NUTRIENT DIGESTIBILITY

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Cottonseed meal (CSM) is a by-product of oil extraction from cotton seeds and is a moderately rich source of protein (30.2-56 %). A recent review concluded that CSM is an acceptable ingredient in poultry diets (Świątkiewicz et al., 2016). However, the use of CSM as a protein source in poultry diets is limited due to the presence of gossypol, variation in nutrient composition and a relatively low lysine level compared to soybean meal, all of which have negative effects on the growth performance of broiler chickens (Nagalakshmi et al., 2007). Enzyme supplementation to improve the nutritive value of feedstuffs for poultry is a regular practice. However, there is scant literature on the use of microbial enzymes to improve the nutritive value and utilization of CSM in poultry feed. A new-generation microbial composite product of xylanase and beta-glucanase (Danisco Animal Nutrition, Marlborough, UK), was examined in the current study, with the aim of increasing CSM levels in broiler chicken diets.

Visceral organ development, meat yield, digestive enzyme activities and nutrient digestibility were assessed in broiler chickens offered diets containing constant levels of CSM throughout the production cycle (d1-d35) and supplemented with microbial enzymes. Nine isocaloric and isonitrogenous diets were basically formulated from wheat/sorghum/soybean meal. Three levels of cottonseed meal (0 without, 6 % in starter, grower and finisher or 12 % in all three growth periods) were supplemented with three levels of xylanase and β-glucanase blend (Axtra XB) at 0, 250 or 500 g/tonne of diet, targeting the arabinoxylans and β-glucans found in grains and CSM. At d10, results showed that the weight of the small intestine and the combined weight of gizzard and proventriculus were increased ($P < 0.01$) due to inclusion of CSM in the diet. The relative weights of thighs and drumsticks increased linearly ($P < 0.05$) with increasing CSM level in the diet. There was an interaction ($P < 0.05$) between CSM and microbial enzyme on starch digestibility. Addition of the enzyme blend improved ($P < 0.05$) protein and energy digestibilities, with better results observed when 250 mg/kg was added. A slight reduction ($P < 0.05$) in dry matter digestibility was observed in the groups raised on CSM-containing diets. Adding microbial enzyme blend up to 250 mg/kg diet increased ($P < 0.05$) the activities of maltase, sucrase, aminopeptidase, chymotrypsin and lipase at d10, while at d24, sucrase, alkaline phosphatase, aminopeptidase, trypsin and chymotrypsin activities were also improved ($P < 0.05$) . On the other hand, feeding a high level of CSM (12 %) 1-10 days post-hatch negatively affected ($P < 0.05$) the activities of aminopeptidase, trypsin and chymotrypsin.

The results demonstrate that feeding CSM to broiler chickens did not adversely affect meat yield and nutrient digestibility. Furthermore, adding xylanase and beta-glucanase blend to CSM-containing diets enhanced the activities of endogenous enzymes and digestibility of nutrients. The current study found that a high level of CSM may not be suitable for young birds.

Nagalakshmi DSV, Rama R, Panda AK, & Sastry VRB (2007) *J. Poult. Sci*. **44:** 119-134.
Świątkiewicz S, Arczewska-Włosek A & Józefiak D (2016) *World Poult. Sci. J.* **72:** 473-484.

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ASSESSING APPARENT, TRUE AND STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY OF COTTONSEED MEAL-CONTAINING DIETS WITH OR WITHOUT A MICROBIAL ENZYME BLEND

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With approximately 30.2-56% crude protein (CP), cottonseed meal (CSM) is regarded as one of the closest alternative protein sources to soybean meal (SBM). It is used at relatively low levels to replace soybean meal in diets for broiler chickens (Swiatkiewicz et al., 2016). While its value at such low levels has been well established, the potential for using CSM at higher levels when fed as such or supplemented with microbial enzymes is not well understood. Similarly, there have been relatively few studies on the digestibility of amino acids in CSM-containing diets, particularly when supplemented with microbial enzymes (Nagalakshmi et al, 2007). The objective of this study was to examine the effect of a new-generation composite enzyme product (xylanase/β-glucanase activities) on apparent (AID), true (TID) and standardized ileal amino acid digestibility (SID) in diets containing moderately high levels of CSM, fed to broiler chickens from hatch to 35d. Four isocaloric and isonitrogenous basal diets were formulated from wheat/sorghum/soybean meal. Cottonseed meal was included at 0, 4, 5 or 6 % in the starter diets; at 0, 8, 10 or 12 % in the grower diets, and at 0, 12, 15 or 18 % in the finisher diets, with or without supplementation with 100 mg per kg diet of xylanase and β-glucanase blend (Danisco Animal Nutrition, Marlborough, UK). The ninth diet was a nitrogen-free reference diet (NFD), which was fed to birds from d19 to d24. Each treatment was randomly assigned to 6 replicates (10 birds each).

Apparent ileal digestibility of arginine increased linearly ($P < 0.013$) with increasing level of CSM. The AID of histidine, leucine, lysine, alanine, glycine and tyrosine was also improved ($P < 0.05$) by enzyme supplementation. There was a significant interaction ($P < 0.05$) between CSM level and enzyme on the TID of all measured dietary amino acids arginine, methionine, serine and tyrosine except ($P > 0.05$). Cottonseed meal decreased ($P < 0.05$) the TID of almost all amino acids, except for arginine, phenylalanine, valine, glutamic acid and proline. Enzyme incorporation more than offset ($P < 0.01$) the reduction in TID of amino acids caused by CSM inclusion. An interaction ($P < 0.01$) was observed between CSM and enzyme on SID of methionine. Increasing the CSM inclusion level significantly decreased SID of histidine, lysine, methionine, alanine, glycine, serine, and tyrosine. Supplementing CSM diets with the enzyme product increased ($P < 0.05$) the SID of all the indispensable AA and most of the dispensable AA but had no effect ($P > 0.05$) on SID of histidine and phenylalanine.

The results showed that supplementing CSM-containing diets with the test microbial enzymes improved the ileal AA digestibility, enabling a higher inclusion of CSM in diets for broiler chickens.

Nagalakshmi DSV, Rama R, Panda AK & Sastry VRB (2007) *J. Poult. Sci*. **44:** 119-134.
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YEAST CELL WALLS SUPPORT HIGHER GROWTH THAN WHOLE YEAST WHEN FED IN BROILER CHICKEN DIETS

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Summary

The objective of this study was to measure the performance response of broiler chickens to diets containing inactive whole yeast and corresponding yeast cell walls. Nine diets based on maize and soybean were offered to 486 Ross 308 broiler chickens from 0 to 35d. The test diets contained inactive dried whole yeast at four levels: (0.5, 1.0, 1.5 and 2.0 g/kg diet) or insoluble, dried and purified yeast cell wall component at the same four levels. These were compared with a control diet (without yeast supplementation) in a 2 x 4 + 1 factorial design. Feed and water were offered *ad libitum*. Feed intake (FI) and body weight gain (BWG) were measured on d10, d24 and d35, while FCR (corrected for mortality) was calculated from FI and BWG. On d10 and d24, one bird per replicate was electrically stunned, killed by cervical dislocation and key visceral organs were weighed. At d35, two birds per replicate were similarly euthanised and dissected to obtain the relative weight of breast, thighs and drumsticks. There was progressive improvement ($p < 0.05$) in BWG, FCR and weight of small intestine at d10 and d24 for birds on the higher levels of both whole yeast and yeast cell walls compared to birds on the control diet. At day 35, there was also significant improvement ($p < 0.05$) in BWG, FCR, small intestinal weight, dressing percentage of carcass, and absolute and relative breast weight for broiler chickens fed higher levels of whole yeast and yeast cell walls compared to birds on the control diet. Although there was no significant ($P > 0.05$) difference between birds on whole yeast or yeast cell wall diets, birds in the 2 g yeast cell wall/kg diet group tended to perform better in terms of BWG, FCR, small intestinal development, dressing percentage of carcass, as well as in absolute and relative breast weight. It can be concluded that supplementation of diets with inactive whole yeast and yeast cell walls at 2.0 g/kg diet improved broiler chicken performance, with the cell wall components being superior to the whole yeast.

I. INTRODUCTION

Non-therapeutic in-feed usage of antibiotics in animal diets has been implicated in development of antibiotic-resistant bacteria, some of which are zoonotic and pathogenic to humans (Stanton, 2013). Based on this fact, the European Union placed a ban on such usage of antibiotics 12 years ago (European commission, 2005; Castanon, 2007). However, the continuous incidence of antibiotic resistance in other regions is fueling the consideration and legislation of a global ban on in-feed antibiotics usage in animal production (Laximinarayan et al., 2013). A global ban on non-therapeutic antibiotic usage in poultry production without a suitable alternative will have adverse effects on the global poultry production. Based on this premise, researchers and poultry feed industries globally have intensified efforts towards identification of suitable alternatives to in-feed antibiotics. The growth-promoting and immunomodulatory potentials of polysaccharides such as β-1-3 glucan and manno oligosaccharides are well documented and gaining research interest (Roto et al., 2015).

Yeast (*Saccharomyces cerevisiae*) contains β-1-3 glucan and manno oligosaccharides in their cell wall, therefore making it a suitable potential alternative to in-feed antibiotics for broiler chickens. Results, however, have been inconsistent and many yeast-based products

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have been used without proper evaluation (Gao et al., 2009). Rather than consider the efficacy of yeast as an alternative to antibiotics alone, evaluation should also be based on quality, efficacy and optimum level of yeast inclusion. Therefore, this study was conducted to investigate the effect of whole yeast and yeast cell wall products at different levels on the performance of broiler chickens.

II. MATERIALS AND METHODS

Four hundred and eighty six day old Ross 308 broilers were obtained and used during the trial in a 2 x 4+1 factorial design conducted in completely randomized experimental design. Nine diets based on maize and soybean were offered to the 486 Ross 308 broiler chickens from 0 to 35d.

