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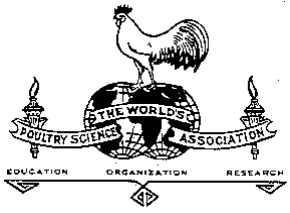

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(Australian Branch)

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PRACTICAL VIEWS ON GLOBAL MEAT CHICKEN NUTRITION

R. KLEYN[1]

Summary

The demand for poultry meat will increase because of population growth and changing socio-economic factors. Resources will become constrained and changing consumer perceptions will result in pressure to produce poultry meat in a sustainable manner. Sustainability is a multi-faceted concept and it is not feasible to select only those aspects that suit a particular narrative. Improvements in technical efficiency are the best way to increase the sustainability of meat production. Much of this responsibility is shouldered by the geneticists, but nutritionists and producers have a role to play. We should focus on improving nutrient utilisation, making optimum use of the ingredients at hand, and rethinking our various approaches to feed specifications. Although our decision making will be reinforced by enhanced data resources, these will not replace the nutritionist as the ultimate decision maker.

I. INTRODUCTION

The global supply of poultry products will need to double by 2050 if we are to meet the aspiration of all people to be food secure. We will need to produce more poultry meat in the face of changing consumer perceptions and constrained resources. Consumers expect this to be achieved in a sustainable manner. The industry has the skills to achieve this. Genotypes continue to improve; production methods and business models are evolving; and we have enhanced our knowledge of poultry health and nutrition. However, our industry will need to develop by offering alternative products and by changing or improving production methods. These changes will occur in the foreseeable future.

The overarching consideration will be sustainability. The definition of sustainability is straightforward – sustainable systems should meet the needs of the current generation without compromising the ability of future generations to meet their own needs. In practice, sustainability is a concept with multiple facets, namely environmental, social and economic. Measuring sustainability is difficult because it depends on which metric is chosen for use, and often it can only be accurately determined in hindsight. Achieving alignment in one area often leads to failure in another, which is a challenge faced by broiler producers and legislators. For example, alternative production systems such as free-range and organic result in an apparent improvement in welfare, but at considerable cost to the environment (Williams et al., 2009).

There is a divide between global citizens. In developed countries, wealthy citizens consume poultry meat produced by 'integrators', delivered via well-developed supply chains, and sold through supermarkets and fast service restaurants. However, it is estimated that there are 2.5 billion people who depend on small farms for their livelihood and food security (FAO, 2013). These farmers have no access to supply chains, either for inputs or for the sale of product. Yet meat consumption is aspirational and poultry forms an important component of dietary protein supply. Most consumers desire access to cheap, safe animal protein, yet our industry is obliged to meet the changing consumer demands of more developed markets. Both market channels will need to be supplied in a sustainable way. Against this background, the primary focus of this paper is the nutritional interventions that can be applied to enable our industry to fulfil its mandate to ensure food security, sustainably.

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II. SUSTAINABILITY AND DEMAND

Sustainability is neither a single entity, nor can we choose those aspects that suit our own particular beliefs. From an environmental viewpoint, sustainability impacts the entire poultry supply chain, causing pollution and ecological degradation. Social aspects of sustainability encompass both human and animal well-being. The compact of the five freedoms of welfare should be applied to all animals, while human health – including exposure to antibiotic-resistant bacteria and the well-being of poultry producers – needs to be considered (FAO, 2012). Paradoxically, most consumers are more concerned about their own well-being through the consumption of 'natural' products than about animal welfare (Magkos et al., 2006; Bray and Ankeny, 2018). The final and seminal aspect of sustainability is financial viability, which is the key enabler of all production systems.

From a nutritional perspective, all the objectives of sustainability should be aligned. The more efficiently chickens utilise feed, the more viable the feeding operation becomes, with a reduced carbon footprint through a lower demand for resources. Appropriate nutrition will also impact on bird welfare. The poultry industry believes that bird welfare and food safety are better when conventional systems are used, but this is not a view shared by wealthy consumers in the developed world. Public opinion is that 'organic' is natural, healthy and sustainable, and that use of medication and intensive farming is bad. Many of these beliefs are based on perception and misinformation, often created by the poultry industry itself, which has used 'Hormone free', 'Drug free' and 'Free range' as marketing slogans for decades. The danger of consumers imbibing harmful drug residues from eating poultry products, and the possible contribution of these drugs leading to an increase in drug-resistant bacteria, are more a perception than a reality (Cervantes, 2015). Regardless of the truth, failure to meet consumer demands will result in their rejection of our products. This happened in Norway, for example, where it was perceived that the use of ionophores was undesirable. The reduced demand for poultry meat ultimately led to the voluntary withdrawal of ionophores from all broiler diets (Kaldhusdal, 2018).

Alternative production systems place a higher burden on the environment than conventional systems (Williams et al., 2009). Petersen (2017) estimates that, if only one-third of the US broiler industry were to switch to the use of slower-growing breeds, an additional three million hectares/year of land would be required to grow the necessary feed ingredients, while, "large scale antibiotic-free production will increase the industry's carbon footprint" (Smith, 2016). More affluent people tend to eat fewer grains and more meat and high-value foods (FAO, 2013; Hofstrand, 2014). The price of poultry substitutes, for example fish and beef, are likely to increase disproportionately, further fuelling demand for poultry. The poultry industry's innovative approach to product development has also led to increased demand. A rising demand for poultry products with specific quality and food safety attributes is likely, probably linked to increased levels of affluence (Narrod *et al.*, 2012). In essence, the poultry industry will be expected to produce increased quantities of different product types, sustainably, without access to some technologies it has used for decades, and still make profits. Improved growth performance and feed efficiency will have a greater impact on sustainability than any other factor. Avendaño et al. (2017) state that the feed conversion ratio of broilers is improving by two to three points per annum. By simple calculation, a two-kilogram broiler, grown a decade from now, will require about 500 g less feed than it does today. This will reduce the environmental impact and make broiler production more financially robust. In short, chicken is the most sustainable meat option available (Henriksen, 2018).

III. IMPROVED FEED UTILISATION

The utilisation of feed chemicals by the broiler relies on a complex web of 'cross-feeding'. This involves the substrates contained in the diet, the birds' endogenous enzymes, enzymes produced by the gut microflora, and the few exogenous enzymes added to the feed. The use of antibiotic growth promoters (AGPs) masks imbalances in the gut microflora to a certain extent, although we still do not fully understand their mode of action (Broom, 2018). In future, we will need to unravel the complexity of the relationship between the broiler, its GIT microflora, the diet being consumed and the additives used.

Improving nutrient utilisation involves far more than simply enhancing dietary digestibility and some 400 kcal/kg of energy, 70% of phosphorus and between 10 and 20% of the essential amino acids in a typical broiler diet are not utilised. Indigestible substrates offer a resource to the nutritionist. Exogenous enzymes enhance digestion of substrates but also break down some of the anti-nutritional factors that occur in typical diets, rendering them harmless. This leads to reduced inflammation and enhanced nutrient uptake (Niewold, 2007). In addition, enzymes prevent the nutrients that escape digestion from becoming a source of nutriment for the GIT microflora. A broiler with a healthy, well-functioning GIT and a stable gut microflora will utilise its diet more effectively, resulting in enhanced digestibility. A well-developed gizzard leads to improved energy utilisation (Truong et al., 2017). Undigested nutrients represent a food source for the GIT microflora and may induce a shift to more proteolytic bacteria, which can lead to enteritis.

IV. PROTEIN

Modern genotypes require more protein and less energy per unit of growth than their predecessors. The efficiency of utilisation of protein is unlikely to change, but proportionally less protein will be used for maintenance purposes and more for protein-rich tissue production, such as breast meat. Future protein supplies will be more constrained than energy (Leeson, 2018), which is likely to increase the cost of protein. However, the efficiency of broiler production will enable our industry to afford more expensive protein when compared to less efficient competitors.

High dietary crude protein (CP) levels in broiler diets place a burden on the environment. Broilers that consume high protein diets emit more nitrogen (N) and ammonia. Ammonia is emitted from the manure through the breakdown of undigested protein and uric acid. It is responsible for water pollution (eutrophication) and soil acidification (Belloir et al., 2017). Legislators in Europe have placed limits on the levels of nitrogen allowed in poultry manure. Precise protein nutrition is beneficial to the sustainability of broiler meat production (Lambert and Corrent, 2018). For example, avoid feeding ingredients that are refractory to digestion (heat damage) or retard gut health and increase disease challenge (inflation and immunity demand protein). Simply reducing dietary CP is a strategy that will have both economic and environmental outcomes. It involves the use of enzymes and crystalline amino acid, or perhaps a simple reduction in feed specifications. Alhotan and Pesti (2016) emphasise that it is important to meet the non-essential amino acid requirements. They demonstrate that requirements for growth and feed conversion differ, but that an ideal ratio between the amino acid level of the diet and its true protein content (TP) exists. Practically, dietary protein may be reduced to a point that impairs performance and loses opportunity.

Belloir et al. (2017) evaluated the impact of a reduction in dietary CP in broilers (Table 1). The feed conversion ratio (FCR) and the breast meat yield were depressed in low CP diets, while abdominal fat increased. The N utilisation data was also of interest. N retention efficiency increased with a reduction in CP, and N excretion reduced. Each 1% reduction in

dietary CP between 19–16% CP decreased N excretion by 13%. Litter moisture also decreased with reduced protein.

Table 1 - A summary of the performance characteristics, carcass yield and N utilisation of Ross PM3 male broilers between 21 and 35 days of age fed with diets differing in CP (after Belloir et al., 2017).

	Crude protein content				
	190 (g/kg)	180 (g/kg)	170 (g/kg)	160 (g/kg)	150 (g/kg)
Gain (g) BWG	1479	1496	1494	1446	1478
Feed intake (g)	2430	2477	2472	2459	2528
FCR	1.64[b]	1.65[b]	1.65[b]	1.69[a]	1.71[a]
Breast meat (% BW)	20.1	20.2	20.8	20.5	19.5
Abdominal fat (% BW)	2.16[b]	2.30[ab]	2.45[a]	2.61[a]	2.51[a]
N retention efficiency %*	60.48	63.66	66.84	70.02	73.2
N excretion (g/kg BWG)*	19.36	17.24	15.12	13.00	10.88
Manure N (g N/kg DM)*	32.7	31.19	29.68	28.17	26.66
Litter moisture (%)*	46.17	44.97	43.77	42.57	41.27

Means within columns not sharing common superscripts (a, b) are significantly different ($P < 0.05$).
Note*: These values were calculated from the published regression equations.

Evonik (2017) has illustrated how opportunity may be lost by feeding low protein diets (Table 2). Modern broilers are highly responsive to an increased level of dietary protein in terms of body weight, FCR and breast meat yield. This effect appears to be independent of dietary energy content. Different levels of energy and balanced protein (measured as dietary SID Lys) were fed to male Ross PM3 broilers from 21 to 35 days of age. These data demonstrate that higher protein levels lead to improved yields of high value products, with improved feed efficiency. Clearly a compromise is required. We know that the birds respond to higher dietary protein, yet – from an environmental perspective – this is precisely what we need to avoid.

V. ENERGY

It is unlikely that the efficiency of energy utilisation for absolute growth will change (Lopez and Leeson, 2005; Tallentire et al., 2016), but the sooner a bird reaches target weight, the smaller the proportion of energy used for maintenance purposes will be. Our role as nutritionists is to meet the bird's demand for sufficient calories on each day of the production cycle. As can be seen from Table 2, the broiler has the ability to maintain its energy intake regardless of the energy level of the diet. In commercial situations, however, it is not this simple since the pressure created by high stocking densities and limited feeding space prevents broilers from consuming enough feed to meet their energy demands. As a rule, energy intake increases as dietary energy levels rise, with a concomitant increase in field performance. It may not be possible to reduce the energy requirement of the bird, but it is possible to feed diets of different nutrient density in order to utilise cheap ingredients and maximise financial returns. Care should be taken not to focus on producing meat at least cost; rather, we should concentrate on maximising returns (profit).

The real challenge is to calculate energy balance in the broiler. To achieve this, an energy system is required, both to equitably quantify one ingredient relative to another and to enumerate the bird's requirements. In theory, the values determined should be linear and additive. Any system should be straightforward, cost-effective and repeatable across laboratories. Parsons (2011) believes that the metabolisable energy (ME) system will be the primary and preferred measurement of energy in the foreseeable future; indeed, most commercial feed is formulated on this basis. Mateos et al., (2018) lament that despite abundant research, no simple procedure exists to evaluate the energy content of ingredients and diets.

Table 2 - The effect of increasing levels of metabolisable energy and amino acid density levels (as determined by the SID Lys content) on selected growth performance and carcass traits of male Ross 308 broilers from 21 to 37 days of age (after Evonik, 2017).

	Body weight (g)	Feed intake (g/d)	FCR	SID Lys intake (mg/day)	TME intake (kcal/day)	Carcass as % of body weight	Breast as % of body weight
TME_n (MJ/kg)							
12.00	2375^{ab}	177.3^{a}	1.69^{a}	–	473	64.98^{b}	18.31^{a}
12.60	2393^{a}	170.7^{b}	1.61^{b}	–	480	66.10^{a}	18.20^{a}
13.10	2355^{ab}	162.7^{c}	1.57^{c}	–	518	65.63^{a}	17.88^{ab}
13.60	2323^{b}	157.0^{d}	1.54^{d}	–	476	65.47^{ab}	17.49^{b}
SID Lysine (g/kg)							
8.5	2205^{b}	162.2^{b}	1.71^{a}	1378	–	64.56^{c}	16.39^{c}
10	2420^{a}	170.0^{a}	1.57^{b}	1700	–	65.64^{b}	18.43^{b}
11.5	2459^{a}	168.0^{a}	1.52^{c}	1932	–	66.45^{a}	19.09^{a}

Means within columns not sharing common superscripts (a, b) are significantly different ($P < 0.05$).

Choct (2017) argues strongly that the use of ME is limiting and that any efforts spent on a workable net energy (NE) system are justified. NE gives a closer representation of 'true' or usable energy, but it does have various shortcomings. It is complicated, time-consuming, expensive to analyse and, as yet, it has no standardised method. In addition, NE systems apply equations that utilise ME as a starting point. Thus, any errors in the 'base' ME values are automatically carried over. NE systems mostly use CP as a measure to calculate the expected heat increment (HI) of a diet. Not all N in the diets is amino nitrogen so perhaps this is an over-simplification. In addition, crystalline amino acids are high in nitrogen and therefore high in crude protein, but they have a close to zero HI in digestion since they are already in their basic form. This is relevant in modern poultry diets where the use of synthetic, crystalline amino acids is increasing. The NE system, as with all systems, fails to account for the non-linearity of fat and fibre addition to broiler diets (Leeson and Summers, 2005; Mateos et al., 2012).

Of interest, is the use of near-infrared spectroscopy (NIRS) technology to predict the chemical composition of an ingredient and hence to predict ME indirectly or, more interestingly, to directly predict AME_n (Hughes et al., 2016). The advantages of NIRS are that it is fast, cheap to run and gives repeatable results. Its limitation is that it is only as good as the calibration used in its set up. In addition, if a flawed energy system is used to calibrate the machine, this will be carried across to all results. The nutritionist is required to decide which energy values to use when formulating, and slavish adherence to a single source of information can be problematic.

The perfect energy system does not exist. This may be because the energy content of a diet is not a property of the diet itself, but rather a property of the bird consuming that diet. However, even if a system is not perfect, it only has to be better than the approach it supersedes. We should accept the flaws of the ME system while we strive for something better, a standardised NE system.

VI. MINERALS

Minerals comprise a small proportion of the diet, but their importance to the birds cannot be overlooked. Dietary supplementation with high levels of inorganic trace minerals is expensive and may be harmful to the environment. Organic minerals are compounds in which minerals are covalently complexed with organic ligands. They are less reactive than mineral salts, but can be supplemented at lower concentrations than sulphates and oxides without impacting on

bird performance (M'Sadeq et al., 2018). Currently, organic minerals are an expensive option but, in future, their use will increase.

Phosphorus (P) and calcium (Ca), deserve special attention. Not only are the global supplies of P constrained, but the way in which P is managed in poultry diets is a key component of sustainability from an environmental perspective. Nutritional strategies can improve the effective use of P, to avoid overfeeding and minimize P excretion (Rousseau et al., 2016). Current research would indicate that the P requirements broilers are lower than mostly used in practice, and the use of phytase allows levels to be reduced still further (Faridi et al., 2015; Kim et al., 2017 and Cheng et al., 2018). These findings are in stark contrast to the recommendations of the primary breeding company and to general commercial practice. New recommendations have been published by INRA (Khaksar et al., 2017) and (Angel, 2018). Although these recommendations differ from each other, they are consistent in that they recommend low levels of both minerals in grower and finisher diets. Phytase doses will likely increase, resulting in more complete degradation of the phytate plant material. Implementation of these strategies will lead to reduction in demand for P and will also decrease the polluting levels of P in broilers manure. Our current approach when formulating diets is to use some measure of available P and the total Ca. Clearly, we would do a better job of formulation if we were to use a measure of available Ca, and then formulate accordingly (Angel, 2017; Ravindran, 2018).

VII. DISCUSSION

As clichéd as the term 'precision nutrition' is, it is the goal that nutritionists strive for. If we are able to meet the nutrient requirement of each individual in a flock, on each day of the production cycle, we will enhance our ability to produce poultry meat in a sustainable manner. Bear in mind that well-performing flocks will have different requirements to poorer flocks. This is challenging when most operations use only three or four phases of feed throughout the broiler cycle. In order to achieve the lofty ambition of precision nutrition, ingredients will need to be quantified more accurately than they are at present. Formulating diets by using a single value for energy ignores the fact that we are unsure of how to measure energy or to cope with variability in the first place. The non-linear nature of fat and fibre addition to bird diets will need to be accommodated.

The efforts made by nutritionists will need to be matched by improvements in feed manufacture. Some form of real-time energy determination and formulation adjustment would be helpful. Disparate parcels of ingredients will need to be identified and preserved, before being precision weighed and mixed. These suggestions will bring about an unimaginable level of management complexity and cost in terms of ingredient purchasing, storage management and logistics. On farms, the bulk of management resources are likely to be utilised in deciding which diet to feed to which flock.

Clearly a more pragmatic approach will be required. Nutritionists will have to make considered decisions about ingredient energy and nutrient content. Decisions about feed specifications will also need to be made, bearing in mind sustainability, the cost and availability of ingredients, farm management, the end products and, importantly, net returns. Feeding programmes will have to be designed to ensure that bird requirements are met, while minimising harmful pollutants. These programmes will have to match the logistics of specific farms, as determined by feed bin size, delivery vehicle capacity and bird numbers. Feed millers will need to manage their ingredients more adroitly, for example by using multiple bins for key ingredients, but probably not by changing formulations on an hourly basis.

The feeding and nutrition of meat chickens have never been more complex and the advances are going to continue. Rather than expecting computer systems to make our lives

easier, professionals in the broiler industry will be required to make more decisions than ever before. Although we are a long way from the goal of precision feeding, the use of the information already at our disposal will ensure that the broiler industry moves towards sustainability in every aspect.

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XYLO-OLIGOSACCHARIDES AND XYLANASES IMPROVE THE PERFORMANCE OF BROILERS FED WHEAT-BASED DIETS

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Summary

Xylanases are almost universally added to commercial wheat-based broiler diets, to improve bird performance and reduce welfare problems associated with wet litter. Recent research suggests that at least some of this improvement in performance can be attributed to the xylanase-mediated release of xylo-oligosaccharides (XOS) in the gut. This trial was designed to determine the influence of adding xylanase alone or with XOS to wheat-based diets on performance, water intake and carcass yield of broilers to 34 days. The results indicated that the xylanase could improve bird performance and reduce water intake and mortality. Addition of short-chain XOS to the xylanase supplemented diets further improved 24 day weight gain.

I. INTRODUCTION

Xylanases have been used in the poultry industry for 30 years, initially to improve litter quality in wheat-based diets but later to also improve performance or reduce costs in wheat, corn, sorghum and barley based diets. It is widely accepted that these benefits are the result of the enzyme partly degrading the insoluble cell wall arabinoxylans to release the enclosed nutrients and the soluble arabinoxylans to reduce digesta viscosity, hence improving nutrient digestibility. It is also known that degrading this fibre polysaccharide to free arabinose and xylose can be detrimental to broiler performance (Schutte, 1991), but that producing XOS can increase energy availability to the host and encourage a more effective fibre-degrading gut microbiota (Courtin *et al*, 2008; Bedford and Apajalahti, 2018). The present trial was designed to test if adding xylanases, alone or with XOS, to a wheat-based diet would influence broiler performance, carcass yield and water intake.

II. METHODS

A randomized complete block design was employed with 12 pen replicates (6 males, 6 females) of 24 birds (Ross 308) per diet. Birds were fed wheat/SBM-based diets in a three phase program. All plant-origin feedstuffs were analysed by NIR prior to diet formulation, with diets pelleted at 80-85^{o}C. All diets contained 500 FTU/kg of a phytase (Quantum Blue, AB Vista), with matrix values of 0.68 MJ/kg, 0.25 g/kg digLys, 1.65 g/kg Ca and 1.5 g/kg avP applied. Excluding these matrix values, the ME, crude protein, digLys, Ca and avP contents of the starter diets (1-13 days) were 12.6 MJ/kg, 236 g/kg, 11.5g/kg, 8.6 g/kg and 4.3g/kg, the grower diets (14-24 days) were 12.9 MJ/kg, 218 g/kg, 10.5 g/kg, 7.6 g/kg and 3.8 g/kg, and the finisher diets (25-34 days) were 13.2 MJ/kg, 204 g/kg, 9.9 g/kg, 6.6 g/kg and 3.3 g/kg, respectively. The Control diets were fed alone or supplemented with 16,000 BXU/kg xylanase (Econase XT, AB Vista) or the blend containing 16,000 BXU/kg xylanase and XOS (Signis, AB Vista).

Weight gain, feed and water intake, mortality and carcass yield were recorded up to 34 days, with feed conversion ratio (FCR), weight-corrected FCR (BWcFCR), water to feed ratio and European Production Efficiency Factor (EPEF = ((ADG×livability)/FCR)×100) calculated. Data were submitted to a two-way analysis of variance (diet x sex) using JMP Pro

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14 statistical software. Least square means were compared using Students t-test, with significance reported at $P < 0.05$.

Table 1 - Performance in broilers fed wheat-based diets unsupplemented (Control) or supplemented with xylanase (16,000 BXU/kg) or the blend containing xylanase plus xylo-oligosaccharides (XOS).

	Control	C + Xylanase	C + Xylanase +XOS	P-value Diet	P-value Sex
Weight gain (d 1-24, g)	1086[b]	1091[b]	1126[a]	<0.02	<0.01
FCR (d 1-24, g:g)	1.42[a]	1.39[b]	1.39[b]	<0.01	0.03
Weight gain (d 1-34, g)	2010	1960	2025	0.23	<0.01
FCR (d 1-34, g:g)	1.57	1.55	1.53	0.19	0.29
BWcFCR (d 1-34, g:g)	1.57	1.57	1.53	0.32	<0.01
Mortality (d 1-34, %)	3.49[b]	1.14[a]	0.83[a]	<0.03	0.72
EPEF (d 34)	364	367	387	0.09	<0.01
Carcass yield (g/kg 34 d BW)	637	640	642	0.42	0.66
Water intake (d 1-34, g)	6057[a]	5754[b]	5907[ab]	<0.01	<0.01
Water:feed (d 1-34, g:g)	1.94	1.89	1.90	0.12	<0.01

[a-b]$P < 0.05$

III. RESULTS

Analysis of all diets confirmed that nutrient contents and enzyme (phytase and xylanase) activities were as expected. The wheat used in this trial was relatively high in dietary fibre (119 g/kg, including 103 g/kg non-starch polysaccharides (NSP) and 16 g/kg lignin), total (68 g/kg) and soluble (21 g/kg) arabinoxylans. The diets were calculated to have 131-132 g/kg dietary fibre, 106-108 g/kg total NSP, 11-13 g/kg soluble and 40-48 g/kg total arabinoxylan.

There were no diet x sex interactions, and xylanase inclusion reduced ($P < 0.03$) 34-day mortality (Table 1). At the end of the grower period (24 days) supplementation with xylanase + XOS improved ($P < 0.02$) weight gain by approximately 40g relative to the other two diets, while both the xylanase and xylanase + XOS diets improved ($P < 0.01$) FCR by 3-points relative to the Control. By 34 days these differences were no longer statistically significant ($P = 0.19$). However, due to numerically better overall performance and lower mortality, the xylanase + XOS diets tended to give a better ($P = 0.09$) EPEF (387) than the Control diets (364), with the xylanase diets intermediate. The xylanase only diets reduced ($P < 0.01$) water intake to 34 days relative to the Control diets, potentially leading to an improvement in litter quality, but the water:feed ratio did not differ between diets ($P = 0.12$). Carcass yield, as a fraction of live weight, was not influenced by diet.

IV. DISCUSSION

Insoluble arabinoxylans are relatively resistant to degradation in the monogastric digestive tract, although the soluble fraction will be extensively degraded (Graham *et al*, 1988). Adding a xylanase to the feed will partly solubilize the insoluble xylans and degrade the soluble fraction to smaller fragments. The xylanase used in this trial can degrade *in vitro* arabinoxylans to short-chain oligosaccharides, primarily with a degree of polymerization (DP) of 3-8. However, *in vivo* it is likely that the action of feed xylanases will produce longer-chain soluble arabinoxylan fragments in the posterior gut that are then fermented by the microbiota in the lower gut, producing volatile fatty acids and thus improving the overall gut function. Fibre fermentation and its by-products could account for some of the performance benefits seen in this and other

trials. This fermentation of XOS can lead to increased caecal butyric acid levels (Graham *et al*, 2004).

Research over the past decade has established that supplementing broiler diets with XOS (DP mainly >5) can improve performance (Courtin *et al*, 2008), and this has been attributed to a 'prebiotic' effect. However, a recent trial (Bedford and Apajalahti, 2018) has indicated that short chain XOS can stimulate the ability of the gut microbiota to hydrolyze dietary xylans.

V. CONCLUSION

The current trial suggests that a combination of short-chain xylo-oligosaccharides, to stimulate microbial fibre degradation, and a xylanase to partly degrade fibre, can act together to improve nutrient digestibility, make the fibre more susceptible to microbial degradation, and thus improve broiler performance.

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EFFECTS OF DIFFERENT LEVELS OF FAST DIGESTIBLE STARCH, PROTEIN, AND FIBRE ON BROILER GROWTH PERFORMANCE

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Summary

Energy and protein digestibility by broilers is a dynamic process, yet static digestibility values are used in feed ingredient evaluation and diet formulation. Dietary fibre also supplies energy to the diet, albeit a miniscule amount in comparison with the non-nutritive effects it has on broiler performance. Therefore, an experiment was conducted to test the hypothesis that similar digestion rates of starch and protein may improve broiler performance and that different fibre fermentation kinetics will interact with different protein and starch digestion rates, thus, influencing broiler performance. A Box-Benken design was utilized to determine the optimum performance response when broilers were fed 3 different concentrations (low, medium, high) of fast digestible starch, protein, and fibre. Results indicated that greater concentrations of fast digestible protein fed to broilers increased average daily feed intake (ADFI) and, subsequently, average daily gain (ADG). Increased fast digestible fibre had the greatest influence on improving broiler feed conversion ratio (FCR). It is warranted, therefore, to utilize digestion kinetics of protein, starch, and dietary fibre in feed ingredient evaluation and diet formulation.

I. INTRODUCTION

Improved FCR was observed in broiler chickens fed a slow digestible starch diet compared with a fast digestible starch diet (Weurding et al., 2001). Furthermore, Enting et al. (2005) indicated that a slow digestible starch diet fed to broiler chickens can spare amino acids (AA). Finally, Liu et al. (2017) and Truong et al. (2017) concluded that starch digestion kinetics play a role in broiler performance, but that protein digestion kinetics may be more important than starch digestion kinetics. Taken together, it can be inferred that starch and protein digestion kinetics should be utilized in diet formulation to optimize broiler efficiency. It is also hypothesized that dietary fibre may impact the digestion kinetics of both starch and protein because of its influence on digesta viscosity and passage rate. Therefore, an experiment was conducted to assess the influence of 3 different levels (low, medium, high) and combinations of fast digestible starch, protein, and fibre on broiler growth performance. It can be inferred that a diet formulated to contain a low concentration of fast digestible starch, but a similar concentration of total starch, provided a diet with a greater quantity of slow digestible starch.

II. MATERIALS AND METHODS

a) Experimental Design

A total of 4.800 one-day-old, male Ross 308 broiler chickens were housed in 120 floor pens with 40 birds per pen at the Trouw Nutrition Poultry Research Centre (Casarrubios del Monte, Toledo, Spain). Broilers were randomly allotted to pens that were assigned to 4 experimental blocks placed in 4 equal quadrants of the barn. Twelve experimental diets were formulated to create a response curve, while a thirteenth experimental diet was replicated 6 times within each block to estimate experimental error according to a Box-Behnken design. Diet 13 was the median combination of fast digestible starch, protein, and fibre. This design was replicated 8 times in total, providing 8

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replicate pens per each of the 12 dietary treatments and 24 replicate pens per the median dietary treatment.

There were 3 experimental phases; d 0 to 10, 10 to 28, and 28 to 42, the starter, grower, and finisher phases, respectively. Birds and feed remaining in feeders were weighed at the end of each phase and ADFI, ADG, and FCR were calculated.

b) Experimental Diets

The 13 experimental diets were selected to create an optimum response surface to estimate broiler performance for every combination of fast digestible starch, protein, and fibre. The 3 levels were equally spaced in concentrations of fast digestible starch, protein, and fibre to provide a low (L), medium (M), or high (H) concentration of fast digestible starch, protein, and fibre, respectively. The medium level of fast digestible starch, protein, and fibre was determined as the typical level of fast digestible starch, protein, and fibre in commercial broiler diets and the low and high levels were then equally spaced from the medium level.

Experimental diets were formulated to be isocaloric (starter = 11.7 MJ AMEn/kg; grower = 12.0 MJ AMEn/kg; finisher = 12.2 MJ AMEn/kg), isonitrogenous (starter = 220 g/kg CP; grower = 205 g/kg CP; finisher = 190 g/kg), equal in apparent faecal digestible Lys (starter = 11.5 g/kg; grower = 10.6 g/kg; finisher = 9.8 g/kg), Ca and digestible P and met or exceeded current requirement estimates for all other nutrients (Trouw Nutrition Broiler Recommendations, 2011). Starter diets were fed as a crumble, while others were pelleted.

Feed ingredients were selected based on their ability to achieve the desired concentrations of fast digestible starch, protein, and fibre in the experimental diets (Table 1; grower and finisher diets not shown, but ingredient composition was similar to starter diets). The quantities of fast digestible starch and protein in feed ingredients were determined using an *in vitro* digestion assay adapted from Boisen and Fernández (1997) and Englyst et al. (2000). The quantity of fast digestible fibre was estimated using digestibility coefficients found in literature and adapted from the procedure to fractionate fibre by Jaworski and Stein (2017) after analysis for acid detergent lignin, ADF, NDF, soluble dietary fibre, and insoluble dietary fibre using the Ankom^{2000} Fiber Analyzer and the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technologies, Macedon, NY).

c) Statistical Analysis

Data were analyzed using the GLIMMIX procedure of SAS (SAS 9.4, Cary, NC) and effect plots were generated with proc PLM. Response surfaces and subsequent performance optimization were generated with proc RSREG. A probability level of less than 5% was considered significant.

III. RESULTS

Models used showed no lack of fit ($P > 0.05$). Fast digestible protein had a greater impact on ADFI than fast digestible starch and fibre. On d 10 and 18, an interaction was observed in which increased fast digestible starch and decreased fast digestible fibre increased ADFI, producing an optimum response.

Increased fast digestible protein increased ADG, indicated by the computed fit at the greatest level of fast digestible protein (Figure 1). Increased fast digestible fibre increased ADG, with optimums near the maximum level of fast digestible fibre.

Broiler FCR was improved by increased concentrations of fast digestible fibre, while fast digestible protein had little effect on broiler FCR. The optimum FCR response was determined at a medium level of fast digestible starch.

Table 1 - Ingredient composition and calculated AME and nutrients in experimental starter diets.

Diet	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
Fast digestible starch	L	L	L	L	M	M	M	M	M	H	H	H	H
Fast digestible protein	L	M	M	H	L	L	M	H	H	L	M	M	H
Fast digestible fibre	M	L	H	M	L	H	M	L	H	M	L	H	M
Ingredients (g/kg)													
Maize	414	579	125	289	584	222	289	549	0	392	554	98	260
Wheat	0	0	153	153	0	0	83	46	236	0	46	83	129
Barley	133	0	248	115	33	325	206	26	321	225	59	398	232
Soy protein conc.	178	83	131	36	177	199	121	12	74	198	106	141	50
Wheat gluten meal	0	30	1	31	0	0	14	66	15	0	36	14	50
Rapeseed meal	146	99	73	26	87	120	73	26	0	61	14	47	0
Sunflower meal	0	8	50	58	0	0	25	8	75	0	0	25	25
Soybean meal	23	134	88	200	65	0	103	207	169	42	137	80	176
Sugar beet pulp	20	0	20	0	0	31	0	0	0	11	0	11	0
Soybean oil	54	32	77	55	21	70	51	21	74	37	10	67	40
MonoCa P	11	11	11	12	11	10	11	13	11	11	12	11	12
Limestone	6	7	6	7	7	6	7	8	7	7	8	7	8
Vit-min premix	5	5	5	5	5	5	5	5	5	5	5	5	5
Sodium bicarbonate	3	4	3	4	3	3	3	4	4	3	3	4	4
L-Lys HCl	2	3	3	4	2	2	3	4	3	2	3	3	4
DL-Met	2	2	2	2	2	2	2	2	2	2	2	2	2
L-Thr	0	0	0	1	0	0	0	1	1	0	0	0	1
L-Arg	0	0	0	0	0	0	0	0	0	0	0	0	0
Salt	1	1	1	1	1	1	1	0	1	1	1	1	1
Phytase	1	1	1	1	1	1	1	1	1	1	1	1	1
Carbohydrase	1	1	1	1	1	1	1	1	1	1	1	1	1
Calculated													
AMEn, MJ	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7
DM, analyzed	898	892	907	900	889	902	899	892	908	901	892	904	897
Crude protein, analyzed	222	220	221	214	220	218	219	214	218	219	219	218	212
Fast digestible starch	125	125	125	125	135	135	135	135	135	145	145	145	145
Fast digestible protein	110	130	130	150	110	110	130	150	150	110	130	130	150
Fast digestible fibre	28	21	35	28	21	35	28	21	35	28	21	35	28

IV. DISCUSSION

This study was conducted to test the influence of 3 different combinations and levels of fast digestible protein, starch, and fibre based on previous research indicating that the site and rate of starch and protein digestion influences broiler performance (Truong et al., 2017; Moss et al., 2018). We hypothesize that the 'ileal brake' theory may be involved in this response because a high concentration of fast digestible starch and protein increased ADFI, suggesting that there was less undigested starch and protein in ileal digesta, therefore, increasing rate of passage and ADFI. This finding suggests that the synchronization of starch and protein digestion kinetics advantage broiler ADFI. However, an optimal FCR was produced with a medium concentration of fast digestible starch. This response is similar to those of Weurding et al. (2001) and Moss et al. (2018) that indicated a high concentration of fast digestible starch depressed protein digestion rates and, therefore, increased broiler ADFI and FCR.

Surprisingly, increased concentrations of fast digestible fibre improved broiler FCR. Rich sources of fast digestible fibre in this study were barley, wheat, soybean meal, and sugar beet pulp. It is hypothesized that rate of passage was reduced and, therefore, digestion dynamics were slowed, favoring greater digestion capacity with increased concentrations of fast digestible fibre. Also, this will increase microbial fermentation and, subsequently, provide the broiler with energy in the form of volatile fatty acids (VFA). Therefore, starch and fibre digestion kinetics may also need to be

optimized to provide a correct energy supply between glucose and VFA for both broiler muscle protein synthesis and intestinal function and health.

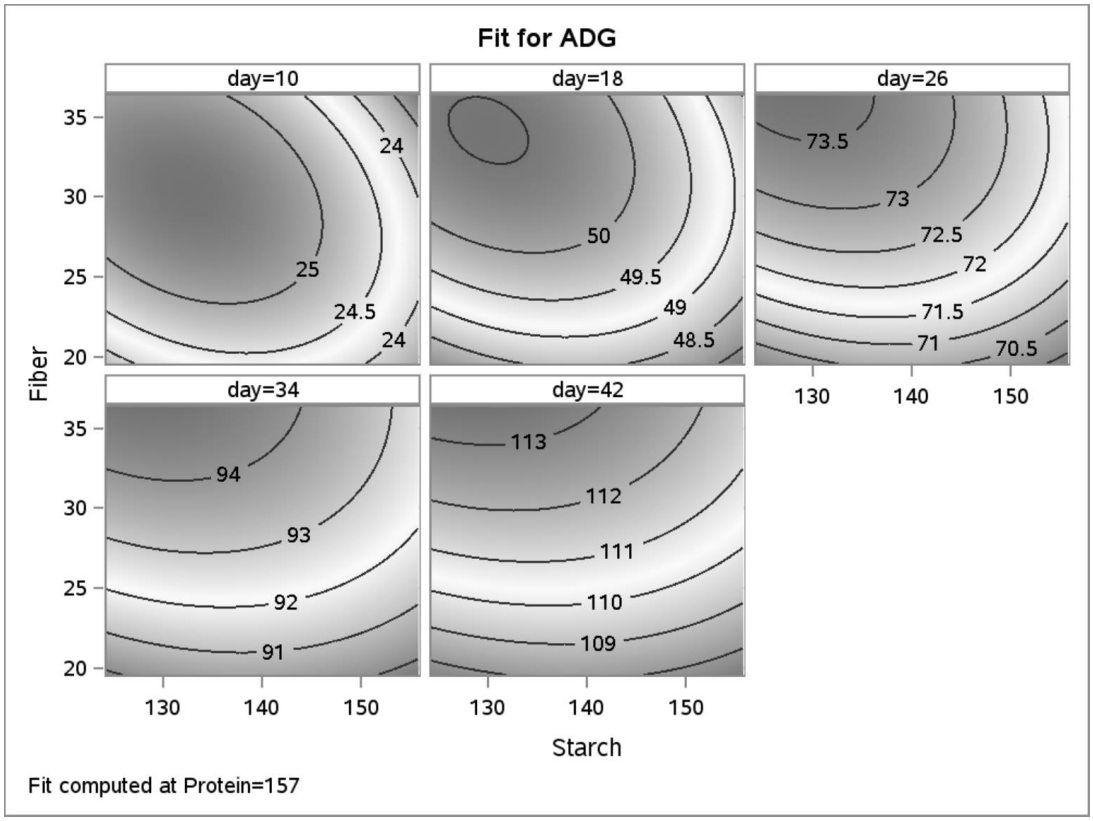

Figure 1 - Influence of fast digestible starch, protein and fibre on ADG in broiler chickens from d 0 – 42 of age.

In conclusion, this study provides further evidence that starch and protein digestion kinetics influence broiler growth performance. Furthermore, dietary fibre and in this case, fast digestible fibre, also play a role in the digestion kinetics of protein and starch as well as energy supply in broiler diets. Results from this study indicate that future research investigating digestion kinetics of protein and starch and the role of dietary fibre in digestion kinetics in broilers is warranted.

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DIGESTION RATES OF STARCH BUT NOT PROTEIN VARY IN COMMON CEREAL GRAINS USED IN BROILER DIETS

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Both glucose and amino acids are essential for muscle protein deposition and feed conversion efficiency. Total tract nitrogen retention was reported to be influenced by protein and starch digestion in broiler chickens (Liu et al., 2013). Embracing the concept of digestive dynamics and applying it in practical diet formulation requires understanding of the variations of protein and starch digestion rates in different ingredients. This present study evaluated protein and starch digestion rates of 18 cereal grains, including sorghum (7), wheat (4), corn (2), barley (3), and triticale (2), in male broiler chickens from 21 – 28 days post-hatch. Experimental diets included the test grain and soybean meal and were formulated to be iso-energetic and iso-nitrogenous but without synthetic amino acids. Grains were hammer-milled through 4.0 mm screen before mixing and cold-pelleting. On day 28, a total of 648 Ross 308 male chicks (6 cages per treatment, 6 birds per cage) were euthanised (intra-venous injection of sodium pentobarbitone), and samples of digesta were taken from the proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI) and distal ileum (DI) and pooled for each cage. The calculations of apparent digestibilities, mean retention time and digestion rates of protein and starch were conducted as described previously (Liu *et al.*, 2013). There were more variations in protein digestibilities in the jejunum than ileum in broiler chickens. The CV of protein digestibilities among different ingredients were 8, 6, 2 and 2 in PJ, DJ, PI and DI, respectively; whereas the CV of starch digestibilities among different ingredients were 7, 7, 6 and 6 in PJ, DJ, PI and DI, respectively (Figure 1). There were no significant differences between protein digestion rates among the 18 cereal grains; however, the starch digestion rates varied by student *t*-test (P = 0.048). On average, wheat had the highest starch digestion rate (0.118 min^{-1}), followed by barley (0.104 min^{-1}), triticale (0.093 min^{-1}), corn (0.087 min^{-1}) and sorghum (0.075 min^{-1}). The synchrony between glucose and amino acid absorption is essential for optimal feed conversion and nutrient utilisation, especially in reduced protein diets with high inclusions of crystalline amino acids. The present study showed the variations in starch digestion rates in different feed grains. Future consideration needs to take into account the differences of protein digestion rates in common protein ingredients.

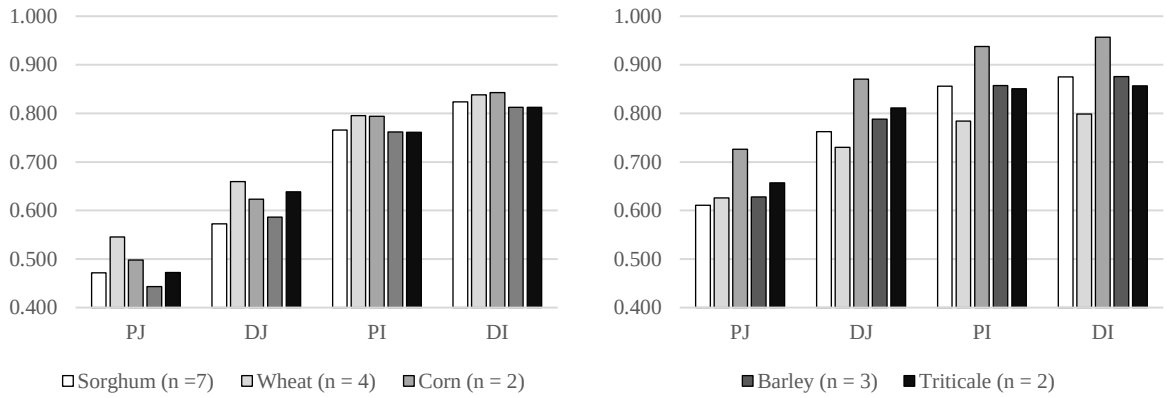

Figure 1 - Apparent digestibility coefficients of protein (left) and starch (right) in broiler chickens at 28 days post-hatch.

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VIEWS ON IDEAL OR LOWER PROTEIN LEVELS FOR MEAT CHICKENS AND APPROACHES TO SECOND TIER AMINO ACIDS

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Summary

The use of feed-grade amino acids (AA) such as DL-Met, L-Lys, and L-Thr, has enabled poultry nutritionists to meet the needs of the three most essential AA while decreasing both crude protein (CP) content and diet cost to produce broilers more efficiently. This strategy allows diets to contain lower inclusion of AA-contributing ingredients, which are known to play a central role in diet cost. Furthermore, lowering dietary CP content can also be advantageous in reducing nitrogen excretion and ammonia emissions. This review will provide an understanding of the role of optimizing Thr, Val, and Ile ratios in reduced CP diets that will allow nutritionists to meet performance objectives.

I. INTRODUCTION

Diet cost represents approximately 60 to 70% of total live production cost of broilers (Donohue and Cunningham, 2009). Price volatility of AA-contributing ingredients may largely impact diet cost and subsequently live production cost. Soybean meal has been considered the "golden standard" intact source of AA due to its excellent AA profile for poultry. Price volatility of soybean meal has created interest in utilizing alternative intact AA sources (canola meal, lupins, meat and bone meal) in conjunction with cereal grains having higher AA content than corn in diet formulation to reduce diet cost. In addition, the importance of accurate digestible AA values for feedstuffs should not be under estimated to ensure optimum performance in meat chickens. The addition of feed-grade AA can provide partial replacement of intact protein sources resulting in decreased CP content and lower dietary cost. When supplementing DL-Met and L-Lys to decrease CP content in diet formulation, less limiting AA concentrations (Thr, Val, Ile, Arg, and Trp) may be lower than diets containing higher CP content resulting in poor growth performance and carcass characteristics of broilers (Corzo et al., 2007). Therefore, maintaining adequate concentrations of these less limiting AA in reduced CP diets may help alleviate poor growth performance of broilers.

II. REDUCED CRUDE PROTEIN DIETS

Despite the benefits from lowering dietary CP content, previous studies have reported sub-optimal growth performance when broilers were provided reduced CP diets (Kerr and Kidd, 1999; Rezaei et al., 2004; Dean et al., 2006; Hernandez et al., 2012). Hernandez et al. (2012) examined the effects of feeding broilers reduced CP diets from 23.0 to 20.0% with increments of 1.5 percentage points from 8 to 21 d of age. These researchers noted that broilers fed the 21.5% CP diet had similar body weight (BW) gain and feed conversion ratio (FCR) to those consuming the 23.0% CP diet. However, broilers consuming the 20.0% CP diet had decreased BW gain and increased FCR by 9.4 and 9.6%, respectively. Similarly, Dean et al. (2006) observed a linear decrease in gain to feed ratio of broilers from 1 to 18 d of age when gradually decreasing dietary CP content by increments of 1.5 percentage points from 22 to 16.2%.

Poor growth performance of broilers fed reduced CP diets may also be observed in older broilers. From 28 to 45 d of age, decreasing CP content in broiler diets from 19.4 to 16.7% reduced average daily gain while also increasing FCR by 2.3 and 3.3%, respectively

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(Kerr and Kidd, 1999). The magnitude of lowering CP content can also be displayed in carcass characteristics of broilers. Rezaei et al. (2004) conducted a 6-wk trial to evaluate the effects of feeding reduced CP diets on growth performance and carcass characteristics of broilers. From 1 to 3 wk of age and 3 to 6 wk of age, the reduced CP diets were formulated to contain 17.8 and 16.1% CP, while the high CP diets were formulated to contain 20.8 and 18.1% CP, respectively. Results demonstrated that BW gain of broilers consuming the reduced CP diets was 5.5% lower compared with birds provided the high CP diets resulting in a 2.4% decrease in carcass weight. Additionally, breast meat yield was decreased and abdominal fat percentage increased by 6.7 and 35.4%, respectively, as dietary CP content was reduced by 3.0 and 2.0 percentage points in broiler diets from 0 to 3 and 3 to 6 wk of age, respectively.

The inclusion of DL-Met, L-Lys, and L-Thr has enabled nutritionists to formulate diets containing lower CP. However, when focusing solely on these AA, less limiting AA concentrations, such as Val, Ile, and Arg may be below optimum concentrations. Insufficient less limiting AA concentrations may produce confounding effects when research aimed at evaluating the effects of lowering dietary CP content on growth performance of broilers is conducted. Prior research demonstrated that the reduction of dietary CP content from 23 to 20% in diets fed to broilers from 7 to 21 d of age resulted in decreased concentrations of Val, Ile, Arg, and Trp by 15, 16, 10, and 18%, respectively, in the 20% CP diet (Pinchasov et al., 1990). Similarly, Waldroup et al. (2005) noted approximately 10% reductions in Val, Ile, Arg, and Trp concentrations when lowering dietary CP content from 22 to 18% leading to an 8.7% increase in FCR of broilers. Adding a mixture of essential AA (Gly, L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu) to the 18% CP diet to obtain similar concentrations in the 22% CP diet resulted in broilers having similar FCR compared with birds fed the 22% CP diet. However, this strategy did not yield similar results when applied to a 20% or 16% CP diet indicating that other factors may influence poor growth performance of broilers (Waldroup et al., 2005). Kriseldi et al. (2018) evaluated reduced CP diets formulated to maintaining adequate AA concentrations on growth performance and nitrogen balance of broilers from 1 to 21 d of age in two experiments. Crystalline AA were added sequentially in the order of limitation (L-Val, Gly, L-Ile, L-Arg, L-Trp, L-His, and L-Phe) in the experimental diets to decrease CP content by approximately 4 percentage points compared with a positive control diet containing DL-Met, L-Lys, and L-Thr. The sequential additions of AA with DL-Met through L-Trp allowed 4.0 and 2.2 percentage point reductions in CP from 1 to 14 and 1 to 21 d of age (Figure 1), respectively, without adversely impacting growth performance compared with a positive control diet containing DL-Met, L-Lys, and L-Thr.

III. DIGESTIBLE THREONINE

Threonine is the 3rd limiting amino acid for broilers fed corn-soybean meal based diets (Baker et al., 2002). The primary functions of threonine include lean meat accretion, feather development, mucin synthesis, and enzyme formation (Kidd and Kerr, 1996, Kidd, 2000, Horn et al., 2009). Ideal AA ratio concept is a popular method to express AA requirements relative to Lys. Utilising L-Thr in concert with ideal AA ratio concept in diet formulation allows nutritionists to meet an optimum digestible Thr to Lys ratio while maintaining cost effective diets. Formulating diets at a digestible Thr to Lys ratio below the broiler's need for Thr limits their genetic potential in meeting performance objectives. Digestible Thr to Lys ratio has been reported to vary among various production periods with ratios ranging from 0.65 to 0.70 (Rostagno et al., 2011, Mehri et al., 2012). In commercial practice, digestible Thr to Lys ratios ranging from 0.63 to 0.68 have been utilised with lower digestible Thr to Lys ratios to reduce feed cost, whereas higher ratios were implemented to optimize growth performance and meat yield.

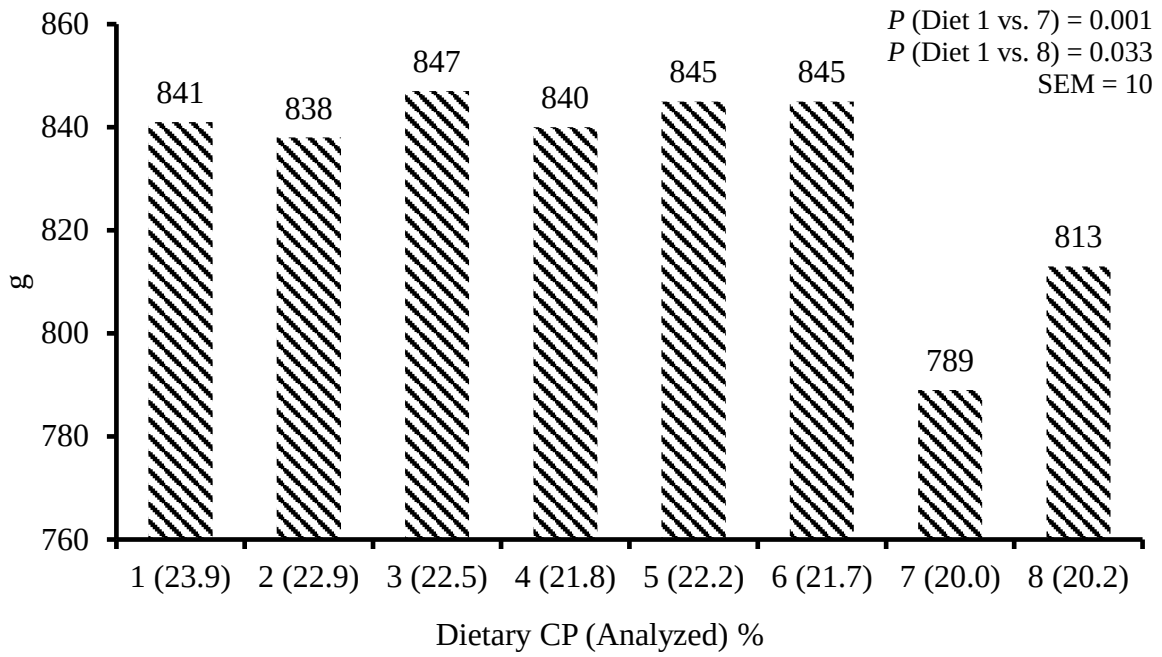

Figure 1 - Effects of sequential addition of limiting amino acids to reduce crude protein on BW gain of broilers from 1 to 21 d of age. Diet 1 was supplemented with DL-Met, L-Lys, and L-Thr; Diet 2: Diet 1 + L-Val; Diet 3: Diet 2 +, Gly, Diet 4: Diet 3 + L-Ile; Diet 5: Diet 4 + L-Arg; Diet 6: Diet 5 + L-Trp; Diet 7: Diet 6 + L-His; Diet 8: Diet 7 + L-Phe. Pre-planned orthogonal contrast was conducted for each dietary treatment vs. Diet 1. Only contrasts with significant differences were shown ($P < 0.05$) (Kriseldi et al., 2018).

Over the last several years, our laboratory has attempted to define the optimum digestible Thr to Lys ratio to optimize growth rate and meat yield in broilers from 1 to 49 d of age through a series of experiments due to the variation across published research and the inconsistent ratios used in commercial practice. Dozier et al. (2015) evaluated digestible Thr to Lys ratio in Hubbard × Cobb 500 male chicks from 1 to 14 d of age. Eight digestible Thr to Lys ratios ranging from 0.55 to 0.76 in increments of 0.03 were fed throughout the experimental period. Using broken-line methodology, digestible Thr to Lys ratios were determined at 0.70 and 0.68, respectively, for BW gain and FCR (Figure 2). Corzo et al. (2009a) determined digestible Lys and Thr requirements simultaneously in Ross × Ross female broilers from 14 to 28 d of age. Digestible Thr to Lys ratios were determined by dividing the Thr requirement by the Lys requirement for each response criterion. Six digestible Thr and Lys concentrations were fed with digestible Thr ranging from 0.46 to 0.86% and digestible Lys concentrations varied from 0.84 to 1.24% in increments of 0.08% for each AA. Digestible Thr requirements ranged from 0.73 to 0.79% and digestible Lys requirements varied from 1.06 to 1.11% depending upon the response criteria, which resulted in digestible Thr to Lys ratios of 0.69 and 0.70, respectively, for BW gain and FCR. Two experiments were conducted to examine growth and meat yield responses of male broilers fed diets varying in digestible Thr to Lys ratio from 21 to 35 (experiment 1) and 35 to 49 (experiment 2) d of age (Dozier et al., 2016). Calculated digestible Thr to Lys ratios ranged from 0.512 to 0.806 in increments of 0.040 (Hubbard × Cobb 500) and 0.552 to 0.793 in increments of 0.035 (Ross × Ross 708). In experiment 1 (from 21 to 35 d of age), optimum digestible Thr to Lys ratios for male Hubbard × Cobb broilers were estimated at 0.68 and 0.67 for BW gain and FCR from 21 to 35 d of age. In experiment 2 (from 35 to 49 d of age), optimum digestible Thr to Lys ratios of Ross × Ross 708 were determined at 0.63 and 0.68 for FCR with linear and quadratic broken-line models, respectively. Meat weights and yields were not affected by the dietary treatments in either experiment. These data indicated that a digestible Thr to Lys ratio of 0.67 to 0.68 optimizes

growth performance of broilers throughout various phases of production. Dietary Thr affects FCR more consistently than meat yield responses. Supplementation of L-Thr can be used effectively to reduce diet cost with the reduction of soybean meal without compromising broiler performance.

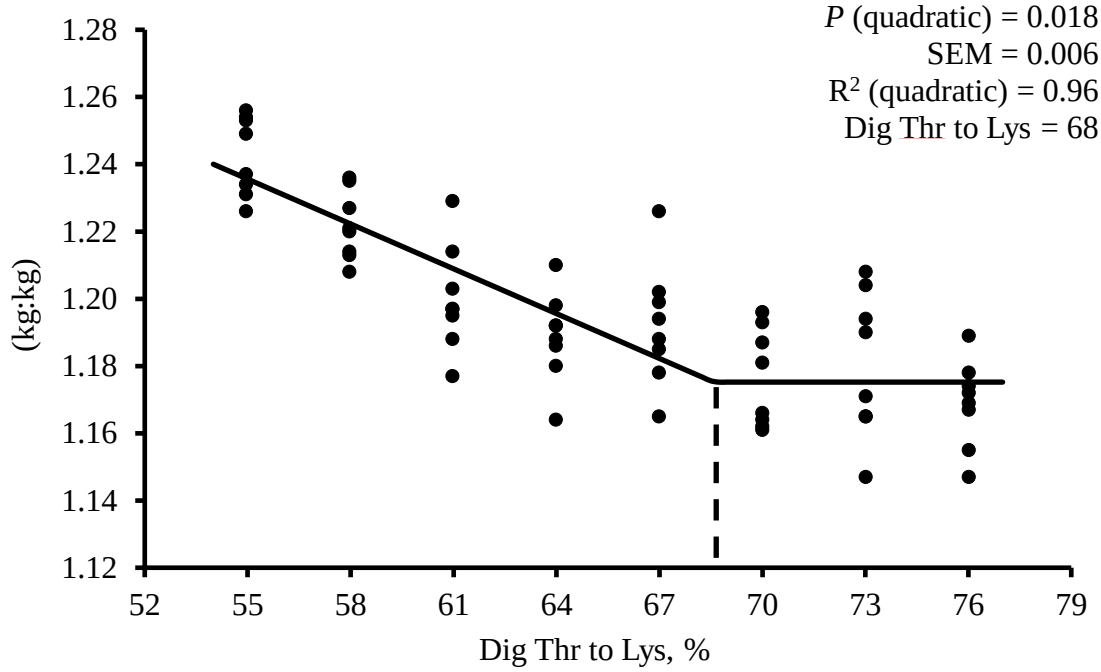

Figure 2 - Effects of digestible Thr to Lys concentrations on feed conversion ratio of broilers from 1 to 14 d of age (Dozier et al., 2015).

IV. DIGESTIBLE VAL

Valine is the 4th limiting AA for broilers fed diets utilising AA-contributing ingredients of vegetable origin (Baker et al., 2002; Thornton et al., 2006; Corzo et al., 2009b). Digestible Val to Lys ratio has been reported to vary among production periods with ratios ranging from 0.74 to 0.78 (Corzo 2007, 2008; Rostagno et al., 2011). Corzo et al. (2011) have reported acceptable performance with the inclusion of L-Val into broiler diets. Price spread among cereal grains, supplemental oil/fat, and protein meals determines the value of L-Val. Kidd and Hackenhaar (2005) reported that Val is the fourth limiting AA with broilers fed corn-soybean meal and wheat-soybean meal based diets utilising formulation scenarios with various ingredients. Growth rate and FCR are more sensitive response criteria than breast meat yield as digestible Val approaches the requirement (Corzo et al., 2007, 2008). Feed-grade production of L-Val has increased due to the demand in swine production and recently it has been entering into some broiler formulations due to the popularity of "all-vegetable" diets in antibiotic-free production. L-Valine has entered into formulation of broiler diets containing ingredients of vegetable origin. Utilizing L-Val in concert with the ideal AA ratio concept in diet formulation allows nutritionists to meet an optimum digestible Val to Lys ratio while decreasing nitrogen excretion and maintaining cost effective diets. Formulating diets at a digestible Val to Lys ratio below the broiler's need for Val limits their genetic potential in meeting performance objectives. Corzo et al. (2011) have reported acceptable performance with the inclusion of L-Val into broiler diets. Corzo et al. (2007) conducted a series of experiments to determine the fourth limiting AA of Ross × Ross 708 male broilers fed diets containing ingredients of vegetable origin and to delineate the digestible Val to Lys ratio from 21 to 42 d of age. In order

to confirm that Val is the fourth limiting AA of diets consisting of ingredients of vegetable origin, corn-soybean meal diets were formulated to be marginal in dietary Ile, Val, Arg, and Gly plus Ser. Supplementing Val to the negative control diet (marginal in Val, Ile, Arg, and Gly) led to similar BW gain of broilers fed the positive control diet, but adding Ile, Arg, or Gly to the negative control diet had lower ($P \leq 0.05$) BW gain compared with the birds fed the positive control diet. These results indicated that Val is the fourth limited AA for broilers fed diets containing corn and soybean meal. In a subsequent study, corn-peanut meal negative control diet was formulated to be adequate in all AA with the exception of Val (0.59% digestible Val).

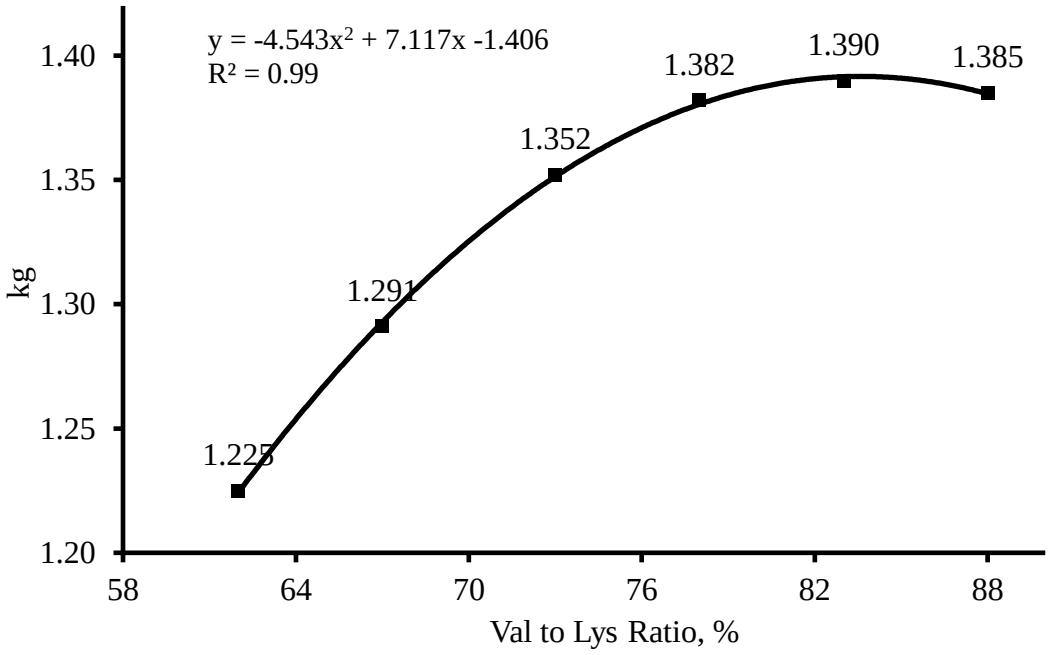

Figure 3 - Effects of dietary Val to Lys ratio on body weight gain of broilers from 21 to 42 d of age (Corzo et al., 2007).

L-Valine was added to the negative control diet to achieve six digestible Val treatments ranging from 0.59 to 0.84% in 0.06% increments. Digestible Val to Lys ratio was determined to be 0.78, 0.77, and 0.74 for BW gain (Figure 3), total breast meat weight, and total breast meat yield. An optimum ratio was not determined for feed conversion ratio. Corzo et al. (2008) evaluated dietary Val requirements (total) of Ross × Ross male broilers with three experiments from 0 to 14, 14 to 28, and 28 to 42 d of age and determined the requirements to be 1.00, 0.95, and 0.85%, respectively. Tavernari et al. (2013) examined the digestible Val to Lys ratio in Cobb 500 male broilers from 8 to 21 and 30 to 43 d of age in two independent experiments. In each experiment, seven experimental diets were provided to broilers that consisted of six diets ranging in digestible Val to Lys ratio (0.69 to 0.84 – 8 to 21 d of age; 0.70 to 0.85 – 30 to 43 d of age) and a positive control diet. Optimum Val to Lys ratio for BW gain and FCR were determined to be 0.77 and 0.75 and 0.75 and 0.77 from 8 to 21 and 30 to 43 d of age, respectively. Moreover, Berres et al. (2011) ascertained the digestible Val requirement of Cobb × Cobb 500 male broilers from 21 to 42 d of age. Seven digestible Val concentrations were fed ranging from 0.71 to 0.97% in increments of 0.04-0.05%. Based on broken-line methodology, digestible Val requirements were estimated at 0.82 and 0.81%, respectively, for BW gain and FCR. No dietary treatment differences were observed for carcass and breast meat yields. Data from these experiments indicate that the digestible Val to Lys ratio of 0.77 to 0.78 optimizes

growth performance and meat yield of broilers. Feed-grade Val can be added to diets approximating 0.52 kg/ton without any adverse response in growth performance and breast meat yield of broilers (Corzo et al., 2011). Increasing L-Val supplementation at or beyond 0.78 kg/ton resulted in poor growth performance and meat yield. Optimum ratios of Ile, Arg, and Trp were not maintained purposely to determine the maximum inclusion of L-Val without supplementing these AA. Breast meat yield was adversely affected at L-Val supplementation of 1.04 and 1.30 kg/ton compared with the control-fed birds and this reduction in breast meat yield may be related to a decrease in the Ile ratio with the highest inclusion rates of L-Val.

V. DIGESTIBLE ISOLEUCINE

Dietary Ile is considered the fifth limiting AA in diets containing ingredients from vegetable origin. The addition of poultry meal or a meat blend product to the diet can result in Ile becoming fourth limiting. Dozier et al. (2011) determined that Val and Ile become co-limiting for broilers fed diets containing animal protein meals from 2.5% inclusion. With animal protein meal inclusion exceeding 5% of the diet, Ile may become the fourth limiting AA. With L-Val being available for diet formulation, digestible Ile becomes the pressure point in least-cost formulation to optimize performance objectives. Kidd et al. (2000) determined that feeding broilers diets containing a 10% reduction of the Ile requirement (NRC, 1994) resulted in a 0.46% reduction in breast meat yield compared with birds fed diets formulated to contain Ile at 100% of NRC 1994 recommendations. Dozier et al. (2012) examined interactive effects of digestible Val and Ile ratios to Lys on growth performance and meat yields of broilers from 28 to 42 d of age. Increasing digestible Ile to Lys ratio from 0.63 to 0.73 led to a 0.4% higher total breast meat yield (21.6 vs. 22.0%). Hence, it is important to employ an adequate dietary Ile concentration in least-cost formulation to optimize meat yields. de Castro Tavernari et al. (2012) evaluated digestible Ile to Lys ratio of Cobb male broilers from 7 to 21 and 30 to 43 d of age in two experiments. Optimum digestible ratios were estimated using linear response plateau and quadratic responses with a regression analysis. Digestible Ile ratio estimates varied due to response criterion and statistical analysis. Overall, digestible Ile ratios were estimated at 0.66 and 0.68, respectively, for broilers from 7 to 21 and 30 to 43 d of age. Digestible Ile ratios determined from the quadratic responses ranged from 0.68 to 0.70 and 0.72 to 0.75, respectively, from 7 to 21 and 30 to 43 d of age. Rostagno et al. (2011) has reported the digestible Ile to Lys ratio as 0.67 and 0.68, respectively, for broilers from 1 to 21 and 22 to 42 d of age for optimum growth performance. In addition, Kidd et al. (2004) determined the total Ile requirements to vary between 0.67 to 0.71%, 0.64 to 0.66%, and 0.55 to 0.66%, respectively, from 18 to 30, 30 to 42, and 42 to 56 d of age. The variation in the Ile requirement was due to response criteria of interest (BW gain, FCR, and carcass characteristics). Maintaining an adequate Ile minimum in diet formulation is needed to optimize growth performance and meat yield of broilers.

VI. CONCLUSIONS

Feeding reduced CP diets can achieve acceptable performance when formulating to optimum ratios of digestible Thr (0.67-0.68), Val (0.76-0.78), and Ile (0.67-0.69). Ingredient composition and price spread among cereal grains and oilseed meals along with oil/fat prices will dictate the cost savings with using feed-grade L-Thr and Val in diet formulations. Further reductions in CP can be achieved beyond using L-Val if essential AA concentrations are maintained. Digestible Lys specifications and the associated AA ratios will determine the amount of reduction in CP that can be achieved without compromising growth performance. However, Gly may be semi-essential with low CP diets fed during the starter period and may be considered when feeding diets consisting of ingredients from vegetable origin.

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PERFORMANCE AND INTESTINAL PERMEABILITY OF BROILERS FED LOW PROTEIN DIETS SUPPLEMENTED WITH GLYCINE, GLUTAMINE OR ARGININE

R. BAREKATAIN[1,2], P.V. CHRYSTAL[3], K. CHOUSALKAR[2] and S. GILANI[2]

A previous study highlighted differences between a low protein (LP) diet and a higher concentration of amino acids (AA) for intestinal permeability and performance (Barekatain et al. 2018). It is hypothesised that individual AA such as glycine (Gly), glutamine (Gln) and arginine (Arg) may improve gut health and barrier function of birds fed LP diets. To test this hypothesis in a broiler study, two basal diets were prepared as follows: 1) a standard diet (SD) containing 225 g/kg crude protein (CP) in grower and 202 g/kg CP in finisher and 2) a LP diet containing 202 g/kg and 176 g/kg CP in grower and finisher diets. The LP diets were supplemented with synthetic AA to match the SD. The SD was based on wheat, sorghum, soybean meal and meat and bone meal. Tested AA were added to the LP diet to make five treatments. Gln and Gly were added at 10 g/kg while Arg was added at 5 g/kg. For general performance, male off-sex Ross 308 broilers (n=300) were assigned to grower (d 7-21) and finisher diets (d 22-35) each replicated six times. To evaluate the role of tested AA on gut permeability, an additional 96 birds receiving LP diets supplemented with Gln, Gly and Arg were transferred to individual metabolism cages on day 13. This part of the study comprised a 4 x 2 factorial arrangement with factors being LP diets and injection of dexamethasone (DEX), a synthetic glucocorticoid, to induce 'leaky gut'. The injections were given at 0.5 mg/kg on d 14, 16, 18 and 20 to half of the birds (12 birds per diet). The fluorescein isothiocyanate dextran (FITC-d) method was utilized as the gut permeability test on d 21. Birds on the LP diet consumed more feed and had a higher weight gain (WG) compared to SD (Table 1). Adding Gln to LP diet reduced feed intake and WG to the level observed for the standard diet with no positive effect on feed conversion ratio (FCR). Birds fed the Gly diet had similar WG to the LP diet. Additional Arg improved FCR compared to both standard and LP diets. There was a tendency ($P = 0.086$) for Arg and Gly diets to reduce the FITC-d concentration in serum which suggests a lower gut permeability associated with Arg treatment followed by Gly fed birds (data not shown). DEX independently increased FITC-d concentration in serum for all dietary treatments ($P < 0.001$). To conclude, results show that LP diets can improve bird performance, with Arg and Gly being beneficial to both performance and intestinal barrier of broilers.

Table 1 - Growth performance of broilers from 7 to 35 days of age.

	Feed intake	Weight gain (g/bird)	FCR
Treatments	d 7-35	d 7-35	d 7-35
Standard diet	3224^{b}	2122^{c}	1.527^{a}
Low protein	3500^{a}	2302^{a}	1.521^{a}
Low protein + 10 g/kg Gln	3262^{b}	2137^{bc}	1.519^{a}
Low protein + 10 g/kg Gly	3402^{ab}	2254^{abc}	1.510^{ab}
Low protein + 5 g/kg Arg	3375^{ab}	2274^{ab}	1.485^{b}
SEM	27.17	20.14	0.0039
P value	0.0443	0.0435	0.0389

^{a,b,c} Means in a column not sharing a same superscript differ significantly.

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LOW PROTEIN DIETS DOWNREGULATE HEPATIC ENZYMES RESPONSIBLE FOR NONESSENTIAL AMINO ACID SYNTHESIS IN BROILERS

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Low protein (LP) diets have clear benefits for the poultry industry with regards to health, welfare and production. Dean et al., (2006) found that supplementing LP diets with glycine (Gly) and essential amino acids (AAs) can maintain performance similar to that seen on a standard protein (SP) diet. Further research into this concept proposes that supplementation of Gly precursors such as threonine (Thr) and serine (Ser) can overcome Gly deficiency in LP diets. Enzymes enabling these conversions include threonine dehydrogenase (TDH) and glycine C-acetyltransferase (GLYA) which convert Thr to Gly, and serine hydroxymethyltransferase (SHMT) which is responsible for the interconversion of Ser and Gly. The aim of this study was therefore to investigate the regulation of these enzymes under different concentrations of Gly, Ser and Thr in LP diets. Eight dietary treatments were investigated that included a SP diet (227 g/kg crude protein (CP)) and seven LP diets (196 g/kg CP) with Gly, Ser or Thr supplemented at two levels (156 and 180 g/kg Gly equivalence). Day-old off-sex male Ross 308 chicks (n = 528) were fed a common starter diet *ad libitum* containing wheat, sorghum, soybean meal and meat and bone meal from d 0 to 7 and grower treatments until d 21. On d 7, chicks of were allocated into 48 pens of equal weight, resulting in six replicate pens per treatment of 11 chicks. Essential AAs were supplemented when considered limiting using AMINOChick®2.0 software. Due to their involvement in Gly synthesis and degradation, the expression of mRNA for genes encoding hepatic enzymes; TDH, GLYA, SHMT, glycine decarboxylase (GDC) and xanthine dehydrogenase (XDH) was determined in liver tissues on d 21 by real-time quantitative PCR. The geNorm module in qBase+ software was employed to determine the two most stable genes; glyceraldehyde 3-phosphate dehydrogenase and tyrosine 3-monooxygenase, (Hellemans et al., 2007) to normalise the hepatic target genes. The output data were subjected to one-way ANOVA with LSD test at the level of $P < 0.05$ using SPSS statistics version 22. Reducing CP by 30 g/kg downregulated mRNA expression of TDH, GLYA, and XDH in the liver ($P < 0.05$). GDC mRNA showed a tendency ($P = 0.054$) for downregulation by LP treatments, however, no difference was seen between the LP treatment with 180 g/kg Gly and the SP treatment, indicating that the dietary Gly level was adequate at this age. No differential expression of SHMT was seen between treatments. The increase in dietary Thr did not affect the expression of TDH or GLYA. Higher dietary Thr (17.5 g/kg) upregulated the expression of XDH ($P = 0.032$), suggesting an increase in nitrogen excretion. Reducing CP by 30 g/kg reduced the expression of enzymes involved in Thr metabolism, regardless of dietary Thr level. Expression of SHMT was not effected by dietary CP but was downregulated with Ser supplementation. Due to a reduction of hepatic enzyme expression in LP diets, *in vivo* reactions theoretically cannot meet Gly requirements when supplementing with the precursors Ser and Thr.

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THE IMPACT OF DIETARY ELECTROLYTE BALANCE ON MALE BROILER PERFORMANCE OFFERED REDUCED CRUDE PROTEIN DIETS

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Summary

Broiler chickens aged from 14 to 35 days post-hatch were offered iso-energetic diets formulated to crude protein levels of 200, 188, 172 and 156 g/kg. These diets were formulated to a constant dietary electrolyte balance of 230 mEq/ kg. An additional fifth diet was formulated to contain 156 g/kg crude protein and a dietary electrolyte balance of 120 mEq/kg. FCR linearly increased from 1.495 to 1.625 as dietary CP declined. In addition, relative fat-pad weights also increased in a linear fashion from 7.26 to 12.30 g/kg of body weight and water to feed intake ratios declined from 2.15 to 1.85. At the lowest crude protein level of 156 g/kg, there were no differences in any of the performance parameters measured when dietary electrolyte balance was reduced and these findings are discussed.

I. INTRODUCTION

Dietary electrolyte balance (DEB) is defined by the interrelationship between sodium (Na^+), potassium (K^+) and chloride (Cl^-) where DEB = Na^++K^+-Cl^- as mEq/kg and it plays an influential role in homeostasis of the body fluids. Borges et al. (2004) investigated DEB in two experiments and concluded that there was a quadratic effect on weight gain and feed conversion efficiency (FCE) when the DEB was increased by the supplementation of Na^+ alone. Feed intake was maximised at a DEB of 264 mEq/kg, when Na^+ level was increased in the diet and, 213 mEq/kg when both K^+ and Na^+ levels were increased in the diet. These authors reported that the ideal DEB, obtained by the manipulation of both the Na^+ and Cl^- levels, was between 202 and 235 mEq/kg. In more recent work, Borges et al. (2011) reported a wide range of acceptable DEB's in the literature; from 200 to 350 mEq/kg, depending on environmental temperature, humidity and other factors and have suggested that the optimum DEB is around 250 mEq/kg of feed.

Teeter and Belay, (1996) reported that the maintenance of blood pH and CO_2 levels were critical to growth rate in broilers and noted that the buffering systems within the bird ensure that pH is usually maintained at optimal physiological levels. However, these authors added that in extreme conditions, when demand for electrolytes is elevated, maintaining buffering capacity may have an adverse effect on other physiological conditions. In broiler chickens, an electrolyte imbalance is known to affect the metabolism of a number of the essential amino acids, particularly arginine and lysine (Kim et al., 1989; Riley and Austic, 1989). In earlier work, Austic and Calvert (1981) concluded that lysine, arginine, glutamic acid and glutamine play an important role in the regulation of acid-base balance.

In reduced crude protein (CP) broiler diets, DEB is often overlooked or ignored. A reduction of CP in diets based on maize (or wheat) and soyabean meal, reduces dietary levels of K^+ due to a reduction of soyabean meal inclusion and may also increase Cl^- level from synthetic sources of AA's (Borges et al., 2011; Lambert and Corrent, 2018). The combination of lower CP and lower K^+ reduced water intake by 1.4%, decreased the water to feed intake

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ratio and lead to a reduction of litter moisture by 2.2% per CP percentage point (Lambert and Corrent, 2018). In recent work by Belloir et al. (2017), DEB was not considered but, by calculating DEB from the ingredients used in this study, the DEB declined in a linear manner from 209 mEq/kg, in the 190 g/kg CP diet, to 127 mEq/kg in the 150 g/kg CP diet and may partly explain why the diets below 170 g/kg CP had a significantly worse FCR ($P < 0.01$).

In Murakami et al. (2003), the effect of DEB on chick performance in reduced CP diets supplemented with free amino acids was investigated. A non-significant linear response in weight gain (g; $r = 0.96$) and feed intake (g; $r = 0.98$) was observed in broilers aged from 1 to 21 days post-hatch when DEB was increased from 200 to 320 mEq/kg. However, a significant ($P < 0.05$) negative linear relationship ($r = -0.89$) was observed for FCR over the range of DEB tested in these broilers. In broilers aged 21 to 42 days post-hatch, these authors observed significant ($P < 0.05$) quadratic responses to DEB for body weight gain (g; $r = 0.89$) feed intake (g; $r = 0.64$) and FCR ($r = -0.95$). The reported optimum DEB levels for weight gain and feed intake were 230 and 270 mEq/kg and the best FCR was achieved at 168.9 g/kg CP and 245 mEq/kg. Therefore, the purpose of this paper is to report on the effect of DEB using sodium chloride, sodium bicarbonate and potassium carbonate to either maintain DEB at a set level or allow it to decline in reduced CP diets.

II. METHODOLOGY

As part of a larger feeding study, a total of 210 male, off-sex, Ross 308 chickens were offered maize-soy diets formulated to contain 200, 188, 172 and 156 g/kg CP and DEB of 230 mEq/kg from 14 to 35 days post-hatch with an additional diet of 156 g/kg CP allowing DEB to reduce to 120 mEq/kg (Table 1). Diets were formulated to iso-energetic levels of 12.85 MJ/kg (apparent metabolisable energy) and standardised ileal digestible (SID) lysine of 11 g/kg. Essential SID amino acid (AA) ratios were maintained across all diets using recommendations from AMINODat 5.0, Platinum (Evonik Industries, Germany). Each dietary treatment was offered to 7 replicate cages of 6 birds per cage. Broiler growth performance, feed intake, relative abdominal fat-pad weights and water to feed ratios were recorded. The experimental data was analysed via the JMP 13 Statistics program (SAS Institute). The conduct of the feeding study fully complied with specific guidelines (2016/973) approved by the Animal Ethics Committee of the University of Sydney.