The diets consisted of control (without yeast supplementation), whole yeast (WY) at four levels: (0.5, 1.0, 1.5 and 2.0 g/kg), and yeast cell walls (YCW) at the same four levels. The WY product is a fine inactive baker's yeast protein-rich powder while the YCW product is an insoluble, purified and dried cell wall component with high levels of glucans and mannans, derived from pure culture yeast of the species *S. cerevisiae*. Both were supplied by AB Vista (UK). Feed and water were offered *ad libitum*. Feed intake (FI) and body weight gain (BWG) were measured on d10, d24 and d35, while mortality-corrected FCR was calculated from FI and BWG. On d10 and d24, one bird per replicate was electrically stunned, killed by cervical dislocation and key visceral organs were weighed. At d35, two birds per replicate were similarly euthanised and dissected to obtain the relative weight of breast, thighs and drumsticks.

Data were analysed using the general linear model of Minitab 17. Differences between mean values were established using Fisher's least significant test, while differences between treatments were compared using orthogonal probability contrasts. Significance was declared at $p \leq 0.05$.

III. RESULTS AND DISCUSSION

The effects of treatments on growth performance of broiler chickens fed whole yeast and yeast cell wall components are summarized in Table 1. The results show that, throughout the experiment, no significant ($P > 0.05$) differences were observed in feed intake across the treatment groups. However, there was progressive improvement ($p < 0.05$) in BWG, FCR and weight of small intestine (SI) at d10 and d24 for birds on the higher levels of both whole yeast and yeast cell walls compared to birds on the control diet. Although all groups consumed similar quantities of feed, the growth-promoting effect inherent in the yeast groups appears to have led to better nutrient absorption, BWG and improved FCR of broiler chicken fed whole yeast and yeast cell wall especially as the levels increased. The results of the present study are in agreement with reports by Gao et al. (2008) of similar improvements in BWG, FCR and intestinal weight of Arbor Acres broiler chickens fed diets containing 2.5 g/kg yeast culture in a 42-day trial.

At day 35, there was also significant ($p < 0.05$) improvement in BWG, FCR, SI weight for broiler chickens fed higher levels of whole yeast and yeast cell walls compared to birds on the control diet. (Table 1). The better BWG, FCR and increase in weight of the SI could be due to enhanced enzymatic activities caused by polysaccharides and unidentified growth-promoting agents present in the yeast and these activities became more pronounced as the level of whole yeast and yeast cell wall increased, resulting in improved performance even though the birds consumed similar amount of feed. This result is in agreement with the reports by Rajput et al. (2013) and Mutassim, 2013 who observed similar increases in

BWG, FCR and SI weight of broiler chickens fed diets containing the yeast, *Saccharomyces bourladii* and yeast culture at 3 kg/t.

Table 1 - Effect of yeast supplementation on response of broilers chickens during 10, 24 and 35 days of age.

	Treatment	Control	WY (g/kg)				YCW (g/kg)				SEM	P-value
	Levels	0	0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0		
Day 1-10	F.I	335	326	319	309	321	318	324	316	313	3.25	0.78
	BWG	278^{b}	285^{b}	289^{b}	287^{b}	303^{a}	286^{b}	304^{a}	305^{a}	306^{a}	1.94	0.01
	FCR	1.18^{a}	1.14^{ab}	1.11^{ab}	1.08^{bc}	1.06^{bc}	1.12^{abc}	1.06^{bc}	1.04^{c}	1.02^{c}	0.01	0.03
Day 1-24	F.I	1651	1649	1604	1629	1633	1653	1637	1635	1671	8.71	0.86
	BWG	1273^{c}	1275^{c}	1302^{bc}	1318^{bc}	1345^{a}	1284^{c}	1320^{bc}	1340^{a}	1372^{a}	7.11	0.01
	FCR	1.28^{a}	1.29^{a}	1.23^{b}	1.24^{b}	1.21^{b}	1.29^{a}	1.24^{b}	1.22^{b}	1.21^{b}	0.01	0.01
Day 1-35	F.I	3230	3133	3142	3030	3075	3207	3197	3187	3216	19.00	0.16
	BWG	2209^{c}	2225^{c}	2317^{bc}	2318^{bc}	2368^{ab}	2294^{bc}	2336^{bc}	2414^{ab}	2494^{a}	21.2	0.01
	FCR	1.47^{a}	1.40^{ab}	1.36^{bcd}	1.31^{d}	1.30^{d}	1.40^{abc}	1.37^{bcd}	1.32^{cd}	1.29^{d}	0.01	0.01

Probability levels of orthogonal contrast

	Day 1-10			Day 1-24			Day 1-35		
Comparison	FI	BWG	FCR	FI	BWG	FCR	FI	BWG	FCR
Control vs Yeast	0.11	0.001	0.006	0.66	0.02	0.001	0.16	0.03	0.001
Control vs WY	0.15	0.01	0.03	0.46	0.04	0.001	0.06	0.04	0.001
Control vs YCW	0.11	0.001	0.002	0.93	0.01	0.001	0.64	0.002	0.001
WY vs YCW	0.83	0.01	0.12	0.31	0.11	0.67	0.51	0.03	0.92

Means in a column not sharing a common superscript differ ($p < 0.05$); WY = Whole yeast; YCW = Yeast cell wall.

Table 2 - Day 35 dressing percent and relative meat cut parts weights of broilers fed different dietary treatments.

Treatment	Dressing percent	Breast	Thigh	Drumstick
Control (Zero Yeast)	73.76^{c}	212^{c}	102	90.13
Whole yeast (g/kg)				
0.5	73.77^{c}	212^{c}	102	89.90
1.0	75.77^{bc}	217^{bc}	103	88.00
1.5	76.45^{abc}	219^{abc}	103	86.00
2.0	76.66^{ab}	233^{ab}	103	87.00
Yeast cell wall (g/kg)				
0.5	75.54^{bc}	210^{c}	102	88.00
1.0	77.54^{abc}	217^{bc}	103	91.00
1.5	81.78^{a}	230^{ab}	103	88.63
2.0	81.91^{a}	234^{a}	103	88.00
SEM	0.75	2.12	0.99	0.87
P-value	0.04	0.02	0.98	0.84
Probability levels of orthogonal contrasts				
Control vs Yeast	0.07	0.04	0.95	0.99
Control vs WY	0.25	0.13	0.95	0.96
Control vs YCW	0.02	0.02	0.96	0.97
WY vs YCW	0.06	0.10	0.99	0.99

Means in a column not sharing a common superscript differ ($p < 0.05$). WY = Whole yeast. YCW = Yeast cell wall.

Table 2 shows the relative % meat cut parts of broiler chickens fed diets containing whole yeast and yeast cell wall components. There were no significant ($P > 0.05$) differences in thigh and drumstick weights. As the level of whole yeast and yeast cell wall increased, there was a progressive increase ($p < 0.05$) in the dressing % and the relative breast weight.

Birds on 2.0 g/kg YCW diet had better dressing % and the relative breast meat yield than birds in the control and the WY groups. These results tend to follow the overall trend observed in BWG and FCR throughout the experiment. According to Nawaz et al. (2015), dressing % and relative breast weight are influenced by dietary supplementation with probiotics. Khan (2001) also reported an improvement in dressing percentage of broilers fed distillery yeast sludge.

In conclusion, supplementation of diets with inactive whole yeast and yeast cell walls at 2.0 g/kg diet improved broiler chicken performance, with the cell wall components being superior to the whole yeast.

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UPREGULATED PROVENTRICULAR PEPSINOGENS AND IMPROVED FEED EFFICIENCY IN BROILERS BY THE COMBINATION OF SUPPLEMENTED SUGARCANE BAGASSE AND COARSELY GROUND CORN IN PELLETED DIETS

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Measures to improve bird performance have been sought due to the imminent phase out of in-feed antibiotics in poultry and continued demand for higher poultry feeding efficiency. Promotion of gizzard development by physical structure of feed ingredients or addition of dietary fibre is one such strategy with the hypothesis that larger ingredient particles and higher fibre enhance digestive enzyme secretion and feed efficiency in broilers (Kheravii et al., 2017; and Svihus, 2011). This study was conducted to evaluate the effect of increased particle size of corn and inclusion of sugarcane bagasse (SB) on mRNA expression of genes encoding pepsinogen A and C, and feed conversion ratio (FCR). A total of 336 Ross 308 male broilers were assigned in a 2 × 2 factorial arrangement of treatments with 2 particle sizes (coarse 3576 µm or fine 1113 µm geometric mean diameter) and 2 levels of SB (0 g/kg or 20 g/kg). Each treatment had 6 replicate pens of 14 birds. All of the 4 diets based on corn, soybean meal, and meat meal were formulated to meet the minimum nutrient profiles of the Ross 308 specifications. Herein, the composition of diets was diluted when 20 g of SB /kg was added over the top of the complete feed. All the diets were thoroughly mixed and cold-pelleted (65◦C). Birds were fed in the phases of starter (d 0 to 10), and grower (d 10 to 24). FCR was measured from d 10-24. The mRNA expression of genes encoding pepsinogen A and C were measured from one bird per pen at d 24. The analysis of gene expression was performed with qBase+ that applied an arithmetic mean method to transform logarithmic Cq value to linear relative quantity using exponential function for relative quantification of genes (Hellemans et al., 2007) and the output data were exported to SPSS statistics version 22 (IBM SPSS, UK) for further analysis. The data were analysed using the General Linear Models (GLM) procedure for the main effect of particle sizes, and SB supplementation along with their interactions and correlations between FCR and the expression levels of these genes were conducted using the Correlate module of SPSS statistics version 22. Differences between mean values were determined using LSD test at the level of $P < 0.05$. During d 10-24, a significant particle size × SB interaction was observed for FCR ($P < 0.01$). The birds fed CC (coarsely ground corn) with 2% SB had lower FCR than those fed CC without SB. A particle size × SB interaction was observed for both expression of pepsinogen A and C ($P < 0.01$). Further, expression of pepsinogen A ($P < 0.01$ and $R = -0.53$) and C ($P < 0.01$ and $R = -0.59$) were negatively correlated with FCR on d 24. These findings suggest that the combination of CC and SB in broiler diets is beneficial to broiler performance through the upregulation of both proventricular pepsinogen A and C.