III. RESULTS

Overall weight gains from 14 to 35 days were 1902 g with an average feed intake of 2951 g and an average FCR of 1.552. The Ross 308 standards (2014) for male broilers for the same ages are 1795 g/bird for weight gain, 2965 g/bird for feed intake and an FCR of 1.652. The results achieved therefore exceeded the breed standards by 6.0% for weight gain and 6.1 % for FCR (Table 2). Reducing dietary CP had no effect on weight gain whilst feed intake increased in a linear manner ($r = -0.156$; $P = 0.018$). Consequently FCR worsened as dietary CP reduced with significantly ($P < 0.001$) inferior FCR at the lowest dietary CP levels (treatments 4D and 5E). Water intake and feed intake were recorded between days 31 to 33 post-hatch (data not shown) and there was a non-significant ($P = 0.160$) trend to decreasing water intake as CP declined. However, combined with a significant increase in feed intake over this period ($P = 0.034$) the water to feed intake ratio (water:feed) decreased significantly as protein declined, in a linear manner ($r = 0.444$; $P < 0.001$). Excreta dry matter content ranged from a minimum of 184.9 g/kg in treatment 2B to a maximum of 201.2 in treatment 3C and was not significant ($P = 0.390$) suggesting that the volume of excreta output declines as dietary CP reduces. A reduction in DEB between the lowest CP diets (treatments 4D and 5E) had no impact on all of the performance parameters and relative fat-pad weights measured (Table 2).

Table 1 - Composition of dietary treatments 1A to 5E.

Diet Feed ingredient (g/kg)	1A	2B	3C	4D	5E
Maize	560	602	659	718	727
Soybean meal	329	289	233	171	170
Vegetable oil	49.7	42.7	32.8	22.4	19.4
Lysine HCl	1.622	2.850	4.558	6.454	6.476
Methionine	2.897	3.249	3.742	4.296	4.288
Threonine	0.974	1.533	2.311	3.178	3.181
Tryptophan	-	-	0.202	0.533	0.537
Valine	0.673	1.364	2.326	3.400	3.401
Arginine	-	0.454	2.080	3.886	3.903
Isoleucine	0.235	0.930	1.898	2.974	2.982
Leucine	-	-	-	1.239	1.220
Histidine	-	-	-	0.319	0.317
Sodium chloride	4.009	2.426	0.222	-	4.253
Sodium bicarbonate	0.010	2.401	5.730	6.187	-
Potassium carbonate	-	-	-	2.615	-
Limestone	7.25	7.17	7.06	6.93	6.94
Dicalcium phosphate	20.29	20.91	21.77	22.75	22.73
Choline chloride (60%)	0.900	0.900	0.900	0.900	0.900
Celite	20.0	20.0	20.0	20.0	20.0
Vitamin-mineral premix	2.00	2.00	2.00	2.00	2.00
Formulated Levels					
Protein (N x 6.25, g/kg)	200	188	172	156	156
DEB (mEq/kg)	230	230	230	230	120
Na^+ (g/kg)	18.0	18.0	18.0	18.0	18.0
K^+ (g/kg)	9.30	8.53	7.45	7.72	6.24
Cl^- (g/kg)	3.06	2.35	1.37	1.62	4.18

Table 2 - Effect of dietary treatments on growth performance and relative abdominal fat-pad weights in male broilers from 14 to 35 days post-hatch.

Treatment (analysed CP g/kg)	Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Water: Feed	Relative fat-pad weight (g/kg)
1A (204)	1934	2888	1.495[a]	2.150[a]	7.26[a]
2B (183)	1931	2896	1.500[a]	2.175[a]	8.49[a]
3C (174)	1912	2907	1.522[a]	1.911[b]	10.13[b]
4D (157)	1864	3036	1.629[b]	1.881[b]	12.40[c]
5E (156)	1869	3027	1.621[b]	1.820[b]	12.21[c]
SEM	40.48	55.32	0.0181	0.0824	0.4955
Significance	P = 0.603	P = 0.069	P < 0.001	P <0.001	P < 0.001
LSD (P < 0.05)	-	-	0.0733	0.161	1.430
Linear effect	r = 0.077 P = 0.106	r = -0.156 P = 0.018	r = -0.546 P < 0.001	r = 0.444 P<0.001	r = -0.732 P < 0.001

[a,b,c,d] Means within a column not sharing a common superscript are significantly different at the value shown in the table.

IV. DISCUSSION

Whilst, in the context of normal CP diets, the range of acceptable DEB levels reported in the literature are wide, few studies have investigated the effect of DEB in reduced CP diets. In the

present study a reduced CP diet with a low DEB was included as a single treatment to determine if there was any effect of DEB on broiler performance in broilers offered reduced dietary CP. In reduced CP diets with large amounts of supplemented crystalline AA's, the current study was unable to demonstrate any effect of DEB and this may be partly due to better AA balance in modern broiler diets and the source of supplemental Na^+ and K^+ as salts rather than organic acids, which is in agreement with Austic and Calvert (1981), who also observed no response when salts of these ions were used (as chloride and/or sulphate). This suggests that the molecular structure of the electrolyte should also be considered in DEB studies and that the source of electrolyte ions plays an important role. The confounding effect of changing dietary pH with the use of metabolisable organic acids and the use of hydrochloric acid to adjust Cl^- levels on broiler performance was not considered in the report from these authors but cannot be ignored.

In Murakami et al. (2003), the lack of significant responses for weight gain and feed intake in broilers aged 1 to 21 days post-hatch is at odds with the excellent regression coefficients for these parameters in this study. In addition, potassium chloride (KCl) was used to adjust the DEB as soyabean meal was reduced and, replacing the K^+ in this manner leads to high Cl^- levels and concomitantly low Na^+ levels in many of the test diets. An excess of Cl^- and/or diets deficient in Na^+ may lead to erroneous conclusions regarding the influence of DEB in reduced CP diets.

Belloir et al. (2017), concluded that a reduction of the dietary CP content by several CP percentage points is possible in growing-finishing broilers, without consideration of DEB with positive implications for the sustainability of broiler production. From the present study, it can be concluded that a reduction in DEB below the reported optimum levels is not a limiting factor in reduced CP diets supplemented with crystalline EAA's. However, it is prudent to maintain DEB levels at accepted optimum levels of 225 to 250 mEq/kg for future broiler trials with reduced CP diets.

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ECONOMICS OF LOW PROTEIN BROILER DIETS: A FORMULATION EXERCISE

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Summary

This paper investigates the costs of wheat-based broiler diets with twenty g/kg lower protein than current through a formulation exercise. In the context of this exercise, this means protein of 187, 176 and 170 g/kg for grower, finisher 1 and finisher 2, respectively. Current prices of valine, arginine and isoleucine would allow these lower protein diets to be formulated at $10-12/t higher priced than their unrestricted protein comparisons. It was found that valine, arginine and isoleucine prices would need to be reduced by around 50% to be able to formulate these lower protein diets at costs similar to the high protein ones. The effects of formulating and producing lower protein diets would mean large increases in lysine, methionine and threonine usage; the use of valine, arginine and isoleucine, higher levels of wheat, lower levels of added oil, and lower levels of soybean meal. It is suggested that, at some point commercial broiler companies may try these lower protein diets to investigate potential benefits on performance, litter quality and odour remediation, starting with finisher 2.

I. INTRODUCTION

Papers have been presented at the past two APSS meetings on the subject of low protein diets (Kidd and Choct, 2017; Hilliar and Swick, 2018; Lambert and Corrent, 2018). Few, if any, have taken note of the economics of such diets. This paper is an examination of the costs of low protein diets. A brief history of amino acids is worth reviewing. Each time a limiting amino acid became available commercially, and was used, the protein level of broiler diets was reduced. Methionine and its hydroxy analogue were the first amino acid sources to be made commercially available in the late 1950's (Gordon and Sizer, 1955). If broiler diets are formulated with the methionine and methionine + cysteine requirements used today, but without D,L-methionine, it would result in protein levels of about 28%! Lysine became commercially available in the 1960s (Waldroup and Harms, 1963) and threonine in the 1980s (Edmonds et al., 1985). In the 2000s valine, arginine and isoleucine became commercially available. However due to cost, only small amounts are used. Benefits for lower protein diets are suggested as reduced water consumption, drier litter, better foot pad health, environmental benefits and improved FCR (Garland, 2018) and lower odour production (Sharma et al., 2016). Excess protein may be associated with necrotic enteritis (Drew et al., 2004).

Each time a new amino acid became available and was used, the level of soybean meal has been reduced. Essentially, what is happening is that soybean meal is replaced with amino acids. This will continue to be the case when we find ways to use valine, arginine and isoleucine. This exercise has been done with wheat-based diets for Australia. If this exercise was done in the USA or other locations using corn-soy diets, the numbers would be quite different, but the conclusions would probably be similar. Which amino acids are available? The amino acids LMT (lysine, methionine and threonine) are widely used in Australian broiler diets. They are priced in the range of $2-4/kg. Small amounts of valine and glycine are also being used. Demand for valine in the market has increased since June 2018. Other amino acids that are available but not widely used in broiler diets due to price are valine, arginine and isoleucine (VAI). Prices used for these amino acids in this exercise are $8.5, 13.3 and 18.9/kg respectively. These are prices in the Australian marketplace over the past 12 months.

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Tryptophan does not appear to be limiting in wheat-based diets, and as such is not considered in this exercise. However, in corn-based diets seeking a 30 g/kg reduction in protein, crystalline tryptophan would be required.

There is a reasonable knowledge of the absolute requirements for lysine, methionine, methionine + cysteine, tryptophan, threonine, arginine, isoleucine and valine. Requirements for amino acids other than lysine are normally calculated based on the ideal protein ratio (Baker and Han, 1994). For this exercise, the 2018 recommendations for Cobb 500 have been used.
The requirement for the "non-essential" amino acid glycine is not clear. Requirements for glycine and glycine + serine are not well understood. Several researchers have shown improvements in broiler performance with added glycine in lower protein diets not containing meat and bone meal (MBM) (Hilliar et al., 2017; Dean et al., 2006). MBM is high in glycine, and it is unlikely that diets containing MBM would need added glycine. Should MBM not be used, the inclusion of 500 grams- 1 kg glycine/t might be advisable. At less than $3/kg, this would not be expensive.

II. FORMULATION EXERCISE

A least cost formulation exercise was conducted, using wheat-based diets, with MBM, canola meal and soybean meal as the protein sources, and tallow as the supplemental oil. Ingredient prices (AUD) for June 2018, for Eastern Australia were used. Xylanase and phytase enzymes were used with recommended nutrient matrices. Formulations are based on Cobb 500 recommendations for SID amino acids (Cobb-Vantress 2018). Diets are shown in Table 1.
The starter diet was not included in this exercise since this diet makes up only about 10% of total feed used. The starter diet may be a special case where increased levels of high quality protein may be advantageous to growth and have little potential issues on litter quality, health, odour production and/or economics.

Table 1 - Ideal protein digestible amino acid specifications used for diets (Cobb-Vantress 2018).

Dig lysine, g/kg	Starter 12.2	Grower 11.2	Finisher 1 10.2	Finisher 2 9.7
Lysine,%	100	100	100	100
Methionine, %	38	40	41	41
M+C, %	75	76	78	78
Tryptophan, %	16	16	18	18
Threonine, %	68	65	65	65
Arginine, %	105	105	105	105
Isoleucine, %	63	64	65	66
Valine, %	73	75	75	75

The formulations were established without any protein restrictions. Note the protein levels are 207, 196 and 190 g/kg respectively as shown in Table 2. Then protein was restricted by 20 g/kg, to 187, 176 and 170 g/kg in grower, finisher 1 and finisher 2 respectively. Protein restriction was achieved by placing a number in the "protein maximum" column in the formulation program, for example 187 g/kg for the grower diet. Formulations were done by 3 methods: 1) No protein restrictions, 2) Protein restricted to 20 g/kg lower than 1. 3) Protein restricted as in method 2, but with prices of arginine, valine and isoleucine reduced by 50%.

III. RESULTS

Results of the formulation exercise are shown in Table 2 and summarised in Table 3. Grower diets are $468.63/t without protein restriction, $480.99/t with protein restriction and current

VAI prices, and $467.10/t with VAI at 50% of current prices. Finisher 1 diets are $450.17/t without protein restriction, $462.03/t with protein restriction and current VAI prices, and $448.43/t with protein restriction and VAI at 50% of current prices. Finisher 2 diets are $441.63/t without protein restriction, $451.80/t with protein restriction and current VAI prices, and $439.21/t with protein restriction and VAI at 50% of current prices. This suggests the protein reduced diets will cost $10-12/t more with current VAI prices. Costs of VAI would need to come down by 50% of current prices, for the lower protein diets to be of equal price to the higher protein ones.

Table 2 - Broiler diet formulations (g/kg unless otherwise noted).

Ingredients	Cost,	Grower			Finisher 1			Finisher 2		
	$/t	1	2	3	1	2	3	1	2	3
Wheat 11	360	675.6	719.5	736.5	687.5	731.0	747.8	709.7	752.4	768.6
SBM Arg	720	195	172	136	132	110	74	113	92	56
MBM 50	500	30	0	30	29	0	30	29	0	30
Canola ml sol.	425	75	75	75	120	120	120	120	120	120
Tallow	700	13	10	4	23	20	14	20	17	12
Salt	260	1.3	1.8	1.3	0.5	0.9	0.5	0.5	0.9	0.4
Na bicarb	560	1	1	1	1	1	1	1	1	1
Limestone	75	1	8	2	0	6	0	0	6	0
L-lysine HCL	1690	2.9	4.3	4.7	2.8	4.1	4.5	2.70	4.0	4.3
D,L-met	3660	1.9	2.2	2.4	1.5	1.7	1.9	1.30	1.5	1.6
L-thr	2190	0.7	1.4	1.5	0.4	1.1	1.2	0.40	1.0	1.1
L-valine	8500	0	0.6	0.7	0	0.2	0.3	0	0.2	0.3
L-arginine	13300	0	1.3	1.6	0	1.2	1.6	0	1.2	1.5
L-ile	18900	0	0.1	0.5	0	0.3	0.6	0	0.2	0.6
Choline Cl 60	1440	0.4	0.6	0.6	0.1	0.3	0.4	0.2	0.4	0.4
Xylanase [1]	20000	0.1	0.1	0.1	0.1	0.1	0.1	0.10	0.1	0.1
Phytase [2]	21000	0.1	0.1	0.1	0.1	0.1	0.1	0.10	0.1	0.1
Vitamins, TM	5000	2	2	2	2	2	2	2	2	2
Total, kg		1000	1000	1000	1000	1000	1000	1000	1000	1000
Total cost, $/t		468.63	480.9	467.10	450.17	462.03	448.43	441.63	451.80	439.21
Protein, %		20.7	18.7	18.7	19.6	17.6	17.6	19.0	17.0	17.0
Nutrients										
ME, MJ/kg		12.76	12.76	12.76	12.97	12.97	12.97	12.97	12.97	12.97
Protein, g/kg		207	187	187	196	196	196	190	170	170
SID, g/kg										
Lysine		11.2	11.2	11.2	10.2	10.2	1.02	9.7	9.7	9.7
Methionine		4.5	4.5	4.5	4.2	4.2	4.2	4.0	4.0	4.0
M+C		8.5	8.5	8.5	8.0	8.0	8.0	7.6	7.6	7.6
Tryptophan		1.8	1.8	1.8	1.8	1.8	1.8	1.7	1.7	1.7
Threonine		7.3	7.3	7.3	6.6	6.6	6.6	6.3	6.3	6.3
Arginine		11.8	11.8	11.8	10.7	10.7	10.7	10.2	10.2	10.2
Isoleucine		7.2	7.2	7.2	6.7	6.7	6.7	6.4	6.4	6.4
Valine		8.5	8.5	8.5	7.6	7.6	7.6	7.3	7.3	7.3
Calcium, g/kg		6.5	6.5	6.5	6.0	6.0	6.0	5.0	5.0	5.0
Avail P, g/kg		3.5	3.5	3.5	3.0	3.0	3.0	2.5	2.5	2.5
Sodium, g/kg		1.8	1.8	1.8	1.5	1.5	1.5	1.5	1.5	1.5
Choline, mg/kg		1450	1450	1450	1250	1250	1250	1250	1250	1250

[1] Econase XT 25 P, ABVista UK,
[2] Quantum Blue 10 G, ABVista UK 10000 FTU

Table 3 - Summary of formulation results.

Item	Grower			Finisher 1			Finisher 2		
	1	2[1]	3[2]	1	2[1]	3[2]	1	2[1]	3[2]
Total cost, $/t	468.63	480.99	467.10	450.17	462.03	448.43	441.63	451.80	439.21
Protein, g/kg	207	187	187	196	176	176	190	170	170
Dig. Lysine, g/kg	11.2	11.2	11.2	10.2	10.2	10.2	9.7	9.7	9.7

[1] Prices for valine, arginine and isoleucine $8.5, $13.3 and $18.9/kg.
[2] Prices for valine, arginine and isoleucine $4.25, $6.65 and $ 9.45/kg.

There are significant ingredient effects of these low protein diets. These are: 1) wheat levels are increased, 2) soybean meal levels are decreased, 3) levels of canola meal are unchanged, 4) added oil (tallow) levels are reduced by almost 50%, 4) MBM remains about the same, 5) amounts of lysine, methionine and threonine are greatly increased. On average of grower, finisher 1 and finisher 2, lysine addition is increased by 60%, methionine by 25% and threonine by almost 300%, 5) valine, arginine and isoleucine are used in these lower protein diets, at levels of 0.2-0.7 kg/t for valine, 1.2-1.6 kg/t for arginine, and 0.1-0.6 kg/t for isoleucine. Arginine is used at the highest level.

IV. DISCUSSION

It is clear that, if lower protein diets are to be produced, the amino acids valine, arginine and isoleucine will be needed. As shown in this exercise, current prices of these 3 amino acids prevent the formulation of lower protein diets at equal cost to higher protein ones. Therefore, the key to lower protein diets is dependent on future prices for these 3 amino acids.

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GLYCINE DYNAMICS IN LOW CRUDE PROTEIN BROILER DIETS

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Summary

The mounting demand of animal proteins for an expanding global population in the face of limited natural resources shall be guided by the responsibility to increase productivity while minimizing environmental impact. Leaving conventional animal feeding methods in the past and shifting to well established modern dietary strategies could play a substantial role in securing a smaller ecological footprint from animal production. This means lowering dietary crude protein (CP) while supplementing essential amino acids (AA) to cover the nutritional requirements of the broilers.

I. INTRODUCTION

Growing emphasis on environmental regulation requires global animal production to adopt strategies like feeding low CP diets to minimize nitrogen excretion. However, in some of the animal feeding studies, lowering dietary CP beyond a certain level showed undesirable effects on growth performance and carcass quality of broilers. A number of explanatory approaches is being debated as the possible reasons for the consequences of lowering dietary CP on broiler performance. The difference in the optimal ratio of essential AA between experimental diets (Kobayashi et al., 2013), specific non-essential AA (Corzo et al., 2004) and utilization of free AA compared to peptide bound AA (Namroud et al., 2008) are among the approaches mostly discussed. In this context, concentration of dietary glycine equivalent (Gly_{equi}) and the levels of metabolic precursors of glycine (Gly) have gained significant attention over the years in optimizing dietary needs to maximize animal performance.

II. MATERIALS AND METHODS

This short paper reviews the advances in knowledge concerning low CP diets predominantly focusing on the published studies from the last two decades concerning factors influencing the response to specific non-essential AA's Gly and Ser in broiler diets.

III. RESULTS AND DISCUSSION

The potential of Gly to improve growth has been known for decades and the metabolic interconversion of Gly and serine (Ser) is not limited for poultry. Therefore, Gly and Ser are usually assessed together to determine the physiological value of a diet. Most studies use the sum of the concentrations of both Gly and Ser to capture the analogous effect of these AA. This does not account for the fact that dietary Ser only has the same effect as Gly on an equimolar basis. Consequently, Gly_{equi} was proposed as a reference unit, which is calculated as the sum of the concentration of Gly and the molar equivalent of the Ser concentration (Gly_{equi} = Gly + 0.7143 x Ser). Waguespack et al. (2009) conducted three feeding studies to determine the Gly+Ser requirement of broilers in a low CP corn-soy based diet. A single slope break point analysis of the pooled feed conversion ratio (FCR) from the three trials estimated the Gly+Ser requirement to be 21 g/kg (Figure 1).

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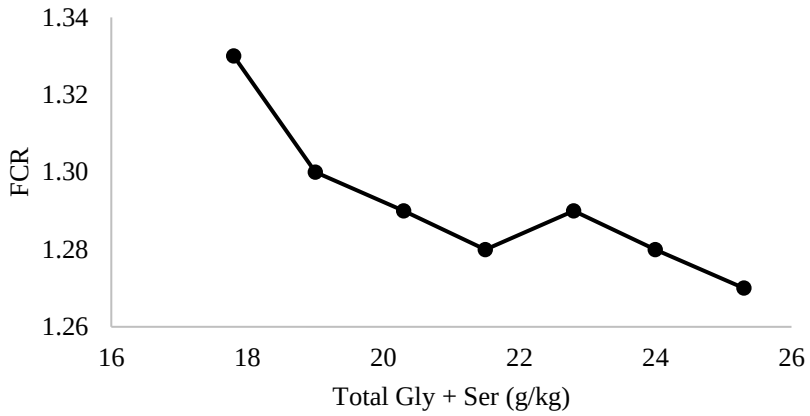

Figure 1 - Pooled FCR of broilers from 3 trials at different levels of dietary Gly+Ser in 1-18 days old broilers (adapted from Waguespack et al., 2009).

Multiple researchers have suggested different Gly+Ser requirements over the last two decades. However, comparing the dietary Gly_{equi} values recommended by these dose response studies (Corzo et al., 2004, Powell et al., 2011) is difficult as the authors defined the Gly_{equi} requirements as the dietary concentrations that led to a certain percentage of maximum response. Seigert et al. (2015) conducted a meta-analysis of a performance database from 9626 broilers covering 10 peer-reviewed studies and 11 experiments for varying dietary levels of Gly from 1-21 days (d) of age. The study revealed that the response to dietary Gly_{equi} is highly variable for average daily gain (ADG) and gain to feed ratio (G:F) with 95% of the maximum response achieved at Gly_{equi} concentrations ranging from 11.9 to 19.3 g/kg and from 11.4 to over 23.6 g/kg for ADG and G:F, respectively.

Few of the earlier studies demonstrated that broilers fail to achieve maximum performance when dietary CP is lowered by more than three numerical points even though all known nutrient requirements were met (Hussein et al., 2001, Bregendahl et al., 2002). However, Yuan et al. (2012) evaluated the effect of supplementing Gly to low CP diets in Cobb male broilers and concluded that diets low in CP (<190 g/kg) resulted in decreased body weight (BW) when the Gly + Ser level fell below 17.1 g/kg and increased FCR at levels lower than 18.7 g/kg. The addition of Gly to these diets to a minimum level of 20 g/kg significantly improved performance and was similar in effect to that of diets with 220 g/kg CP. The reduction in performance with low CP diets reported in the above-cited studies may well be due to the underestimation of Gly levels in the diet. Hilliar et al. (2017) reported similar improvement in response with Gly supplementation in diets with CP levels 185 g/kg and 165 g/kg during grower and finisher stages, respectively. Gly supplemented low CP diets showed 7 points improvement in feed efficiency and 254 g improvement in body weight gain (BWG) compared to non-supplemented low CP diets. A dose response trial with low CP diets (177 g/kg and 165 g/kg for grower and finisher respectively), covering five levels of digestible Gly+Ser ranging from 12.4 to 15.7 g/kg and 11.4 to 14.9 g/kg in grower and finisher diets, was done at Wageningen university. The researchers did not find any noticeable effect on production performance neither with three numerical points of CP reduction nor with added Gly compared to the diets with CP level of 209 g/kg and 199 g/kg in grower and finisher stages (van Harn et al., 2018). The authors concluded that a digestible Gly+Ser levels of 12.4 g/kg and 11.4 g/kg in grower and finisher phase, respectively, is sufficient in low CP diets. In contrast, Ospina-Rojas et al. (2013) reported a linear increase of BWG and G:F of broilers from 21-35 d with increasing dietary total Gly+Ser concentration of 14.7 g/kg to 17.7 g/kg in low CP diets. Although birds endogenously synthesize Gly, 40% of the total requirement of this AA must come from the diet (Graber and Baker, 1973). This might be even more relevant in the context

of today's fast-growing broilers as the synthesis of Gly may fall short to meet specific requirements for protein accretion, high endogenous losses, and needs related to other metabolic processes.

Differences in the optimal dietary concentrations of Gly_{equi} in broiler diets observed in the reported studies may well be explained by the different dietary levels of endogenous Gly precursors of which Thr and choline are quantitatively most important. When diets are merely satisfying essential AA requirements, there is a need to better understand the relationship between Gly_{equi} with Thr. Study by Seigert et al. (2015) showed that certain levels of G:F and ADG can be achieved with distinct combinations of Gly_{equi} and Thr. An increase in dietary Thr levels reduced the Gly_{equi} concentration required to achieve certain response levels. Corzo et al. (2009) in their animal feeding study showed that given the diets are formulated to contain lower CP values, overcoming marginal levels of dietary Gly may be accomplished by allowing moderate excesses of dietary Thr. In this study, dietary interactions were observed for BWG, carcass and breast meat weight. All of these parameters showed improvements with increasing dietary Thr in combination with low dietary Gly + Ser levels (Table 1). However, this reported Gly sparing effect of Thr was not in agreement with the observation from van Harn et al. (2018) wherein supplementing 0.7 g/kg additional Thr to low CP diet supplemented with Gly did not show any beneficial effect on the growth performance of male broilers. Since the growing chick requires a dietary source of Gly at all stages of development, it is of interest to consider contributors to the bird's endogenous pool of glycine while formulating diet.

Table 1 - Growth performance of male broilers fed different levels of Gly+Ser and Thr from 21-42 d (adapted from Corzo et al., 2009).

Total Gly+Ser (g/kg)	Digestible Thr (g/kg)	BWG (g)	Carcass weight (g)	Breast weight (g)
1.55	5.7	1760^{b}	1617^{b}	451^{b}
1.55	6.1	1801^{ab}	1682^{ab}	462^{ab}
1.55	6.5	1847^{a}	1702^{a}	477^{a}
1.65	5.7	1871^{a}	1734^{a}	486^{a}
1.65	6.1	1812^{ab}	1669^{ab}	470^{ab}
1.65	6.5	1811^{ab}	1673^{ab}	466^{ab}

A high potential conversion of choline to Gly is reported in mammals (Melendez-Hevia et al., 2009). However, despite the obvious role of Gly in poultry nutrition, very limited information is available on the interactive effect of these nutrients on the animal requirements. Siegert et al. (2015) looked at the magnitude of mutual replacement effects of Gly_{equi}, Thr, and choline using a quadratic regression model. At a fixed choline concentration of 1.05 g/kg DM, the Thr requirement at 95% of maximum G:F ranged from 8.2 to 9.3 g/kg DM when the Gly_{equi} concentration varied between 19.5 and 22.9 g/kg DM (Figure 2a). Likewise, at a fixed Gly_{equi} concentration of 19.5 g/kg DM, the Thr requirement at 95% of maximum G:F ranged from 8.8 to 9.5 g/kg DM when the choline concentration varied between 1.03 and 1.72 g/kg DM (Figure 2b).

IV. CONCLUSION

In conclusion, the evidence outlined in this paper contributes to further optimization of the dietary Gly_{equi} concentration as well as the other dietary nutrients influencing the response to Gly_{equi}. This enables reducing the dietary CP content without adverse effects on broiler growth performance and concomitantly minimizing the impact of animal production on the environment.

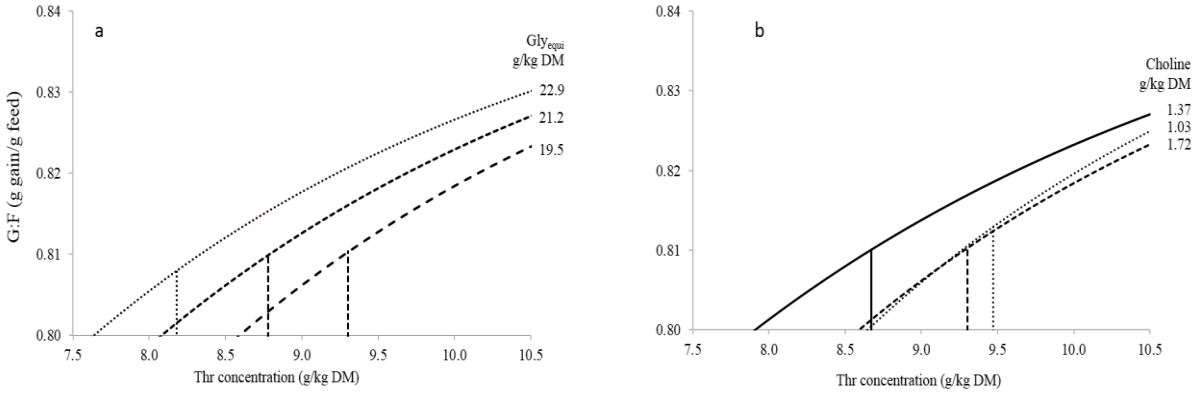

Figure 2 - Dietary Thr concentration on G:F at varying Gly_{equi} and choline concentration in 7-21 days old broilers (adapted from Seigert et al., 2015).

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THE RELEVANCE OF STARCH-PROTEIN DIGESTIVE DYNAMICS IN CRUDE PROTEIN-REDUCED BROILER DIETS

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Summary

Broiler chickens were offered diets formulated to contain 200, 188, 172 and 156 g/kg crude protein (CP) from 14 to 35 days post-hatch. Feed conversion ratio (FCR) increased linearly from 1.495 to 1.629 as dietary CP declined with a corresponding increase in distal jejunal starch-protein disappearance rate ratios from 2.08 to 3.17. These two parameters were quadratically related ($r = 0.838$; $P < 0.001$) and there was a linear relationship ($r = 0.605$; $P < 0.001$) between relative fat-pad weights and FCR. Increasing starch-protein disappearance rate ratios are detrimental for birds offered CP-reduced diets and the implications of these outcomes are discussed.

I. INTRODUCTION

Crude protein-reduced broiler diets have the potential to provide tangible advantages in respect of economics, bird welfare, flock health and the environment. Moderate reductions in dietary crude protein (CP) levels can be achieved without compromising broiler performance when coupled with judicious synthetic amino acid inclusions. However, there appears to be a threshold where further CP reductions negatively influence performance, especially FCR and this is associated with increased fat deposition (Belloir et al., 2017). Moss et al. (2018) demonstrated that starch is influential in the context of CP-reduced diets to the extent that glucose and amino acids may be competing for intestinal uptakes via their respective Na^+-dependent transport systems given atypically high dietary starch contents. There has been a considerable focus on non-essential synthetic amino acids, including glycine and serine (Dean et al., 2006; Siegert and Rodehutscord, 2017), in the quest to lower the CP threshold. However, such specific approaches may be neglecting the importance of starch and starch-protein digestive dynamics (Liu and Selle, 2017; Selle and Liu, 2018). Thus the purpose of this paper is to report an investigation into starch-protein digestive dynamics in the context of crude protein-reduced diets.

II. METHODOLOGY

A total of 168 male, off-sex, Ross 308 chickens were offered maize-soy diets formulated to contain 200, 188, 172 and 156 g/kg crude protein from 14 to 35 days post-hatch as shown in Table 1. Each dietary treatment was offered to 7 replicate cages of 6 birds per cage. Growth performance, relative abdominal fat-pad weights, apparent digestibility coefficients of starch and protein (N) in the distal jejunum were determined by standard procedures as outlined in Moss et al. (2018). Disappearance rates (g/bird/day) were calculated from daily feed intakes, analysed dietary concentrations and digestibility coefficients of starch and protein. Experimental data were analysed via the SPSS Statistics 24 program (IBM Corporation. Somers, NY). The feeding study fully complied with specific guidelines (2016/973) approved by the Animal Ethics Committee of the University of Sydney.

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Table 1 - Composition of dietary treatments.

Diet Ingredient (g/kg)	200 g/kg CP	188 g/kg CP	172 g/kg CP	156 g/kg CP
Maize	560	602	659	718
Soybean meal	329	289	233	171
Vegetable oil	49.7	42.7	32.8	22.4
Lysine HCl	1.622	2.850	4.558	6.454
Methionine	2.897	3.249	3.742	4.296
Threonine	0.974	1.533	2.311	3.178
Tryptophan	-	-	0.202	0.533
Valine	0.673	1.364	2.326	3.400
Arginine	-	0.454	2.080	3.886
Isoleucine	0.235	0.930	1.898	2.974
Leucine	-	-	-	1.239
Histidine	-	-	-	0.319
Sodium chloride	4.009	2.426	0.222	-
Sodium bicarbonate	0.010	2.401	5.730	6.187
Potassium carbonate	-	-	-	2.615
Limestone	7.25	7.17	7.06	6.93
Dicalcium phosphate	20.29	20.91	21.77	22.75
Choline chloride (60%)	0.900	0.900	0.900	0.900
Celite	20.0	20.0	20.0	20.0
Vitamin-mineral premix	2.00	2.00	2.00	2.00
Analysed contents				
Starch	303	322	356	399
Protein (N x 6.25)	196	191	181	155

III. RESULTS

The performance objectives for Ross 308 birds from 14 to 35 days post-hatch call for weight gains of 1795 g/bird, feed intakes of 2965 g/bird and an FCR of 1.652. As can be deduced from Table 2, overall weight gains (1911 g/kg) and FCR (1.536) exceeded these objectives by 6.5% and 7.0%, respectively, in this study. Reducing dietary crude protein did not influence weight gain; however, feed intakes were linearly increased ($P < 0.025$) as dietary CP declined. Consequently, birds offered the lowest CP diet (156 g/kg CP) had significantly higher FCR than their counterparts; for example, the transition from the 172 g/kg CP to the 156 g/kg CP diet significantly compromised FCR by 7.03% (1.629 versus 1.522; $P < 0.0001$) on the basis of a pair-wise comparison. Collectively, reducing dietary CP compromised FCR in a linear manner ($r = -0.738$; $P < 0.001$); similarly, reducing dietary CP linearly increased relative abdominal fat-pad weights ($r = -0.840$; $P < 0.001$). Distal jejunal starch digestibility coefficients ranged from 0.909 in the 200 g/kg CP diet to 0.926 in the 156 g/kg CP diet without any significant treatment effects (data not shown). Alternatively, protein digestibility coefficients ranged from 0.674 in diet 200 g/kg CP to 0.754 in diet 156 g/kg CP with a significant linear effect ($r = -0.678$; $P < 0.001$). Starch disappearance rates in the distal jejunum increased remarkably by 41.0% from 37.8 to 53.3 g/bird/day) as CP was reduced from 200 to 156 g/kg and the linear effect was highly significant ($r = -0.933$; $P < 0.001$). In contrast, protein disappearance rates decreased modestly by 7.65% (18.3 versus 16.9 g/bird/day) but the linear effect was significant ($r = 0.382$; $P < 0.05$). As a consequence the starch:protein disappearance rate ratio in birds offered 200 and 156 g/kg CP diets linearly increased ($r = -0.903$; $P < 0.001$) by 52.4%, from 2.08 to 3.17, respectively.

Table 2 - Effect of dietary treatments on growth performance, relative abdominal fat-pad weights, starch and protein disappearance rates (g/bird/day) in the distal jejunum and starch:protein (N) disappearance rate ratios in broilers from 14 to 35 days post-hatch.

Treatment (g/kg CP)	Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Relative fat-pad weight (g/kg)	Starch disappearance	Protein disappearance	Disappearance rate ratio
200	1934	2888	1.495a	7.26c	37.8a	18.3ab	2.08a
188	1931	2896	1.500a	8.49c	40.4b	18.9b	2.14a
172	1912	2907	1.522a	10.13b	45.3c	18.5b	2.45b
156	1864	3036	1.629b	12.40a	53.3d	16.9a	3.17c
SEM	36.31	42.25	0.0152	0.5011	0.8198	0.6333	0.0535
Significance	0.510	0.068	< 0.001	< 0.001	< 0.001	0.043	< 0.001
LSD (P < 0.05)	-	-	0.0444	1.463	2.39	1.48	0.156
Linear effect	r = 0.282 P = 0.146	r = -0.433 P = 0.021	r = -0.738 P < 0.001	r = -0.840 P < 0.001	r = -0.933 P < 0.001	r = 0.382 P = 0.045	r = -0.908 P < 0.001

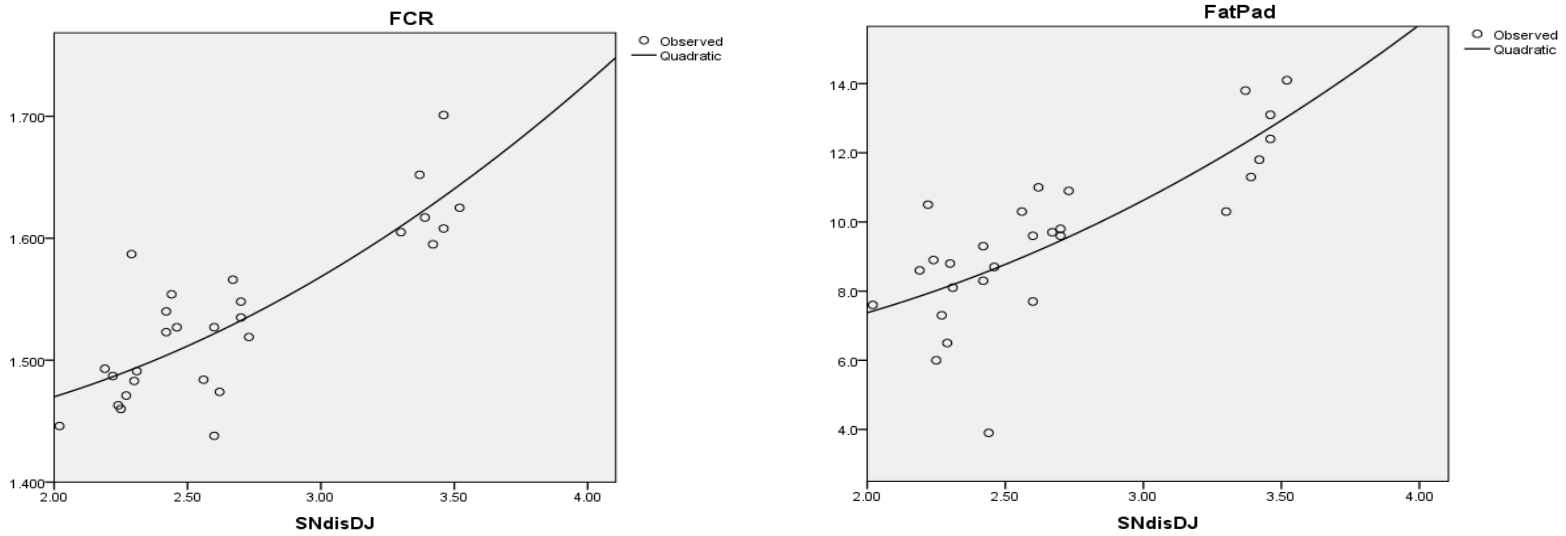

Figure 1 - Quadratic relationships between distal jejunal starch:protein disappearance rate ratios with FCR (r = 0.838; P < 0.001) and relative abdominal fat-pad weights (r = 0.786; P < 0.001).

IV. DISCUSSION

This focus on starch and protein digestive dynamics stems from a fundamental premise that an ideal balance of glucose and amino acids should be provided at sites of skeletal muscle protein synthesis to promote efficient growth and to evade losses of surplus nutrients. That starch:protein disappearance rate ratios are indicative of weight gain and feed conversion efficiency in broiler chickens was demonstrated in the Sydenham et al. (2017) study where ideal disappearance rate ratios in the proximal jejunum for maximal weight gains and minimal FCR were deduced from significant quadratic regression equations.

Reductions in dietary CP are usually achieved by increasing the feed grain content at the expense of the protein meal and in the present study maize increased from 560 to 718 g/kg and soybean meal decreased from 329 to 171 g/kg when Diets 1A and 4D are compared. Consequently, analysed starch concentrations increased by 31.7% (399 versus 303 g/kg) and protein (N) decreased by 20.9% (155 versus 196 g/kg) with the transition from 200 to 156 g/kg CP diets. Therefore, it is not surprising that these quite radical modifications to the diet formulations impacted on starch-protein digestive dynamics. Importantly, in the present study, increasing starch:protein (N) disappearance rate ratios in distal jejunum quadratically influenced FCR ($r = 0.819$; $P < 0.001$) and relative abdominal fat-pad weights ($r = 0.794$; $P < 0.001$), as illustrated in Figure 1. As the ratio widened feed conversion efficiency deteriorated accompanied by heavier fat-pad weights and it is relevant that there was a positive linear relationship ($r = 0.605$; $P < 0.001$) between fat-pad weights and FCR in the present study. The quadratic equation for FCR and distal jejunal disappearance rate ratios is as follows:

$$y_{(FCR)} = 1.456 + 0.0305*\text{ratio}^2 - 0.0540*\text{ratio}.$$

While these significant relationships are not conclusive, they do imply that quite blatant increases in digestion and absorption of starch and glucose relative to that of protein and amino acids in the context of CP-reduced diets are adversely influencing starch-protein digestive dynamics. Moreover, this imbalance is, in turn, almost certainly compromising feed conversion efficiency, which is being reflected in heavier fat-pad weights. In conclusion, this study would suggest that CP reductions in broiler diets may be better achieved by more modest feed grain increases in the formulation which would be facilitated by the partial substitution of soybean meal with feedstuffs with lesser protein contents. Limiting dietary starch levels or feed grain quantities in CP-reduced diets may permit a lower CP threshold to be realised without compromised growth performance and increased fat deposition.

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PREDICTION OF DIGESTIBLE LYSINE REQUIREMENT IN BROILER CHICKENS FROM 14 TO 35 DAYS POST-HATCH BY THREE DIFFERENT MODELS

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Accurate estimation of the broiler's lysine (Lys) requirement is vital because it is the second limiting amino acid (AA) in poultry diets and, when the ideal protein ratio concept is applied, the essential amino acids (EAA) are expressed as ratios to Lys. It is necessary to regularly update AA requirements for modern broiler chickens in order to accommodate their genetic progress. However, defining a nutrient requirement is difficult because of variations in age and sex of animals and choice of statistical response models (Baker, 1986). The present study compared three statistical models evaluating the digestible (dig) Lys response for growth performance of male and female Cobb MV × 500 broilers during the grower phase.

Dietary treatments consisted of 6 concentrations of dig Lys ranging from 0.84 to 1.29 % in increments of 0.09%. Experimental diets were based on maize, soybean meal and peanut meal. All diets were balanced for EAA by using the ideal protein ratio approach. Each of the 6 diets was offered to both male and female broilers from 14-35 days post-hatch and each treatment was offered to 6 floor pens with 12 birds per pen or a total of 864 birds. Three models, including quadratic polynomial (QP), linear broken-line (LB) and quadratic broken-line (QBL), were fitted to estimate the optimal level of dig Lys to maximize broiler growth performance.

In male birds, the Lys requirement for maximum weight gain was 1.03% (BL model), 1.14% (QBL) and 1.13% (QP). In female birds, the Lys requirement for maximum weight gain was 1.15% (BL) and 1.29% (QBL) but the QP model was not significant. The dig Lys requirement for optimal FCR using the QP model was estimated to be 1.21 % for females and 1.23% for males. However, the BL model predicted that the dig Lys requirement for optimal FCR was 1.10 % for females and 1.22% for males; whereas, for QBL model, the dig Lys requirement for optimal FCR was 1.29% for both male and female birds. In general, the BL model generated the lowest dig Lys requirement in comparison to the QP and QBL models. Also, the optimal dietary dig Lys concentration for maximal profit may differ due to fluctuations in ingredient and chicken meat prices. Therefore, the dietary dig Lys recommendations reported in the literature should be applied with caution in practice.

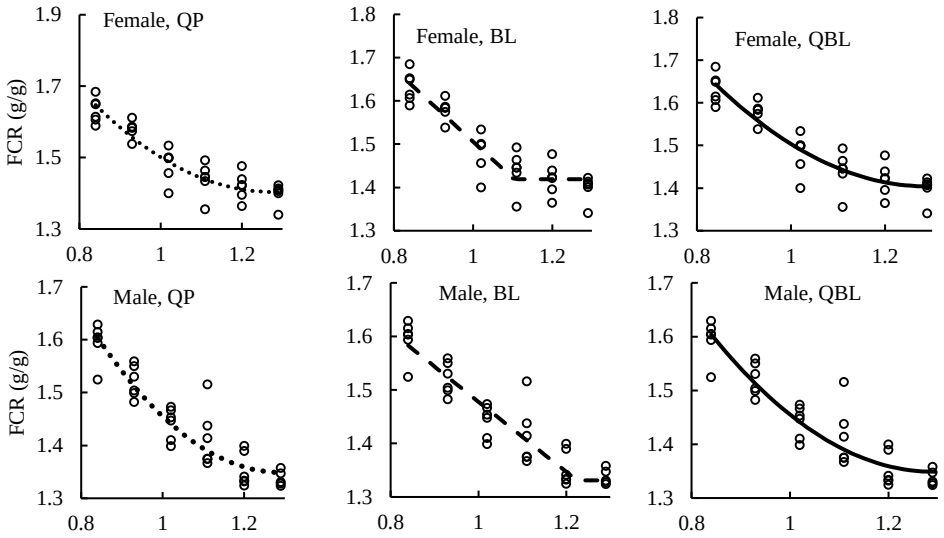

Figure 1 - Prediction of lysine requirment in male and female birds by three different models.

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EFFECTS OF SUPPLEMENTAL XYLANASE, CEREAL GRAIN SOURCE, AND AGE ON CAECAL VOLATILE FATTY ACID CONCENTRATIONS OF BROILERS FROM 14 TO 42 DAYS OF AGE

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Supplemental xylanase may be used in diet formulation to reduce diet cost, mitigate the anti-nutritive effects of arabinoxylans, increase energy utilization, and improve growth performance of broilers. Xylanase has been shown to partially depolymerize arabinoxylans, which can reduce intestinal viscosity, reduce nutrient encapsulation, and modulate the intestinal microflora of broilers (Choct et al., 1999; Bedford, 1995; Bedford and Cowieson, 2012). In addition, products of xylanase hydrolysis, arabinoxylo- and xylo-oligosaccharides, have been reported to stimulate a prebiotic effect, increasing broiler caecal fermentative capacity, and volatile fatty acid (VFA) production (Masey O'Neill et al., 2014). However, factors such as cereal grain source and bird age affect the mode of action of xylanase.

An experiment was conducted to assess the effects of supplemental xylanase, cereal grain source, and age on caecal VFA concentrations of Ross × Ross 708 male broilers during weekly intervals from 14 to 42 d of age. One thousand five hundred day-old chicks were randomly distributed into 60 floor pens (25 chicks/pen; 0.078 m^2) and fed 1 of 4 dietary treatments from 1 to 14, 15 to 28, and 29 to 42 d of age with 15 replicate pens per treatment. Dietary treatments consisted of a 2 × 2 factorial arrangement with 2 cereal grain sources (corn- or wheat-based) and 2 supplemental xylanase inclusions (with or without) as the main factors. Caecal contents were collected and pooled from 4 broilers per pen at 14, 21, 28, 35, and 42 d of age for VFA analysis.

Cereal grain source and supplemental xylanase interacted ($P < 0.05$) to affect butyric (14 and 21 d of age) and total VFA (21 d of age) concentrations. Broilers fed corn-based diets with and without xylanase and broilers fed the wheat-based diet with xylanase exhibited higher concentrations of butyric and total VFA than broilers fed wheat-based diet without xylanase. Cereal grain source ($P < 0.05$) influenced propionic, isobutyric, butyric, isovaleric, valeric, and isocaproic acid concentrations at 14, 21, 28, 35, and 42 d of age. Broilers fed corn-based diets had higher ($P < 0.05$) concentrations of propionic, isobutyric, isovaleric, valeric, and isocaproic acids than birds fed wheat-based diets from 14 to 42 d of age. Conversely, broilers fed wheat-based diets had higher ($P < 0.05$) concentrations of butyric acid at 28, 35, and 42 d of age compared with broilers fed corn-based diets. All individual and total VFA concentrations increased ($P < 0.05$) linearly from 14 to 42 d of age. Age and cereal grain interactive effects ($P < 0.05$) were observed with propionic, isobutyric, butyric, isovaleric, and valeric acid concentrations.

These results indicate that broiler caecal VFA concentrations are influenced by cereal grain source and bird age. However, inconsistent effects of xylanase supplementation on broiler caecal VFA concentrations demonstrate that future research evaluating factors such as substrate availability, gastrointestinal environment and age, xylanase inhibitors, microflora composition, immunological and stress conditions, and health are warranted.

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NIRS STUDY ON NUTRITIONAL PROFILES OF 100 SOYBEAN MEAL SAMPLES FROM USA AND BRAZIL

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Summary

This paper compares the nutrient profile of soybean meal (SBM) samples from the USA and Brazil. Proximate analysis, total amino acids, standardized ileal digestibility (SID) of amino acids, apparent metabolizable energy (AME), and phytic phosphorus were estimated using near infrared reflectance spectroscopy (NIRS) with calibrations derived from chemical analyses and *in vivo* determinations. The study found nutritional profiles of both SBM origins to be similar. A significant difference was observed in AME content in favour of US meal (9.59 ± 0.075 MJ/kg vs. 9.38 ± 0.084 MJ/kg, $P < 0.05$), whereas SBM from Brazil contained slightly higher level of digestible lysine (25.4 vs. 24.6 g/kg, $P > 0.05$). In addition, US meals displayed lower variation in terms of crude protein, AME, crude fibre etc.

I. INTRODUCTION

Soybean meal is the most widely used protein source in poultry diets, because of its high levels of crude protein, high digestibility and consistency compared to other protein ingredients. However, its nutritional value is affected by several factors, such as genetic selection (Palacios et al., 2004; Loeffler et al., 2013), production or planting environment (van Kempen et al., 2002; Goldflus et al., 2006) and crushing process, which affect the concentration of both nutrients and anti-nutritional factors (ANF). There are numerous studies on the quality and consistency of SBM. Maitri & Hurburgh (2007) compared quality of soybeans and soybean meals from US and other origins. Grieshop and Fahey (2001) reported soybeans from China contained a higher crude protein and a lower lipid level than those from Brazil. Karr-Lilienthal et al (2004) reported soybean meals produced in Argentina and Brazil have lower true TAA digestibility than US SBM and Karr-Lilienthal et al. (2005) reported crushing procedures and conditions affect nutritional value.

Today, US and Brazil are the two dominant sources in terms of soybean production and exportation. The livestock industry has a keen interest in quantifying the variation of soybean and soybean meals. The objective of this study was to survey the quality of SBM from the US and Brazil, especially for their metabolisable energy and SID amino acids which have not been well defined due to complexity in determination.

II. MATERIALS & METHODS

In this study, to eliminate differences derived from processing conditions, raw soybeans were imported directly from US and Brazil, crushed and produced by the same SBM manufacturer in Vietnam. Imported soybeans were crushed within 10 days. Five samples from each origin were randomly collected during each day's crushing. Consequently, a total of 100 SBM samples was obtained, in which 50 samples originated from US and the rest 50 from Brazil. Nutritional values including proximate analysis, total amino acids, standardized ileal digestibility of amino acid and apparent metabolizable energy (AME) values were evaluated using Adisseo's near infrared reflectance spectroscopy (NIRS) predictive equations derived from *in vivo* digestibility tests. In parallel, the SBM samples were analysed for the contents of proximate nutrients by [a] third party laboratory in Vietnam.

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III. NIRS PREDICTION FOR NUTRIAL CONTENTS IN SBM

Feasibility of using NIRS to predict nutrition content has been proven by previous studies. Adisseo Precise Nutrition Evaluation (PNE) services integrated both *in vivo* expertise and NIRS technology to offer on-line NIR platform since 2012. Available parameters include AME, total and standardized ileal digestible amino acids, total, phytic and available phosphorous for poultry and pigs, and proximate nutrients. Digestibility coefficients were determined *in vivo*, using the model of adult caecectomized ISA Brown cockerels, with caeca surgically removed in order to minimise intestinal microflora interference. A Precise Nutrition Evaluation (PNE) AME database was obtained through *in vivo* measurements using 3-week-old male broilers, following the European reference method (Bourdillon et al., 1990) with *ad libitum* feeding and total excreta collection.

The proximate nutrients of SBM samples were determined by both NIRS and laboratory as shown in Table 1. Consistent results obtained by NIRS and laboratory tests demonstrate the reliability of using NIRS to predict the nutritional values. As the dry matter content of samples was similar, nutrient values on a dry matter basis were not calculated. The proximate compositions are similar for SBM from US and Brazil origins, except for protein content which is slightly higher for Brazil SBM (467.7 g/kg, as fed) compared to US SBM (460.7 g/kg, as fed), but with no statistical difference ($P > 0.05$). In addition, total phosphorus and phytic phosphorus have been found similar when comparing SBM from US and Brazil.

Table 1 - Proximate compositions of US and Brazil originated SBM samples.

	US SBM (N = 50) g/kg as fed			Brazil SBM (N = 50) g/kg as fed			P value
	NIRS		Lab	NIRS		Lab	NIRS
	Mean	Std	Mean	Mean	Std	Mean	
Ash	60.6	0.8	60.8	60.7	0.8	60.0	NS
Fat	13.0	0.4	-	12.4	1.7	-	NS
Crude fiber	42.3	1.7	42.6	40.2	2.4	42.9	NS
Dry matter	874.6	1.3	874.3	875.0	0.8	874.9	NS
Crude protein	460.7	1.8	465.4	467.7	2.3	465.8	NS
Total phosphorus (P)	6.3	0.1	-	6.4	0.1	-	NS
Phytic phosphorus (PP)	4.2	0.1	-	4.1	0.1	-	NS

NS – not significant

The concentrations of essential amino acids of SBM from US and Brazil origins were found to be similar and consistent (Table 2), particularly when the amino acids concentrations were expressed in percentage of crude protein. The concentration differences ranged between -0.04 to 0.11 (in % of crude protein), with no statistical difference ($P > 0.05$).

Coefficients for SID of amino acids reflected similar values when comparing US SBM and Brazil SBM (Table 3). Lysine digestibilities were found at 84.8 % and 85.7 % for US SBM and Brazil SBM respectively, which is in line with common reference (Sauvant et al., 2004) and methionine digestibilities were 89.7 % and 89.4 % for US SBM and Brazil, respectively. Similar AA digestibilities may be due to the same processing conditions used in this study since the digestibility of AA is influenced largely by the adequacy of heat-processing to destroy or reduce the ANF, especially trypsin inhibitors.

Table 2 - Concentration of essential amino acids of SBM samples from US and Brazil origins.

TAA	US SBM (N = 50)		Brazil SBM (N = 50)			US SBM (N = 50)	Brazil SBM (N = 50)
	g/kg as fed					in % of crude protein	
	Mean	Std	Mean	Std	P value	Mean	Mean
Crude Protein	460.7		467.7			-	-
Lysine	29.04	0.21	29.62	0.15	NS	6.30	6.33
Methionine	6.14	0.05	6.22	0.05	NS	1.33	1.33
Cystine	6.85	0.05	6.86	0.05	NS	1.49	1.47
Threonine	18.54	0.12	18.87	0.13	NS	4.02	4.03
Tryptophan	6.36	0.05	6.51	0.05	NS	1.38	1.39
Valine	23.33	0.12	23.74	0.10	NS	5.06	5.08
Isoleucine	22.67	0.16	23.21	0.14	NS	4.92	4.96
Leucine	35.87	0.24	36.63	0.18	NS	7.79	7.83
Histidine	11.83	0.08	12.04	0.05	NS	2.57	2.57
Arginine	34.44	0.38	34.39	0.20	NS	7.48	7.35

NS – not significant

Table 3 - Coefficients of standardised ileal digestibility of essential amino acids for SBM from US and Brazil origins.

	Coefficients of standardized ileal digestibility of amino acids					
SID AA	US SBM (n=50)		Brazil SBM (n=50)			
	Mean	Std	Mean	Std	Diff, %	P value
Lysine	84.8	0.6	85.7	0.4	0.9	NS
Methionine	89.7	0.3	89.4	0.3	-0.3	NS
Cystine	77.2	0.4	77.3	0.1	0.1	NS
Threonine	81.7	0.3	82.2	0.2	0.5	NS
Tryptophan	84.4	0.3	85.1	0.3	0.7	NS
Valine	83.4	0.2	83.4	0.1	0.0	NS
Isoleucine	88.4	0.2	88.5	0.2	0.1	NS
Leucine	88.0	0.2	87.7	0.2	-0.3	NS
Histidine	88.4	0.1	88.3	0.2	-0.1	NS
Arginine	91.6	0.1	91.6	0.2	0.0	NS

NS – not significant

Besides AA digestibility, metabolizable energy has a high impact on animal performance and consequently on profitability. Significant differences were observed in the apparent metabolizable energy (AME) related to soybean origin: Average AME 9.59 MJ/kg for US SBM vs. 9.39 MJ/kg for Brazil SBM ($P<0.05$). Similarly, nitrogen corrected AME (AMEn) for US SBM was 8.83 MJ/kg (Figure 1), higher than Brazil SBM at 8.59 MJ/kg ($P< 0.05$).

In addition, US SBM had higher consistency for most of the nutrient contents compared with Brazil SBM. Except for dry matter content, smaller standard deviation values were observed for US SBM (1.80 g/kg for CP and 0.075 MJ/kg for AME), compared to Brazil SBM (2.25 g/kg for CP and 0.084 MJ/kg for AME). The low variability in the US samples is probably due to the low genetic variability among current US soybean cultivars (van Kempen et al., 2002).

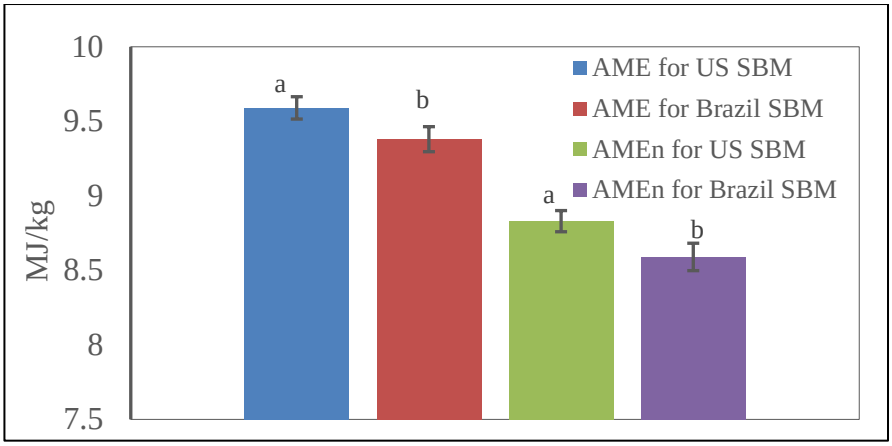

Figure 1 - AME and AMEn values with standard deviation for SBM from US and Brazil. [a and b] values with different superscripts are significantly ($P < 0.05$) different from each other.

IV. CONCLUSIONS

The NIRS technique has unique advantages of being non-destructive and rapid in screening the quality of any given feed ingredient. Predicting from the *in vivo* referenced NIRS calibrations, this study demonstrated that soybean meal from US origin has higher AME and AMEn values than soybean meal from Brazil. Overall, US originated soybean meal also showed better consistency for most nutrients with smaller standard deviation values, which highlighted the potential economic benefit of SBM from the US.

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EFFECTS OF ESSENTIAL OILS AND ENCAPSULATED BUTYRIC ACID ON GROWTH PERFORMANCE, INTESTINAL MICROFLORA AND SERUM LIPID PROFILE OF JAPANESE QUAILS

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Summary

A total of 360 one-d-old Japanese quail chicks was used in a 35-d trial to investigate effects of supplementing essential oil (EO) with or without encapsulated butyric acid (BA) on growth performance, intestinal microbiota and serum metabolites. Quail chicks were randomly allocated to 1 of 4 experimental diets with 6 replicate pens per diet and 15 chicks in each pen in a completely randomized design. The experimental diets were; 1) a corn-soybean basal diet (CON), 2) EO group (CON + 300 mg/kg essential oil), 3) BA group (CON + 500 mg/kg encapsulated butyric acid), and 4) MIX group (CON + 300 mg/kg essential oil and 500 mg/kg encapsulated butyric acid). Birds fed diets supplemented with EO and MIX had greater body weight gain (BW gain) and a lower feed conversion ratio (FCR) than the CON birds ($P < 0.05$). The plasma concentrations of total cholesterol and LDL-cholesterol in quails fed the EO, BA, and MIX diets were lower than the CON birds ($P < 0.05$). The lactic acid bacteria count was lower in the caeca of quails fed the CON diet than the other experimental dietary groups, while the coliform bacteria count in the ileum and caeca were greater in CON group ($P < 0.05$). The results of the current study suggest that the dietary supplementation of either EO or BA and particularly the MIX improves growth performance, gut microflora balance and serum cholesterol of Japanese quails.

I. INTRODUCTION

Antibiotics as growth promoters (AGPs) have been extensively used at sub-therapeutic levels in poultry production to prevent diseases and promote growth performance. However, the persistent use of antibiotics can increase antibiotic-resistant bacteria and antibiotic residue accumulation in the animal products as well as potentially transferring resistant strains to humans through the food chain (Jazi et al., 2018a). Consequently, the poultry industry has been forced to seek alternatives to AGPs in poultry feeds. Essential oils (EO) extracted from aromatic plants as feed additives, have demonstrated benefits on growth performance, as well as antibacterial and antioxidant effects for birds (Du et al., 2015). In addition, some studies have shown that the use of EO reduces serum cholesterol in broiler chickens (Chowdhury et al., 2018). Butyric acid (BA), a short chain organic acid, has shown beneficial effect in increasing the production of mucin and antimicrobial peptides which enhances the development of intestinal epithelium and defense barriers (Fernandez-Rubio et al., 2009). Encapsulated organic acids which are active in the entire digestive tract have a higher efficacy than free organic acids in reducing pathogen bacteria proliferation in the gastrointestinal tract (Levy et al., 2015). The present research was designed to determine the efficacy of EO, encapsulated BA, and the synergistic effects of the 2 additives (MIX) on growth performance, intestinal microflora and serum lipid profile of Japanese quails.

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II. METHOD

Three hundred and sixty one-day-old healthy Japanese quail chicks were obtained from a local hatchery, weighed and randomly allocated to 1 of 4 experimental groups with 6 replicate pens of 15 birds each with an initial body weight of 10.53 ± 1.17 g. The experimental diets were as follows: 1) basal diet as control, (CON), 2) basal diet + 300 mg essential oil/kg of diet (EO; CRINA® Poultry; DSM Nutritional Products Ltd., Switzerland, containing 29% active components such as thymol, piperine, and eugenol), 3) basal diet + 500 mg butyric acid/kg of feed (BA; ButiPEARL; Kemin Industries, Herentals, Belgium, including 50% butyrate salt), and 4) basal diet + 300 mg/kg EO and 500 mg/kg BA (MIX). Nutritional requirements of the Japanese quails were adopted from National Research Council (NRC, 1994) tables. The rearing conditions and lighting, and temperature program were based on those described by Jazi et al. (2018b). Feed intake (FI), body weight (BW) gain, and feed conversion rate (FCR) were recorded for d 1-21, d 21-35, and d 1-35 periods. Microbiological examinations were carried out according to the method described by Jazi et al. (2018a, b). Briefly, enumeration of lactic acid bacteria (LAB), coliforms and total anaerobic bacteria (TAB) were carried out on de Man Rogosa and Sharpe agar, violet red bile agar and plate count agar, respectively. To measure serum lipid profile, at d 35, 12 birds from each experimental group were randomly selected and blood samples were collected from the wing vein (Jazi et al., 2018b). All data were subjected to one-way ANOVA as a completely randomized design using the GLM procedure of SAS (SAS, 2003). Tukey's HSD test was used to make pairwise comparisons between means, where appropriate. Statistical significance was declared at $P < 0.05$. Data represent means of 6 replicates per treatment.

III. RESULTS

For the d 1-21 period, birds fed the diets supplemented with EO, BA, and particularly MIX had greater BW gain and lower FCR than the birds on the CON diet (Table 1; $P < 0.05$). Likewise, for the d 21-35 and the total growth period, BW gain was greater and FCR was lower in birds fed the diets supplemented with EO and MIX compared to the birds fed the CON and the BA diets ($P < 0.05$).

Table 1 - Effect of essential oil and butyric acid on Japanese quail's growth performance[1].

	Treatment[3]				
Item[2]	CON	EO	BA	MIX	*P*-value
1 to 21 d					
BW gain (g/bird)	88.7±3.68^{c}	102.9±4.45^{ab}	99.8±3.27^{b}	112.2±3.67^{a}	0.002
Feed intake (g/bird)	232.1±14.88	246.2±8.91	240.9±14.04	250.5±14.51	0.21
FCR (g/g)	2.62±0.07^{a}	2.40±0.15^{b}	2.42±0.07^{b}	2.23±0.06^{c}	0.001
22 to 35 d					
BW gain (g/bird)	84.5±2.92^{c}	97.2±3.21^{ab}	94.8±2.63^{b}	100.9±2.89^{a}	<0.01
Feed intake (g/bird)	316.3±10.23	332.7±16.80	319.1±10.55	337.6±13.84	0.20
FCR (g/g)	3.74±0.05^{a}	3.42±0.09^{b}	3.36±0.04^{b}	3.34±0.05^{b}	<0.01
1 to 35 d					
BW gain (g/bird)	173.3±6.54^{c}	200.1±7.21^{ab}	194.6±6.72^{b}	213.1±6.80^{a}	<0.01
Feed intake (g/bird)	548.9±23.16	578.9±24.17	560.0±24.51	588.1±18.29	0.15
FCR (g/g)	3.16±0.03^{a}	2.89±0.08^{b}	2.88±0.03^{b}	2.76±0.07^{c}	<0.01

[a-b]Means with different superscripts in each row are statistically different ($P < 0.05$).
[2]BW gain = body weight gain; FCR = feed conversion ratio.
[3]CON = control; EO = essential oil; BA = butyric acid; MIX = EO plus BA.

However, no differences were observed in feed intake of birds. The LAB population was greater in the ileum and caeca of birds fed the diets containing EO, BA, and MIX when

compared to CON (Table 2; P < 0.05). Also, birds fed the additives had a lower count of coliforms in the ileum and caeca content (P < 0.05).

Concentrations of the total cholesterol and the LDL-cholesterol in the serum of birds fed with EO, BA or MIX were lower than the CON birds (Table 3; P < 0.05). Experimental diets had no effect on the concentrations of triglycerides, HDL and LDL-cholesterol of birds.

Table 2 - Effects of essential oil and butyric acid on gut microbiota composition.

	Treatment[2]				
Item[1]	CON	EO	BA	MIX	*P*-value
Ileum					
LAB (log_{10} cfu/g)	7.08±0.24	7.53±0.52	7.36±0.53	7.68±0.40	0.13
Coliform (log_{10} cfu/g)	5.56±0.45^{a}	4.99±0.30^{b}	5.12±0.37^{ab}	4.67±0.41^{b}	0.007
TAB (log_{10} cfu/g)	7.77±0.31	7.58±0.38	7.71±0.29	7.66±0.37	0.79
Caeca					
LAB (log_{10} cfu/g)	7.25±0.31^{c}	7.67±0.34^{ab}	7.40±0.22^{bc}	7.81±0.20^{a}	0.001
Coliform (log_{10} cfu/g)	6.06±0.25^{a}	5.44±0.35^{bc}	5.61±0.24^{b}	5.07±0.40^{c}	0.005
TAB (log_{10} cfu/g)	8.01±0.35	7.97±0.30	7.91±0.19	7.79±0.23	0.69

$^{a-b}$Means with different superscripts in each row are statistically different (P < 0.05).
[1]LAB= lactic acid bacteria
[3]CON = control; EO = essential oil; BA = butyric acid; MIX = EO plus BA

Table 3 - Effects of essential oil and butyric acid on blood metabolites

	Treatment[2]				
Item[1]	CON	EO	BA	MIX	*P*-value
Cholesterol	170.1±13.90^{a}	143.7±12.53^{bc}	149.2±8.62^{b}	137.4±12.55^{c}	0.001
Triglycerides	97.4±10.62	89.9±11.15	95.7±7.12	91.9±9.22	0.49
HDL-C	85.6±9.81	84.5±8.66	87.1±4.71	81.4±7.85	0.54
LDL-C	64.9±4.08^{a}	41.2±4.34^{bc}	42.9±5.32^{b}	37.6±5.69^{c}	0.001
VLDL-C	19.4±2.12	17.9±2.23	19.1±1.42	18.3±1.84	0.40

$^{a-b}$Means with different superscripts in each row are statistically different (P < 0.05).
[1]HDL = high density lipoprotein; LDL = low density lipoprotein; VLDL = very low density lipoprotein;
[3]CON = control; EO = essential oil; BA = butyric acid; MIX = EO plus BA

IV. DISCUSSION

The results of the current study showed that all the experimental diets improved the growth performance of birds compared to the CON diet. However, birds fed the MIX diet had the greatest BW gain and lowest FCR among the experimental groups, which indicates the synergistic effect of the feed additives used in the present study. Similarly, Levy et al. (2015) reported that supplementing encapsulated BA to the diet improved BW gain and FCR compared to the control. Jazi et al. (2018a) have also reported that BA supplementation improved the growth performance of broilers challenged with *Salmonella*. In a recent study, however, it has been reported that the use of non-encapsulated organic acids has no effect on the growth performance of quail chicks (Jazi et al., 2018b). Lack of growth response to the added free organic acids is probably due to quick absorption of these acids in the upper gastrointestinal tract such as the crop, while the encapsulated organic acids can reach the hind gut (Levy et al., 2015). The positive effects of BA supplements on bird's growth performance is likely due to their ability to lower the pH of the gastrointestinal tract and increase the proliferation of beneficial microbes such as *Lactobacillus* (Jazi et al., 2018a). Our results are in line with the results of various studies showing the benefits of the use of EO in improving the growth performance of birds (Jang et al., 2007; Park and Kim. 2018). Improved growth performance of the birds can be due to the stimulating effects of EO on digestive enzymes,

antibacterial activity, balancing the microbial flora of the digestive tract, and improving the intestinal morphology (Jang et al., 2007; Chowdhury et al., 2018).

Dietary supplements resulted in an increase in caeca lactic acid bacteria counts and a decrease in ileum and caeca coliforms counts. Du et al. (2015) reported that EO (thymol and carvacrol) exhibited a strong antibacterial activity against gram-negative bacteria such as *E. coli*, *Salmonella* strains, and *C. perfringens*. Park and Kim (2018) demonstrated that the addition of EO to broiler diets increased *Lactobacillus* counts and decreased *E. coli* populations in the ileum. As a potential mechanism of antimicrobial activity, the EO may stimulate mucus secretion into the intestinal lumen and create a disturbance to the bacterial cell membrane through disintegrating the outer membrane causing leakage of intracellular materials (Du et al., 2015). Similarly, Jazi et al. (2018a) showed that feeding encapsulated BA in broiler chicks increased *Lactobacillus* and decreased *Bifidobacterium* counts in the caeca. Organic acids provide the optimum conditions for the growth and proliferation of beneficial bacteria through increasing the acidity of the gastrointestinal tract (Jazi et al., 2018a). Beneficial bacteria will consequently reduce the pH of the gut by producing short-chain fatty acids, creating a natural defense against pathogenic bacteria.

In the present study, concentrations of total cholesterol and LDL-cholesterol of birds fed the experimental diets were lower than the control group. Recently, Chowdhury et al. (2018) reported that feeding diets containing EO reduced the serum concentrations of cholesterol and LDL-cholesterol in broiler chicks. Lea et al., (2003) have shown that EO have inhibitory effects on 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase enzyme activity. This enzyme is responsible for the synthesis of liver cholesterol. In addition, the increase in LAB reduces the intestinal pH and deconjugated bile salts which impairs the intestinal absorption of bile salts and therefore increases the fecal excretion of bile salts. As a result, the liver turns a greater amount of cholesterol to bile to reconnect the intestinal-liver cycle of bile salts. Cholesterol concentration, therefore, is reduced in the blood and tissues (Jazi et al. 2018b).

The results obtained in the present research indicate that the use of EO, with or without BA improves growth performance, increases caeca lactic acid bacteria counts, and reduces ileum and caeca coliform counts as well as serum cholesterol level. In conclusion, based on the synergistic effects of EO and BA, the 2 additives could be considered as an alternative to antibiotics.

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UPDATE ON INGREDIENT CALCIUM DIGESTIBILITY: IMPACT OF PRESENCE AND SOURCE OF PHYTATE AND SOURCE OF CALCIUM

C.R. ANGEL[1]

Summary

The work to be presented focuses on improving our understanding of some of the potential interactions between phytate source and concentration as well as of calcium sources and particle size on standardized ileal digestibility (SID) of Ca and P in diets with or without phytase. 1. From work comparing Ca sources (limestone vs bone meal at the same particle size) Ca source has a profound effect on SID Ca and P. Based on solubility of these two sources, Ca reactivity (solubility at low pH) may be the most important factor involved in the phytate Ca interactions. 2. Ca digestibility is affected by presence or absence of phytate in the basal diet used. Work with purified, zero phytate diets, will be difficult to implement in a formulation system that is based on additivity. 3. Phytate source impacts both SID Ca and P. Two phytate sources, corn or SBM were used at the same concentration (2.3 g/kg phytate P) if phytate P (PP) and SID Ca and P in diets with the same concentration and source of limestone, differed. This suggests that as diets are formulated closer to requirements, phytase matrix values should start to reflect the effect of phytase on the specific phytate containing ingredients used in the diet. That is, phytase matrix should vary with formula ingredient use. 4. Particle size of limestone has a profound effect on SID Ca of limestone and SID P of the diet the limestone is added to, both in the presence or absence of phytase.

I. INTRODUCTION

As we learn more about the negative impacts of calcium (Ca) on the availability of phosphorus (P) in broiler diets, it highlights how little we know about Ca requirements, digestibility of Ca in ingredients and optimal Ca to P ratios. The interactions through which Ca exerts some of its negative effects can be direct or through chelation's with phytate and the latter having a profound impact on the efficacy of phytase and on P digestibility. Historically the ratio of Ca to P has been defined as total Ca to total P or total Ca to available P in the diet. Currently we have measures of "availability" of P in ingredients (available, digestible and retainable P) but we continue to use total Ca to formulate poultry diets and to define Ca requirements. Our inability to define ingredient Ca digestibilities, provide accurate estimates of digestible Ca matrix values for phytases, and importantly corroborate digestible Ca needs of the animal hinder us in being able to formulate and use both Ca and P effectively. For the animal, what is important are the amounts of Ca and P that can be digested, absorbed and potentially used for deposition in body tissues as well as for metabolic needs. Retainabilities of Ca and P are directly and quickly impacted by digestible Ca (dCa) and P (dP) ratios. At requirements dCa and dP values are very close to retainable values (Jimenez Moreno et al., 2013). In order to fully implement a system that allows us to feed closer to P requirements and reduce P excretions, we need to understand the digestibility of Ca in ingredients and factors that affect these and ultimately, work towards a dCa to dP system. A general review of this literature was published in 2017 (Li et al., 2017).

Several papers have been published in recent years on methods as well as ingredient Ca digestibilities (Anwar et al., 2015, 2016 a, b, c; Anwar, 2017; Proszkowiec-Weglarz et al., 2013 a, b). Data from Anwar (2017) shows that as particle size decreases from a 1 to 2mm

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limestone to <0.5mm limestone, true Ca digestibility decrease from 62 to 38%. Similar results were found in work done at the University of Maryland (Angel, 2018) where Ca digestibility decreased as particle size of the limestone decreased.

The goal would be to define a range of optimal dCa to dP ratios for diet formulation that require that dCa and dP needs of the broiler and ingredient SID Ca and P be defined. A better understanding is needed of the impact of other factors that affect the ability of the animal to digest and/or absorb Ca and P, such as the physical form and type of concentrated Ca containing ingredients such as limestone and their solubility; use and concentration of phytase, phytate source and concentration, inclusion and concentrations of vitamin D or its metabolites as well as other feed additives, age, and diet ingredient selection to name just a few. Because we still do not understand the role that specific phytate or Ca sources or phytate concentration play in the interactions that occurs between Ca, P, phytate and phytase, the work that will be discussed was done to develop a better understanding of these interactions in order to start building towards a dCa to dP formulation system.

II. METHOD AND DISCUSSION

The objective of this work was to determine the impact of: 1. Presence or absence of phytate on SID Ca; 2. Source of calcium on SID Ca and P; 3. Source of phytate on SID Ca and P using one source of Ca; 4. Particle size of limestone.

A digestibility methodology developed at the University of Maryland (Angel, 2013 and 2017; Angel et al., 2013, 2014, 16; Proszkowiec-Weglarz et al., 2013 a and b) was utilized in this work. This method was chosen because it allows for the determination of Ca and P digestibilities concurrently. It was felt that because of the close and high impact interactions that occur between Ca and phytate, it was necessary to do the work in the presence of phytate as well as in the presence or absence of phytase. Despite publications talking about phytate free nutrition (reviewed by Cowieson et al., 2016) what is often disregarded is that today there is no evidence that all the phytate can be removed quickly enough in the proventriculus and gizzard such that all potential chelation's (with mineral cations and soluble proteins) can be avoided 100%. In this study either 0 or 1000 U/kg of a commercial *Buttiauxella* spp. phytase expressed in *Trichoderma reesei* was used.

All animal work was approved by the Institutional Animal Care and Use Committee of University of Maryland. One-day old Ross 708 male broiler chicks were obtained from a local commercial hatchery. Broilers were fed a commercial prestarter (hatch to 7 days) and starter diets (8 d to 20 d of age) and then fed the experimental diets for 36 h.

Thirteen treatments (diets) were tested (Table 1). Three basal diets were mixed plus a nitrogen/Ca/and P free diet in order to manufacture the 13 experimental diets. All basals contained a micro mineral and vitamin premix as well as salt, sodium bicarbonate, choline chloride, soy oil and an indigestible marker (premix of TiO2 and corn added at 20 g/kg to the basal to achieve a 5.8 g/kg Ti concentration in the final diet). Basal 1 contained corn and corn germ in proportions to yield 2.3 g/kg PP (2.34 g/ kg PP by analysis). This basal made up 956.7 g/kg of the final experimental treatments (1 through 8). For diets 1 and 2 containing no added limestone, ground silica (celite) was used as the filler to achieve 100%. It was then mixed, subdivided into 2 equal parts and phytase added from an analyzed batch to obtain 1000 U/kg. Limestone from 1 batch that was ground to achieve either 0.8 (diets 3 and 4) or 0.15 (diets 5 and 6) mm geometric mean diameter (GMD) was added to achieve 70 g/kg Ca in the final diet with only 2.4% Ca coming from the basal. Diets 7 and 8 contained bone meal, ground to a 0.152 mm GMD, added to the basal to achieve 70 g/kg Ca from bone meal in the final diet. Basal 2 contained SBM and corn, mixed to yield a 2.3 g/kg PP diet with 65% of the PP coming from SBM and 35% from corn. The same procedure as describe

previously was used to make the 0 and 1000 U phytase/kg diets (9 and 10). The nitrogen/Ca/P free (NF diet) diet was mixed in one batch and subdivided into 3 parts. It was used as the basis for 3 treatments: for treatment 13, the basal diet was fed as mixed to determine endogenous losses; and for treatments 11 and 12, the same limestone of 0.8 and 0.15 mm was added to the NF diet. These last 2 diets also contained mono sodium phosphate to achieve in similar P concentration as the phytate containing diets.

Table 1 - Treatments: calcium (Ca), phosphorus (P), phytate phosphorus (PP), phytase and calcium source.