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IMPROVING SOYBEAN DIGESTIBILITY WITH MONO-COMPONENT PROTEASE

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Summary

The usefulness of exogenous protease to improve the nutritional value of soybean meal has been recently demonstrated and is thought to be linked to a reduction in the antinutritional effect of various proteinaceous antinutrients e.g. trypsin inhibitor and antigenic protein as well as to a general improvement in the integrity of the intestinal tract (mucin and tight junctions). Importantly, a clear link has been established between the inherent quality of the soybean meal (amino acid and energy digestibility) and the magnitude and consistency of the effect of protease which highlights the need for concurrent raw material surveillance to optimize value creation. Exogenous protease should also be systematically integrated into existing enzyme solutions (phytases and carbohydrases) to maximise effect without double-accounting in feed formulation assumptions. The present paper attempts to draw together some key threads in the microbial protease narrative to provide some insight into the value and mode of action of protease in soybean meal and recommendations to optimize return on investment.

I. INTRODUCTION

Exogenous feed enzymes were first introduced as commercially-relevant feed additives in the 1980s with an initial focus on reduction of the antinutritional effects of high molecular weight soluble pentosans in wheat- and barley-based diets for young broiler chickens (Bedford & Partridge, 2010). Exogenous phytase was launched in the early 1990s to increase the digestibility of organic phosphorus and to reduce the antinutritional effects of phytic acid (Selle & Ravindran, 2007). Subsequently there have been a plethora of additional feed enzyme launches including amylase, pectinase, alpha-galactosidase, beta-mannanase, protease and others (Bedford & Partridge, 2010). In general, these enzyme classes do not compete with one another for substrate but the cumulative response to their addition for a given diet tends to be sub-additive due to overlap in effect on released nutrients e.g. energy, amino acids or phosphorus (Cowieson, 2010). Importantly, the magnitude and consistency of the response to enzyme addition is linked to the inherent digestibility of the diet to which it is added with elevated effect in ingredients or nutrients with a lower digestibility and vice versa (Cowieson, 2010).

Microbial protease is a relative new-comer to the global feed enzyme market, especially when considered as a specific mono-component activity (Cowieson & Roos, 2016). In common with its predecessors, the effect of protease on the digestibility of various dietary ingredients is higher when inherent digestibility is low. For example, Douglas et al. (2000) noted a direct correlation between the ileal digestible energy content of various batches of soybean meal and the beneficial effect of an admixture of xylanase, amylase and protease. Otherwise, the effect of exogenous protease in animal nutrition shares little with alternative exogenous enzymes and there is considerable opportunity for substantial value creation through its use in concert with alternative enzyme classes. The major mechanism of action of exogenous proteases include reduction in the severity of effect of proteinaceous antinutrients e.g. trypsin inhibitors or antigenic proteins, improvement in the solubility and digestibility of dietary protein (and adjacent nutrients such as fat), reduction in the flow of

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endogenous protein in the intestine e.g. mucin and various beneficial effects on gut health e.g. increase tight junction integrity and intestinal tensile strength (Cowieson & Roos, 2016). A major source of dietary proteinaceous antinutrients (and, certainly, dietary protein *per se*) is soybean meal and this has been shown to vary considerably in chemical and mechanical characteristics (Dozier & Hess, 2011; Cowieson et al., 2017). The interaction between exogenous protease and soybean meal has not been systematically explored but a reasonably large body of evidence exists to suggest that benefits vary in line with the inherent quality of the meal, processing methods (and severity thereof) and the concentration of various antinutrients therein. It was therefore the purpose of the present paper to briefly outline the usefulness of exogenous protease to enhance the nutritional value of soybean meal for poultry and to suggest factors that may increase or decrease mean effect.

II. EXOGENOUS PROTEASE AND SOYBEAN MEAL

Following the pioneering work of Lewis and Baker in the 1950s (Lewis et al. 1955; Baker et al. 1956), who were able to show potential of exogenous protease sources in pigs, specific benefits of protease for soybean meal were not conclusively demonstrated until the early 1990s (Cowieson & Roos, 2016). Initially, these beneficial effects were focused on reducing the antinutritional effects of trypsin inhibitors in soybean meal and on examination of various classes of proteases e.g. alkaline vs. acidic. More recently, the effects of exogenous proteases on general animal performance, nutrient digestibility, gut health and intestinal physiology have been explored and reported. For example, Cowieson & Roos (2014) conducted a meta-analysis of the effect of exogenous protease on the apparent ileal digestibility of amino acids from 25 independently conducted studies and noted that the effect magnitude was directly linked to the inherent digestibility of amino acids in the control groups (Fig. 1). This relationship underlines the importance of quality control in raw material selection and use and suggests that microbial protease will reduce variance in the digestibility of protein in animal nutrition. This general relationship between ileal amino acid digestibility and the corresponding value of exogenous protease has recently been confirmed in soybean meal and full-fat soy meal (Cowieson et al. 2017; Table 1) where the inherent digestibility of various amino acids and also AMEn was well correlated for leveraging the scale of response to exogenous protease. It is therefore recommended that some estimate of soybean meal quality is available in order to optimize the value creation of exogenous protease in animal diets.

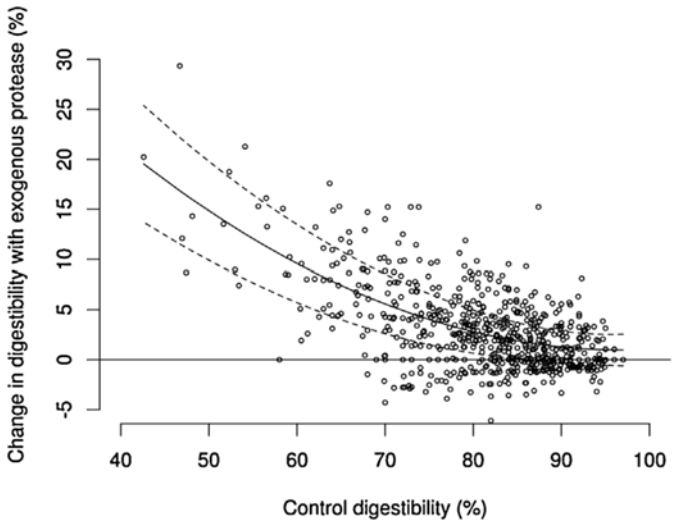

Figure 1 - Correlation between the inherent digestibility of amino acids in the control diet and the effect of a mono-component microbial protease on the same (Cowieson & Roos, 2014).

Table 1 - Effect of the inherent digestibility of selected amino acids and energy in FFSB and SBM on the effect of exogenous protease on the same (adapted from Cowieson et al. 2017).

Nutrient	Inherent digestibility Effect on protease - SBM	Inherent digestibility Effect on protease - FFSB
SID EAA	$P < 0.001$; $r^2 = 0.34$	$P < 0.001$; $r^2 = 0.16$
SID NEAA	$P < 0.001$; $r^2 = 0.49$	$P < 0.05$; $r^2 = 0.07$
SID Met	$P < 0.001$; $r^2 = 0.46$	$P < 0.001$; $r^2 = 0.74$
SID Cys	$P < 0.001$; $r^2 = 0.24$	$P < 0.05$; $r^2 = 0.06$
SID Lys	$P < 0.001$; $r^2 = 0.22$	NS; $r^2 = 0.01$
SID Thr	$P < 0.001$; $r^2 = 0.24$	$P < 0.001$; $r^2 = 0.19$
SID Val	$P < 0.001$; $r^2 = 0.24$	$P < 0.001$; $r^2 = 0.20$
SID Ile	$P < 0.001$; $r^2 = 0.23$	$P < 0.001$; $r^2 = 0.18$
SID Gly	$P < 0.001$; $r^2 = 0.53$	$P < 0.001$; $r^2 = 0.18$
AMEn	$P < 0.001$; $r^2 = 0.25$	$P < 0.001$; $r^2 = 0.50$

III. EXTRA-PROTEINACEOUS EFFECTS OF MICROBIAL PROTEASE

Soybean meal, as with many other feed ingredients, contains several antinutritional compounds that vary in nutritional significance. Trypsin inhibitors, lectins, antigenic and recalcitrant proteins are implicated in various intestinal perturbances and contribute to variance in the nutritional value of soy products, especially for neonates (Cowieson & Roos, 2016). In addition to direct hydrolysis of proteinaceous antinutrients (Rooke et al., 1998), exogenous proteases are able to enhance the resilience of the intestinal tract, putatively through improved tight junction and mucin integrity (Cowieson & Roos, 2016). Exogenous proteases have also been shown to increase the digestibility of energy and fat in poultry diets which suggest a generic enhancement of nutrient solubility and digestion following their addition (Cowieson & Roos, 2016).

IV. CONCLUSIONS

Exogenous proteases, at least as an explicit mono-component activity, are a relative new-comer to the global feed enzyme market. However, their use has expanded substantially since their introduction and their effect extends far beyond ‘simple’ improvement in the digestibility of dietary protein to benefits in gut integrity, environmental sustainability and the digestibility of non-proteinaceous macro-nutrients such as lipid. The link between the inherent quality of the raw material and the degree of protease effect should be further explored and exploited via articulation between nutrient release recommendations and rapid raw material quality surveillance. In conclusion, the usefulness of exogenous protease to enhance the digestibility of soybean meal and full-fat soy (as well as many alternative feed ingredients) is clear. Adequate characterization of raw material quality and inherent nutritive value is an important consideration when assigning nutrient matrix values to a protease; however, such products are able to reduce variability in the digestible nutrient value of raw materials of unquantified quality. Further to this concept, integration of exogenous protease with adjacent enzyme technology such as phytase and carbohydrase is recommended for maximum consistency of response and value creation.