Trt/ diet	Source of Ca added[1]	Phytase, U/kg		Anal[2] PP	Source of PP[3]	Anal Ca[4]	Anal P[4]	Particle size of added Ca,	# of reps[5]
		Fml[6]	Anal[7]	g/kg				GMD[8], mm	
1	None	0	< 50	2.34	Corn	0.24	2.90	NA	7
2	None	1000	967	2.34	Corn	0.24	2.90	NA	7
3	$CaCO_3$	0	< 50	2.34	Corn	7.13	2.91	0.80	14
4	$CaCO_3$	1000	1021	2.34	Corn	7.13	2.91	0.80	14
5	$CaCO_3$	0	< 50	2.34	Corn	7.15	2.90	0.15	8
6	$CaCO_3$	1000	998	2.34	Corn	7.15	2.90	0.15	7
7	Bone[9]	0	< 50	2.34	Corn	7.29	5.05	0.15	7
8	Bone	1000	1015	2.34	Corn	7.29	5.05	0.15	7
9	$CaCO_3$	0	< 50	2.20[10]	SBM/corn[10]	7.73	3.01	0.80	7
10	$CaCO_3$	1000	1015	2.20	SBM/corn	7.73	3.01	0.80	7
11	$CaCO_3$	0	< 50	0	none[11]	7.83	3.06	0.80	7
12	$CaCO_3$	0	< 50	0	none	7.83	3.06	0.80	7
13[12]	None	0	<50	0	none	0	0	0	6

[1]Cerne Calcium Company, Fort Dodge Fine & Granular Products (2123 200th Street, Ft Dodge, IA 50501). [2] Analyzed phytic acid x 28.18 = phytate P. [3] Source of phytate P (PP) for corn was a mix of corn grain and corn germ. [4] Analyzed calcium and phosphorus (triplicate analysis). All analyzed values are given as is. [5] Number of replicates used. Each replicate pen had 4 birds. [6] Formulated (Fml) phytase added based on analyzed batch concentration. [7] Analyzed phytase concentration done in duplicate. [8]Geometric mean diameter (GMD). [9]Bone from swine meat meal ground to 0.15 mm GMD. [10] 65% of the PP in this diet came from SBM. [11]In diets 11 and 12 a semi purified diet was used where the P source was mono sodium phosphate. [12]Nitrogen and calcium and phosphorus free diet for endogenous loss determination.

The experimental diets were fed to battery pens of 4 birds for 36 h between day 20 and 22 of age. All birds within a pen were sampled for distal ileal digesta collection. The birds were first anesthetized with a gas mixture of 35% CO_2, 35% N_2, and 30% O_2 (2 to 3 min) and then killed with CO_2 (2 to 3 min). The content in the distal ileum, the distal half from Meckel's diverticulum to 3 cm above the ileo-cecal junction, were collected

Dry matter in all samples was determined by drying overnight in a 100°C oven. Ingredients, feed and ileal samples were analyzed for Ca and P after acid digestion using inductively coupled plasma atomic emission spectrometry (ICP-AES; AOAC, 1999). Phytate-P in the basal diet was analyzed based on the method of Vinjamoori et al. (2004). Titanium dioxide concentrations in diets and ileal content were determined as described by Short et al. (1996). Phytase in diets was determined with an assay based on that of Engelen et al. (2001). Particle size and distribution were determined in all ingredients using the ASAE method S319.4, 2008.

Data were analyzed by the GLM procedure of JMP 12 (SAS Institute, 2015). Trt were considered as a fixed effect and pen within a block as a random effect. Tukey's (Tukey, 1949) adjustment was applied in all pair-wise comparisons to protect *P*-values. Pen within a block was the experimental unit and all calculations were generated based on pen averages. Significance was declared at $P < 0.05$. Specific contrasts were done to determine main effects and interactions.

III. RESULTS AND DISCUSSION

SID Ca and P for all Trt are presented in Table 2. Results of specific contrasts are discussed but not included in tables.

This experiment was designed to answer several questions. Results of digestibility will be presented based on the main questions posed.

a) Does phytate source affect digestibility of Ca and P in the presence or absence of phytase and a limestone from one source? There was a main effect ($P < 0.01$) of phytate source and phytase on SID Ca but no ($P>0.05$) interaction. Limestone Ca digestibility was higher in diets containing phytate originating from SBM (1.5 g/kg)/corn (0.8 g/kg) vs that in diets with phytate only from corn (main effect means 559.2 and 491.8 g/kg , respectively). This suggests that the chelation of reactive Ca (ionized, soluble Ca) is greater with phytate from corn as compared to that of SBM. Phytase inclusion at 1000 U/kg increased SID Ca from 461.6 to 589.4 g/kg with no phytate source x phytase interaction seen. This points to an improvement in SID Ca with phytase addition being similar regardless of phytate source. There were main and interaction effects ($P < 0.01$) of phytate source and phytase on SID P. In the absence of phytase, SID P was greater when the diet contained 1.5 g/kg of its PP (2.3 g/kg) from SBM as compared to that when the diet contained all PP from corn. There was a greater improvement in the SID P in the presence of phytase when dietary phytate originated from both SBM and corn vs phytate originating only from corn (910.2 and 874.7 g/kg, respectively). This suggests that the effects of phytate on SID P, and similarly, the magnitude of the phytase effect on SID P, are dependent on the source of phytate in the diet. It will be important in the future, to evaluate the effect of more phytate sources on Ca digestibility from limestones and on the impact of phytase source and level.

b) Does Ca source added to a diet with a defined source and concentration of phytate, affect digestibility of Ca and P in the presence or absence of phytase? There were main and interaction effects ($P < 0.01$) of Ca source (limestone or bone) and phytase on both SID Ca and P. Irrespective of phytase inclusion, SID Ca was greater ($P < 0.01$) when Ca originated from bone vs limestone. However, phytase only increased the SID Ca when Ca originated from limestone, but had no effect on SID Ca when the Ca originated from bone. These data suggest an important and large difference in the response in SID Ca from phytase that is dependent on Ca source and is potentially related to the reactivity (solubility at pH at or below 3) of the Ca in the different Ca source. It is important to note that even though PP concentration was the same in these diets, total P was much higher in the diet containing bone (5.05 g/kg) vs that containing limestone (2.91 g/kg) and this may have a confounding effect. The source of Ca also affected the magnitude of response in SID P from phytase with a substantially smaller improvement in SID P of 165.7 g/kg from phytase when the dietary Ca originated from bone meal vs. an improvement in SID P of 467.6 g/kg when the sole Ca source was from limestone. P concentration in the diet can affect P digestibility (Sommerfeld., et al 2018). The Ca-source dependent differences in the improvement in the SID Ca and P following phytase addition would suggest that phytase matrix values may be dependent on the source of dietary Ca and are substantially smaller when the Ca originates from bone vs. limestone.

c) Is the effect of Ca particle size on digestibility of Ca and P similar when diets contain phytate from a defined source, or no phytate? A reduction in limestone particle size reduced ($P < 0.01$) SID Ca in both diets containing 2.3 phytate from corn, as well as in phytate free diets. There was no interaction of limestone particle size and presence

of PP on SID Ca. When the phytate free diets were used SID Ca for limestone was higher ($P < 0.01$) as compared to SID Ca of the same level and source of limestone when this was added to diets containing 2.3 g/kg PP from corn (main effect means of 785.9 vs 382.8 g/kg, respectively).In contrast to results on SID Ca, there was an interaction ($P < 0.01$) of Ca particle size and PP concentration on SID P. A reduction in limestone particle size from 0.8 mm to 0.151 mm reduced SID P from 240.3 to 140.8 g/kg, but had no effect on SID P in diets without phytate (914.1 vs 918.4 g/kg, respectively).

d) What is the effect of limestone particle size? SID Ca was affected ($P < 0.01$ by particle size and phytase but no interaction effect was seen. A reduction in particle size of a limestone from the same batch from 0.80 or 0.15 mm GMD decreased SID Ca from 491.8 to 380.9 g/kg ($P < 0.01$, main effect means) while phytase addition at 1000 FTU increased the SID Ca from 382.8 to 490.0 g/kg. Although the improvement in SID Ca from phytase was numerically greater when diets contained the 0.8 mm limestone (123.7 g/kg) vs the 0.151 mm GMD (90.6 g/kg), there was no interaction of limestone particle size and phytase addition ($P = 0.1189$). In contrast, there was an interaction ($P < 0.01$) of limestone particle size and phytase on SID P, with a substantially greater improvement in SID P when phytase was added (634.4 g/kg) in diets containing the 0.8 mm GMD limestone vs 467.6 g/kg when the 0.151 mm GMD limestone was included. This shows that the magnitude of the phytase response on SID P was affected by limestone particle size. A reduction in the limestone particle size from 0.8 mm to 0.151 mm GMD reduced SID P to a greater extent when diets contained phytase than in the absence of phytase.

Table 2 - Treatment effects (one way ANOVA) on standardized ileal digestible (SID) Ca and P as well as on digestible Ca (dCa) and digestible (dP).

Diet	Ca source[1]	Ca GMD, mm	Phytase	Phytate Source	PP[2]	SID Ca[3]	SID P[3]	dCa[4]	dP[4]
						g/kg			
1	None	NA[5]	0	Corn[6]	2.3	100.6^{g}	721.8^{b}	0.03^{e}	2.28^{d}
2	None	NA	1000	Corn	2.3	175.5^{f}	911.3^{a}	0.05^{e}	2.87^{bc}
3	LM[7]	0.80	0	Corn	2.3	430.0^{e}	240.3^{g}	3.33^{d}	0.77^{f}
4	LM	0.80	1000	Corn	2.3	553.7^{d}	874.7^{a}	4.29^{c}	2.80^{c}
5	LM	0.15	0	Corn	2.3	335.6^{i}	140.8^{h}	2.61^{i}	0.44^{i}
6	LM	0.15	1000	Corn	2.3	426.2^{e}	608.4^{d}	3.31^{d}	1.91^{e}
7	Bone[8]	0.15	0	Corn	2.3	686.9^{b}	505.0^{e}	5.44^{b}	2.77^{c}
8	Bone	0.15	1000	Corn	2.3	704.1^{b}	670.7^{c}	5.58^{b}	3.68^{a}
9	LM	0.80	0	Corn/SBM[9]	2.2	493.3^{e}	324.6^{f}	4.15^{c}	1.06^{f}
10	LM	0.80	1000	Corn/SBM	2.2	625.0^{c}	91.0.2^{a}	5.26^{c}	2.98^{b}
11[10]	LM	0.80	0	None	0	842.9^{a}	914.2^{a}	6.63^{a}	2.85^{b}
12[10]	LM	0.15	0	None	0	728.9^{b}	918.4^{a}	5.73^{b}	2.86^{b}
P value						< 0.001	< 0.001	< 0.001	< 0.001
SEM						11.96	11.06	0.092	0.040

$^{a-h}$ Means within a column with different superscript letters differ ($P < 0.05$) based on Tukey HDS means separation. [1] Calcium source is either the same limestone with 2 different particle sizes or pork bone (0.150 mm particle size). Ca was added at 70 g/kg from the test source. [2] Analyzed phytic acid x 28.18 = phytate P. [3]Standardized ileal Ca or P digestibilities were determined by using endogenous Ca and P losses determined with 6 pens in this experiment (105.6 and 190.8 mg per kg DM intake, respectively) to take digestibilities from apparent to standardized. [4] dCa and dP determined by multiplying analyzed Ca or P by the digestibility of that nutrient. [5] Not applicable. [6] All corn diet (corn and corn germ) to achieve a 2.3 g/kg PP diet. [7] LM is limestone. [8] Bone removed from a not ground pork meat and bone meal. The separated bone was cleaned and ground to a 0.150 mm particle size. [9] 65% of the phytate P came from SBM. [10] Purified diet with monosodium phosphate equal to that in the corn based diet with 2.3 g/kg phytate P from corn.

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DIETARY HYDROXY-SELENOMETHIONINE HELPS FINISHING BROILERS TO COPE WITH HEAT STRESS

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The induction of oxidative stress by heat exposure, as well as the relationship between selenium (Se), selenoproteins and oxidative stress, is well described in the scientific literature (Habibian et al.,2015). Zhao et al. (2017) showed that hydroxy-selenomethionine (OH-SeMet) differently affected the expression of the selenogenome as compared to sodium selenite (SS). Hence, it was hypothesised that dietary supplementation of OH-SeMet may benefit heat stressed finishing broilers due to specific regulation of the selenogenome.

A total of 720-day-old male Ross 308 broilers was randomly assigned to 3 treatments (12 replicates; 20 birds each). Experimental diets were: no supplemental Se (NC), SS at 0.3 mg Se/kg and OH-SeMet at 0.3 mg Se/kg. A chronic cyclic heat stress challenge (temperature increased to 34℃ for 6h, daily; RH = 50-60%) was applied in the finisher phase (25-39d; Akbarian et al., 2014). Growth performance parameters were recorded per pen. At day 39, in serum or plasma (one bird per pen), total Se, glutathione peroxidase activity (GPx) and tri-iodothyronine (T3) were measured.

Table 1 - Performance and blood traits of finishing broiler fed different selenium sources (25-39 d).

	Growth performance 25-39 days				Blood paramenters		
Treatment	BW (d 25)	ADG (g/d)	FCR	Mortality (%)	Se plasma (µg/L)	Plasma GPX (U/g Hb)	Serum T3 (µg/L)
NC	1601	102.3	1.612 a	8.7	52 c	84 b	1.08 b
Selenite	1581	104.4	1.618 a	9.4	147 b	219 a	1.08 b
OH-SeMet	1580	105.1	1.561 b	5.2	178 a	211 a	1.34 a
P-value	0.289	0.313	0.026	0.264	< 0.001	< 0.001	0.01

In the finisher phase (Table 1), OH-SeMet significantly decreased FCR as compared to NC and SS (P = 0.026). Mortality, which increased substantially but not statistically significantly with cyclic heat stress, tended to be lowest in the treatment with OH-SeMet; 3.5 and 4.2% lower than NC and SS, respectively. Se supplementation significantly increased plasma total Se and OH-SeMet fed birds showed a higher level than SS fed ones (P < 0.001). GPx was significantly higher in Se supplemented diets but not different between OH-SeMet and SS. OH-SeMet significantly maintained serum level of T3 as compared to NC and SS (P < 0.01). In conclusion, compared with SS, OH-SeMet improved FCR of heat stressed finisher broilers, but it remains to be established whether differential effects on the expression of various selenoproteins might be involved.

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SOURCES AND LEVELS OF COPPER INFLUENCE BROILER PERFORMANCE AND CARCASS CHARACTERISTICS

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Copper (Cu) is an essential component involved in a wide variety of biochemical processes. It must be supplemented in the trace mineral premix as the amount present in raw materials is inadequate to meet broiler requirements. At levels higher than nutritional requirements (up to 250 mg/kg), dietary Cu addition enhances growth performance by altering gut microbiota and enhancing nutrient absorption. Such high levels may irritate the gizzard and result in high Cu levels in the poultry litter (Fisher et al., 1973). The hydroxy form of copper, copper hydroxychloride (IBC) is less soluble than $CuSO_4$, thus it is less reactive with other feed components such as vitamins and fats and might be expected to not irritate tissues. Its higher bioavailability allows the producer to achieve similar or improved performance, at reduced levels of trace mineral inclusion (Richards et al., 2010).

The current study was designed to compare nutritional and growth promoting levels of IBC with $CuSO_4$ on performance and carcass characteristics in broiler chickens. A total of 864 Ross 308 male day-old chicks were fed wheat-SBM based starter diets from day 1-14 and the grower diets from day 14-35. Birds had *ad libitum* access to feed and water throughout the trial period. Eight dietary treatments were replicated six times in floor pens with hardwood shaving litter: negative control (NC) treatment contained no supplemental Cu, 15 mg/kg Cu from $CuSO_4$, 200 mg/kg Cu from $CuSO_4$, 15 mg/kg Cu as IBC, 50 mg/kg Cu as IBC, 100 mg/kg Cu as IBC, 150 mg/kg Cu as IBC and 200 mg/kg Cu as IBC. Body weight gain (BWG), livability (LIV) and feed intake (FI) were measured from d0-35 and used to calculate adjusted feed conversion ratio (FCR). Carcass evaluation and footpad scoring were done on day 35 on 3 randomly selected birds per pen by weighing carcass components and a visual inspection footpad score from 0 to 9.

No difference in starting weight of chicks was recorded ($P > 0.05$). The highest BWG (2580 g) and lowest FCR (1.365 g/g) were observed in birds receiving 200 mg/kg Cu as IBC, followed by 100 mg/kg Cu as IBC (2558 g and 1.375 g/g) compared to NC (2458 g and 1.447 g/g, $P < 0.01$). Birds fed 200 mg/kg IBC gained more weight (77 g, $P < 0.01$) and had lower FCR (3.2 points, $P < 0.01$) compared to those fed 200 mg/kg Cu as $CuSO_4$. Feed intake (average 3514.3 g/bird) and LIV (average 98.6 %) were not significantly affected by Cu levels or sources ($P > 0.05$). Carcass characteristics including breast, thigh, drumstick yield and fat pad were not affected by copper level or source ($P > 0.05$). Foot pad lesions were not affected by Cu source or level ($P > 0.05$).

It can be concluded that supplementation of copper from IBC was more efficacious than sulfate in promoting growth performance of broiler chickens and the inclusion of 200 mg/kg IBC resulted in the highest feed efficiency.

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IN VITRO EVALUATION OF XYLO-OLIGOSACCHARIDE PRODUCTION FROM DIFFERENT BATCHES OF WHEAT WITH AND WITHOUT XYLANASE

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Supplementing poultry diets with xylanase partially depolymerizes the xylans present in the dietary cereals, reducing the number of sugars in the molecular chains. The resulting oligosaccharides can be selectively fermented by beneficial intestinal bacteria, resulting in improved nutrient utilization (De Maesschalck *et al*., 2015). The study aim was to investigate the production of xylo-oligosaccharides (XOS) in different batches of Australian wheat, in the presence or absence of xylanase. Five different batches of wheat were selected from NSW, VIC, QLD, WA and SA. Samples were exposed to a 2-step *in vitro* digestion assay that simulated gastric (pH 3.5 with 0.1M HCl and pepsin) and small intestine (pH 6 with 1M NaOH and pancreatin) phases of digestion in broiler chickens. Either 0 or 16,000 BXU/g xylanase (Econase XT 25, AB Vista Feed Ingredients, UK) was added. The samples were centrifuged and resulting supernatant prepared for analysis of XOS composition, along with standards of xylobiose (X_2), xylotriose (X_3), xylotetraose (X_4) and xylopentaose (X_5) (Morgan *et al.*, 2018). The standards and samples were analysed on a Shimadzu LCMS-8050 with a C18 HPLC column by electrospray ionization.

Xylanase inclusion diversely impacted XOS production across the wheat varieties (Table 1), demonstrating how growth conditions impact their susceptibility to degradation in the gastrointestinal tract environment, and where in the tract the majority of XOS production occurs. The greatest abundance of XOS was produced from wheat from NSW, wheat from QLD seemed to be degraded substantially during the gizzard phase but no further in the ileal, and wheat from WA produced the most X_2 and X_3. This study highlights a new and important facet of cereal NSP content and highlights the need to focus xylanase research on determining the optimal XOS production rate and location to generate the best prebiotic effects in wheat based diets.

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Table 1 - Total proportion of xylan in wheat samples converted into xylo-oligosaccharides (g/100g).

Location	Xylanase (BXU/g)		X_2	X_3	X_4	X_5	Total X_2-X_5
NSW	0	Gizzard	0.02	0.02	0.00	0.00	0.04
		Ileum	0.05	0.04	0.05	0.11	0.24
	16,000	Gizzard	0.58	0.71	0.59	3.46	5.34
		Ileum	1.33	1.31	1.07	8.37	12.08
VIC	0	Gizzard	0.02	0.02	0.00	0.00	0.04
		Ileum	0.03	0.01	0.00	0.05	0.08
	16,000	Gizzard	0.75	0.78	0.63	4.17	6.32
		Ileum	1.22	1.16	0.88	6.37	9.63
QLD	0	Gizzard	0.03	0.02	0.00	0.00	0.05
		Ileum	0.04	0.01	0.00	0.00	0.06
	16,000	Gizzard	0.77	2.68	0.64	3.94	8.02
		Ileum	1.32	1.23	0.89	5.35	8.78
WA	0	Gizzard	0.03	0.02	0.00	0.00	0.04
		Ileum	0.03	0.01	0.00	0.00	0.05
	16,000	Gizzard	0.80	0.79	0.62	3.60	5.82
		Ileum	1.68	1.76	1.19	6.36	10.99
SA	0	Gizzard	0.05	0.02	0.00	0.00	0.07
		Ileum	0.05	0.05	0.00	0.00	0.10
	16,000	Gizzard	1.01	1.12	1.17	5.01	8.32
		Ileum	1.49	1.80	1.34	5.79	10.42

PRELIMINARY INDICATIONS THAT EXOGENOUS PHYTASE INFLUENCES AMINO ACID AND GLUCOSE CATABOLISM IN THE GUT MUCOSA

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Summary

Wheat-soybean meal diets containing 0, 500, 1000 and 2000 FTU/kg phytase included over the top were offered to 192 male Ross 308 chicks (8 replicate cages) from 7 to 28 days post-hatch to determine the influence of exogenous phytase on concentrations of glutamic acid + glutamine and glucose in portal plasma and the effects of 500 FTU phytase on portal plasma amino acid concentrations. 500 FTU/kg phytase significantly ($P < 0.05$) increased plasma concentrations of isoleucine, leucine, lysine, phenylalanine, tryptophan, valine and serine. Additionally, phytase inclusions linearly reduced concentrations of glutamic acid plus glutamine ($r = -0.363$; $P < 0.05$) and logarithmically increased plasma glucose levels ($R^2 = 0.127$; $P < 0.05$). Glutamic acid plus glutamine to glucose concentration ratios in portal plasma were related to both FCR ($R^2 = 0.374$; $P < 0.005$) and phytase inclusions ($R^2 = 0.252$; $P < 0.05$). Thus, phytase may be manipulating the metabolic fate of glucose and amino acids within the gut mucosa; thereby contributing to improvements in feed efficiency. These findings indicate that further investigations are justified.

I. INTRODUCTION

Exogenous phytase improved ileal amino acid digestibility by an average of 5% based on 745 observations across 24 studies in a systematic review (Cowieson et al., 2017). Nevertheless, the post-enteral availability of amino acids is ultimately determined by their transition across enterocytes in the gut mucosa. Amino acids may be denied access to the portal circulation because they may enter numerous anabolic pathways, or they may be catabolized for energy provision. Thus, the catabolism of amino acids within the gut mucosa is of importance and glutamate/glutamine and glucose are the primary energy substrates (Watford et al., 1979). It follows that the 'catabolic ratio' of glutamate plus glutamine to glucose within enterocytes may be manipulated by dietary strategies to derive energy more efficiently. Previously, 500 FTU/kg phytase increased the average digestibility coefficient of 16 amino acids by 49.7% (0.720 versus 0.481) in the proximal jejunum, 20.1% (0.801 versus 0.667) in the distal jejunum, 9.07% (0.878 versus 0.805) in the proximal ileum and 7.24% (0.904 versus 0.843) in the distal ileum (Truong et al., 2015). The authors concluded that phytase generates a 'proximal shift' in sites of amino acid absorption which may spare amino acids from catabolism in the gut mucosa.

To determine the extent of amino acid catabolism within the gut mucosa, net amino acid concentrations within portal blood were measured previously in pigs (Stoll et al., 1998). Therefore, the present study determined the influence of exogenous phytase inclusion in poultry diets on gross concentrations of amino acids and glucose within the portal circulation.

II. MATERIALS AND METHODS

Wheat-soybean meal based broiler diets were formulated to standard specifications and supplemented with 0, 500, 1000 and 2000 FTU/kg phytase (*Buttiauxella sp.* expressed in *Trichoderma reesei;* Axtra® PHY, Danisco Animal Nutrition, Marlborough, UK) added over

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the top. Diets were offered to eight replicate cages (6 birds/cage) or a total of 192 male Ross 308 chicks from 7 to 28 days post-hatch. The accuracy of the phytase inclusions was confirmed by analysis using the standard method of Engelen et al. (1994). Blood samples were taken from the anterior mesenteric vein from three birds per cage following euthanasia at 28 days post-hatch. Blood samples were centrifuged and the decanted plasma stored at −80ºC prior to analysis. Concentrations of 16 proteinogenic amino acids in plasma were determined using methods described in Selle et al. (2016). Growth performance, nutrient utilisation and amino acid digestibility were also determined as described in Moss et al. (2018) (data not shown). 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, birds outperformed 2014 Ross 308 objectives (values given in parentheses) with a mean weight gain of 1436 g/bird (1387 g/bird), feed intake of 2045 g/bird (2052 g/bird) and an FCR of 1.426 (1.479) from 7 to 28 days post-hatch. The effects of phytase inclusions on glutamic acid plus glutamine and glucose concentrations (µg/mL) in portal plasma and growth performance are shown in Table 1. Phytase inclusion significantly improved weight gain and FCR but did not influence feed intake. Phytase inclusion did not influence glutamic acid and glutamine concentrations via a one-way ANOVA; however, phytase linearly reduced concentrations of glutamic acid plus glutamine in the portal circulation ($r = -0.363$; $P < 0.05$). Similarly, phytase did not influence glucose concentrations via an ANOVA; however, there was a significant logarithmic relationship where phytase increased plasma glucose concentrations in portal blood ($R^2 = 0.127$; $P < 0.05$). The effects of 500 FTU phytase on essential and non-essential amino acid concentrations (µg/mL) in portal plasma are shown in Table 2 where phytase inclusion increased the concentration of seven amino acids.

Table 1 - Effects of phytase inclusion on glutamate + glutamine and glucose concentrations (µg/mL) in portal blood and growth performance.

Treatment	Glu + Gln	Glucose	Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)
Control	287.0	18.8	1370[a]	2017	1.472[b]
500 FTU	280.1	21.1	1485[b]	2074	1.397[a]
1000 FTU	238.0	21.4	1440[b]	2036	1.417[ab]
2000 FTU	248.7	21.7	1448[b]	2054	1.419[ab]
SEM	15.27	1.07	22.62	32.51	0.0194
Significance (P =)	0.089	0.230	0.011	0.636	0.047
LSD (P < 0.05)	-	-	65.5	-	0.0558

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

Table 2 - Effects of 500 FTU phytase on essential and non-essential amino acid concentrations (µg/mL) in portal blood.

Treatment	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp
Control	81.61	21.70	22.95[a]	36.18[a]	29.39[a]	13.23	27.34[a]	85.01	6.68[a]
500 FTU	85.87	22.47	27.03[b]	42.70[b]	41.03[b]	14.09	31.10[b]	72.19	8.56[b]
SEM	3.617	1.608	1.021	1.451	2.931	0.570	1.016	5.784	0.376
Significance (P =)	0.421	0.740	0.015	0.007	0.015	0.306	0.021	0.142	0.004
LSD (P < 0.05)	-	-	3.119	4.433	8.954	-	3.103	-	1.147

Treatment	Val	Ala	Asp + Asn	Gly	Pro	Ser	Tyr	Mean
Control	36.1[a]	95.7	55.5	69.2	76.5	60.6[a]	45.7	58.0
500 FTU	40.9[b]	97.1	59.9	71.2	74.3	71.5[b]	44.3	59.8
SEM	1.50	7.12	3.06	4.49	4.27	3.41	2.58	2.95
Significance (P =)	0.043	0.892	0.328	0.752	0.727	0.041	0.707	0.676
LSD (P < 0.05)	4.58	-	-	-	-	10.40	-	-

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

IV. DISCUSSION

Phytase inclusion of 500 FTU/kg generated the largest weight gain and most efficient feed conversion in comparison with the control. Therefore, it is relevant that at the standard 500 FTU/kg inclusion, phytase significantly increased concentrations of free amino acids in portal blood; including isoleucine (17.8%), leucine (18.0%), lysine (39.6%), phenylalanine (13.8%), tryptophan (28.1%), valine (13.2%) and serine (18.0%). Glucose and amino acids (particularly glutamate/glutamine) are critical energy sources for the intestinal mucosa as they may be catabolised to provide energy in avian enterocytes (Watford *et al.*, 1979). Whether these energy sources are open to nutritional manipulation is unknown (Reeds et al., 2000). In the present study, phytase inclusion linearly reduced concentrations of glutamic acid and glutamine in the portal circulation ($r = -0.363$; $P = 0.049$) and increased plasma glucose levels by a logarithmic relationship ($R^2 = 0.127$; $P = 0.045$) in portal plasma. Thus, it appears that the energy source utilised by the intestinal mucosa may be subject to manipulation by phytase inclusion. Phytase may be sparing glucose and perhaps other amino acids from catabolism and increasing their entry into the portal vein by favoring glutamate/glutamine for catabolism. These relationships alone do not prove causation and more research is required. However, it is instructive that the ratio of glutamate plus glutamine to glucose concentrations within portal blood is significantly related phytase inclusion ($R^2 = 0.252$; $P < 0.05$) and FCR ($R^2 = 0.374$; $P < 0.005$), where the more efficient FCR was generated in birds with relatively more glucose present within portal plasma (Figure 1). The likelihood is that manipulation of the energy source of the gut mucosa with phytase inclusion has contributed to improvements in feed efficiency in the present study. Therefore, it appears that phytase can influence the metabolic fates of amino acids and glucose in the gut mucosa. Phytase may be sparing glucose and other amino acids from catabolism as reflected in the reduced entry of glutamic acid/glutamate into the portal circulation. Clearly, additional studies are required to confirm this important observation.

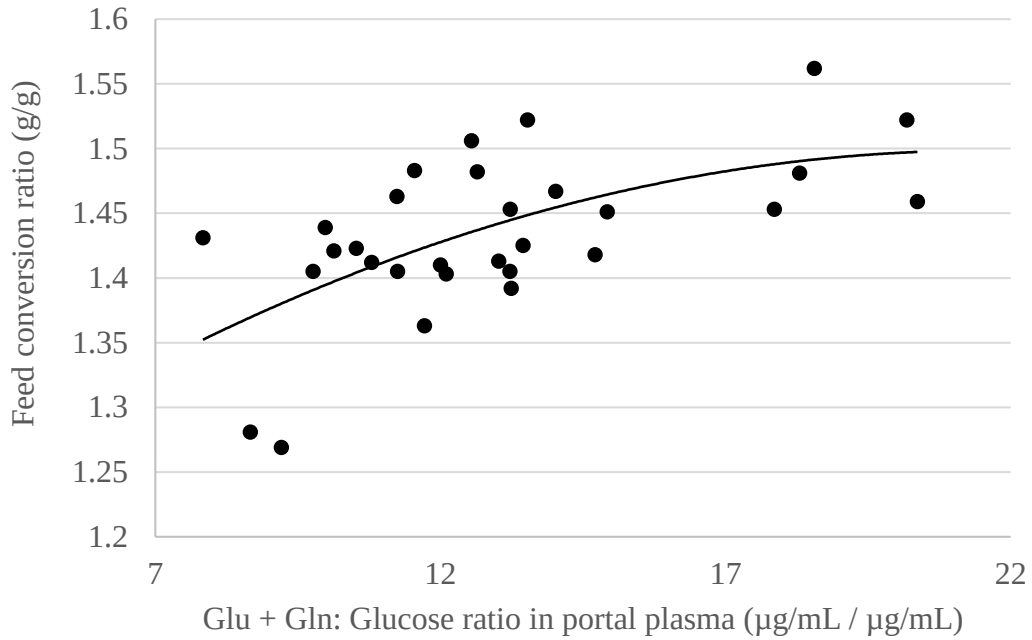

Figure 1 - The quadratic relationship between the ratio of glutamate plus glutamine to glucose concentrations within portal blood and feed conversion ratio ($R^2 = 0.374$; $P < 0.005$) in birds offered diets containing 0, 500, 1000 and 2000 FTU/kg phytase activity.

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PATH TO THE 100 WEEK AGE LAYER HEN IN CAGE FREE SYSTEMS

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Summary

Egg producers are always looking for ways to improve efficiency as, nowadays, the genetic potential of layers is targeting an extended age of production of 100 weeks, making the industry more sustainable. Australia is moving towards increased cage free production and there will be differences compared to the traditional production in cages in how to achieve this goal. Achieving the extended production in cage free production should be based in 5 principles of nutrition: the proper development of the body of the pullet during the first 5-6 weeks of life using the proper starter feed; the feed intake capacity using a developer feed; the right energy and amino acid intake to avoid deficiencies and behaviour issues; a calcium and phosphorus balanced nutrition based on needs; and the proper structure of the feed where the bird cannot select based on particle size. Each of these principles will facilitate the path to achieving layer hens in cage free production at 100 weeks of age.

I. INTRODUCTION

Right now, there are two different models of production in the world and they are based on the income of the people. We have 7% of the population that lives on more than US$50 per day (Pew Research Centre, 2015) and the percentage of food expenses is a minimal part of their income. These rich countries are moving towards production where cages are banned and the birds need to be provided with space outside of the barn. However, 71% of the world population lives on less than US$10 per day and these countries are investing in closing the birds in, getting them into cages and making production automated to improve productivity.

It is a fact well documented in the literature that the birds in cage-free production need higher levels of nutrients (Brainer et al., 2015) to achieve the same production as caged birds. These needs will require a higher demand for raw materials, and therefore more natural resources like soil, water, transport energy and fertilizer. This increased requirement for resources contradicts the concept of sustainability itself. Sustainable development has been defined by FAO as "*the management and conservation of the natural resource base, and the orientation of technological and institutional change in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations defined as the maximization of the natural resources available in our planet earth"* (FAO Council 1989). Somehow, this point has been forgotten or put aside when moving to cage free production by rich countries without thinking about the rest of the world.

Genetic companies are working towards maximising egg production. Since genetic selection commenced, the number of eggs per hen has increased due to improved laying rate of the bird as it gets older. As these birds can keep producing for a longer time, we also must adjust our nutrition to provide the nutrients to allow it to happen. In cage-free production, there are 5 points where we must focus our attention: the starter feed to achieve the body weight at 5-6 weeks of age; feed intake capacity at the start of lay with the developer feed; correct levels of energy and amino acids in the feed during production; Ca and P nutrition; and feed structure.

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II. STARTER FEED

Until the 6th week of age, the pullet is developing the carcass that will determine what kind of layer we will have in the future. If we want to achieve production to 100 weeks, it is necessary to achieve the target body weight recommended in the genetic guidelines. There is a clear correlation between flocks that did not achieve the body weight target at the 5-6th week and early cull in production due to several problems.

The starter feed should be a high energy and amino acid feed. Energy is a limiting factor in the growth of the pullets during the early stages (Guzman et al., 2015). Increased energy in the starter diet has a positive correlation with body weight that can be achieved in the birds. The starter feed of pullets has been mash feed; however, recent research and practical experience is showing that using crumble type of feed improves the body weight of pullets in the early stages (Table 1) and it can be a useful tool when there are challenges at the farm for achieving the desired body weight (Guzman et al., 2015).

Table 1 - Effect of feed presentation during the first 5 weeks of age of brown pullets.

	Daily feed intake (g)	Average Daily Gain (g)
Mash	21.5	9.2
Crumble	21.2	9.9
DS (n=30)	0.59	0.32
P <	**	***

III. FEED INTAKE CAPACITY

At the start of the production, there is a peak of needs, associated with body weight growth and the production of the first egg. At the start of lay, we see a growth of protein tissue, mainly related to sexual maturity and bone growth that is almost as great as what happens during carcass development. This bone development is critical for having good egg shell quality as the hens get older, as it will provide up to 30% of the Ca of the egg shell when the supply of Ca in the diet is not sufficient.

At the start of production, the birds are challenged as the needs are high and the feed intake is far from the average feed intake of a mature layer hen. Therefore, it is important to produce a pullet with a high feed intake capacity before production commences. Therefore, we need to develop the feed intake capacity using a certain amount of fiber in the pullet feeds, focussing this use in the developer feed.

There is plenty of early literature (Kondra et al., 1974) in which the addition of fibre has been shown to increase gut size. However, fibre had a bad reputation for reducing performance or increasing the growth of pathogens. Recent research shows that not all fibre is the same and that the type, level and source will have different effects. We need to understand that all fiber is not the same and we should probably move from the Crude Fiber (CF) concept to Neutro Detergent Fiber (NDF) values in feed formulation, once we establish the requirements. The NDF values might give more accurate information about the expected effect we will get in the development of feed intake. However, so far, information is limited so recommendations are given in CF bases by the genetic companies.

The addition of fiber will reduce the time for the bird to achieve adult feed intake; therefore it will be easier to achieve the right body weight during the start of lay.

IV. ENERGY AND AMINO ACIDS IN PRODUCTION

Energy and amino acids are two major factors affecting the performance of the layer hen and are the most expensive part of the feed. The energy needs of layers are driven mainly by the

maintenance need and that is determined by body weight of the bird. For layers with the same production of egg mass, body weight has a significant effect on the daily energy needs (Table 2). This body weight effect is not usually considered when formulation is conducted.

Table 2 - Effect of body weight on the daily needs of kcal for maintenance for a 60-gg mass performance.

BW (gr)	Maintenance needs (kcal/day)	Egg mass needs (Kcal/day)	Total (kcal/ day)
1800	184	120	304
1850	189	120	309
1900	194	120	314
1950	199	120	319
2000	204	120	323

As there are differences among breeds and flocks in body weight, it is necessary to have information about body weight in order to adjust the feed formulation. Historically we have not worried much about it, but relied on the capacity of the layer to self-regulate the feed intake based on its needs. To a certain degree, this could be valid when the birds are in a closed house where the temperature can be controlled and the birds have no option but to eat what is in front of them in the feeder. However, in cage free production, the birds may be exposed to high temperatures that will reduce feed intake, and what and where the bird eats is out of our control. In cage-free production, there will be an increase in the maintenance need due to the activity of the birds and the temperature at the farm. There will be a big impact on the maintenance needs, and in the energy intake too, as high temperatures will reduce the energy intake. The reported daily energy needs are very variable depending on the source of information; there are numbers of 5-15% increase compared to cage housing. The type of housing, barn or free range, and the temperature at the farm are some of the main reasons for this big variation in energy recommendations.

The amino acid needs are driven mainly by the egg mass production, which is around 80% of the total amino acid intake requirement. If we review the egg mass produced by layer hens, we see that egg mass starts dropping beyond 50 weeks of age. The needs of these birds at 50 weeks is not decreasing as it was in the old genetic stock; the work on longevity conducted by the geneticists has extended the high needs for amino acids because egg mass production does not decrease as it used to. However, it is a common practice to change to a more diluted feed after week 45. In these situations, we hope the bird can obtain the necessary nutrients by increasing feed intake, but sometimes what we ask of the bird might not be possible. The drop in amino acids, the housing and the feed intake capacity will be some of the reasons for birds not obtaining the amino acids they need. The reaction of the modern hen will be to sacrifice feather covering, egg size and even health, as long as the egg is produced. In the field, we can see late mortality without specific symptoms that I think are related to insufficient amino acids and effect of the oxidative stress.

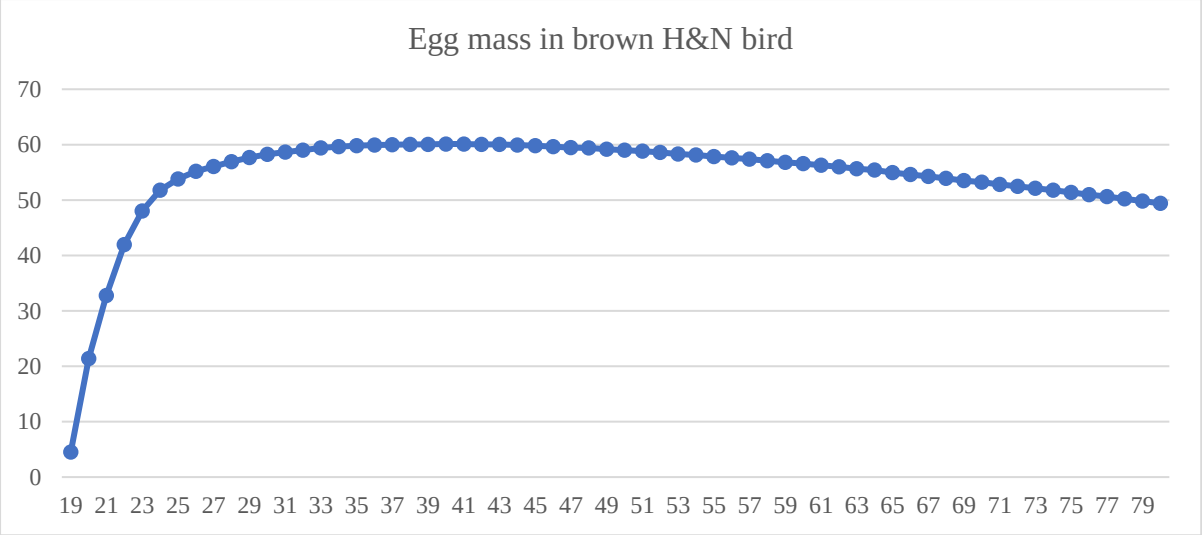

Figure 1 - Egg mass production (g/day) of brown layer.

Oxidative stress is a physiological challenge that occurs when the antioxidant system of the birds is overwhelmed by the production of free radicals. The production of free radicals is increased in birds with high metabolic rate, temperature stress, disease, oxidised fat and mineral and vitamin deficiencies. In cage free production, we have a high performing bird exposed to temperature changes and more pathogens than before; therefore, they might be much more impacted. The actual practice of changing to a lower density diet without considering the egg mass being produced could explain some of the issues when we try to achieve 100 weeks of production.

V. CALCIUM AND PHOSPHORUS NUTRITION

The calcium and phosphorus in layer diets is key to good egg shell quality as the hen gets older. There must be a balance of the added levels and the source of them. Phosphorus has an important role; its deficiency can decrease growth during the pullet phase and at the start of the production. After the peak of production, its requirements progressively decrease. In a meta-analysis conducted by Ahmadi et al. (2012), the needs for nonphytic phosphorus were shown to be 220 mg/day after the peak of the production. An excess of phosphorus in the diet is a concern for sustainability and it has an impact on the absorption of calcium by the hen. It is well documented that excess phosphorus will impact calcium absorption (Chandramoni et al., 1998)

The calcium level is becoming as important as how it is provided in the diet. Egg shell formation takes 20 hours, most of which happens during the night. During the night, the source of calcium for the egg shell will come from whatever is left in the gut and from bone reabsorption. If we want to keep layer hens up to 100 weeks of production, we need to reduce the calcium supplied from the bones, so we need to provide calcium in the feed, with large particle size (>3mm) for 70-80% of the calcium supplied in the diet, and with delayed solubility so it will be longer in the gut, even during the night time. There will always be some calcium provided from bone reabsorption. Therefore, it must be replaced during the morning by bone mineralisation so there will be certain needs for calcium in the morning that can be provided by fine limestone (1mm). However, it should not be very fine because the layers will not eat fine particles due to selection of particles in the feed.

VI. FEED STRUCTURE

Birds prefer to eat large particles before eating the fines. When birds are in cages, this selection capacity is reduced and with a good management of the feed delivery, we can minimise this activity even if we do not have the correct feed structure. When the birds are out of cages, we cannot control this selection of particular particle size so it is important to make a feed as homogenous as possible were the bird cannot select. Making a homogenous feed is not easy; the layer feed raw materials are at the extremes with large particles such as the grains and calcium and very fine particles such as the synthetic amino acids, vitamins, minerals and trace minerals. Therefore, there is an important job to do by the feed mill and the type of the raw materials we select.

In the feed mill, it is important to have a pre-grinding facility where we can decide which raw material should or should not be ground as the first step. At the grinding equipment, a roller mill will help us to have a more uniform cut of the grains and reduce the fines during their milling. In the selection of the raw materials, there are also important decisions we can make; they might increase the feed cost but it will improve the performance of the birds as they will be eating what they need. These decisions are about reducing the dustiness of the feed. We can add as many liquids as possible in replacement of the fine particles and we can have a minimum of oil in the diet. The oil will help to bind the fine particles, so we will reduce the fine particles (<5 mm) significantly. However, despite the additional cost that might be involved, this practice can produce some additional benefits (Table 3) as was shown in the study by Safaa et al. (2008).

Table 3 - Effect of added fat in the egg production.

	1%	3%	SEM
Lay (g)	77.0[a]	79.3[b]	0.84
Egg weight (g)	64.9[a]	66.3[b]	0.28
Feed intake	117	118	0.83
FCR (kg/kg)	2.36[a]	2.26[b]	0.02

Achieving 100 weeks of production will be a matter of improving the actual practices on egg production and, in cage free production, it will need additional management practices as we will no longer have as much control over the birds as they will be out of cages.

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LAYING PERFORMANCE, EGG QUALITY AND FEED STABILITY IN RESPONSE TO REPLACEMENT OF INORGANIC ZINC, COPPER AND MANGANESE WITH HYRDOXYCHLORIDE SOURCES IN HY-LINE LAYER HEN'S DIET

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Traditionally, Zn, Cu and Mn have been added to poultry diets in the form of inorganic salts, such as sulphate, to meet requirements and prevent deficiencies. The sulphate sources of trace minerals have low bioavailability, are highly water soluble and reactive in the feed and digestive tract (Ma et al., 2011). Hydroxychloride minerals (HyC) are a class of naturally occurring minerals with fully defined crystalline structure, where the crystal is held together by a series of covalent bonds between the metal ion, multiple hydroxyl groups and the chloride ions. Compared to inorganic salts, HyC minerals have been found to be less water soluble, more bioavailable and exhibit less oxidant promoting activity in the feed (Perez et al., 2017).

The present study was designed to investigate the effect of replacing $ZnSO_4$, $MnSO_4$ and $CuSO_4$ (INO) with HyC sources on egg production, egg quality parameters and feed stability in Hyline brown commercial layers during post-peak production. Zn, Mn and Cu were supplemented at 80, 80 and 15 ppm, respectively. There were 600 Hyline brown layer hens (45-wk old) distributed into 300 cages, with 10 cages (20 birds) considered as one replicate (15 replicates per treatment). The experimental diets were offered for 12 weeks, (45 to 57 weeks of age) during which feed consumption and egg production were recorded on a weekly and daily basis, respectively. External and internal egg quality parameters were analysed at the end of weeks 2, 6 and 12 of the feeding trial using 25% of eggs from each replicate. A composite representative of feed samples were collected and stored in farm conditions for four weeks and then analysed for fat oxidation.

As shown in Table 1, HyC diet improved hen-day egg production, FCR and egg mass ($P < 0.01$). There was no significant effect of diets on feed intake and egg weight. Shell reflectivity percentage improved by 1.1% in the HyC treatment. Other external and internal egg quality parameters tested, including shell thickness and breaking strength, shell, yolk and albumen percentage, albumen height, Haugh unit and yolk colour score, were not affected by mineral sources ($P > 0.05$). Inclusion of HyC Zn, Cu and Mn reduced the extent of oxidation in the feed, as indicated by lower peroxide (0.7 vs. 1.5 mEq/kg), higher free fatty acids (0.95 vs. 0.78 %w/w) and higher iodine values (4.0 vs. 3.0 g/100g) measured in the feed samples. Based on the current findings it could be concluded that replacing sulphate sources of Zn, Cu and Mn with hydroxychloride sources in layer diets can improve feed stability, egg production rate and FCR.

Table 1 - Laying performance and eggshell reflectivity precentage in response to dietary treatments.

Item	Feed Intake (g/b/d)	HDEP (%)	Egg Weight (g/egg)	Egg Mass (g/d)	FCR (g feed/g egg)	Shell Reflectivity (%)
Inorganic	114.3	90.3[b]	63.1	57.0[b]	2.019[a]	19.4 [a]
HyC	114.8	92.6[a]	63.2	58.6[a]	1.973[b]	18.3 [b]
SEM	0.449	0.496	0.211	0.361	0.012	0.397
P-value	0.397	0.002	0.726	0.005	0.016	0.001

[a–b] values in a column with no common superscripts differ significantly ($P \leq 0.01$).

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MODES OF ACTION OF PHYTOGENICS TO SUPPORT LAYING HEN PERFORMANCE

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Summary

Phytogenic feed additives (standardised blends of plant-derived bio-active compounds) have big potential to improve laying hen performance via diverse modes of action (like stimulating secretions of digestive juices, enhancing transport proteins in enterocytes, and anti-oxidant and anti-inflammatory effects). These natural feed additives can increase production performance and resilience of high producing laying hens against intestinal and environmental challenges. In this paper, modes of action have been elucidated, with a focus on anti-oxidant and anti-inflammatory effects and on heat tolerance.

I. INTRODUCTION

Over the last few decades, laying hen performance potential has largely been improved by genetic selection: egg production increased from 11.8 to 21.0 kg eggs per hen housed, FCR reduced from 3.29 to 1.96, mortality from 19.1 to 5.6% and body weight from 2.42 to 2.20 kg between 1970 and 2000 (Jeroch, 2011). Production cycles of 100 weeks without moulting will become commercially feasible based on improved laying persistence, although egg shell quality is still a key limitation, especially in older brown layers. Global egg production increased from 41.0 to 69.8 million tons between 1994 and 2014; 20.2 million tons of this increase (70%) was realised in the Asia-Pacific region (Windhorst, 2017).

The enormous increase in performance potential can only be realised with optimum nutrition, management and health. Studies have shown that highly productive layers of commercial breeds perform considerably better under optimal conditions than indigenous breeds, whereas performance is similar under low-input and environmentally harsh conditions (Pym, 2013). Therefore, trends like increased poultry production in (sub)tropical regions and changing from cage to non-cage housing systems, like barn and free-range systems in temperate zones, will challenge the production environment for these highly productive layers. This will require additional measures to improve bird resilience under sub-optimal conditions. Feed additives can improve nutrient digestion and utilization and support hen performance. At the same time, feed additives based on plant-derived bio-active compounds, like essential oils, herb extracts, oleoresins, tannins, and saponins, are still a rather newcomer to laying hen nutrition. They can improve hen production, not only by increased nutrient digestion, but also by supporting hens' resilience against environmental and intestinal health challenges, e.g. via anti-oxidant and anti-inflammatory effects. However, it is important to standardise the content of the main active compounds in these natural feed additives to 1) ensure minimum effective concentrations, and 2) prevent too high concentrations of plant components that have potential anti-nutritional effects, like tannins and saponins.

II. NUTRIENT DIGESTION AND EGG PRODUCTION

The effect of a combination of pungent and bitter substances, essential oils and saponins (marketed as Biostrong®510, Delacon, Austria) on nutrient digestibility and production performance was tested in Lohmann Brown laying hens from 22 to 29 weeks of age (24 individually housed birds per treatment). Birds were fed a corn (42.7%) / wheat (25.0%) /

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soybean meal (19.0%)-based mash diet, supplemented with phytase and xylanase. The basal diet was optimised to meet the birds' nutrient requirements and produced as one batch. Half of the diet was supplemented with the aforementioned phytogenic feed additive (PFA) composition (150 g/MT), whereas the other half was fed without. Production performances were measured weekly. At 29 weeks of age, ileal digestibility of crude protein, amino acids, fat, calcium and phosphorus was measured (pooled samples of three birds per treatment). Results are given in Table 1.

Table 1 - Production performance from 22 to 29 weeks of age and ileal nutrient digestibility values at 29 weeks of age in brown laying hens, fed a control diet as such or supplemented with a phytogenic feed additive (PFA).

	Control		PFA		
	Mean	SD	Mean	SD	P
	Production performance				
ADFI, g	116.9	4.2	117.4	3.2	NS
Nº of eggs/h	47.5	2.3	49.1	1.6	**
Egg mass, g/h	2724	148	2848	116	***
Egg weight, g/egg	57.3	2.2	58.1	1.4	NS
FCR[1)]	2.406	0.108	2.312	0.104	**
	AID of nutrients and amino acids				
Crude protein	70.2	3.2	75.4	2.0	*
Lysine	74.1	2.9	80.2	1.8	*
Methionine	78.5	2.9	82.3	2.7	NS
Threonine	59.2	4.0	69.2	2.8	*
Total AA	73.2	3.0	78.5	1.9	*
Crude fat	70.4	4.0	73.5	2.0	NS
Calcium	70.2	3.8	72.0	2.2	NS
Phosphorus	58.4	1.8	59.2	1.1	NS

NS: Not significant; *: P<0.05; **: P<0.01; ***: P<0.001

[1)] High FCR values are due to onset of lay: Average FCR from 25 to 29 weeks of age were 2.261 and 2.142 for the control and PFA supplemented diet, respectively.

The PFA improved apparent ileal nutrient digestibility values significantly for protein and amino acids and numerically for the other nutrients, resulting in improved egg production and FCR. Positive effects of phytogenics on nutrient digestibility are related to increased activities of pancreatic and brush border enzymes by pungent and bitter substances in the feed (Platel and Srinavasan, 2004). Malayoglu et al. (2010) and Hashemipour et al. (2013) confirmed enhanced pancreatic enzyme activities in the intestine of 21-day old broilers by dietary supplementation of oregano essential oils or a thymol/carvacrol mixture, respectively. Reyer et al. (2018) found increased jejunal mRNA levels of peptide and amino acid transporters in 21-day old broilers after supplementing their diets with an essential oil blend from star anise, rosemary, thyme and oregano, quillaja saponins or a combinations, which are all part of Biostrong®510. These results could be further corroborated in an *in vitro* study with Caco-2 cells, incubated with the same phytogenic actives, showing a significantly increased transporter recruitment of SGLT1 and PEPT1 to the membrane by these phytogenics.

III. HEAT STRESS AND INTESTINAL INTEGRITY

Studies on the efficacy of phytogenic actives are often focused on anti-oxidant and anti-inflammatory effects. These modes of action confer the birds' resilience to heat stress and are closely linked with intestinal integrity (Liu et al. 2016). Mignon-Grasteau et al. (2015) concluded in a meta-analysis based on 131 published papers that chronic heat stress in laying

hens mainly affected feed intake, hen-day egg production, egg mass and egg shell strength (Table 2). The impact of heat stress was dependent on hen genotype, age and group size. In their study it was shown feed intake is already affected when ambient temperatures exceed 25°C.

Table 2 - Mean production performance of laying hens housed at thermoneutrality (15-29C) and under heat stress (30-35C), after Mignon-Grasteau et al. (2015).

	Thermo-neutral	Heat-stressed	Delta (%)	P
Feed intake, g/d	112.8	87.3	-22.6	***
Egg production rate, %[1]	86.9	77.1	-11.3	***
Egg weight, g	58.1	53.9	-7.2	***
Egg mass, g/d	48.5	44.1	-9.0	***
FCR[1]	2.313	2.224	-3.9	***
Shell strength, g	3513	3009	-11.3	***
Shell thickness, mm	0.363	0.344	-5.3	***

[1] On transformed scale; *** P<0.001

High ambient temperatures induced oxidative stress in Hy-line brown hens, as shown by increased malondialdehyde levels in plasma, liver tissue and egg yolk. Heat stress inhibited follicle development mainly manifested as reduced egg production rate and ovary weight. In addition, progesterone production in granulosa cells, and follicle development were reduced and reactive oxygen species (ROS) levels increased (Yang et al., 2012). Adverse effects of heat stress could be alleviated by dietary supplementation with 250 IU/kg vitamin E as anti-oxidant (Yang et al., 2012). Others have shown that phytogenic actives like curcumin, ameliorated heat stress in quail via modulation of the Nrf2/HO-1 pathway and cellular repair mechanisms like heat shock protein (HSP) 70 levels (Sahin at el., 2012), whereas Mueller et al. (2012) showed in 35-day old broilers that sulforaphane (from broccoli extract), and essential oils from turmeric, oregano, thyme and rosemary stimulate the Nrf-2 driven anti-oxidant response element (ARE)-regulated genes, resulting in increased systemic anti-oxidant capacity. Apart from anti-oxidant efficacy, curcumin also exhibits anti-inflammatory effects via reduction of the inflammatory transcription factor nF-κß (Sahin et al., 2012), thereby affecting the oxidation-inflammation cascade. Anti-inflammatory effects of these phytogenic actives enable development of alternative natural feed additives promoting intestinal health as shown in broilers (Van der Klis et al, 2018).

Heat stress mediated damage to the intestinal barrier is responsible for increased morbidity and mortality of animals due to uncontrolled transfer of endotoxins across the intestinal wall (Pearce et al. 2013). Varasteh et al. (2015) indicated that HSPs play a crucial role in maintaining the localization of tight junction (TJ) proteins. During heat stress, an upregulation of TJ gene expression is observed to compensate for the loss of damaged TJ proteins (Pearce et al. 2013, Liu et al. 2016, Varasteh et al. 2015). Rosemary oil, as part of an essential oil mixture (Reyer et al. 2018) showed distinct benefits on preservation of transepithelial electrical resistance in CaCo2 cells, cultivated in transwell-dishes (Figure 1) in heat-stressed Caco-2 monolayers.

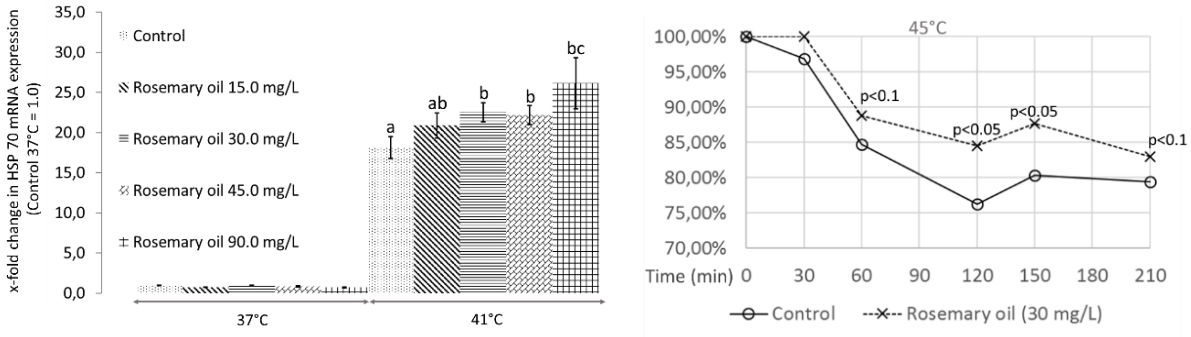

Figure 1 - Left side: Support of heat shock response in heat-stressed CaCo2 monolayers through addition of different concentrations of rosemary oil. Right side: Development of initial transepithelial electrical resistance (TEER) in heat-stressed CaCo2 cells, cultivated in transwell dishes, without phytogenic substances (Control) or with the addition of 30 mg rosemary oil/L culture medium.

IV. CONCLUSIONS

Phytogenics (plant-derived bio-active compounds) have clear potential to support hen production by increased nutrient digestion and by anti-oxidant and anti-inflammatory effects. The latter are important modes of action to improve hens' resilience against environmental and intestinal health challenges. Formulating phytogenic feed additives requires extensive *in vitro* screening, dose optimization and *in vivo* validation. Standardization of the contents of the main active compounds in these natural feed additives is crucial to ensure efficacy.

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RELATIONSHIP BETWEEN PRODUCTION TRAITS AND EGG QUALITY OF INDIVIDUAL ISA BROWN HENS

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Summary

For maximum efficiency in egg production to be attained, laying hens must maintain consistency in production traits and quality of eggs produced. However, there is a lack of information on the relationship between production traits and egg quality in laying hens. A six-week study was conducted to investigate how production traits are associated with egg quality in individually caged ISA Brown hens. Forty-five birds, aged 25 weeks, were randomly selected from a population of 450 hens and monitored for average daily feed intake (ADFI), egg production and egg quality. Eggs were collected from all experimental hens (n=45) once a week over the six-week experimental period and were assessed for egg quality. Egg quality and production data from individual hens were investigated using correlation analysis. The results of the study showed that feed conversion ratio (FCR) was negatively correlated with albumen weight (r = -0.50; $P < 0.01$) and positively associated with yolk percentage (r = 0.41; $P < 0.01$). There was a moderate positive relationship between egg mass and albumen height (r = 0.32, $P \leq 0.05$) and between egg mass and yolk height (r = 0.44, $P < 0.01$). Yolk colour was seen to be positively related with both FCR (r = 0.38, $P \leq 0.05$) and ADFI (r = 0.38, $P \leq 0.05$). There was no significant relationship observed between body weight (BW) and egg weight (r = 0.09, $P > 0.05$) or egg mass (r = 0.14), and between FCR and Haugh unit (r = 0.03, $P > 0.05$). The findings of this study show that there are important associations between some production and egg quality traits in 25-week-old ISA Brown hens. It will be useful to explore the extent and depth of these relationships with larger sample sizes and across different hen laying periods.

I. INTRODUCTION

The profitability of the egg industry at commercial farm level is influenced by key production traits such as feed consumption, feed efficiency and egg quality. These measures of profitability are primarily controlled by genetics, but are also influenced by environmental factors leading up to the onset of lay and beyond. Consequently, inconsistency in voluntary feed intake and hen body weight can result in variations in feed efficiency, egg production and egg quality (Roberts et al., 2015). Recent studies suggest that important relationships exist between egg quality and some production traits such as feed intake, FCR and body weight, in mid lay hens (Akter et al, 2018), whereby hens ranked as feed inefficient lay eggs with lower Haugh unit scores. While many studies have focused on describing the genetic causes of variation in production traits in hens (Liu et al, 2018), there is an information gap on how production traits such as feed intake, laying persistency and feed efficiency relates to egg quality in individually caged ISA Brown hens and little work has been done to describe the extent and effect of these relationships. The objective of this study was to investigate the relationships between variations in production traits of individual laying hens and the quality of eggs produced from the same hens.

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II. METHODOLOGY

Without any exclusion criteria, forty-five individually caged ISA Brown hens, aged 25 weeks, were randomly selected from a 450-layer hen observational study and monitored for six weeks for feed intake, egg production, egg weight and egg quality characteristics. Hens were individually caged in 25 × 50 × 50 cm layer cages. Shed temperature, photoperiod and lighting were managed according to the ISA Brown management guide. A wheat and soybean based layer mash diet containing 16% protein was offered *ad libitum* in metal feeders. Water was offered *ad libitum* through automatic drinkers. Egg production and egg weights were recorded daily. Feed intake was calculated weekly as feed offered minus feed refusals and egg mass and FCR were calculated as well. Eggs from the experimental hens were collected once weekly over the six-week experimental period and analysed for egg quality assessment. Albumen width was measured using a graduated calliper, Kincrome (Australia). Albumen height was measured using an albumen height gauge (Technical Services and Supplies – York, United Kingdom) and yolk height was measured using an AMES tripod micrometre, Waltham, USA. Yolk colour was determined using a DSM Yolk Colour Fan, Switzerland, 2005 – HMB. The albumen was separated from the yolk and each was weighed separately using a digital weighing scale. The Haugh unit score was calculated using the formula $100 \times \log_{10} (h - 1.7 \times w^{0.37} + 7.6)$, where h = albumen height (mm), w= egg weight (g) (Şekeroğlu & Altuntaş, 2009). All data collected for the six-week period were pooled and analysed using the SAS software, Version 8 of the SAS System for 2011. Pearson and Spearman correlation coefficients output for production traits and egg quality measurements was generated using the proc univariate procedure in SAS and coefficient values were considered significant if the P – value was ≤ 0.05.

III. RESULTS

The correlation results of the relationships between production traits and egg quality are presented in Table 1.

Table 1 - Correlation (*r*) between production performance and egg quality parameters of ISA Brown hens (*n* = 45).

	Egg weight	Yolk colour	Albumen height	Yolk %	Albumen weight	Haugh unit	Yolk height	Albumen width
Body weight	0.09	0.22	-0.09	0.14	-0.05	-0.11	0.01	0.10
ADFI	0.06	0.38*	0.18	0.14	-0.04	0.18	0.15	-0.14
Production lay	-0.08	0.05	0.09	0.06	-0.08	0.11	0.23	-0.07
Egg weight	1.00***	-0.18	0.34*	-0.55***	0.94***	0.20	0.42**	0.24
Egg mass	0.75***	-0.08	0.32*	-0.43**	0.70***	0.22	0.44**	0.18
FCR	-0.43**	0.38*	-0.03	0.41**	-0.50**	0.03	-0.09	-0.25

***** - coefficients with a *P* value of ≤ 0.05; ****** - *P* < 0.01; ******* - *P* < 0.001; FCR: Feed conversion ratio; ADFI: Average Daily Feed Intake

The average FCR of the six-week measurement period was negatively correlated with albumen weight, (r = -0.50, P < 0.01) and positively associated with yolk percentage (r = 0.41, P < 0.01), as graphically represented in Figures 1 and 2. There was a slight positive relationship between egg mass and albumen height (r = 0.32, $P \leq 0.05$) as shown in Figure 3. Further, a significant positive correlation was observed between egg weight and albumen weight (r = 0.94, P = < 0.001), and conversely, a negative correlation *(*r = -0.55, P < 0.001), was observed for egg weight and yolk percentage.

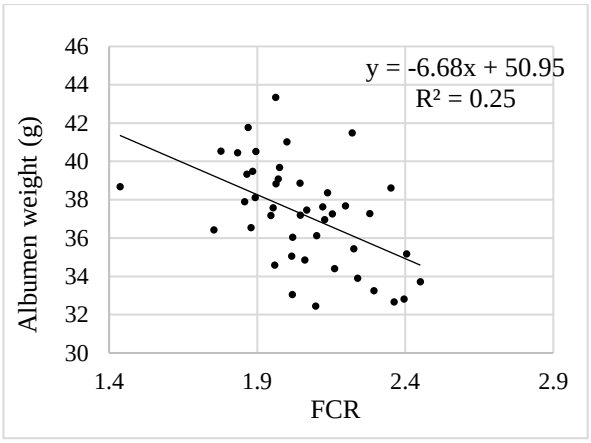

Figure 1 - Association between albumen weight and feed conversion ratio of individually caged ISA Brown hens.

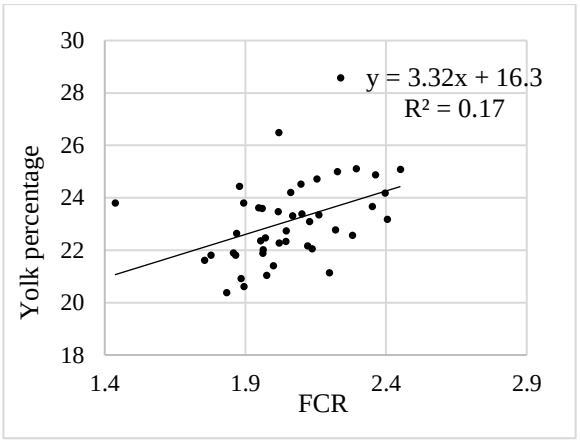

Figure 2 - Association between yolk percentage and feed conversion ratio (FCR) of individually caged ISA Brown hens.

Yolk colour was also seen to be positively related with feed intake ($r = 0.38$; $P \leq 0.05$). There was no significant relationship observed for egg weight and body weight, ($r = 0.09$, $P > 0.05$), as shown in Figure 4, nor between FCR and Haugh unit ($r = 0.03$, $P > 0.05$).

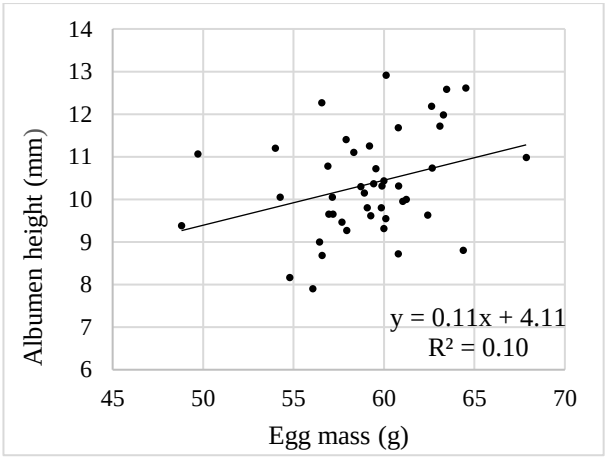

Figure 3 - Association between albumen height and egg mass of individually caged ISA Brown hens.

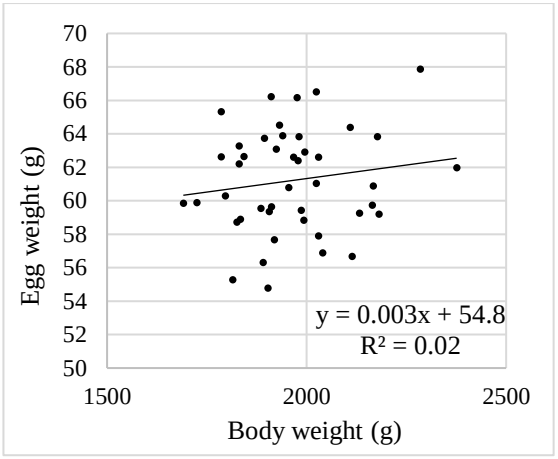

Figure 4 - Association between egg weight and hen body weight of individually caged ISA Brown hens.

IV. DISCUSSION AND CONCLUSION

The objective of this study was to investigate the relationships between production traits of individual laying hens and egg quality. From the results, FCR was seen to be negatively correlated with albumen weight and positively associated with yolk percentage. The weight of the egg albumen is accepted to be directly related with egg quality, as a generous amount of albumen is required to protect the egg yolk from shock damage and microbial contamination. As the feed efficiency worsened, the amount of albumen reduced while the yolk took up more of the egg mass. This is in agreement with Akter et al. (2018) who reported that more efficient hens (hens with a lower FCR) produced eggs which had higher albumen weights and quality, compared to eggs from hens having a higher FCR. Our results showed a negative association between FCR and egg weight a significant positive correlation was observed between egg weight and albumen weight; and conversely, a negative relationship between egg weight and yolk percentage. and a positive significant association between egg weight and albumen weight. The average weight of a chicken egg is around 60g, with 58% of the total weight being

contributed by the albumen (Washburn, 1979). The negative relationship observed between FCR and egg weight therefore suggests that low feed efficient hens (hens having a higher feed conversion ratio) produce smaller eggs which invariably have a lesser albumen weight and consequently, may be of a lesser quality. The results of the study also reported a slight positive relationship between egg mass and albumen height and a moderate relationship between egg mass and yolk height. The height of the albumen, associated with Haugh unit, and the height of the yolk of an egg are indicators of its quality. The higher the heights of the albumen and yolk, the higher the quality of the egg. This agrees with Şekeroğlu & Altuntaş (2009), who reported that albumen and yolk heights increased with an increase in egg weight. Yolk colour was seen to be positively related with the average daily feed intake. Yolk colour is influenced by xanthophylls, which are naturally present in certain feed ingredients or supplied artificially to the hens in the diet. This relationship therefore agrees that as hens consumed more feed, the amount of xanthophyll deposited in the yolk increased, hence the darker the yolk colour score. The non-significant relationship between body weight and egg weight suggests that body weight, even though it varied amongst hens, has little or no causal relationship with egg weight in early lay hens. There was no significant relationship observed for FCR and Haugh unit. Significant relationships were demonstrated between production traits and egg quality of individual 25-week-old ISA Brown hens, but, there was no relationship observed for the body weight of hens and the eggs weight. Further investigation with larger sample sizes, across the different hen laying periods is suggested as this may provide better understanding of the links between production traits and the quality of egg produced.

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LAYER NUTRITION – A FUTURE VISION

R. KLEYN[1]

Summary

The potential of laying hens continues to improve, and the birds that will populate our farms by 2020 will be capable of producing 500 eggs at 100 weeks of age. Knowing exactly how to feed these hens will be a challenge as we will not have gained access to test material before they arrive. It is possible that new genomic methodologies will help to overcome some of the issues, such as oviduct degeneration, but there is no assurance of this. Although overall egg number will increase, bird size will remain largely unchanged and daily egg mass output will probably be lower. Nutrient requirements may well decrease, and eating patterns will change. The challenge will be to maintain hen health and the production of quality, saleable eggs until 100 weeks of age. How this will be achieved is largely speculative.

I. INTRODUCTION

Forecasting is fraught with difficulties associated with poor data and unknown unknowns. Fortunately, in the case of laying hens, this is not true. We have an excellent history of how our birds have improved over the past decade (Table 1), and a fair idea of what can be expected in future breeding programmes used by the primary breeding companies. Our understanding is that the requirements for energy and protein per unit of output have remained unchanged and that different genotypes utilise dietary components in a similar manner (Morris and Njuru, 1990; Lopez and Leeson, 2005; Kimiaeitala et al., 2017). The real challenge will be how to feed birds that are widely called 'long-life' layers. These hens are expected to lay 500 eggs by 100 weeks of age and are likely to be on farms by 2020 (de Keijze, 2014). Indeed, some reports of this target being achieved in practice are already filtering through. These birds will be in production before we can take the opportunity to determine their peculiarities and specific nutrient shortfalls. Trials carried out in the past may no longer be applicable as the genotypes used are not representative of the modern bird. This paper will deal briefly with the matter of genetic change and how this will alter the nutrient requirements of hens. More importantly, some speculation about how we should feed the long-life layer will be raised.

II. GENOTYPE AND NUTRIENT REQUIREMENT

Continuous selection for improved egg production is the pre-eminent selection criterion applied to laying hens, with an annual increase of two to three eggs expected. The key parameter is improved persistency, which entails selecting birds that lay longer clutches of eggs (Preisinger, 2018; Rubinoff, 2018). Egg size has decreased, which is a conscious decision made to ensure egg shell quality late in lay. Although egg size increases with age, this is not accompanied by a proportional increase in shell weight, leading to decreased shell thickness. Egg mass output has increased during the entire laying period, accompanied by lower feed intakes that lead to improved feed efficiency. Feed usage has dropped by 22% per egg produced over the past 20 years. Alternative production systems add a new dimension, and diets will need to be formulated to ensure nutrient intake under different conditions. Hens can cope well under alternative systems, provided that they are permitted to consume adequate feed (Pottgüter, 2013).

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Table 1 - Some historic data for the Hy-Line Brown, taken from the Management Guides of the company (average figures used).

Characteristic	2002–2004	2016
Feed consumed in rear	6	5.75
Body weight 17 weeks (kg)	1.43	1.40
Age at 50% production (days)	145	140
Body weight 60 weeks (kg)	2.0	1.96
Feed intake at 60 weeks (g/d)	116	112
Hen-housed eggs (60 weeks) (%)	249	285
Hen-housed production at 60 weeks (%)	83	85
Hen-housed eggs (110 weeks)		508
Average egg weight 60 weeks (g)	66.3	63.7
Egg output at 60 weeks (g/bird d)	55	54.1
Cumulative egg output 60 weeks (kg)	15.4	15.5
Kg feed/kg eggs	2.06	1.87

At first glance it would be easy to assume that the nutrient requirements of laying hens have increased as flock egg output improves. However, bear in mind that although the modern bird has been selected for increased persistency, it still lays a single egg daily. The hen's nutrient requirements should be considered on a daily basis. Since our hens are at least the same size, if not even a little smaller, with a reduced daily egg output (see Table 1), it is probable that daily nutrient requirements may have decreased rather than increased. Rubinoff (2018) reports that the birds we will see on our farms by 2020 will be slightly heavier.

The modern bird purportedly consumes less feed, but it is difficult to be sure of this from published data. Practically, for example, a large commercial operation in South Africa, using the Hy-Line Brown, is achieving an average feed intake of 116 g/bird/d, so feed intakes may not have declined significantly. Birds coming in to peak production are sometimes not capable of consuming enough feed, forcing them to draw on body fat reserves as an energy source. If this is inadequate, flocks exhibit a typical 'post-peak' dip. This represents a major problem for modern layer genotypes (Pottgüter, 2016) and impacts on the lifetime performance of the bird. Feeding low-density, high-fibre rearing feeds helps to train young hens to achieve higher feed intakes and ensures adequate carcass fat deposition. Practical experience has taught us that little can be done to the diets offered in the layer house to overcome this consumption problem as its origin is during rearing. In the case of broiler breeders, high protein diets during rearing result in reduced carcass fat (Van Emous et al., 2014; Soumeh et al., 2018). It is likely that a similar pattern will exist in laying hens, which means that the use of high protein diets during rear may be the incorrect strategy to follow.

III. ENERGY REQUIREMENTS

The energy requirements of laying hens will continue to be driven by the need for maintenance (determined by body weight), egg output and feather cover. Glatz (1998) demonstrated that poorly feathered brown egg layers consumed 19% more feed than birds with good feather cover. It has long been understood that laying hens are able to meet their energy requirements by simply adjusting their feed intake (Morris, 1967; Kleyn and Gous, 1988). Recent research indicates that this adjustment may not be perfect, and that energy intake tends to increase slightly with higher dietary energy levels. This may or may not have a link with social interaction or competition within the cages. To all intents and purposes, however, the layer of the future is likely to be able to consume adequate feed to meet its energy requirements. Considerable interest has been shown in the use of split feeding, where diets fed in the morning are rich in energy, and those fed in the afternoon are rich in protein and Ca. Despite this, hens

seem capable of regulating their feed intake from the various diets to achieve the same energy and nutrient intake as birds on a continuous diet. Little advantage is to be gained from split feeding as birds do not appear to be able to adjust their feed intake based on a fraction of a day (Traineau *et al*., 2015).

IV. PROTEIN REQUIREMENTS

The requirements for protein and amino acids are less well understood, which subjects the inexperienced nutritionist to many different opinions on the requisite quantities. It is perhaps worth starting this discussion by dealing with those aspects that are known:

- The provision of the correct level of essential amino acids in the diet is of concern. The inclusion of a crude protein minimum is unlikely to lead to increased egg numbers, but it will increase egg size. The rule of thumb suggests that, for each additional gram of protein a bird consumes, the egg size will increase by 1.4 g.
- We have a reasonable idea of what a correct amino acid profile should look like.
- We should always be profit driven when deciding on dietary levels of amino acid. It is unlikely that a single recommendation will ever be correct for all circumstance. Differences in the cost of ingredients and the value of the egg produced preclude this outcome.

Achieving adequate protein intake will be less of a challenge than might be expected, as the birds are likely to have a reduced requirement on a daily basis, coupled with an ability to simply consume more feed. The danger lies in overfeeding protein, which will cause the egg size to increase with the concomitant shell quality problems. Paradoxically, the production of big eggs will become more difficult because the innate ability of hens to produce large eggs has decreased.

V. CALCIUM AND PHOSPHORUS

During their lifetime, hens will deposit 30 to 40 times as much Ca in egg shells as is present in their own skeleton. The shell is formed in the uterus as an extracellular process, governed by the proteins responsible for Ca transport and by the establishment of the pH gradient needed for crystal formation. Some proteins are secreted and integrated into the shell where they regulate the calcification process and become part of the organic shell matrix. Approximately 5.5 g of Ca carbonate is deposited into each eggshell in a 17–20-hour period, making it one of the fastest bio-mineralisation processes known. The P content of the eggshell is small (20 mg) when compared to the egg content (120 mg).

During the later stages of rearing, Ca and P are deposited into the medullary bone (as crystalline hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) while, during the laying cycle, Ca is 'withdrawn' from the skeleton. Early interventions stimulate bone development during the rearing stage. Neijat et al. (2018) found that birds reared in an aviary system (as opposed to cages) had a heavier total bone weight. Dietary nutrient levels may be less important than management. Jing et al. (2018) demonstrated that a low P feeding regime had no effect on growth parameters or bone characteristics. Notably, the diets used in this study did not contain phytase.

Maturity of the medullary bone begins when the oestrogen levels rise at the onset of sexual maturity (when the wattles colour up) and is completed by about 30 weeks of age. The magnitude of daily Ca requirements cannot be met by dietary sources alone, forcing the hen into a daily ritual of bone remodelling. Regardless of dietary Ca supply, hens use this reserve to supply up to 1 g of Ca per day (Leeson, 2017). During shell formation, 60–75% of Ca in the shell is of dietary origin and the remainder is drawn from skeletal stores. If there is insufficient bone reserve, egg shell quality declines fairly quickly. Issues of Ca depletion in the bird may have more to do with metabolic Ca insufficiency rather than dietary deficiency (Fosnaught, 2009).

Mineral nutrition is complicated because hens are able to utilise minerals differentially, depending on their dietary level. Clunies et al. (1993) were able to measure Ca retention in laying hens in the range 36–62%, depending on the Ca level in the diet. It is often erroneously believed that only dietary Ca has any bearing on shell quality. Therefore, when shell quality problems arise, it is tempting to increase dietary Ca and, thus, limestone in the diet. Improvement may be observed, but more often there is none. Increasing dietary Ca in late-lay may increase shell strength and bone strength if the bird retains adequate levels of Ca in the medullary bone. Pongmanee et al. (2018) demonstrated that supplementing layer diets with 600 FTU of phytase (twice the normal level) increased bone strength and prevented bone loss throughout the laying cycle.

Broiler chickens can adapt to the dietary challenges brought about by low dietary Ca by improving Ca uptake at a later stage. This improvement is achieved through modulation of certain genes that encode intestinal Ca and P transporters (Rousseau et al., 2016). Leeson (2017) suggests that the same phenomenon may exist in laying hens. This was supported by the finding of Ieda et al. (1999) that feeding low Ca diets doubled the levels of Ca binding protein (CaBP-D28k) mRNA, in the intestine. If the young hen is fed too much Ca (no more than about 3.5 g/day is required), the Ca uptake system appears to lose its ability to become more efficient in the face of increased demand. This aspect is of particular importance when feeding hens during extended lay, when hens are required to become more efficient utilisers of Ca.