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PERFORMANCE PARAMETERS AND TISSUE ZINC DEPOSITION IN BROILER CHICKENS IN RESPONSE TO DIFFERENT LEVELS OF HYDROXY SOURCE OF ZINC

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Zinc (Zn) is a fundamental trace mineral for growth, development and health in broilers. The form of Zn has a direct impact on its biological function in broiler chickens (Bun et al., 2011). Hydroxy forms, Zn hydroxychloride (IBZ) can potentially offer advantages over other inorganic and organic sources. This study was designed to determine the optimum level of IBZ in broiler diets, by evaluating the effect of different levels on growth performance and zinc deposition in tissues. A total of 864 one-day-old male Ross 308 chicks were housed in floor pens on pine shaving litter and were randomly assigned to seven treatments, resulting in seven replicates of 18 chicks per pen. Diets were wheat-soybean meal based and birds had *ad libitum* access to feed and water throughout the trial period. The positive control (PC) treatment was supplemented with inorganic zinc (50 mg/kg ZnO and 50 mg/kg ZnSO4) and the negative control treatment (NC) contained no supplemental Zn. The remaining 5 treatments contained Zn as IBZ at 20, 40, 60, 80, 100 mg/kg. The treatments were fed from d0 to d35, as starter (d0 - d14) and grower (d14 - d35). Body weight gain (BWG), livability (LIV) and feed intake (FI) were measured from d0-35 and used to calculate adjusted feed conversion ratio (FCR). Tibias from three birds from each pen were collected on d14 and liver samples from three birds each pen were collected on d35. Zinc concentration was analyzed in the samples by ICP-OES on an individual bird basis. The results showed that FCR significantly improved for birds offered 100 mg/kg IBZ compared to those fed the PC treatment or treatment with 20 mg/kg IBZ ($P < 0.001$). Body weight gain from d0-35 was higher in birds fed IBZ at 80 and 100 mg/kg compared to those fed the NC treatment and IBZ at 20 mg/kg ($P < 0.001$). Zn source and level had no impact on FI, LIV or Zn concentration in the liver ($P > 0.05$). Tibia Zn concentration was significantly lower ($P < 0.001$) in birds on the NC treatment compared to those on any other treatment, and was numerically highest in birds fed 100 mg/kg IBZ. In conclusion, feeding broilers 40 - 60 mg/kg IBZ support performance parameters similar to that of 100 mg/kg inorganic Zn in the diet. Also, supplementation of 100 mg/kg IBZ results in better feed efficiency.

Table 1 – Growth performance parameters and zinc concentration in liver and tibia of broiler chickens.

	PC	NC	IBZ 20 mg/kg	IBZ 40 mg/kg	IBZ 60 mg/kg	IBZ 80 mg/kg	IBZ 100 mg/kg	SEM	*P* value
BWG 0-35 d	2530^{ab}	2397^{c}	2440^{bc}	2532^{ab}	2521^{ab}	2572^{a}	2614^{a}	29.96	0.001
FI 0-35 d	3620	3523	3534	3609	3558	3642	3653	44.22	0.237
FCR 0-35d	1.430^{bc}	1.470^{a}	1.448^{ab}	1.425^{bcd}	1.411^{cd}	1.415^{cd}	1.397^{d}	0.0066	0.001
LIV % 0-35 d	97.9	98.2	97.3	94.6	94.6	94.6	96.4	1.76	0.564
Liver Zn ug/g	82.12	80.82	82.21	84.20	82.74	85.13	88.90	1.83	0.070
Tibia Zn ug/g	436^{a}	367^{b}	427^{a}	425^{a}	435^{a}	431^{a}	450^{a}	6.40	0.001

$^{a-d}$ means in the same row with different superscripts are significantly different ($P < 0.05$)

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POTENTIAL EFFECTS OF A PHYTOGENIC FEED ADDITIVE ON CARCASS AND MEAT TRAITS IN BROILERS COMPARED TO AN ANTIBIOTIC GROWTH PROMOTER

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Plant origin (phytogenic) feed additives (PFA), comprising herbs, spices, essential oils, plant extracts and derived products have gained considerable interest in view of the worldwide ban on use of antibiotic growth promoters (AGPs) in food animals. These products have the ability to improve performance by maintaining a healthy gut environment (Windisch et al., 2008). They have also been reported to influence carcass and meat quality characteristics in agricultural livestock (Isabel and Santos, 2009; Hong et al., 2012). The present study was aimed at comparing the effects of a PFA and an AGP on carcass and meat traits in broiler chickens.

A 39 day trial was conducted with an as-hatched flock of 432 day-old Cobb 400 broilers. Chicks were randomly assigned to three dietary treatments with 12 replications per treatment and 12 birds per replicate. The three dietary treatments consisted of a control (basal diet only), AGP (basal diet + 225 mg/kg bacitracin methylene disalicylate), and PFA (basal diet + PFA Digestarom® Poultry 150 mg/kg). Birds received maize-soybean meal based diets from one to seven days (starter), eight to 21 days (grower) and 22 to 39 days (finisher). On day 39, 12 birds having body weight closest to the mean weight of the group were selected from each dietary treatment, weighed and killed by cervical dislocation, followed by exsanguination. Carcass yield and yields of breast, thighs and drumsticks relative to live weight (g/kg) were measured. Breast fillets were stored at 4^{o}C for 24 hours for determination of pH, drip loss, moisture and crude protein contents. Moisture in meat was determined in triplicate by drying ground breast meat sample at 98^{o}C for 24 hours. Meat protein (Nitrogen x 6.25) was determined in duplicate by the Kjeldahl method (AOAC, 2005). Drip loss and pH were determined according to Qiao et al. (2007). Pieces of breast fillet were stored at 4^{o}C for 48 hours and the loss of weight as the percentage of the original sample weight was determined. Meat pH was measured at six different locations across the sample surface with a pH meter (AB15, Thermo Fisher scientific, Waltham, MA). The average represented the ultimate pH of the sample.

Except for the yield of drumsticks, which was higher in the AGP and PFA groups as compared to the control ($P = 0.002$), the carcass traits generally remained unaffected by the diets ($P > 0.05$). Relative organ weights were also not affected due to supplementation of AGP and PFA to the diet ($P > 0.05$); however, weight of the viscera decreased ($P = 0.004$) in the dietary groups receiving AGP and PFA supplementation. The reduction in viscera weight by dietary supplementation of AGP and PFA implied a reduction in the energy required to maintain the gut, thereby leaving more energy available for productive processes such as body weight gain (BWG) and better feed conversion ratio (FCR) as reflected in the trial with significantly higher BWG and reduced FCR in PFA and AGP groups ($P = 0.001$). No significant effect of the diets was observed on drip loss and pH of meat although the PFA group tended to have a lower meat pH compared to the other two groups ($P > 0.05$).

The PFA evaluated in this study was equally effective to the AGP used, as far as the carcass and meat traits of the birds are concerned when added to a maize-soybean meal-based coccidiostat-free broiler diet and thus can serve as an alternative to the AGPs in broiler production.

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RELATIONSHIP OF CALCIUM, PHOSPHORUS AND PHYTASE TO BROILER GROWTH PERFORMANCE FROM DAY 1 TO 21.

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It has been shown that an ileal digestible phosphorus (IDP) value of 2.0 g/kg can support optimum bird performance (Li et al., 2017). However, the relationship of this value to dietary Ca concentration and phytase supplementation is still unclear. The aim of this study was to further clarify these interrelationships.

Four hundred, day old, Ross, male broiler chicks were fed experimental diets based on wheat, sorghum, canola meal, soybean meal and meat and bone meal. The diets contained 1 level of IDP (2.0 g/kg) and 4 concentrations of Ca (3.5, 4.0, 4.5 & 5.0 g/kg) either with or without phytase (Axtra PHY 10000 TPT, 500 FTU/kg diet, Feedworks, Australia) supplementation. Each experimental diet was fed to 5 replicate pens with 10 birds per replicate: starter diet from days 1 to 14 and the grower diet from days 15 to 21 post-hatch. All birds had free access to feed and water. Body weight and feed intake were recorded weekly and the feed conversion ratio (FCR) was calculated.

Table 1 - Broiler body weight at day 21, feed intake and FCR from day 1 to 21.

Diet	Body weight (g/b)		Feed intake (g/b/d)		FCR (g feed/g gain)	
Ca (g/kg)	-Phytase	+Phytase	-Phytase	+Phytase	-Phytase	+Phytase
3.5	970^{ab}	1008^{a}	52^{b}	54^{a}	1.20	1.20
4.0	930^{bc}	1022^{a}	50^{bc}	55^{a}	1.20	1.21
4.5	914^{bc}	1005^{a}	49^{c}	54^{a}	1.21	1.19
5.0	889^{c}	1024^{a}	48^{c}	55^{a}	1.22	1.19

^{a,b,c,} Means under the same parameter with different superscripts differ (P<0.05)

The results (Table 1) indicate that the dietary Ca concentration had a significant effect on chick performance. Without phytase supplementation, feed intake and body weights were lower as the Ca to P ratio increased ($P < 0.05$). However, with phytase supplementation the detrimental effect of a high Ca to P ratio was alleviated. The results indicate that phytase reduced the negative impact of Ca on body weight (P=0.025) and feed intake (P=0.02). Similar numerical differences were seen with FCR. These results highlight the importance of delineating the bioavailability of both Ca and P in relation to phytase supplementation of current broiler diets.

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RESPONSE OF FINISHER BROILERS TO REDUCED DIETARY CALCIUM AND PHOSPHORUS CONCENTRATIONS WITH PHYTASE ADDITION

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It has been demonstrated that the phosphorus (P) requirements of modern broilers are significantly lower than historical industry practice (Li et al., 2017). However, it is also apparent from these studies that the P requirement is dependent on the dietary calcium (Ca) concentration and whether the diet is supplemented with phytase. Most of the contemporary research has been conducted in the starter phase, but this feeding trial has been conducted in the finisher phase (days 22- 49) to determine the influence of the addition of microbial phytase to diets containing different concentrations of ileal digestible phosphorus (IDP) and dietary Ca on growth performance in broilers fed a low IDP diet.

Eight hundred, day-old, Ross, male broiler chicks were fed a commercial diet to 21 days of age. Commencing on day 22, the experiment was a 2×4×2 factorial design with experimental diets based on wheat and sorghum; diets contained either 2.5 or 3.0 g/kg of IDP with 3.5, 4.5, 5.5 or 6.5 g/kg Ca. All the diets were prepared with or without phytase (Axtra PHY, 10000 TPT, 500 FTU/kg diet, Feedworks, Australia) supplementation. Each of the experimental diets was fed to 5 replicate pens with 10 birds per pen; grower diet, days 22 to 28; finisher diet, days 29 to 49. Body weight and feed intake were recorded weekly and FCR was calculated.

The result showed that birds fed the diet with phytase supplementation had higher ($P < 0.05$) body weight and feed intake than birds consuming the same IDP and Ca levels without phytase supplemented. Phytase also improved feed efficiency, as the FCR of phytase groups was significantly better ($P < 0.05$) than the non-phytase groups. Interestingly, IDP and Ca levels did not influence growth performance and feed efficiency among the treatments ($P > 0.05$); diets containing 2.5 g/kg IDP and 6.5 g/kg Ca with phytase supplemented showed the best numerical growth performance.

It can be concluded from this data that IDP values can be reduced in finisher diets. It also appears that the finisher broiler is less sensitive to the interrelationship between dietary Ca and P concentrations than the starter chick (Li et al., 2017).

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DIETARY CONCENTRATIONS OF PHOSPHORUS AND CALCIUM AND BROILER PERFORMANCE THROUGHOUT THE GROWING CYCLE

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Phosphorus (P) is a non-renewable resource and will become increasingly scarce during this century. Nevertheless, excessive usage of P in the poultry industry results in additional costs and also increased P excretion, causing eutrophication and land erosion. Tackling these problems requires better understanding of P bioavailability and the P requirements of the bird. Furthermore, the evolution of phytase technology has necessitated a reevaluation of P and calcium (Ca) requirements for poultry. An important consideration when studying P is dietary Ca concentration and, in this study, different dietary concentrations of both minerals were evaluated throughout the 49 day broiler growth cycle.

Four hundred, day old, Ross 308, male broiler chicks were fed experimental diets based on a sorghum-wheat blend and soybean meal to which were added xylanase (Axtra XB 201 TPT, 100g/metric tonne, Feedworks, Australia) and phytase (Axtra PHY 10000 TPT, 500 FTU/kg diet, Feedworks, Australia). The diets contained combinations of Ca and P that had been shown in our previous experiments (Li et al. 2017) to support optimum broiler performance. Ileal digestible P (IDP) concentrations of 2.0 and 2.5 g/kg were each combined with two Ca concentrations (4.5 and 6.5 g/kg). The highest concentration of IDP fed was 3.0 g/kg in combination with Ca of 3.5, 4.5, 5.5 or 6.5 g/kg. The same concentrations of both minerals were maintained in the starter, grower and finisher diets. Each experimental diet was fed to 5 replicates with 10 birds/replicate. No statistical differences ($P>0.05$) were found in final body weight (4554g), feed intake (140 g/bird/day) or feed conversion ratio (1.52) between the 8 treatments, indicating that an IDP value of 2.0g/kg combined with 4.5g/kg Ca would support optimum broiler performance. In a subsequent study (Cheng et al. 2017), lower concentrations supported optimum performance from day 1-21 post-hatch.

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PERFORMANCE AND CAECAL METABOLITE COMPOSITION OF BROILERS FED LOW AND HIGH PROTEIN DIETS SUPPLEMENTED WITH FEED ADDITIVES

N.K. SHARMA[1], M. CHOCT[1], S. WU[1] and R.A. SWICK[1]

Growth promoting effects of zinc bacitracin, *Bacillus*-based probiotics and a blend of *Yucca* and *Quillaja* saponin in broilers have been well documented (Engberg et al. 2000; Cheeke, 2009; Jeong and Kim, 2014). These in-feed additives are commonly used in broiler diets but their effects at different dietary crude protein (CP) concentrations remain unclear. This study was conducted to study the effect of these additives when added to low and high protein diets on performance and caecal metabolite composition of broilers.

Day-old Ross 308 male broiler chicks (n = 576) were assigned to eight dietary treatments with six replicate pens per treatment and 12 birds per pen. A 2 × 4 factorial arrangement of treatments was employed. Factors were: diet (low CP, high CP) and additive (none, antibiotic, probiotic, saponin). The CP contents in low and high protein diets were: starter phase (210 g/kg vs 260 g/kg), grower phase (195 g/kg vs 240 g/kg), and finisher phase (184 g/kg vs 230 g/kg). The low CP diet was supplemented with crystalline amino acids including L-valine, L-isoleucine and L-arginine, L-lysine, D,L-methionine and L-threonine. The in-feed antibiotic used was zinc bacitracin (Albac 150, Zoetis) at 330 g/t of feed (50 mg/kg), the probiotic was a blend of three *Bacillus subtilis* strains (Enviva Pro, Dupont Animal Nutrition) at 500g/ton and the saponin was a blend of *Yucca schidigera* and *Quillaja saponaria* (Nutrafito Plus, Desert King International) and used at 150 g/t of feed. Data were subjected to two-way ANOVA (using JMP software v.8) and means were separated by Tukey's HSD test at $P < 0.05$. The results showed that the birds fed the low CP diet had higher feed intake (FI) and weight gain (WG) at all phases compared to those fed the high CP diet ($P < 0.01$). The addition of antibiotic, probiotic or saponin to either of the diets had no effect on FI at all the phases ($P > 0.05$). Antibiotic, but not the other additives, increased WG by 8.2 % on d 10 ($P < 0.05$). A diet × additive interaction was detected for WG and FCR during other phases. On d 24 and 34, addition of antibiotic to the low CP diet increased WG of birds by 6.1 and 4.9 % ($P < 0.05$) and decreased FCR by 4 points each ($P < 0.05$), but WG and FCR were not affected when antibiotic was added to the high CP diet ($P > 0.05$). Interestingly, when probiotic was added to the high CP diet, improvements in WG of 3.6 % and 2.6 % over the control were observed on d 24 and 34 respectively but the effect was not observed when probiotic was added to the low CP diet. The birds fed the low CP diet had lower concentration of isovaleric acid in the caecal contents compared to those fed the high CP diet ($P < 0.05$). There was diet × additive interaction for isobutyric acid and branched chain fatty acids (BCFA) in the caecal contents. The birds fed the high CP diet without additives had higher concentrations of isobutyric acid and BCFA in the caecal contents compared to all other treatments ($P < 0.05$). Addition of antibiotic, probiotic or saponin to the high CP diet, but not to the low CP diet, reduced the concentrations of isobutyric acid and BCFA ($P < 0.05$). The concentration of H_2S in caecal contents also tended ($P = 0.054$) to change with different dietary treatments with the highest concentration observed in the birds fed the high CP diet.

Thus, zinc bacitracin may improve broiler performance when added to a low protein diet and *Bacillus subtilis* based probiotic may give better results when added to a diet with high protein content.

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IMMUNE RESPONSE FOLLOWING *ASCARIDIA GALLI* INFECTION IN FREE RANGE LAYING HENS

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A recent study suggests that artificial *Ascardia galli* infection has no effect on performance and egg quality of free-range laying hens from the point of lay until 40 weeks of age (Sharma et al., 2017). However, the lower hepatic lipid reserve of infected hens observed in the same study indicated the potential for *A. galli* to affect production at a later stage. In this study, serum and yolk antibody levels of *A. galli* were measured to investigate the immune response to natural infection in hens with different infection levels.

Sixteen week old Lohmann brown laying hens (n=200) were divided into 4 treatments with 5 replicates of 10 hens per pen. Hens had access to the range between 9:00 am and 5:00 pm. Hens of group 1 served as negative controls (NC) and ranged on a decontaminated range, hens of group 2 (low) and 3 (medium) ranged on an area previously contaminated using hens that were artificially infected with 250 and 1000 *A. galli* eggs/hen respectively. Hens of group 4 (positive control-PC) were artificially inoculated with 1000 *A. galli* eggs/hen and ranged on an area that was previously contaminated using hens that were artificially infected with 1000 *A. galli* eggs/hen. Infection intensity of all treatment groups was measured by counting *A. galli* eggs in the hen excreta weekly, as well as by counting mature *A. galli* worms in the hens' intestines after sacrificing them at 30 weeks of age. Serum and egg yolk were collected and analysed for anti-*A. galli* antibody (Ab) using ELISA method before range access at 16 weeks of age, and after range access at 20, 25 and 30 weeks of age. Data were analysed using JMP statistical software version 8 (SAS Institute Inc, Cary, NC). The experimental unit was the pen. Data were subjected to a two-way ANOVA with repeated measures, and difference between group means were assessed by Tukey's HSD test at a probability level of 0.05. Fixed effects used were treatment, age and their interation.