The strong interaction between Ca and P cannot be ignored. Diets low in available phosphorus lead to reduced blood P concentrations which stimulate the synthesis of the active vitamin D_3 metabolite, $1.25(OH)_2$. This in turn stimulates intestinal P absorption, as well as intestinal Ca absorption, even when blood Ca levels are normal (Wideman, 2015). Substantially lower levels of dietary P than are commonly applied in commercial diets have been researched (Snow et al., 2005). Lambert et al. (2014) found that a retained P intake of 2.6 g/kg was adequate until 65 weeks of age; thereafter, this intake needed to increase to 2.8 g/kg. Both values are far lower than recommendations by Hy-Line (2014). Ahmadi and Rodehutscord (2012) reported that the addition of phytase (300 FTU/kg) lowered the laying bird's requirement for dietary P.

VI. FEEDING THE LONG-LIFE LAYER

By 80 weeks of age, it is estimated that the bird will have produced 24.1 kg of eggs, comprising 7.1 kg of yolk and 2.1 kg of shell (after Hy-Line, 2016). At this point, we would expect the birds to produce an egg a day (the best birds will achieve this) for a further 20 weeks. Clearly, feeding the long-life layer will be a challenge. The greatest hurdle will be maintaining egg quality with regard to both its internal characteristics and shell quality. Using genomic tools, breeders will be able to address the issues of declining albumen quality, egg shell colour, shell breaking strength, bone quality (although this is difficult to measure) and, importantly, the weight and efficiency of the oviduct (De Keijze, 2014; Bain et al., 2016).

Managing the long-life layer begins before the hen is 'old'. Nutritional support will be required throughout the hen's life, preventing post-peak dips for example. It is difficult to know when birds can be considered old but, for the purposes of this paper, it will be assumed to be 60 weeks of age. From a physiological perspective, the areas that will require attention are the maintenance of good plumage, the maintenance of skeletal integrity including Ca reserves, the maintenance of a fully functional oviduct, and the management of the health and function of the bird's liver. Liver management will be the key aspect in supporting production and egg quality (Rutten, 2018). Nutritionists will be required to focus on providing the correct levels of nutrients throughout the production cycle. Although the requirements of the individual hen will

not have changed much, feed specifications and feeding programmes may need to be adapted as eating patterns change and flock output (persistence, in particular) increases.

As old age approaches, the cells in the hypothalamus that control the levels of oestrogen produced by the hen become less efficient. Oestrogen plays a crucial role in the maintenance and performance of the medullary bone, as well as the growth and maintenance of the oviduct. The net effect of the decline in oestrogen production is a depletion of medullary Ca and an oviduct that is damage-prone and less well developed (Bain et al., 2016). Differences between individuals in this regard show that phenotype extends to the physiological level, which is what the geneticists will be able to focus on in order to improve both aspects (Dunn, 2013).

During rearing, the pullet is required to develop robust organs and a healthy, well-functioning gastro-intestinal tract. Structurally, the bird will have an ideal frame size and body weight, with the correct body composition (sufficient fat reserve). Importantly, the Ca deposits in the medullary bone should be maximised. Once the bird begins to produce eggs, it is difficult to replenish the Ca reserves in the medullary bone and, once the circulating oestrogen levels begin to decline, replenishment of these reserves becomes more difficult. Thus, Ca reserves decline steadily as the bird ages. At the point where they become depleted, osteoporosis occurs, shell quality declines and production ultimately ceases, regardless of dietary Ca levels. Any insult to the bird's Ca supply during early lay will deplete lifetime skeletal reserves. If dietary Ca levels are too high in early life, there is a possibility that the bird will not respond to increased Ca levels later, although this needs to be confirmed experimentally.

Maintaining good plumage and the reduction of feather pecking are further issues that require our attention throughout the life span of the hen. Feather pecking is as much a behavioural problem as a nutritional one. The addition of some fibrous material to the diet is known to reduce feather pecking, as is the rearing of birds at a low light intensity. In addition, slightly elevated dietary methionine, tryptophan and glycine have helped to maintain plumage in laying hens, or at least to reduce injurious pecking (Prescilla et al., 2018). A project in the Netherlands aims to unravel the underlying physiological mechanisms of feather pecking. This will include such aspects as satiety and the activation of the gut-brain axis, as related to nutrient supply and density. Eating and foraging behaviour impact on the motivation for feather pecking which will also be investigated. The intention of the project is to develop practical dietary strategies during rearing to reduce feather-pecking behaviour in later life (Mens et al., 2018).

The liver provides most of the nutrients for the development of the yolk and albumen. It plays a support role in shell formation through its provision of the lipoprotein component of the shell, which contributes to shell tensile strength and elasticity (Pottgüter, 2016). The maintenance of a healthy, functional liver is a challenge. Fatty liver haemorrhagic syndrome (FLHS) is a major issue, particularly in the late-layer. The balance between hepatic synthesis and secretion of lipids is of vital concern in the regulation of hepatic and extrahepatic fat deposition in hens. Liver fat accumulation can be increased by many factors, including nutrition, housing conditions and inflammatory challenges (Shini, 2014).

This subject has received little attention and our understanding is far from complete. Knowledge of liver function is clouded because we draw conclusions from the examination of dead hens – not their live, healthy counterparts. ln order to avoid FLHS and relieve pressure on the liver, at least a proportion of the dietary energy should be supplied as lipid throughout the laying production cycle. Nutrients that promote liver function and the export of fat from the liver are termed lipotropic nutrients. These include methionine, choline, inositol (elevated by the use of phytase), Vitamin B_{12}, biotin, tryptophan, carnitine and selenium. Supplementation with these nutrients has been used as a treatment for FLHS with variable success (Hy-Line, 2017). Betaine is also used to relieve liver metabolism (Pottgüter, 2016). Dietary 25(OH)D_3 and vitamin E have also been shown to limit FLHS in layers (Bouvarel and Nys, 2013). Prevention of FLHS will be more successful than treatment, as is the case with

most nutritional or metabolic conditions. This will require a focus on liver health throughout the bird's life, which is difficult to measure as it requires bird sacrifice. Perhaps the biomarker technology used in human medicine could be applied in this case?

From a nutritional perspective, it seems logical that increasing dietary Ca levels may be beneficial. The recommendation is to increase dietary Ca levels during late-lay to achieve a consumption of about 4.5 or 5 g/hen/day, but research results in this regard are conflicted. Kershavarz and Nakajima (1993) found no benefit from feeding birds a 'step-up' Ca regime, while Star *et al*. (2013) perceived no difference between birds fed diets with 3.5 g/kg or 3.9 g/kg. However, An et al. (2016) found that the percentage of cracked eggs in aged laying hens (70 weeks old) declined as Ca levels were increased, while Nascimento et al. (2014) showed that a high Ca level can have significant detrimental effects on feed conversion and production. It is suggested that the results are confounded by the level of the Ca reserve contained in the medullary bone of the hen.

Practical experience has taught us that the Ca source is more important than dietary levels *per se.* The use of larger, less soluble particles creates a supply of Ca during the hours of darkness, which is when the bird needs it most for shell formation. Rather than increasing dietary Ca levels, the daily feeding of coarse grit may be more effective. Another alternative is to implement a split feeding regime, with diets high in Ca being fed in the afternoon. However, Molnár et al. (2018) found that split feeding did not maintain shell quality, although it did improve relative shell weight. Al-Zahrani and Roberts (2015) showed that the addition of 1 g of 25(OH)D_3 resulted in the highest shell weight, percentage of shell, shell thickness and lowest shell deformation in Hy-Line Brown layers (aged 19–80 weeks), but 0.5 g/ton did not have a significant outcome. Most commercial diets probably contain more than enough phosphorus. In the late-layer, the real danger lies in feeding too much P since high levels in the blood inhibit the mobilisation of Ca from bone.

VII. CONCLUSIONS

The next generation of laying hens will have reduced daily nutrient requirements but may have different feed intake patterns. Under normal circumstances – even using disparate farming systems - hens will be able to achieve their genetic potential provided that they are able to consume adequate levels of nutrients and energy. While we have a fair idea of the essential energy requirements of a laying bird, there is still much debate about the level of amino acid provision. Any decision should be based on the economics of egg production, but we need to bear in mind that it is probably pointless to feed for large eggs when the genetics of the bird have been modified to produce smaller eggs. Remarkably, the requirements for the key minerals Ca and P have not yet been resolved. It appears that we are overfeeding P and that, until such time as we have a better understanding of the dynamics of the Ca reserves in the medullary bones of laying hens, Ca provision will be more guesswork than science.

The real challenge will be in feeding the long-life layer. Current recommendations are based on hypothesis and conjecture and have yet to be tested. Many of the proposals put forward may simply be too expensive for commercial systems. Some issues, namely the degeneration of the oviduct and the way in which Ca is deposited in the medullary bone, will be addressed by the geneticists. The prevention of FLHS will continue to be an issue for as long as we fail to track liver health and function throughout the bird's life. The management of feather cover involves the establishment of good plumage, which is largely determined by nutrient provision and addressing the behavioural aspects of feather pecking. This may be feed related, but it also has much to do with farm management and the bird's innate behaviour. The modern laying hen is an efficient and robust bird, with an almost unbelievable propensity to

produce eggs. The challenges we face in designing feeding programmes for the bird will be overshadowed by the expected improvements in performance.

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THE IMPORTANCE OF GUT MICROBIOTA IN CHICKENS WITH PARTICULAR EMPHASIS ON THE FIELD SITUATION

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Summary

Intestinal systems of living organisms are inhabited by a dense community of microorganisms dominated by bacteria but also containing archea, fungi, protozoa and viruses. The microorganisms within these communities exist in a symbiotic relationship with the host and are collectively known as microbiota. Recent expansion in knowledge about the influence of microbiota on health and disease started a major research revolution in the area. In poultry research, a number of ground-breaking publications have significantly expanded our knowledge and understanding of poultry health. In this mini-review, we focus on poultry gut heath issues and propose solutions for improving birds' health by refining their intestinal microbiota health and stability.

I. INTRODUCTION

The recent major drop in the cost of DNA sequencing, combined with advances in the annotation of 16S rRNA gene microbial databases and analysis tools, have all contributed to the "golden age of microbial ecology" (Oakley et al., 2014). Poultry researchers have leveraged and applied these advances to define approaches that may be taken to manipulate microbiota to improve a number of poultry production steps. One of the major issues in poultry microbiota research is the extent of flock to flock variability which makes both health and nutritional treatments difficult to reproduce among the flocks (Stanley et al., 2013b). A key contributor to this variation is removal of maternal microbiota transfer via use of modern hatching practices. Removal of antibiotic growth promoters left the industry looking for alternative approaches to control pathogen growth, and new methods for improving intestinal microbiota membership. It is very difficult to change a mature intestinal microbial community; therefore, the first days of bird's life are likely to be the key intervention point to permanently reducing pathogen load and encouraging beneficial bacteria (Stanley et al., 2014). In addition, maintaining low levels of stress and operating production systems close to optimal are prerequisites for better bird health. As in other symbiotic relationships, host and microbiota have a two way relationship, if the host suffers the microbiota will not be left unchallenged.

II. FIRST DAYS OF LIFE

Until recently we assumed that chicks are fully sterile *in ovo*. Recently, that opinion has been challenged and a low level colonisation before birth is being suggested for both chickens (Kizerwetter-Swida and Binek, 2008) and humans and other mammals (Perez-Munoz et al.,, 2017) alike. Moreover, if the *in ovo* colonisers of the bird embryo include opportunistic pathogens, the embryo is likely to die before hatch (Jahantigh, 2010), similar to the spontaneous abortion due to foetal infection in humans (Perez-Munoz et al., 2017). There is an abundance of studies that have used *in ovo* probiotic injection to improve immunity and vigour of hatchlings without any negative effects on hatching rate and with a number of health benefits reported (Pender et al., 2017; de Oliveira et al., 2014).

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Despite possible low-level colonisation *in ovo*, major steps towards formation of gut microbiota starts immediately post hatch and is determined by the bacteria present in the chick's immediate environment during the first days of life. Unlike in humans where the microbiota is considered stable and formed by the toddler age, the timeline for microbiota establishment in broiler chickens is significantly reduced and well established during the first week post-hatch (Apajalahti et al., 2016), remains reasonably constant until 30 days of age (Lu et al., 2003); however, slowly increasing in richness and still capable of acquiring new members (Donaldson et al., 2017). Mature microbiota attains a level of stability and the ability to resist change, unlike the immature developing microbiota where significant perturbations, such as antibiotic administration or pathogen exposure, may lead to lifelong health consequences (Zhou et al., 2018). For example, very low levels of *Salmonella* are needed to permanently colonise the intestinal tract at a young age while old birds show significant resistance to *Salmonella* colonisation. However, transfer of microbiota from older to young chicks increases their resistance to colonisation (Oakley et al., 2014).

The knowledge of rapid colonisation and maturation of microbiota in chicken strongly suggests that it is of utmost priority to reduce pathogen load and increase beneficial bacteria load during the first week of chick's life. Both of these aspects are crucial for intestinal and overall health; just reducing pathogens via maintenance of a very clean environment (Stanley et al., 2013b) is more damaging to the microbiota than early overloading of the bird with beneficial probiotic strains without any pathogen control, which, depending on strains used, can even be beneficial (Baldwin et al., 2018). The inherent variability of poultry microbiota is much more pronounced than in mammalian species due to nearly complete removal of maternal influence. Hatching birds in very clean hatcheries without the presence of poultry adapted microbiota leads to random colonisation and high flock to flock variation (Stanley et al., 2013b). There are currently no published data on the dynamics of natural microbiota seeding from mother hen to the chicks.

Exposure of birds to the microbiota of previous well performing flocks reduces the effects of random colonisation and microbiota variability among the birds but does not fully transfer donor microbiota and performance, mostly depending on the ability of specific core microbiota species to colonise and persist (Donaldson et al., 2017). In the United States, bacterial diversity is encouraged by litter re-use from the previous flock, providing adequate and rich inoculum to the new flock. The litter carryover would be detrimental if the previous flock was of mildly compromised heath due to latent low level pathogen infection which could then be amplified in the subsequent flock due to early exposure. Regardless, high levels of hygiene in the first days and depletion of maternal microbiota adapted to the chicken host over thousands of years appears to be an unforeseen and undesirable outcome of modern day hatchery practices.

III. SPATIAL AND TEMPORAL FLUCTUATIONS

Introducing significant changes to microbiota does not really need a very influential variable. Even the most insignificant treatments will cause temporary change in gut microbiota. It is well recognized in human research that gut microbiota of each individual changes slightly on a daily bases. In chickens, faecal microbiota is so variable (Stanley et al., 2015; Stanley et al., 2013b) that even replicates of a single sample can show variation and faecal matter is often visibly non-homogenous. Faecal microbiota from the same bird will vary slightly due to periodic emptying of different gut sections; however, core faecal microbiota remains fairly constant (Stanley et al., 2015). The caeca is the gastrointestinal site with the most diverse microbiota in birds and is more stable in composition than the faecal microbiota; it has therefore been a preferred sampling site when birds are sacrificed at the end of a trial.

There is a range of host factors that influence development and stability of intestinal microbiota. The majority of studies about poultry microbiota have been done in broilers, generally in birds less than 42 days of age, so the available information represents the microbiota of younger chickens. Considerable difference is reported between broilers and layers. However, to our knowledge, there has only been one published study comparing broilers and layers hatched together and reared in the same facility, using randomised design, on the same feed and environment (Han et al., 2016), showing that there are significant genetic breed influences between layers and broilers, on intestinal microbiota composition (Ranjitkar et al., 2016; Videnska et al., 2014). Bird sex is also a strong variable when considering microbiota studies, extremely so in sexually mature birds (Wilkinson et al. 2016).

Environmental factors (reviewed by Kers et al., 2018) such as housing, including individual rearing conditions and ambient environmental conditions as well as the type of production system, can influence microbiota composition. Access to free range opens a path for ingestion of soil and insect associated microbiota. Other factors shown to influence microbiota profiles in poultry include litter quality, type and management, lighting schedule, access to feed, climate and quality of temperature and humidity control.

IV. MANIPULATION THROUGH NUTRITION

Feed is the source of nutrients and energy for both host and microbiota growth and homeostasis, and consequently a very strong microbiota modifier. Macronutrients have a very significant influence on microbiota composition: proteins induce expansion of bacteria taxa that are very different to the ones that prefer to grow on carbohydrates. Poultry nutrition is already well-designed for the optimum productivity in both layers and broilers and manipulation of poultry health through manipulation of poultry microbiota can be achieved via feed additives. The most notable example is addition of Antibiotic Growth Promoters (AGPs), a practice that was used in the poultry industry for over 6 decades (Dibner and Richards, 2005). The primary function of AGP addition was demonstrated productivity improvement and control of enteric pathogens like *Clostridium perfringens*. Although AGPs were used in low, sub-therapeutic doses and were different from antibiotics used in human medicine, recent concerns over possible linkage between AGP use in the livestock industries and the rising level of antibiotic resistance in human pathogens has resulted in a ban on the use of AGPs in many countries and the Australian poultry industry is voluntarily removing AGPs from a majority of farms. The need for pathogen control, especially fear of necrotic enteritis outbreaks, is encouraging the industry to look for alternatives to AGPs with proven ability to control major poultry pathogens. Pathogen control leads to better bird health and often results in better performance. Herbs and spices with known antimicrobial properties (Cetin et al., 2016; Scocco et al., 2017) as well as other natural products such as biochar, bentonite and zeolites (Prasai et al., 2016) are also being tested as pathogen controlling feed additives and giving promising results. However, the major microbiota modifying additives in poultry feed are still probiotics and prebiotics.

VI. PROBIOTICS AND PREBIOTICS

Probiotics are widely used in agriculture for their proven health benefits; however, individual farmers report very variable success using commercial products. This is expected knowing that intestinal microbiota can vary at a phylum level even in flocks of the same genetics, reared under same conditions using the same feed batch (Stanley et al., 2013b). Flocks with microbiota differences at such high magnitude would respond differently to probiotic administration in feed. Commercial probiotics targeting the livestock market include *Lactobacillus*, *Enterococcus*, *Bifidobacterium* and *Streptococcus* species (Fuller, 1989); however *Bacillus* is

emerging as a more reliable alternative because of its favourable resistance to feed preparation procedures, and independent of viability issues due to its sporulation nature.

One of the major benefits of probiotic action is their ability to assist the host microbiota during pathogen invasion. In order to launch successful infection, pathogens must either colonise or, if latently present, activate and expand. The host, including the symbiotic microbiota, will also launch a range of defences (Patterson & Burkholder, 2000). Probiotics may also assist via the mechanism of competitive exclusion and the production of antibacterial products such as very efficient antibacterials– bacteriocins as well as toxic metabolites such as hydrogen peroxide. The host will launch immune responses to prevent pathogen translocation from the gut and often amplify rapid periodic emptying of the small intestine which leads to pathogens being washed out (Patterson and Burkholder, 2003).

There is an abundance of research showing beneficial effects of probiotics in *in vitro* assays against pathogens and in controlled bird trials. However, running a chicken trial in a research facility environment often uses administration of freshly grown probiotics or a batch validated by the industry for high probiotic isolate viability. Birds are generally grown under optimal, ethics committee approved conditions, which exclude extreme stress. Sometimes those birds are challenged with a pathogen to inspect the efficiency of the probiotic strain/product. In reality, on the farm, birds are often not under such ideal conditions, exposed to more stress, and harbouring very different microbiota in their gut. These differences, especially in layers, in our own experience from sampling a number of major layer farms, are of a very high magnitude. Probiotics thus immediately encounter very different intestinal environments in terms of the inhabitants and their metabolite driven chemistry. In addition to that, the viability of commercial probiotic products and the ability to withstand acid and bile varies greatly due to the intense processing, such as freeze drying (Dodoo et al., 2017) which can alter not just viability but also their probiotic properties (Archacka et al., 2019).

Viability as well as acid/bile tolerance is overcome by the use of sporulating *Bacillus* probiotics and the research on benefits of such probiotic formulations intensified in the last few years. Despite the obvious *Bacillus* production advantage, there is no need to give up on the development of beneficial lactic acid bacteria and bifidobacteria species. Preservation methods need to be further optimised towards the targeted place of delivery (Dodoo et al., 2017), for example, although they may be spread through multiple gut sections, *Lactobacillus* often targets the small intestine while *Bifidobacterium* use less digestible polysaccharides and the more anaerobic environment in the large intestine. Probiotics are suggested to be more effective when combined with prebiotics – so called synbiotics (Mookiah et al., 2014). Prebiotics are dietary carbohydrates, particularly oligosaccharides and polysaccharides, resistant to intestinal enzymatic digestion but fermentable by intestinal microbiota. They selectively modulate the microbial populations or activity of intestinal bacteria, promoting health and wellbeing of the host (Gibson et al., 2004; Gibson and Roberfroid, 1995). Prebiotics are a nutritional source for beneficial intestinal microbiota that produce short chain fatty acids (SCFAs). Appropriate delivery combined with specific probiotic optimised prebiotic mixes will result not only in better gut heath and pathogen reduction but will also benefit the productivity of birds (Angelakis, 2017). Healthy intestinal microbiota is characterised by a high beneficial/pathogenic species ratio. Probiotics administration can help maintain that ratio, especially if the probiotics are delivered from the first days of life, which can allow them to colonise first and, most importantly, colonise intestinal mucosa and remain permanently protective (Baldwin et al., 2018).

VII. MICROBIOTA AND HOST METABOLISM

The demonstrated correlation between intestinal microbiota and growth and productivity indicates that the microbiota can positively influence the metabolism of the host (Stanley et al., 2012; Stanley et al., 2013a; Stanley et al., 2016). Short Chain Fatty Acids (SCFAs) (Byrne et al., 2015) and bile salts (Krogdahl, 1985; Maldonado-Valderrama et al., 2011) are two of the major metabolites mediating the impact of microbiota on the host metabolism. Intestinal microbiota produces SCFAs by fermentation of digestive-enzyme-resistant non-starch polysaccharides. Although SCFAs affect body weight gain and adiposity in mice (Ridaura et al., 2013; Liou et al., 2013), possibly through appetite regulation and energy homeostasis, their effects on feed intake and energy metabolism in chicken are yet to be fully revealed. However, the beneficial effects of SCFAs as antimicrobials and growth promoters have been studied to some extent.

Bile salts (bile acids conjugated to glycine or taurine) also affect energy homeostasis (Watanabe et al., 2006; Watanabe et al., 2012). Host and intestinal microbiota, through their bile salt hydrolase (BSH) activity, together determine the size of the bile salts pool in the intestine. Although the role of bile salts in lipid, cholesterol and glucose metabolism in mammals has been well studied (Thomas et al., 2008; Joyce and Gahan, 2016), these metabolites have received less attention in poultry research. One of the suggested modes of action of AGPs to improve growth rate in poultry is lowering of BSH activity in the intestine (Guban et al., 2006; Feighner and Dashkevicz, 1987). As we are gradually phasing out AGPs from poultry diets, non-antibiotic BSH inhibitors could be another area of research to find alternatives for AGPs.

VIII. STRESSFUL LIFE OF CHICKENS

Intestinal microbiota influences the gut-brain axis and can be altered when the host is exposed to periods of stress. Both broilers and layers can be exposed to periods of metabolic stress by factors such as oscillations in environmental conditions, sudden loud noises, transportation, beak trimming, intestinal pathogens, feed change, to name a few. Broilers are, however, harvested at a young age, while in layers environmental challenges can persist over longer periods of time. Living in a large flock, small disturbances in social hierarchies, inability to use a preferred nesting box, and other social behaviour induced anxiety can be a problem with layers. Based on the abundance of human research on the influence of anxiety on gut microbiota, as well as certain microbiota causing and amplifying anxiety (Neufeld et al., 2011), it is reasonable to suggest that this connection is valid in chickens. Stresses can also lead to intestinal permeability issues and this has recently becoming a topic of interest in poultry research with a number of gut permeability models being investigated by poultry researchers (Gilani et al., 2017; Kuttappan et al., 2015).

VIII. CONCLUSIONS

The last decade of intensive research into poultry microbiota has resulted in a large scientific literature and knowledge repository now being available to poultry researchers and the industry. Despite the inherent complexity of microbiota-host interactions, we are now well into a stage of transition towards applying this knowledge in solving some major issues in poultry production, such as pathogen control, bird immunity and AGP removal. Removing or controlling flock to flock and bird to bird microbiota variation via early post-hatch microbiota remodelling, appears to be one of the ways towards growing healthier more productive birds.

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HOST AND MICROBIAL BIOMARKERS FOR INTESTINAL HEALTH AND DISEASE IN BROILERS

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Summary

When broilers are produced without in-feed and preventive antibiotics, gut health syndromes, diseases and associated performance losses are rather common. While it is easy to diagnose diseases such as coccidiosis and clinical necrotic enteritis, more subtle gut health disorders affecting animal performance are more difficult to diagnose. Poor performance is often associated with changes in gut morphology such as reduced villus length as well as increased inflammatory conditions. This is associated with microbial shifts in the intestinal tract, a condition also referred to as dysbiosis. Veterinarians diagnose this condition by macroscopically evaluating gut wall appearance, which is time-consuming and invasive. Because of the technological developments in meta-omics, (host and bacterial) proteins, (mainly bacterial) metabolites and (bacterial) DNA sequences correlating with gut health can be identified. Host biomarkers for gut health found in faecal material are related to cellular damage, leakage of serum proteins in the intestinal contents and inflammation. These may be (semi-) quantified using ELISA or even dipstick methods on-farm. DNA sequences can be quantified using qPCR methods that are also currently available in carry-on equipment. These tools can be used to determine the gut health status of animals and to predict animal performance. They can be used to make a decision whether or not to apply feed additives or other interventions that promote gut health.

I. INTRODUCTION

One of the most sustainable types of production of animal protein is chicken meat production. Chicken production needs less feed consumption per kilogram of produced meat and uses less land and water for both farming and feed production. The major reason for this is the continuous improvement of animal performance, reflected in an ever decreasing feed conversion (kg feed consumed per kg body weight) and reduced time to achieve market body weight (Zuidhof et al., 2014). Continuous improvements in performance parameters include genetic selection for high-performing chicken lines, technological developments in hatching and housing conditions, and feed optimization and management practices that support (intestinal) health. Among the latter, the use of antimicrobial growth promoters is a practice that has been banned in many countries worldwide but the use of therapeutic antimicrobials in the animal production industries is still high, though decreasing. This has created a situation in which the animal and its microbiota are experiencing a big change, as the animal breeds have been used for more than 50 years almost exclusively in a production system where antimicrobial usage was common practice. Reducing or stopping this practice has resulted in different diseases and syndromes, most of which are of intestinal origin. Indeed, about 60% of therapeutic antibiotic usage in broilers is to control intestinal diseases. The move away from antimicrobials has led to increasing concerns about gut health. Bacterial diseases, enteritis, dysbiosis, and poor digestibility are a consequence resulting in poor growth performance of birds. In fact, all these entities have common denominators in the form of microbial shifts that go hand in hand with epithelial permeability increases, inflammation and thus performance

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losses, and are often related to nutrient excesses in the intestine or feed-derived issues (poorly digestible nutrients, excess of energy or protein levels). The most important intestinal disease entities and syndromes in broilers, with a performance effect, are briefly described in the next paragraph.

II. INTESTINAL DISEASES AND SYNDROMES IN BROILERS

The most severe example of a disease that has emerged in broiler chickens after the ban on growth-promoting antibiotics in animal feed is necrotic enteritis, which imposes a significant economic burden on the poultry industry worldwide (Skinner et al., 2010; Kaldhusdal et al., 2016). This disease is typically caused by nutritional excesses in the gut as well as predisposing epithelial defects caused by mycotoxins and coccidia, and it is occurring in animals with the highest body weight gain, so clearly related to production parameters (Moore, 2016; own unpublished data). The causative agents of necrotic enteritis are netB-toxin containing *Clostridium perfringens* (type G) strains (Rood et al., 2018). Necrotic enteritis can occur as an acute clinical form which is characterized by a sudden increase in mortality, and as a subclinical form which results in a lower weight at slaughter age. In both cases, macroscopic necrotic lesions are found at the mucosa of the small intestine upon necropsy, and thus the intestinal barrier is compromised and severe mucosal inflammation occurs (Prescott et al., 2016).

A disease syndrome that has clearly emerged in the EU broiler industry simultaneously with the ban of growth promoting antibiotics is the so-called 'dysbacteriosis'. This is a poorly described condition of the gut and may or not be a synonym for conditions such as 'wet litter', 'non-specific bacterial enteritis', 'small intestinal bacterial overgrowth', 'malabsorption', and many more. The common clinical denominator is thinning and ballooning of the small intestine, increased water content of faeces and reduced digestibility of feed with undigested residues visible in the faeces (Teirlynck et al., 2011; Ducatelle et al., 2015). In many cases, this is linked to increased feed conversion, decreased body weight and thus poor performance. Moreover, wet litter leads to various additional disease conditions such as pododermatitis, breast blisters and 'hock burn', which are criteria used to evaluate animal welfare. It is generally believed that 'dysbacteriosis' is a condition in which the interaction between the gut microbiota and the host is impaired, such that the gut health is not optimal. All this is probably influenced by nutrition and it is suggested that the altered composition of the gut microbiota induces changes in the gut wall, including morphological changes (villus length decreases, crypt depth increases, epithelial cell damage, …) and inflammatory reactions (infiltration of immune cells in the wall). The combination of a suboptimal microbiota combined with effects on the gut wall would then most likely interfere with digestive processes, eventually leading to poor performance, and induce enteritis.

As broilers often have gut barrier integrity issues (increased permeability), toxins, feed antigens, but also bacterial products and bacteria can cross this barrier and spread systemically. This also aids locomotory diseases that are a consequence of both the high body weight gain and the pressure this puts on the skeleton of the animal, but also of bacteria that attach to bones at different sites in the body. Indeed, lameness in broiler chickens is a significant animal welfare problem, which is increasingly occurring (up to 1% of all animals). Bacterial chondronecrosis with osteomyelitis (BCO) is a disease characterized by bacterial infection in rapidly growing bones under repeated mechanical stress and typically occurs in tibiae, femora and the thoracic vertebrae (Wideman, 2016). The terminology is often confusing and names such as 'kinky back', spondylitis, spondylolisthesis, femoral head necrosis and others are given to describe similar or the same syndromes. It is assumed that bacteria cross the intestinal barrier, enter the bloodstream and hematogenously spread to osteochondritic clefts or to microfractures at the growth plates. When colonizing the growth plates, the bacteria are rather

inaccessible to antibiotics and the host immune system, enabling them to induce necrosis. Bacteria that are found in BCO lesions are commensal intestinal bacteria that have translocated through the intestinal epithelium and have spread systemically. Bacterial genera and species that are isolated from BCO cases are, amongst others, opportunistic bacteria including staphylococci, *Escherichia coli*, and enterococci. These kind of disease entities are thus again originating from high performance and at least partly have an intestinal origin. It has been shown that probiotics can affect BCO, again pointing to the intestine as origin of the bacteria that cause the disease (Wideman et al., 2015).

III. MEASURING INTESTINAL HEALTH IN BROILERS

Intestinal health is a term that is not yet clearly defined, despite being a focus of major research efforts in the last decade, both in human and in veterinary medicine. It can be described at different levels. In the past, one has used different indirect systems to measure gut health, such as the water content of faecal material. At macroscopic level, optimal gut health can refer to a condition in which there are no observable changes in gut wall appearance as compared to a normal condition. While this is very clear in conditions in which gross lesions are seen, such as in necrotic enteritis and coccidiosis cases, this is less clear and even invisible in conditions that might cause microscopic alterations that affect performance. A method to score gut wall appearance has been validated previously (Teirlynck et al., 2011) and is used by veterinarians for broiler chickens. In this system, in total, 10 parameters are assessed and scored 0 when absent or 1 when present during visual inspection of the intestinal wall at autopsy after which the animal will receive a total score between 0 and 10. Zero represents a normal gastrointestinal tract and 10 the most severe form of dysbiosis. The parameters are (1) 'ballooning' of the gut; (2) inflammation, redness, of the serosa and/or mucosal side of the gut, cranial to the Meckel diverticulum; (3) macroscopically visible and tangible fragile small intestine cranial to the Meckel diverticulum; (4) loss of turgor in longitudinal cutting of the intestine cranial to the Meckel diverticulum within the 3 seconds after incision; (5) abnormal occurrence of the intestinal content (excess mucus, orange content, gas) cranial to the Meckel diverticulum; (6,7,8,9) are identical to (2,3,4,5) but caudal to the Meckel diverticulum and (10) is presence of undigested particles caudal to the ileo-cecal junction. A low gut wall appearance score thus indicates good gut health. This system however is rather subjective, as it depends on the person who performs the scoring and it is influenced by specific factors such as diet type (e.g. meal vs pellet with regard to the presence of undigested feed particles). It has, however, been described that the score is associated with histological parameters under certain conditions, including villus length and infiltration of immune cells in the gut wall (Teirlynck et al., 2011). These parameters are much more objective, quantitative and clearly associated with gut health, as they relate to the epithelial surface (villus length) and thus digestibility, and with inflammation. As such, they are associated with intestinal insults that damage the epithelial lining and thus affect performance. Although histological parameters have value in evaluating gut health, these are mainly of importance under experimental conditions (e.g. when testing interventions) and are more difficult to use in field conditions, given their invasive and time-consuming nature. Optimal gut health could thus also be defined as a condition in which no microscopically visible alterations are seen. Other invasive biomarkers are mainly ones that can be found in blood. Acute phase protein (APP) production in the liver can be the result of intestinal bacteria that cause inflammation in the gut, and thus cytokine production by epithelial and immune cells that is sensed by liver cells that produce APP. It can also be the consequence of translocation of bacteria and their products through the gut wall reaching the liver, so that hepatocytes secrete the APP. APP can be measured in serum, but the production of APPs can be triggered anywhere in the body of the animal and not specifically the gut (Langhorst et al., 2008;

Eckersall and Bell, 2010). Other biomarkers that could potentially be found in the serum are of microbial origin. Increases in intestinal permeability in poor gut health conditions would lead to translocation of bacteria, LPS and even metabolites such as D-lactate, that can be found in serum. These markers however do not seem to be very reliable because of multiple reasons, including intrinsic differences in the intestinal concentrations on itself that may cause variability in the serum levels (Ducatelle et al., 2018 for more detailed references).

Non-invasive markers are preferred in the field and ideally they should be based on faecal material as this is easy to collect. In addition, mixed faecal samples can be taken so that the gut health status of the whole flock can be evaluated. These markers can be microbial or host-derived. Microbial markers originate from the observation that gut health problems often are associated with shifts in the microbial composition. This has been described in detail for human inflammatory bowel disease, in which *Enterobacteriaceae* have been associated with inflammation and butyrate producing bacteria from the *Ruminococcaceae* family, such as the genus *Faecalibacterium*, have been shown to be depleted in the faeces of diseased individuals (Machiels et al., 2018; Rivera-Chavez et al., 2017). While changes in microbial composition are clear in the case of severe intestinal inflammation, differences can be much more subtle in intestinal disorders with a much less clear phenotype, such as irritable bowel syndrome in humans. The same is true for chickens, in which rather well-described microbial composition shifts have been described in the gut of animals with necrotic enteritis, but, despite numerous studies, it is not easy to identify OTUs that are correlated with intestinal health and animal performance (Stanley et al., 2016). Our group has conducted a number of studies using intestinal inflammation models to describe 16S rDNA sequences that have a correlation with intestinal health (i.e. villus length, immune cell infiltration in the gut wall, and performance) and some general patterns of beneficial and harmful microbial groups can be extracted from these data. Examples are correlations between the reduced abundance of *Faecalibacterium prauznitzii* and *Butyricicoccus pullicaecorum* and conditions that increase the villus length and decrease the $CD3^+$ T-cell infiltration in the small intestinal wall of broilers under experimental challenge conditions (unpublished), but many more relevant OTU changes occur. In addition to the use of microbial composition and taxa, one could also use functional genes or metabolites as markers. The most well-known example of a beneficial microbial metabolite is butyrate, and functional genes such as the butyryl-CoA:acetate CoA-transferase can be used to quantify the abundance of butyrate producing bacteria in faecal samples (Onrust et al., 2015; De Maesschalck et al., 2015). A lot of other metabolites are involved in gut health and this is a domain in which much progress can be made. Measurements of epithelial permeability can be done using oral administration of compounds that pass through the epithelial layer when damaged and thus can be measured in serum (e.g. FITC-dextran, lactulose/rhamnose) (Ducatelle et al., 2018 for references). This is not applicable to field conditions. Host biomarkers for gut health should ideally be associated with gut function, such as digestibility, cellular damage and inflammation, amongst others. In humans, calprotectin, a neutrophil granule protein, is used to quantify gut inflammation and is very useful to assess the severity of intestinal inflammation (Ayling and Kok, 2018). For poultry, our group recently identified a similar protein biomarker in colonic content of animals from an inflammation model. Other markers were identified and were related to inflammation, serum leakage, epithelial cell and tight junction damage. Currently, these are being brought to a practical field assay using ELISA or dipstick assays. An example is ovotransferrin, a protein that is produced in the liver and thus only reaches the faeces when serum leaks through the epithelial cells. The concentration of ovotransferrin increases with the severity of necrotic enteritis and coccidiosis infections, and has been associated with gut damage in not yet published dysbiosis models (Goossens et al., 2018).

IV. APPLICATIONS AND PERSPECTIVES

Easy-to-measure biomarkers in faecal samples are of value for the poultry industry for various reasons. First, they can be used to measure gut health in field conditions and justify interventions to promote gut health. These can be the administration of antimicrobials when animals suffer from a diagnosed bacterial infection but can also be the supplementation of a gut-health promoting feed additive when the animals are not having symptoms. The latter is often the case, and prediction of poor performance will likely be the most important driver for using gut health biomarker tools. Ideally, diagnostic tests for gut health result in the choice of specific feed additives or changes in feed management, depending on the parameter that is affected. Another important application of gut health biomarkers is efficacy testing of newly developed feed additives by the industry. While faecal biomarker proteins (host proteins) and microbial markers (taxa and metabolic pathway genes) have been identified in experimental models (necrotic enteritis, gut inflammation, and more), field applications are likely not to be straightforward for various reasons. Apart from the technical aspects (dipstick or ELISA development), field conditions are very different in different regions worldwide and it might be complex as a lot of preventive antimicrobials are still used. It will be a challenge to convince poultry producers to use a diagnostic tool to justify use of antimicrobials, as the latter are often a cheap and easy certainty for production performance. Educating the poultry production industry on the risks of antimicrobial usage, antimicrobial resistance, and the need to use a strategy of measuring gut health and using non-antibiotic prevention methods is already occurring but needs further effort.

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TRANSCRIPTOMIC MODIFICATIONS CAUSED BY SUBCLINICAL NECROTIC ENTERITIS IN BROILER CHICKENS

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Phasing out of in-feed antibiotics in the poultry industry has caused necrotic enteritis (NE) to become a primary concern in commercial poultry production in many countries. Damage to the intestinal mucosa caused by this disease leads to impaired broiler productivity. To gain a better understanding of the impact of NE on transcriptome changes, a whole transcriptome sequencing (RNA-seq) was performed on jejunal RNA of challenged broilers to identify the pathways affected by this disease.

For this study, jejunal tissue samples were obtained from a previous experiment conducted by Rodgers et al. (2015) from NE challenged and unchallenged broilers (Ross 308 males). Briefly, to induce NE, birds in the challenge group were inoculated with 1 mL mixed *Eimeria* species on day 9 followed by 1 mL of approximately 10^8 CFU of a pathogenic strain of *C. perfringens* type A on days 14 and 15. Birds in both groups were fed similar wheat-based diets. On day 16, one bird from each pen was randomly selected and euthanised to collect 2 cm of jejunum for RNA extraction. RNA-seq was performed by AGRF© using the extracted RNA of four birds of each experimental group. cDNA libraries were synthesised following the TruSeq RNA library preparation protocol and sequenced on an Illumina HiSeq200 platform. After quality assessment, cleaned reads were mapped to the reference genome *Gallus gallus* (Ensembl) using TopHat2. The differentially expressed genes (DE) were identified by contrast control and treatment using a TMM normalization in edgeR and the voom function in limma (adjust P-value ≤ 0.05 and $\log_2 FC \geq 1$). The DE genes were used to perform functional analysis in DAVID and to find protein-protein interactions with STRING.

A total of 377 genes was differentially expressed; from those genes 207 were up-regulated and 170 were down-regulated. In this study, PPAR signaling, essential in the regulation of cellular differentiation, metabolism and fatty acid degradation was the enriched pathway. The challenged birds showed upregulation of LCK ($P < 0.01$), TMEM173 ($P < 0.01$), and ZAP70 ($P < 0.05$) which are all related to T-cell function and inflammation. From the protein-protein interaction network, downregulation of genes ECI2 (necessary in fatty acid degradation) ($P < 0.001$), ALDH2 (takes part in different amino acid pathways and also glycogenesis) ($P < 0.001$) and EHHADH (essential in many different pathways such as bile acid biosynthesis, fatty acid degradation and also amino acid metabolism) ($P < 0.01$) was observed in the challenge group. These proteins seem to have an important role during NE infection by interacting with multiple genes. It could be concluded that NE challenge affects different pathways and protein signaling, especially related to digestion and inflammatory responses, both reducing nutrient absorption that can subsequently be the reason for impaired growth in NE challenged birds.

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EFFECTS OF DIETARY PROTEIN AND BALANCED AMINO ACID LEVELS ON BIRDS UNDER SUB-CLINICAL NECROTIC ENTERITIS CHALLENGE

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One benefit of low protein (LP) diets is improving gut health in a future of antibiotic-growth-promoter free diets. Drew *et al*., (2004) found an interaction between dietary crude protein (CP) and proliferation of *Clostridium perfringens*, a pathogenic bacterium identified to cause clinical and sub-clinical necrotic enteritis (NE), which costs the poultry industry US$6 billion dollars annually (Wade and Keyburn, 2015). Keerqin *et al*., (2017) observed improvements in body weight with excess digestible amino acids (AAs) from intact protein in birds challenged with sub-clinical NE. The current study aimed to investigate the effects of CP increasing dietary AAs on the performance of birds challenged with sub-clinical NE. Day-old male Ross 308 breeders (n = 648) were fed a common starter diet *ad libitum* from d 0 - 7 followed by grower treatment diets until d 21. All diets were wheat based with sorghum, soybean meal and canola oil; essential amino acids were supplemented when limiting according to AMINOChick®2.0 AA recommendations; glycine was supplemented to 15 g/kg glycine + serine. A 2 x 2 x 2 factorial arrangement was used to determine the effects of NE challenge, standard (SP) and LP diets, and 100 and 115% dietary AAs. Four isonitrogenous and isocaloric dietary treatments were used, including a SP diet at 216 g/kg CP and a LP diet at 171 g/kg CP with AAs at 100 and 115%. On d 7 all birds were allocated to pens of equal weight with 14 and 13 birds per pen in the challenged and unchallenged pens respectively. Challenge birds received 1ml *per os* of *Eimeria spp*. at d 9; followed by viable growth of (approx. 10^8 CFU/mL) *C. perfringens* EHE-NE18 *per os* 1 mL/bird consecutively at d 14 and 15 to induce sub-clinical NE. Unchallenged birds received sham treatments of PBS and sterile broth at d 9 and d 14 and 15 respectively. Weekly pen and feeder weights were recorded and on d 16 two birds per pen were euthanised to determine jejunal lesion score. Data underwent statistical analysis (Minitab® 17.1.0) using a stepwise general linear model ANOVA with statistical significance determined using Tukey post-hoc test. Lesion scoring data were distributed non-normally, therefore were analysed using Kruskal-Wallis nonparametric test to test for significance. Challenged birds had significantly ($P < 0.001$) lower body weight gain (627 vs 881 g), feed intake (956 vs 1121 g) and higher feed conversion (1.530 vs 1.277). The challenge was further confirmed by the presence of jejunal lesions ($P < 0.001$) in challenged birds. Reduction of CP significantly lowered body weight gain (984 vs 813 g) in the unchallenged group. However, no significant difference occurred within the challenged group between SP and LP treatments (640 vs 614 g). The supplementation of excess AA showed no significant difference in performance ($P > 0.05$) at this stage of growth within CP or challenge groups. Reducing CP to 171 g/kg from 216 g/kg or supplementing 115% AAs did not suppress the effects of sub-clinical NE in broilers as of d 21.

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THE INFLUENCE OF TWO DIETARY CALCIUM AND PHYTASE LEVELS ON THE PERFORMANCE OF BROILER CHICKENS CHALLENGED WITH NECROTIC ENTERITIS

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Calcium is an important cation in chicken diets, being the most abundant element in the body of the chicken. Its functions include: mineralization of bones, blood clotting, enzyme activation, neuromuscular function, muscle contraction, and intracellular signaling. However, high dietary Ca is a limiting factor for phytase efficacy and the formation of insoluble Ca-phytate complexes decreases Ca and P availability. Furthermore, Williams (2005) has shown high dietary Ca concentration in the gastrointestinal tract to be associated with pathogenesis of necrotic enteritis (NE). This study investigated the hypothesis that high dietary calcium would decrease phytase efficacy and decrease performance in chickens either challenged or unchallenged with NE. Ross 308 male broiler breeder (n=768) were randomly distributed to 8 treatments in a factorial arrangement. Factors were: calcium level (0.6 or 1.0%), phytase level (500 or 1500 FTU/kg) (Quantum Blue^{TM}, AB Vista, Malborough, UK) and NE challenge (no or yes). There were 48 pens, 16 birds per pen and 6 replicates per treatment. Performance was measured weekly with the exception of feed intake (FI) and feed conversion ratio (FCR) which were not measured on d 21. The FCR was corrected for mortality (FCRc). Half of the birds (384) were challenged with 5000 unattenuated sporulated oocysts each of *E. acervulina*, *E. brunetti* and *E. maxima* (Bioproperties Pty Ltd) on d 9, and 10^8 CFU per mL of *C. perfringens* strain EHE-NE18 (known to express NetB toxin, CSIRO) on d 14 and again on d 15.

Body weight was reduced as a result of challenge for every time point measured post challenge ($P < 0.05$), being 10.7% lower on d 42. Challenge also increased FCRc on d 28 ($P < 0.001$) and d 35 ($P < 0.01$). There was no Ca effect on body weight or gain at any time point ($P > 0.05$). Low and high Ca FCRc was on d 14, 1.205 vs 1.176 ($P < 0.05$), d 28, 1.556 vs 1.507 ($P < 0.05$) and d 35, 1.478 vs 1.399 ($P < 0.05$). Low and high Ca FI was: d 28, 2259 vs 2161 g ($P < 0.05$) and d 35, 3205 vs 3052 g ($P < 0.05$). Body weight (BW) was higher in birds fed the high phytase level compared to the low phytase level: d 14, by 23g ($P < 0.05$), d 21, by 41g ($P < 0.05$), d 28, by 66g ($P < 0.05$), d 35, by 100g ($P < 0.05$), d42, by 69g ($P < 0.05$). Birds fed the high phytase level had greater livability than those fed low phytase on d 0-21, 89.9 vs 92.3%, ($P < 0.05$). Birds fed the diet with high phytase and high Ca had the lowest FCRc, d 7, 1.079 ($P < 0.05$) prior to NE challenge. Birds not challenged with NE and fed high phytase and high Ca had the highest weight gain on d 42. The lowest weight gain was observed in NE challenged birds fed diets with low phytase and low Ca on d 42.

The results reject the hypothesis that high calcium decreases phytase efficacy and performance of birds challenged or unchallenged with NE. The result evaluated as FCR (FI to live bird wt) resulted in the same conclusion.

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ALTERNATIVE FEED INGREDIENTS FOR POULTRY DIETS: CHALLENGES AND PROSPECTS

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Summary

Poultry production is the fastest-growing animal protein industry globally. Production of poultry products, especially of chicken meat, is expected to surge substantially in the future to meet the increasing global preference for healthy meat. This predicted future growth will have a profound effect on the demand for conventional ingredients, and will, considering the finite available resources, pose ever-increasing pressure on the poultry feed market. The search for alternative raw materials and strategies to maximise their use, although not a new phenomenon, will continue to gain more attention to address this challenge. However, identifying the potential alternative ingredients and overcoming their nutritional challenges are complex issues and require multi-dimensional strategies.

I. INTRODUCTION

A number of factors including the global population growth, shift towards high protein diets, finite land availability for crop production, climate uncertainties, increase in fossil fuel prices and diversion of feed ingredients, especially of cereals, to biofuel manufacture presents challenges to the global feed supply. This is a major threat for sustainable poultry production, in both the developing nations, as they move towards animal protein food regimen due to rising incomes, and developed countries that rely heavily upon a limited number of soybean meal producing nations (USA, Argentina and Brazil) (Fitches et al., 2017). According to forecasts, the demand for poultry feed will increase at a compound annual growth rate of 7.1% from 2018 to 2027, with the sale volume expected to grow beyond 890 million metric tonnes by 2027. Therefore, it is clear that the future requirements for traditional feed ingredients (maize and soybean meal) cannot be met, even according to optimistic forecasts. As a result, sourcing of quality and sustainable feed ingredients at acceptable prices has become the major challenge for the industry. The most obvious strategy is to search for potential alternative raw materials. Each region has its own range of alternative (or unconventional) ingredients and their feeding value has been researched locally. The commercial use of these ingredients, however, has been limited due to constraints imposed by several nutritional, technical and socio-economic aspects. The intention of this paper is not to review the available literature on individual ingredients that could be potentially used, but rather to provide a critical overview of limiting factors and strategies to maximise their use in poultry diets. The focus will be particularly on four main aspects: (i) definition of the term 'alternative' feed ingredients; (ii) limitations to use of these raw materials in poultry nutrition; (iii) strategies to overcome the nutritional challenges posed by alternative ingredients, and (iv) future prospects.

II. DEFINITION OF THE ALTERNATIVE FEED INGREDIENTS

Besides the ever-increasing demand for quality conventional ingredients, scarcity in supply of these ingredients, market volatility and rising prices (sometimes unprecedented) have always encouraged poultry nutritionists to explore the usefulness of locally available ingredients, so-called "alternative feed ingredients". An alternative, also known as "non-traditional", raw

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material is normally referred to as a feed ingredient that is available locally, but not regularly included in commercial poultry diets, and its nutritional value and optimum inclusion level are not well defined (Dale, 2008). However, it must be noted that the term 'alternative' is a relative expression, depending on the geographical region and the time period, and it is therefore difficult to draw a clear distinction between traditional and non-traditional feed ingredients. Feed ingredients that may be classified as non-traditional in one region, may actually be traditional and widely used in others. For instance, in the United States, Brazil and China (the top three global producers of chicken meat) and many Asian countries where maize is the cereal of choice (traditionally and economically), other cereals such as wheat, barley or sorghum are considered as viable alternative ingredients. Conversely, wheat remains the most commonly used grain in European Union, Canada, Australia and New Zealand with other cereals as potential alternatives during the times of scarcity or rising wheat price. Another example is palm kernel meal (PKM), which is a non-traditional feedstuff in Western Africa, but an increasingly normal feedstuff for poultry in Southeast Asia, especially in pullet and layer diets. Some ingredients may have started as non-traditional but are now being used increasingly in commercial poultry diets. Therefore, a broad definition of an alternative ingredient should also take into account the time period and region. Alternative ingredients, depending on whether or not they are previously known to the industry, can be classified to non-traditional or novel ingredients. Non-traditional ingredients constitute those that have been used only locally and their potential as a global feed ingredient has not been evaluated and acknowledged. A novel feed ingredient, however, can be defined as an ingredient previously unknown to the feed industry that has never been used on a commercial scale.

III. LIMITATIONS TO USE OF ALTERNATE RAW MATERIALS IN POULTRY NUTRITION

It is widely recognised that existing potential feed resources worldwide, particularly in developing countries, have been largely overlooked in many circumstances, and either unutilised and wasted or used inefficiently. Most of these alternative ingredients have obvious potential, but their use has been negligible owing to constraints imposed by nutritional, technical and socio-economic factors (Ravindran and Blair, 1991):

1- Nutritional aspects: variability in nutrient composition and quality; limited information on digestible amino acid (AA) and metabolisable energy content; presence of anti-nutritional and/or toxic factors; high fibre content; low level of energy and poor protein quality; need for nutrient supplementation (added cost); poor palatability.

2- Technical aspects: seasonal and unreliable supply; physical characteristics (bulkiness, powdery texture, dustiness); logistic issues (collection, storage and delivery), requirements for further and relatively expensive processing (de-hulling, drying, physical and thermal processing); limited research and development facilities for determining nutrient availability and optimum inclusion levels in poultry diets.

3- Socio-economic aspects: competition for human consumption; lack of desire from farmers to plant these crops due to low prices relative to other arable crops; high cost per unit of energy or limiting AA relative to conventional sources.

Three major criteria determining the regular use of an alternative ingredient in commercial diets are: i) it must be available in economic quantities, even if its availability is seasonal, ii) the price must be competitive against conventional ingredients, and iii) its nutritive value, variation and digestibility must be understood.

IV. STRATEGIES TO OVERCOME THE NUTRITIONAL CHALLENGES POSED BY ALTERNATIVE INGREDIENTS

Before the application of alternative ingredients can be considered in the commercial poultry production, most, if not all, of their limitations must be resolved. Several possibilities are available for improving the feeding value and increasing the inclusion levels of alternative feedstuffs: a) feed evaluation, with focus on energy and digestible AA, b) formulation of diets based on digestible AA, rather than total AA, c) use of synthetic AA to balance their specifications, d) supplementation with commercial exogenous enzymes to improve nutrient and energy utilisation, and e) application of feed processing techniques.

a) Feed evaluation

Difficulty in assessing the nutritive value of an ingredient, due to the lack or scarcity of appropriate research or analytical facilities, is a major factor discouraging poultry feed manufacturers from considering the use of alternative ingredients. There has been keen interest in evaluating alternative feed resources over the years, especially from developing countries. However, given that only limited published data are available on digestible AA and apparent metabolisable energy (AME) of alternative feed ingredients, routine feed evaluation and continuous update of matrix values are crucial for the efficient use of these ingredients. One of the major challenges for proper feed evaluation is the lack of a standard procedure to be used in the *in vivo* assays. An example could be the use of different methods for measurement of AA digestibility of the feed ingredients for poultry. The digestibility of AA in feed ingredients can be measured using three different methods, namely direct, difference and regression methods (Lemme et al., 2004). The direct method, due to the simplicity of the assay and calculations, is the most commonly used assay (Ravindran and Bryden, 1999). Our recent study with PKM as an unconventional ingredient (Abdollahi et al., 2015), showed that AA digestibilities determined using the direct method were substantially lower than the corresponding coefficients determined by the difference method. This finding suggests that values determined by the direct method might underestimate the AA digestibility of low-protein materials that includes the majority of alternative ingredients.

The use of an appropriate energy system is another critical issue because of the importance of energy to diet cost and bird performance. In a recent review of the procedures used for estimation of the energy content of ingredients for poultry, Mateos et al. (2018) reported extreme variation among published data on the nitrogen-corrected AME (AMEn) content of the feed ingredients. Interestingly, the reported differences in AMEn (MJ/kg) are greater for unconventional ingredients such as sorghum (1.42), barley (1.97), full fat soybean (2.13), rapeseed meal (2.64), sunflower meal (2.85), maize DDGS (3.18) and broken (polished) rice (3.56) than for the conventional raw materials like maize and wheat (1.05) and soybean meal (1.63). Therefore, although there is a wealth of data on nutritive value of ingredients for poultry, these values vary widely for most of the ingredients, in particular the alternative ingredients. The discrepancies among the available data might be due to various reasons but the reported variability justifies the urgent need for consensus on the methodology for energy evaluation, especially of non-traditional raw materials (Mateos et al., 2018). Another major constraint in the endeavour to better define nutrient quality is the lack of rapid tests to determine digestible AA content or AMEn.

b) Formulation of diets based on digestible amino acids

When fibrous and poorly digested ingredients are considered for use, feed formulation on the basis of metabolisable energy and digestible AA is a prerequisite. Digestibility of AA is not

the same in all ingredients; they are well digested in some ingredients while the digestibility is lower in others. The use of digestible AA is particularly relevant to situations where diet formulations consist of a range of alternative ingredients that are poorly digestible. A good example in this regard is the use of PKM in broiler diets. In a recent study (Abdollahi et al., 2015), we evaluated the AME and digestible AA content of a PKM sample using broiler studies. Poor digestibility of protein and AA in the PKM confirmed that formulating PKM-containing diets based on crude protein or total AA content will fail to meet the birds' requirement for a balanced diet and impair the growth performance of broilers. In a follow-up study (Abdollahi et al., 2016), where AME and digestible AA contents of PKM were used to formulate the broiler diets, it was shown that PKM can be included in broiler diets up to 16% with no deleterious effects on growth performance. Using digestible AA contents of alternative raw materials when formulating broiler diets holds promise to increase the range and inclusion levels of these feedstuffs in practical diets, despite the fact that they may contain less than optimal AA profiles and are poorly digested.

c) Use of synthetic amino acids to balance amino acid specifications

The differences in AA digestibility of ingredients can be effectively harnessed to improve the precision of feed formulations and meet the AA requirements. Nutritionists can achieve this effectively nowadays because of the availability and use of feed grade essential AA in synthetic forms. In order to improve the feed efficiency, reduce the nitrogen and ammonia emissions and ensure sustainable poultry production, there has been a huge interest to use reduced protein diets supplemented with synthetic AA. Despite these possibilities, there is no doubt that there would be a limit on how low we can go with dietary protein levels in poultry diets, and the further use of synthetic AA beyond this threshold might not be feasible once the minimum threshold is reached. However, the use of alternative raw materials, due to their high variability and most probably less balanced AA composition and poor digestibility compared to conventional feed ingredients, would motivate the continuous development and use of synthetic AA. When alternative ingredients are included special attention must be given to threonine supplementation. Most alternative ingredients, owing to the presence of high fibre and anti-nutrients and their effect on mucin secretion, will increase endogenous protein losses in the gut. Mucin is rich in threonine and the resultant effect will be increased threonine requirement.

d) Supplementation with commercial exogenous enzymes

Commercial application of biotechnology and acceptance of feed additives in poultry nutrition during the last two decades has offered vast opportunities to enhance nutrient utilisation, feed efficiency and productivity. Perhaps the most important additive to enter the poultry feed market is exogenous feed enzymes. The availability of glycanases (xylanases and glucanases) in the 1990's has effectively overcome the anti-nutritive effects of non-starch polysaccharides (NSP) and enabled the increased use of viscous grains such as wheat and barley in poultry diets. In relation to alternative ingredients, feed enzymes can (i) enable the use of certain ingredients (which otherwise may not be possible), (ii) remove nutritional constraints and enable higher inclusion levels, (iii) increase the range of ingredients used in feed formulations, and (iv) reduce variability in the nutritive value between batches. Enzyme addition, in particular, uplifts the value of poor quality samples and reduces the variation among the samples of a given ingredient.

Enzyme technology will undoubtedly continue to offer new opportunities for the application of alternative and novel feed ingredients in poultry diets. However, a prudent enzyme selection and development strategy will be needed to ensure optimal enzyme match

with its intended substrate. The unique feature of enzymes is that they act only on a specific site on a specific substrate. In terms of alternate ingredients, the implication is that the exact nature of the substrate must be known. However, despite advances in analytical techniques, the chemistry and structure of most target substrates (especially in alternative ingredients) are still poorly defined, which remains a constraint to finding suitable enzymes.

e) Application of feed processing techniques

Feed and ingredient processing, including extrusion and pelleting, can facilitate the use and higher inclusion of alternative raw materials in poultry diets, mostly through inactivation of anti-nutritional factors, break-down of cell wall matrix as a result of the physical stress, especially in fibrous materials, and provision of greater accessibility of encapsulated cellular contents to digestive enzymes. The latter effect was clearly shown by Saunders et al. (1969) in steam-pelleted wheat bran. Microscopic examination of chicken excreta showed that the proportion of empty aleurone cells (utilised contents) was greater and the level of unutilised (residual) protein by birds decreased in the pellet diet than the mash diet.

Extrusion cooking, a high-temperature/short-time thermal treatment, although it has not been used commonly in commercial poultry feed production due to its high capital investment costs, may be a useful processing technique for some non-conventional feed ingredients. Extrusion of improved faba bean cultivars, even though they contain low tannin contents, has been shown to enhance their nutritional value in broilers through reducing the phytate phosphorus (PP), trypsin inhibitor activity (TIA) and resistant starch (RS) contents (Hejdysz et al., 2016). These researchers reported that extrusion of 5 different faba beans decreased the concentration of RS by 94% (10 vs. 182 g/kg), TIA by 50% (0.3 vs. 0.6 g/kg) and PP by 51% (1.9 vs. 3.9 g/kg) compared to raw faba beans. Extruded faba beans were also characterised by higher starch digestibility (0.970 vs. 0.773), fat retention (0.989 vs. 0.872) and AMEn (14.95 vs. 10.79 MJ/kg).

The fundamental objective of pelleting at the time of introduction to the feed industry in the mid-1920′s was to convert fibrous, bulky and less palatable blends of feed ingredients into compact pellets that facilitate easy prehension (Coffey et al., 2016). One of the most momentous changes in broiler diets since the introduction of pelleting has been the shift from fibrous, textured and poorly digestible ingredients, to low fibre, texture-less and nutrient-enriched diets. This transition not only hinders the development, functionality and health of the gastrointestinal tract, but also constrains some of the advantages of pelleting. Our recent studies with maize- (Abdollahi et al., 2018) and wheat-based (unpublished data) diets, have clearly shown that the pellet-associated benefits were more pronounced in low nutrient density diets containing alternative ingredients such as wheat bran, PKM and canola meal, than high density diets based on conventional ingredients. Pelleting can attenuate the negative effects of feeding bulky, fibrous and less nutrient dense diets on broiler growth performance and facilitate higher incorporation of less desirable alternative ingredients into practical broiler diets. However, this potential of the pelleting process is not being efficiently harnessed due to the global shift towards high density diets that demands the use of conventional feed ingredients.

V. FUTURE PROSPECTS FOR ALTERNATIVE FEED INGREDIENTS

Local market conditions and production objectives are major drivers for the application and setting the inclusion levels of alternative ingredients in poultry diets. In countries such as Australia and New Zealand where the poultry production aims towards maximising the biological performance rather than economic performance, the use of alternative raw materials, unless there is scarcity in the supply of conventional feed ingredients, might not be so attractive. However, in some parts of the world where the industry follows a business model of economic-

oriented goals rather than maximum biological productivity, alternative ingredients can make a useful contribution to poultry diets and be included even beyond maximum inclusion limits. Whilst the immediate prospects for the use of alternative ingredients will be in semi-commercial poultry units that employ some degree of on-farm feed mixing, the greatest potential for using these ingredients is in the feeding of layers, irrespective of the production system. Owing to physiological differences, pullets and laying hens are more tolerant to high fibre, poor-quality ingredients and nutritional challenges than fast-growing modern broilers. Rice bran is a good example of this tolerance that can be used at maximum levels of only 10 percent in broiler diets, but may be safely incorporated into pullet and layer diets at levels of up to 30 percent.

There is a potential for fibrous alternative sources to improve the upper gut development and functionality in modern broilers. Enhancing the feed structure through the inclusion of ingredients with insoluble fibre sources holds a promise to mitigate the concerns around the sub-optimal functionality of the upper part of the digestive tract and gut health that has been an issue with feeding highly processed diets and exacerbated in light of antibiotic free poultry production. Layers not only are able to better utilise high fibre ingredients, but a recent study (Abdollahi et al., 2017) also showed that the function of the digestive tract in layers is even more sensitive to feed structure than for broilers and pullets, emphasising the importance and need for high fibre raw materials to be included in layer feeds.

The ban on the use of non-organic feed ingredients in organic poultry production has made this production system an appropriate sector for the use of alternative ingredients. Free range production systems, developed to meet the consumer demand for natural feeding and better bird welfare, may also serve as a sector for the application of alternative raw materials. Slow growing broiler strains, although not the best choice for sustainable agriculture, are becoming increasingly popular especially in Europe and can be a potential market for the utilisation of alternative ingredients.

VI. ALTERNATIVE ENERGY AND PROTEIN SOURCES

With the exception of soybean meal which remains the sovereign protein source in poultry diets globally, other ingredients, including maize and wheat, might be considered as alternative ingredients depending on the geographical location. Although there is an extensive list of raw materials of plant or animal origin that can be used in poultry feeds in different regions, only some of these potential ingredients are highlighted in Table 1. This list is by no means an exhaustive one, but mentions some of the overlooked or undervalued ingredients that have promise for use in poultry diets on a more regular basis.

VII. CONCLUDING REMARKS

Demand for poultry products will continue to escalate over the coming decades. This high demand, along with the fact that chicken is the most sustainable farm animal species, will create an opportunistic scenario for the poultry industry. However, the ever-growing poultry industry should address, among others, the challenge of supplying adequate and sustainable feed resources. The poultry industry should not wait for a crisis, but be prepared, as global feed resources are limited, to meet the quest for sustainable production.

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Table 1 - Some promising alternative ingredients for use in poultry diets.

Feedstuff	GP (MMT)	Major producers	Potentials/ comments	Limitations	Stratgeies to improve the application	Maximum inclusion level
Barley	144	EU, Russia, Ukraine,	Resistant to drought and saline soils	Low palatability and energy content, high fibre content and soluble NSP	Pelleting, enzyme application	28% in broilers and 70% in layers
Cassava	280	Nigeria, Thailand, Indonesia, Brazil, Ghana	High in highly digestible starch, excellent energy source	Low in protein (<3%), high cyanogenic glucosides, dustiness and bulkiness	Detoxification, pelleting, synthetic pigments for egg yolk pigmentation	20% in broilers and 30 to 50% in layer diets
Rice bran	76	India, China, Japan	Relatively high energy	High fibre and phytate (86% of total P), rancidity problems, trypsin inhibitor	Heat processing, phytase inclusion, pelleting	20% in broilers and 40% in layers
Palm kernel meal	10	Indonesia, Malaysia, Thailand, Nigeria	Moderate energy content (7.8-11.1 MJ/kg), source of functional fibre, medium protein	High fibre, low AA digestibility, grittiness and low palatibility	Pelleting, formulation based on digetible AA content	16% in broilers and 30% in layers
Rapeseed meal	76[1]	China, India, Canada, EU, Japan	Good source of protein (36-38%) and sulphur-containing AA	ANFs, glucosinolates and erucic acid, low lysine content	Plant breeding, formulation based on digetible AA content	8% in broiler starters and 24% in broiler growers and layers
Sunflower meal	50[1]	Ukraine, Russia, Argentina, China	Good source of protein (36-40%), rich in sulphur-containing AA, no major ANFs	Lower lysine content than SBM, bulkiness due to high fibre	Dehulling, formulation based on digetible AA content, pelleting	Replacing half the soybean meal in broiler and layer diets
Cottonseed meal	44[1]	India, China, Pakistan, Brazil, Usbekistan	Good source of protein (40%)	High fibre, less energy, AA (with moderate digestibility) content than SBM, presence of gossypol	Formulation based on digetible AA content, addition of ferrous sulphate	Replacing 40% of the protein from SBM in broilers, limit use in layers
Grain legumes	121	Canada, Western Australia, Eastern Europe	Moderate to good source of protein (15-40%) and energy	ANFs such as tannins, trypsin inhibitors, oligosaccharides, phytate, resistant starch, low in methionine	Extrusion cooking, phytase inclusion	20-30% when processed
Insect meal	-	-	Ability to grow on a range of organic wastes, high levels of lysine, threonine, methionine	Application in poultry feed is not allowed in EU, lack of proper data on AA digestibility	Proper feed evaluation	-
Macro and micro algae	-	-	No need to arable land, very high yields, high protein (30-50%)	Lack of information, large variation in nutritive value, high NSP and minerals	Proper feed evaluation, drying, formulation based on digestible AA	5 to 15%

AA, amino acids; ANFs, anti-nutritional factors; GP, global production; MMT, milion metric tons; NSP, non-starch polysaccharides; P, phosphorus; SBM, soybean meal.

[1] Global seed output.

ASSESSMENT OF THE USE OF PERCHES TO IMPROVE LEG STRENGTH IN AUSTRALIAN FAST GROWING MEAT CHICKENS

D.V. PHIBBS[1], P.J. GROVES[1] and W.I. MUIR[1]

Poor leg health in Australian fast growing meat chickens is a major welfare issue, resultant from long-term genetic selection for bird size and growth rate (Bessei 2006). Anecdotal evidence and preliminary data suggest perches may have the potential to improve leg health, decreasing the prevalence of associated leg disorders and lameness (Groves and Muir 2013). The following study sought to assess the potential role of perches in improving leg health in the context of Australia's fast growing meat chicken industry.

Day old chicks, both Ross 308 and Cobb 500 were randomly allocated into one of four treatments within a 2 by 2 factorial for breed with and without a perch. There were 6 pens per treatment, and 42 birds per pen (density 28kg/m^2). Perches were an A-frame, with two levels, one 15cm, the second 30cm above the ground, each providing 1 metre of perch. Observations of activity and spatial distribution and perching behaviour were recorded every two days at five time points throughout the six-week study. At 35 days of age (d) the standing ability of 9 visually male birds per pen (54 per treatment) was assessed in a latency to lie (LTL) test, after which a sample of blood was collected to determine serum Ca, P and Mg, and assessments of breast blisters, hock marks and footpad dermatitis were completed. At 42 d, a further 9 visually male birds per pen underwent the LTL test. Blood was taken, each bird was then humanely euthanased and breast, hock and footpads were assessed in addition to tibial dyschondroplasia (TD). Bird weights and feed conversion ratio (FCR) were measured throughout.

Birds used the perches from 6 d to 42 d, with 1.25% of birds using the perches at any given time. Perch use peaked at 38 d and in the final two weeks of the study, female birds used the perches more than males ($P<0.001$). Perches did not affect bird spatial distribution ($p=0.78$), nor litter moisture ($p=0.059$), but Cobb birds were observed resting less than Ross ($p=0.001$). Bird weights were not impacted by the presence of perches nor were they significantly different between strains ($p=0.186$). Ross FCR was lower than Cobb ($p=0.02$).

The perches had minimal impact on leg health. LTL times showed no effect of perches at either 35 d ($p=0.58$) or 42 d ($p=0.87$). Serum levels were largely unaffected by treatment, although notably, Cobb birds with access to perches had lower serum magnesium at 35 ($p=0.01$) and 42 d ($p=0.001$). Ross birds experienced more footpad dermatitis than Cobb at 35 d (0.031) and 42 d (0.045). Overall, the prevalence of TD was low (0.5% birds sampled).

These LTL results are contradictory to those observed by Groves and Muir (2013) and are being retested in subsequent studies. Evaluation of the relationship between the amount of perch space with leg strength and bird physiology are also being evaluated.

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FLOCK UNIFORMITY AND SAMPLE SIZE REQUIREMENTS FOR ACCURATE PREDICTION OF LIVE WEIGHT DURING MIXED-SEX REARING OF CHICKENS

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Summary

Poor flock uniformity presents challenges to the processing of birds and meeting customer requirements. Flock uniformity is calculated on farm through the weighing of birds; however, paradoxically the required number of birds weighed varies depending on uniformity. This report discusses a desk-top study that investigated variation in live weights of even and uneven mixed-sex flocks at weekly intervals from four to eight weeks of age. A dynamic listing of individual bird weights in a flock of 50,000 mixed-sex chickens was constructed in a Microsoft Excel spreadsheet. The coefficients of variation (CV) were assumed to be 5 and 10% for even and uneven flocks, respectively, and male and female weights were normally distributed. When combined to emulate a mixed-sex flock, weights were no longer normally distributed. Furthermore, the uneven mixed-sex flock had larger numbers of under- and over-weight birds compared with the even flock. The report includes tables of sample sizes required to predict live weight in even and uneven mixed-sex flocks to varying degrees of accuracy.

I. INTRODUCTION

Flock uniformity is a key performance indicator and economic driver in commercial practice. Hughes et al. (2017) pointed out that Australian losses could exceed AUD$127M per annum, assuming that 5% of throughput totalling 1,159,602 tonnes chicken meat per annum was downgraded by 40% due to out-of-range weight specifications. From these brief comments it is easy to see that flock uniformity in live weight is a very important matter. This raises questions about how uniformity is assessed, what is a benchmark figure for flock uniformity in commercial practice, and what influences post-hatch uniformity.

Flock uniformity can be expressed as the coefficient of variation (CV) in live weight, with increased CV values synonymous with decreased uniformity, or in other words, a wider spread in live weights above and below the flock average. Here CV is calculated as statistical variance in live weight expressed as a percentage of the mean value of the flock. Commercial facts and figures are hard to ascertain, but it is clear that flock uniformity can decrease if close attention is not paid to incubation, nutrition, vaccination, husbandry, health and hygiene. For example, Ciftci and Ercan (2003) reported CV for live weight increasing from 8.7 to over 10% when males were fed less homogeneous feed. Madsen and Pedersen (2010) reported CV for live weight increasing from 6.7% to 9.5% when DL-methionine supplementation was reduced from 1.2 g/kg to 0.8 g/kg, and CV for breast meat yield rising from 8.7% to 11.7 % with this reduction of 0.4 g/kg DL-methionine supplementation. Hughes et al. (2017) reported proxy estimates of CV for live weight derived from three large scale chicken growth experiments ranging from 6.6% for males (3,157 g/bird) and 6.1% for females (2,714 g/bird) at 5 weeks of age, 7.3% for males (3,620 g/bird) and 8.8% for females (3,044 g/bird) at 6 weeks of age, and 6.7% for males (4,286 g/bird) at 7 weeks of age. CVs were sensitive to breed, sex, diet formulation and source of feed, with each of these factors influencing uniformity in live weight. Hughes et al. (2017) concluded that an optimistic estimate of achievable uniformity in live weight of meat chickens under experimental conditions was about 6%.

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This report discusses a desk-top study that investigates variation in live weights of even and uneven mixed-sex flocks at weekly intervals from four to eight weeks of age. It also discusses the optimal number of chickens to be weighed individually to accurately predict flock weight.

II. MATERIALS AND METHODS

The data used in this investigation are summarised in Table 1. The mean values for live weight of male and female chickens were taken from tables of performance objectives for Cobb500^{TM} chickens published by Cobb-Vantress (2018). It was assumed that within sex and age, live weight of individual chickens in the flock was normally distributed as observed in experimental flocks (Hughes et al. 2017). Standard deviations for male and female chickens were calculated from SD = CV * Mean / 100. A dynamic listing of individual bird weights in a flock of 50,000 mixed-sex chickens was constructed in a Microsoft Excel spreadsheet. Individual weights for males and females were calculated using NORMINV and RAND functions. Means and standard deviations from mixed-sex chickens were calculated from pooled data from the hypothetical flock of 50,000 chickens.

Table 1 - Means and standard deviations (SD) for male, female and mixed-sex chickens from four to eight weeks of age.

Sex		Flock age:				
		4 weeks	5 weeks	6 weeks	7 weeks	8 weeks
Male	Mean	1,675	2,392	3,147	3,891	4,576
	SD for 5% CV	84	120	157	195	229
	SD for 10% CV	168	239	315	389	458
Female	Mean	1,554	2,153	2,757	3,342	3,878
	SD for 5% CV	78	108	138	167	194
	SD for 10% CV	155	215	276	334	388
Mixed	Mean	1,615	2,273	2,952	3,617	4,227
	SD for 5% CV	101	165	246	328	408
	SD for 10% CV	174	257	354	451	552

Samples of various sizes comprised of equal numbers of male and female chickens were selected at random from individual weights of birds in the hypothetical flock of 50,000 chickens. This process was iterated for each age group from four to eight weeks of age, and for flocks described as even (relatively high uniformity, CV 5%) and uneven (relatively low uniformity, CV 10%). The SAS procedure UNIVAR ((SAS Institute Inc., Cary, NC, USA)) was used to test the normality of live weight distributions in male, female and mixed-sex groupings in even and uneven flocks at various ages, as shown in Table 1.

III. RESULTS

The hypothetical mixed-sex flock of 50,000 birds contained equal numbers of male and female chickens. Weights within male and female groupings were normally distributed, but when combined, weights were found by statistical analysis to be non-normal in distribution. This was particularly evident for the even flock (CV 5%), which can be seen as bi-modal in nature (Figure 1). It is also clear from Figure 1 that the uneven mixed-sex flock had larger numbers of underweight and overweight birds compared with the even flock.

The sample sizes required to accurately predict flock average weight at various ages from 4-8 weeks are shown in Tables 2 and 3 for even and uneven mixed-sex flocks, respectively. For example, if the processor is prepared to accept an error of 100 g/bird in flock average weight at six weeks of age, then a minimum sample size of 25 birds is required for a mixed-sex flock with CV of 5% (Table 2). For an uneven flock with CV 10%, the minimum sample size is 50 birds (Table 3). If tighter tolerances are required by the processor, then sample sizes need to increase accordingly.

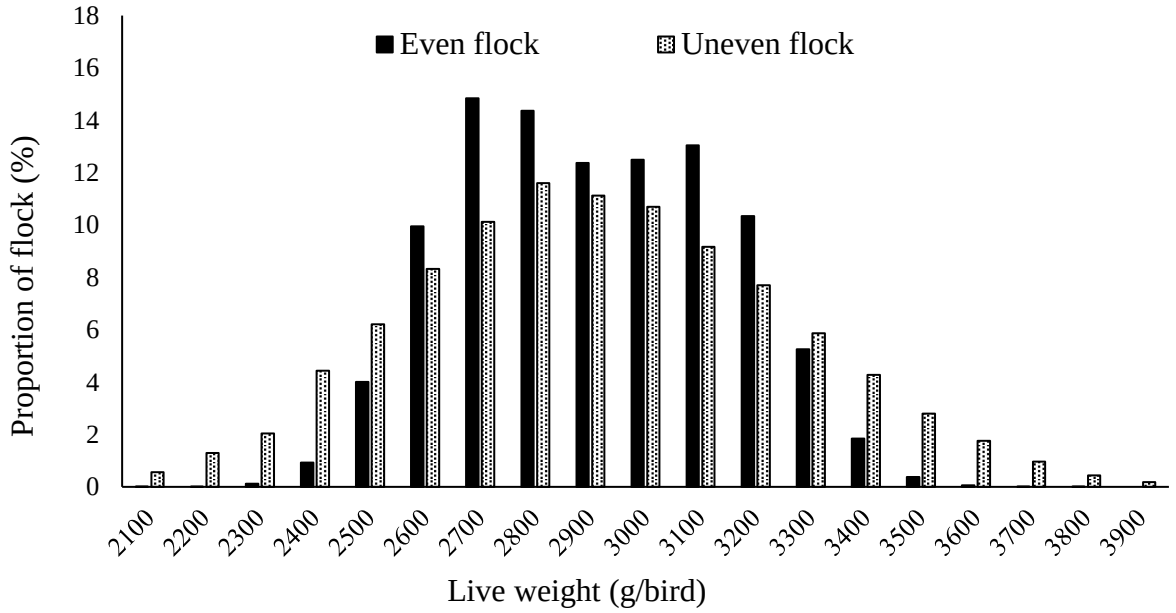

Figure 1 - Spread of live weights for even (CV 5%) and uneven (CV 10%) mixed-sex flocks 42 days of age. Average live weight for both flocks was 2,952 g/bird. Standard deviations were ±235 and ±342 g/bird for even and uneven flocks, respectively.

Table 2 - Estimates of sample size required for even flocks defined as having a coefficient of variation of 5% for each of males and females raised together. Tolerance is the difference between flock and sample means with an error rate of 1 in 20.

Sample size	Tolerances:				
	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks
25	40	65	96	129	161
50	28	46	68	91	113
100	20	32	48	64	80
200	14	23	34	46	56
500	9	14	21	29	36
1000	6	10	15	20	25
Mean weight	1,615	2,273	2,952	3,617	4,227

Table 3 - Estimates of sample size required for uneven flocks defined as having a coefficient of variation of 10% for each of males and females raised together. Tolerance is the difference between flock and sample means with an error rate of 1 in 20.

Sample size	Tolerances:				
	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks
25	68	101	139	179	216
50	48	71	98	126	151
100	34	60	70	90	108
200	24	35	49	63	76
500	15	22	31	40	48
1000	11	16	22	28	34
Mean weight	1,615	2,273	2,952	3,617	4,227

IV. DISCUSSION

The results from this desktop analysis of live weight distribution in flocks of various ages and differing in uniformity was based on data being normally distributed. It is possible that frequency distributions of live weight in commercial flocks are negatively skewed or leaning to the right. That is, the mode and median weights are greater than the mean weight, and there will be a higher proportion of under-weight birds. If that is the case, then skewness will affect on-farm sample sizes required for accurate estimation of live weight of commercial flocks.

There is anecdotal evidence of a decrease in uniformity in commercial flocks in recent times in Australia. A plausible explanation is that both Cobb 500 and Ross 308 broilers are no longer feather-sexable as the breeders have reversed the cross. One reason given for this reversal is that the use of automated equipment in hatcheries has outpaced the manual feather sexing procedure, so it is no longer feasible to continue single-sex rearing of chickens. The current study shows that mixed-sex rearing could result in a wider distribution of bird weights that is arguably no longer normally distributed which gives the impression of reduced uniformity. It seems less likely that other factors which affect flock uniformity, such as paying close attention to incubation, nutrition, vaccination, husbandry, health and hygiene, will have contributed to this wider variation in live weight.

V. CONCLUSIONS

Mixed-sex rearing could contribute to an apparent reduction in flock uniformity, and larger proportions of under- and over-weight birds sent for processing. Optimising sample weighing of live birds on farms might assist flock managers to identify underlying reasons for reduced uniformity and processors to optimise the order in which flocks are processed to reduce down-grades.

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EFFECT OF DIFFERENT DILUTION RATES ON FERTILITY OF CHICKEN SPERMATOZOA AND ITS STORAGE

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Summary

Under natural mating, with a ratio of hen:cocks of 8:1 fertility rates are still low. Artificial insemination (AI) technology can be adopted and is the most effective means for economic poultry production and genetic improvement. The effect of different dilution rates of freshly ejaculated white leghorn chicken (WLH) semen was investigated using CARI poultry semen diluent. Healthy adult males and 180 healthy adult hens from the same hatch were taken randomly and maintained in individual cages under uniform husbandry conditions. The hens were divided equally and randomly into 9 groups. Good quality pooled fresh semen samples were collected by massage method and diluted with CARI poultry semen diluent at different dilution rates. Using the freshly ejaculated semen, superior fertility was obtained from 2 to 6 days post insemination with dilution rate of 1:1 and the dilution rate of semen was associated with the gradual reduction in fertility. In another study, fertilizing ability of CARI poultry semen diluent was examined at different storage periods at 8±1°C in WLH and Kadaknath chicken. Adult healthy males (20) and females (60) from each breed were selected randomly and maintained under similar conditions. Fertility was higher in WLH than in Kadaknath chickens but was not significantly affected in either breed by storage for 24 or 48 hours. From this study, it can be concluded that freshly ejaculated chicken semen can be extended up to 1:7 in CARI diluent without critical loss in fertility. Further, this diluent can be used for 24 or 48 hrs storage of chicken semen with maintenance of high fertility.

I. INTRODUCTION

Using AI, the services of a single superior male can be extended to a large number (more than 250) of females instead of 8 hens (natural mating). AI in avian species expresses better fertility than natural mating (Saeki and Nagomi, 1964, Mohan *et al.*, 2016). AI increases overall fertility and hatchability with reduced cost of production per day old chick (Brillard, 2003). Adopting AI needs fewer males which saves feed, labour, space, maintenance and operating costs but requires the immediate dilution of chicken semen because it is highly concentrated, containing 6 (roosters) to 12 (toms) billion spermatozoa/ml (Donoghue and Wishart, 2000) and has a low volume. There is the risk of sperm being killed by dehydration resulting from evaporation of water at room temperature from highly concentrated semen unless semen is diluted (Lake and Stewart, 1978) and semen diluent can increase the number of hens that can be inseminated by the semen provided by a single superior male.

Various semen diluents are available as reported in the literature but CARI poultry semen diluent produces good results (Beulah, 2017). Therefore, CARI diluent developed in our laboratory (Mohan *et al.*, 2017) was selected to investigate the appropriate dilution rate of this diluent for optimum fertility. Further, the fertilizing ability of this diluent was also examined at 0, 24 and 48 hrs of storage of diluted semen.

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II. MATERIALS AND METHODS

Healthy adult males (20) and females (180) from the same hatch of WLH chicken were taken randomly and maintained in individual cages under uniform husbandry conditions. Good quality semen samples were collected by the Burrows and Quinn (1937) method. For examining the fertility in fresh samples, pooled semen (sperm concentration 5.73×10^9/ml) was placed in 9 round bottom glass tubes of 5 ml capacity (length=7cm, diameter =1cm). All the tubes containing varying semen volume were diluted with CARI diluent at the rate of 1:1, 1:3, 1:5, 1:7, 1:9, 1:11, 1:13, 1:15 and 1:17 to achieve the final fixed volume of 3 ml in each tube (Table 1). Immediately after dilution, the sperm concentration was examined using a haemocytometer.

Artificial insemination with freshly ejaculated semen was carried out by intravaginal insemination using an AI gun (IMV, France) in 9 different groups each of 20 hens. Fertile eggs were collected from 2-6 days of post insemination of hens and examined by candling at the 9^{th} day of incubation. Break out studies were performed for the fertility assessment where fertility was not clear by candling. In another study, 20 healthy adult males and 60 females from the same hatch of WLH (exotic) and Kadaknath (native) chicken were taken randomly and maintained in individual cages under uniform husbandry conditions as in the above experiment. All hens in this study were divided equally into 3 groups (20 each). The first group of hens received the freshly ejaculated semen (0 hr stored) diluted (1:2) with CARI poultry semen diluent and served as control. The second and third groups of birds received the semen diluted (1:2) with CARI poultry semen diluent and stored in 5 ml capacity glass vial at 8±1°C for 24 and 48 hrs respectively before insemination of hens. A.I. and evaluation of fertility were conducted as described in experiment 1. Data were analysed as per the standard methods.

III. RESULTS AND DISCUSSION

Data on the effect of different dilution rates on fertilizing ability of freshly ejaculated chicken spermatozoa are presented in Table 2. Superior fertility was obtained from 2 to 6 days post insemination for the dilution rate of 1:1 (143.00), 1:3 (71.50) 1:5 (47.75) and 1:7 (35.75 million (m) sperm/AI) which ranged from 91 to 94%. With higher dilution rates i.e. 1:9 (28.60 m sperm/AI) and 1:11 (23.83 m sperm/A.I.), fertilizing ability of sperm reduced to 87.23 and 85.13% respectively. Further increases in the dilution rate like 1:13 (20.42 m sperm/AI), 1:15 (17.88 m sperm/AI) and 1:17 (15.89×10^6 m sperm/AI), reduced fertility further at 81.15, 78.16 and 75.60% respectively. According to this study, a minimum 36 million sperm (1:7) are required for good fertility in chickens. The results of this study are similar to the work carried out by Beulah (2017), who used freshly ejaculated diluted chicken semen with CARI diluent and recorded above 90 % fertility up to 1:8 (29.70×10^6 sperms/A.I.) dilution rate and 79% fertility at 1:18 (14.70 m sperm/AI). The reduction of fertility with higher dilution rate is associated with the decrease in number of spermatozoa per unit volume. The minimum number of sperm required per AI is 45 to 90 million /hen for good fertility in chickens (Rowell and Cooper, 1960, Kim et al., 1974) although Etches (1996) advocated 100 million sperm /hen/AI. Results on the effect of CARI diluent on storage of chicken semen at 24 or 48 hrs are presented in Table 3. In the exotic breed of chicken, fertility rates at 0 (fresh/ control), 24 and 48 hrs were 93.10±3.90, 91.20±4.20 and 89.72±4.61% respectively and in native breeds 82.31±2.56, 81.61±3.77 and 80.24±3.44% respectively. These data indicate that no significant difference was found in fertility among the three storage periods in both breeds. However, a lower ($P < 0.05$) fertility was found in Kadaknath as compared with the WLH breed. This may be due to breed to breed variation. Mohan et al. (2011) also reported higher fertility in WLH than Kadaknath. Poor fertility in native fowl during storage of spermatozoa may be associated with more dead and morphologically abnormal spermatozoa than for WLH. High fertility depended

more on semen quality than semen quantity (Kamar, 1960). This investigation indicated that chicken semen can be stored (8±1°C) for 24 or 48 hrs in CARI diluent and still express high fertility similar to the freshly ejaculated semen (Table 3). However, earlier studies indicated that chicken semen can be stored for 24 hrs at 0-5°C (Lake, 1960, Van Wambeke, 1967, Sexton, 1977, Lake and Ravie, 1979). This suggested that the composition of the CARI poultry semen diluent may differ from others and preserve the chicken semen at 8±1°C (instead of 0-5°C) up to 48 hrs without impairing the fertility (89.72±4.60%). Adequate data are not available on 48 hr stored semen to compare with ours. However, Lake (1960) reported 47 % fertility after 48 hrs storage of semen at 0-2°C, nearly half that of the present study.

Table 1 - Sperm concentration/AI at different dilution rates using CARI poultry semen diluent.

Dilution rate	Semen volume (ml)	Diluent (ml)	Total volume (ml)	Sperm concentration/AI ($\times 10^6$/0.05 ml)
1:1	1.50	1.50	3.00	143.25
1:3	0.75	2.25	3.00	71.63
1:5	0.50	2.50	3.00	47.75
1:7	0.38	2.62	3.00	35.75
1:9	0.30	2.70	3.00	28.60
1:11	0.25	2.75	3.00	23.83
1:13	0.21	2.79	3.00	20.42
1:15	0.19	2.81	3.00	17.80
1:17	0.17	2.83	3.00	15.89

Table 2 - Effect of different dilution rates on fertilizing ability of fresh chicken spermatozoa (Mean±SEM, n=20).

Days (Post-insemination)	Dilution rate								
	1:1	1:3	1:5	1:7	1:9	1:11	1:13	1:15	1:17
Day-2	97.87 ±0.82	93.87 ±2.60	92.15 ±2.73	92.33 ±2.58	88.57 ±3.53	86.34 ±2.07	88.37 ±2.03	86.67 ±2.28	84.67 ±3.57
Day-3	93.52 ±1.73	96.14 ±0.97	95.23 ±1.40	90.27 ±2.80	90.67 ±2.07	86.03 ±2.92	89.99 ±2.31	90.00 ±1.63	84.60 ±1.80
Day-4	90.61 ±1.47	90.83 ±2.17	92.60 ±1.53	90.00 ±2.52	86.40 ±2.36	80.26 ±2.16	89.67 ±2.15	84.33 ±2.21	70.66 ±2.27
Day-5	94.45 ±3.65	95.60 ±2.43	92.17 ±2.30	90.67 ±4.35	85.63 ±2.66	90.03 ±2.68	77.40 ±2.41	70.13 ±2.08	72.67 ±2.08
Day-6	93.83 ±2.64	90.47 ±3.05	90.07 ±1.58	92.10 ±4.06	84.87 ±5.20	83.00 ±2.46	60.33 ±2.31	59.67 ±2.60	65.33 ±3.54
Average (Day 2-6)	94.06^{a} ±0.57	93.38^{ab} ±0.29	92.44^{b} ±0.37	91.14^{b} ±0.84	87.23^{c} ±1.47	85.13^{c} ±0.68	81.15^{d} ±0.47	78.16^{de} ±0.87	75.60^{e} ±2.33

Mean values with different superscript (abcde) within rows differs significantly ($P \leq 0.05$)

Table 3 - Effect of various storage temperatures on fertilizing ability of chicken spermatozoa using CARI poultry semen diluent (Mean±SEM, n=20).

Breed	0 hr	24hr	48 hr
WLH	93.10 ± 3.90^{a}	91.20 ± 4.20^{a}	89.72 ± 4.61^{a}
Kadaknath	83.31 ± 2.56^{b}	81.61 ± 3.77^{b}	80.24 ± 3.44^{b}

^{ab}Mean values bearing different superscript in column (a,b) differs significantly ($P \leq 0.05$)

IV. CONCLUSION

Using CARI diluent, freshly ejaculated chicken semen can be extended up to 1:7 without significant loss in fertility. This diluent can be used for 24 or 48 hrs storage of chicken semen with high fertility.

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DEVELOPMENT OF COCCIDIAL VACCINAL IMMUNITY IS NOT IMPAIRED BY FEEDING OREGANO ESSENTIAL OIL

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Summary

Coccidial vaccination is an alternative to medication programmes, although vaccination can lead to impaired performance. The objective of this study was to determine the effect of co-administration of an oregano essential oil (OEO) product in feed and an attenuated *Eimeria* vaccine on performance and immunity of vaccinated birds. A total of 1,750 Cobb broilers was randomly allocated to 5 treatment groups (7 replicates/treatment 50 birds/replicate): CON – salinomycin (66 g/t); VACC – HatchPac Cocci III (Merial, USA) single dose Day 0; OEO – OEO (300 g/t); OEOD10 - OEO (300 g/t) from Day 10; SAL/OEO – salinomycin (66 g/t), OEO (150 g/t). Birds in OEO, OEOD10 and SAL/OEO were vaccinated on Day 0 with the anticoccidial vaccine. With the exception of VACC and OEOD10, in-feed treatments were fed the entire study, 0-42 days. Birds were reared in floor pens on clean litter. Litter samples were collected weekly for oocyst enumeration. On Day 21, 5 birds/pen were moved to cages (35 birds/treatment, 7 replicates) and challenged orally with a mixed *Eimeria* infection. Coccidia-free birds were used as a positive control (POS) (5 birds/cage, 7 replicates). Birds were fed an untreated ration for the challenge phase. On Day 27 birds were euthanised, weight recorded and coccidial lesion scored (LS). Coccidia were detected in the litter of SAL from 10 days indicating contamination. Body weight gain in VACC was significantly lower than SAL/OEO; all other treatments did not differ significantly. Feed conversion ratio was significantly higher in both VACC and OEOD10 versus SAL and SAL/OEO while VACC, OEO and OEOD10 did not differ significantly. Lesion scores were significantly lower in all treatments versus POS while LS in SAL and SAL/OEO was significantly higher than VACC. Oocyst enumeration profile demonstrated a peak in VACC on Day 21 that was absent in all other treatments. In conclusion, the inclusion of OEO in poultry feed from Day 0 or Day 10, either alone or in combination with salinomycin, and fed to birds vaccinated with an attenuated *Eimeria* vaccine on Day 0 had no adverse effect on the development of coccidial immunity. The combination of OEO and salinomycin significantly lowered Day 0-42 FCR versus birds receiving vaccine or OEO alone. The use of OEO in combination with an *Eimeria* vaccine or an ionophore offers another potential tool in the management of coccidiosis.

I. INTRODUCTION

Management of coccidiosis has traditionally been via medication or vaccination. Anticoccidial programmes typically comprise a chemical and an ionophore component. Ionophores are under increasing regulatory and consumer scrutiny for their antimicrobial activity. Coccidial vaccines comprise *Eimeria* strains that are either attenuated (typically via repeated passage) or non-pathogenic or unmodified strains of field isolates. Coccidial vaccines have been proposed to restore anticoccidial drug sensitivity to circulating *Eimeria* populations in a poultry house (Chapman and Jeffers, 2014). However, coccidial vaccines have been shown to impair bird performance (Waldenstat, 2013). Oregano essential oil (OEO) based products have demonstrated compatibility with coccidial vaccines, although determination of immune status following challenge was not determined (Waldenstat, 2013).

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The objective of the study was to determine the effect of a commercial oregano essential oil product on the development of immunity following vaccination with an attenuated coccidial vaccine.

II. METHOD

A total of 1,750 Cobb 500 male chicks was allocated to 6 treatment groups, with 7 replicates per treatment as per Table 1. Each replicate (experimental unit) comprised 50 birds. Feed and water were available *ad libitum*. Feed rations were corn/soya-based and free of all medication with the exception of treatment 1. Feed for all other treatments contained the test material as per Table 1. On Day 0, all birds in treatments 2-5 received a single dose of *Eimeria* vaccine, HatchPak Cocci III (Merial, USA) via hatchery-spray. Birds were reared on earth floor pens on 10 cm deep clean litter (wood shavings) until Day 42. Average body weight gain was determined on Day 28 and 42 and FCR calculated. Mortality was determined daily and European Production Efficiency Factor (EPEF) calculated. Litter samples were collected weekly from each pen and the number of oocysts enumerated via salt flotation.

For immunity assessment, 5 birds were randomly selected from each pen on Day 21 and transferred to wire-floor cages, 7 cages/treatment. Each bird was challenged orally with 1 ml of mixed *Eimeria* (~100,000 oocysts *E. acervulina*, ~75,000 *E. maxima* and ~50,000 *E. tenella*). A separate group of birds which were naïve and coccidian-free (5 birds/cage, 7 cages) were also inoculated and acted as a positive control group (POS) for the immunity assessment only. On Day 27, all birds were euthanised and scored for coccidial lesions according to the system of Johnson and Reid (1970).

Data were analyzed by ANOVA using Minitab v14 (Minitab Inc, USA) and statistical significance declared at $P \leq 0.05$.

Table 1 - Experimental design.

Treatment	Inclusion	Details	Duration (Days)	Coccidial vaccination
SAL	66 g/tonne	Salinomycin	0-42	
VACC	-	HatchPak Cocci III	0	✓
OEO	300 g/tonne	Oregano essential oil*	0-42	✓
OEOD10-42	300 g/tonne	Oregano essential oil*	10-42	✓
SAL/OEO	66 g/tonne 150 g/tonne	Salinomycin Oregano essential oil*	0-42	✓

* Orego-Stim (Anpario plc, UK)

III. RESULTS

There was no significant effect of treatment on Day 28 body weight gain, EPEF or mortality (Table 2). Bodyweight gain on Day 42 in VACC was significantly lower than SAL/OEO, 2.420 and 2.586 kg, respectively. All other treatments did not differ significantly from VACC or SAL/OEO. Feed conversion ratio was significantly higher in both VACC and OEOD10 versus SAL and SAL/OEO, while FCR in OEO, OEOD10 and VACC only differed numerically. Feed conversion ratio in OEO (1.718) was also significantly lower versus SAL/OEO (1.663). The average lesion score in POS was significantly higher than all other treatments. Additionally, the average lesion score in VACC was significantly lower than SAL and SAL/OEO, 1.34, 1.68 and 1.71 respectively but VACC only differed numerically from OEO (1.47) and OEOD10 (1.65). SAL/OEO, SAL, OEO and OEOD10 did not differ significantly.

Table 2 - Zootechnical performance.

Treatment	Body weight gain (kg)		FCR*		Mortality (%)	EPEF	Average lesion score
	28	42	Day 28	Day 42			
SAL	0.630	2.555^{ab}	1.467^{b}	1.683^{bc}	15.0	307	1.68^{b}
VACC	0.617	2.420^{b}	1.507^{a}	1.755^{a}	10.7	293	1.34^{c}
OEO	0.622	2.477^{ab}	1.494^{ab}	1.718^{ab}	12.9	299	1.47^{bc}
OEOD10	0.608	2.506^{ab}	1.551^{a}	1.733^{a}	7.9	317	1.65^{bc}
SAL/OEO	0.645	2.586^{a}	1.488^{b}	1.663^{c}	13.6	320	1.71^{b}
POS	-	-	-	-	-	-	2.6^{a}
SEM	0.0105	0.0195	0.0055	0.0074	1.18	5.3	0.041
Significance (P)	0.866	P < 0.05	P < 0.01	P < 0.01	P > 0.05	0.477	P < 0.01

*FCR corrected for mortality. Column data having different superscripts are significantly different (P≤0.05).

A peak in litter oocyst counts was observed in OEO and OEOD10 on Day 14 followed 7 days later by a peak in VACC on Day 21 (Figure 1). The peak in oocyst counts observed in OEO and OEOD10 were approximately 60-70% lower than VACC. Litter oocyst counts continued to increase in SAL/OEO from Day 7 until the end of the study. Coccidial contamination in SAL was detected on Day 14 and oocyst counts continued to increase until peaking on Day 35.

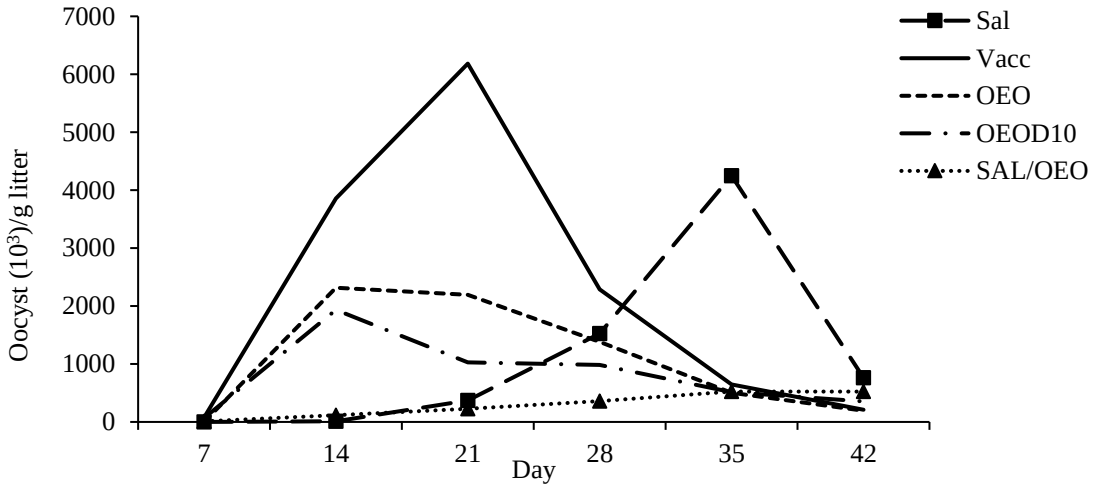

Figure 1 - Weekly litter oocyst counts.

IV. DISCUSSION

The inclusion of a commercial OEO product did not interfere with the development of immunity following vaccination with an attenuated coccidial vaccine as determined by lesion scores. Body weight gain in birds fed OEO was numerically but not statistically higher than vaccinated birds without OEO. Similarly, Küçükyilmaz et al. (2012) did not demonstrate a performance benefit in birds vaccinated with the coccidial vaccine Livacox and fed essential oils of laurel leaf, oregano and lavender. Oregano essential oil has been shown to lower oocyst counts in the litter from vaccinated birds but not stop oocyst recycling completely (Waldenstat, 2013), as was observed in the current study. In the current study, OEO fed to birds 10 days after vaccination did not significantly affect performance or immunity. Indeed, FCR and body weight gain were numerically better than VACC despite poorer performance at 28 days, suggesting performance recovery due to compensatory growth. OEO is known to improve

immune and antioxidant status in addition to balancing the microbiota (Giannenas et al., 2016; Idris et al., 2017), biological processes known to be important in the host response to *Eimeria* infection. The combination of OEO and salinomycin appeared highly effective at inhibiting coccidial recycling in litter with a minimal impact upon the development of coccidial immunity. Furthermore, such a combination also appeared to have a positive impact on performance. Bozkurt et al. (2016) did not report any synergy between OEO and monensin in *E. tenella* challenged birds, despite using 24 ppm OEO versus 15 ppm in the current study. However, presentation of the OEO in Bozkurt et al. (2016) was substantially different from the mineral carrier used in the current study A comparison with salinomycin in the current study was difficult due to contamination of the treatment group (most likely from the earth floor). In conclusion, the inclusion of OEO in poultry feed from Day 0 or Day 10, either alone or in combination with salinomycin, numerically improved the performance of birds vaccinated with an attenuated *Eimeria* vaccine on Day 0 with no adverse effect on the development of coccidial immunity. The use of OEO in combination with an *Eimeria* vaccine or ionophore offers another potential tool in the management of coccidiosis.

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PROTECTIVE MECHANISMS OF REFINED FUNCTIONAL CARBOHYDRATES AGAINST *SALMONELLA* TYPHIMURIUM INVOLVE INHIBITION OF INFLAMMATORY RESPONSE AND ACTIVATION OF APOPTOSIS RELATED REGULATORS

M. SINGH[1], C. O'SHEA[2], K. GAO[3], S. WILLIAMSON[4], S. SHARPE[4] and P.J. GROVES[1]

Summary

Prebiotics in the form of refined functional carbohydrates (RFCs) from enzymatically hydrolyzed yeast have been associated with control of pathogens and enhancement of immune response in chickens. In this study, we investigated the underlying immunological mechanisms of the action of prebiotics against colonization of the intestine by *Salmonella* Typhimurium in sexually mature hens. Birds received RFCs in feed (100g/MT) from day 1 of age and, subsequently, received *Salmonella* serovar Typhimurium at week 17 of age. Caecal tonsils were removed 4 days post-challenge, RNA was extracted and hybridized to GeneChip™ Chicken Gene 1.1 ST Array Plate to compare gene expression profiles of control vs RFCs fed hens. A total of 472 gene transcripts was found to be differentially expressed, with interferon signaling, pattern recognition receptors (PRRS), Th1 and Th2 pathways, role of receptors for recognition of bacteria and JAK/Stat signaling pathways inhibited in RFCs fed hens. The modulatory effects of prebiotics on immune responses may be attributed to adhesion of pathogenic bacteria onto the surface of RFCs leading to inhibition of pro-inflammatory signaling pathways and activation of apoptotic regulators, consequently leading to reduced translocation of bacteria. Thus, RFCs can benefit hens at sexual maturity by boosting the immune response while also providing protection from *Salmonella* infection.

I. INTRODUCTION

Salmonella serovars can invade the host by inducing their own uptake into intestinal epithelial cells. This uptake is induced by virulence proteins delivered into cytoplasm of infected cells by a specialized mechanism known as type III protein secretion system (TTSS) (Bertelsen et al., 2003). These proteins activate signaling pathways involved in cytoskeleton rearrangements and cellular uptake processes (Galán and Zhou, 2000). Besides facilitating the invasion process, the interaction between invading pathogen and host epithelium also leads to activation of genes with pro-inflammatory functions (Hobbie et al., 1997). The ability of *Salmonella* to cause macrophage programmed cell death or apoptosis, may be important for the initiation of infection, bacterial survival, and escape of the host immune response (Monack et al., 1996). Enzymatic hydrolysis of yeasts produces Refined Functional Carbohydrates (RFCs) that show activity against gram negative bacteria. Earlier studies have shown that the RFCs can agglutinate as well as prevent adherence of several species of *Salmonella* to the intestinal epithelium preventing it from colonizing in the gastro-intestinal tract (Walker et al., 2017, 2018). Hens at sexual maturity are known to exhibit immunosuppression allowing for considerable surges in *Salmonella* prevalence in poultry houses (Johnston et al., 2012). The present study aims to investigate the effect of continuous feeding of RFCs on the host's response to infection. Using the transcriptomics approach, gene expression profiles were

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generated to identify transcript regulators and pathways that control the response of 17-week-old hens, fed diets with or without RFCs, four days post infection with *Salmonella* Typhimurium.

II. MATERIALS AND METHODS

Ninety-six 1-day old commercial brown egg layer strain chickens were reared in separate floor pens (24 birds per pen, 2 pens per treatment group). Birds received the same basic ration of pullet starter and grower rations (produced at University of Sydney feed mill). Treatment groups were untreated Controls and continuous RFCs in feed (100g/MT) from day old. Oral challenge with 10^6 CFU of *Salmonella* Typhimurium DT 135 was given to 16 birds per group at 17 weeks and then to 8 birds per group at 20, 25, 35 and 45 weeks of age and cloacal swabs used to detect *Salmonella* prevalence using specific PCR. Of the 16 birds per group challenged at 17 weeks, eight birds per treatment were euthanised at 4- and 10-days post infection respectively and their caecal tonsils were harvested and stored in Invitrogen™ RNAlater™ Stabilization Solution (Qiagen, Valencia, CA, USA) according to the manufacturer's protocols at –20°C until analysed. The caecal tonsils were chosen as it contains as much as 45.7% of the lymph nodules, and as such, are the main source of immune function in chicken guts. Based on PCR results at 4-days post infection, four birds were selected per group and RNA was isolated from their caecal tonsil tissue with a Qiagen RNeasy mini kit and quantity and quality were assessed with a NanoDrop ND-1000spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and an Agilent Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). Labelled cRNA for all samples was prepared using the Ambion (Paisley, UK) WT Expression Kit following the manufacturers' instructions. Labelled cRNA was hybridised to GeneChip™ Chicken Gene 1.1 ST Array Plate for 16 h at 45°C, followed by washing, staining and finally scanning in an Affymetrix GeneAtlas® Imaging Station and Fluidics Station.

The Affymetrix CEL files were imported into Applied Biosystems™ Transcriptome Analysis Console (TAC) version 4.0 to perform quality analysis checks. Summary statistics were computed after the Robust Multi-Array (RMA) normalization and significance analysis was performed using the Limma (Linear Models for Microarray Data; Smyth, 2005) within the TAC 4.0. To identify the statistically significant changes in gene expression, microarray data were subjected to gene filtering based on a combination of a log 2 -fold change at ±1.3 as cut off and P value ≤0.05. Lists generated by pairwise comparisons were used as input into the bioinformatics tool Ingenuity Pathway Analysis (IPA, Ingenuity Systems, USA), which was used to interpret the gene expression data in the context of biological processes and networks.

III. RESULTS

Of the 18,214 gene transcripts that were included in the gene chip array, a total of 472 gene transcripts (163 up-regulated and 309 down-regulated) were found to be differentially expressed, at log 2 -fold change of ±1.3 and $P < 0.05$, in samples from sexually mature birds fed RFCs when compared to Control. The top functional annotations associated with this list of genes were: host response to infection (number of transcripts, n=22), inflammatory response (n=22), carbohydrate metabolism (n=25) and cell signalling (n=24). Inflammatory response network analysis identified molecules in the interferon signalling, pattern recognition receptors (PRRS) triggering innate immunity, Th1 and Th2 pathways indicating both pro-inflammatory and anti-inflammatory functions, role of receptors for recognition of bacteria, apoptosis signalling and JAK/Stat signalling all of which were inhibited in expression (Fig 1). Of the top upstream regulators, IFNA2, Interferon alpha and PRL were Inhibited while TRIM24 and MAPK1 were activated. The biological functions that were upregulated in birds fed control diets as compared to RFCs fed, were pro-inflammation, transmembrane helix/integral

component of the membrane (n=11) and secretion (n=8) indicating increased gut modulation and bacterial translocation.

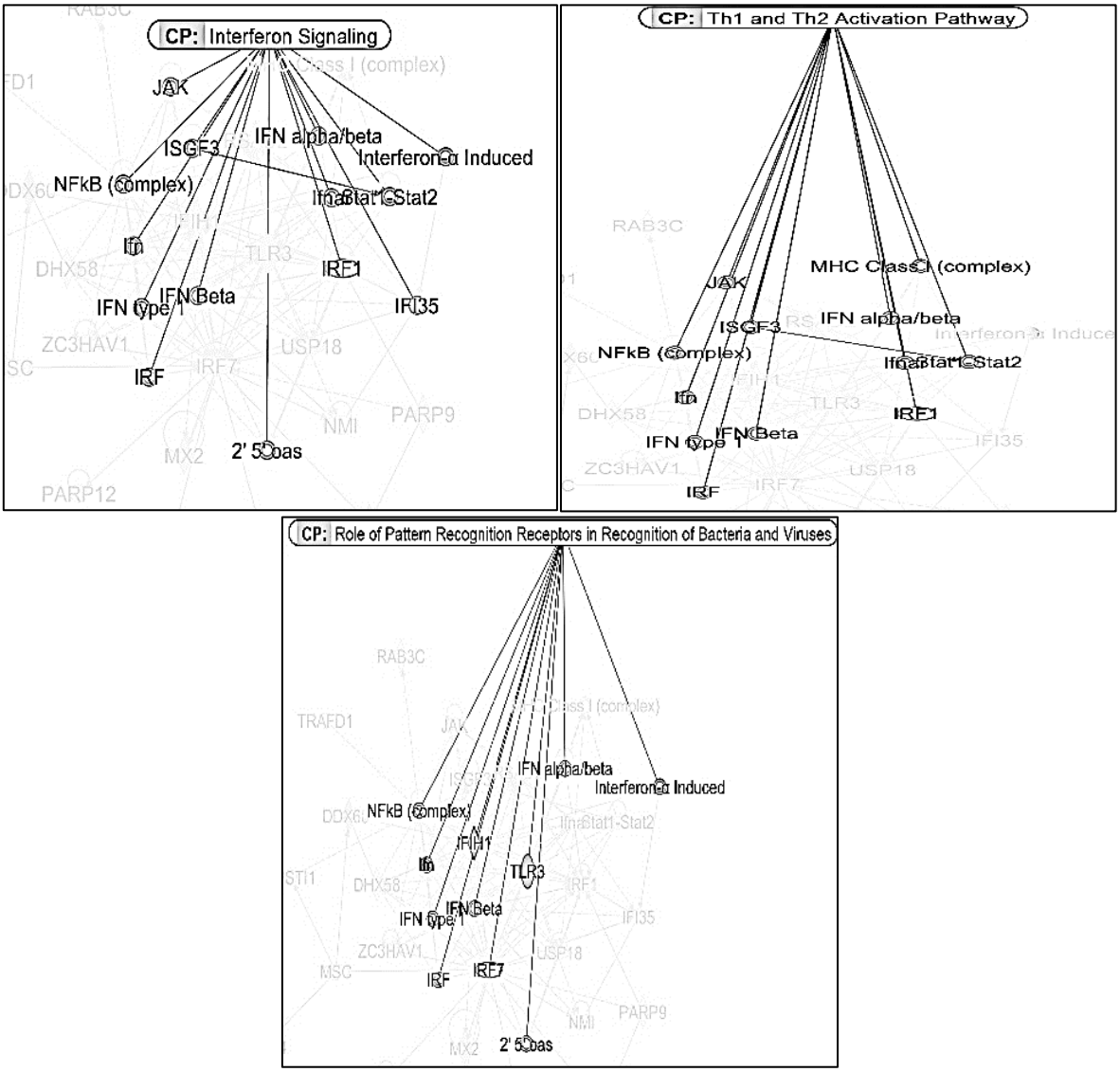

Figure 1 - Network analysis showing relationship between molecules in Inteferon signalling, Th1 and Th2 activation, and Pattern recognition pathways responsible for inhibited inflamatory response in RFCs fed hens as compared to control.

IV. DISCUSSION

Microarray technology has the advantage of simultaneously investigating the expression of thousands of genes, to enable the study of gene interactions and signal pathways. *Salmonella* infection in control samples was shown to activate relevant intracellular signalling pathways associated with pro-inflammatory cytokines production, modulation of gut with proliferation through epithelial adhesion and secretory pathways all leading to consequent inflammation, ulceration and translocation of the bacteria. Treatment with RFCs, however, showed decreased activation of inflammatory and host response to infection, carbohydrate metabolism, gastrointestinal disease and apoptosis associated signalling pathways. This was associated with decreased bacterial translocation and decreased systemic inflammation.

The top regulators identified within these pathways were either responsible for the protective response seen by the addition of RFCs or represented the consequence of reduced

infection with this treatment. Type I interferons (IFNs) activate intracellular host response cascades and influence the development of innate and adaptive immune responses. IFN signalling activates the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway, leading to transcription of IFN-stimulated genes (ISGs). Down regulation of IFNA2 indicates the host-pathogen interaction and the decreased regulation in response of cells to this signalling pathway, thus calibrating host defences while limiting tissue damage and preventing autoimmunity (Robinson et al., 2012). Tripartite motif-containing (TRIM) superfamily are expressed in response to IFNs and are involved in a broad range of biological processes that are associated with innate immunity. TRIM24 associated with precision autophagy, that leads to recognition and delivery of invasive cellular components to lysosomes for degradation was upregulated in response to RFCs treatment, indicating the clearing mechanisms of *Salmonella* (Allton et al., 2009). Inhibition of interferon alpha (IFN-α) is related to host survival while inhibition of PRL indicated lowered proliferation, suppression of apoptosis and decreased cytokine production by modulation of immune response to recognise reduction in infection (López-Meza et al., 2010). Activation of the Mitogen-Activated Protein Kinases MAPK1 involves a signalling cascade leading to early activated response to stimuli and the resultant clearance (Hobbie et al., 1997).

The modulatory effects of prebiotics on host response to infection can partly be attributed to altering of immune system related gene expression. RFCs are seen to have effects on cytokine gene expression, including the genes encoding for pro-inflammatory cytokines and T helper (Th1and Th2) cytokines thus boosting the immune function of sexually mature hens. Moreover, the increased apoptotic functions in the presence of prebiotic decreases the numbers of colonised bacteria in the caeca of chicks. The adhesion of pathogenic bacteria onto the surface of yeast derived RFCs instead of intestinal receptors could also be responsible for decreased activation of pro-inflammatory signalling pathways, and, consequently, translocation of bacteria.

Prebiotics like RFCs can benefit during sexual maturity in hen by boosting the immune response while also providing protection from infection. Unravelling the mechanisms involved in these positive outcomes helps to develop targeted feeding strategies. It will be useful to look at the immune modulatory effects at latter time points to look at the longer lasting effects of the prebiotic during period of lay and beyond in hens.

ACKNOWLEDGEMENTS: This research was funded by Australian Eggs. Help from the PRF and Birling team is also acknowledged.

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GENOMIC AND PATHOGENICITY STUDIES ON *CAMPYLOBACTER HEPATICUS*, THE AGENT RESPONSIBLE FOR SPOTTY LIVER DISEASE IN CHICKENS

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Summary

Spotty Liver Disease (SLD) causes significant economic losses to the poultry industry, causing mortality of up to 11% for up to 6 weeks and up to 25% reduction in egg production. The cause of the disease was recently identified as *Campylobacter hepaticus*. To investigate possible mechanisms of pathogenesis, we studied multiple genomes of *C. hepaticus* isolated from different parts of Australia. By comparison to the HV10 reference genome, the Northern QLD isolates 19L and 54L showed higher variation than the other isolates from Southern parts of Australia including Victoria, New South Wales and South Australia. Some isolates carry plasmids that encode tetracycline resistance. Challenge studies in chickens found that different *C. hepaticus* isolates have different levels of virulence.

I. INTRODUCTION

Campylobacter hepaticus currently causes significant economic losses to the poultry industry, as it is the cause of spotty liver disease (SLD) in chickens (Van *et al.*, 2016, 2017a). The clinical manifestations of SLD include the formation of gray/white lesions in the liver, an increase in mortality rate in a flock, and reduction in egg production. It has been sporadically reported over the last 60 years, first from the United States, then in other countries, including Australia. The disease has become increasingly common in Australia over the last decade and is now considered one of the most significant health challenges in the egg industry (Grimes and Reece, 2011). *Campylobacter hepaticus* was identified and characterised from Australian cases of SLD in 2016 (Van *et al.*, 2016). This work built on the report of isolation of a novel *Campylobacter* isolated from UK cases of SLD (Crawshaw *et al.*, 2015). In 2017, *C. hepaticus* was definitively shown to be the cause of SLD (Van *et al.*, 2017a, Van *et al.*, 2016, Van *et al.*, 2017b).

C. hepaticus is most closely related to the foodborne pathogens *C. jejuni* and *C. coli*. It is anticipated that *C. hepaticus* must harbour genes which are responsible for its ability to cause SLD in chickens, but these are yet to be discovered.

To investigate the bacterium's biology and pathogenicity, we compared the whole genome sequences of 16 Australian isolates of *C. hepaticus*, together with nine *C. hepaticus* isolates from outbreaks in the United Kingdom. We also investigated the plasmid content of these Australian isolates and carried out animal challenge studies with selected Australian isolates to determine their comparative levels of virulence.

II. METHODS

a) Isolation and genomic sequencing of isolates

C. hepaticus was isolated from bile and/or liver samples from outbreaks throughout Australia using the methods described in Van et al. (2017a). DNA of 16 Australian isolates was extracted and sequenced on an Illumina MiSeq Sequencer at RMIT University (Van et al., 2016),

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whereas the sequencing data of nine isolates from UK was obtained online (Petrovska et al 2017). Genomes were assembled using the A5Miseq pipeline (Coil et al., 2015), producing draft genomes for all isolates. For the type strain HV10, a complete finished genome was obtained using sequence data from both Illumina and PacBio platforms (accession CP031611.1). All assemblies and read sets were deposited in NCBI (Bioproject PRJNA485661). The Compare Sequence feature of the SEED viewer was used to compare sequence identity of the annotated genes of all strains.

b) Presence of plasmids in Australian isolates

Sequence assembly contigs with genes annotated as suspected plasmid elements were Blasted against the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Significant matches were identified with >98% coverage and identity to previously characterised plasmids from other *Campylobacter sp*. Selected isolates identified as carrying plasmids bearing antibiotic resistant genes were grown on horse blood agar plates supplemented with antibiotic to confirm their antibiotic resistant characteristics.

c) Animal trials to assess virulence levels of *C. hepaticus* isolates

Two *C. hepaticus* challenge trials of 26-week old Hy-Line layer hens were carried out to investigate the comparative levels of virulence of selected isolates. The animal experimentation was approved by the Wildlife and Small Institutions Animal Ethics Committee of the Victorian Department of Economic Development, Jobs, Transport and Resources (approval number 14.16). The challenges were carried out as described in Van *et al.*, 2017a. The birds were challenged by direct oral gavage with 1 x 10^9 CFU or 10^8 CFU of the relevant *C. hepaticus* strain in 1 ml of Brucella broth. Isolates from Victoria (HV10), Queensland (19L) and New South Wales (44L) were used in the first trial. A second animal trial was performed using HV10 and 44L isolates to confirm the findings of the first trial. Unchallenged control chickens were given 1 ml of uninoculated Brucella broth. Birds were scored as SLD positive if the liver had typical SLD lesions. Fisher's exact test was used to find statistically significant differences in the number of SLD positive birds.

III. RESULTS

Genome sizes of the *C. hepaticus* isolates ranged from 1.481-1.535 Mb and the GC content ranged from 27.9 to 28.2%. The genomes contained 1,472 to 1,555 predicted protein coding sequences. By comparison to the HV10 reference genome, the UK isolates showed some variation in genome sequences and the Northern QLD isolates 19L and 54L showed higher variation than the other Southern Australian isolates. The protein analysis coincides with the genome analysis with regards to the comparative relationship between isolates (Fig. 1).

Six out of 16 Australian isolates contained plasmids. Five isolates from Vic (ACE1, ACE8659, ACEM3A, 84B, 27L) contain a *C. jejuni* pCJDM210-like plasmid (>98% identity) and one isolate from South Australia (SAJune18) contained a *C. coli* pCC31-like plasmid (100% identity). Both plasmid types contain a tetracycline resistant gene, *tet(O).* These plasmid-containing isolates grew on horse blood agar plates containing 30 µg/mL of tetracycline, whereas plasmid negative isolates such as HV10 and NSW44L isolates did not grow.

Two animal trials were carried out to examine the relative degree of virulence of a number of isolates. In the first trial, at the same challenge dose of 10^9 CFU, the isolate HV10 (Victoria) showed a similar level of virulence as the isolate 54L (QLD), as judged by the

percentage of challenged birds that had visible liver lesions. At a challenge dose of 10^8 CFU, strain NSW44L (NSW) showed a higher level of virulence than the strain HV10, in terms of disease severity and percentage of SLD-positive birds, as all 8 birds of the former group contained 200-1000 SLD typical white spots, while only half of the latter group of birds have that similar disease severity. A second trial was carried out to confirm the greater pathogenicity of the NSW44L strain compared to the HV10 strain. As expected, when birds were challenged with the same dose, NSW44L caused disease in more birds than the HV10 strain (p=0.03) (Table 1).

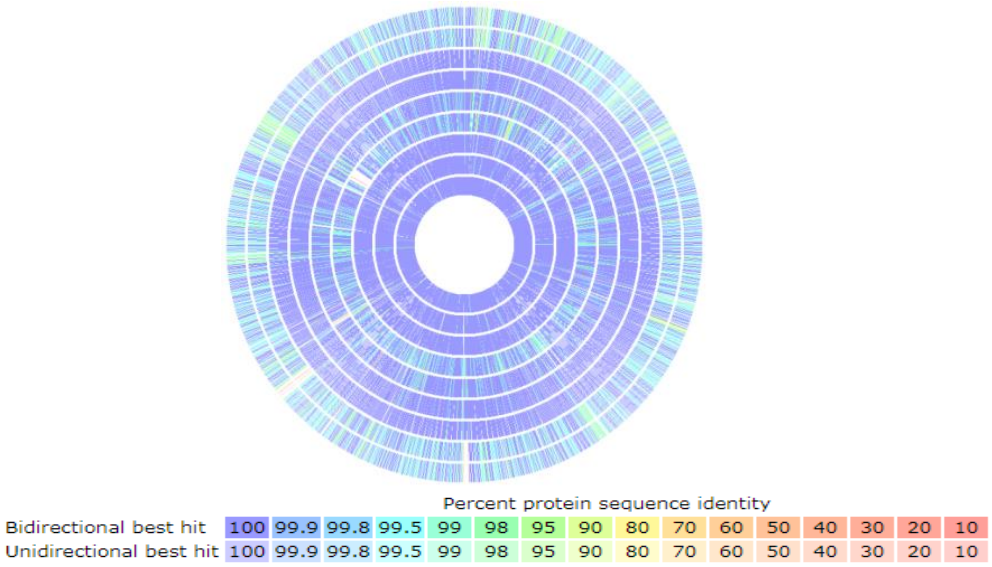

Figure 1 - Comparison of predicted *C. hepaticus* proteins from different isolates with HV10 was used as the reference sequence. From the most inner to outer rings are VicJune18 (Vic isolate), SAJune18, SA32L (SA isolates), S12-0322, S11-0036 (UK isolates), NSW44L (NSW isolate), ACE1 (Vic), 54L and 19L (QLD isolates). Percent protein sequence identity is expressed by colour coding provided in the legend.

Table 1 - Percentage of SLD-positive birds after challenge with *C. hepaticus* isolates.

Challenged doses	Trial 1		
	Isolates (State of Origin)		
	HV10 (Vic)	NSW44L (NSW)	19L (QLD)
Dose 10^9 CFU	76.6% (7/8)	ND	76.6% (7/8)
Dose 10^8 CFU	62.5% (5/8)	100% (8/8)	ND
	Trial 2		
Dose 10^8 CFU	8.3% (1/12)	58.3% (7/12)	ND

Genome comparison between NSW44L and HV10 showed 12 genes were present in NSW44L but absent in HV10 (eight of these genes were hypothetical proteins, and the remaining were genes encoding trimethylamine-N-oxide reductase, beta lactamase, possible lipoprotein, and *YidD)*, plus there were various genes with less than 100% identity between the two isolates.

IV. DISCUSSION

Genome and proteome comparison showed that the isolates from the Southern region (Vic, NSW and SA) were more closely related to each other than to the isolates from the Northern

region (QLD). This indicates that *C. hepaticus* clonal populations are geographically confined, although more isolates need to be collected to confirm this finding.

The genome sequences of all isolates were examined for plasmid content as plasmids may play an important role in dissemination of antibiotic resistance genes. Plasmids were found in isolates from Victoria and SA. They contained two different types of plasmid; isolates from Victoria contained *C. jejuni* pCJDM210L-like plasmids, and the SAJune18 isolate from SA contained a *C. coli* pCC31-like plasmid. Both plasmids contained a tetracycline resistant gene, *tet(O).* Selected isolates were also shown to phenotypically express tetracycline resistance as they grew well on tetracycline containing plates in the laboratory. It seems that both *C. jejuni* and *C. coli* can pass antibiotic resistance plasmids to *C. hepaticus*, as the UK isolates also have the *C. coli* pCC31-like plasmid (Petrovska *et al.*, 2017), which has 89% query coverage and 100% identity to Australian pCC31-like plasmids. The presence of Tet resistant plasmid in *C. hepaticus* isolated from SA might be the reason why the flock from which this strain was isolated did not respond to chlortetracycline treatment (Kapil Chousalkar, personal communication). The C. *coli* pCC31 plasmid has been shown to be conjugative (Batchelor *et al.*, 2004), therefore *C. hepaticus* plasmids could be disseminated to important human and animal pathogenic bacteria.

Three isolates from different states were selected for the first challenge trial (HV10 from Victoria, NSW 44L from NSW, and 19L from QLD) and it was shown that the NSW44L strain was more virulent than the type strain HV10, while HV10 showed a similar level of virulence as the isolate 54L. Genome comparison between HV10 and NSW44L isolates showed some unique genes within the NSW44L isolate and these may play a role in the greater virulence of NSW44L. However, to confirm this finding, substantial further work is needed, for example the construction and testing of targeted mutants of potential virulence genes to determine their involvement in disease pathogenesis.

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FIELD VACCINATION AGAINST ILT IN BROILER CHICKENS: LACK OF CONSISTENCY

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Summary

When vaccination against infectious laryngotracheitis (ILT) is necessary in broiler flocks, mass administration techniques are required due to the sheer numbers of birds involved. This is usually done by drinking water administration even though ILT vaccines are generally registered for individual eye drop application. Often the results are variable, with occasional outbreaks occurring in vaccinated flocks or rolling vaccine "reactions" occurring, sometimes involving clinical signs and even mortality. A field evaluation study was performed during a field outbreak using collection of tracheal swabs from birds in vaccinated sheds. DNA extracted from swab samples was submitted for quantitative real time PCR (qPCR) to detect and quantify ILT virus (ILTV) at the University of New England (UNE). The first shed examined used 70 individually identified birds which were retrieved at 7 post vaccination time points. A further 7 sheds were then followed with 40-45 randomly selected birds at 4, 7-8, 12-13 and 25-26 days post vaccination. Vaccination procedures and timings of administration were recorded. Patterns of vaccine "take" were compared across factors recorded at vaccination day to look for putative risk factors for poor or better vaccination success. ILT virus recovery varied markedly between sheds but there were patterns observed between poor and somewhat better results. Factors showing statistical association with these patterns included use of a dye product to stabilize water as a risk factor and longer time of water stabilization with a skim milk product prior to vaccine addition as a protective factor. There was also an almost significant (P=0.07) association of chick source with vaccine take pattern. Results have implications for vaccine application and suggest a possible monitoring process for vaccination success.

I. INTRODUCTION

Field success of mass ILT vaccination in young meat chickens is often accompanied by reports of vaccine reactions, often rolling through the flock for some time, and even apparent vaccination failure with wild strain outbreaks in vaccinated flocks. This is in spite of laboratory challenge studies often describing good protection of vaccines against an artificial challenge with the field outbreak strain (Arzey and Arzey, 1993). Laboratory studies (Rodrigues-Avila et al., 2007; Coppo et al., 2012a and 2012b) have described post vaccination (pv) recoveries of vaccine virus in directly vaccinated birds and in-contact birds. Using the Serva ILT vaccine strain (MSD Nobilis; Class 7 virus), these studies have shown that directly vaccinated birds (eye drop route) have a peak tracheal viral copy number at day 4 pv, while birds placed in contact at day of vaccination show a delayed peak at day 8 pv. Birds vaccinated by drinking water in these laboratories however show a peak tracheal ILT viral titre at day 8 pv (Coppo et al., 2012b), which would tend to indicate that there are some birds receiving contact with the virus by spread from other birds. Further, Rodrigues-Avila et al. (2007) showed that birds placed in contact with Serva vaccinated birds at various times showed intermittent ILT viral

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recovery from trachea for extended periods with intermittent levels of mucosal antibody in the trachea cited as being responsible for this pattern. Robertson and Egerton (1981) demonstrated that ILT vaccine virus must make contact with a respiratory target tissue (e.g. conjunctiva, nasal passages, and trachea) to establish an infection and replicate and thus stimulate immunity but contact only with the mouth, tongue, pharynx or oesophagus was ineffective. These authors also showed that drinking water application only achieved this respiratory contact by accident, with less than 30% of birds showing such contact, and only with a limited amount of the vaccine administered. de Wit (2013) further notes that for ILT vaccination via drinking water to be as effective as eye drop, multiple doses (10 to 100 times) of the vaccine are required.
To evaluate some aspects of what actually occurs during a field mass vaccination application of ILT vaccine in broiler chickens, a study was performed to look at sequential ILT vaccine virus detection in tracheas in several flocks.

II. METHOD

Study 1: A natural ILT outbreak induced by a wild ILT strain classified as Class 9 began in the greater Sydney region in mid-2017. The local poultry companies began vaccinating broilers in this region with Class 7 (Serva) strain vaccine. A single shed on a 5-shed farm that was being vaccinated was selected at random for the initial study. Vaccination was administered in drinking water using skim milk powder as a stabilizer at 9 days of age. Seventy chicks were selected at random from various locations in the shed 1 day pv. These had individual tracheal swabs collected and they were individually identified with coded permanent pen markings on each shank and their wings were sprayed with blue stockmark to enable them to be located at the next sampling day. The birds were retrieved on each of days 4, 8, 12, 18, 21 and 26 pv when tracheal swabs were again collected and the identification markings refreshed. Apart from one bird which died from a non-ILT cause on day 15 pv, every bird was retrieved at every sampling occasion. DNA was extracted from swabs at Birling Avian Laboratories (BAL) and samples submitted to UNE for ILT qPCR.

Study 2: Based on the findings of study 1, a sample size of 40 was calculated as statistically able to provide a valid estimate of prevalence of ILT detectability from tracheas and another 7 sheds were selected across three farms. Forty or 45 birds were randomly selected at each of days 4, 7-8, 12-13 or 25 pv and tracheal swabs were collected from each and again submitted for qPCR to enumerate ILTV genome copy number (Roy et al., 2015).
In all sheds, vaccination procedures were documented, including times between critical procedures during administration and choice of water stabilizer used. Two sheds on the final farm agreed to use a dye preparation as stabilizer as a comparison to a skim milk product (either powder or liquid).

Point and period prevalence of ILT viral detection in tracheas was calculated at each sampling time. Statistical analyses were conducted using STATISTICA v6 software comparing prevalence across vaccination administration factors.

III. RESULTS AND DISCUSSION

In study 1 with repeat sampling of the same birds, the incidence of birds (i.e. those first showing a positive result on each day/ number remaining negative at the previous day) with positive tracheal detection of ILT virus was only 2 birds positive at day 4pv (2.85%), 4 at day 8 pv (6.06%), 13 birds at day 12 pv (20.3%), 13 birds at day 18 pv (25.4%), 10 birds at day 21 pv (27.0%) and 18 at day 25 pv (66.6%). Period prevalence (i.e. the total number of birds with ILT virus detectable from tracheal swabs on each sampling day) was 2.9%, 8.6%, 27.1%, 46.4%, 60.9% and 87.0% at each time point respectively. Thus, from a very few number of birds positive at the first sampling, the numbers of birds showing detectable virus grew slowly

over time. There were still 9 birds (13%) in the sampling group which never showed ILT viral detection up to day 26 pv (or 34 days of age when depopulation of the flock began). Also many birds revealed an intermittent presence of ILT virus in their tracheas over time. Quantification of viral DNA in tracheas over time for this study is shown in Figure 1. This intermittent pattern is very evident and it can be seen that viral loads did not peak at 8 days, as would have been expected from laboratory studies using drinking water administration of Serva strain vaccine (Rodrigues-Avila et al., 2007; Coppo et al., 2012b). It was obvious that bird to bird transmission was mostly responsible for the eventual vaccine spread through this flock. All positive isolates on day 25 pv were typed at BAL and all proved to be Class 7, consistent with the vaccine strain. No Class 9 strain was detected in this flock. It was concluded that the uptake of vaccine virus from the actual drinking water application in this flock was very poor.

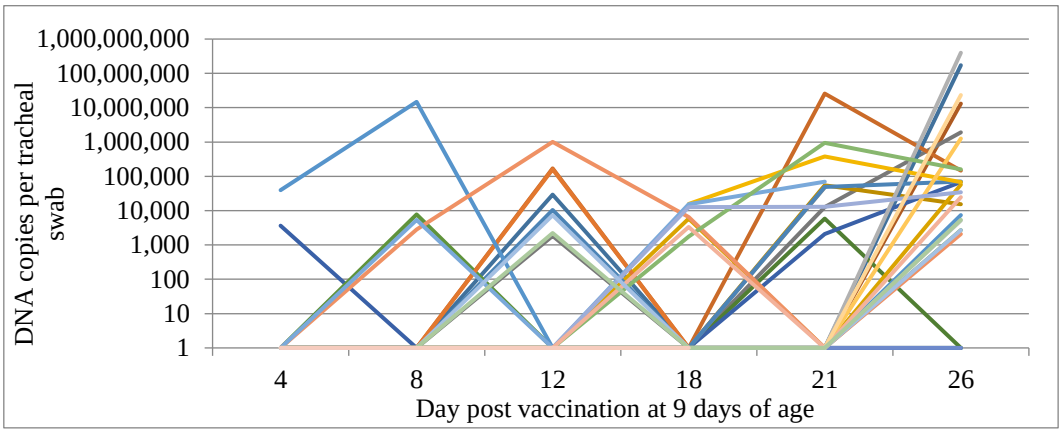

Figure 1 - ILT viral genomes per 5 µL reaction following vaccination in sequentially sampled birds in Study 1. Each line represents a single sequentially measured bird.

Period prevalence from study 2 (and including results from study 1) for detection of ILT viral DNA from tracheal swabs is shown in Figure 2. Two obviously different patterns of apparent success of vaccination could be discerned from Figure 2, with 3 flocks showing a "poor" uptake and 5 flocks showing an earlier "better" uptake. None of the flocks could really be regarded as having a "good" vaccine uptake from the drinking water administration (the prevalence of tracheal ILT DNA at day 4 pv varied from 2.8 to only 52%). The flocks in the "better" category had significant proportions of the flock exposed to the vaccine virus by 12-13 days pv (i.e. around 21-23 days of age). Most field outbreaks are reported after 30 days of age and hence, given an incubation time of 6-8 days (AV Dis ref), spreading wild virus infection may be occurring around 22-24 days. Flocks with high vaccine prevalence after 12-13 days pv therefore may have significant protection but those in the "poor" category would still have large numbers of susceptible birds at this age. This may explain the field observation of vaccine failures. Also, bird to bird transfer of vaccine strains can lead to reversion to virulence, and the slower spreading of vaccine virus in the "poor" take flocks may be responsible for the observed vaccine "reactions" in the field.

Contingency table analysis across the two vaccine "take" patterns showed significant associations of the "poor" takes with the use of the dye stabilizer product as compared to skim milk products and with shorter stabilization time before vaccine was added. There was also a possible association with hatchery source of the chicks (P=0.07) with prevalence of tracheal ILT DNA detection at 12-13 days, but this needs to be further explored.

Drinking water administration of ILT vaccine continues to be a gamble. Uptake of the vaccine virus from this mass vaccination route appears to be fraught with inconsistency and studies aimed at understanding which techniques result in better vaccine uptake need to be expanded in order to improve vaccinal protection for susceptible broilers in the face of

outbreaks. This was a restricted study in terms of sheds studied so wider observational studies aimed at confirming the putative risk factors identified here are necessary to validate the conclusions on risk factors.

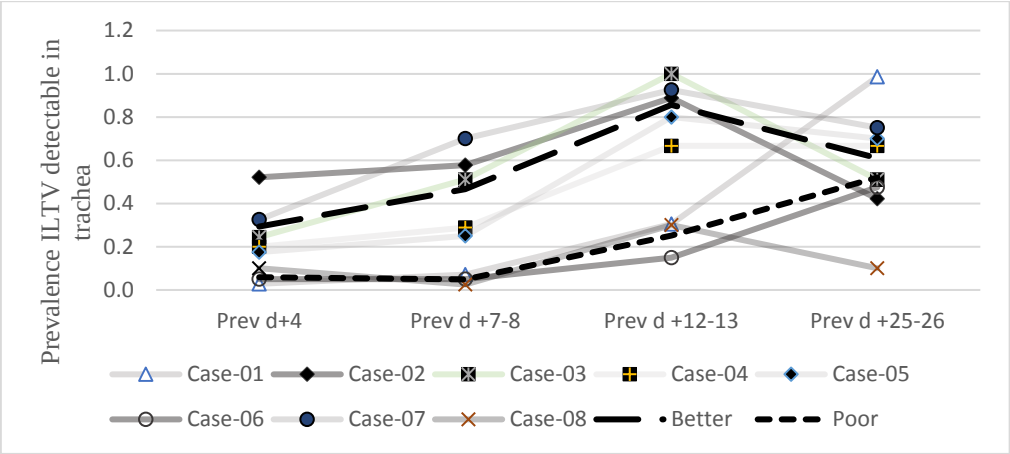

Figure 2 - Point prevalence of ILT viral DNA detection in birds sampled over the eight sheds from both studies. Dashed lines show division of the outcomes into poor or better vaccination success.

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TEMPORAL VARIATION OF ILTV AND MDV VIRAL GENOME IN DUST SAMPLES AFTER VACCINATION IN A LAYER FLOCK

T.V. NGUYEN[1], M. AHADUZZAMAN[1], D. CAMPBELL[2], P.F. GERBER[1] and S.W. WALKDEN-BROWN[1]

Summary

An experiment with two phases, a pullet raising phase and a laying phase, was conducted to monitor the temporal variation of infectious laryngotracheitis virus (ILTV) and Marek's disease virus (MDV) vaccinal load in dust samples. These were collected weekly by settle plate and scrapings from the wall and horizontal surfaces from placement at day old to 40 weeks of age. The chickens were vaccinated against MDV (Rispens) at day old and with ILTV in water at 6 weeks (A20 strain) and by eye drop at 12 weeks of age (SA2 strain). The genome copy (GC) number of ILTV and Rispens in the dust samples were measured by using qPCR. ILTV was detected in dust 4 weeks after the first ILTV vaccination and viral load peaked 2 weeks after the second vaccination. ILTV became undetectable by 8 weeks after the 2nd vaccination apart from one positive sample at week 26. MDV was detected in dust one week after vaccination, peaked 3 weeks post vaccination and was detectable at a high level until week 17, when levels started to decline. There was no difference between settle plate and scraped samples in ILTV GC but, for MDV, higher GC were found in scraped samples later in the experiment probably reflecting accumulation of old dust. The findings suggest that the settle plate method better reflects the current level of vaccine virus in dust while the scrape method likely represents a cumulative and historical record of shedding over a period of time. Assessment of viral GC in a dust sample post vaccination is a promising candidate for a practical, routine method of indirectly assessing vaccine virus take for these viruses in commercial layer flocks, consistent with findings for ILTV vaccine take in broilers (Ahaduzzaman et al., 2019).

I. INTRODUCTION

Marek's disease virus (MDV) and infectious laryngotracheitis virus (ILTV) are two economically important viruses of subfamily *Alphaherpesvirinae*. MDV is an extremely contagious virus which can cause severe oncogenic changes in viscera and tissues and high mortality in chicken while ILTV causes serious respiratory disease in chickens. Routine surveillance of MDV in poultry dust by quantitative PCR (qPCR) has been successfully used by industry for monitoring MDV presence in Australia (Walkden-Brown et al., 2013) and in the USA (Kennedy et al., 2017). Dust samples can be collected on settle plates or scraped from barn surfaces. Kennedy et al. (2017) suggest that both sampling methods could be biased, but the spatial artefacts from settle plates could be avoided by using the scraped method. This same approach could be used in commercial chicken flocks to investigate vaccination success for other pathogens. In fact, Roy et al. (2015) has shown that ILTV vaccine strains can be found at high levels in dust samples under experimental conditions. This study was therefore designed to investigate MDV and ILTV vaccine viral load following vaccination in dust samples collected by settle plates or scraped from surfaces. Variation in the weight of dust collected over time was also analysed.

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II. METHOD

The experiment used 1700 Hy-Line Brown chicks raised from day old and included two phases, pullet raising (0 to 16 weeks of age) and laying (17 to 40 weeks of age) in different poultry houses and conditions. During the pullet raising phase chicks were reared in a shed with three separate rooms with no common air space. In each room, around 570 Hy-Line Brown chicks were raised from day old. Each room was 6.25 x 9.78 m, with two doors, two circulating fans, 1 roof fan and 4 double mini-vents. The air was pulled into the shed through double mini-vents and exhausted through the roof fans. Chicks were reared on rice hulls for bedding. At day old, chicks were vaccinated against MDV serotype 1 Rispens CVI988 (Vaxsafe RIS, Bioproperties Pty Ltd) by subcutaneous injection. The birds were vaccinated against ILTV with A20 strain (Poulvac Laryngo A20) by drinking water at 6 weeks of age and with SA-2 (Poulvac Laryngo SA2) by eye drop at 12 weeks.

At 16 weeks of age, the pullets were moved to an experimental free-range laying shed with rice hulls as bedding. Within the shed, birds were housed in 9 chicken mesh and shade cloth sided pens with 154 chickens/pen in a space of 17.28 m^2. The pens shared a common air space, but 4 pens had exhaust fan in them while the remaining 5 did not. Each pen had a single chicken mesh door and 3 pop-holes that connected the pen with a free-range area outside. The pop-holes were opened between 9 h and 18 h from 25 weeks of age onwards.

Dust samples were collected from settle plates (surface area 520 cm^2) suspended at a height of approximately 1.4 m. Dust samples were collected weekly from 4 sites per pen in the pullet raising phase and 2 sites per pen in the laying phase. A single pooled dust sample from all sites was created each week. Samples were stored in plastic ziplock bags at -20^{o}C.

Following thorough mixing of dust pools, DNA was extracted from 5 mg of dust using the Bio-line ISOLATE II Genomic DNA kit (Roy et al., 2015). Extracts were tested by qPCR for ILTV (Callison et al., 2007) and MDV (Islam et al., 2006). Results were reported in log10 genomic copies (GC). Statistical analyses were conducted using JMP 13 (SAS Institute Inc., Cary NC, USA). The effects of week of age and sampling method on dust weight and viral GC were tested using appropriate analyses of variance (ANOVA).

III. RESULTS

The weight of dust collected from settle plates increased rapidly during the pullet raising phase while it was lower during the laying phase, when birds were at a lower density (Figure 1). In the pullet raising phase, the weight of dust per plate rapidly increased from 316 mg at week 1 and peaked at week 13 with 6538 mg ($P < 0.0001$). In both periods there was a highly significant effect of bird age on dust weight ($P < 0.0001$).

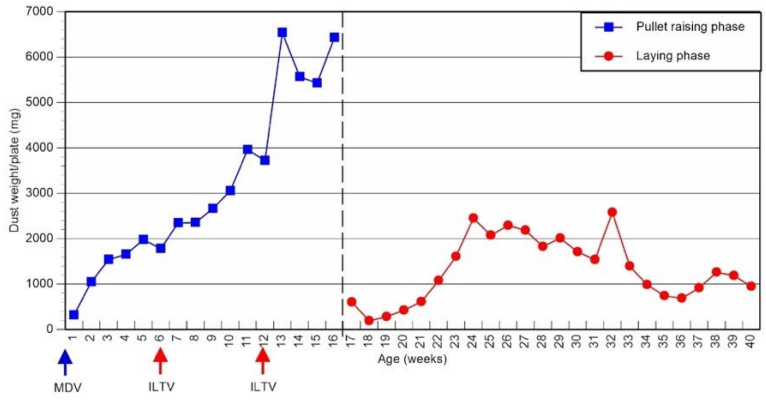

Figure 1 - Mean of dust weight collected from individual settle plate samples during the pullet raising phase from weeks 1 to 16 of and laying phase from weeks 17 to 40 of age.

In the pullet raising phase, ILTV was first detected in dust at week 10 and peaked at week 14, 3 weeks after second vaccination (Figure 2). There was a significant effect of bird age on ILTV GC ($P < 0.0001$). During the laying phase ILTV was detected in both settle plate and scraped samples from weeks 17-19 becoming negative thereafter apart from a positive settle plate sample at week 26. There was no significant difference in the number of ILTV positive samples ($P = 0.28$) or ILTV GC ($P = 0.30$) between settle plate and scraped samples.

MDV GC in dust also varied significantly with chicken age during the pullet raising phase ($P < 0.0001$). The first positive detection was at week 1 with a peak in week 3 followed by a slow decline over the next 12 weeks (Figure 2). In the laying phase, MDV GC in settle plate samples reduced rapidly. This decline was not evident in scraped samples resulting in a significantly higher number of MDV positive samples ($P < 0.01$) and MDV GC ($P < 0.0001$, 2.15 and 4.82 respectively) compared to settle plate samples.

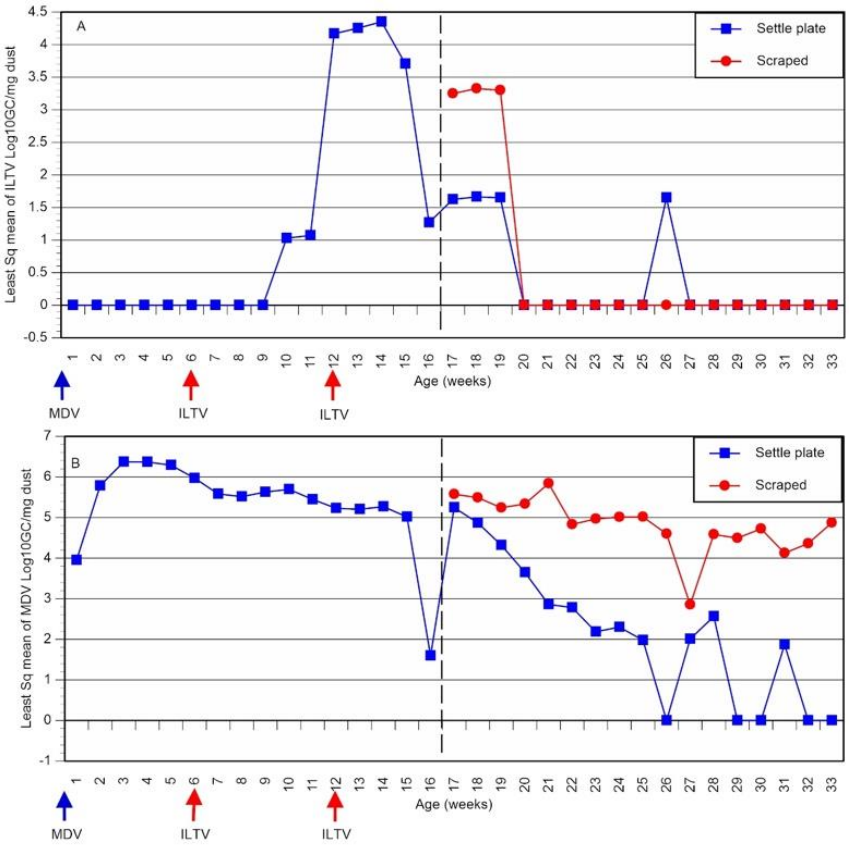

Figure 2 - Log10 GC/mg of ILTV (A, top figure) or MDV (B, lower figure) in pooled settle plate or scraped dust samples over time following vaccination.

IV. DISCUSSION

The amount of dust collected at all times was far in excess of the 5mg used for DNA extraction, so that collection intervals of less than one week would be possible, particularly from two weeks of age onwards. The dust in poultry houses is a complex combination of materials including feed and avian excreta such as faeces, skin and feather (Ralapanawe et al., 2016) with animals themselves the most important dust source (Ellen et al., 2000). The increase in dust production during the raising phase reflects the increased breakdown of litter materials and increase in bird excretion. The marked reduction in dust amount during the laying phase is probably due the lower bird density in this phase with fluctuations due to differences in ventilation rate and range use by the chickens.

With regard to ILTV detection in dust, there was a low shedding after water vaccination at week 6 (A20 strain), but a higher shedding after eye drop vaccination at week 12 (SA2 strain). These differences may be due to the different vaccines used and to the different vaccination methods, with eye drop vaccination resulting in a better vaccination success. The dust results are consistent with the initial report of Roy et al. (2015) of ILTV detection in isolator dust from vaccinated birds. Ahaduzzaman et al (2019) have shown that ILTV GC levels in dust following ILT vaccination of broilers in water relate well to variable vaccine take assessed in individual birds as reported by Groves et al., (2019). The origin of the virus in the dust is unclear but Roy et al., (2015) reported high levels of ILTV GC in the faeces following vaccination and this is one potential source. Overall, these results indicate that measurement of ILTV in dust 1-3 weeks after vaccination may be an effective method of assessing vaccination success in layers. The low sample number (one pooled sample), ease of collection and transport (no cold chain required) and moderate cost (approx $100) would assist in making it practical under commercial conditions. The single positive at week 26 may represent a release from latency and this requires further investigation.

With regard to MDV there was a peak in viral load 3 weeks post vaccination but with a much more prolonged shedding period of some 25 weeks. The decline in MDV shedding from week 17 detected in settle plate samples was masked in the scraped samples which appear to provide a mean viral load value over the period of dust accumulation at the site, rather than the current levels of MDV in dust. This may explain the longer persistence of MDV Rispens shedding in scraped samples reported by Ralapanawe et al. (2016). For either virus, dust samples collected in settle plate over a fixed period of time have the advantage of reflecting current viral load in dust samples, when compared to scraped dust samples that may reflect historical accumulation.

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SPATIAL AND TEMPORAL VARIATION IN INFECTIOUS LARYNGOTRACHEITIS VIRAL GENOME IN BROILER FLOCK DUST POST VACCINATION

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Summary

Infectious laryngotracheitis (ILT) is an economically important disease of chickens that is endemic in several important poultry production regions of Australia. Outbreaks commonly occur in meat chicken flocks and mass vaccination, usually in water, is used to control the disease and limit its spread during outbreaks. Vaccination with live virus via water and nipple drinkers requires stringent adherence to protocols to ensure success. Evaluation of vaccination success is not performed due to a lack of practical and economic methodologies. ILT vaccine virus has been shown to be detectable in dust following vaccination in experimental studies and offers a potential method of assessing vaccination success. The pattern of vaccinal infectious laryngotracheitis virus (vILTV) detection in commercial poultry dust following vaccination has not been defined in commercial flocks to date and this is the purpose of this study. We report the longitudinal profile of vILTV genome copies (GC) in dust following vaccination of 8 flocks/sheds of commercial meat chickens on four farms. vILTV GC could be detected in poultry dust after vaccination using quantitative real-time polymerase chain reaction (qPCR). There was considerable variation between flocks in the pattern observed and this variation was associated with vaccination success measured in individual birds. There was no significant effect of sampling location within sheds on vILTV GC ($P = 0.90$). Results suggest that measurement of vILTV GC in a single pooled sample at days post vaccination 7 or 8 may enable discrimination between vaccination success and failure and provide practical means of monitoring vaccination.

I. INTRODUCTION

Infectious laryngotracheitis (ILT) is an important respiratory disease of chickens caused by ILT virus (ILTV, *Gallid herpesvirus 1*) that infects the upper respiratory tract and conjunctiva resulting in high morbidity and sometimes mortality (García et al., 2013). Vaccination with live attenuated vaccines administered by eye drop is generally effective: however for commercial meat chickens mass vaccination in drinking water via nipple drinkers is generally practiced. This method increases the risk of vaccination failure due to the lack of vaccine contact with the upper respiratory tract and conjunctival tissues (Robertson and Egerton, 1981; Menendez et al., 2014). Due to the lack of practical and economically viable methods, no evaluation of vaccination success is undertaken following mass vaccination of commercial meat chickens. A method for monitoring of vaccinal or wild type strains of a related herpesvirus, Marek's disease virus, in poultry dust by real-time quantitative PCR (qPCR) has been successfully implemented by the poultry industry (Walkden-Brown et al., 2013; Kennedy et al., 2017). The feasibility of using this method to determine vaccination success for other poultry vaccines needs to be determined. A previous study has shown that high ILTV genomic copies (GC) are detected in faeces and dust after ILT vaccination by oral inoculation (Roy et

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al., 2015). This study aims to determine under commercial conditions: 1. the level and duration of ILTV detection in dust at various times following water based ILT vaccination, and 2. if sampling location within a shed influences vILTV GC detection.

II. METHODS

During a natural outbreak of ILT in the greater Sydney region, preventive mass vaccination with the Serva strain of ILTV vaccine (Nobilis ILT, MSD) in meat chickens was implemented. Flocks in four conventional and four tunnel ventilated meat chicken sheds on 4 farms were included in the study. Water vaccination was conducted by the farmers at 7 - 14 days of age. Two to six settle plates were installed in each shed on the day of vaccination. The settle plates comprised an L-shaped piece of flat metal with a plastic plate bolted to the horizontal section. The vertical portion was attached to vertical wires in the shed using a clamp mechanism. A second plate sat in the first plate and could be easily removed for dust collection into pre-weighed in plastic zip-lock bags. The surface area of the settle plate was 520 cm^2. Dust samples were collected from each plate at 4, 7-8, 12-13 and 25 days post vaccination. Samples were weighed in the bags and stored at -20°C until needed. DNA was extracted from 5 mg of dust from each sample using the ISOLATE II Genomic DNA kit (Roy et al., 2015). Extracted DNA was tested for ILTV by qPCR targeting the gC gene (Callison et al., 2007; Roy et al., 2015). Results were reported as ILTV log10 genomic copies (GC) per mg of dust based on a plasmid preparation of the target sequence (Roy et al., 2015).

Statistical analyses were performed using JMP v.14 software (SAS Institute, Cary, NC, USA). Analysis of variance was used with viral GC fitted as the variable effect and settle plate location, shed type, and day post vaccination (dpv) fitted as fixed effects. Data are presented as least-squares means (LSM±SEM). Chi-square test was used for effect of location on ILTV GC detection rate. $P < 0.05$ was considered statistically significant.

III. RESULTS

The proportion of positive samples varied between flocks and day post vaccination (Table 1) ($P < 0.001$). Overall, two ILTV detection patterns were observed, flocks in which positive samples were first detected at 7-8 dpv and positive thereafter (Flocks 2, 3, 4, 5 and 7) and flocks in which samples were first consistently positive at or later than 12-13 dpv (Flocks 1, 6 and 8). Unusually, in Flock 1, 33% of dust samples were positive for ILTV GC at 4 dpv but then negative until 25 dpv. Of the 32 combinations of sample time × flock, 27 (84%) all dust samples were uniformly positive or negative. A mixture of positive and negative samples was mostly observed when ILTV GC load was low or decreasing (Table 1, Figure 1).

Table 1 - Proportion of ILTV positive samples in dust on days post vaccination 4 to 25 in eight meat chicken flocks (positive sample times are bolded).

Flock	Day post vaccination				P-value
	4	7-8	12-13	25	
1	**0.33 (2/6)**	0.00 (0/6)	0.00 (0/6)	**0.83 (5/6)**	0.69
2	0.00 (0/2)	**1.00 (1/1)**	**1.00 (2/2)**	**1.00 (2/2)**	1.00
3	0.00 (0/2)	**1.00 (1/1)**	**1.00 (2/2)**	**1.00 (2/2)**	1.00
4	0.00 (0/2)	**1.00 (2/2)**	**1.00 (2/2)**	**1.00 (2/2)**	1.00
5	0.00 (0/4)	**1.00 (4/4)**	**1.00 (4/4)**	**0.75 (3/4)**	0.21
6	0.00 (0/4)	0.00 (0/4)	**1.00 (4/4)**	**1.00 (4/4)**	1.00
7	0.00 (0/4)	**1.00 (4/4)**	**1.00 (4/4)**	**1.00 (4/4)**	1.00
8	0.00 (0/4)	0.00 (0/4)	**0.75 (3/4)**	**0.50 (2/4)**	0.02

Overall, settle plate location had no impact in the ILTV GC detection rates (P = 0.90) except for Flock 1 on 4 dpv and 25dpv; Flock 5 on 25dpv; and Flock 8 on 13dpv and 25dpv (Table 1). All dust samples collected from Flocks 2 to 4 and Flocks 6 and 7 at a given dpv were consistently positive or negative for the duration of the study. For the remaining flocks, the detection rates for a given dpv were between 33% and 83% (Table 1).

Likewise, ILTV GC varied between flocks and dpv (P < 0.001) (Figure 1). At 7-8 dpv, five flocks (2 - 5 and 7) exhibited ILTV $\log_{10}$ GC between 3.11 and 7.8 with high values persisting until the end of the study at 25 dpv. The remaining three flocks (1, 6 and 8) had low ILTV $\log_{10}$ GC (0 - 0.09) before 12-13 dpv, with rapid increase afterwards.