Hens of the medium infection group had higher worm counts in the intestine (43.9±3.96) and excreta egg counts (3437±459 eggs/g) compared to hens of the low infection group (23.8±3.96 and 1820±449 eggs/g) but similar worm numbers compared to hens of the PC (34.4±3.96 and 2918±474 eggs/g; $P < 0.01$). An interaction was detected between infection level and time for serum and yolk Ab ($P < 0.01$). While serum Ab levels were not different in hens between all groups before exposure ($P > 0.05$), they gradually increased overtime after exposing to the contaminated ranges. Only hens of the NC group maintained serum Ab constant all the time ($P > 0.05$). At 20 weeks, higher serum Ab was observed in hens of the medium (OD 1.07±0.03) and PC (OD 1.17±0.03) compared to the low (OD 0.38±0.03) ($P < 0.01$) group. At 25 weeks, serum Ab was similar in hens of low and medium infection groups but significantly lower compared to PC ($P < 0.01$). At 30 weeks, serum Ab levels were similar in the hens of low, medium and PC group ($P < 0.01$). Yolk Ab followed the same pattern except for medium infection group where yolk Ab remained same at 25 or 30 weeks of age (OD 0.72±0.04 and 0.74±0.04, respectively).

These findings suggest that hens naturally infected with *A. galli* produce both serum and yolk Ab at different levels depending on infection intensity and duration of exposure. Presence of *A. galli* eggs in excreta throughout the experiment suggests that increasing Ab levels in serum may not protect hens from continuing *A. galli* infection.

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A REVIEW OF THE REGULATORY FRAMEWORK RELATING TO BACKYARD POULTRY IN SYDNEY AND SURROUNDING SUBURBS

S. ALFRED[1], M. SINGH[1] and R. ALDERS[1]

There is an increasing trend of raising backyard poultry in urban and suburban settings across Australia, with Australian Egg Corporation Ltd. reporting backyard chicken coops accounting for almost 12 per cent of the nation's total egg production and 393 million eggs produced in metropolitan and regional gardens. However, there is no national or state legislation/policy in place currently for backyard poultry keeping and the issue is managed by local governments at a council level who may publish regulations and guidelines which are not often monitored or enforced. This study reviewed the regulatory framework that exists currently for keeping backyard poultry in the local councils of Sydney. The study also determined the level of this knowledge as understood by local veterinary practitioners.

The 43 Councils within Sydney were investigated to determine existing regulations. Eighty-one percent of the councils had no regulations pertaining to the housing of backyard chickens, 53% councils however had guidelines in place, 58% outlined housing requirements, 30% had a rooster ban in place, and 25% had restriction on bird numbers. Only three of the 43 councils issued fines for violation while only two councils provided information on vaccination requirements.

On classifying the councils into three regions (Inner City (N = 22), Suburbia (N = 13) and Outer Urban (N = 8)), it was evident that increased numbers of councils in the suburban stratum provided a regulatory framework (Figure 1).

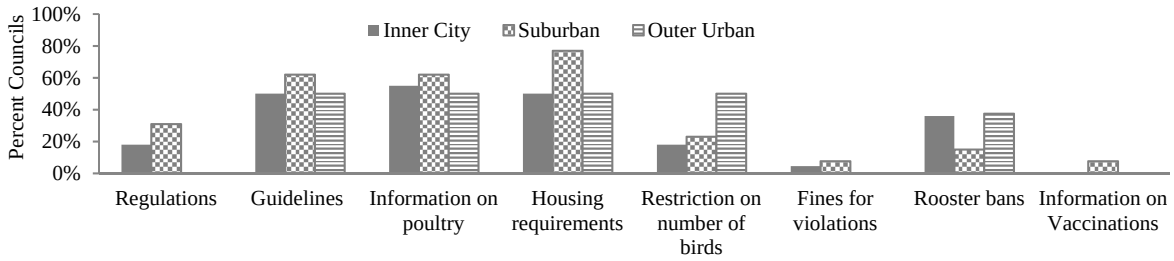

Figure 1 - Council level data regarding backyard poultry keeping in Sydney and surrounding suburbs.

An online questionnaire (containing 19 questions) developed using SurveyMonkey was sent to 24 listed avian veterinarians to determine their knowledge regarding regulations for backyard poultry keeping. Response data highlighted poultry visits were getting increasingly frequent, mainly for investigation of infectious diseases, respiratory problems and peritonitis. Knowledge of regulations at the local government level was acknowledged by 63% of respondents; yet, 75% of them did not provide this information to clients; however, 60% were comfortable with advising clients of biosecurity practices. All respondents were aware of notifiable diseases and the protocol for reporting notifiable disease outbreak. However, only 75% were aware that the Department of Agriculture provided a free service to determine the presence of a notifiable disease in private flocks. All veterinarians knew senior experts to confer with when faced with decision making and 87.5% conducted post mortem examinations on dead poultry and used disposal methods already employed by the clinic for the other animals.

Legislation for backyard poultry keeping needs to be developed and reviewed at council, state and federal levels in Australia in order to protect public health and the commercial poultry industry.

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BIRDS FOUND OUTSIDE SHEDS SHOW LESS FEATHER DAMAGE THAN BIRDS FOUND IN SHEDS

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An observational study conducted on three commercial layer farms showed that environmental enrichment increases number of birds on the range (see Dekoning et al. in this APSS Proceedings). In the same study, we aimed to determine if plumage damage score is different between birds found inside versus outside sheds. The farms were stocked with Hy-Line Brown flocks (all beak-trimmed); Farm-1 & Farm-3 had one shed each and Farm-2 had two sheds (all 3 farms & four flocks with a fixed range). Farms 1 & 3 had flock sizes of 3,000 and 11,700 hens, respectively, at a stocking density of 1,500/ha. Farm-2, had two flocks, each with 10,000 hens: Farm 2-1 at 10,000 hens/ha and Farm 2-2 at 1,500 hens/ha. Plumage damage score was conducted on 100 random birds inside and 100 random birds outside each shed. The AssureWel (http://www.assurewel.org) score system was used: 0 = no or minimal feather loss; 1 = slight feather loss; and 2 = moderate/severe feather loss. Plumage was scored (without catching) across five body parts: head/neck, back, base of tail/around preen gland, tail and wings. Plumage score per hen was summed (maximum of 10; 2 x 5). All farms were visited once a month for six months. In general, the average total plumage damage increased with age ($P<0.05$); outdoor birds had significantly less ($P<0.05$) damage than birds found inside (Fig. 1). The most severely affected body part was the neck region. When the total plumage score was categorized for the level of damage: No (0), Low (1-3), Medium (4-6) and Severe (≥7), well over 50% of birds on the range had No to Low damage across all farms. Furthermore, Flock 2-2, the oldest flock, had 80% of birds on the range with No to Low plumage damage. Conversely, there were fewer range birds in the Medium to Severe plumage damage range. We conclude that attracting birds out onto the range minimizes the level and severity of plumage damage in layer birds. Further, the benefit did not diminish with age of hens.

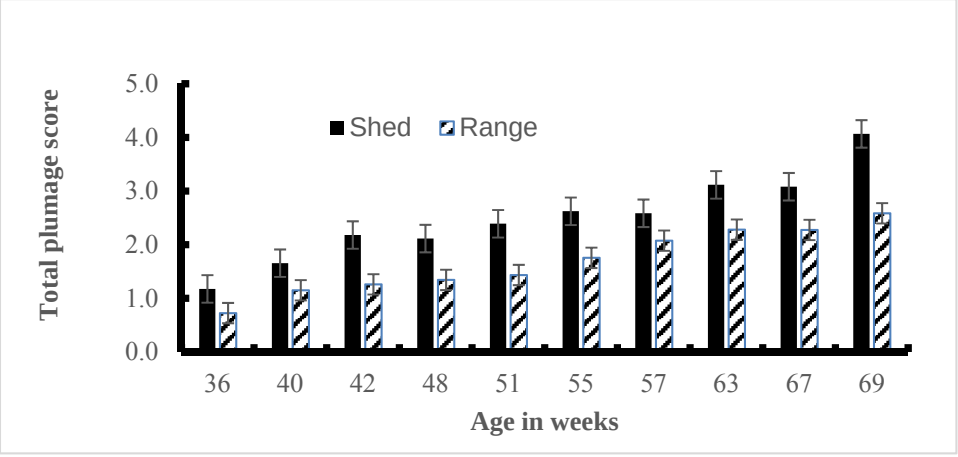

Figure 1 - Total plumage score observed on hens found inside and outside sheds across farms.

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PREFERENCES FOR PREEN OIL CONSTITUENTS MAY HELP EXPLAIN FEATHER-EATING BEHAVIOUR IN HENS

S. CHO[1] and E. ROURA[1]

Feather pecking is synonymous with economic and major welfare problems in the poultry industry and is positively associated with feather ingestion. In a choice-feeding experiment, laying hens showed a stronger preference for unwashed compared to washed feathers (McKeegan and Savory, 2001). The attraction toward unwashed feathers could be related to the preen oil produced by the uropygial glands located dorsally at the base of the tail. Preen oil covers the feathers during the process of preening. The main fatty acids present in the oil are lauric, myristic, palmitic, and stearic acids but the relative amounts seem to change with age and diet. For example, the long chain fatty acids (palmitic and stearic acids) increase with age while the medium and short chain fatty acids decrease (Sandilands et al., 2004). Therefore, it is possible that changes in fatty acid composition in the preen oil, relative to age and/or individual birds drive the attraction ultimately causing feather pecking. However, specific fatty acid preferences and appetites in birds have rarely been studied. Thus, the aim of this work was to assess differences in specific fatty acid preferences between feather eating (FE) and non-feather eating birds (NFE).