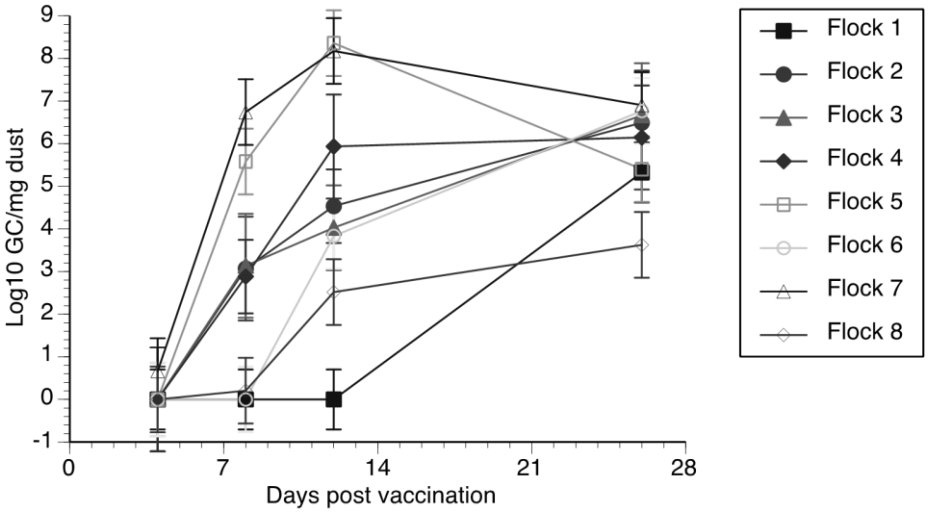

Figure 1 - ILTV GC in dust by day post vaccination in eight meat chicken flocks (LSM ± SEM). Note days 7-8 and 12-13 combined for analysis and shown on days 8 and 12 in this figure.

IV. DISCUSSION

ILTV vaccine GC were detected in dust samples from 4 to 25 dpv. At 4 dpv, most samples were negative for ILTV but the number of positive samples increased at variable rates over time. The results are consistent with Roy et al. (2015), who reported ILTV GC detection in dust samples from 7 to 28 dpv in birds orally vaccinated with ILTV SA2 and A20 strains.

A rapid increase of ILTV GC was observed shortly after vaccination with the ILTV Serva strain in five of eight flocks which is suggestive of vaccination success, whereas absence of detection of ILTV at 7-8 dpi or detection of very low levels in the other 3 flocks is an indication of a low level of early vaccination take. This difference between the flocks reflected real differences in vaccine take in these flocks detected by tracheal swab measurements of individual birds (Groves et al., 2018). However, the variation in vaccination take observed in the present studies highlights risk of vaccination failure with water application through nipple drinkers due to the lack of vaccine contact with the upper respiratory tract and conjunctival tissues (Robertson and Egerton, 1981; Menendez et al., 2014). Taken together, these results suggest that the best point of differentiation among flocks with adequate or poor vaccine take is at 7-8 dpv. Peak of ILTV GC detection at later time points would indicate secondary bird to bird transmission resulting from non-uniform initial vaccination. It should be noted that the tracheal swab data of Groves et al., (2018) revealed that in only one flock were more than 50% of chickens ILTV positive at 4 dpv so even the best vaccination events in this experiment may have been sub-optimal. Very low initial vaccination take could explain the low ILTV GC detection rates at 4 dpv on Flock 1 followed by negative results until 25 dpv.

Regarding sampling location, no significant variation was observed in the detection rates for ILTV GC; therefore, a sample from a location in the shed was as good as from any

other. Kennedy et al. (2017) suggested that collection of dust sample from poultry sheds using settle plates could be biased as distribution of virus in a shed may not uniform. Although location had no significant effect in this study, as not all samples collected from at a given day were positive, pooling samples from two or more dust plates per flock would maximise ILTV GC detection and overcome possible differences in virus distribution in a shed, particularly at low levels of GC.

In this study, collection of dust samples using polypropylene plastic settle plates provided a convenient sampling method for vaccine and pathogen monitoring thus would be suitable for use under commercial conditions. Similar but relatively expensive metal settle plates have been used by Skóra et al. (2016) for evaluating microbial contaminations in poultry dust. The design optimisation of the settle plates described in this study provides a device for dust collection that is inexpensive, durable, easy to manufacture and convenient to handle in commercial poultry settings.

In conclusion, ILTV is detectable in commercial meat chicken dust most readily from 7-8 dpv and the presence of significant GC load at 7-8 dpv appears to be a reasonable basis for differentiating adequate and poor initial vaccine take. It is possible that earlier sampling may be required to differentiate poor from adequate take and this is worthy of further investigation. The lack of spatial variation within a shed suggests that choosing a random sampling location within a shed will not affect virus detection but pooling samples from two to four settle plates would be ideal for maximising sensitivity.

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MANAGEMENT AND HEALTH OF STATIONARY AND NOMADIC DUCKS IN THE COASTAL AND HAOR AREAS OF BANGLADESH

MD.E. HOSSAIN[1], MD.A. HOQUE[1], G. FOURNIE[2], G.B. DAS[1] and J. HENNING[3]

Summary

A cross-sectional survey was conducted between February and June 2016 to explore the management and health status of stationary and nomadic duck flocks in the coastal and Haor areas of Bangladesh. Stationary duck flocks (N=70) were smaller (mean: 6.5 birds) than nomadic duck flocks (N=10, mean: 152 birds). Indigenous ducks were the most common type of duck breed in stationary flocks (95.7%) and the Khaki Campbell breed was predominant in nomadic flocks (80.0%). Indian Runner ducks were reared only by the stationary duck farmers (14.0%). Egg production for consumption was the sole reason for nomadic duck production, whereas the production of ducklings was the predominant reason for raising ducks in the stationary flocks. Paddy rice was the main feed for nomadic ducks (100.0%), while rice polish was the major supplementary feed for stationary ducks (81.4%). Scavenging feeds for nomadic ducks included more insects (100.0%) and worms (90.0%) compared to stationary ducks (50.0% and 60.0% respectively). Sudden deaths were more common in nomadic duck flocks (100%) compared to stationary flocks (40.0%) ($P < 0.001$). Anthelmintics were more frequently used in nomadic duck flocks (40.0%) compared to stationary flocks (8.6%) ($P = 0.019$). Vaccination against duck plague was more common in nomadic ducks (70.0%) compared to stationary flocks (25.7%) ($P = 0.009$). Overall, the two management systems fulfill different purposes: whereas nomadic ducks are raised by entrepreneurs in more business-like settings, stationary duck rearing is mainly conducted to support farm income or for home consumption.

I. INTRODUCTION

In Bangladesh, duck flock management is practiced using two systems: viz. stationary and nomadic. 'Nomadic ducks' are those which scavenge in post-harvest paddy fields during the day and stay in temporary enclosures or tents overnight near the scavenging area (Henning et al., 2012). Stationary or household ducks are kept overnight near the farmer's house; they travel only over short distances and are often reared by families in subsistence farming systems (Ghosh et al., 2012). Increased productivity of stationary and nomadic ducks has the potential to reduce malnutrition and increase family income (Pervin et al., 2013).

Duck raising has some benefits over chicken raising: ducks easily adapt to a wide range of environments (Rahman et al., 2009); they lay more eggs and produce more meat than indigenous chickens (Rahman et al., 2007); and women and aged people are able to manage ducks easily. However, there is limited scientific information available regarding the management and health of stationary and nomadic ducks in Bangladesh. This study was undertaken to describe the management and health status of stationary and nomadic ducks in the coastal and Haor areas of Bangladesh.

II. METHODOLOGY

A cross-sectional survey was conducted in stationary and nomadic duck owning households using a structured questionnaire between February and June 2016. Low-lying upazilas (sub-districts) of Chittagong (coastal area) and Moulavibazar (Haor area) districts in Bangladesh were selected for the present study according to the highest density of ducks and availability of water bodies (BBS, 2012). Eight stationary duck households per village across 8 villages in the Chittagong district and

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four stationary duck households per village across 4 villages in the Moulvibazar Districts were selected; thus a total 80 stationary duck households was involved in this study (64 in Chittagong and 16 in Moulavibazar). In the Moulvibazar district, six households having nomadic duck flocks were selected from the four villages in the Barlekha upazila and four additional households were selected from Vatera from the Kulaura upazila having nomadic duck flocks.

Five interviewers (veterinary students and a researcher from CVASU) were trained in interviewing techniques and conducted the interviews. A questionnaire was developed containing closed and open-ended questions. It explored the flock structure, housing systems, feeding, predation, health care, disease prevention, and vaccination of stationary and nomadic ducks. The questionnaire was pre-tested with 8 stationary and 1 nomadic duck farmers, none of whom were included in the main study. A total of 81 questions was modified following pilot testing, with ambiguous questions being eliminated or modified. Institutional approval for conducting interviews with the stationary and nomadic duck owners was obtained from The University of Queensland, Human Research Ethics Committee (No. 2016000342). Data analysis was conducted in STATA (Stata/SE 14.1, Stata Statistical Software, Stata Corporation, College Station, TX, USA). Descriptive and summary statistics were produced. Proportions of duck management factors were compared between stationary and nomadic ducks using Fisher's exact test. A probability value of <0.05 was considered significant.

III. RESULTS

A total of 10 stationary duck flock owners who had no ducks at the time of the interview were excluded from the analysis. Nomadic duck flocks (N=10) were larger than stationary flocks (N=70) with mean (median, range) flock sizes of 6.5 (5, 2-18) and 152.0 (132.5, 65-300), respectively. Indigenous ducks were the most common type of duck in the stationary flocks (95.7%; N=67) and Khaki Campbell was the predominant breed in nomadic flocks (80.0%; N=8). Indian Runner ducks were reared only by the stationary duck farmers (14.0%; N=5). Overall, 75.7% (N=53) of the stationary duck flocks raised ducks together with other poultry, while none of the nomadic flocks had other poultry.

Housing of stationary and nomadic ducks differed considerably. Nomadic ducks were provided with temporary enclosures near the scavenging areas made from fishing nets (100.0%; N=10), while only a few of the stationary flocks use this type of material (14.3%; N=10) ($P < 0.001$). In general, stationary duck farmers provided solid houses, made from corrugated iron, bamboo, wood, mud or bricks.

Paddy rice was the main feed source for all nomadic ducks (100%; N=10) but not for stationary flocks (11.4%; N=8), while rice polish was the major supplementary feed for stationary ducks (81.4%; N=57) but not for nomadic flocks (30%; N=3). In addition to paddy, the majority of the nomadic farmers (60.0%; N=6), but very few stationary duck farmers (8.6%; N=6) provided commercial feeds for their ducks ($P < 0.001$). The latter group provided cooked rice (80%), and some rice husk (14.3%), kitchen waste (4.3%), wheat bran (2.0%) and homemade feed (2.0%), whereas none of these were provided to nomadic flocks.

Scavenging food was more abundant in the rainy season (Jun-Aug) for stationary ducks (74.0%; N=52) and in the winter for nomadic ducks (60.0%; N=6). Scavenging feeds for nomadic ducks included more insects (100.0%, N=10), worms (90.0%, N=9) compared to stationary ducks (60.0%, N=42; 50%, N=35), but similar amount of fish and snails (97-100%).

Nomadic flocks were only raised for the production of table eggs whereas hatching egg production was the main reason for keeping stationary ducks (68.6%; N=48) since usually both sexes were present in the flock. All nomadic duck farmers (100.0%; N=10), but only 7.1% of stationary duck farmers (N=5) bought ducklings from government hatcheries ($P < 0.001$). Purchase of fertile eggs from neighbors to produce ducklings for restocking their own flock was more common in stationary (54.3%; N=38) compared to nomadic flocks (10.0%; N=1) ($P = 0.005$).

Sudden death of ducks was experienced by all nomadic duck flock owners, but only in 40.0% (N=28) of stationary duck flocks ($P < 0.001$). Use of anthelmintics was more common in

nomadic (40.0%; N=4) compared to stationary duck flocks (8.6%; N=6) (P = 0.018) (Table 1). A significantly higher proportion of nomadic duck flock owners vaccinated against duck plague (70.0%; N=7) compared to stationary duck flock owners (25.7%; N=18) (P = 0.009).

Table 1 - Disease prevention characteristics of stationary (N=70) and nomadic ducks (N=10) in the coastal and Haor areas of Bangladesh.

Characteristics of disease prevention	Stationary Ducks %Yes (N)	Nomadic Ducks %Yes (N)	P
Do you use anthelmintics in YOUR ducks?	8.6 (6)	40.0 (4)	0.020
Do you use antibiotics for YOUR ducks?	40.0 (28)	70.0 (7)	0.100
Who advised you on the USE of antibiotics?			
Pharmacy (Drug store)	5.7 (4)	10.0 (1)	0.500
Local doctor	34.3 (24)	60.0 (6)	0.160
Veterinary hospital	0.0 (0)	30.0 (1)	0.130
For what symptoms do you use antibiotics?			
Paralysis	15.7 (11)	20.0 (2)	0.660
Neck twisting	1.4 (1)	10.0 (1)	0.360
Diarrhoea	8.6 (6)	10.0 (1)	1.000
Drowsiness	5.7 (4)	20.0 (2)	0.580
Fever	2.9 (2)	0.0 (0)	1.000
Not eating	4.3 (3)	10.0 (1)	0.420
Do you vaccinate YOUR ducks against duck plague?	25.7 (18)	70.0 (7)	0.010
Who conducted the vaccinations of YOUR ducks?			
Local doctor	24.3 (17)	70.0 (7)	0.010
Myself	1.4 (1)	0.0 (0)	1.000

Both stationary and nomadic duck farmers reported the existence of a wide range of predators. Crows (*Corvus brachyrhynchos*) and a range of wild cat species were the most important predators for both stationary and nomadic duck flocks (Figure 1).

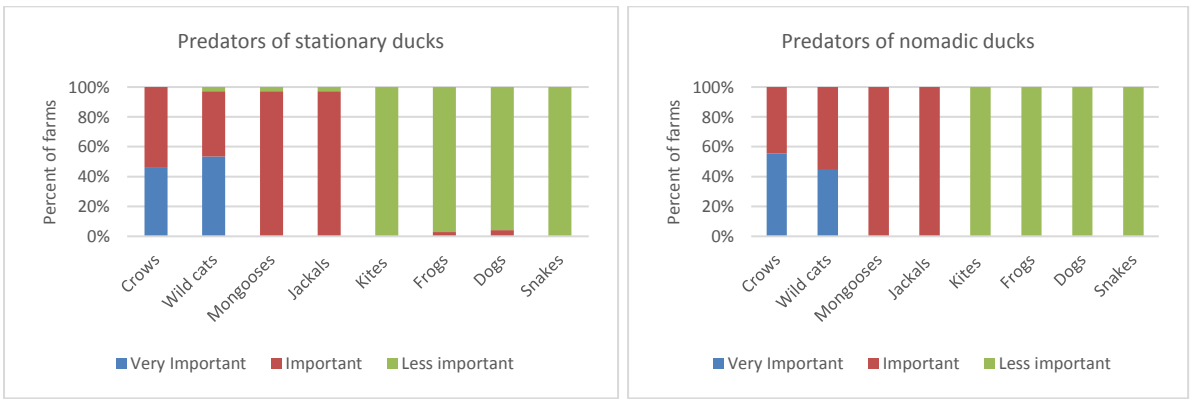

Figure 1 - Importance of predators for stationary (N=70) and nomadic ducks (N=10) in the coastal and Haor areas of Bangladesh.

IV. DISCUSSION

The preference of stationary duck farmers for indigenous breeds is due to their belief that these breeds are more resistant to common predators and diseases (Baki et al., 1993) and that these birds are suitable for both egg and meat production. In contrast, nomadic flocks were dominated by the high egg producing Khaki Campbell breed.

Few of the stationary duck farmers (only 8.6%) provided commercial feeds because of their expense making such provision not cost-effective for free range, medium to low egg producing scavenging type of ducks. The stationary duck farmers produced ducklings from hatching eggs of

their own stock, instead of buying ducklings from government hatcheries, because it is a general belief that ducklings purchased from hatcheries are less resistant to prevailing duck diseases (Baki et al., 1993). Since nomadic duck farmers rear a large number of ducks, they bought ducklings from hatcheries, mainly through middleman, and ensured that the ducks are vaccinated routinely. Snails and fish were the most abundant scavenging feeds that were available to ducks. In fact, ducks are very good foragers and help control weeds and pests in crop fields (Manda et al., 1993). Young ducklings from hatch to about three weeks of age prefer tadpoles, insects and worms, as they are palatable and a very good source of animal protein, whereas older birds like to seek paddy, shrimps, snails, insects, small fishes and weeds from the field (Nind and Tu, 1998). Scavenging feeds are plentiful during the rainy season (May-July), and scavenging is common for stationary ducks. However, this season is less favorable for nomadic ducks since the Haor areas are flooded and ducks cannot scavenge there. However, late winter is another peak season both for stationary and nomadic ducks. During this time, floodwaters have declined providing a high concentration of worms, insects, snails and fishes (Kabir et al., 2007).

Predator attacks are a major constraint to duck farming in Bangladesh (Hoque et al., 2010). Some predators feed solely on duck eggs, while others target ducklings or adults. Abundance of excessive predators and losses of many young are distressing for duck farmers and farmers often change their management in response to the increased predation risk (Zimmer et al., 2011), such as modifying the duration of scavenging or using different scavenging habitats. However, this sometimes results in a shift to less suitable habitats for nomadic ducks with less access to food (Lima, 1998).

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ANTIMICROBIAL STEWARDSHIP – THE PATH TO LEAST RESISTANCE

S.W. PAGE[1] and D.J. TROTT[2]

Summary

Antimicrobial resistance is now accepted as a global public health priority and an important emerging animal health issue. Antimicrobial use contributes to the selection of antimicrobial resistance and consequently only necessary high quality use of antimicrobial agents is considered appropriate. The Australian meat chicken and egg industries are historically low users of antimicrobial agents and recent surveys of antimicrobial resistance in bacterial commensal species isolated from meat chickens and the environment of laying sheds have revealed very low levels of antimicrobial resistance. Despite this very favourable position the implementation of formal and systematic antimicrobial stewardship plans will support the continued low frequency of antimicrobial use and antimicrobial resistance.

I. INTRODUCTION

Antimicrobial resistance (AMR) is considered one of the biggest threats to human and animal health today (Australian Government, 2015; O'Neill 2016) and all users of antimicrobial agents have a responsibility to ensure that these agents are only used when necessary.

The Australian poultry industry takes the issue of AMR very seriously and has a long history of developing and introducing initiatives to enhance infection prevention and control and to encourage restriction of antimicrobial use to essential situations (Hewson, 2018). The codes of practice and guidelines introduced progressively since the 1980s have evolved into the antimicrobial stewardship (AMS) plans of the 2000s. AMS and good stewardship practice (GSP) concerns much more than just judicious or prudent use of antimicrobial agents (Lloyd and Page 2018). Indeed the current focus is on continuous improvement and ways to refine, reduce and replace antimicrobial use (Page et al., 2014) while maintaining the highest standards of bird health, allowing close alignment with the Australian (Australian Government, 2015) and global (WHO, 2017) strategies for AMR and antimicrobial use minimisation.

II. ANTIMICROBIAL STEWARDSHIP

The long history of conservative regulation and use of antimicrobial agents in the Australian poultry industry has resulted in the unique situation where many of the critically important antimicrobial classes used widely in poultry production outside Australia have never been available. For example, the focus of the British Poultry Council (BPC) AMS programme has been the reduction or elimination of the use of fluoroquinolones, third generation cephalosporins and colistin – all antimicrobial classes never approved for use in Australian poultry. Thus Australian AMS programmes can focus on more advanced aspects of stewardship. However, the aims of Australian AMS coincide with those of the British and there is great confluence with the statement of BPC Chairman, John Reed, who concluded after reviewing the 2017 AMS programme (BPC 2017) that "Our farmers and veterinarians need antibiotics in their toolbox to treat sick birds – zero use is not an option – and we will protect the health and welfare of our birds. We will safeguard the efficacy of antibiotics as part of sustainable food production, and we will continue to feed the nation."

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But what is AMS? One of the clearest descriptions is that provided by Guardabassi and Prescott (2015) who define AMS as "the multifaceted and dynamic approaches required to sustain the clinical efficacy of antimicrobials by optimizing drug use, choice, dosing, duration, and route of administration, while minimizing the emergence of resistance and other adverse effects". That AMS is multifaceted means that it is complex and involves many elements and requires clear thinking. The dynamic approach reflects the fact that, just like AMR, AMS is not a stationary practice; it is forever changing and the direction of change, especially that of continuous improvement, is guided by the multifaceted AMS team. Optimising drug use, choice, dosing, duration and route of administration is very challenging as it is inevitably not a fixed and predictable equation, it does not mean 'one dose suits all'; each circumstance may require a different approach, which may also include no antimicrobial use. Minimising the emergence of AMR is a necessary and demanding goal, but one for which there is insufficient guidance. Only by monitoring responses to treatment or non-treatment and undertaking surveillance of AMR can any insight into resistance minimisation be gleaned.

The 5R framework for AMS was developed to provide a systematic and comprehensive approach to AMS planning, implementation and monitoring (Lloyd and Page, 2018; Page et al., 2014; Prescott and Boerlin 2016; Weese et al., 2013) to allow a potentially complex process to be both practical and effective. The 5R include Responsibility, Review, Refine, Replace and Reduce. AMS is a continuous process with a goal of defining and applying best practice, AMR minimisation and optimal control of animal health. Good Stewardship Practice (GSP) describes the development, implementation and continuous improvement of the AMS plan (ASP).

The first Australian Veterinary Antimicrobial Stewardship AMS Conference was held in November 2018 and provided a comprehensive overview of the current situation (AVAMS 2018) and initiatives introduced by the various livestock industries as well as actions and plans in companion animal practice.

III. ANTIMICROBIAL RESISTANCE

Supported by funding from the Australian Department of Agriculture and Water Resources, the Australian Chicken Meat Federation recently published the results of a national study of surveillance for AMR in enteric commensals and pathogens in Australian meat chickens (ACMF, 2018). The study was comprehensive and followed international standards for design and analysis. Importantly, it was observed that resistance to antimicrobials considered of critical importance to human health (ASTAG, 2018; WHO, 2017) was very low in commensal bacteria from Australian meat chickens – highlighting the effectiveness of past and present AMS initiatives.

Two examples demonstrate the stark contrast in resistance levels in Australia with those elsewhere, for example comparing results from Australia with those from the EU and UK. EFSA & ECDC (2018) published the European Union summary report on AMR in zoonotic and indicator bacteria from humans, animals and food in 2016. With respect to campylobacter, for the 3,117 *Campylobacter jejuni* isolates from broilers reported by 24 member states, the overall observed level of resistance to ciprofloxacin was 66.9% and to nalidixic acid was 61.7%. In the UK (VARSS, 2017) ciprofloxacin resistance was reported in 40.6% of *C. jejuni* isolates from broilers. These results contrast with those observed in the Australian study where only 14.8% of *C. jejuni* were found to carry ciprofloxacin resistance. This low level of resistance was unexpected and the first report of such resistance in *C. jejuni* isolated from Australian poultry. Fluoroquinolones (FQ) (the class to which ciprofloxacin belongs) have never been approved for use in Australian livestock including poultry. The finding of FQ resistance is highly unlikely to be the result of FQ use in poultry, rather the appearance of this

resistance may ultimately be found to have arisen in humans and subsequently transferred to poultry – highlighting the need for vigilance and the need for biosecurity to encompass poultry workers.

The second example of contrasts between resistances found in Australia and elsewhere relates to *Escherichia coli*. None of the *E. coli* isolates from Australian poultry demonstrated resistance to ceftiofur, colistin, florfenicol, chloramphenicol or gentamicin (ACMF, 2018). Two isolates (1%) demonstrated reduced susceptibility to ciprofloxacin but these isolates were not considered clinically resistant. In the UK (VMD, 2017) resistance to the FQ ciprofloxacin was observed in 21.6% of *E. coli* isolates recovered from caecal contents of healthy broilers at slaughter. In the European Union, EFSA & ECDC (2018) reported "for broilers, the highest overall resistance levels observed in the reporting MSs were to the quinolones, i.e. nalidixic acid (59.8%) and ciprofloxacin (64.0%), and to ampicillin (58.0%), sulfamethoxazole (49.9%), tetracycline (47.1%) and trimethoprim (40.7%). Levels of resistance to the third-generation cephalosporins, cefotaxime and ceftazidime, were similar at 4.0% and 3.6%, respectively."

A proof-of-concept AMR study of *Salmonella* isolates obtained from Australian layer shed environments has also recently been completed (Trott, unpublished). The susceptibility of 307 isolates collected over the period 2015 to 2018 was assessed against a panel of 16 antimicrobial agents. Overall, a very low frequency of resistance was observed. Remarkably, 295 isolates (96.1%) displayed no evidence of phenotypic resistance to any tested antimicrobial, while 8, 1 and 2 isolates were respectively resistant to 1, 2 or 3 antimicrobial agents.

The enviable status of Australian poultry meat with respect to AMR was supported by the recent publication of McLellan et al. (2018) who found no evidence of acquired multidrug resistance in Gram-negative bacteria isolated from raw chicken drumsticks obtained from 30 retail outlets in Melbourne.

IV. CONCLUSION

The progressive introduction by the Australian poultry industry over many decades of infection prevention and control measures (including vaccination programmes and biosecurity initiatives), combined with high standards of husbandry, nutrition and environmental controls, and buttressed by a conservative regulatory system that has not permitted the approval in poultry of many antimicrobial agents now considered of critical importance in human medicine (including colistin, fluoroquinolones and 3rd and 4th generation cephalosporins), is undoubtedly a significant contributor to the low levels of AMR now evident in isolates from meat chickens and from layer environments. However, complacency is not an option. Continued vigilance reinforced by AMS programs will help protect the rare environment of low AMR, allowing early identification of any changes in resistance status as alerts to investigate risk mitigation measures, or assurance of the effectiveness of production practices.

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USE OF BEST PRACTICE HOT BLADE TRIMMING WHEN INFRARED BEAK TREATMENT IS UNAVAILABLE

G.A. RUNGE[1] and P.C. GLATZ[2]

Summary

It is well known that Infrared Beak Treatment (IRBT) is a superior method of beak tipping (McKeegan, 2016; Glatz and Runge, 2017) and is applied to more than 93% of layer chicks hatched in Australia. For smaller hatcheries, the treatment cost per chicken makes it uneconomic to use an IRBT machine. Small hatcheries either apply a hot blade trim (HB) at day old or advise their clients to HB trim at five to 10 days with a follow up retrim at 10 weeks if required to control cannibalism and feather pecking. Poultry can only be treated with IRBT at day old. If tipped beaks need to be retrimmed, the HB method is the only practical option available on commercial farms. Sometimes the beaks of IRBT birds require a second trim at 10 weeks due to excessive beak regrowth which increases the risk of feather pecking and cannibalism. This occurs if the treatment applied is incorrect due to changes in the birds' environment or genetics (new generation of breeders). The amount of treatment applied is determined by the chick type, strain, housing system and ventilation, farm latitude (temperature and outdoor light intensity) and feeding system.

I. INTRODUCTION

HB trimming is still used worldwide for retrimming birds on farm where required to reduce the risk of or control serious outbreaks of cannibalism and feather pecking. In the review by Glatz (2000) other methods of trimming included a gas beak trimming machine, an electric soldering iron and a cold blade. These methods are only practical to use when retrimming birds on smallholder farms as they are too slow to use on a large number of birds on a commercial farm. This paper aims to reaffirm the research which identified best practice HB trimming that was included in previous Australian Model Codes of Practice for the Welfare of Animals – Domestic Poultry (Welfare Code) in 1992, 1995 and 2002. HB trimming has been banned in many European countries and IRBT in some because of the concerns from animal rights organisations and consumers. It was clear from the early research, that IRBT was a superior method of tipping (Glatz, 2005) and this was acknowledged in later research by McKeegan & Philbe (2012). Since then, there has been little attempt to repeat the work which led to development of best practice HB beak trimming as defined in the 1992, 1995 and 2002 Welfare Codes. Instead, most of the beak tipping research has been about understanding the effects of IRBT on beak physiology and growth and defining best practice IRBT. This research (Dennis, 2009 & 2012; Carruthers, 2012) included comparisons made with the standard HB method used in the country where the research was conducted. Gabrush (2011) did compare the severity of HB beak trimming for day old chicks using the guide-plate on the trimming machine which has 3-hole sizes (4, 5 and 6 mm in diameter). HB treatment was successful in effecting the degree of beak shortening with beak length being 14, 31 and 39% shorter than the control treatment at 11 weeks of age. Histological examination of the beaks did not find neuromas, supporting the best practice method for HB trimming reported in the 1992, 1995 and 2002 Welfare Codes.

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II. HOT BLADE TRIMMING

Research by Glatz (1987, 1990) had identified that HB trimming birds at hatch and removing half of the upper beak and one third of the lower resulted in less stress to birds and better performance than beak trimming birds at older ages. Glatz (1990) showed that chickens trimmed at hatch have better feed conversion than those trimmed at 10 or 42 days of age. Hester and Shea-Moore, (2003) also reported that better egg production is obtained when birds are HB trimmed earlier.

The initial pain reactions to HB trimming can be divided into 3 phases; painless, acute, and chronic. Acute pain (2h to a few days) results from stimulation of nociceptors (pain receptors) in the tip of the beak and follows a pain free period (up to 26h) normally associated with action by the birds' endogenous analgesia system (Cheng, 2005). Neuromas form in the beak when axons are severed because of beak trimming (Lunam, 2005). They may develop as scattered micro neuromas which regress (one-half upper beak, one third lower beak trim) or in the case of severe beak trimming (two-thirds of upper beak; one-half of lower beak) neuromas may persist and discharge action potentials that may be perceived by the bird as chronic pain.

III. INCIDENCE OF NEUROMAS

Lunam et al. (1996) used the recommendation from Glatz (1987, 1990) to beak trim birds at hatch with a hot blade. The incidence of neuromas at 10 and 70 weeks-of-age in untrimmed birds was compared with chicks given a moderate trim (i.e. one half the upper beak removed (3 mm) and one-third of the lower beak removed (2.5 mm) versus birds with a severe trim where two-thirds of the upper beak (4 mm) and half of the lower beak (3mm) was removed. The results were consistent with other work (Keunzel, 2007) and indicate that neuromas do form after trimming but resolved in birds moderately trimmed, with sensory corpuscles still present in the upper and lower beak. Neuromas (consisting of masses of disorganised tangles of nerve fibres) only persisted in birds that had been severely trimmed and may discharge ectopic spontaneous action potentials perceived as pain.

The moderately trimmed birds had a 25% reduction in beak length compared to control birds at 70 weeks while the severely trimmed birds had a reduction of 50-65% in beak length as compared with control birds. Beak trimming younger birds (less than one week of age) appears to avoid the long-term chronic pain that can occur in the stump of the beak when older birds are trimmed (Lunam et al, 1996) and suggests that beak-trimming at an early age decreases the formation of scar tissue and reduces the risk of neuroma development. A likely explanation for this is that the beak of young birds has a greater capacity for regeneration compared to the regenerative ability of older birds.

A case was then made by Gentle (1998) who had previously recommended a ban on HB trimming to continue HB beak trimming in young birds using moderate trimming (Gentle, 1988) as previously recommended by Glatz (1987,1990) and Lunam et al. (1996).

Work by Glatz (1987, 1990) was included in the 1992 Welfare Code recommending that HB trimming of half of the upper beak and one third of the lower beak be practiced on chicks early in life. Gentle et al (1997) suggested that anything less than 50% top beak removal results in extensive re-growth and this can lead to outbreaks of feather pecking and cannibalism after beak trimming and hence the need to retrim birds if the one-third rule is adopted. To investigate impact of retrimming, an anatomical and behavioural study examined the effects of moderate hot blade beak-trimming of chickens (as specified above) on the day of hatch and re-trimming of 2 mm of the upper and lower beak at 14 weeks-of-age (Lunam, 2005). Beak trimming was conducted per industry standards for beak trim accreditation (Bourke et al., 2002a, b). A heated blade on a commercial electric beak trimming machine (Lyon Electric Company) cut and cauterised half the upper beak and one-third of the lower beak for 2s. At 14

weeks of age chickens were re-trimmed using a heated blade that removed 2 mm of the upper and lower beak. The wound was cauterised with the heated blade for 2s.

Sensory receptors and individual nerve fibres were observed near the tips of the retrimmed upper and lower beaks at 28 weeks-of-age. In the tip of the lower beak, large Herbst corpuscles were present and many nerve bundles traversed the dermis between the mandibular bone and epidermis of the beak tip. At 66 weeks-of-age, sensory receptors and nerve fibres were observed in the dermis at the beak tip (Lunam, 2005). The hens returned to normal feeding and pecking behaviours by 66 weeks-of-age (Jongman et al. 2008) and supported the microanatomy that the sensory input to the beak is restored after retrimming.

IV. BEAK TRIMMING CODE OF PRACTICE

The early findings on hot blade trimming by Glatz (1987, 1990) were recognised in the 1992 Australian Model Code of Practice for the Welfare of Animals. Domestic Poultry Edition 2 (and again in Edition 3, 1995) (see page 13 Management Practices (12.2.1 and 12.2.2) where one half of the upper beak and one third of the lower beak may be removed in day old to 10-day old birds) and were supported by the later findings of Lunam et al. (1996) and Gentle (1988).

In the 2002 Australian Model Code of Practice for the Welfare of Animals, Domestic Poultry, it specified that beak trimming of birds be conducted using an accredited trainer under an accredited training program in accordance with an agreed accreditation standard. The co-author of this paper was requested by RIRDC's Egg Program to develop an accreditation standard and publish a training manual for HB trimmers and worked with TAFE (NSW), VIAS and PIRSA (see Bourke et al., 2002) to develop the standards.

V. CONCLUSION

IRBT is the most popular method to beak tip poultry in Australia. The HB method is used for re-trimming flocks during the rearing period if beaks have regrown after treatment at hatch with the infrared method. Where IRBT is not available at small hatcheries, HB trimming is used for the first trim and a second trim where required. The standard developed for hot blade beak trimmers was referred to in the 1992, 1995 and 2002 Australian Welfare Codes and involved removal of no more than half the beak length and to provide a step to the lower beak for birds trimmed at day old to 10-12 days-of age (see P. 68 of Bourke et al. 2002b). This standard is still used today.

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SLOWER GROWING BROILERS SHOWED HIGHER APPETITE FOR ALANINE

S. NIKNAFS[1], M. FORTES[1] and E. ROURA[1]

A balanced broiler chicken diet requires essential (EAA) and non-essential (NEAA) amino acids to attain maximum growth. However, the optimal ratio of these two dietary AA groups may change with different growth rates but this is not fully understood (Heger, 2003). In addition, commercial broiler feeds are formulated based on average flock requirements of EAA, hence neglecting NEAA ratios and potential individual variations. Consequently, it is expected that commercial broiler diets for slower growing birds may contain excess EAA relative to NEAA, and vice versa for fast growing birds. Reportedly, when given adequate choices, chickens have the ability to select diets based on their nutritional requirements, particularly AA (Zuberbuehler et al., 2002). Therefore, we hypothesized that in an attempt to optimize their EAA/NEAA, slow and fast growers fed a commercial balanced diet will show higher specific appetites for NEAA than EAA.

A two-choice feeding regime was developed to test AA preferences in chickens, consisting of offering two identical feeders: one contained standard feed and the other standard feed supplemented with AA mix. Four treatments were used: T1 a control with no supplement (standard feed in both containers or feed: feed); T2 with an EAA mix (feed: feed + Lys, Met, Thr), T3 with a NEAA mix (feed: feed+ Ala, Asp, Asn), and T4 with a non-limiting EAA mix (feed: feed + Cys, Ser, His). These treatments were tested using 48 slow and 48 fast growers during two weeks (week 5 and 6). In addition, we compared the gene expression and protein synthesis profiles in proventriculus and duodenum between 6 slow and 6 fast growers selected from a flock of 600 at week 6.

In a two-choice model, if the bird consumes the same amount from each feeder, the preference value is 50% or not significantly different from 50% in a t-test. Results showed that the preference for NEAA (Ala, Asp, Asn) was significantly higher than 50% neutral value in slow growers (57.4%; $P<0.05$) but not in fast growers. In contrast, the preference for EAA (Lys, Met, Thr) was significantly lower than 50% (35.1%; $P<0.05$) in the same group. In addition, slow compared to fast growers showed a significantly ($P<0.05$) lower EAA/NEAA ratio (0.577 vs 0.595, respectively). The analysis of gut-expressed genes revealed that slow growers had an increased rate of Ala catabolism. α-gustducin, which is downstream signalling of Ala sensor in chickens (T1R1/T1R3), and AA transporters (SLC38A1 and SLC1A2) were downregulated in slow growers ($P<0.05$) suggesting a potential lower uptake of the NEAA tested from the gut content. In addition, the proteomic analysis showed a low level of glucose transporters (SCL2A2 and GLUT4) in slow compared to fast growers ($P<0.05$). Furthermore, the glycolysis rate in slow growers was downregulated. All of the glycolytic pathway described above resulted in a low production of pyruvate, the only substrate for biosynthesis of Ala in the cell (hence the main reason why Ala is a NEAA). In conclusion, Ala catabolism in slow growers is upregulated, whereas Ala biosynthesis, sensing, and transporting were downregulated. This contributed to develop an appetite and higher consumption of Ala in slow growing birds.

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DETERMINING A BUFFER DISTANCE BETWEEN AUSTRALIAN COMMERCIAL CHICKEN FARMS AND WATER BODIES TO MINIMISE WILD BIRD PRESENCE ON FARM

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Summary

The aim of this study was to determine the appropriate buffer distance between commercial chicken farms and water bodies to safeguard chicken farms against possible interactions with waterfowl and shorebirds. Sixty-four layer and broiler chicken farms in New South Wales (NSW) and Queensland (QLD) were selected for the study. The distance between a chicken shed and five closest water bodies was measured using Google Maps. On-farm questionnaire data specifying the presence or absence of waterfowl and shorebirds around feed storage and range areas was correlated with the distance measurements. Logistic regression analysis indicated a higher probability of encountering waterfowl outside shed areas if the closest water body was 100-200 m away from the shed (P = 0.003). This result could aid the development of a biosecurity buffer distance to guide future farm planning and encourage the implementation of more stringent biosecurity strategies in farms with proximal water bodies.

I. INTRODUCTION

Avian influenza viruses (AIVs), subdivided into Low Pathogenicity Avian Influenza (LPAI) and Highly Pathogenic Avian Influenza (HPAI), are diseases of major concern to domestic chickens worldwide (Hansbro et al., 2010). LPAI is known to circulate naturally in wild birds of the order Anseriformes (waterfowl) and Charadriiformes (shorebirds) and can potentially mutate into HPAI once transmitted to chickens (East et al., 2008). Reducing direct and indirect contact between wild birds and commercial chickens is of paramount importance to the commercial chicken industry due to the risk of AIV spillover. As surface water is an attractive habitat for wild birds, chicken sheds proximal to water bodies may be at an increased risk of virus transmission. This study aimed to determine an appropriate buffer distance between commercial chicken farms and open surface water (including dams, rivers and creeks) to minimise waterfowl and shorebird presence on future farms, and to identify current farms at risk of AIV incursion.

II. MATERIALS AND METHODS

Sixty-four layer and broiler chicken farms in NSW and QLD (subset from the AIRisk survey, Scott et al., 2017), were located using satellite images on Google Maps (Google Inc., 2018, California, USA). The ‘measure distance’ tool was utilised to determine the distance between the closest edge of a chicken shed on each farm to the edge of the closest water body. On-farm questionnaire data specifying the presence or absence of waterfowl and shorebirds (yes/no) around farm sheds and range areas was correlated with the distance measurements. Logistic regression (Genstat, 2011, Hemel Hempstead, UK) was used to predict the relationship between waterfowl and shorebird observations around feed storage areas, the distance between the chicken shed and the closest water body, and the type of farm. Further models were included for predictions for range areas.

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III. RESULTS AND DISCUSSION

Regression analysis revealed a statistically significant (P = 0.03) relationship between the distance of the closest water body to the chicken shed and the sighting of waterfowl at feed storage areas. Farms which have the closest water body 101-200 m to the shed have a 56% predicted chance of sighting waterfowl at feed storage areas (P = 0.003). The predicted value at feed storage areas tended to decrease if this distance was over 200 m, although this difference was not statistically significant (P > 0.05). This finding suggests that, although waterfowl are attracted to feed areas adjacent to chicken sheds, they may be unwilling to stray further than 200 m from a water source to get there. Surprisingly, the chance of sighting a waterfowl at feed storage areas decreased if the closest water body was under 101 m away (P > 0.05). However, for shorebirds, this relationship was not significant (P = 0.21) (Table 1).

Table 1 - Prediction values for sighting waterfowl or shorebirds around feed storage areas at the distance (m) to the nearest water body.

	Waterfowl (df = 3, P = 0.03)		Shorebird (df = 3, P = 0.21)	
Distance	Prediction	s.e.	Prediction	s.e.
0-50	0.06	0.04	0.06	0.04
51-100	0.20	0.09	0.15	0.08
101-200	0.56	0.17	0.33	0.16
200+	0.25	0.15	0.25	0.15

There was no significant relationship between the distance of the closest water body to the shed and sighting a waterfowl or shorebird on range areas of free-range farms (P > 0.05). This may indicate a tendency for wild birds to avoid large masses of chickens congregated at one site, perhaps to elude competition or aggression. Overgrazed, bare pastures present on free-range sites could also be a potential factor for the low observation of wild birds by the farmers (Scott et al., 2018). Our study suggests a minimum biosecurity buffer distance of 200 m from feed storage areas to the closest open water source to decrease numbers of on-farm waterfowl, although further research with a larger sample size is warranted. In addition, camera traps could be stationed on-farm to measure the frequency of wild bird visits over a longer period of time. The type of feed or vegetation on the range, on-farm biosecurity protocols relating to open water resources, feed spill protocols and use of wild bird deterrence mechanisms are additional factors that could be considered in future studies.

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AUSTRALIAN POULTRY SCIENCE SYMPOSIUM: REFLECTING ON THIRTY YEARS OF SCIENCE COMMUNICATION

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Summary

Since its inception, thirty years ago, the Australian Poultry Science Symposium (APSS) has continued to grow and provide a venue for knowledge exchange of the latest developments and trends in the application of science to the poultry industry. It has achieved its aim to become an important international poultry science meeting. In so doing, APSS has showcased Australian poultry research and provided an invaluable opportunity for young scientists to communicate their achievements and to network.

I. INTRODUCTION

The first Australian Poultry Science Symposium (APSS) occurred in February 1989 and has continued annually at that time, since then. Until 2016, APSS was held at the University of Sydney but is now held in a prestigious Sydney hotel. This year, 2019 marks the 30th occasion on which APSS has been held but it is 31 years since the inaugural meeting. The discrepancy occurs because the World's Poultry Congress occurred in Brisbane in 2008 and it was decided not to hold the symposium that year. It is timely to recall briefly some pertinent events in the history of APSS and the role that it has played in Australia in both poultry science research and the scientific advancement of poultry production in Australia.

II. HISTORY

In 1987 the President of the Australian Branch of WPSA, Dr Bruce Sheldon, approached the Poultry Research Foundation (PRF) within the University of Sydney with a proposal to combine resources and organise an annual national conference. The PRF had been conducting a very successful annual meeting for a number of years and the plan was to expand that meeting into an annual APSS, commencing in 1989. Professors Derick Balnave and Frank Annison (2014) have provided a detailed historical account of the formation of the APSS. The aim was to present an annual conference that would attain the status of a national/international meeting. To this end all submitted papers were to be refereed. A high priority was to maintain scientific merit while providing information of relevance to all sectors of the poultry industry. Special provision was to be provided for presentations by postgraduate students.

There can be no doubt that APSS has achieved these aims and in this regard special mention must be made of Derick Balnave and Frank Annison who represented the University and PRF, Dr Bob Pym who represented WPSA, and Dr Balkar Bains who was the President of PRF (1981-1998). Their combined efforts in the formative years of APSS established the foundation and traditions for the ongoing success of the Symposium. The international status achieved by the APSS was demonstrated by the comments of the respected journalist William A. Dudley-Cash writing in the US magazine "Feedstuffs" in April 2001. He stated, "one of the most outstanding poultry science symposiums is sponsored by the Poultry Research Foundation of the University of Sydney and the Australian Branch of the World's Poultry Science Association". It is not widely known that both the Australasian Dairy Science Symposium (established 2004) and the Australasian Equine Science Symposium (established

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2006) are modelled on APSS. This is further testament to the successful formulae developed by APSS.

III. KNOWLEDGE EXCHANGE

In discussing papers presented at APSS, no distinction is made between invited, long or short (abstracts) papers. Throughout the history of the APSS, some 1,684 papers have been published of which 293 (17.4%) were invited contributions. Most invited speakers have come from universities or research institutions in North America and Europe. Some invited speakers have been employed by the major companies that service the global poultry industry.

At the first symposium in 1989, 29 papers were presented and the greatest number of papers (84) was presented in 2017. Over the years a similar range of topics has been covered (Table 1), largely reflecting research in Australia. A snapshot of topics by year is shown in Table 1 and the categories or topics are those used in *Poultry Science*; the exception is that Feedstuffs, Additives and Toxicology has been separated from Metabolism and Nutrition and Meat and Egg Science added. Papers were only allocated to one category but a number could have been assigned to up to three categories.

Table 1 - Percentage of studies on topics published in APSS in 1995, 2005 and 2015.

Topic/Category	1995 (n = 66)	2005 (n = 76)	2015 (n = 73)
Welfare and Behaviour	9.09	7.35	19.4
Genetics and Genomics	1.52	2.94	1.49
Immunology, Health and Disease	4.55	32.35	7.46
Management and Production	13.64	19.12	2.99
Metabolism and Nutrition	7.58	14.71	10.45
Feedstuffs, Additives and Toxicology	36.36	36.76	35.82
Meat and Egg Science	18.18	2.94	16.42
Microbiology and Food Safety	-	-	7.46
Molecular and Cell Biology	-	7.35	-
Physiology, Reproduction and Development	13.64	-	5.97

Note: Each article was assigned to only one topic

Although the Symposium covers a wide breadth of topics, it is dominated by nutrition with 40-50% of papers dealing with that aspect of poultry production that accounts for 60-70% of production costs. In some years, feed enzyme papers have dominated this area and the first enzyme paper at APSS appears in the second proceedings (Classen and Campbell, 1990). By comparison, the percentage of papers in other categories is small with the exception of disease related papers that increase when the Australian Veterinary Poultry Association holds an overlapping meeting in Sydney. The other area that has gradually increased is welfare, reflecting both research funding for this area and growing public concern. It is interesting to note that the majority of papers categorised as meat and eggs relate to egg and eggshell quality.

Over the years, invited papers, which are an up to date review, have covered all the topics listed in Table 1. However, often the short papers contain cutting edge results. As John McLeish commented, "One cannot give enough recognition to the short papers presented at APSS and included in the Proceedings. In many instances, the 1-page papers are precursors to full papers in peer-reviewed journals but they have already been presented at APSS, in some cases several years in advance of their full publication. A vast amount of research work has been extended to the poultry industries…." (McLeish, 2014).

On occasion, papers have been presented that have provided a historical perspective on a topic which has highlighted how advances in science are built incrementally on the shoulders

of others. These have included papers on infectious bronchitis (Cumming, 1994), genetics (Hunton, 1997), feed milling (Darling, 1998) and the poultry industry (Kerin, 2013). Professor Rob Cumming, University of New England, attended all the early meetings of APSS and could be relied upon to generate discussion in all areas of poultry science. Interestingly, Mr John Darling had been instrumental in the formation of the PRF and was its initial Chairman (now known as President, 1959-1969). He and his family had been involved in the stock feed industry when it commenced as a sideline to flour milling in the 1950s. Dr Peter Hunton, from Canada, was former global President of WPSA and the Honourable John Kerin is former Commonwealth Minister of Agriculture, poultry farmer and Chairman of the Poultry CRC.

Volume 23 (2012) is unique in that it contains addresses by the Presidents of the two global poultry associations; WPSA (Dr Bob Pym, University of Queensland) and the World Veterinary Poultry Association (Dr Trevor Bagust, University of Melbourne) (Bagust, 2012; Pym, 2012). In that year, the President of both organisations was an Australian, a situation that is unlikely to occur again.

IV. RECOGNITION

The Australian Branch of WPSA has presented the Australian Poultry Award, annually since 1964. With the growing importance of the APSS the Australian Branch of WPSA arranged for this presentation to be made at the official APSS dinner. This award is made to an Australian resident who has made an outstanding contribution to poultry science or to the Australian poultry industry.

Shortly after his death in 1982, the WPSA with the support of the PRF introduced the WPSA Syd Wilkins Prize. Syd Wilkins was a major figure in the Australian poultry scene being President of the Australian Branch of the WPSA, a Vice-President of the world body of WPSA and for many years Deputy President of the PRF. The award was presented for excellence in poultry research conducted by a young poultry scientist in Australia and the recipient presented at APSS.

On a number of occasions, time has been set aside at the Symposium to recognise the contribution of poultry industry identities who have recently died; Rob Cumming (Bryden *et al.,* 2003), Bruce Sheldon (Pym *et al.,* 2004), Jack Ingham (Fairbrother, 2004) and John Barnett (Cronin *et al.,* 2011).

V. COMMITTEES AND SPONSORS

The ongoing success of APSS has been largely due to two committees (Table 2). The Organising Committee, which comprises members from PRF, WPSA and industry, has overall responsibility for the meeting and in attracting sponsorship. The excellent presentation of the Proceedings has been the responsibility of the Editorial Committee, whose chair edits the Proceedings. The Chairs of these Committees are listed in Table 2 and they deserve our special thanks, as their efforts that have been pivotal to the continued success of APSS.

The PRF secretary, who also coordinates the day-to-day conference organisation, ably supports both committees. Mrs Deirdre Pudney and Mrs Noelene West provided efficient and tireless support for the first thirteen years of APSS. Mrs Pudney retired from the Foundation in 2001 followed by Mrs West in 2003. Since then, Mrs Jo-Ann Geist has continued to fill this demanding and supportive role in similarly efficient manner.

Table 2 - Chairpersons of the Organising and Editorial Committees of the Australian Poultry Science Symposium, 1989-2018.

Year	Organising Committee	Editorial Committee
1989-91	D. Balnave	D. Balnave
1992-94	D. Balnave	R.J. Johnson
1995-97	D. Balnave	D. Balnave
1998	D. Balnave	R.A.E. Pym
1999	D. Balnave	D.J. Farrell
2000	D. Balnave	R.A.E. Pym
2001	D. Balnave	D. Balnave
2002-03	W.L. Bryden	R.A.E. Pym
2004-06	T.A. Scott	T.A. Scott
2007	T.A. Scott	R.A.E. Pym
2008	W.L. Bryden	P.H. Selle
2009-10	P.J. Groves	P.H. Selle
2011-13	A.J. Cowieson	J.R. Roberts
2014	A.J. Cowieson	P.H. Selle
2015-17	P.J. Groves	P.H. Selle
2018	P.J. Groves	J.R. Roberts

A significant contribution to the ongoing success of APSS has been sponsorship by industry companies, both local and international. The level of sponsorship has increased over time, which reflects the standing of APSS with the industry. In particular, the financial support received from the Australian Chicken Meat and Egg Research (later Australian Egg Corporation Limited, now Australian Eggs) Councils was extremely important in establishing the symposia and their contributions are worthy of particular mention. Both groups have sponsored all APSS symposia.

VI. NETWORKING

For many attending APSS, it is an excellent opportunity to network. Informal meetings during session breaks, meetings organised by companies, or attending the pre-registration informal debate or forum on the Sunday evening prior to the official opening of the symposium, are features of APSS. The symposium gala dinner was held in St John’s or St Andrew’s Colleges of the University of Sydney followed by a nightcap at the Prince Alfred Hotel for many years. The dinner now alternates between various prestigious non-University venues in Sydney or on Sydney Harbour.

VII. REFLECTION

Over the last thirty years, APSS has continued to grow and provide a venue for knowledge exchange of the latest developments and trends in the poultry industry. It has become an important international poultry science meeting. In so doing, APSS has showcased Australian poultry research and provided an invaluable opportunity for young scientists to communicate their achievements.

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EXOGENOUS EMULSIFIERS AND MULTI-CARBOHYDRASE IMPROVED GROWTH PERFORMANCE OF BROILER CHICKENS FED REDUCED ENERGY DIETS

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Dietary energy is vital in animal nutrition as it is a major cost component in animal diets. Dietary fat and oil contribute high levels of energy into the diet, and different fat types affect growth performance of fast growing broiler chickens (Meng et al., 2004). Furthermore, decreased fat digestion and absorption were reported in young broiler chickens (Al-Marzooqi and Leeson, 2000). Addition of emulsifier and multi-carbohydrase resulted in improved energy availability and growth performance in broiler chickens (Upadhaya et al., 2018; Mohammadigheisar et al., 2018). There is a limited number of studies that have shown the performance benefits with the combination of emulsifier and multi-carbohydrase enzymes in broiler chickens. Therefore, this study was conducted to examine the effects of exogenous emulsifiers and multi-carbohydrase supplementation, as a combination, into a reduced energy density diet on growth performance and visceral organ weights of broiler chickens from hatch to 21 days.

168 one-day-old Ross male broiler chickens were allocated in a completely randomized design to 24 pens and each pen assigned to one of four treatments to give six replications with seven birds in each cage. Dietary treatments were: positive control with recommended energy level (PC), negative control with 100 kcal/kg lower energy than PC (NC), NC+CSL (0.05% calcium stearoyl-2 lactylate as the emulsifier) and NC+CSL+M (0.05% multi-carbohydrase). Corn and soybean-meal-based control diets containing soybean oil were formulated to meet the Ross 308 nutrition specification (Aviagen, 2014). Diets were provided on an *ad-libitum* basis in a mash form. Emulsifiers and multi-carbohydrase were top-dressed onto the basal diet to make dietary treatments. Growth performance and visceral organ weights were measured on day 21. Our results revealed that emulsifier and multi-carbohydrase supplementation into low energy density diets improved ($P < 0.05$) the body weight (5%), weight gain (4%), and feed efficiency (10%) of the broiler chickens compared to the broiler chickens fed NC diets from hatch to 21 days. However, the diet containing emulsifier and multi-carbohydrase did not affect ($P > 0.05$) visceral organ weights of broiler chickens fed low energy density diets. In conclusion, our results indicated that a combination of emulsifier and multi-carbohydrase in reduced energy diets has the ability to improve growth performance of broiler chickens by curtailing the negative impact of low energy in the diets.

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PERFORMANCE OF BROILERS FED DIETS WITH HIGH AND LOW NET ENERGY BUT SIMILAR METABOLISABLE ENERGY

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Summary

A semi-commercial scale feeding study was conducted to compare formulations of broiler diets based on predicted net energy (NE) and metabolisable energy (ME). It was hypothesised that formulating to NE may result in more efficient use of dietary energy, promoting optimal performance response in the birds. Dietary treatments contained higher or lower NE but with similar ME. Chickens fed the higher NE formulated diet had higher NE cost per weight gain (WG) and also higher productive energy (NEp) per body weight (BW) compared with birds fed the low NE diet. This resulted in a poorer performance in the high NE birds, such as lower WG, higher fat pad weight and decreased thigh meat yields. The results from this experiment did not confirm a beneficial effect of formulating diet based on NE, most likely due to lower protein level in the NE diet coupled with only a small increase in the NE content of the diet.

I. INTRODUCTION

The most important cost component of broiler feed relates to energy, as this may account for up to 75% of feed costs (van der Klis & Kwakernaak, 2008). Therefore, more accurate evaluation of feed energy in broiler production is imperative for environmental and financial reasons. The metabolisable energy (ME) system is currently the preferred measure for determining energy utilisation in poultry feed. However, this system does not give a complete picture of the amount of total energy actually available to the bird for maintenance, growth or production (MacLeod, 2002). It has been postulated that broiler feed formulation based on net energy (NE) may be more accurate and cost-effective than the current ME system, as has been shown in ruminants and pigs (Noblet et al., 1994). However, this system has yet to be successfully assessed in poultry and is thus not yet ready for use in commercial poultry diet formulation. Researchers at UNE have developed the equations for predicting NE in diets using closed-circuit chambers, and these equations have been validated in a range of diets (Wu et al., 2018). A controlled large-scale experiment is, however, required to validate and confirm whether NE formulation is advantageous over ME based formulation. The aim of this study was to evaluate whether a high NE diet is advantageous compared to a low NE diet with similar ME, based on bird performance and meat yield.

II. MATERIALS AND METHODS

Two experiments were undertaken with commercial feather sexed Ross 308 chicks. Experiment 1 involved a NE study in sealed respiratory chambers, whereas experiment 2 involved a semi-commercial floor pen feeding study. In experiment 1, a total of 40 day-old chicks obtained from two different batches was randomly assigned into 2 runs of 10 closed-circuit respiratory chambers per run, with two birds per chamber (one male and one female). This ensured there were 10 replications per dietary treatment, with 2 birds per replicates. The two dietary treatments, based on wheat, corn and soybean meal, were formulated to contain similar ME values with one higher and another lower in NE, where crude protein (CP) and

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ether extract (EE) varied according to their contributions to NE as reported in Wu et al., (2018). Amino acid spectrum used was obtained using AminoChic 2.0 software. The high NE diet contained 19% CP and 7.5% EE. The low NE diet was formulated to contain 24% CP and 6.4% EE. A common starter diet was fed to all birds from d0 to d14 and the dietary treatments were introduced from d14 to d28. The NE run was undertaken from d25 to d28, during which total excreta were collected daily. Birds and feeds were weighed to determine body weight gain (WG), feed intake (FI) and feed conversion ratio (FCR). Additionally, oxygen bottles were weighed, KOH samples taken, and resulting oxygen consumed and CO_2 produced were measured on a daily basis (Wu et al. 2018). Heat production was calculated using the modified Brouwer equation (Brouwer, 1965; McLean, 1972):

$$\text{Total heat (kcal)} = 3.866 \times O_2 \text{ consumed (L)} + 1.200 \times CO_2 \text{ exhaled (L)}$$

Table 1 - Composition and calculated major nutrients of grower-finisher diets (d14-d35).

Ingredient (%)	High NE Diet	Low NE Diet
Corn	42.9	10.0
Wheat Pollard	17.0	6.2
Full Fat Soybean Meal	14.9	5.0
Canola Meal Cold 11%	11.0	5.0
Wheat	5.0	40.2
Canola Oil	2.8	3.5
Soybean Meal	2	27.1
Dical Phos 18P/21Ca	1.197	1.237
Limestone	1.084	1.039
L-Lysine HCl 78.4	0.240	
Na Bicarb	0.200	0.200
DL-Methionine	0.176	
Mineral Conc. 1.0 kg/mt (0.1%)	0.100	0.100
Vitamin Conc. 0.7 kg/mt (0.07%)	0.070	0.070
Rovabio® Advance	0.005	0.005
Calculated Major Nutrients	Amount	Amount
ME Poultry (mj/kg)	12.97	12.97
NE Broiler (mj/kg)	9.82	9.64
Crude Protein (%)	19	24
Crude Fat (%)	7.50	6.36
d Arg pou (%)	1.09	1.45
d Lys pou (%)	0.99	1.12
d Met pou (%)	0.46	0.41
d M+C pou (%)	0.74	0.74
d Ile pou (%)	0.70	0.91
d Thr pou (%)	0.64	0.74
d Val pou (%)	0.79	1.01
Calcium (%)	0.80	0.80
Av. Phosphorus (%)	0.40	0.40

In experiment 2, diet formulations were the same as those featured in experiment 1. A total of 720 day-old chicks was randomly distributed into 72 floor pens. Birds were reared according to Ross 308 standard and the mortality rate was less than 3%. The study had a 2 × 2 factorial arrangement; the assessed factors included dietary treatment (higher NE vs. low NE diet) and gender (male and female). Birds were randomly allocated to dietary treatments from d14 to d35, with 18 replicates of 10 birds per replicates. A common starter diet was fed to all

birds from d0 to d14 and the dietary treatments from d14 to d35. The weight of birds and feed per pen were recorded weekly from d14 to d35 to determine WG, FI and FCR. On day 35, abdominal fat pad weight and meat yield was measured in three birds per pen, to evaluate carcass characteristics.

III. RESULTS

Table 2 shows that birds fed the high NE diet had reduced N intake ($P < 0.01$) and retained N ($P < 0.05$) per bird, while the N efficiency (ratio of N retained to N intake) was significantly increased ($P < 0.01$). Other parameters were not statistical different ($P > 0.05$), although respiratory quotient (RQ) tended to be increased by feeding the high NE diet ($P = 0.055$).

Table 2 - Net energy trial results between high and low NE diets with similar ME diet.

Factor	ME (MJ/kg)	NE (MJ/kg)	NE/ME (%)	RE/b/d (kJ)	N_i /b/d (g)	N_f /b/d (g)	N_f/N_i (%)	RQ
Low NE diet	15.02	11.43	76.1	758	4.85[a]	3.09[a]	63.7[b]	0.99
High NE diet	15.07	11.58	76.8	804	3.93[b]	2.77[b]	70.4[a]	1.01
SEM	0.10	0.09	0.0	35	0.15	0.09	0.0	0.01
P-value								
Diet	0.773	0.403	0.323	0.386	0.000	0.018	0.000	0.055
Run	0.033	0.161	0.513	0.001	0.001	0.001	0.721	0.125

Data are expressed as means. Values within a row that do not share a common letter are significantly different ($P \leq 0.05$).
ME: metabolisable energy; NE: net energy; RE: retained energy; N_i: nitrogen intake; N_f: nitrogen fixed; RQ: respiratory quotient.

As shown in Table 3, the high NE diet increased ME utilisation per g WG ($P < 0.05$), NE utilisation per g WG ($P < 0.05$) and productive NE (NEp) per g WG ($P < 0.01$). This diet also decreased ($P < 0.05$) the WG and significantly increased ($P < 0.01$) the fat pad weight. However, FCR, FI and breast yield were not affected ($P > 0.05$) by dietary treatment. No dietary treatment by gender interaction was observed for any of the measured parameters. All measurements were lower in the females ($P < 0.01$), except thigh yield which showed no differences ($P > 0.05$) between males and females.

IV. DISCUSSION

The results from this study illustrate an increase in NE utilisation per WG in birds fed diets high in NE, with approximately 3% higher cost of NE/BW compared to the low NE diet. This resulted in a comparatively increased NEp at approximately 6.5% in the birds fed the high NE diet. This was associated with significantly higher relative abdominal fat pad weight in the high NE diet birds, 27% heavier than for the low NE diet birds. In fact, the high NE diet contained a lower protein concentration (19% CP) while the low NE diet contained 24% CP. Adipose fat accretion is promoted by an increase in energy:protein ratio, as well as protein concentration lower than normal requirements, whereas an increase in CP leads to lean muscles and impaired feed efficiency (Buyse et al., 1992; Collin et al., 2003). Therefore, the increased fat deposition in chickens fed the high NE diet may be explained by the fact that these chickens overconsumed feed (high feed consumption per g of WG compared with the low NE group) in an attempt to meet their protein requirements for sustaining their growth potential. By doing so, they consumed more energy which increased fat deposition (Buyse et al., 1992; Collin et al., 2003). However, this finding is not supported by the results of MacLeod (1990), who reported that the control of energy intake takes priority over AA intake. The lean carcass observed in the low NE diet fed birds is in agreement with the findings of Leeson *et al.* (1996), who reported that birds deposit less carcass fat when there is either a decrease in energy intake or an increase in

protein intake. In this study, the growth rate of the high NE diet birds was approximately 2.5% lower than those fed the low NE diet. The poor performance in birds fed the diet with low protein concentration is consistent with earlier findings and might suggest a deficiency of non-essential AA, such as glycine (Buyse et al., 1992).

Table 3 - Effect of high and low NE diets with similar ME on broiler performance from d14 to d35.

Factor		WG/b (g)	FCR (DM)	FI/b/d (g DM)	ME/WG (kJ/g)	NE/WG (kJ/g)	NEp/BW (kJ/g)	Fat pad (%)	Breast (%)	Thigh (%)
Diet	Low NE	1912^{a}	1.43	130	21.41^{b}	16.30^{b}	9.26^{b}	0.76^{b}	20.8	9.8^{a}
	High NE	1866^{b}	1.45	129	21.84^{a}	16.78^{a}	9.90^{a}	1.04^{a}	20.9	9.4^{b}
Gender	F	1769^{b}	1.48^{a}	125^{b}	22.33^{a}	17.08^{a}	9.89^{a}	0.98^{a}	21.2^{a}	9.5
	M	2010^{a}	1.39^{b}	133^{a}	20.92^{b}	16.00^{b}	9.27^{b}	0.82^{b}	20.4^{b}	9.7
SEM		18	0.01	1	0.13	0.10	0.07	0.03	0.1	0.05
P-value										
Diet		0.017	0.084	0. 531	0.036	0.002	0.000	0.000	0.608	0.000
Gender		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.212
Diet*Gender		0.145	0.147	0.988	0.150	0.160	0.204	0.310	0.518	0.133

Data are expressed as means. Values within a row that do not share a common letter are significantly different ($P \leq 0.05$).
WG/b: weight gain per bird over d14-35; FCR: feed conversion ratio as dry matter basis; DM: dry matter; FI/b/d: feed intake per day per day; ME: metabolisable energy; NE: net energy; NEp: energy available for productive purposes.

The results of this study illustrate that feeding chickens diets with high NE but low CP yielded negative results which does not confirm a beneficial effect of formulating diet on only NE. This is apparently due to the substantial difference in CP concentration, yet only slightly higher NE. As the NE value of feed is affected by the CP and EE content of the ingredients, the dietary composition has to be adjusted to achieve different NE level. This may be an issue for formulating diet based on NE and consideration needs to be taken to minimise nutrient deficiency for such formulation. Therefore, further study on NE validation using diets with limited change of CP levels should be considered to make sure all diets have equal growth performance, possibly by change of fat level.

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EVALUATION OF THE EFFICACY OF A MULTI-CARBOHYDRASE AND PHYTASE COMPLEX ON CORN-WHEAT-SOYBEAN MEAL-BASED DIETS WITH VARYING LEVELS IN METABOLIZABLE ENERGY, DIGESTIBLE AMINO ACIDS, AVAILABLE PHOSPHORUS, AND CALCIUM

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Summary

Efficacy of a global enzyme solution containing a multi-carbohydrase and phytase complex (MCPC) was evaluated on growth performance and feed efficiency of broilers fed diets with varying levels in apparent metabolizable energy (ME), digestible amino acids (dAA), and available phosphorus and calcium (avP&Ca) from 10 to 42 days of age (d). Diets fed included a corn-wheat-wheat bran-soybean meal-soy hull-based were formulated to be included in a 3-3 Box-Behnken design with different levels of digestible lysine (dLys) (10.0, 9.6, 9.3, 9.1 g/kg), ME (13.06, 12.54, 12.21, 11.89 MJ/kg), Ca (6.8, 5.8, 4.7 g/kg) and avP (3.3, 2.1, 1.2 g/kg).). Impact for each diet was considered fixed in relation to an iso-substrate formulation (i.e. arabinoxylan and phytate). Feed intake (FI), body weight gain (BWG) and FCR were described by third order polynomial equations (R^2 = 0.99, 0.98 and 0.81, respectively) with inter connection between variables. Available P was the most important factor affecting FI (quadratic) and also heavily affected BWG and FCR. Decrease of avP from 0.33 to 0.12% in control diets resulted in decrease by 25, 33 and 12% of FI, BWG and FCR, respectively. Energy and dLys were secondarily affecting these performance parameters. Inclusion of MCPC alleviated the impact of avP on FI, BWG and FCR in connection with phytase activity and associated P released. As a result, ME and dLys became the most important factors affecting BWG and FCR. The ME was negatively related to FI ($r = -0.89$, $P < 0.001$). Similarly, dLys content was positively correlated to BWG ($r = 0.74$, $P < 0.001$). Both parameters affected FCR with -0.83 and -0.85 for ME and dLys, respectively. Overall, MCPC usage enabled significant reductions in each of ME and dAA, and avP in broiler diets. The effect of MCPC might be associated with nutrient released 0.56 MJ ME/kg, 0.06% units of dAA and 0.15% units of avP&Ca.

I. INTRODUCTION

Metabolizable energy (ME), digestible amino acids (dAA) and available phosphorus (avP) are the largest and most expensive components in broiler diets, considerable fractions of which still pass through the digestive tract undigested and are lost via excretion (Ravindran et al., 2013), hence, emphasizing the need for higher nutrient usage efficiency than current practice. Ability of carbohydrases to degrade non-starch polysaccharides and liberate caged starch and protein (Cozannet et al., 2017) and of phytase to degrade phytate to increase availability of P and Ca (Amerah et al., 2014) have been established. The drive to further increase nutrient usage efficiency necessitates higher efficiency of exogenous enzymes usage. Combined usage of both carbohydrases and phytase in diets is hypothesised to enable significant reductions in ME, dAA, avP, and Ca with no adverse effects on growth performance and feed efficiency of the birds. Efficacy of a global enzyme solution, which consists of a multi-carbohydrase and phytase complex (MCPC), to release ME, dAA, avP, and Ca was evaluated throughout performance and feed efficiency effects on broilers from 10 to 42 d. The objective of the current study was

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to evaluate the effect of MCPC on growth performance and feed efficiency of broilers fed diets with different levels of ME, dAA and avP.

II. MATERIALS AND METHODS

The trial was conducted on 3,840 Ross 308 broiler chicks, housed using a partial block design (Box-Behnken) with 20 birds per pen and 192 pens and fed common starter diet from 0 to 10 d and one of 24 grower and finisher experimental diets from 10 to 28 d and 28 to 42 d, respectively. Diets were corn-wheat-wheat bran-soybean meal-soy hulls-based and included a positive control (PC) diet (12.98 MJ ME, 11.1 g/kg dLys, 3.5 g/kg avP, and 7.5 g/kg Ca and 13.19 MJ ME, 8.8 g/kg dLys, 3.0 g/kg avP, and 6.0 g/kg Ca during grower and finisher phase, respectively) and 11 negative control (NC) diets based on optimized factorial combinations of 3 reduced ME levels (-4.0, -6.5, -9.0%) × 3 reduced dAA levels (-4.0, -6.5, -9.0%) × 2 reduced avP levels (balanced with Ca; PC-36, -70%) with MCPC supplementation at 0 (NC-) or 100 g/ton of feed (NC+). The associated levels were dLys (10.0, 9.6, 9.3, 9.1 g/kg), ME (13.06, 12.54, 12.21, 11.89 MJ/kg), Ca (6.8, 5.8, 4.7 g/kg) and avP (3.3, 2.1, 1.2 g/kg). The MCPC (Rovabio® Advance Phy, Adisseo France SAS, Antony France) consisted of a multi-carbohydrase from *Talaromyces versatilis* (xylanases / beta-glucanases / arabinofuranosidases 1,250 / 850 / 9,250 U/kg of feed) and a phytase (1,000 FTU/kg of feed) sourced from *Buttiauxiella spp*.

The growth performance and feed efficiency, BW and feed intake were measured at 10, 28, and 42 d for calculation of mortality-corrected 10 to 42 BWG, FI, and FCR. Generated data were analyzed using Proc NLIN. Experimental diets allowed precise evaluation of ME, dLys and avP on performance (i.e. BWG, FI and FCR). MCPC effect was estimated using the equation established and converted into ME, dAA and avP improvement. Response surface was fitted by first-, second-, or third-degree polynomial regressions. Pen mean was used as the experimental unit and a 5% level of probability was considered to be significant.

III. RESULTS

Performance results are summarized in Table 1, FI, BWG and FCR varied largely by 15, 17 and 4% coefficient of variation, respectively. A model was developed based on individual results. The surface response for BW and FCR is presented for 2 levels of avP. Equations suggested for the control diet a large impact of avP on animal performance with correlation coefficient equal to 0.84, 0.91 and -0.75 for FI, BWG and FCR ($P < 0.001$). Decrease of avP from 0.33 to 0.12% for control diets resulted in decrease by 25, 33 and 11% of FI, BWG, and FCR, respectively. As a consequence, avP effect on FI was quadratic with an inflection point at 0.26% avP. Secondly, ME and dAA had an important role on intake and feed efficiency. Decrease of ME by 9% was associated with decrease of 12% of BWG. Decrease of dAA by 9% was associated with decrease of 3% of FCR. Interaction between ME and dAA was also important with largest impact of dAA deficiency in high ME diets compared with low ME diet.

The effect of MCPC was highly significant for all parameters with 16, 20 and -4% for intake, gain and FCR, respectively. Significant interactions were found between diet and MCPC. Minimum-maximum variation values were 0 - 44, 2 - 53 and -2 - -6% for FI, BWG and FCR, respectively. Primary discrimination between enzyme responses was related with avP reformulation. Using MCPC, the correlation between avP and FI BWG and FCR became not significant ($r = -0.34$, 0.43 and -0.54 respectively). In contrast to unsupplemented MCPC diets, ME and dAA became primary drivers of FI and BWG ($r = -0.89$ and 0.74, $P < 0.001$, respectively). The MCPC restored FI and BWG at levels not significantly different from those observed for PC. Therefore, FCR was only partly restored for diets reduced in ME, dAA and avP. Also, MCPC supplementation only completely alleviated the increased FCR by NC1+,

NC2+ and NC4+ diets; partially alleviated the increased FCR by NC6+, NC8+ and NC11+; with no effect of the enzyme usage for FCR of other NC+ diets, relative to the PC and respective NC- diets.

Based on the previous relationship established between animal performance and control diet nutrient content, ME dAA and avP released by MCPC might be estimated at 0.56 MJ/kg and 0.06% units and 0.15% units, respectively. The established relationship between diet nutrient content (ME, dAA, avP) and the MCPC effect precisely described the broiler response with high R square between predicted and observed values for BWG, FI and FCR (R^2 = 0.98, 0.99 and 0.81, respectively).

IV. DISCUSSION

This study is a primary work to further define the interactions among nutrients for broiler performance, as demonstrated by Sharma et al. (2017). The fitted models clearly illustrate a main deleterious effect of P deficiency on each indicator of growth performance. In broilers, P deficiency results in a loss of appetite (Underwood and Suttle, 1999) and reduced growth. Restoration of FI by MCPC supplementation indicates the effectiveness of phytase to release phosphorus and reach FI similar to those of animal fed at the requirement or event be above the requirement. This finding is similar to those of Letourneau et al. (2010) who found a positive effect of phytase enzyme on FI and the effect was negatively correlated with the amount of avP in the diet. Interaction was also found with dietary phytate but this effect was not tested in present experiment.

Table 1 - Effects of MCPC on growth performance and feed efficiency of broilers fed varying deficiency levels of ME, dAA and avP from 10 to 42 days of age.

	Reduction levels, %			BW initial, g	Experimental diets effects (10 to 42 day of age)[2]					
					Control diets			Supplemented diets		
Diets	ME	dAA	avP[1]		Gain, g	Intake, g	FCR	Gain, g	Intake, g	FCR
PC	0.0	0.0	0.0	304	3171^{ab}	5015^{bc}	1.582^{kl}	3237^{a}	5019^{de}	1.551^{l}
NC1	-4.0	-4.0	-36	300	2994^{cdef}	5021^{de}	1.677^{efghi}	3191^{ab}	5122^{bcde}	1.605^{jkl}
NC2	-6.5	-4.0	-70	303	2238^{g}	3886^{f}	1.736^{bcde}	3144^{abc}	5138^{bcde}	1.634^{ijk}
NC3	-9.0	-4.0	-36	294	2978^{def}	5076^{bcde}	1.705^{efg}	3190^{ab}	5326^{ab}	1.669^{fghi}
NC4	-4.0	-6.5	-70	301	2067^{h}	3554^{g}	1.718^{defg}	3100^{abcde}	5088^{bcde}	1.641^{fhijk}
NC5	-6.5	-6.5	-36	298	2964^{def}	5026^{cde}	1.696^{efgh}	3093^{abcde}	5154^{bcde}	1.667^{ghi}
NC6	-9.0	-6.5	-70	312	2122^{gh}	3805^{fg}	1.793^{ab}	3150^{ab}	5301^{ab}	1.683^{defgi}
NC7	-4.0	-9.0	-36	299	2948^{ef}	4980^{e}	1.689^{efghi}	3100^{abcde}	5153^{bcde}	1.663^{ghij}
NC8	-4.0	-9.0	-70	312	2052^{h}	3659^{fg}	1.783^{abc}	3057^{bcdef}	5134^{bcde}	1.680^{efghi}
NC9	-6.5	-9.0	-36	311	2943^{ef}	5092^{bcde}	1.731^{cdef}	3115^{abcd}	5246^{abcd}	1.685^{efghi}
NC10	-9.0	-9.0	-36	307	2915^{f}	5154^{bcde}	1.769^{abcd}	3079^{bcde}	5281^{abc}	1.716^{defg}
NC11	-9.0	-9.0	-70	305	2076^{gh}	3772^{fg}	1.817^{a}	3186^{ab}	5429^{a}	1.705^{efgh}

[1]Dietary Ca levels balanced with avP; Ca was reduced by 0.10 and 0.21% units of the PC diet at 0.12 and 0.23% unit of avP, respectively.
[2]dataset (n=192) were analyzed by variance analysis using diets (n=12), enzyme inclusion (n=2), room (n=2), block (n=4) and interaction enzyme diets (n=24) as fixed effect. Significant effect of diet were observed for all parameters

The AA requirements of growing broilers have been frequently estimated using an empirical method (Mack et al., 1999). These studies evaluating requirements did not allow evaluation of interaction between digestible amino acid requirement and other nutrient such as avP and ME. The results suggest an interaction between avP and dAA for FCR and ME and dAA for FI. Inclusion of MCPC in the diet with effects on BWG and FCR of the birds might be partly associated with dLys and other amino acid release. These finding are in accordance with previous results obtained with phytase (Walk et al, 2013) or with carbohydrase (Cozannet et al., 2017).

Finally, the broiler chicken seems immune to the effects of variable diet energy level on general growth and development. Contrary to the observations of Newcombe and Summers (1984), results from the current study suggest that the broiler has a remarkable ability to control energy intake when offered diets of varying energy content. As expected, negative relationships have been found between ME content of the diet and FI. This finding is in line with previous results of Leeson et al. (1996). Hence, exogenous enzymes remove the nutrient encapsulating effect of NSP in broiler diets, thereby improving nutrient access for endogenous enzymes and enhancing overall feed digestibility (i.e., starch, fat, and CP; Meng et al., 2005). As a result, the ME content of the diets also improved via gross energy from digestible nutrients including amino acids previously described.

V. CONCLUSION

This study affirmed that significant reductions in ME, dAA and avP are essential to optimize the benefits of substrate degrading efficacy of enzymes on performance and efficiency of broilers. Therefore, interrelationships among dietary nutrients might also be considered. Imbalance among nutrients might be associated with higher decrease of performance than expected and use of additives might even result in detrimental effects accentuating imbalance, hence the inconsistent effect of the MCPC. This study presents an interesting design for evaluation of animal requirement and MCPC matrix value. Equations of present experiment were the result of one single trial and required further data set to be validated. Many miscellaneous points have not been considered in connection with missing freedom degree. In the following development of such disposal, it might be interesting to test such as amounts of Ca and their interactions with avP or to amounts of substrates such as arabinoxylans or phytate.

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DL-METHIONINE AND L-METHIONINE ARE EQUALLY EFFICIENT IN BROILERS

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Summary

In this study, the relative bioavailability value (RBV) of a new L-methionine source with 90% purity (L-Met90) was determined compared to DL-methionine diluted to a corresponding purity of 90% (DL-Met90) in male broilers from 1 to 34 days of age. A total of 1,800 day old Ross 308 broilers were fed starter (d 1 to 10), grower (11 to 26) and finisher (27 to 34) diets. Each phase comprised 17 dietary treatments including a basal diet deficient in standardized ileal digestible (SID) Met+Cys without supplemental Met; and 8 increasing levels of either L-Met90 and DL-Met90. Met sources were added in all phases on weight-to-weight basis at 0.00, 0.30, 0.60, 0.90, 1.20, 1.50, 2.10, 2.70 and 3.60 g/kg. For RBV determination, growth performance and carcass data were subjected to multi-exponential regression analysis. No significant differences were found for any of the RBV estimates of L-Met90 compared to DL-Met90 during any of the feeding phases. These results demonstrate that the new L-Met source with 90% purity is only as efficient as a diluted DL-Met to the corresponding purity in broilers.

I. INTRODUCTION

Commercial poultry diets are routinely supplemented with methionine (Met) sources to precisely meet their Met+Cys specifications. Globally, dry DL-methionine (DL-Met, 99% purity) is the most commonly used Met source followed by methionine hydroxy analogue products (MHA-FA liquid, 88% purity and dry MHA-Ca, 84% purity) and L-methionine (L-Met, 99% purity). During recent years, numerous studies designed to determine the replacement ratio of L- and DL-Met products have shown that L-Met and DL-Met are 100% equally efficient in broilers (Baker 1994; Ribeiro et al., 2005; Baker 2006; Dilger and Baker, 2007). Although the 100% nutritional equivalence of L- and DL-Met has been well-documented, few other publications claimed higher bioavailability for L-Met (Shen et al. 2014; Park et al., 2017). Furthermore, a new L-Met source with a minimum content of 90% L-Met has been introduced to the market claiming that it can replace DL-Met (99% purity) on 1:1 product-to-product basis because of its higher bioavailability. Therefore, a study was conducted to determine the bioavailability of L-Met90 compared to diluted DL-Met to 90% purity in male broilers from 1 to 34 d of age.

II. MATERIALS AND METHODS

A total of 1,800 d-old male Ross 308 broilers were allocated to 90 floor pens of 20 broilers each. Each pen (~ 3 m^2) was equipped with a bell drinker and a round feeder. Light and temperature regimes were managed according to the breeder's recommendations and complying with EU welfare legislation. Broilers were fed a 3-phase feeding schedule with starter (d 1 to 10), grower (d 11 to 26), and finisher phases (d 27 to 34). Starter feeds were produced in crumbles while grower and finisher diets (3.0 mm) were steam pelleted. Feed and water were supplied ad libitum throughout the experimental period. Each phase comprised 17 treatments including a basal diet deficient in SID Met+Cys without supplemental Met, and 8 increasing levels of either L-Met90 or DL-Met diluted to 90% purity – DL-Met90. Starch was used to dilute DL-Met to a Met content of 90%. Met sources were added in all phases on

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weight-to-weight basis at: 0.00, 0.30, 0.60, 0.90, 1.20, 1.50, 2.10, 2.70 and 3.60 g/kg. There were 10 replicate pens for the basal treatment and 5 replicate pens for each of the Met supplemented treatments (2 to 17). Diets were formulated to meet or exceed amino acid recommendations by AMINOChick® 2.0, except for SID Met and Met+Cys. Main ingredients were analyzed by AMINONIR® and results were used for diet formulation. In addition, Met sources were analyzed for Met purity. The SID Met+Cys levels of the starter, grower, and finisher basal diets were 6.1, 5.3 and 5.1 g/kg, respectively, as shown in Table 1. Growth performance variables were evaluated for each feeding phase. On day 34, four birds close to average pen weight were selected for carcass evaluation. After cooling, carcass yields (CY) were determined, prior to fileting for breast meat yield measurement (breast without skin and bone). Growth performance and carcass data were subjected to multi-exponential regression analysis using the nonlinear-regression procedure described by Littell et al. (1997). The significance of the RBV estimate was defined by the values of the 95% confidence intervals. No significant difference between L-Met90 and DLM90 is declared if the approximate 95% confidence interval includes the value of 100%, whereas a confidence interval completely above 100% would indicate statistical superiority of L-Met90.

Table 1 - Dietary ingredients and calculated nutrient composition (g/kg) of the experimental diets.

	Starter d 1 to 10	Grower d 11 to 26	Finisher d 27 to 34
Ingredients:			
Corn	491.6	527	547.8
Soybean meal (46% CP)	357.5	287.8	259.5
Peas	50	100	100
Corn gluten meal	20	---	---
Limestone	13.7	12.9	11.7
Soybean oil	36.6	45.8	56.3
Monocalcium phosphate	17.6	15.1	13.5
Salt (NaCl)	2.4	2.7	2.7
Premix	5	5	5
L-Lysine HCl	2.3	1.1	1
L-Threonine	0.8	0.5	0.5
L-Valine	0.5	0.3	0.2
Choline chloride, 60%	0.2	0.3	0.4
Sodium bicarbonate	1.8	1.5	1.4
Calculated content[1]			
AMEn (MJ/kg)	12.55	12.97	13.38
Crude protein	231.3 (230.7)	203.6 (197.2)	191.1 (185.2)
SID Lys[1]	12.8	10.9	10.1
SID Met	3.1	2.7	2.5
SID M+C	6.1	5.3	5.1
SID Thr	8.1	7	6.6
SID Trp	2.4	2.1	1.9
SID Val	10.1	8.7	8.1
SID Ile	8.7	7.6	7.1
Total Lys	14.2 (13.9)	12.1 (12.0)	11.3 (11.0)
Total Met+Cys	7.1 (7.2)	6.2 (6.0)	5.9 (5.6)
Calcium	9.6	8.7	7.9
Available P	5	4.4	4

[1]SID: standardized ileal digestible. Number in parenthesis represent analyzed values.

III. RESULTS AND DISCUSSION

Analyzed values of the experimental diets from all dietary phases were in close agreement with the calculated values (Table 1). The commercial lot of L-Met90 used in the study was analyzed to contain a purity of 93%, which is greater than its minimum guarantee content of 90% L-Met. Therefore, the analyzed values for both supplemental Met sources (DL-Met90 and L-Met90) for each phase were used to calculate the total intake of supplemental Met and used for RBV determination.

Increasing levels of either L-Met90 or DL-Met90 significantly improved growth performance and carcass yields compared to the basal diet. Relative to the basal diet, the highest Met addition improved body weight gain (BWG) by 79 and 76% and reduced feed conversion ratio (FCR) by 22 and 21% for DL-Met90 and L-Met90, respectively. Similarly, CY and breast meat yield as percentage of carcass weight were improved by 7 and 7% and 51 and 51% for DL-Met90 and L-Met90, respectively. These relative responses demonstrate that the basal diets were clearly deficient in dietary Met+Cys, and that both Met sources showed a common plateau at the highest Met addition. Therefore, data fit well for multi-exponential regression analysis for RBV determination

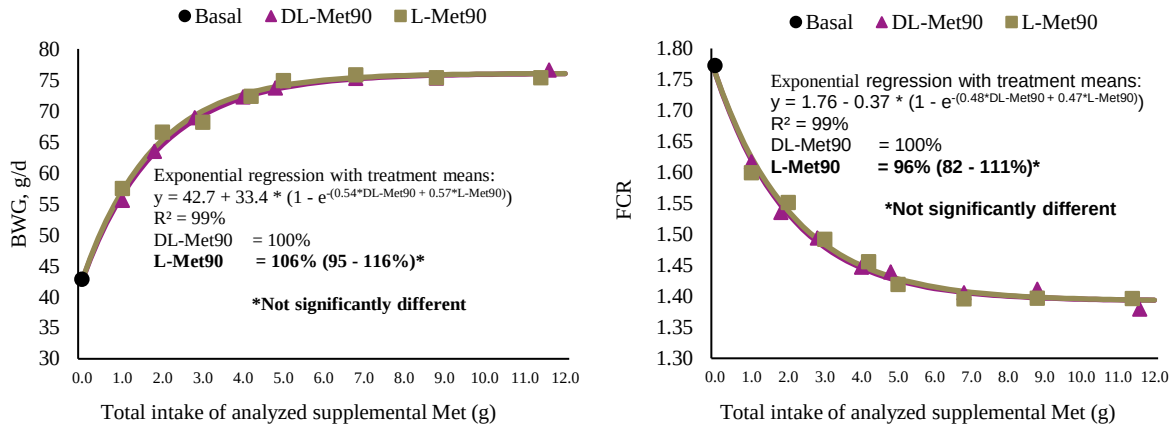

Figure 1 - Relative bioavailability of L-Met90 compared to DL-Met90 based on body weight gain (BWG, left) and feed conversion ratio (FCR, right) from 1 to 34 days of age.

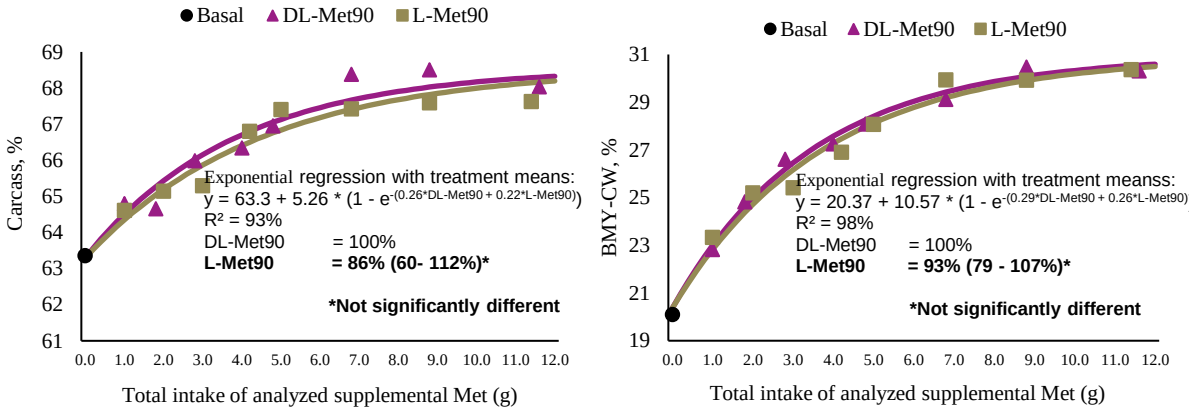

Figure 2 - Relative bioavailability of L-Met90 compared to DL-Met90 based on carcass yield (left) and breast weight as percentage of carcass weight (BMY-CW, right) at 34 days of age.

Overall responses (d 1 to 34) to increasing levels of DL-Met90 and L-Met90 are shown in Figures 1 and 2 for growth performance and carcass yield, respectively. Multi-exponential

regression analysis revealed that L-Met90 was 106 and 96% as efficacious as DL-Met90 for BWG and FCR, respectively. Based on carcass traits, L-Met90 was estimated to be 86, and 93% as efficacious as DL-Met90 for CY and breast meat weight as percentage of carcass weight, respectively. However, based on the confidence intervals none of the RBV estimates were significantly different from 100%.

No significant differences were found for any of the RBV estimates of L-Met90 compared to DLM90 for any of the feeding periods. These results demonstrate that the new L-Met source with a minimum purity of 90% L-Met is equally efficient as a diluted DL-Met to the corresponding purity of 90% in broilers from 1 to 34 d of age. These results are consistent with previous reports (Baker 1994; Ribeiro et al., 2005; Baker 2006; Dilger and Baker, 2007) that have demonstrated the same nutritional value of L-Met (99%) and DL-Met (99%) in broilers.

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DIETARY XYLANASE IMPROVES GROWTH PERFORMANCE AND COST SAVINGS IN BROILER CHICKENS FED A CORN-SOYBEAN BASED DIET

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The increased demand to reduce production costs, as well as concerns about the environment, have resulted in pressure on poultry producers to increase dietary energy utilisation and to improve feed efficiency. Usually, technologies such as exogenous enzymes that are supplemented in the diets have been one of the main pathways used to reach these goals. Xylanase is well known to produce positive effects on growth performance of birds fed diets based on non-starch polysaccharide (NPS) rich cereals, helping the birds to overcome the anti-nutritional effects of NSP by reducing the intestinal viscosity and improving energy digestibility (González-Ortiz et al., 2016). However, although corn-based diets are widely used in poultry in many regions, there is still a lack of information about the effect of xylanase on this type of diet. The present xylanase has high activity on the insoluble portion of arabinoxylans, which are present in large quantities in corn and soybean; therefore, it could improve the release of the nutrients from these grains. For this purpose, the present study aimed to evaluate the effects of a bacterial xylanase on growth performance of broilers fed a corn-soybean based diet.

A total of 1,440 male broiler chickens Cobb 500 was allocated to floor pens from 1 to 42 d of age. The experiment followed a randomised designed, with 8 replicates of 60 birds for each one of the 3 treatments: standard diet (STD), diet with 150 kcal/kg reduction in metabolisable energy (RED) and RED diet + 100 g/t of *Bacillus subtilis* xylanase (RED+XYL; Jefo & Puratos). It was intended to create a substantial drop in the growth performance between the STD and RED treatments; therefore, the RED diets were formulated with an uplift greater (150 kg/kcal) than the recommended (80 kcal/kg) when using this particular xylanase in corn-soybean meal-based diets. Average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) were evaluated by feeding phase (1-21, 22-35 and 36-42 d of age). The feed cost per kg of live weight was calculated in the end of the trial considering the market price for the xylanase studied. The data were analysed by ANOVA and treatment means were separated by the Duncan test when $P < 0.05$.

There was no effect of the treatments on ADFI. Birds fed the RED diet had the worst response for ADG and FCR from 14 d of age to the end of the trial ($P < 0.05$). There was a reduction of 4.6% ($P < 0.05$) on ADG of birds fed RED diet throughout the overall rearing period, compared to the STD diet. However, the addition of the xylanase in the RED diet was effective in partially recovering the growth response ($P < 0.05$; 49% for ADG and 45% for FCR), which is in accordance with the recommended uplift (80 kcal/kg). The lowest cost/kg of live weight was observed for the RED+XYL diet (difference of USD 0.08 in comparison to the RED diet and USD 0.21 to the STD diet). The results showed that there is an opportunity to improve the average daily gain and feed conversion ratio of broilers chickens fed a corn-soybean based diets with reduced energy, when the diet is supplemented with the xylanase studied. The addition of the enzyme in the reduced energy diets can be used to allow savings on feed costs.

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RESPONSE OF BROILER CHICKENS TO FEED FORM AND MICROBIAL ENZYME SUPPLEMENTATION ON TANZANIAN-TYPE DIETS

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Summary

This study investigated the effect of feed form (mash vs. pellet) and microbial enzyme supplementation on the performance of broiler chickens fed Tanzanian-type diets from 0 to 35 days of age. A total of 480 unsexed day-old Ross 308 broiler chickens was offered eight diets made from feed ingredients that are commonly used in Tanzania, including maize, soybean, cottonseed and fish meals. Diets were fed as either mash or pellets and supplemented with one of two enzymes, a composite carbohydrase (Axtra XB) or phytase (Quantum Blue, QB), or a combination of the two enzymes, in a compleely randomized design with a 2×4 factorial arrangement. Each of the eight treatments was replicated six times, with 10 birds per replicate. Feed intake (FI) was significantly ($P < 0.05$) higher in birds fed the pelleted diets at d24 and d35, and increased further ($P < 0.05$) when enzymes were supplemented individually and in combination. Body weight gain (BWG) increased ($P < 0.001$) in the birds fed the pelleted diets. Enzyme supplementation increased ($P < 0.05$) BWG at d24 and d35. Feed conversion ratio (FCR) was reduced ($P < 0.001$) in chickens fed on the pelleted diets compared to the mash-fed birds. Birds provided with a combination of Axtra XB and QB responded with lower ($P < 0.01$) FCR. The relative weights of breast, thighs and drumsticks were significantly ($P < 0.001$) higher in birds fed pelleted diets, and were increased further when the diets were supplemented with Axtra XB, QB and a combination of the two enzymes. The findings from the current study will help small-to-medium scale (SM) farmers to improve their feed formulation in order to improve the productivity of the sector.

I. INTRODUCTION

In Tanzania, a majority of the SM farmers use mash diets for feeding broiler chickens, and this has resulted in lower performance. The feed industry in Tanzania is less developed due to lack of analytical facilities, low quality of ingredients and lack of government policy enforcement on feed quality (Geerts 2014). Improving feed quality through physical processing and microbial enzyme supplementation can enhance broiler performance. Feeding pellets improves nutrient density, reduces wastage during feeding and reduces feed selection by the birds (Glover *et al.* 2015). Grinding and pelleting disrupt the physical structure of dietary ingredients, thus exposing the feeds to the action of digestive enzymes, enabling high performance of broilers (Ghobadi and Karimi 2012). The application of microbial enzymes in poultry diets is important because poultry lack endogenous enzymes that can break cell wall structures from vegetable sources in feed ingredients (Ghobadi and Karimi 2012). Adding microbial enzymes will improve nutrient availability and also reduce costs related to production by increasing the productivity of chickens in Tanzania.

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II. MATERIALS AND METHODS

A total of 480 day-old unsexed Ross 308 broiler chickens was randomly assigned to a 2 × 4 factorial arrangement of treatments, with two feed forms (mash and pellet), without enzyme, or supplemented (at 100 mg/kg) with Axtra XB (xylanase and beta-glucanase composite), QB (phytase) or a combination of Axtra XB + QB. Eight dietary treatments were compounded mainly from maize, soybean, cottonseed and fish meals. Birds were allocated to six replicates per treatment (10 birds per replicate) and housed in a climate-controlled deep-litter system with feed and water supplied *ad libitum* from 0 to 35 days. Birds were housed at the Centre for Animal Research and Teaching, University of New England, Armidale, Australia and raised in three phases, starter (d1-10), grower (d11-24) and finisher (d25-35). Measurements of the feed intake (FI) and body weight gain (BWG) were taken on d10, d24 and d35 to calculate feed conversion ratio (FCR). On d35 two birds per replicate were also euthanised and dissected to obtain the dressing percentage as well as absolute and relative weights of breast, thighs and drumsticks. Data were analysed using the general linear model of Minitab version 17 (Minitab 2014). Least square means were computed using the Tukey pairwise comparisons test, and considered to be significant at $P \leq 0.05$.

III. RESULTS

Broiler gross performance (FI, BWG and FCR) results are shown in Table 1. Feed intake to d24 and d35 was higher ($P < 0.05$) for birds raised on pelleted diets compared to mash diets. Microbial enzyme supplementation increased FI ($P < 0.05$) at d24 and d35, with higher FI observed when a combination of Axtra XB and QB was used. The BWG to d24 and d35 was increased ($P < 0.001$) when birds were fed pelleted diets and when diets were supplemented with enzymes ($P < 0.001$) at both d24 and d35. The FCR for all age groups of birds was significantly ($P < 0.001$) reduced when feeding pelleted diets. However, enzyme supplementation decreased ($P < 0.007$) FCR only on day 24, with the combination of Axtra XB and QB resulting in the lowest FCR. There was no interaction effect between the main factors (feed form and enzyme supplementation) for any response variables ($P > 0.05$). The dressing percentage and relative weights of breast, thighs and drumsticks were superior ($P < 0.001$) in birds fed pelleted diets and the diets supplemented with enzymes, although the relative weight of drumsticks was not affected ($P > 0.05$) by enzyme (Table 2).

Table 1 - Effect of feed form and microbial enzyme supplementation on feed intake (FI), weight gain (WG), and feed conversion ratio (FCR) between hatch and 10, 24 and 35 days.

Feed form	Enzymes	d1-10			d1-24			d1-35		
		FI	WG	FCR	FI	WG	FCR	FI	WG	FCR
Mash		274	269^{b}	1.025^{a}	1541^{b}	1272^{b}	1.243^{a}	3370^{b}	2343^{b}	1.529^{a}
Pellet		287	323^{a}	0.842^{b}	1605^{a}	1395^{a}	1.123^{b}	3598^{b}	2503^{a}	1.356^{b}
	No enzyme	275	300	0.955	1524^{b}	1317^{ab}	1.252^{a}	3323^{b}	2364^{b}	1.470
	Axtra XB	275	297	0.917	1598^{ab}	1376^{a}	1.158^{b}	3382^{b}	2498^{a}	1.472
	QB	291	293	0.911	1546^{ab}	1292^{c}	1.172^{b}	3492^{b}	2464^{ab}	1.397
	AxtraXB+QB	282	293	0.950	1626^{a}	1349^{ab}	1.148^{b}	3741^{b}	2366^{b}	1.430
Source of variation										
Feed form		0.071	0.001	0.001	0.005	0.001	0.001	0.001	0.001	0.001
Enzyme		0.285	0.744	0.402	0.007	0.005	0.007	0.001	0.025	0.24
Feed form × Enzyme		0.104	0.746	0.671	0.173	0.245	0.466	0.560	0.926	0.219

$^{(a-c)}$Means with different superscripts within the columns are significantly different ($P < 0.05$). SEM = Standard error of the means, each value represents the mean of 6 replicates (10 birds per replicate). AxtraXB (Xylanase and beta-glucanase composite) a dry feed microbial enzyme, QB = Quantum Blue (phytase enzyme).

Table 2 - Effect of feed form and enzyme supplementation on dressing percentage and relative weight (g/kg) of broiler parts meat yield.

Feed form	Enzyme	Dressing percent	Breast	Thighs	Drumsticks
Mash		70.2[b]	210.6[b]	96.8[b]	86.9[b]
Pellet		75.3[a]	223.3[a]	103.3[a]	92.5[a]
	No enzyme	70.3[b]	208.0[c]	96.6[b]	87.2
	Axtra XB	72.6[ab]	221.8[a]	100.3[ab]	90.8
	QB	73.3[ab]	214.6[b]	102.0[a]	89.4
	Axtra XB+QB	74.9[a]	223.4[a]	101.4[ab]	91.5
Source of variation					
Feed form		0.001	0.001	0.001	0.001
Enzyme		0.002	0.001	0.028	0.148
Feed form × Enzyme		0.249	0.584	0.456	0.028

[(a-c)] Means with different superscripts within the columns are significantly different ($P < 0.05$), SEM = Standard error of the means. Each value represents the mean of 6 replicates (10 birds per replicate). Axtra XB (Xylanase and beta-glucanase composite) a dry feed microbial enzyme, QB = Quantum Blue (phytase enzyme). DP = Dressing percentage.

IV. DISCUSSION

The improved gross performance of broiler chickens consuming pelleted diets with or without enzyme supplementation could be due to increased nutrient availability, improved digestibility, reduced feed wastage and energy spent by bird during eating (Serrano et al. 2012; Abdollahi et al. 2013). These results agree with the findings of Truong et al. (2015) who reported that pelleting diets increases FI, reduces feed selection and improves nutrient digestibility. The result is also in line with those obtained by Lv *et al.* (2015) who found that broiler chickens fed with pelleted diets had better performance for the entire period of study. The improved BWG in this study could be due to the increase in feed intake (Amerah et al., 2008). The poorer performance in mash-fed birds might be a reflection of reduced ability of birds to eat bulkier, and possibly less palatable diets, which would result in lower nutrient availability (Brickett et al. 2007). The FCR values observed in this study are to some extent similar to those reported by Mabelebele et al. (2018) who found that broiler chickens fed pelleted diets had better growth performance and FCR than mash-fed broiler chickens.

The increased dressing percentage and meat yield (breast, thighs and drumsticks) of birds could be directly related to increased body weight as a result of feeding pelleted diets and or enzyme supplementation. Futhermore, pelleted diets improve the ingestion and passage rate of feeds leading to increased growth of bird and body parts weight (Amerah et al. 2007a). The heavy breast and thighs in this study are related to enzyme addition, which is similar to those reported by Erdaw et al. (2015) and Abdallh et al. (2017) who who observed that adding microbial enzymes to broiler diets increases weight gain and meat yield.

It can be concluded that adopting and applying these technologies will be beneficial to the SM poultry farmers in Tanzania. It will be necessary to assess the cost-benefit analysis of the diets when fed in Tanzania.

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APPARENT METABOLIZABLE ENERGY AND ENERGY UTILIZATION BY BROILER CHICKENS AS AFFECTED BY FEED FORM AND MICROBIAL ENZYME SUPPLEMENTATION OF TANZANIAN-TYPE DIETS

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Summary

A 2 x 4 factorial study was conducted to assess the apparent metabolizable energy (AME) and energy utilization in broiler chickens fed pelleted or mash diets, with or without enzyme supplementation. Eight diets were made based on maize, soybean, cotton seed and fish meal, ingredients that are typically used by small-to-medium (SM) scale poultry farmers in Tanzania. The energy value and utilization of such diets have never been assessed by the users due to lack of facilities. Four hundred and eighty day-old unsexed Ross 308 broiler chickens were fed as-is or the diets were supplemented with microbial enzymes, Axtra XB (xylanase and β-gluconase), Quantum Blue (QB - phytase) or a combination of the two enzymes, at 100 mg/kg. Apparent metabolizable energy was higher in birds supplied with pelleted diets than those fed mash diets and was increased by enzyme supplementation. Metabolizable energy intake (MEI), net energy for production (NEp) and energy retained as protein were also higher in the birds that consumed the pelleted diets. Enzyme supplementation increased the energy retained as protein but had no effect on MEI or NEp. These results indicate that pelleting diets increased the AME and energy utilization, with further improvements due to enzyme supplementation. The results of the current study will save as a reference and basis for improvement of the SM sector in Tanzania.

I. INTRODUCTION

The optimum utilization of nutrients, especially energy and protein, by poultry is essential in commercial production. The efficiency of feed utilization has been improved through genetic development (Rege 1994), alongside feed processing and use of feed additives, especially microbial enzymes. Energy utilization in poultry can be measured by assessing metabolizable energy or net energy, which may be for production (NEp) or for maintenance (NEm). The NEp is regarded as a more sensitive measure of energy utilization by the chickens receiving enzyme because it considers the efficiency of utilization of ME for growth. The utilization of feeds can be enhanced by pelleting the diets as well as enzyme supplementation. Birds fed on pellets use less energy for feeding, thus reserving the available energy for growth (Amerah *et al.* 2008). The diets that were tested in this study represent typical diets that are utilized by small-to-medium scale (SM) broiler producers in Tanzania, which generally are poorly formulated and have never been evaluated to determine their nutritive value. This study represents an attempt to develop suitable diets for that sector, with the additional benefits of data on energy values and utilization of such energy.

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II. MATERIALS AND METHODS

A total of 480 day-old unsexed Ross 308 broiler chicks was randomly allocated according to a 2x4 factorial design. There were 2 feed forms (mash or pellet) and four microbial enzyme treatments (none, AxtraXB, QB and AxtraXB+QB). Each treatment was replicated six times, with 10 birds per replicate. Birds were raised in deep litter pens in climate-controlled rooms at the Centre for Animal Research and Teaching, University of New England, Australia. Eight dietary treatments were formulated from maize, soybean, cottonseed and fish meal, which are commonly used by poultry producers in Tanzania. Diets were fed as-is or supplemented with 100 mg/kg of either Axtra XB (Danisco Animal Nutrition, UK), providing 250 units of β-glucanase and 2,500 units of xylanase per kg of the formulated feeds, or 100 mg/kg of Quantum Blue (AB Vista, Marlborough,UK) providing 500 FTU, or a mixture of the two enzymes (50 mg/kg of each). These treatments were used throughout the production cycle (d0-35). Titanium dioxide (TiO_2) was added to the grower diets at 0.5 % as an indigestible marker in order to assess the AME and metabolizable energy intake (MEI). Twelve day-old chicks were electrically stunned, killed by cervical dislocation, minced and later analysed to obtain the baseline data of energy, fat and protein contents. Excreta was collected from d19-21 using aluminium foil sheets. The excreta samples were pooled by replicate (per pen), mixed and stored at -20 ℃. Two birds per cage were killed at d24 and immediately stored intact at -20 ºC. The intact day-old and adult carcasses (d24) were minced and freeze-dried. Ileal digesta samples were collected from another two birds at d24 of age. They were then ground (0.5 mm) and analysed for DM, CP, GE and total fat. Excreta samples were freeze-dried, ground and analysed for GE and TiO_2 contents.

The AME was calculated according to Olukosi et al. (2008):

AME (MJ/kg) = GEdiet – [GEexcreta × (Tdiet /Texcreta)], where GEdiet = diet gross energy (MJ/kg); GEexcreta = excreta GE (MJ/kg); Tdiet = titanium dioxide concentration of diets (g/kg of DM intake); and Texcreta = concentration of titanium dioxide in the excreta (g/kg of DM intake).

The NEp was calculated by subtracting initial carcass GE from the final carcass GE. The MEI was calculated by multiplying AME by bird FI value. The efficiency of ME use for energy retention was calculated as (KRE) = NEp/MEI, while efficiency of ME use for protein retention was calculated as (KREprotein) = REprotein/MEI. Heat production was calculated by subtracting NEp from MEI. Diets were analysed for CP and gross energy. Data were analysed by the general linear model (GLM) procedure of Minitab statistical software version 17 (Minitab 2014).

III. RESULTS

The effects of feed form and microbial enzyme supplementation on AME and energy utilization are presented in Table 1. There was no interaction between feed form and enzyme supplementation for either AME or the energy utilization measurements. Birds fed the pelleted diets had higher ($P < 0.001$) AME than those fed the mash diets. Microbial enzyme supplementation increased ($P < 0.005$) AME, with the best result observed when Axtra XB and QB were combined in the diet. Metabolizable energy intake, NEp and the energy retained as protein were comparatively improved ($P < 0.002$) when pelleted diets were fed. Adding microbial enzymes to the diets increased ($P < 0.01$) energy retained as protein, coupled with a slight increase in MEI and efficiencies of ME usage. Neither feed form nor microbial enzyme addition had significant effect ($P > 0.05$) on the heat production (HP), energy retained as fat and efficiency of ME used for lipid retention.

IV. DISCUSSION

The increased AME, MEI, NEp and energy retained as protein in the group of birds fed pelleted diets compared to those fed mash could be due to the fact that pelleting diets reduces feed wastage and particle selection during consumption. Furthermore, birds use less energy when eating pelleted diets, thus conserving more energy, some of which could be lost when fed mash diets (Serrano *et al.* 2012). Pellet processing conditions, including pressing, heating and addition of moisture might deactivate anti-nutritive factors (ANF) and improve the palatability of diets, leading to an improvement in nutrient availability, particularly energy, for the bird (Abdollahi *et al.* 2013). The current results are supported by the findings of Greenwood *et al.* (2004), who reported that pelleting diets makes more energy available to birds and hence proportionally increases dietary energy in order to achieve maximum utilization and retention. Similar enzymes have been shown to reduce the negative effects of ANF and improvement in the activities of endogenous enzyme activities (Cowieson *et al.* 2006). The results of this study suggest that the test diets would be better fed as pellet and would benefit further from supplementation with microbial enzyme supplements.

Table 1 - Metabolizable energy intake, energy utilization and efficiency of metabolizable energy use for energy, lipid and protein retention of broiler chickens as affected by feed form and microbial enzyme supplementation.

Feed form	Enzyme	AME	MEI (kJ/d)	Energy utilization (kJ/d)		Energy (kJ/d) retained as:		Efficiency of ME use for:		
				NEp	HP	Protein	Fat	Energy	Protein	Lipid
Mash		12.3^{b}	790.2^{b}	444.6^{b}	345.6	233.0^{b}	247.4	0.54^{b}	0.28^{b}	0.30
Pellet		13.0^{a}	851.6^{a}	499.8^{a}	351.8	256.0^{a}	262.9	0.62^{a}	0.32^{a}	0.33
	No enzyme	12.1^{b}	797.6	455.9	341.7	241.9^{ab}	247.9	0.55	0.29	0.30
	Axtra XB	12.7^{ab}	817.7	484.0	333.7	250.7^{a}	256.2	0.58	0.30	0.31
	QB	12.7^{ab}	818.5	457.3	361.1	232.2^{b}	247.3	0.59	0.30	0.32
	Axtra XB+BQ	13.0^{a}	849.8	491.5	358.2	253.2^{a}	269.4	0.61	0.31	0.33
Source of variation										
Feed form		0.001	0.002	0.001	0.783	0.001	0.138	0.001	0.001	0.065
Enzyme		0.005	0.285	0.191	0.795	0.010	0.397	0.391	0.523	0.429
Feed form × Enzyme		0.947	0.399	0.455	0.245	0.419	0.698	0.446	0.348	0.348

($^{a-b}$) Means with different superscripts within the columns are different ($P < 0.05$), Values are means of 6 replicates (10 birds per replicate). Axtra XB = Xylanase and beta-glucanase composite, QB = Quantum Blue (Phytase enzyme), SEM = Standard error of the mean, AME = Apparent metabolizable energy, MEI = Metabolizable energy intake, NEp. = Net energy for production, HP = Heat production.

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ENDOGENOUS ENZYME ACTIVITIES AND ENERGY UTILISATION OF BROILER CHICKENS FED SORGHUM-BASED DIETS SUPPLEMENTED WITH PHYTASE AND CARBOHYDRASES.

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Summary

This study was conducted to evaluate the endogenous enzyme activities and energy utilisation of broiler chickens fed sorghum-based diets supplemented with phytase and carbohydrases. The birds were housed in cages in climate-controlled rooms. The jejunum and pancreas were collected at 10 and 24 d for analysis of endogenous digestive enzyme activities. Birds were also sampled at hatch and 24 d and analysed for gross energy, fat and crude protein contents. The data were used to calculate heat production, net energy of production and efficiency of energy utilisation. There were improvements in digestive enzyme activities and utilisation of energy, in terms of metabolisable energy and net energy of production (NEp), suggesting the suitability of the exogenous test enzymes for use in sorghum-based diets.

I. INTRODUCTION

A recent on Australian sorghum by Selle et al. (2017) that sorghum produced in Australia is used almost exclusively for feed, especially cattle, pigs and poultry. The objective of the present study is to assess the response of broiler chickens to diets based on sorghum, when supplemented with a combination of enzymes, targeting different substrates.

II. MATERIALS AND METHODS

A total of 648 male and female Ross 308 broiler chickens was randomly assigned in a 3 × 2 × 2 factorial arrangement of treatments [3 doses of phytase none, standard (100 mg/kg) and superdose (300 mg/kg)] × 2 doses of xylanase and of β-glucanase [none and standard (100 mg/kg)] in a completely randomised 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 as crumble (1-10 d), grower as pellet (11-24 d) and finisher as pellet (25-35 d). The test diets contained 60, 64 and 68 % of sorghum in the starter, grower and finisher respectively and were formulated to meet the specifications recommended for the Ross 308 broiler chickens (Aviagen, 2014). A sub-sample of 10 day-old chicks was euthanised by cervical dislocation, minced and analysed to provide baseline data on body composition (gross energy, crude protein and fat contents). At d 24 two birds per pen were randomly selected, euthanised by cervical dislocation and processed (chopped, minced and freeze-dried) and used to determine carcass energy, protein and fat. On d 10 and d 24 one bird was randomly selected from each cage, electrically stunned and euthanised by cervical dislocation. These were dissected to obtain the whole pancreas and anterior jejunum (4-5 cm long) and used to determine the endogenous enzyme activities. Another 2 birds were similarly slaughtered at d 24 and processed as described for the birds collected at d 0, to determine the energy, protein and fat contents of the intact carcass. The data from d 24 were related to the baseline data obtained from the day-old chicks, to calculate the heat production (HP), NEp and efficiency of utilisation of metabolisable

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energy. Between d 25 and d 35 birds were fed finisher diets to measure meat parts yield. A general linear model procedure was used to analyse the collected data (Minitab Inc., 2013).

III. RESULTS AND DISCUSSION

There was an interaction ($P < 0.003$) between phytase, xylanase and β-glucanase on chymotrypsin activity at d 10. Addition of phytase increased ($P < 0.02$) pancreatic protein content, trypsin activity and general proteolytic activity. At d 24 pancreatic protein content and enzyme activities (chymotrypsin, trypsin and general proteolytic activity) also responded ($P < 0.02$) to interactions between phytase and β-glucanase. General proteolytic activity was increased ($P < 0.004$) in the groups supplemented with phytase. At d 10, there was no interaction between the factors on the activities of jejunal membrane-bound enzymes but the activities of maltase, sucrase and alkaline phosphatase were increased ($P < 0.004$) with phytase inclusion. Phytase supplementation also increased ($P < 0.01$) the activities of jejunal sucrase and aminopeptidase at d 24

At d 24, there was an interaction ($P < 0.03$) between phytase, xylanase and β-glucanase on apparent metabolisable energy (AME) content. The interaction between xylanase and β-glucanase on energy retained as fat and as protein and on efficiency of metabolisable energy use for lipid retention were significant ($P < 0.01$). Addition of phytase to the diets increased ($P < 0.001$) the NEp, AME, energy retained as fat, energy retained as protein, and the efficiency of metabolisable energy for energy, lipid and protein retention. The efficiency of utilisation of metabolisable energy for energy retention was also increased ($P < 0.05$) with β-glucanase supplementation.

IV. CONCLUSIONS

The test enzymes are suitable for use in sorghum-based diets and can be increased the enzyme activities in jejunal and pancreas. Their effects on energy use warrant this recommendation.

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Table 1 - Effect of diets fed on pancreatic protein concentration (mg/g tissue) and enzyme activities (µmol/mg protein/min) at 10 and 24 days of age.[1]

Phytase levels	Xylanase levels	β-glucanase levels	Day 10					Day 24				
			Protein	ChymoT[2]	Trypsin	GPA[3]	Lipase	Protein	ChymoT[2]	Trypsin	GPA[3]	Lipase
None	None	None	94.6	7.40^{ab}	4.94	1.30	6.13	80.2^{ab}	4.00^{a}	5.79^{ab}	0.79^{ab}	9.14
	Optimum	None	76.3	8.31^{a}	4.75	1.33	6.77	71.0^{c}	3.78^{ab}	5.47^{ab}	0.72^{ab}	8.25
	None	Optimum	75.2	8.56^{a}	5.39	1.42	6.92	79.8^{ab}	3.12^{b}	4.74^{b}	0.66^{b}	7.63
	Optimum	Optimum	89.5	6.94^{b}	5.08	1.23	6.46	78.4^{bc}	3.62^{ab}	5.91^{ab}	0.73^{ab}	7.01
Optimum	None	None	104.5	7.42^{ab}	4.85	1.25	4.67	71.8^{c}	4.62^{a}	6.27^{a}	0.82^{ab}	9.15
	Optimum	None	97.8	6.90^{b}	4.27	1.24	4.99	69.0^{c}	4.37^{a}	5.86^{ab}	0.85^{ab}	7.50
	None	Optimum	111.7	6.82^{b}	4.41	1.26	5.55	90.5^{a}	3.13^{b}	4.70^{b}	0.75^{ab}	6.73
	Optimum	Optimum	106.1	7.76^{ab}	4.70	1.26	5.17	82.8^{ab}	3.81^{ab}	5.49^{ab}	0.75^{ab}	6.50
Superdosing	None	None	123.2	7.65^{ab}	5.31	1.69	3.94	78.2^{bc}	3.01^{b}	5.27^{ab}	0.76^{ab}	7.23
	Optimum	None	135.4	7.29^{ab}	5.26	1.65	4.26	80.2^{ab}	3.79^{ab}	5.40^{ab}	0.78^{ab}	7.36
	None	Optimum	135.8	7.29^{ab}	5.23	1.67	3.65	74.9^{bc}	4.12^{a}	6.11^{a}	0.91^{a}	7.59
	Optimum	Optimum	125.4	7.21^{ab}	4.91	1.65	4.19	67.3^{c}	3.52^{ab}	6.13^{a}	0.89^{a}	7.82
SEM			*3.75*	*0.12*	*0.09*	*0.03*	*0.17*	*1.76*	*0.11*	*0.12*	*0.01*	*0.26*
Main effects:												
None			83.9^{c}	7.80	5.04^{ab}	1.32^{b}	6.57^{a}	77.4	3.62	5.48	0.73^{b}	8.01
Optimum			105.0^{b}	7.23	4.56^{b}	1.26^{b}	5.10^{b}	78.6	3.98	5.58	0.78^{ab}	7.47
Superdosing			129.9^{a}	7.36	5.18^{a}	1.67^{a}	4.01^{c}	75.2	3.61	5.73	0.83^{a}	7.50
	None		107.5	7.52	5.02	1.43	5.14	79.2	3.67	5.48	0.78	7.91
	Optimum		105.1	7.40	4.83	1.40	5.31	74.8	3.81	5.71	0.79	7.41
		None	105.3	7.50	4.90	1.41	5.13	75.1	3.93	5.68	0.79	8.10
		Optimum	107.3	7.43	4.95	1.41	5.32	79.0	3.56	5.51	0.78	7.21
Source of variation												
Phytase			*0.001*	*0.12*	*0.02*	*0.001*	*0.001*	*0.72*	*0.25*	*0.66*	*0.004*	*0.65*
Xylanase			*0.71*	*0.60*	*0.30*	*0.17*	*0.50*	*0.21*	*0.47*	*0.31*	*0.85*	*0.35*
β-glucanase			*0.75*	*0.78*	*0.75*	*0.92*	*0.43*	*0.27*	*0.07*	*0.46*	*0.80*	*0.10*
Phytase × Xylanase			*0.90*	*0.59*	*0.97*	*0.53*	*0.72*	*0.95*	*0.97*	*0.82*	*0.96*	*0.66*
Phytase × β-glucanase			*0.78*	*0.82*	*0.40*	*0.92*	*0.49*	*0.02*	*0.02*	*0.01*	*0.001*	*0.22*
Xylanase × β-glucanase			*0.77*	*0.58*	*0.68*	*0.29*	*0.28*	*0.75*	*0.84*	*0.06*	*0.55*	*0.58*
Phytase × Xylanase × β-glucanase			*0.21*	*0.003*	*0.41*	*0.12*	*0.53*	*0.57*	*0.05*	*0.31*	*0.24*	*0.86*

[a,b,c]Mean values with different superscripts within the columns are different ($p < 0.05$). [1]Values are means of 6 replicates (9 birds each cage). [2]ChymoT; Chymotrypsin, [3]GPA; General proteolytic activity. SEM = Standard error of means.

Table 2 - AME[1], AMEI[2] & utilisation of energy by broiler chickens on sorghum-based diets supplemented phytase, xylanase and β-glucanase at 24d[3].

Phytase levels	Xylanase levels	β-glucanase levels	NE_P[4]	AME[1]	HP[5]	MEI[2]	RE_f[6]	RE_P[7]	K_{RE}[8]	K_{Ref}[9]	K_{Rep}[10]
None	None	None	693.3	14.1^{b}	1273.4	1966.7	315.3^{b}	514.8^{ab}	0.35	0.16^{b}	0.26
	Optimum	None	705.9	14.1^{b}	1218.3	1924.1	348.3^{b}	515.5^{ab}	0.37	0.18^{ab}	0.27
	None	Optimum	672.2	14.1^{b}	1288.9	1961.1	362.0^{ab}	481.3^{b}	0.34	0.18^{ab}	0.25
	Optimum	Optimum	742.0	14.1^{b}	1211.3	1953.3	368.3^{ab}	500.1^{ab}	0.38	0.19^{ab}	0.26
Optimum	None	None	739.7	14.7^{ab}	1246.7	1986.4	310.2^{b}	544.4^{a}	0.37	0.16^{b}	0.27
	Optimum	None	694.4	14.7^{ab}	1322.3	2016.7	349.2^{b}	509.4^{ab}	0.35	0.17^{b}	0.25
	None	Optimum	726.2	14.6^{ab}	1217.3	1943.5	371.1^{ab}	514.5^{ab}	0.37	0.19^{ab}	0.26
	Optimum	Optimum	731.5	14.5^{ab}	1246.3	1977.8	322.5^{b}	549.2^{a}	0.37	0.16^{b}	0.28
Superdose	None	None	790.3	14.9^{a}	1039.9	1830.1	409.6^{a}	538.0^{a}	0.43	0.22^{a}	0.30
	Optimum	None	836.8	14.8^{a}	974.7	1811.5	433.8^{a}	528.2^{ab}	0.46	0.25^{a}	0.29
	None	Optimum	849.1	14.9^{a}	937.0	1786.0	431.6^{a}	533.8^{a}	0.48	0.24^{a}	0.30
	Optimum	Optimum	859.4	15.0^{a}	925.6	1784.9	414.3^{a}	540.9^{a}	0.48	0.23^{a}	0.30
SEM			*9.7*	*0.04*	*20.0*	*14.1*	*6.55*	*3.57*	*0.01*	*0.005*	*0.003*
Main effects											
None			703.3^{b}	14.1^{c}	1248.0^{a}	1951.3^{a}	348.5^{b}	502.9^{b}	0.36^{b}	0.18^{b}	0.26^{b}
Optimum			722.9^{b}	14.6^{b}	1258.2^{a}	1981.1^{a}	338.3^{b}	529.4^{a}	0.37^{b}	0.17^{b}	0.27^{b}
Superdose			833.9^{a}	14.9^{a}	969.3^{b}	1803.1^{b}	422.3^{a}	535.2^{a}	0.46^{a}	0.24^{a}	0.30^{a}
	None		745.1	14.5	1167.2	1912.3	366.6	521.1	0.39	0.19	0.27
	Optimum		761.6	14.5	1149.7	1911.4	372.7	523.9	0.40	0.20	0.28
		None	743.4	14.5	1179.2	1922.6	361.1	525.0	0.39^{a}	0.19	0.27
		Optimum	763.4	14.5	1137.7	1901.1	378.3	520.0	0.40^{a}	0.20	0.27
Source of variance											
Phytase			*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*
Xylanase			*0.23*	*0.78*	*0.48*	*0.97*	*0.51*	*0.64*	*0.21*	*0.37*	*0.68*
β-glucanase			*0.15*	*0.75*	*0.10*	*0.35*	*0.07*	*0.40*	*0.05*	*0.09*	*0.92*
Phytase × Xylanase			*0.17*	*0.29*	*0.13*	*0.56*	*0.55*	*0.71*	*0.09*	*0.38*	*0.60*
Phytase × β-glucanase			*0.56*	*0.001*	*0.39*	*0.58*	*0.37*	*0.08*	*0.33*	*0.46*	*0.12*
Xylanase × β-glucanase			*0.39*	*0.98*	*0.92*	*0.68*	*0.01*	*0.01*	*0.65*	*0.004*	*0.12*
Phytase × Xylanase × β-glucanase			*0.31*	*0.03*	*0.69*	*0.96*	*0.38*	*0.13*	*0.37*	*0.54*	*0.36*

[a,b,c]Mean values with different superscripts within the columns are different ($p < 0.05$). [1]Apparen metabolisable energy. [2]Metabolisable energy intake. [3]Values are means of 6 replicates (9 birds each). [4]Net energy production.. [5]Heat production.. [6]Energy retained as fat. [7]Energy retained as protein. [8]Efficiency of metabolisable energy use for energy retention. [9]Efficiency of metabolisable energy use for lipid retention. [10]Efficiency of metabolisable energy use for protein retention. SEM = Standard error of mean.

ENDOGENOUS ENZYME ACTIVITIES AND ENERGY UTILISATION OF BROILER CHICKENS FED MAIZE-BASED DIETS SUPPLEMENTED WITH PHYTASE AND CARBOHYDRASES.

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Summary

This study was aimed at assessing endogenous enzyme activities and utilisation of metabolisable energy by broiler chickens fed maize-based diets supplemented with phytase and carbohydrases. Birds were raised in cages in climate-controlled rooms. The jejunum and pancreas were collected at 10 and 24 d for analysis of endogenous digestive enzyme activities. Birds were also sampled at hatch and 24 d and analysed for gross energy, fat and crude protein contents. The data were used to calculate heat production, net energy of production and efficiency of energy utilisation. In the current study, the tested enzymes increased the activities of some of the endogenous enzymes and energy utilisation, and can be suggested for use in maize-based diets.

I. INTRODUCTION

Maize is the main cereal grain used in poultry nutrition in many parts of the world. Maize quality has been found to be variable around the world and diets containing maize could be improved through supplementation with some microbial enzymes (Cowieson, 2005). The objective of this study was to assess the response of broiler chickens on diets containing maize and supplemented with some enzymes. The activities of endogenous enzymes and utilisation of dietary energy were assessed.

II. MATERIALS AND METHODS

A total of 648 male and female Ross 308 broiler chickens were randomly assigned, in a 3 × 2 × 2 [Three doses of phytase none, standard (100 mg/kg) and superdose (300 mg/kg)] × two doses of and of β-glucanase [none and standard (100 mg/kg)] full factorial study in a completely randomised 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 as crumble (1-10 d), grower as pellet (11-24 d) and finisher as pellet (25-35 d). The test diets contained 60, 64 and 68 % of maize in starter, grower and finisher respectively and formulated to meet the specifications recommended in the Ross 308 broiler (Aviagen, 2014). A sub-sample of 10 day-old chicks was euthanised by cervical dislocation and minced to provide baseline data on body composition (gross energy, crude protein and fat contents). At 24 days, two birds per pen were randomly selected, slaughtered by cervical dislocation and processed (chopped, minced and freeze-dried) to determine carcass energy, protein and fat. On days 10 and 24, one bird was randomly selected from each cage, electrically stunned and euthanised by cervical dislocation to obtain the whole pancreas and anterior jejunum (4-5 cm long) and used to determine endogenous enzyme activities. Another 2 birds were similarly euthanised by cervical dislocation at 24 days and processed as described for the birds collected at d 0, to determine the energy, protein and fat contents of the intact carcass. The data from d 24 were related to the

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baseline data obtained from the d 0, to calculate the heat production (HP), net energy of production (NEp) and efficiency of utilisation of metabolisable energy. The remains birds were raised to 35d to measure meat yield. A general linear model procedure was used to analyse the collected data (Minitab Inc., 2013).

III. RESULTS AND DISCUSSION

There was an interaction ($P < 0.005$) between the test enzymes and chymotrypsin activity in the pancreas at 10 d (Table 1). Pancreatic protein content was increased ($P < 0.001$) by supplemental phytase at standard and superdose level. At d 24, there was an increase ($P < 0.01$) in the activities of trypsin, general proteolytic activity and lipase due to phytase supplementation. Proteolytic activity was also improved ($P < 0.01$) through supplementation of β-glucanase. The activities of jejunal maltase, sucrase and alkaline phosphatase at 10 d were increased ($P < 0.001$) with phytase supplementation but there was no interaction between the microbial test enzymes. At 24 d, supplementation with phytase resulted in an increase ($P < 0.001$) in the activities of maltase, alkaline phosphatase and aminopeptidase.

There was an interaction ($P < 0.01$) between xylanase and β-glucanase on the utilisation of apparent metabolisable energy (AME) retained as fat and protein, and efficiency of AME used for lipid retention. There was also interaction ($P < 0.01$) between phytase and β-glucanase on the efficiency of AME used for protein retention. Supplementation of phytase improved ($P < 0.03$) the AME content, AME intake, NEp, HP, energy retained as fat and as protein. Xylanase supplementation also increased ($P < 0.01$) AME contents, and AME intake, while the NEp and AME contents were improved ($P < 0.05$) by supplementation with β-glucanase (Table 2).

IV. CONCLUSIONS

Supplementation with the test microbial enzymes increased the AME energy and NEp while interactions were observed between xylanase and β-glucanase. Dietary exogenous enzymes also increased the activities of some of the endogenous enzymes.

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Table 1 - Effect of diets containing different levels of phytase, xylanase and β-glucanase on pancreatic protein concentration (mg/g tissue) and enzyme activities (µmol/mg protein/min) at 10 and 24 days of age.[1]

			Day 10					Day 24				
Phytase levels	Xylanase levels	β-glucanase levels	Protein	Chymo T[2]	Trypsin	GPA[3]	Lipase	Protein	Chymo T[2]	Trypsin	GPA[3]	Lipase
None	None	None	69.0	7.83^{ab}	4.70	0.78	8.48	101.8	3.85	5.82	0.63	5.31
	Optimum	None	65.2	8.98^{a}	4.56	0.79	8.88	84.5	3.27	5.83	0.65	5.02
	None	Optimum	62.6	9.31^{a}	5.25	0.90	9.03	79.2	3.80	5.61	0.72	5.25
	Optimum	Optimum	70.1	7.19^{b}	4.88	0.72	7.97	93.0	2.99	5.00	0.72	5.10
Optimum	None	None	84.0	7.82^{ab}	4.55	0.72	7.30	94.5	3.19	4.90	0.70	9.10
	Optimum	None	83.8	7.16^{b}	3.84	0.70	6.92	87.4	3.56	5.52	0.71	8.99
	None	Optimum	84.6	7.06^{b}	4.01	0.72	7.31	94.3	4.42	6.01	0.81	9.72
	Optimum	Optimum	83.1	8.27^{ab}	4.38	0.72	7.85	92.0	3.37	5.03	0.79	9.69
Superdosing	None	None	81.1	8.64^{ab}	4.97	0.80	5.22	74.0	4.47	6.55	0.94	9.18
	Optimum	None	78.2	8.01^{ab}	4.90	0.77	5.41	83.2	3.96	5.96	0.88	7.88
	None	Optimum	85.0	8.05^{ab}	4.85	0.77	5.70	81.7	3.83	6.58	0.92	8.84
	Optimum	Optimum	88.8	7.87^{ab}	4.37	0.78	5.32	79.2	3.79	6.05	0.92	7.76
SEM			*1.49*	*0.17*	*0.12*	*0.01*	*0.19*	*2.62*	*0.12*	*0.12*	*0.02*	*0.26*
Main effects:												
None			66.7^{b}	8.33	4.85	0.80^{a}	8.59^{a}	92.0	3.48	5.57^{b}	0.68^{c}	5.17^{c}
Optimum			83.9^{a}	7.58	4.19	0.72^{b}	7.35^{b}	89.7	3.63	5.36^{b}	0.75^{b}	9.38^{a}
Superdosing			83.4^{a}	8.14	4.77	0.78^{ab}	5.41^{c}	79.5	4.01	6.29^{a}	0.92^{a}	8.42^{b}
	None		77.8	8.12	4.72	0.78	7.18	87.6	3.93	5.91	0.79	7.9
	Optimum		78.2	7.91	4.49	0.75	7.06	86.6	3.49	5.56	0.78	7.4
		None	76.9	8.08	4.59	0.76	7.04	87.6	3.72	5.76	0.75^{b}	7.6
		Optimum	79.0	7.96	4.62	0.77	7.20	86.6	3.70	5.71	0.81^{a}	7.7
Source of variation												
Phytase			*0.001*	*0.14*	*0.07*	*0.02*	*0.001*	*0.14*	*0.14*	*0.01*	*0.001*	*0.001*
Xylanase			*0.87*	*0.52*	*0.35*	*0.19*	*0.57*	*0.85*	*0.06*	*0.13*	*0.69*	*0.10*
β-glucanase			*0.39*	*0.71*	*0.89*	*0.73*	*0.43*	*0.85*	*0.93*	*0.82*	*0.01*	*0.63*
Phytase × Xylanase			*0.89*	*0.57*	*0.98*	*0.30*	*0.70*	*0.82*	*0.71*	*0.78*	*0.79*	*0.25*
Phytase × β-glucanase			*0.35*	*0.78*	*0.47*	*0.83*	*0.43*	*0.73*	*0.23*	*0.33*	*0.28*	*0.45*
Xylanase × β-glucanase			*0.24*	*0.62*	*0.77*	*0.34*	*0.36*	*0.46*	*0.38*	*0.12*	*0.99*	*0.80*
Phytase × Xylanase × β-glucanase			*0.56*	*0.005*	*0.41*	*0.08*	*0.06*	*0.26*	*0.22*	*0.34*	*0.66*	*0.99*

[a,b,c]Mean values with different superscripts within the columns are different ($p < 0.05$). [1]Values are means of 6 replicates (9 birds each cage). [2]ChymoT; Chymotrypsin, [3]GPA; General proteolytic activity. SEM = Standard error of means.

Table 2 - AME[1], AMEI[2] & utilisation of energy by birds on maize-based diets supplemented phytase, xylanase and β-glucanase at 24d[3].

Phytase levels	Xylanase levels	β-glucanase levels	NE_P[4]	AME[1]	HP[5]	MEI[1]	RE_f[6]	RE_P[7]	K_{RE}[8]	K_{Ref}[9]	K_{Rep}[10]
None	None	None	709.0	13.61	1228.6^{b}	1937.6	306.0^{bc}	553.7^{ab}	0.37	0.16^{ab}	0.29^{a}
	Optimum	None	749.2	13.87	1325.9^{ab}	2075.1	339.1^{abc}	554.4^{ab}	0.35	0.16^{ab}	0.27^{ab}
	None	Optimum	751.7	14.08	1392.0^{ab}	2143.7	352.7^{abc}	520.2^{b}	0.35	0.16^{ab}	0.24^{b}
	Optimum	Optimum	721.5	14.13	1439.5^{ab}	2161.0	359.0^{abc}	539.0^{ab}	0.34	0.17^{ab}	0.25^{ab}
Optimum	None	None	708.2	14.20	1432.3^{ab}	2140.4	294.4^{c}	576.2^{a}	0.33	0.14^{b}	0.27^{ab}
	Optimum	None	768.2	14.27	1402.1^{ab}	2170.4	333.4^{abc}	541.2^{ab}	0.35	0.15^{ab}	0.25^{ab}
	None	Optimum	789.5	14.40	1236.6^{b}	2026.0	355.4^{abc}	546.3^{ab}	0.39	0.18^{a}	0.27^{ab}
	Optimum	Optimum	804.1	14.56	1364.7^{ab}	2168.8	306.7^{bc}	581.1^{a}	0.37	0.14^{ab}	0.27^{ab}
Superdose	None	None	870.7	15.05	1485.6^{ab}	2356.3	371.8^{abc}	556.3^{ab}	0.37	0.16^{ab}	0.24^{b}
	Optimum	None	876.6	15.17	1544.7^{ab}	2421.3	409.3^{a}	546.5^{ab}	0.36	0.17^{ab}	0.23^{b}
	None	Optimum	900.9	15.27	1520.2^{ab}	2421.1	393.8^{a}	552.1^{ab}	0.37	0.16^{ab}	0.23^{b}
	Optimum	Optimum	943.6	15.72	1655.1^{a}	2598.7	376.5^{ab}	559.1^{ab}	0.37	0.15^{ab}	0.22^{b}
SEM			*12.7*	*0.08*	*24.0*	*27.6*	*6.0*	*3.3*	*0.01*	*0.002*	*0.004*
Main effects											
None			732.8^{b}	13.92^{c}	1346.5^{b}	2079.3^{b}	339.2^{b}	541.8^{b}	0.35	0.16	0.26^{a}
Optimum			767.5^{b}	14.36^{b}	1358.9^{b}	2126.4^{b}	322.5^{b}	561.2^{a}	0.36	0.15	0.27^{a}
Superdose			897.9^{a}	15.30^{a}	1551.4^{a}	2449.4^{a}	387.8^{a}	553.5^{ab}	0.37	0.16	0.23^{b}
	None		788.3	14.44^{b}	1382.6	2170.9^{b}	345.7	550.8	0.36	0.16	0.26
	Optimum		810.5	14.62^{a}	1455.3	2265.9^{a}	354.0	553.6	0.36	0.16	0.25
		None	780.3^{a}	14.36^{b}	1403.2	2183.5	342.3	554.7	0.36	0.16	0.26
		Optimum	818.5^{a}	14.69^{a}	1434.7	2253.2	357.4	549.6	0.37	0.16	0.25
Source of variance											
Phytase			*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.03*	*0.34*	*0.20*	*0.001*
Xylanase			*0.25*	*0.01*	*0.09*	*0.01*	*0.38*	*0.64*	*0.44*	*0.56*	*0.09*
β-glucanase			*0.05*	*0.001*	*0.46*	*0.07*	*0.12*	*0.40*	*0.32*	*0.56*	*0.09*
Phytase × Xylanase			*0.79*	*0.50*	*0.90*	*0.88*	*0.57*	*0.71*	*0.72*	*0.53*	*0.91*
Phytase × β-glucanase			*0.52*	*0.63*	*0.05*	*0.06*	*0.25*	*0.08*	*0.09*	*0.11*	*0.01*
Xylanase × β-glucanase			*0.49*	*0.57*	*0.47*	*0.64*	*0.004*	*0.01*	*0.51*	*0.004*	*0.20*
Phytase × Xylanase × β-glucanase			*0.50*	*0.42*	*0.60*	*0.34*	*0.43*	*0.13*	*0.59*	*0.14*	*0.58*

[a,b,c]Mean values with different superscripts within the columns are different ($p < 0.05$). [1]Apparen metabolisable energy. [2]Metabolisable energy intake. [3]Values are means of 6 replicates (9 birds each). [4]Net energy production. [5]Heat production. [6]Energy retained as fat. [7]Energy retained as protein. [8]Efficiency of metabolisable energy use for energy retention. [9]Efficiency of metabolisable energy use for lipid retention. [10]Efficiency of metabolisable energy use for protein retention. SEM = Standard error of means

EFFECT OF PHOSPHORYLATED TOCOPHEROL MIXTURE ON GROWTH PERFORMANCE AND MEAT QUALITY IN BROILER CHICKENS

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This study investigated the effect of a novel phosphorylated tocopherol mixture (TPM) on the growth performance and meat quality in broiler chickens reared in normal temperatures (NT) or cyclical high temperatures (CHT). Three hundred and sixty Ross 308-day old broilers were housed in groups of 5 in cages (n = 12/treatment). From day of placement until d 35, broilers were assigned to 1 of 3 vitamin E-adequate, wheat and soybean meal-based diets containing TPM at 0 (Control; 20ppm of Vit E), 10 or 20 mg/kg diet. Experimental diets were formulated by commercial nutritionists (ACE Livestock Consulting, Australia) to reflect industry norms. From 21-35 d of age, birds were exposed to either normal temperatures (NT; 22±1°C; 60% RH) or cyclical high temperature (CHT; 32±1°C; 8 h; 80-90% RH and 16 h at 22±1°C; 60% RH).

There was no interaction between CHT and TPM on growth performance. The body weight (BW) of birds assigned to the CHT treatment was lower on d 35 when compared with the birds assigned to the NT treatment. There was an effect of TPM inclusion on final (d 35) BW. Birds offered the CD + TPM 10 had an increased BW (2511 g/b) when compared with the CD group (2454 g/b; P = 0.016). There was significant effect of CHT on average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR). Birds assigned to the CHT treatment had decreased ADFI (163 vs 167 g; 21-35 d; P = 0.015), decreased ADG (105 vs 110 g; 21-35 d; P < 0.001 and overall; 68.6 vs 70.1 g; P = 0.021) and increased FCR (1.55 vs 1.51; 21-35 d; P = 0.002 and overall; 1.47 vs 1.45; P = 0.001) when compared with the CD group. There was an effect of TPM inclusion on growth performance. Birds offered the CD + TPM 10 diet had increased ADG (110 vs 107 g; 21-35 d; P = 0.027 and overall; 71 vs 69 g; P = 0.010) when compared with the CD group, and decreased FCR (1.33 vs 1.36; 0-21 d; P = 0.024) when compared with the TPM 20 diet. The growth performance of birds offered the CD + TPM 20 was not different when compared with the CD group. Meat quality was determined on 1 bird per cage (d 35). Birds assigned to the CHT treatment had increased core breast muscle temperature at 24 h (11.1 vs 10.0 ^{0}C; P < 0.001), decreased pH at 24 h (5.98 vs 6.07; P = 0.003) and decreased drip loss after 5 days (4.0 vs 5.3%; P < 0.001) when compared with birds assigned to the NT treatment. The shear force value of cooked breast meat from the birds assigned to the CHT treatment was higher (tougher; 3545 vs 2827 g; P < 0.001) than birds assigned to the NT treatment. There was no effect of CHT or diet on breast colour at d 0 or d 7. There was a non-significant decrease (P = 0.057) in drip loss of birds assigned to the CD + TPM 10 diet when compared with the CD group.

In conclusion, CHT suppressed growth performance and reduced tenderness value of breast meat in broiler chickens. Broilers offered a diet containing TPM at 10 ppm improved growth performance over the whole growing periods in both NT and CHT regimes and tended to reduce drip loss following storage of breast muscle.

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HISTORICAL FLAWS OF METABOLISABLE ENERGY BIOASSAYS FOR POULTRY – A MINI REVIEW

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Dietary energy available to animals is key to formulating feed as it is required for all aspects of animal life. In poultry, apparent (AME) and true (TME) metabolisable energy (ME) values have been developed for feed formulation with or without correction for nitrogen balance. Over the past 50 years, the accuracy of ME systems has been an ongoing debate and the data produced from different bioassays have been found not to be comparable (Farrell, 1999). Overall, the ingredient matrix ME values used in feed formulation are not consistent and to some extent confusing (Mateos, et al., 2018; Pesti and Edwards Jr, 1983). Therefore, a thorough review of the bioassay methods and the values published in the literature becomes necessary.

A recent review discussed the reasons for the discrepancies among AME values from different sources, focusing on factors such as physico-chemical characters of diets and ingredients, heat processing, feed form and particle size, dietary fibre and fat content, antinutritional factors, and supplementation with additives (Mateos et al., 2018). However, aspects related to the experimental design, data analysis and even human errors present in the literature have not been evaluated. Thus, we examined the ME data produced over the past century to elucidate pros and cons of different ME bioassays, the practice of correcting ME to zero nitrogen retention, and other methodological errors such as equations used in the calculations.

It is concluded that accurate ME values have to be generated by *in vitro* bioassays to ensure accurate utilisations of other approaches, namely prediction equations, table values, *in vitro* assays and NIR analysis, to estimate ME of feedstuffs for feed mills. We suggest that the multiple linear regression method or basal diet substitution method, with several levels of the test ingredient, be used to generate ingredient matrix ME values. Until a systematic reevaluation of the ME values of feed ingredients used for poultry, feed formulation will continue to rely on data with large deviations, leading to less than satisfactory practical outcomes in precision feeding.

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INFLUENCE OF INCLUSION LEVEL OF BARLEY IN WHEAT-BASED DIETS AND SUPPLEMENTATION OF CARBOHYDRASE ON GROWTH PERFORMANCE AND NUTRIENT UTILISATION IN BROILER STARTERS

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The influence of inclusion level of barley in wheat-based diets and supplementation of a multi-component non-starch polysaccharide (NSP) degrading enzyme (Ronozyme® Multigrain) on growth performance and nutrient utilisation in broiler starter (d 0 to d 21) was evaluated. The activities of endo-1,4-β-glucanase, endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase in enzyme were 800 BGU/g, 700 BGU/g and 2700 XU/g, respectively. Normal-starch (non-waxy) hulled barley (NSH) was evaluated with five inclusion levels (0, 141, 283, 424 and 565 g/kg, with corresponding wheat inclusion levels of 629, 472, 314, 157, 0 g/kg; as fed basis) and two levels of enzyme supplementation (0 and 150 mg/kg of feed). A 5 × 2 factorial arrangement of 10 treatments was used and all diets were formulated to be equivalent in respect of metabolisable energy and digestible amino acid contents (11.9 MJ/kg AMEn, 12.2 g/kg digestible lysine, 9.6 g/kg Ca, 4.8 g/kg npp). A total of 400, day-old male broiler chicks was allocated to 50 cages (5 cages/treatment; 8 birds/cage). The inclusion level of barley had a significant effect on weight gain (WG; $P < 0.01$), feed intake (FI; $P < 0.001$) and feed conversion ratio (FCR; $P < 0.001$), whereas enzyme effect was significant only for WG ($P < 0.05$) and FCR ($P < 0.001$). Regardless of enzyme supplementation, WG showed a gradual increase up to 283 g/kg inclusion of NSH and reduced with NSH inclusion above this point. Inclusion of NSH at 424 and 565 g/kg significantly ($P < 0.05$) suppressed FI. Increasing levels of NSH, however, resulted in lower ($P < 0.05$) FCR. Enzyme addition increased WG and improved FCR for each level of barley inclusion. Inclusion level of barley and enzyme supplementation had significant effects on coefficients of apparent ileal digestibility (CAID) of starch, nitrogen and fat ($P < 0.001$ to 0.05). Birds fed diets with highest inclusion of wheat or barley showed the lowest and highest CAID, respectively, for all three parameters measured. Digestibility of all parameters was improved by enzyme inclusion ($P < 0.05$) regardless of barley inclusion level. Three major modes of action of NSP-degrading enzymes have been recognised in previous studies; (i) reduction of digesta viscosity (Almirall et al., 1995), (ii) release of encapsulated nutrients via cell wall degradation (Bedford, 1996) and, (iii) improved gut microflora through supply of prebiotic oligosaccharides (González-Ortiz et al., 2017). Inclusion level of barley resulted in significant effects on gizzard pH ($P < 0.05$). Gizzard pH decreased beyond 283 g NSH/kg in the diet corresponding to decreased FI. The current data suggest that, although increasing the inclusion level of NSH up to complete replacement of wheat improved FCR, the best inclusion level of NSH for WG was 283 g/kg of diet. Increasing NSH inclusion improved nutrient utilisation possibly through better gizzard function through better function of gizzard. FCR and nutrient digestibility can benefit from carbohydrase supplementation of wheat- and barley-based diets, irrespective of their inclusion level.

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PHYTIC ACID REDUCTION IN CANOLA AND CAMELINA MEALS BY FUNGAL FERMENTATION FOR POTENTIAL BROILER FEEDING

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Canola and camelina meals have been identified as alternative plant protein sources in place of soybean meal for animal feeding. The use of these protein sources at high inclusion levels is limited due to the presence of antinutritional factors such as phytic acid, glucosinolates, erucic acid, sinapines and tannins which have negative effects on production performance of broiler chickens (Thacker and Widyaratne, 2012). Solid state fermentation is a preferred method for enriching agroindustrial residues since it offers several economical and practical benefits (Vig and Walia, 2001). Reports on the chemical composition of solid state fermented canola and camelina meals, and effects of fermented canola and camelina meals on nutrient digestibility and other performance parameters in broiler chickens, are limited. In a preliminary study, solid state fermentation using *Saccharomyces cerevisiae* was conducted with the aim of reducing phytic acid contents in canola and camelina meals. The effect of length of incubation on phytic acid content was investigated.

Saccharomyces cerevisiae (ATCC 38555) was cultured on yeast malt extract (YME) agar plates. Canola and camelina meals were obtained from a commercial feed mill. Solid state fermentation was conducted in 250 ml Erlenmeyer flasks. Before autoclaving, moisture content was adjusted to 50% with RO water. Flasks with substrates were autoclaved at 121^{o}C for 15 mins, cooled to room temperature and inoculated with spore suspension containing 10^{7} spores/ml. Flasks were incubated at 25^{o}C, and harvested after 3 and 7 days of fermentation. Fermented samples were oven dried at 60^{o}C for 2 days, milled and stored for chemical analysis. pH of the experimental samples initially reduced between day 0 to 3 and slightly increased between day 3 to 7. Phytic acid concentration was analyzed according to the modified colorimetric method of Gao et al. (2007). The fermentation process reduced phytic acid concentration by 25.7% (from 37.4 to 27.8 mg PA/g) in canola meal and 33.7% (from 36.8 to 24.4 mg PA/g) in camelina meal after 7 days of incubation. Further research is ongoing to determine the chemical composition and *in vitro* enzyme activities of canola meal, lupin flour and camelina meal fermented with *Aspergillus sojae* and *Aspergillus ficuum*. Nutrient digestibility and performance studies will also be conducted to determine the suitability of these fermented products for broiler chickens.

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POTENTIAL TO PRODUCE POULTRY FEED FROM FOOD WASTES

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Summary

The annual food waste in Australia is estimated at 7.5 million tonnes with the majority disposed of in landfills. This not only causes significant economic loss but also has a negative environmental impact. This study aimed at investigating the possibility of recycling food waste into feed for poultry. Nutrient contents of various waste streams were evaluated. The food waste collected from the services club and restaurant contained the highest levels of crude protein and crude fat (404 g/kg and 278 g/kg, respectively) while crude protein and crude fat content of food waste originating from bakeries and fruit-vegetable growers were 100 g/kg and 43 g/kg, respectively. The findings indicated that the blended material had an excessive Na concentration (6.5 g/kg), low Ca content (1.0 g/kg) and high/low concentrations of other nutrients relative to broiler grower feed requirements. Further studies are required to investigate the blending of waste streams with other nutrient sources to meet nutrient requirements. Microbial contamination, free fatty acids, oxidation and nutrient digestibility need to be considered before valuable recycled food wastes can be used as a feed source for poultry.

I. INTRODUCTION

Food waste refers to "the discarding or alternative (non-food) use of food that was fit for human consumption - by choice or after the food has been left to spoil or expire as a result of negligence" (FAO, 2015). It is estimated that the global economic loss caused by food waste is US$ 1 trillion annually. The wasted amount of cereals, root crops, fruits and vegetables, fish, oilseeds, meat and dairy products in the food industry has been estimated to be between 20 to 50% each year (FAO, 2015). In Australia, the annual wasted food has been estimated to be 7.5 million tonnes equivalent to a loss of US$8 billion in 2014 (Torrisi, 2014). The Australian national food waste report in 2016 showed that the quantity of food waste sent to landfills was greater than any other disposal system in 2014-2015, representing 58% of total food waste generation (Pickin and Randell, 2016). When food is wasted, the costs related to the production, packaging, delivery, selling and preparation of that food is also lost. Furthermore, food waste ending up in landfills can cause serious environmental impacts (Kawashima, 2004). Salemdeeb et al. (2017) indicated that recycling of food waste as wet or a dry pig feed resulted in better environmental and public health outcomes than other food waste disposal methods such as composting and anaerobic digestion. As the world's population is predicted to increase to 9.8 billion by 2050 (UN, 2017) food waste will increase proportionally suggesting the opportunity to further examine systems for recycling. In some Asian countries like Japan and South Korea, where the demands for animal feed are high, the recycling of food waste as animal feed is popular and is supported by local laws (Gen, 2006; Kim et al., 2011). Among the food waste sources, food dregs like bean curd or shochu dregs are the most common material being used to produce animal feed in Japan, which is followed by misdated food from supermarkets, bread, noodles and similar products (Sugiura et al., 2009). In Australia, although the food waste based feed is a new concept, the use of animal origin protein sources as poultry feed is not restricted

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by government legislation (NSWDPI, 2017) and thus is a potential area to develop. The main objective of the current study was to investigate nutrient levels in Australian food waste streams for use as poultry feed.

II. METHODS

Nine food related businesses, educational institutions and hospitals in the Hawkesbury district, NSW, Australia were requested to participate in the study with food waste collected over a 2 hour period (either 10am to 12 noon or 12 to 2pm) on agreed days. Most of these collections were carried out between March and June 2017. Samples were collected from 9 commercial operations such as, cafes, restaurants and bakeries. Buckets were provided with instructions to fill with kitchen scraps, serving waste and plate scraps. At the end of the 2 hour period, the buckets were collected and transported to the Hawkesbury campus of Western Sydney University in a refrigerated container for processing and producing food waste pellets. Only one sample was collected from each commercial operation. Hence, the consistency of the sample collected from each of the commercial operations was not checked. Collected food waste was screened to remove foreign objects with initial weights of all samples recorded. Food waste suppliers were de-identified and given general classifications, e.g. restaurant, hospital and supermarket. Food waste was heat treated on trays to 90^{o}C with steam for 10 min, then dehydrated at 70°C for 30 hours in a large commercial dehydrator cabinet and ground to pass through a 3 mm screen. The powdered samples were analysed for moisture, crude protein (CP), crude fat (CF), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and phosphorus (P) and blended to produce extruded pellets. Pellets were produced by blending food waste samples with water (56:44) to produce a dough that was then passed through a Bottene pasta extruder to create 3mm x 6mm pellets. Pellets were dehydrated for 24 hours at 70°C. The nutrient content of feed pellet samples including dry matter (DM), moisture, CP, CF, Ca, Mg, Na, K and P and ash content were analysed. This procedure is further described in Australian patent 2018100266 (Boyle, 2018).

III. RESULTS

The nutrient content of food waste samples and extruded pellets are presented in Table 1. The dry matter yield of different food waste sources ranged from 5% (fruit and vegetable waste) to 65% (bakery waste). The CP and CF contents were variable between the waste samples. Food waste collected from the services club and restaurant contained the highest levels of CP and CF (404 g/kg and 278 g/kg, respectively) while CP and CF contents of food waste originating from the bakeries and fruit-vegetable growers were 100 g/kg and 43 g/kg, respectively. Blended pellets were shown to have 187 g/kg CP and 151 g/kg CF (Table 1). The findings also indicated that final blended pellets had a high Na concentration (6.5 g/kg) and a low Ca content (1.0 g/kg) relative to nutrient requirements for meat chickens (Table 1).

IV. DISCUSSION

Feed is the most significant cost of poultry production. Much of this is attributed to the protein/amino acid content (DeGroot, 2014). The results of the current study indicate that food waste may be able to supply a significant amount or all of the protein required for poultry feed. Further testing is required to assess nutrient consistency over time from each source. The protein content of the food waste sample collected from the services club was 404 g/kg being similar to that of other high protein ingredients used in the feed industry. Furthermore, Kwak and Kang (2006) pointed out that significant feed cost reduction (32.9%) can be obtained when

50% of food waste mixture, containing restaurant food waste, bakery by-product and broiler litter, was incorporated into the normal diet for finishing pigs compared to the control ($0.57 vs $0.85/kg weight gain).

Table 1 - Nutrient content of food waste samples and blended pellets (g/kg unless noted).

Sample type[1]	WFW [2] (g)	PFW[3] (%)	CP[4]	CF	DM	Ca	Mg	Na	K	P
1	5992	38	122	122	310	0.5	0.5	4.6	4.1	2.1
2	8950	14	362	278	150	0.6	0.5	10.9	8.9	3.0
3	4744	10	118	35	70	1.0	0.8	1.1	13.4	1.7
4	3175	3	180	178	260	0.6	0.5	6.5	6.6	2.7
5	8783	6	188	43	50	0.9	1.4	2.6	17.6	3.1
6	8874	3	279	226	250	1.6	0.5	11.4	6.4	2.7
7	945	16	404	165	250	0.3	0.6	3.2	5.1	2.7
8	4001	2	225	219	280	1.2	0.5	3.0	4.4	2.2
9	7947	8	100	64	650	0.4	0.3	4.7	2.7	1.1
10	-	-	187	151	949	1.0	0.5	6.5	5.6	2.5
11	-	-	215	-	-	8.7	0.5 - 5.0	1.6 - 2.3	4.0 - 9.0	4.4

[1]Sample type: 1-Café 1, 2-restaurant, 3- Fruit and vegetable market, 4- Café 2 and 3, 5-fruit and vegetable grower, 6-retirement home, 7-services club, 8-educational institute, 9-bakery, 10-pellet, 11-nutrient requirement for meat chicken grower feed based on nutrition specifications for Ross 308 broiler (Aviagen, 2014).
[2]WFW: Amount of food waste collected.
[3]PFW: Percentages of food waste sources used for feed (pellet) formulation.
[4]CP, CF and mineral content of the samples were calculated as on DM basis.

More work needs to be done to determine digestible amino acid content of food waste and the cost of food waste based feed in Australia. In addition, as the quantity of food waste might change between the regions and seasons of the year, further studies on availability of food waste in defined areas is necessary to determine the reliability and sustainability of waste streams as a continuing material source. The fat and Na content of blended food-waste was higher and calcium was lower than broiler grower requirements. Free fatty acids, oxidation and metabolisable energy need to be considered. Variability is an issue that needs consideration as indicated by various research groups (Myer et al., 1999; Yang et al., 1999; Kawashima, 2004; Sugiura et al., 2009). However, solutions to solve this problem have been rarely mentioned in those studies. Processing method might play an important role in maintaining nutritional composition of the food waste. Sayeki et al. (2001) found that chemical composition of food waste dehydrated by fry cooking ranged from 1.2 - 1.8% only. In addition, it has been suggested that, when garbage food waste is collected from numerous origins and blended, nutrient variability will decrease (Kawashima, 2004). It is proposed that the issues associated with nutrient variation in the food waste based feed can be addressed through measurement and blending of various waste streams and incorporation of other ingredients such as amino acids, limestone, phosphate, vitamins, trace minerals, antioxidants and antifungal agents. There is a good agreement between nutrient values obtained in the current study and those reported by Kwak and Kang (2006) who reported dry matter, CP and CF contents of restaurant food waste on DM basis to be 191 g/kg, 220 g/kg and 126 g/kg, respectively and bakery by-product were 890 g/kg, 95 g/kg and 20 g/kg, respectively. Other reports have indicated fat and protein contents of restaurant waste to be higher (17.3% and 25%, respectively) and metabolisable energy level to be lower (2344 Kcal ME/kg) compared to the nutrient requirement for meat chickens (Chae et al., 2000; Aviagen, 2014). In contrast, bakery waste is rich in energy but has low protein and mineral levels (Ensminger et al., 1990). Importantly, Chen et al. (2014) pointed out that the general hygiene and chemical safety of food waste feed produced in China were

good with low risks of pathogen and organic contamination but the product safety related to salt concentration was rather low.

The variable CP and high sodium contents observed in the food waste based feed in this study can be solved by applying processing methods that blends various waste streams to produce an optimum final product to be used on a commercial scale. To achieve this optimum blend, further data collection and analysis are required.

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TOWARDS PRACTICAL METHODS FOR ASSESSING ILT VACCINE TAKE

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Infectious laryngotracheitis (ILT) is an ongoing problem in meat chickens in important production areas of Australia. In response to outbreaks, live vaccines are typically administered at 7-14 days of age in drinking water via nipple drinkers which may not provide optimal contact with susceptible tissues. The efficacy of vaccination is not routinely assessed. As part of a series of experiments investigating the kinetics of ILT virus (ILTV) in meat chickens after water vaccination via nipple drinkers, we investigated different sampling methods for assessing ILTV genome copy number (GC) by qPCR to assess flock status.

The study included 8 flocks (sheds) of meat chickens in Sydney and surrounding areas. The flocks were vaccinated with the Serva strain of ILT vaccine in drinking water at 7-14 days of age (doa) according to the normal protocol for the farm. Individual tracheal swabs from 40-70 birds, and 2-6 dust samples from settle plates were collected at 4, 7-8, 12-13 and 25-26 days post vaccination (dpv). In some flocks, a subset of 10 birds from which the tracheal swabs (TS) were collected also had cloacal (ClS), conjunctival (CoS) and/or choanal cleft (CCS) swabs collected and in one flock, a faecal sample was collected. ILTV GC has been reported at high levels in faeces and dust under experimental conditions (Roy et al., 2015). DNA was extracted from the various samples and subjected to the ILTV specific qPCR to determine GC described by Roy et al. (2015).