Individually caged 96 laying hens were offered a double choice test consisting of two containers, a control feed (ground wheat) and the same supplemented with one of the four main fatty acids in preen oil (i.e. lauric, myristic, palmitic, and stearic). In addition, we tested two mixtures of the same fatty acids at a similar ratio of the preen oil constituents reported for young (MP-young) or old (MP-old) hens to give a more practical perspective to our study. All treatments were tested at 1 and 5% concentrations. At the end of the trial, the hens were euthanised and feather consumption was assessed by necropsy. Preference values (test feed intake divided by total intake) of each treatment were compared to the random choice value of 50%. The significance of the main effect “feather eating”, (FE vs NFE), was assessed using the GLM procedure of SAS.

All laying hens showed strong ($p<0.05$) rejection of 5% lauric and myristic acid addition while no differences were found for palmitic and stearic acids. The results are consistent with previous findings that chickens preferred long-chain over medium-chain fatty acids in the diet (Furuse et al., 1993). Moreover, while the 5% MP-young mix was rejected ($p<0.05$), the MP-old mix was not ($p>0.05$), possibly due to the lower lauric and myristic content of the MP-old compared to the MP-young. Hence, more frequent feather pecking behaviours in older laying hens may be an effect of less lauric and myristic acids in the preen oil. In addition, only FE birds showed rejection of 1% laulic and myristic acids ($p<0.05$) suggesting that FE birds were more sensitive to these two acids. In conclusion, this study suggests that taste, and/or odour of specific fatty acids could be an important factor in the attraction to feathers in FE behaviour. These results add to previous findings in our group strongly advocating dietary approaches to prevent feather pecking.

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INFRA-RED BEAK TRIMMING INFLUENCES, PECKING STONE CONSUMPTION, FEED INTAKE, FEED AND NUTRIENT SELECTION IN FREE RANGE LAYING HENS

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Beak trimming can have serious consequences on hen welfare, health and production. However, compared to hot blade trimming, the infra-red technique has resulted in more uniform beak length and symmetry (Dennis *et al*., 2009). Pecking stones (Analysed Values: 20.50% calcium, 4.30% phosphorus, 5.0% sodium and 2.50% magnesium) have been used in Europe and are reported to blunt hens' beaks and alter pecking behaviour (Glatz and Runge, 2017). The objective of the current study was to evaluate the impact of infra-red beak trimming, pecking stone usage and hen age on feed intake and nutrient selection as well as the evaluation of relationships between beak length and pecking stone consumption, feed intake and nutrient selection.

A total of 18 flocks (Farm A, n = 10 flocks, infrared red beak trimmed hens, 20,000 /flock; Farm B, n = 8 flocks, non-beak trimmed hens, 2000 /flock) were examined. On each farm, hen placement was performed in 2 identical sheds at the same time. Each flock was randomly assigned to either a control group (no pecking stones) or a treatment group (1 pecking stone/1000 hens/10 weeks). At each time point (every 10 weeks from 16 until 66 weeks of age), 10 hens were randomly selected from each flock and individually confined in a holding pen (each holding pen housed 20 hens individually in each flock) amongst their peers. Each holding pen was equipped with 250g of mash feed and *ad libitum* water. Beak length was measured prior to placement while individual body weights were measured prior to and after 24 hours of placement in these holding pens. Feed samples prior to placement and feed residues from each hen were weighed before and after the 24 hour evaluation period and used for feed intake calculation and proximate analyses. Data were analysed in a 2 × 2 factorial arrangement (non-trimmed/ infrared trimmed beaks × pecking stones/no pecking stone availability) using a general linear mixed model. Spearman's rho correlation coefficients were used to examine the relationship between beak length and other measured variables.

Farm type showed trend towards an effect (P = 0.057) on feed intake and a significant effect (P= 0.04) on crude protein consumption. A significant farm × pecking stone effect was observed on aluminum mineral consumption (P = 0.03), similarly a significant effect of farm × age was observed on copper (P = 0.007), sodium (P = 0.01), magnesium (P <0.01), phosphorus (P=<0.01), and zinc (P <0.01) intake. Beak length was positively correlated with crude protein (r = 0.275, P = 0.008), sodium (r = 0.221, P = 0.035) and phosphorus (r = 0.284, P = 0.006) consumption. In addition, beak length was strongly correlated to pecking stone consumption (r = 0.563, P < 0.001) indicating hens with longer beaks consumed significantly more pecking stones.

Although infrared beak trimming is a non-invasive technique it altered the pecking behaviour and nutrient selection.

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USING HYDROXY SELENOMETHIONINE TO ENRICH SELENIUM IN EGGS

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Selenium (Se) is an essential nutrient for both animals and humans. Eggs rich in Se have nutritional significance for both hatching and for human consumption. In the animal industries, selenite has been widely used as Se additive in spite of a number of disadvantages, such as low bio-efficacy, toxicity and interaction with other nutrients. We conducted two studies to compare efficacy of chemically synthesized Hydroxy Selenomethionine (OH-SeMet, Selisseo®) as a new organic Se source for laying hens.

Study 1 used 648 Hyline hens of 18 weeks of age, randomised into 6 treatments, 6 replicates each of 18 birds, 3 birds in a cage. The birds were fed on a typical corn-soybean based diet of mash form. After 1 week of adaptation, the experiment lasted for 24 weeks. The control diet contained no added Se (Negative Control), the remaining 5 treatments were supplemented with selenite 0.3 mg Se/kg (Positive Control) or OH-SeMet (Selisseo®) at 0.1，0.2，0.3 or 0.5 mg Se/kg. The birds had free access to feed and water. At the week 2, 4, 8, 16 and 24, two eggs were collected from each replicate, freeze-dried and analyzed for Se contents. The Se addition had no influence on laying performance (data not shown), but significantly increased Se in eggs, with a clear dose response (Table 1). OH-SeMet was more efficient than selenite and 0.3 mg Se (OH-SeMet)/kg diet led to approximately 30 µg Se in 100 g fresh egg.

Table 1 - Se contents in fresh eggs after dietary Se supplementation.

Se in egg, ppm	Control	OH-SeMet, ppm				Selenite
		0.10	0.20	0.30	0.50	0.30 ppm
2 weeks	0.109 ^{d}	0.157 ^{cd}	0.186 ^{bc}	0.218 ^{b}	0.315 ^{a}	0.198 ^{bc}
4 weeks	0.121 ^{d}	0.178 ^{cd}	0.215 ^{bc}	0.284 ^{b}	0.502 ^{a}	0.202 ^{c}
8 weeks	0.113 ^{e}	0.196 ^{d}	0.287 ^{c}	0.354 ^{b}	0.522 ^{a}	0.304 ^{c}
16 weeks	0.085 ^{d}	0.146 ^{cd}	0.223 ^{c}	0.307 ^{b}	0.507 ^{a}	0.210 ^{c}
24 weeks	0.051 ^{e}	0.205 ^{d}	0.278 ^{c}	0.358 ^{b}	0.514 ^{a}	0.210 ^{d}

*Values in the same row not bearing the same alphabet differ significantly ($P < 0.05$).

Study 2 compared Se transfer efficiency of Selenite (SS), Se-yeast (Sel-Plex® 2000, SY) and Selisseo® (SO), using 160 ISA-Brown birds from 40 weeks of age. The birds were divided into 4 treatments with 8 replicates of 5 hens and the test period lasted for 56 days. At Day 8, Day 14 and Day 56, one egg was collected from each replicate, for Se analyses. Table 2 shows average Se contents in dry eggs collected at Day 56.

Table 2 - Efficiency of Se enrichment in eggs by 3 Se sources at 0.2 ppm*.

	Control	Selenite	Se-Yeast	OH-SeMet
Se in dry egg, ppm	0.26 ^{d}	0.73 ^{c}	0.90 ^{b}	1.16 ^{a}

*Values sharing the same superscript differ significantly ($P < 0.05$).

The results confirmed that organic Se (Se-Yeast and OH-SeMet) is more efficient in transferring Se into eggs than selenite. OH-SeMet is 28.8% more efficient than Se-Yeast. An addition of 0.3 mg Se as OH-SeMet/kg can produce eggs with Se 30-40 µg/100 g fresh egg.

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THE EFFECT OF HEN AGE, STORAGE PERIOD AND TEMPERATURE ON EGG QUALITY IN LAYER HENS

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Extensive research has investigated the effects of production systems on egg quality; however, confounding problems exist with many of the experimental designs (Chung and Lee, 2014). Insufficient consideration is given to the influence of genotype, hen age, ambient temperature and stress on egg quality. Eliminating the ambiguity created by these effects is crucial when evaluating the effect of production system on egg quality. The present study aims to identify limits to the hen age, so that age is not a confounding factor when evaluating the effect of different production systems on egg quality.

All eggs were obtained from Isa Brown hens that had been reared on a single farm. During production, the hens were housed in a single barn shed. Hens of different ages were housed in individual units of 2500 birds. Eggs were collected on one single day from hens of four different age groups (21, 30, 50 and 63 weeks). The eggs were stored in a refrigerator at 4°C, in a cool room temperature at 15°C or in a room temperature at 22°C over a 28 day period. Twenty eggs were sampled at the time of collection and then after storage for 7, 14, 21 and 28 days. At each sampling time conventional measures of egg quality, egg weight, Haugh unit (HU), albumen index (AI) and yolk index (YI) were made but here the data analysis concentrated on differences in HU. Data were analyzed using the REML linear mixed model function of Genstat® 17th edition.

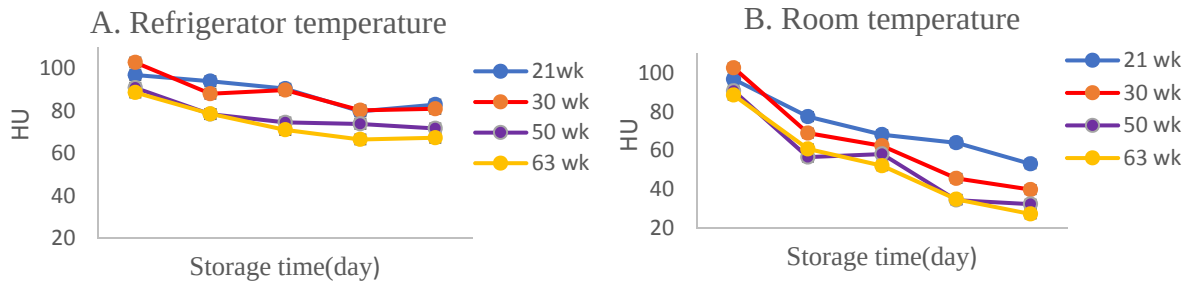

Figure 1 - Effects of hen age and storage time on HU at A) refrigerator temperature, B) room temperature.

The effect of hen age on HU measurements was significant but it was influenced by storage temperature and storage time as the three way interaction between these was significant ($P < 0.001$). The HU changes at the lowest (4°C) and highest (22°C) temperature are given in Figure 1. For all flocks the HU significantly decreased between lay and the end of storage ($P < 0.05$). The decline in HU was more pronounced as the storage temperature increased ($P < 0.05$). At lay, the 50 wk (90.8) and 63 wk (88.6) flocks had similar HU measures and were lower than for the 21 (96.8) and 30 (102.7) wk flocks (SEM 2.32: $P < 0.05$). At all storage temperatures and times, the HU was similar for the 50 and 63 wk flocks but lower than the younger flocks ($P < 0.05$). In conclusion, when evaluating the effect of egg production systems on egg quality, use of Isa Brown hens between at 50 and 63 weeks of age, would eliminate the influence hen age has on such evaluations.

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ANALYSIS OF ANTI-*ASCARIDIA GALLI* ANTIBODY LEVELS IN EGG YOLK TO DETECT PARASITE INFECTION IN COMMERCIAL LAYING HENS

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In recent years, in response to consumer concerns regarding welfare of birds, there has been a move from caged to free-range production systems. This change has resulted in increased exposure of hens to pathogens including parasites, which can compromise the welfare of the animal (Wongrak et al., 2015). Amongst helminths, *Ascaridia galli* is the most abundant nematode in poultry, and can cause significant economic losses and negative impacts on bird health and welfare (Daş et al., 2010). Early detection of *A. galli* infection is important to allow effective treatment to be administered before irreparable damage occurs to the hosts' intestines. However, the typical method of detecting *A. galli* infection is based on counting *A. galli* eggs in excreta samples and is only possible when the worms are mature (Martín-Pacho et al., 2005). Meanwhile, alternative methods, such as serological tests, require collection of blood which is labour intensive and more intrusive for the birds (Beck et al., 2003). Thus, the current study was undertaken to determine if anti-*A. galli* antibodies in egg yolks can be used to detect *A. galli* infection in layers, and to compare yolk antibodies from caged and free-range production systems.

Six eggs and pooled excreta samples were randomly collected from 3 caged, 2 barn-housed and 4 free-range flocks in Australia. All farms housed Isa Brown hens which were at least 60 weeks of age at the time of sampling. Eggs were processed to determine anti-*A. galli* antibody level in the egg yolks using an in-house ELISA assay. Excreta samples were assessed to determine numbers of worm eggs using standard procedures. Significant differences in egg yolk anti-*A. galli* antibody levels were observed in flocks within and between production systems. Free-range flocks had significantly higher egg yolk anti-*A. galli* antibody levels than did cage flocks (0.50 ± 0.08 versus 0.16 ± 0.03 OD units; $P < 0.001$). However, low levels of anti-*A. galli* antibodies were also detected in eggs obtained from some free-range flocks. The antibody level of barn-laid eggs was similar to that of the free-range eggs (0.49 ± 0.09 versus 0.50 ± 0.08 OD units). The results obtained from the excreta worm egg counting test confirmed that excreta samples from free-range and barn-housed flocks contained significantly higher numbers of *A. galli* eggs compared to samples from caged flocks. The average numbers of *A. galli* eggs per gram of excreta sample from caged, free-range and barn-house flocks were 0 ± 0, 750 ± 239 and 850 ± 600, respectively. However, the worm egg count did not correlate with the levels of anti-*A. galli* antibodies in the hen egg yolk ($P > 0.05$).

These findings suggest that analysis of anti-*A. galli* antibodies in the egg yolk is indicative of worm infections (past or present) in layer flocks.

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THE IMPACT OF EARLY-LIFE INTERVENTION ON MICROBIOTA COMPOSITION IN FREE-RANGE LAYING HENS

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Laying hens need to maintain their health status, including their gastrointestinal health until at least 72 weeks of age. Gut microbiota composition is extremely important to gastrointestinal and overall health. Furthermore, the gut-brain axis enables interactions between the enteric microbiota, the enteric nervous system and the central nervous system (Dinan & Cryan, 2017). The composition of gastrointestinal microbiota can be affected by animal behavior and vice versa (Neufeld et al., 2011; Berthoud, 2008). The purpose of this study was to investigate the impact of early-life intervention on caecal microbiota composition in laying hens.

A total of 300 day-old Hy-Line Brown layer chicks was obtained from a commercial hatchery and randomly allocated into two groups of 150 birds each. Birds in group 1 were reared under standard conditions according to the Hy-Line management guide. Birds in group 2 were reared using unpredictable environmental stimulations. The stimulants included visual and audio noise, toys and equipment, and was applied from 4-21 days of age. From 21 days onwards, hens in both groups were housed under exactly the same conditions. At 12 weeks of age, hens were moved to the same laying facilities and housed in two separate indoor pens with identical resources. All birds received commercial feed and litter from the same source and were maintained by the same personnel. At 21 weeks of age, 12 hens were randomly selected from each group and sacrificed. Caecal content was collected for microbiota analysis. The Australian Genome Research Facility performed microbial diversity profiling from the extracted caecal DNA. Statistical analysis included univariate non-parametric Mann-Whitney tests with adjustments for multiple comparisons using Benjamini-Hochberg adjustments ($P < 0.05$ was considered statistically significant).

At the phylum level, levels of *Actinobacteria* and *Bacteroidetes* were significantly higher in hens from group 2, compared to hens from group 1 ($P = 0.05$). At the order level, significant differences were observed for various groups, with levels of Clostridiales and Campylobacterales significantly higher in hens from group 1, which were reared under standard conditions. Further, differences various levels could be observed.

In conclusion, early-life intervention had a significant impact on caecal microbiota composition.

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INVESTIGATION INTO THE RELATIONSHIP BETWEEN PRODUCTION TRAITS IN INDIVIDUALLY CAGED EARLY-LAY ISA BROWN HENS

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In commercial egg production, production traits such as bodyweight (BW), feed intake and efficiency, and egg quality are targets that are critical to profitability. Assessment of average BW and uniformity are relatively straightforward measurements. Furthermore, it is well known that achieving target BW for the breed standard and minimising BW variation are key to producing greater egg mass and egg quality. It is more difficult to get an understanding of the variation in feed intake, egg production and feed efficiency in flocks and the relationships between these traits. The aim of this study was to evaluate the uniformity in a range of production variables and explore relationships between them.

Three hundred ISA Brown hens were housed individually in cages at point of lay. Hens were offered *ad libitum* access to a common wheat-soybean meal mash diet formulated to meet requirements for ISA Brown hens in early lay. Management conditions were the same for all birds as suggested by the breed standard. At 28 weeks of age, feed intake assessments were carried out weekly, and egg mass and weight daily for 6 weeks. BW was recorded at the conclusion of the experimental period. Hens with an average percentage lay of below 85% (>2 standard deviations less than the average) were excluded from the study (13 birds).

The average BW of the birds at the conclusion of the study was 2 kg, CV 8.3% while the average feed conversion ratio (FCR) was 1.90, CV of 8.2%. Average daily voluntary feed intake was 119g, CV 9.2%. The average percentage lay was 97.5%, CV 3.2%. The average egg weight was 64.4g, CV 6.4%. The average egg mass was 62.8g, CV 6.4%. Pearson's correlations were conducted between production variables of relevance. There was a moderate positive association between final BW and average daily feed intake (ADFI; Fig. 1, r = 0.60, P<0.001) and between BW and FCR (Fig 2, r = 0.44, P<0.001). There was also a positive relationship between BW and egg weight (r = 0.35, P<0.001).

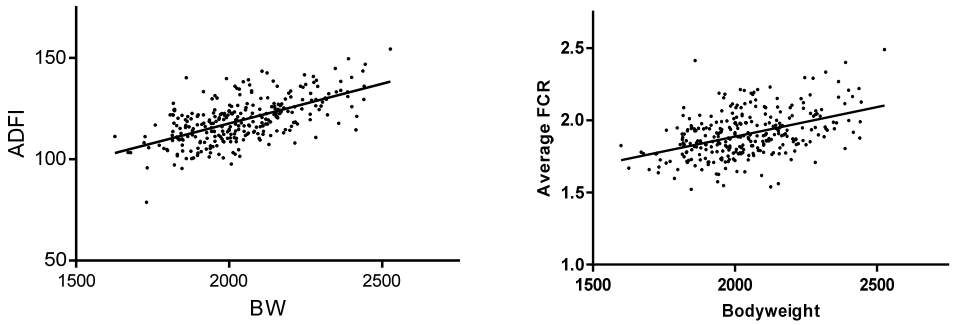

Figure 1 & 2 - Correlation between BW and ADFI and BW and FCR respectively

Using individually housed hens, this study provides some simple data on the uniformity of production targets which are not calculable on a flock basis. The average hen BW was greater than the breed standard at 2kg versus ~1.87-1.9 kg for hens 30-34 weeks of age. There was a positive correlation between BW and FCR which suggests rationale for controlling BW in line with the breed standard.

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