ILTV GC was readily detectable in all sample types but in faeces this was sensitive to the method of DNA extraction. There was wide variation in the proportions of TS positive in the post vaccination period with poor initial "take" of vaccine in 3 of the 8 flocks (Groves P.G. *et al.* these proceedings). This poor take was reflected in low ILTV GC in dust samples at 7-8 dpv suggesting that this could be a useful population level measure of vaccine take (Ahaduzzaman, M. et al. these proceedings). With regard to individual bird measures, CCS provided very similar results to TS both in terms of numbers of birds positive (80% concordance) and in terms of viral load (Linear regression R^2 0.58, $P<0.001$). Virus took longer to appear in CoS but were detectable for longer. ClS and faeces had lower sensitivity of detection of infection, but this was confounded by extraction method.

The results of this study indicate that CCS are a more practical, and less invasive method of detecting ILTV in individual chickens than TS. CoS are also less invasive than TS but risk eye injury and have reduced sensitivity at 4 dpv. Faeces are more difficult to collect and they and ClS and have drawbacks associated with extraction method. At a population level, dust samples offer promise as a marker of vaccination success, being able to differentiate in this study between farms with poor and adequate vaccination takes. Being able to assess this in a single, stable, easily collected and transported sample at 7-8 dpv makes assessment of vaccination take a practical consideration in commercial meat chicken flocks. Testing the utility of these alternative measurements under a wider range of conditions is warranted.

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GROWTH AND TITRATION OF HAEMORRHAGIC ENTERITIS VIRUS OF TURKEYS IN CHICK EMBRYOS

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Haemorrhagic enteritis virus (HEV) is an immunosuppressive adenovirus that causes haemorrhagic enteritis in young turkey poults with increased incidence of secondary bacterial infections, such as colibacillosis (Pierson and Fitzgerald, 2013). Worldwide live vaccines propagated in cell culture or turkeys (crude spleen homogenates) are used to prevent the disease. In Australia, there is currently no licensed HEV vaccine due to inability to import the only cell line known to support HEV propagation (RP19) and to the unavailability of specific pathogen free (SPF) turkeys. The use of a vaccine to confer appropriate flock immunity could decrease the incidence of colibacillosis associated with HEV infection in Australia (Gerber et al., 2017). The main goal of this study was to investigate the feasibility of propagating and titrating HEV in SPF chicken embryos.

A total of 308 SPF viable eggs was used. In experiment 1 the susceptibility of embryos to infection was studied. A total of 127 eggs at 10 days of embryonic age was inoculated with saline (sham-inoculated), non-heat-treated (live) or heat-inactivated (dead) HEV. Allantoic fluid was retrieved at 0, 1, 3, 5 and 7 days post-investigation (dpi) and tested for HEV DNA by a quantitative PCR (qPCR) (Shah et al., 2013). In experiment 2, five HEV stocks were titrated using 181 fertile eggs inoculated with a 10-fold dilution of HEV virus stocks with 5 replicates per dilution/virus.

Inoculation with HEV did not cause visible growth impairment nor lesions in the chicken embryos and chorioallantoic membrane. Overall, there was no difference in the post-inoculation mortality rates among groups sham-inoculated (6/30, 20.0%) or inoculated with live (34/252, 13.4%) or dead (3/26, 6.9%) HEV ($P = 0.58$). The amount of virus recovered in allantoic fluid at 7 dpi of eggs inoculated with live HEV was similar to the inoculated dose, indicating that HEV propagation in chicken embryos is not efficient. Viral DNA was detected in all samples from eggs inoculated with live HEV while no HEV DNA was detected after 3 dpi in eggs inoculated with dead virus. This indicates that it is possible to differentiate between live and dead HEV from virus stocks using a combination of egg inoculation followed by qPCR of allantoic fluid at 7 dpi. Preliminary data from experiment 2 shows that this method is suitable for titration of HEV infectivity in stocks.

In conclusion, HEV infects chicken embryos with low levels of replication without causing gross pathology. The total virus recovery at 7 dpi is similar to the inoculated dose, making the process unsuitable for vaccine production. However, since DNA from heat-treated virus is cleared from chicken embryos within 3 dpi, this method is suitable for titrating HEV infectivity when combined with qPCR detection of viral nucleic acids as the endpoint measurement.

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EFFECTS OF YEAST AND ITS DERIVATIVES ON MEAT YIELD AND HAEMATOLOGICAL INDICES OF BROILER CHICKENS CHALLENGED WITH *SALMONELLA* LIPOPOLYSACHARIDE

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Summary

The present experiment was designed to determine the effect/s of yeast and its derivatives on meat yield and haematological indices of broiler chickens challenged with *Salmonella* lipopolysaccharide (a component of *Salmonella* bacterial cell wall). Eight diets based on maize and soybean were offered to 432 Ross 308 broiler chickens in a 35 days experiment. Each dietary group had six replicates having nine birds each. The eight treatments groups consisted of a negative control (NC) (without supplementation and not challenged); positive control (PC) (without supplementation + LPS challenged); whole yeast + LPS challenged (WYC); yeast cell wall + LPS challenged (YCWC); yeast glucan + LPS challenged (YGC); yeast manno-protein + LPS challenged (YMPC); zinc bacitracin + LPS challenged (ZNBC); Salinomycin + LPS challenged (SalC). All yeast treatments were included at 2 g/kg diet while zinc bacitracin and Salinomycin were included at 0.03 and 0.05 g/kg diet, respectively. At d24, mean corpuscular volume, mean corpuscular haemoglobin concentration, and counts of eosinophils, neutrophils, basophils, red blood cell, haemoglobin and platelets were not significantly affected ($P > 0.05$) by LPS challenge. Birds in the PC group had approximately 25 % ($P < 0.05$) increase in the white blood cell (WBC) count compared to birds in the NC group. However, supplementation with WYC, YCWC YGC, YMPC, and ZNBC, resulted to a lower ($P < 0.05$) WBC count compared to birds in the PC group. Birds in the SalC and PC groups had similar ($P > 0.05$) WBC counts. Relative to birds in the NC group, birds in the PC group had increased ($P < 0.05$) lymphocyte and monocyte counts of 20% and 26%, respectively. The WYC, YCWC, YGC, YMPC and ZNBC-supplemented groups had lower ($P < 0.05$) lymphocyte count of 12% and monocyte count of 13%, compared to birds in the PC group. However, it was observed that birds in the SalC group had similar lymphocyte and monocyte counts to birds in the PC group. Dressing %, the weight of breast, thighs and drumsticks at d35 were depressed by LPS inoculation by approximately 14, 21, 14 and 3.5% ($P < 0.05$) in the PC group compared to the NC group. However, supplementation with WY, YCW, YG, YMP, ZNB and Sal in the challenged groups improved ($P < 0.05$) the dressing %, weight of the breast, thighs and drumsticks relative to the PC group. This present study shows that whole yeast and its derivatives can improve meat yield of broilers and, through its effect on white blood cell, lymphocyte and monocyte counts, may be associated with an amelioration of stress induced by *Salmonella* lipopolysaccharide in broiler chickens. This study also shows that autolyzed yeast, yeast cell wall, and its enzymatically hydrolyzed components when supplemented at 2g/kg diet may serve as a suitable alternative to in-feed antibiotics in broiler production.

I. INTRODUCTION

The non-therapeutic use of antibiotics in animal feed has been banned in some regions of the world (Lekshmi et al. 2017) and there are restrictions on the use of some products in other

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countries, including Australia (APVMA, 2017). This has led to a search for alternatives to antibiotics for use in poultry production. Yeasts and their by-products have been identified as potential alternatives to antibiotics. In a recent study (Ahiwe et al., 2018), we demonstrated the positive effect of dietary supplementation with yeast and its derivatives supplemented at different levels of inclusion on healthy broiler chickens. In the current study, we tested the efficacy of whole yeast, yeast cell, yeast glucan and yeast mannoprotein in protecting broiler chickens that were challenged with a lipopolysacharide (LPS) extract from the cell wall of *Salmonella* Typhimurium, a gram-negative bacterium.

Exposure of healthy chickens to LPS has been shown to induce immunogenic response, non-specific inflammation and toxicity, leading to stress, poor body weight gain and reduced meat yield (Mueller et al. 2004, Abbas et al. 2000). Furthermore, Xie et al. (2000), reported that LPS challenge increased white blood cell (WBC), lymphocyte (LYM) and eosinophil (EOS) counts and several other haematological indices in broiler chickens. The author suggested that the increase in these haematological parameters may be part of the reason for the stress and poor performance observed in birds treated with LPS.

Yeast and several prebiotics have been reported to ameliorate the effect of stress on different parameters of broiler chickens (Silva et al. 2010). However, to the best of our knowledge, research on the effect of yeast and its components on meat yield and haematological indices of broilers challenged with *Salmonella* lipopolysaccharide is scarce. Hence the need for this study.

II. MATERIALS AND METHODS

Eight diets based on maize and soybean were offered to 432 Ross 308 broiler chickens from d0 to d35. Each dietary group had six replicates having nine birds each. The eight treatment groups consisted of a negative control (NC) (without supplementation and not challenged); positive control (PC) (without supplementation + LPS challenged); whole yeast + LPS challenged (WYC); yeast cell wall + LPS challenged (YCWC); yeast glucan + LPS challenged (YGC); yeast manno-protein + LPS challenged (YMPC); zinc bacitracin + LPS challenged (ZNBC); Salinomycin + LPS challenged (SalC). All yeast treatments were included at 2 g/kg diet while zinc bacitracin and Salinomycin were included at 0.03 and 0.05 g/kg diet, respectively. Except for the NC group that was only inoculated with 3 ml (0.9% saline suspension), birds in the other groups were inoculated with 3 ml LPS (made up as 100 µg/ml in 0.9 % saline) intraperitoneally on days 13, 15 and 17.

III. RESULTS AND DISCUSSION

The results showed that, at d24, the mean corpuscular volume, mean corpuscular haemoglobin concentration, and counts of eosinophils, neutrophils, basophils, red blood cell, haemoglobin and platelets were not significantly affected ($P > 0.05$) by LPS challenge. Relative to birds on the NC group, birds in the PC group had approximately 25 % ($P < 0.05$) increase in the white blood cell (WBC) count. However, supplementation with WY, YCW YG, YMP, and ZNB to the challenged group led to a lower ($P < 0.05$) WBC count relative to birds in the PC group. Birds in the SalC group had similar ($P > 0.05$) WBC counts to birds in the PC group. Compared to birds in the NC group, birds in the PC group had increased ($P < 0.05$) lymphocyte and monocyte counts of 20% and 26%, respectively. The WYC, YCWC, YGC, YMPC and ZNBC-supplemented groups had lower ($P < 0.05$) lymphocyte count (12 %), and monocyte count (13 %), compared to birds in the PC group. However, it was observed that birds on the SalC group had similar lymphocyte and monocyte counts to birds in the PC group. Wang et al. (2016) also observed that live yeast could alleviate LPS-induced inflammation, haematological indices and stress in broilers at d27.

Dressing %, the weight of breast, thighs and drumsticks at d35, were depressed ($P < 0.05$) by LPS inoculation by approximately 14, 21, 14 and 3.5 % in the PC group compared to the NC group. However, the dressing %, weight of the breast, thighs and drumsticks was improved ($P < 0.05$) in the WYC, YCWC, YGC, YMPC, ZNBC and SalC groups relative to the PC group. This observation is in agreement with the findings of Faithi et al. (2012), who concluded that yeast culture supplemented at 1.5 g/kg improved carcass yield and humoral immunity of broilers under the stress of challenge with Newcastle disease.

The results of the present study show that supplementation with whole yeast and its derivatives can improve meat yield of broilers and, through its effects on white blood cell, lymphocyte and monocyte counts may be associated with an amelioration of stress induced by *Salmonella* lipopolysaccharide in broiler chickens. It can also be concluded that autolyzed yeast, yeast cell wall, and its enzymatically hydrolyzed components at 2g/kg diet may serve as a suitable alternative to in-feed antibiotics in broiler production.

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MICROBIAL CONTAMINATION ON FRESH AND FROZEN CARCASSES OF BROILER IN MANOKWARI MARKETS, WEST PAPUA INDONESIA

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Chicken meat is a common meat in Manokwari, West Papua, Indonesia and it is sold both in fresh and frozen forms. Fresh carcasses are usually from local producers while frozen carcasses are supplied from other regencies (administrative entities). Fresh carcasses are generally sold in traditional markets and frozen carcasses sold in both traditional markets and supermarkets.

Chicken meat is an ideal medium for many microorganisms especially pathogenic bacteria which lead to spoilage and which can be transferred as foodborne illness (Álvarez-Astorga et al., 2002). Contamination can occur along the food chain including production, processing, distribution, retail marketing and handling or preparation (Kusumaningrum et al., 2012). Retail marketing such as traditional markets is another place that can contaminate carcasses. Traditional markets are identified as crowded and dirty places as the chicken meat is placed in the open air.

This research aims to determine the extent of microbiological contamination on both fresh and frozen carcasses of broilers sold in both traditional markets and supermarkets in Manokwari Regency.

The study included two traditional markets, three supermarkets and ten distributors from which carcasses were collected. All samples were subjected to the following examinations: Total Plate Count (TPC), number of *Escherichia coli*, *Coliform, Staphylococcus aureus,* and the presence of *Salmonella sp. and Campylobacter*. Testing methods were according to the Bacteriological Analytical Manual and SNI 01-2897-2008. The data were analyzed descriptively then further significant difference was tested by Chi-square test.

The results of this study confirmed microbial contamination of broiler carcasses circulating in both traditional markets and supermarkets in Manokwari by positive test results for TPC, *E. coli, Coliform, Streptococcus aureus, Salmonella sp.*, and *Campylobacter sp. E coli* contamination was found at the lowest value of 3.0×10^1 cfu/g and the highest contamination of 1.5×10^4 cfu/g. *Coliform* contamination was found at the lowest value of 1.9×10^2 cfu/g and the highest value of 5.4×10^4 cfu/g. *Staphylococcus aureus* contamination was at the lowest value of 2.1×10^2 cfu/g and the highest value of 7.5×10^2. *Salmonella* and *Campylobacter* contamination were found on two samples, from frozen and fresh carcasses respectively. Fresh carcasses were highly contaminated compared to frozen carcasses. The maximum limit of microbial contamination (BMCM) permitted on fresh chicken meat is less than 1×10^1 cfu/g. Traditional markets had greater contamination of chicken meat compared to supermarkets. Microbial contamination was greater on fresh carcasses sold in traditional markets than on frozen carcasses. Sales of fresh carcassesretain the offal inside the body to show customers that the carcasses are from local fresh chicken. These results can also be used by the animal husbandry Department to control the regulation of meat sales in both traditional and super markets. This study concluded that microbial contamination is found more on fresh carcasses sold in traditional markets than frozen carcass. Distributors who are not obey the safety meat regulation are warned and may have their licenses revoked.

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PRODUCTION, HAEMATOLOGICAL AND IMMUNOLOGICAL ATTRIBUTES OF BROILERS FED DIETS SUPPLEMENTED WITH TAMARIND SEED BASED POLYPHENOLS EXTRACT

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Summary

The present experiment was designed to investigate the effect of tamarind polyphenols under different dietary protein diets (soybean and DDGS) on production, physiological and immunological attributes in broilers. Day old chicks (n=280) were randomly distributed into 7 treatment groups with 5 replicates each containing 8 birds. All birds were provided with feed and water *ad libitum* and maintained in experimental pens during tropical conditions (temperature 34-41°C and 47% humidity). Polyphenols were fed at 125 and 250 ppm with and without distillers dried grains with solubles (DDGS) and antibiotic growth promoter (AGP). One way analysis of variance (ANOVA) was performed to assess the significant difference among treatment groups. Results indicated that experimental diets supplemented with polyphenols (T_2, T_3) alone significantly ($P<0.01$) reduced haemoglobin (Hb) levels compared with the control (T_1), while polyphenols supplemented at 125 and 250 ppm in combination with DDGS significantly improved Hb levels compared with the control (T_4). Administration of polyphenols at 125 and 250 ppm in corn-DDGS diets increased ($P<0.05$) the immune response i.e., foot pad index in response to phytohaemagglutunin (PHA-P), Newcastle vaccination (ND) titres as compared with diets supplemented with DDGS (T_4- control). Production performance did not show a significant difference among treatment groups. In conclusion, polyphenols at 125 and 250 ppm in combination with 5% DDGS improved ($P<0.05$) broiler production especially immunity and erythrocyte membrane resistance under hot climatic conditions.

I. INTRODUCTION

Owing to the increased efficiency, growth rate and reduced mortality achieved, antibiotics have been used continuously in the broiler industry for decades (Cromwell, 2002) and are being questioned for antibiotic resistance. Some of the alternatives include vitamins, minerals, herbal drugs, plant extracts, phytobiotics and antimicrobial peptides and beneficial bacteria (Yadav et al., 2016). Recently, there has been increased interest given to polyphenols from researchers and the wider food industry. Several studies have explored the anti-inflammatory, anti-allergic, immunomodulatory and anti-mutagenic activities, anticancer, and cardiovascular protective effects (McCullough et al., 2012) besides their antimicrobial activity. Polyphenolic tannins exert both positive and negative effects as they possess anti-nutritive properties, but are also beneficial for health due to their antimicrobial, anti-oxidative properties and their ability to stimulate the immune system and various effectors (Quideau et al., 2011). Further, bio-availability of proteins is questionable in polyphenol enriched broiler diets and may vary among protein sources. DDGS which are being used in broiler diets have high energy and protein content which make them an attractive substitute for expensive sources of energy and protein ingredients in poultry feed. The present study was undertaken to investigate the multifaceted effects of tamarind based polyphenol extracts and their combination with different protein sources on haematology, immunity and production of broilers reared from 0 to 42 days under tropical conditions.

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II. MATERIALS AND METHODS

A total of 280 day old straight run commercial broilers (CARIBRO Vishal) were randomly assorted into 7 treatment groups (with 5 replicates each consisting of 8 birds): Basal diet without antibiotic growth promoter (AGP) as control (T_1); Basal diet with 125 (T_2) and 250 ppm (T_3) polyphenols; Basal diet with 5% DDGS replacing 5% soybean meal (T_4); Basal diet with 5% DDGS replacing 5% soybean meal and 125 ppm polyphenols (T_5); Basal diet with 5% DDGS and 250 ppm polyphenols (T_6); Basal diet with 0.025% AGP-bacitracin methylene disalicylate (T_7). Comparisons for T_2, T_3 and T_5, T_6 were made with their respective controls i.e., T_1 and T_4. All birds were maintained under standard managemental conditions and a tropical environment and experimental diets were fed *ad libitum*. Tamarind seeds (TS) that were procured locally were extracted as per Razali et al. (2012) with methanol as a solvent in 1:10. Solvents were then removed using a rotary evaporator and the residues were directly mixed with the feed. For production attributes, body weight (BW), body weight gain (BWG), feed intake (FI) and feed efficiency (FE) and mortality were calculated. Haemoglobin (Hb) and erythrocyte osmotic fragility (EOF) were determined as per Sahli's acid haematin (Sahli, 1909) and Buffenstein et al. (2001) respectively. For humoral immunity, Haemagglutination inhibition (HI) titer (OIE, 2012) and ELISA based antibody titer (IDEXX, USA) to Newcastle disease (ND) were estimated in serum on 14 and 21 dpi. Cutaneous basophilic hypersensitivity (CBH) was evaluated in terms of foot pad index (FPI) for the assessment of cell mediated immunity as per Cheng and Lamount (1988) in response to PHA-P. Results were subjected to one way ANOVA using SPSS 16.00 to assess the significant differences at 5% and 10% level among various groups with tukey's post hoc test.

III. RESULTS AND DISCUSSION

Body weight and body weight gain (fortnightly) were significantly affected by polyphenols in birds fed with corn-soy (T_2, T_3) and corn-DDGS (T_5, T_6) diets to 4 weeks of age. However, final (42 days) live BW and BWG (Tables 1, 2) showed no significant difference among the treatment groups. Aengwanich et al. (2009) also found similar results. Brenes et al. (2010) with grape polyphenols found no significant ($P>0.05$) effect on FI and FE. In contrast, Masek et al. (2014) and Gopi et al. (2017) found higher BWG in polyphenol supplemented groups. The polyphenol source might be the reason for this difference. Sinchaiyakit et al. (2011) demonstrated higher (>30%) amounts of tannic acid content in TS coat husk. Direct interaction of polyphenols (rich in tannins) and some components such as proteins and polysaccharides can occur with binding affecting absorption (Gopi et al., 2017). FE was reduced ($P<0.05$) in T_5 and T_6 compared to their corresponding control and corn-soy diets. Treatment groups T_2 and T_3 had significantly ($P<0.01$) lower Hb levels than the control (T_1), while those supplemented with DDGS based diets (T_2,T_3) significantly ($P<0.05$) improved Hb levels. Polyphenols in corn-soy diets (T_2,T_3) have slightly improved the membrane strength of erythrocytes (Table 3) at 0.5% saline concentration.

Polyphenols in corn-soy diets did not improve FPI (Table 4) in relation to the control (T_1). Polyphenols at 125 ppm (T_2) showed significant ($P<0.01$) reduction in CMI response associated with higher mortality rate (Table 3). T_6 group had improved ($P<0.01$) immunity to ND on 14 and 21 dpvon and immunity to PHA-P ($P<0.01$). The modulating effects of polyphenols on immune function are mediated mainly via the inflammatory responses in macrophages which in turn initiate the production of pro-inflammatory cytokines (Cuevas *et al.*, 2013). It appears that unidentified factors in DDGS might have some role that enhances the polyphenol induced immunity.

Table 1 - Body weight recorded on fortnightly in broilers fed with varying levels of tamarind seed polyphenols and different protein sources (Mean ± SE, n=40).

Treatment Groups	2nd Week	4th Week	6th Week
T_1 (Control without AGP)	$306.15^{b} \pm 7.06$	$861.80^{ab} \pm 17.57$	1627.62 ± 29.23
T_2 (125 mg TS-PE)	$266.55^{a} \pm 5.58$	$831.80^{a} \pm 23.22$	1630.43 ± 31.08
T_3 (250 mg TS-PE)	$308.42^{bc} \pm 5.47$	$891.85^{ab} \pm 21.03$	1632.41 ± 31.36
T_4 (5% DDGS)	475.75 ± 37.05	$1102.25^{e} \pm 53.72$	1817.50 ± 61.59
T_5 (5% DDGS + 125 mg TS-PE)	$330.00^{cd} \pm 4.55$	$918.20^{b} \pm 16.61$	1633.46 ± 25.93
T_6 (5% DDGS + 250 mg TS-PE)	$335.10^{d} \pm 5.96$	$933.00^{b} \pm 23.01$	1671.08 ± 38.64
T_7 (Basal diet with AGP)	$282.25^{a} \pm 6.52$	$853.35^{ab} \pm 25.32$	1654.66 ± 34.00
Mean Standard Deviation	55.97	143.75	197.89
Standard Error Mean	3.57	9.15	12.91
Significance (P-value)	0.00	0.00	0.10

[abc]Means bearing different superscripts within columns differ significantly (P<0.05)

Table 2 - Body weight gain on fortnightly in broilers fed with varying levels of tamarind seed polyphenols and different protein sources (Mean ± SE, n=40).

Treatment Groups	2nd Week	4th Week	6th Week
T_1 (Control without AGP)	$197.10^{b} \pm 5.59$	345.05 ± 14.00	325.20 ± 7.45
T_2 (125 mg TS-PE)	$167.00^{a} \pm 4.81$	362.05 ± 14.71	344.60 ± 6.90
T_3 (250 mg TS-PE)	$198.58^{b} \pm 3.69$	359.16 ± 13.41	338.11 ± 7.65
T_4 (5% DDGS)	$206.05^{b} \pm 3.47$	348.30 ± 10.33	324.48 ± 5.79
T_5 (5% DDGS + 125 mg TS-PE)	$213.75^{b} \pm 4.06$	357.15 ± 13.92	326.50 ± 9.88
T_6 (5% DDGS + 250 mg TS-PE)	$179.40^{a} \pm 5.25$	355.90 ± 15.26	336.18 ± 8.02
T_7 (Basal diet with AGP)	$365.50^{c} \pm 30.30$	341.75 ± 30.53	317.00 ± 11.71
Mean Standard Deviation	46.59	85.49	48.31
Standard Error Mean	2.97	5.45	3.08
Significance (P-value)	0.00	0.97	0.35

[abc]Means bearing different superscripts within columns differ significantly (P<0.05)

Table 3 - Influence of tamarind seed polyphenols extract on Hb and EOF (Mean ± SE, n=6).

Treatment Groups	Haemoglobin (g/dl)	Erythrocyte Osmotic Fragility (% hemolysis rate) at different NaCl concentrations		
		0.3%	0.5%	0.9%
T_1 (Control without AGP)	$10.33^{f} \pm 0.12$	97.77 ± 1.49	27.61 ±2.13	23.32 ±1.50
T_2 (125 mg TS-PE)	$9.75^{e} \pm 0.96$	97.79 ± 1.90	25.57 ±1.99	22.54 ± 0.98
T_3 (250 mg TS-PE)	$9.00^{d} \pm 0.82$	97.77 ± 2.23	23.36 ±1.31	23.44 ± 1.00
T_4 (5% DDGS)	$6.73^{a} \pm 0.39$	93.71 ± 4.04	22.78 ± 2.21	23.08 ± 1.15
T_5 (5% DDGS + 125 mg TS-PE)	$9.28^{de} \pm 0.13$	93.61 ± 3.81	26.51 ± 2.04	23.90 ± 1.34
T_6 (5% DDGS + 250 mg TS-PE)	$8.28^{c} \pm 0.18$	94.33 ± 2.80	33.59 ± 4.93	24.50 ± 1.78
T_7 (Basal diet with AGP)	$7.65^{b} \pm 0.96$	89.57 ± 6.58	24.50 ± 0.92	24.20 ± 1.52
Mean Standard Deviation	0.78	7.04	5.62	2.47
Standard Error Mean	0.39	1.33	1.06	0.47
Significance (P-value)	0.00	0.64	0.09	0.95

[abc]Means bearing different superscripts within columns differ significantly (P<0.05)

Table 4 - Influence of tamarind seed polyphenol extract on cell mediated and humoral immunity in broilers (Mean ± SE, =6).

Treatment groups	Foot pad index (mm)	ND-HI titer (log_2 values)		Antibody titer (log_2 values)	
		Day 14	Day 21	Day 14	Day 21
T_1 (Control without AGP)	$1.02^{bc} \pm 0.07$	$0.73^{b} \pm 0.08$	$0.68^{ab} \pm 0.08$	2.71 ± 0.28	2.51 ± 0.12
T_2 (125 mg TS-PE)	$0.60^{a} \pm 0.11$	$0.45^{a} \pm 0.09$	$0.60^{a} \pm 0.00$	2.92 ± 0.33	2.89 ± 0.31
T_3 (250 mg TS-PE)	$0.84^{ab} \pm 0.16$	$0.60^{ab} \pm 0.00$	$1.05^{abc} \pm 0.15$	2.44 ± 0.31	2.46 ± 0.71
T_4 (5% DDGS)	$0.95^{bc} \pm 0.13$	$0.38^{a} \pm 0.08$	$1.13^{bc} \pm 0.14$	2.33 ± 0.11	2.53 ± 0.09
T_5 (5% DDGS + 125 mg TS-PE)	$1.13^{bc} \pm 0.05$	$0.58^{ab} \pm 0.08$	$1.18^{bc} \pm 0.19$	2.46 ± 0.14	2.54 ± 0.14
T_6 (5% DDGS + 250 mg TS-PE)	$1.25^{c} \pm 0.10$	$1.13^{c} \pm 0.14$	$1.36^{c} \pm 0.26$	2.67 ± 0.42	2.54 ± 0.12
T_7 (Basal diet with AGP)	$0.96^{bc} \pm 0.12$	$0.38^{a} \pm 0.08$	$1.05^{abc} \pm 0.15$	2.44 ± 0.16	3.03 ± 0.22
Mean Standard Deviation	0.28	0.29	0.38	0.51	0.37
Standard Error Mean	0.05	0.06	0.07	0.10	0.70
Significance (P-value)	0.02	0.00	0.03	0.18	0.19

IV. CONCLUSION

Supplementation of polyphenols at 125 and 250 ppm in combination with 5% DDGS based diets improved ($P<0.05$) broiler production especially immunity and erythrocyte membrane resistance under tropical conditions. Polyphenols did not improve production performance.

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THINK BEYOND THE OBVIOUS: EXOGENOUS ENZYMES AS PART OF STRATEGY TO REDUCE USE OF ANTIBIOTICS IN POULTRY PRODUCTION

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Poultry production is entering an era of antibiotic reduction or production without antibiotics. In such a situation, nutrition plays a crucial role and enzymes can help by reducing feed costs; however, enzymes are more than a nutrient matrix. As science of application of enzymes in feed and their effects on digestion kinetics in the gut evolve, it is becoming increasingly clear how enzymes influence the conditions in the gastrointestinal tract (GIT). Dietary challenges posed by fibre content in the diet (especially soluble arabinoxylans) include increased digesta viscosity and transit time, leading to lower nutrient digestibility and absorption. This promotes the proliferation of microflora in the small intestine, competition for available nutrients, and overgrowth of pathogenic bacteria. Additionally, microbial activity promotes reduction in nutrient digestibility, especially of fat, through deconjugation of bile salts. These events can be reversed by the addition of exogenous xylanase; by degrading soluble arabinoxylans the enzyme reduces viscosity, limiting the proliferation of microflora in the small intestine. Furthermore, the degradation of arabinoxylan, by xylanase, in the upper gastrointestinal tract, produces xylo-oligosaccharides which have a prebiotic effect on the beneficial microbial population in the lower GIT. A reduction in growth of pathogenic bacteria in the digesta by the application of xylanase in the diet has been shown in several studies. The beneficial shift in microbial activity in the upper and lower GIT positively impacts gut health, reducing the risk of disease, and consequently the need for antimicrobial treatments. As enzymes work on crucial factors that directly influence gut environment, a positive effect of a combination with feed additives that have direct effects on microbial community and host immune system -such as probiotics and phytomolecules- can be hypothesized.

Therefore it is also being studied in more depth how enzymes help to enhance the performance of other gut health feed additives. The holistic effects of xylanase on the digestion process in the small intestine makes it a fundamental part of the diet, which should also be considered within an overall antibiotic reduction strategy.

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DEVELOPMENT OF INTERACTIVE VISUALISATION SOFTWARE FOR RESEARCH COMMUNICATION

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Tracking and understanding hen movement in commercial free-range flocks is challenging due to complex housing furniture as well as the sheer volume of individual data involved. The purpose of this study was to develop interactive visualisation software that allows users to display recorded data and individualise graphs on demand using existing data. A total of ~9,375 laying hens were housed in three identical commercial free range sheds equipped with an open aviary system. Hens were individually tracked within the sheds as well as on the range from 16 - 72 weeks of age using a custom made RFID system. Hen movements were captured every second generating >1.597 billion records (~1 TB). Data were consolidated in PostgreSQL [v10.5, PostgreSQL Global Development Group] an open source relational database. "Materialised views" were built to summarise information and format data for visualization. Web based software was build using Javascript [v 1.8.5, Netscape Communications, Dulles, US] for both front and back end development. AngularJS [v1.6, Google LLC, Mountain View, US] served as front-end web application framework. D3.js [v5.5.0, M. Bostock & J. Heer, New York, US] was used to render visualisations. We developed interactive web based software (visualhens.une.edu.au) enabling users to visualise the movement and visitation patterns. Users can generate visualisations of:

- Percentage of hens selected that were detected at different locations;
- Origin, destinations and numbers of movements of groups of hens in the aviary system;
- Movements of individual hens to determine if some hens remain in certain locations.

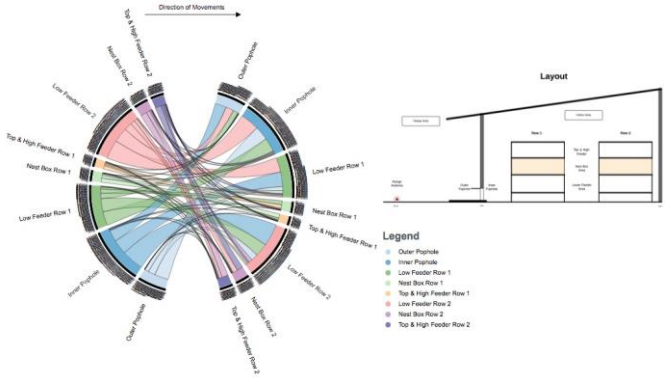

Figure 1 - This visualisation displays the origin, destination and number of movements a group of hens made in the aviary system. The left hand of this interactive diagram shows how many times a specific area was left. On the right hand side is shown how many times a specific area was entered. The thickness of the line indicates how many movements occurred in a specific direction.

Hens can be selected by weight as well as by activity classifications. Temporal selections enable users to visualise specific days and events.

In conclusion, our dataset and web tool can help answer specific questions on range and structure utilisation. Open source tools and relational databases can be harnessed to analyse and visualise large datasets. In future, additional datasets e.g. weather and necropsy data can be added allowing for additional features.

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EFFICACY OF A MICRO-ENCAPSULATED PHYTOGENIC PRODUCT BASED ON CARVACROL, CINNAMALDEHYDE, CAPSAICIN AND CINEOL IN DIETS FOR BROILER AND LAYING HENS - IMPROVEMENTS IN A DOSE DEPENDENT MANNER WITHOUT COMPROMISING SAFETY

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Antimicrobial resistance (AMR) is a major threat to global public health and facilitated through overuse of antibiotics in both human medicine and agriculture. Antibiotic reduction as part of antimicrobial stewardship might be a tool to lower the incidence of AMR. Alternatives like phytogenic feed additives have the potential to reduce the use of antibiotics in poultry farming. To evaluate the capabilities of a commercial phytogenic feed additive (Activo® by EW Nutrition GmbH) feeding studies have been conducted in broilers and in laying hens. One broiler and one laying hen study have been conducted as tolerance studies and two studies have been conducted as dose finding studies.

The first study evaluated the tolerance and dose dependent efficacy of the phytogenic product in diets for broiler chickens from day 1 to day 35 of age (400 animals, 4 treatment groups). Basal starter and grower diets were supplemented with 0, 100 mg/kg, 1000 mg/kg and 10000 mg/kg of the product. Body weight gain, feed intake, feed conversion ratio, health status, and blood parameters were used for demonstrating the tolerance and efficacy of the product.

Compared to the control group, supplementation with 100 mg/kg showed significant improvements in body weight gain in the starter period (+4%) and significant improvements in feed conversion ratio (FCR) in the grower period (+4%), resulting in an overall improvement in FCR of 3%. At 1000 mg/kg supplementation, a significant improvement in FCR of 6% was observed over the entire feeding period. Results recorded for hematological parameters were within the reference range of healthy broiler chickens when feeding up to 10000 mg/kg of the product in feed.

The second study evaluated the dose dependent efficacy and tolerance of the phytogenic additive in laying hens from week 20 to 43 of age (200 animals, 5 treatments). A basal diet for laying hens was supplemented with dose levels of 0, 100, 250, 500, and 5000 mg/kg of the product, respectively. Responses were demonstrated on body weight (gain), feed intake, egg production, FCR and health status throughout the 168 day feeding period. Blood profile measurements were included at the end of the study.

Inclusion levels from 100 mg/kg upwards improved laying performance, egg mass and egg weight in comparison to the control group and reduced FCR compared to the control group. Blood parameters obtained in the layers at the end of the feeding study showed that the product at an inclusion of 5000 mg/kg did not affect the measured parameters, when compared to the control group. Two more studies, one broiler and one laying hen study found dose-dependent effects for the tested additive, where higher inclusion rates led to higher performance improvements.

In conclusion, all four conducted studies revealed that graded inclusion levels of a defined micro encapsulated phytogenic feed additive can significantly increase production parameters of both broiler and laying hens without negatively affecting animal health when incorporated at up to 100 fold the dose recommended for inclusion into diets.

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DIETARY SUPPLEMENTATION OF MONO-GLYCERIDES SHOWED REDUCED MORTALITY AND IMPROVED FEED EFFICIENCY IN BROILERS CHALLENGED WITH CLINICAL NECROTIC ENTERITIS

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Necrotic enteritis (NE) is one of the most important diseases of the world broiler industry which costs over six billion dollars annually. There has been a concerted effort to control and minimise the effects of NE that have arisen since the EU ban of in-feed antibiotics in the poultry industry. Organic acids have been used as alternatives to in-feed antibiotics for maintaining good gut health of poultry by suppressing the growth of pathogenic bacteria, resulting in improved performance (Naseri et al., 2012). The present study investigated the effects of dietary supplementation of a mixture of 1-mono-glycerides (MG) and sodium buffered formic acid (FA) in diets on broiler performance, mortality rate and caecal microflora under a necrotic enteritis challenge. A total of 544 d-old Ross 308 broiler chicks (as hatched) was allotted to 32 pens each stocked with 17 birds. A randomised complete block design was used with 4 treatments replicated 8 times. The treatments included: 1- negative control, without additives; 2- positive control containing salinomycin (0.050%) and zinc bacitracin (0.033%); 3- mixture of 1-mono-glycerides (MG), starter: 0.5%, grower: 0%, finisher: 0%; 4- MG + sodium buffered formic acid (FA), starter: 0.5% MG, grower: 0.3% FA, finisher: 0.3% FA. All diets were wheat, sorghum, soybean meal and meat and bone meal based, formulated to meet Ross 308 nutrient specifications. The NE challenge model followed the method described by Keerqin et al. (2017). Bird performance was measured from d0-10, d11-24, d0-24 and d0-35. NE caused mortality was determined by necropsy and recorded after challenge, while caecal microflora was measured on d16. Data were analysed using the General Linear Models (GLM) of SPSS and male:female ratio determined by feather sexing was set as a covariate for performance data. Birds fed diet-contained antibiotics showed better FCR, higher weight gain and feed intake during the whole period of study ($P < 0.05$) except for d0-10, compared to those fed diets supplemented with or without additives. Birds fed the diet supplemented with 0.5% MG in starter phase and 0.3% FA in grower phase had enhanced FCR (from d10-24 and d0-24) compared to those fed no-additive diet ($P < 0.05$). However, no significant differences were observed on FCR, weight gain and feed intake between birds which received different additives and those without additives during d0-35 ($P > 0.05$). The diet-contained antibiotics reduced the occurrence of mortality due to NE ($P < 0.05$). Birds fed the diet supplemented with 0.5% MG in starter phase reduced the occurrence of mortality due to NE compared with those fed no-additive diet (26.1% vs. 14.8%, $P < 0.05$). Birds fed the diet supplemented with antibiotics had lower counts of *Lactobacillus* spp. compared to those fed the diet without additives and those fed the diet supplemented with 0.5% MG in starter phase following 0.3% FA in grower phase ($P < 0.05$), but did not significantly differ from birds fed 0.5% MG in the starter phase. Birds fed the diet supplemented with different additives had higher counts of *Clostridium perfringens* ($P < 0.05$) compared to the antibiotics group but were not significantly different from those fed the no-additive diet. Total anaerobic bacteria, *Ruminococcus* spp., *Bacillus* spp., and *Bifidobacterium* spp., were not affected by different treatments ($P > 0.05$). These results demonstrated that the MG supplemented in the starter phase and MG in starter phase followed by FA in the grower phase may partially protect birds from clinical necrotic enteritis as indicated by reduced mortality and improved feed efficiency.

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EFFECT OF SELECTED YEAST FRACTION ON THE GROWTH OF *CLOSTRIDIUM PERFRINGENS*: QUANTITATIVE DETERMINATION OF GROWTH INHIBITION AND ADSORPTION CAPACITY

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Yeast cell wall (YCW) fractions have proven effective in reducing the incidence of necrotic enteritis induced by *Clostridium perfringens*. Dietary supplementation with YCWs stimulates the systemic innate immune responses of broiler chickens, suggesting the role of these products in regulating immune homeostasis (Alizadeh et al., 2016). In a proposed mode of action of YCW on bacteria, the branched lateral chains of the mannan-oligosaccharides (MOS) in the YCW structure bind the bacteria and provide alternative sites for the adhesion of pathogens. The ingestion of YCW products might supply competitive attachment sites for the host receptors, thus reducing the risk of pathogenic bacteria colonising the intestinal tract. Bacteria bound to MOS in the intestinal tract can pass through the gut, instead of attaching to host epithelial cells (Caipang and Lazado, 2015). This inhibition mechanism seems to be limited to some specific Gram-negative enteropathogens (*Salmonella* and *E. coli*), although several *in vivo* studies report their effect also on Gram positive pathogens like clostridia (Santovito et al., 2018).

To provide *in vitro* evidence on the antimicrobial effect of YCW on *C. perfringens*, the effectiveness of YCW fractions in inhibiting the growth of several *C. perfringens* strains was quantitatively determined. The bacterium was grown in the presence of different YCW fractions at different concentration levels. The effect of YCW fractions on growth parameters was analysed. One product out of four materials was selected as the best candidate for *C. perfringens* inhibition. The selected product, at an optimal dosage of 1.25 mg/mL, increased the lag phase duration, and reduced the maximum growth rate and the final cell count in a significant manner with respect to the control. The adsorption of the pathogen to YCW was studied using the isotherm adsorption approach. The effect of YCW dosage, incubation time, and bacterial concentration on the adsorption was evaluated. The study proved that the product adsorbed *C. perfringens* cells in a dose and time dependent manner. Equilibrium isotherms showed that the cell adsorption onto the product was fast, stable over the time, and occurred with high affinity and capacity. The selected product sequestered up to ca. 10^4 cells of *C. perfringens* per mg. To the best of our knowledge, this is the first report showing the *in vitro* efficacy of yeast fraction products in inhibiting the growth of *C. perfringens* and reducing the culturable cells by an adsorption process. The *in vitro* approach proposed herein is a powerful tool for studying the adsorption of aerobic or anaerobic pathogens by eubiotics.

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COMPARATIVE EFFICACY OF A NOVEL MULTI-STRAIN *BACILLUS*-BASED DIRECT FED MICROBIAL AND EACH ONE OF ITS SINGLE STRAINS FOR THE CONTROL OF NECROTIC ENTERITIS CAUSED BY *CLOSTRIDIUM PERFRINGENS* IN BROILER CHICKENS

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The use of *Bacillus* species as probiotic supplements is expanding rapidly and these products demonstrate immune stimulation, antimicrobial activities, enzyme production and competitive exclusion as the most prevalent modes of action (Grant et al., 2018; Hmani et. al., 2017; Reis et al., 2017).

The objective of this study was to evaluate the effect of a multi-strain *Bacillus*-based directly fed microbial (DFM) and its three single strains on performance of broilers challenged with a commercial *Eimeria* vaccine and a field isolate of *Clostridium perfringens* known to cause necrotic enteritis (NE) originating from a commercial broiler operation.

The experiment consisted of 2250 day of hatch Cobb 500 male chicks distributed in 45 pens with 50 chicks per pen. The five treatments were replicated in nine blocks, randomized within blocks of five pens each. T1 (control group; challenged birds without DFM); T2 (DSM32324 strain at 8 X 10^5 CFU/g of feed); T3 (DSM32325 strain at 5 X 10^5 CFU/g of feed); T4 (DSM25840 strain at 3 X 10^5 CFU/g of feed) and T5 (the three strains combined at 1.6 X 10^6 CFU/g of feed). The standard diet was corn soy based pelleted feed and was, together with water, provided *ad libitum*. All birds were spray vaccinated with *Eimeria* vaccine on day of hatch. On Days 19, 20, and 21, all pens were challenged with a field isolated *C. perfringens*. Each pen received the same amount of *C. perfringens* (1.0 X 10^8 CFU) (Mathis 2018). On day 21, five birds per pen were sacrificed and examined for the degree of NE lesions. The NE lesion scoring was as follows: 0 for normal looking intestines and up to 3 for extreme NE lesions. Weight gain (WG), feed intake and feed conversion ratio (FCR) were recorded on day 21, 35 and 42. NE lesion scores and % NE mortality were calculated on day 21.

The groups supplemented with DFM's had significantly improved FCR ranging from 1.841-1.898 compared to the control group (1.957) ($P < 0.05$), the multi-strain DFM showing the lowest value (1.841). Furthermore, DFM treatments significantly reduced NE lesion scores (0.5-0.7) and NE related mortality (0.4-1.6%) compared to the control group (1.0 and 4.2% respectively) ($P < 0.05$).

In conclusion, the results of this study showed that inclusion of both the multi-strain *Bacillus* based DFM or its single strains decreased the degree of NE lesion scores and mortality and improved FCR in a sub-clinical NE model. It is hypothesised that the mode of action for this novel probiotic involves antimicrobial activities and competitive exclusion against pathogens.

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PERFORMANCE, INTESTINAL MORPHOLOGY AND ANTIOXIDANT STATUS OF BROILERS FED OREGANO ESSENTIAL OIL OR AN ORGANIC ACID BLEND

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Summary

In this study, the effect of an oregano essential oil, or a blend of formic and propionic acids and essential oil on mineral carriers, on the performance, intestinal morphology and antioxidant status of broilers was investigated. A total of 720 day-old Ross 308 male broilers was allocated to 3 treatments (8 replicates/treatment) and reared for 42 days: CON - basal diet (n=8), OEO – oregano essential oil (300 g/t) (n=8) and OAB - formic acid/propionic acid/oregano essential oil (2 kg/t) (n=8). Zootechnical performance was assessed and intestinal samples were taken on day 42 for determination of gut morphology and cellular proliferation via the proliferating cell nuclear antigen (PCNA) assay. Breast and thigh muscles were taken on day 42, kept at 2-8°C, and malondialdehyde (MDA) concentration determined 0, 3 and 6 days post collection. By 42 days, birds fed OAB had significantly greater body weight gain and lower FCR than both CON and OEO. There was no effect of treatment on villus height, crypt depth or villus height:crypt depth in duodenal, jejunal or ileal samples. PCNA score was higher in OEO than CON treated birds for all tissues. PCNA score did not differ significantly between OEO and OAB in jejunal and ileal samples. Finally, MDA concentrations in breast and thigh muscles were significantly lower in birds fed OEO than CON and OAB on days 0, 3 and 6 post collection. In conclusion, the supplementation of broiler feed with either an oregano essential oil product or a product comprising organic acids, essential oil and plant extracts can improve the performance of broilers. Oregano oil also has the potential to improve meat quality.

I. INTRODUCTION

The use of feed additives such as those based on essential oils or organic acids is increasing in poultry production as a result of a number of factors including legislation on restricting antibiotic use and consumer preferences for more natural, welfare friendly poultry production. Formic and propionic acid products have a strong antimicrobial activity and have been shown to improve bird performance and positively influence the gut microbiota (Emami et al., 2017). Oregano essential oil has antimicrobial and antioxidant properties and has also been shown to improve bird performance (Giannenas et al., 2016). Organic acids can be presented as salts or liquids while studies using oregano have included whole plants, dried leaves and essential oils. Variability in the presentation and supplementation rate of these products necessitates further work to build a sufficient information base to understand the influence these parameters have on performance and how best they can be optimized.

The objective of this study was to investigate the efficacy of two commercial products on the performance, gut morphology and antioxidant status of broilers. The products were an oregano essential oil presented on a mineral carrier (Orego-Stim, Anpario plc, UK) and a mixture of formic and propionic acids, oregano essential oil and plant extract presented on a mineral carrier (Genex, Anpario plc, UK).

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II. METHODS

A total of 720 day-old Ross-308 male chicks was randomly allocated into 3 equal groups with 8 replicates of 30 birds: CON- control group, basal ration; OEO300- basal ration and OEO (300 g/t); OAB- organic acid blend (2 kg/t). Birds were reared in floor pens on clean litter. The study ran for 42 days. Feed and water were available *ad libitum*. Feed rations were presented as a mash and comprised starter, grower and finisher phases. Rations were formulated to meet or exceed to NRC for Poultry recommendations (1994) (Table 1). All feed was free of all antibiotics and anticoccidial medication.

Table 1 - Diet composition of basal diet.

Ingredients	Starter	Grower	Finisher
Wheat (g/kg)	360	470	557
Maize (g/kg)	200	100	50
Soybean meal, 47	350	330	300
Soybean oil (g/kg)	32	40	35
Palm fat (g/kg)	15	20	25
Lysine (g/kg)	2.5	2.2	1.3
Methionine (g/kg)	2.6	2.4	1.7
Vitamins and mineral premix [1] (g/kg)	2.0	2.0	2.0
Crude protein [2], %	22.0	21.0	19.0
Lysine, %	1.3	1.2	1
Methionine+Cystine, %	1.0	0.96	0.94
Calcium [2], %	91.2	90.4	90.2
Phosphorus [2], %	0.71	0.69	0.66
Metabolisable energy, MJ/kg	12.6	13.3	13.5

[1]Supplying per kg feed: 12,000 IU vitamin A, 5,000 IU vitamin D_3, 30 mg vitamin E, 3 mg vitamin K, 5 mg thiamin, 6 mg riboflavin, 6 mg pyridoxine, 0.02 mg vitamin B_{12}, 60 mg niacin, 15 mg pantothenic acid, 1.5 mg folic acid, 0.25 biotin, 10 mg vitamin C, 500 mg choline chloride, 100 mg Zn, 120 mg Mn, 20 mg Fe, 15 mg Cu, 0.2 mg Co, 1 mg I, 0.3 mg Se, and phytase in recommended quantities per kg of diet.
2 Proximate analysis.

Birds were weighed weekly. On day 42, 4 birds/replicate were euthanised and samples taken for the following analysis:

a) Histology

Histology: Sections (1cm) of the duodenum, jejunum and ileum were taken into 10% buffered formalin for morphometric analysis according to Giannenas et al. (2011). Villus height (VH) and crypt depth (CD) were measured.

b) Proliferating cell nuclear antigen (PCNA)

Unstained tissue sections taken were collected for immunohistochemical examination of PCNA, using the avidin-biotin immunoperoxidase method (ABC kit, Vector Laboratories, CA, USA). Positive controls included canine testicular tumors. Normal intestine sections were used as negative controls with the primary antibody replaced by PBS.

c) Antioxidant status

The lipid oxidation of raw breast (*Pectoralis major*) and thigh (*Biceps femoris*) meat during refrigerated storage (2-8°C), was determined as malondialdehyde (MDA), using a modified version described by Buege and Aust (1978). Meat samples were analysed at the time of collection (day 0) and 3 and 6 days later.

Data were analyzed by ANOVA using JMP®, v. 13 (SAS Institute Inc., Cary, NC) and statistical significance declared at $P \leq 0.05$.

III. RESULTS

Body weight gain was significantly higher in OEO than CON on day 21 (P=0.0065), while OAB and CON did not differ significantly (Table 2). Overall, body weight gain was significantly greater in OAB than both CON and OEO by 0.148 and 0.092 kgs respectively (P=0.0006 and P=0.0271, respectively). Feed intake differed significantly between all treatments for 0-28 and 0-42 days. Feed intake for day 0-28 was lowest in OAB while for day 0-42 it was lowest in OEO. FCR for days 0-28 was significantly lower in OEO and OAB than CON (P<0.0001 and P<0.0001, respectively), while OEO and OAB did not differ. For the whole study period of 0-42 days, FCR was significantly different between all treatments. FCR was lowest in OAB (1.34) while FCR in OEO was 39 points lower than CON.

Table 2 - Zootechnical performance.

Treatment	Body weight gain (g)		Feed intake (g)		FCR*		Mortality (%)
	day 0-28	day 0-42	day 0-28	day 0-42	day 0-28	day 0-42	
CON	1335^{b}	2364^{b}	1967^{a}	4072^{a}	1.46^{a}	1.73^{a}	3.75
OEO	1412^{a}	2420^{b}	1856^{b}	3371^{b}	1.31^{b}	1.44^{b}	2.50
OAB	1377^{ab}	2512^{a}	1802^{c}	3406^{c}	1.32^{b}	1.34^{c}	2.90
SEM	18.2	9.6	15.3	65.8	0.017	0.036	0.050
Significance (P)	< 0.01	< 0.01	< 0.0001	< 0.001	< 0.0001	< 0.0001	0.444

*FCR corrected for mortality. Column data having different superscripts are significantly different ($p \leq 0.05$).

Analysis of intestinal samples for villus height, crypt depth and villus height:crypt depth ratio did not identify any significant treatment effect. (Table 3). Duodenal PCNA score was significantly higher in OEO than both CON and OAB (OEO versus CON, P < 0.0001; OEO versus OAB, P = 0.0005). CON and OAB did not differ significantly in duodenal PCNA score. PCNA score was significantly higher in OEO than CON in jejunal (P = 0.002) and ileal samples (P = 0.0152), while CON versus OAB and OEO versus OAB did not differ significantly.

Table 3 - Morphometric analysis and determination of PCNA in intestinal samples.

Sample		Treatment			SEM	Significance (P)
		CON	OEO	OAB		
Duodenum	Villus height (µm)	1592	1765	1712	36.9	0.154
	Crypt depth (µm)	196	198	164	11.9	0.449
	Ratio	8.9	9.52	11.17	0.59	0.278
	PCNA score	15.9^{b}	21.1^{a}	17.5^{b}	0.55	P < 0.001
Jejunum	Villus height (µm)	1286	1455	1377	37.1	0.185
	Crypt depth (µm)	145	144	158	8.8	0.759
	Ratio	9.8	10.6	9.5	0.62	0.747
	PCNA score	19.8^{b}	23.7^{a}	22.2^{ab}	0.53	P < 0.01
Ileum	Villus height (µm)	691	760	734	16.7	0.196
	Crypt depth (µm)	120	120	97	6.2	0.236
	Ratio	6.0	6.8	7.9	0.36	0.104
	PCNA score	22.3^{b}	24.7^{a}	23.6^{ab}	0.38	P < 0.05

IV. DISCUSSION

The supplementation of broiler feed with either a commercial oregano essential oil product or a product based on formic, propionic acid, essential oils and plant extracts significantly improved broiler performance. These findings are in agreement with other authors who demonstrated an improvement in bird performance using products with similar composition (Spais et al., 2002). While organic acids have been shown to increase villus height and other morphometric parameters (Garcia et al., 2007), the organic acid blend used in the current study did not demonstrate such an effect. However, the proportion of acids, dietary inclusion and presentation are all factors that could influence efficacy on intestinal morphology. While oregano supplementation has been shown to increase villus height in birds (Silva et al., 2009), results from the current study did not repeat these findings, although villi were numerically longer in birds fed oregano than controls. PCNA is a co-factor of DNA polymerase-δ, expressed in dividing cells during the growth phase (Bravo et al., 1987). The activity of nutrient assimilation aligns with the proliferative activity of enterocytes, enhancing healthy tissue turnover and maintenance (Garcia et al., 2007). The current study demonstrated an effect of oregano and, to a lesser extent, organic acid/essential oil/plant mixtures on the number of dividing enterocytes. Similarly, Silva et al. (2009) observed an increase in PCNA in intestinal cells of birds fed oregano essential oil. This observation in conjunction with improved bird performance in these treatments suggest nutrient utilisation and perhaps absorption was improved, despite an apparent lack of significant increase in gut villus length. The use of oregano essential oil in feed improved the antioxidant status of the bird as seen by lower MDA levels in breast and thigh muscles, an effect observed previously (Fonseca-García et al., 2017). Improved antioxidant status in the muscle can result in improved meat quality and longer storage time. In conclusion, the supplementation of broiler feed with either a commercial oregano essential oil product or a product comprising organic acids, essential oil and plant extracts can improve the performance of broilers Oregano oil also has the potential to improve meat quality.

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BACILLUS AMYLOLIQUEFACIENS IMPROVES PERFORMANCE AND GUT INTEGRITY IN BROILERS FED LOW PROTEIN DIETS UNDER NECROTIC ENTERITIS CHALLENGE

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Summary

The objective of this study was to investigate how the interaction between feeding probiotic and different dietary protein levels impacts performance, caecal bacterial population, gut permeability and serum uric acid of chickens under subclinical necrotic enteritis (NE) challenge. The study consisted of two 2 × 2 factorial arrangements of treatments and birds were evaluated from 0 to 24 days of age. Initially, birds subjected to NE challenge (yes or no) and probiotic treatment (yes or no) under standard protein feeding regime (21.5 g/kg) were compared; then data from birds fed with two different crude protein levels (standard (21.5 g/kg) or low (19.5 g/kg)) with or without supplementation of probiotic all under NE challenge condition were analysed. Dietary concentration of probiotic was 1 x 10^6 CFU of *Bacillus amyloliquefaciens* CECT 5940 per g of feed. All diets were iso-caloric and formulated to contain same amount of digestible essential amino acids. There were no interaction effects and hence only main effects are presented and discussed. Results indicate that weight gain (WG) and feed conversion ratio (FCR) ($P < 0.05$) were significantly reduced in NE challenged birds. Feeding probiotic increased WG ($P < 0.001$) and feed intake (FI) ($P < 0.01$) and decreased FCR ($P < 0.05$) followed by an increase in caecal *Ruminococcus* numbers under both non-challenge and challenge conditions but the effect was more pronounced under NE challenge. Furthermore, probiotic supplementation decreased FCR and increased caecal *Ruminococcus* population in birds fed low protein diets ($P < 0.01$) Additionally, serum uric acid levels were lower in birds fed low protein diet ($P < 0.001$). These findings suggest that supplementation of *Bacillus amyloliquefacens* CECT 5940 can help to improve performance and/or gut microflora in NE challenged birds fed either standard or low protein diets.

I. INTRODUCTION

The withdrawal of antibiotics has caused an increased prevalence of necrotic enteritis (NE) in broiler chickens, making it the most common disease in the poultry-industry globally. Direct-fed microbials (DFMs) have been shown to have great potential as alternatives to antibiotic growth promoters (AGPs). *Bacillus amyloliquefaciens* produces several extracellular enzymes including cellulase, hemicellulose, amylase, xylanase and proteases. Its application has been reported to enhance digestibility and absorption of nutrients and to improve immune function of the gut (Lee et al., 2008). The objective of this study was to determine to what extent *Bacillus amyloliquefaciens* is able to ameliorate the negative effect of subclinical NE in chickens fed low or standard protein diets.

II. METHOD

A total of 720 d-old Ross 308 chicks (as hatched) were randomly allocated to six treatments, with eight replicates pens per treatment, each with 15 birds. Treatment groups were as listed:

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Table 1 - Experimental treatments.

No.	Treatment
T1	Non-challenged – Standard protein diet
T2	Non-challenged – Standard protein diet + Probiotic
T3	Challenged – Standard protein diet
T4	Challenged – Standard protein diet + Probiotic
T5	Challenged – Low protein diet
T6	Challenged – Low protein diet + Probiotic

Treatments were analysed in two 2 × 2 factorial arrangements to observe the different main effects. The first comparison was subclinical NE challenge (yes or no) and supplementation of probiotic (yes or no) (T1, T2, T3 and T4). The second analysis compared two different crude protein levels (standard (21.5 g/kg) or low (19.5 g/kg)) and supplementation of probiotic (yes or no) all under subclinical NE challenge (T3, T4, T5 and T6). Dietary concentration of probiotic was 1 x 10^6 CFU of *Bacillus amyloliquefaciens* CECT 5940 per g of feed. The experimental diets were fed *ad libitum* for the duration of the trial period, and were fed as starter (d 0-10), and grower (d 11-24). On d 9, birds in the challenged groups were subjected to an oral gavage of 1 ml *Eimeria* strains. Non-challenged group were inoculated with 1 ml of PBS. On days 14 and 15, birds were inoculated with approximately 10^8 CFU of *Clostridium perfringens* NE18 strain, control birds were inoculated with 1mL of sterile thioglycollate broth. All birds and feed were weighed on d 0, 10 and 24. Average body weight gain (WG), average feed intake (FI) and feed conversion ratio (FCR) were calculated taking mortality into account. On d 16, birds were inoculated with 1 ml dilution of FITC-d (4.17 mg/kg body weight) and serum samples were obtained at 2.5 hours post-inoculation, for gut permeability and uric acid analysis. Serum FITC-d was measured by using a microplate reader (Synergy HT, Multi-mode microplate reader, BioTek Instruments, Inc., VT, USA). Caecal digesta samples were also collected on d 16, for bacterial quantification by quantitative PCR (Shannon et al., 2007).

III. RESULTS

Treatment had a significant impact on broiler performance from d 0-24. According to the results presented in Table 2, NE challenge significantly reduced WG and FI and resulted in higher FCR ($P < 0.001$). On the other hand, probiotic significantly improved WG ($P < 0.001$), FI ($P < 0.004$) and FCR ($P < 0.001$) in birds. Serum samples of the challenged birds had lower uric acid concentration ($P < 0.001$) and higher FITC-d content ($P < 0.001$) compared to the unchallenged birds. Furthermore, analysis of the caecal bacterial population showed that NE challenge decreased the number of *Ruminococcus* ($P < 0.001$) and *Bacillus* ($P < 0.01$). However, probiotic addition significantly increased *Bacillus* and *Ruminococcus* population ($P < 0.05$).

Regarding the results in Table 3, birds fed low protein diets had a higher FCR compared to those fed standard protein ($P < 0.001$) and the addition of the probiotic improved the FCR ($P < 0.05$). Low protein diet groups had less uric acid present in the serum ($P < 0.001$) compared to those fed the standard protein diet. Also, *Ruminococcus* population in birds fed low protein diets increased ($P < 0.01$) by probiotic supplementation.

Table 2 - Effect of probiotic and NE challenge on performance of broilers fed with standard protein diets (d0-24), gut integrity, uric acid levels and bacteria population (day 16).

Main effects		WG (g/bird)	FI (g/bird)	FCR	FITC-d[1] (ug/mL)	Uric acid (mg/dL)	Bacteria population (Log_{10})	
							Ruminococcus	*Bacillus*
Probiotic	No	721[b]	959 [b]	1.332 [a]	0.24	8.26	9.85 [b]	7.51
	Yes	795 [a]	1035 [a]	1.307 [b]	0.20	7.77	10.0 [a]	8.02
Challenge	No	829 [a]	1064 [a]	1.284 [b]	0.16 [b]	9.39 [a]	10.2 [a]	8.13 [a]
	Yes	687 [b]	930 [b]	1.353 [a]	0.28 [a]	6.66 [b]	9.7 [b]	7.40 [b]
P- value								
Probiotic		<0.001	0.004	0.005	0.146	0.236	0.046	0.021
Challenge		<0.001	<0.001	<0.001	< 0.001	<0.001	< 0.001	0.002
Challenge×Probiotic		0.527	0.670	0.812	0.632	0.712	0.052	0.405

[a, b, c] means in a column not sharing a common letter are significantly different ($P < 0.05$). [1]fluorescein isothiocyanate dextran.

Table 3 - Effect of low protein diets and probiotic on performance of broilers under necrotic enteritis challenge (d0-24), gut integrity, uric acid levels and bacteria population (day 16).

Main effects		WG (g/bird)	FI (g/bird)	FCR	FITC-d[1] (ug/mL)	Uric acid (mg/dL)	Bacteria population (Log_{10})	
							Ruminococcus	*Bacillus*
Protein[2]	Standard	690	930	1.353 [b]	0.27	6.66 [a]	9.69	7.40
	Low	667	950	1.436 [a]	0.27	5.12 [b]	9.82	7.36
Probiotic	No	665	938	1.410 [a]	0.29	6.11	9.61 [b]	7.17
	Yes	689	941	1.369 [b]	0.26	5.67	9.91 [a]	7.60
P- value								
Protein		0.380	0.520	< 0.001	0.943	< 0.001	0.248	0.891
Probiotic		0.310	0.915	0.022	0.168	0.252	0.007	0.121
Protein×Probiotic		0.117	0.051	0.301	0.584	0.773	0.605	0.347

[a, b, c] means in a column not sharing a common letter are significantly different ($P < 0.05$). [1]fluorescein isothiocyanate dextran. [2] Crude protein levels (standard: 21.5 g/kg and Low: 19.5 g/kg).

IV. DISCUSSION

Disturbances in broiler intestinal microbial population can result in increased levels of pathogenic organisms and this has consistently been found to negatively impact FI, WG and FCR (Remus, et al., 2014). Probiotics have been shown to improve the development and maintenance of a stable gut microbiome in poultry, which leads to reduced enteric disease and improved growth performance (Ducatelle, et al., 2015). *Bacillus amyloliquefaciens* is known to produce enzymes that may contribute for improved digestibility of nutrients (Gangadharan et al., 2008). Therefore, the beneficial effects of the probiotic on broiler performance observed in this study could be attributable to better gut health resulting in better utilisation of nutrients. On the other hand, feeding high protein diets has a negative impact on gut health of birds as a result of increased flow of indigestible protein to the large intestine thus increasing metabolite production (Apajalahti and Vienola, 2016). Serum responses provide some evidence of non-essential nitrogen utilisation by the chicks. Lower uric acid observed in birds fed with low protein diets indicates lower nitrogen excretion compounds. A numerical reduction of uric acid concentration in serum of birds fed the probiotic could also be related to better utilisation of protein and amino acid for synthesis rather than metabolism, as uric acid is the major end-product of protein metabolism in birds. This could also be a reason for improved performance in probiotic fed groups. Probiotics also help to stabilise the intestinal ecosystem and enhance the growth of beneficial bacteria. *Bacillus* and *Ruminococcus* are favourable groups of bacteria that were increased by probiotic supplementation. Production of secondary metabolites and

quorum quenching activity by *B. amyloliquefaciens*, could have supressing effects on the pathogenic bacteria (Doranalli et al., 2017) creating a better growth environment for the beneficial groups. This is reflected in the slight impovment of gut integrity in probiotic supplemented treatments. The positive result could be due to a better gut enviroment and less inflammation and damage to the epithelium cells creating a stronger tight junction in the epithelium. This study concludes that the supplementation of *Bacillus amyloliquefaciens* in diets can help achieve optimal performance through the modulation of gut health and gut bacteria in broilers under subclinical NE challenge, and improve feed efficiency in broilers fed low protein diets.

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ORGANIC ACIDS AND ESSENTIAL OILS COMBINED WITH AN ANTIBIOTIC GROWTH PROMOTER TO IMPROVE GUT HEALTH AND NUTRIENT DIGESTIBILITY IN CHALLENGED BROILERS CHICKENS

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There is increasing pressure for the use of antibiotic growth promoters (AGPs) in poultry diets to be discontinued or reduced. In this context, the search for natural additives such as organic acids (OA) and essential oils (EO) has increased. Previous studies have reported beneficial effects on gut health of supplementing poultry diets with these natural feed additives. As in many countries the use of AGP is still allowed and the adoption of programs combining the effects of AGPs with feed additives is a reality in the field, the objective of this study was to investigate the dietary supplementation of protected organic acids and essential oils P(OA+EO) in combination with an AGP in *Eimeria* spp. and *Clostridium perfringens* challenged broiler chickens.

A total of 256 male broiler chickens Cobb 500 was allocated in cages from 1 to 28 d of age. The experiment followed a randomized design, with 4 replicates of 16 birds for each one of the 4 treatments: negative control (NC), positive control (PC), AGP (Enramycin at 10 ppm) and AGP + P(OA+EO). Except those of the NC group, all birds were challenged with *Eimeria* spp. at 1 d and *Clostridium perfringens* at 11, 12 and 13 d. The diet was corn and soybean-based (Rostagno et al., 2011). Analysis of ileum morphology and CD4+ and CD8+ lymphocyte counts was performed at 7, 14, 21 and 28 d and ileal digestibility at 28 d. Gut health was assessed by the I See Inside scoring system (ISI), according to Kraieski et al. (2017).

Compared to the NC group, the PC group had higher ileum villus necrosis at 14 d ($P < 0.05$), tissue congestion at 21 d ($P < 0.05$), infiltration of inflammatory cells in the lamina propria ($P < 0.05$) at 28 d, as well as CD4+ and CD8+ counts at 7, 14 and 21 d ($P < 0.01$), showing that the challenge was efficient in inducing an inflammatory response. Compared to the PC group, the AGP + P(OA+EO) had lowest ISI score for villus apical necrosis ($P \leq 0.02$) at 14 d, for congestion ($P < 0.01$) at 21 d, for infiltration of inflammatory cells in the lamina propria at 28 d and, higher counts of CD4+ and CD8+ at 7, 14 and 21 d ($P < 0.01$). At 28 d, the ileum cell dynamic of the AGP + P(OA+EO) group suggested a recovery of intestinal mucosa due to an increase of enterocyte proliferation, higher number of CD4+ cells and a reduction of CD8+ cells ($P < 0.01$) compared to the PC group. In addition, the AGP + P(OA+EO) group had higher coefficients of digestibility of DM ($P < 0.01$), CP ($P \leq 0.08$), and energy ($P < 0.01$) compared to all other treatments. The metabolisable energy was also higher for the AGP + P(OA+EO) group (184 kcal difference to the AGP group; $P < 0.01$). The greater number of alterations in the ileum observed in the ISI analysis and by immunohistochemistry for the PC groups compared to the NC group indicates that the challenge model was efficient. The supplementation with a blend of P(OA+EO) combined with an AGP seems to ameliorate the negative effects on gut health parameters caused by the *Eimeria* spp. and *C. perfringens* challenges in addition to improving dietary nutrient digestibility. Therefore, the combination of the additives would be an efficient management in the gradual transition process towards to the total antimicrobial withdrawal.

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SCREENING OF HIND GUT ACTIVE COMPOUNDS WITH AND WITHOUT A FOREGUT ACIDIFIER IN THE ABSENCE OF ANTIBIOTICS

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Summary

The use of antibiotics as growth performance enhancers has been cited as a potential cause of antimicrobial resistance Landers et al. (2012) and World Health Organisation (2018). As such, many regions have banned the use of antibiotics in animal production for prophylactic or growth performance activity. In these jurisdictions, antimicrobial compounds may only be prescribed by a veterinarian for therapeutic purposes and none may be used that are considered important for human health. Many alternative therapies are available as feed additives in the broiler production market as reported by Gadde et al. (2017) and have shown benefits when used both with and without antibiotic intervention. As producers look to a reliable antibiotic alternative, it is clear, as shown by Dibner and Richards (2005), that no single intervention will support the immunity and growth of broilers independently. They also showed that withdrawal of antibiotic growth promoters has the greatest negative effect on feed conversion. This study was designed to look at multiple interventions shown to have some benefit in hind gut health and microbiota modulation; these were then tested both alone and in combination with an effective foregut acidifier. This study found that, of the nine interventions including four in combination, only four performed significantly differently and only one treatment showed improved performance beyond that seen in the antibiotic control. The negative control containing no antibiotics or other additive with antimicrobial function performed as well as the antibiotic treatment, suggesting there was no significant adverse effect of bacterial load in this study. One treatment consisted of a water-based foregut acidifier; this treatment alone, in combination with either a slow release organic acid compound, or copper hydroxy analogue and copper hydroxy analogue alone resulted in less feed required to reach equivalent bodyweight to other treatments in the first 10 days of growth. Performance by 35 day harvest had become equal across all treatments. Foot pad lesions were least evident in antibiotic treatment, fore and hind gut acidifier treatments, essential oil alone and in both copper treatment groups. Tibial head lesions were found half as often in combination fore and hind gut acidification treatment group and were not significantly different from either essential oil alone or copper hydroxy analogue in combination with foregut acidifier. No treatments had significant deviation from the antibiotic control in flock uniformity, antibody titre response, intestinal bacterial enteritis score, carcass weight and dressing percentage, *Lactobacillus* or *Clostridium* counts in ileum and *Salmonella* in litter or litter score in this study.

I. INTRODUCTION

Antibiotics are critical in the treatment of infection due to microbial ingress in both food animal and human populations (Landers et al., 2012). Some classes of antibiotics are becoming less effective due to overuse and microbial adaptation leading to resistance to treatment. The pool of available, effective antibiotics is shrinking as this resistance increases. The European Union, Thailand, Indonesia and Vietnam are some of the largest food producing jurisdictions that have banned the prophylactic use of antibiotics as a growth promoter in all animal production and

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the number of countries expected to follow suit is continuing to grow. There is already restricted use in Mexico, Japan and South Korea and more countries have a planned phase out of antibiotics with China pledging to ban AGPs in the next 5 years. Alternatives to antibiotics are being used and sought to ensure the health and performance of broilers, layers and other farmed species across the world. There are many feed ingredient alternatives that have been tested and shown variable response (Mehdi et al., 2018), yet most studies have been conducted in isolation. This study was designed to look at the effectiveness of four separate additives that have shown improvements in animal performance and hind gut health independently. These additives were tested both as a stand-alone treatment and in combination with an effective water-delivered foregut acidifier. This screening protocol was intended to show which treatment intervention was the most effective in supporting performance, health, environmental burden and bird integrity.

II. METHOD

A total of 1,512 male Arbor Acres Plus broilers was randomly assigned to one of 9 treatments (Table 1). This consisted of 168 birds in each treatment in 8 pens containing 21 birds each. These birds were fed for 35 days and then processed. Birds were housed on re-used litter. Diets were based on corn and soy bean meal containing maximum 7% corn DDGS with 3.4% meat and bone meal. All diets were identical in makeup except the copper methionine hydroxy analogue (MHA) treatments which were balanced for methionine content and had supplemental inorganic copper removed. Treatments three and four included a combination of calcium formate, silicic acid and benzoic acid contained within a vegetable fat matrix at 500ppm for release and activity in the lower gastrointestinal tract. Treatments five and six included a combination of carvacrol and thymol presented in a matrix protected form and dosed at 60ppm in the 14-day starter phase, then 30ppm for the remaining 21 days for release and activity in the lower gastrointestinal tract. Treatments seven and eight included copper in the form of copper MHA at 30ppm. In treatments four, six, eight and nine, water acidification was achieved using a blend of liquid MHA, formic and propionic acid at 0.2 ml per litre to target a drinking water pH of four. Measurements taken at the beginning of the study were litter score, flock uniformity, diet proximate analysis and litter score for *Salmonella* levels. Microbiota analysis was done by sacrificing 1 bird per pen at 21 days and collecting ileum contents. These samples were tested for *Lactobacillus* and *Clostridium* content. Antibody titration against NB (HI test), IB (HI test) and IDB (ELISA) was measured in 2 birds per pen at 28 days. Intestinal health was scored by an independent veterinarian at 35 days on 2 birds per pen at processing. Carcass measurements at processing included carcass yield, foot pad lesions, tibial head lesions and liver weight. Statistical analysis was conducted using Duncan's multiple range test where $P < 0.05$ was considered to be significant.

III. RESULTS

A differentiation in growth performance was found only at 10 days, when feed conversion and feed intake with water acidification alone and combination of water acidification and hind gut organic acid release resulted in lower feed intake ($P < 0.01$) with the combination resulting in greater feed conversion efficiency (FCR, $P<0.05$ Table 2)**.** No treatment resulted in significant improvement to bodyweight, feed conversion or uniformity by day 35 of the study. Results at 21 days when small intestine contents were analysed for *Lactobacillus spp* and *Clostridium perfringens* content showed numerical differences across treatments with copper treatments trending higher for both colonies but no significant differences and with no differences seen between antibiotic and negative control groups.

Table 1 - Treatments.

Treatment	Dietary Inclusion
1	Antibiotic control, contains zinc bacitracin positive control (PC)
2	Negative control contains no antibiotics or additive treatment negative control (NC)
3	Contains lipid matrix organic acid blend 500ppm (ACID HIND)
4	Contains lipid matrix organic acid blend 500ppm, birds received water acidifier target pH of 4 (ACID HIND AND FORE)
5	Contains matrix encapsulated essential oils carvacrol and thymol starter 60ppm, grower/ finisher 30ppm (EO HIND)
6	Contains matrix encapsulated essential oils carvacrol and thymol starter 60ppm, grower/ finisher 30ppm, birds received water acidifier target pH of 4 (EO HIND AND ACID FORE)
7	Contains 30ppm copper as methionine hydroxy analogue chelate (COPPER HIND)
8	Contains 30ppm copper as methionine hydroxy analogue chelate, birds received water acidifier target pH of 4 (COPPER HIND AND ACID FORE)
9	Birds received water acidifier target pH of 4 (ACID FORE)

Table 2 - Feed conversion efficiency.

	FCR 0-10d	FCR 0-35d
T1	1.016	1.348
T2	1.017	1.354
T3	1.014	1.351
T4	1.001**	1.346
T5	1.017	1.352
T6	1.017	1.347
T7	1.007*	1.342
T8	1.007*	1.347
T9	1.006*	1.349

** $p<0.05$ * numerically different not significantly different from other treatments

This suggested little to no challenge to intestinal health; this was reinforced with no difference seen in bacterial enteritis score, antibody titer response or litter *Salmonella* count among treatments. Results at harvest showed no difference between treatments for dressing percentage, or any other parameter other than slightly lighter drumstick by percentage in the antibiotic fed group T1 compared with all other treatments $P < 0.05$. Carcass integrity markers showed numerical deviation with T4 tibia head lesion (THL) score almost half the rate of incidence of that seen in the antibiotic fed group T1 (Table 2). The negative control group T2 THL score was 39% greater than the antibiotic treatment. Copper MHA treatment T7, alone and the combination of fore and hind gut active organic acids T4, had the only numerical advantage over the antibiotic fed birds for the incidence of clean footpads (Table 2).

IV. DISCUSSION

Although the study was conducted in an environment which had been designed to allow space for antimicrobial activity due to a moderate rate of pressure, the birds remained healthy. The birds were housed on re-used litter, offered feed with maximum levels of DDGS and MBM and were held at maximum pen density. This study did give an excellent indication of how a range of products could perform, both alone and in combination, when very little performance pressure was applied. The key indicator of the healthy flock was the equivalent performance of the negative control treatment T2 to the zinc bacitracin fed group T1. The key indicator of the healthy flock was the equivalent performance of the negative control treatment T2 to the

zinc bacitracin fed group T1. The only area of difference between the antibiotic treatment and control was in the tibial head lesion score which might suggest some level of gut barrier failure and the litter *Salmonella* score which was numerically though not significantly higher. The most interesting performance belonged to the water acidification treatment T9, and the effect in combination with a slow release hind gut active organic acid blend T4 and the copper hydroxy analogue chelate T8. Results from this study would suggest that the most consistent performance improvement can be gained by including copper hydroxy analogue, copper hydroxy analogue with an effective foregut acidifier and the combination of water acidifier and hind gut effective organic acid blend. Arbe and Bekker (2017) showed how inclusion of copper methionine hydroxy analogue could increase the performance of broiler birds at lower inclusion levels than inorganic salts and Hassan et al. (2010) showed the benefit of organic acid inclusion without an antibiotic growth promoter which supports the trends seen in this study. The other notable trend was for birds that grew more efficiently during the critical first 10 days T4, T7, T8, T9 to have greater carcass integrity at 35 days, resulting in healthier foot pads and tibia joints with fewer lesions.

Table 3 - Results at harvest.

TRT	Dressing %	Liver weight	Footpad clean, score 0	THL score
T1	76.27	2.87	58.33	0.396
T2	76.15	2.85	50.00	0.646
T3	76.23	2.89	35.42	0.375
T4	76.24	2.91	62.50	0.208
T5	76.04	2.92	54.17	0.292
T6	75.92	2.94	39.58	0.542
T7	76.20	2.78	60.42	0.396
T8	75.61	2.91	56.25	0.313
T9	76.02	2.95	47.92	0.458

THL (Tibial Head Lesion), footpad clean, score 0 shows no sign of injury or dermatological damage

These results have given some indication of the best performing lower gastrointestinal tract candidates which will be used in conjunction with a range of feed sanitisers and acidifiers in stage two of the study to determine the most effective combination.

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IDEAL AMINO ACID PROFILE AND CHICKEN GUT DISTURBANCE: A REVIEW

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Summary

There is considerable interest in the development of low protein diets with supplemental amino acids for broiler chickens due to economic, environmental and bird welfare advantages. However, under commercial feeding conditions, chickens are exposed to challenges of infectious or non-infectious origin. Reduced protein diets may result in amino acids being redistributed away from growth and production processes toward intestinal cells involved in immune and inflammatory responses and an unbalanced supply of amino acids in the diet can be deleterious to the chicken gut immune system. Therefore, an ideal balance of dietary amino acids (AA) is crucial for broiler chicken gut health, particularly under an antibiotic-free production system.

I. INTRODUCTION

There is considerable interest in the development of low protein diets balanced with supplemental amino acids for broiler chickens due to economic, environmental and bird welfare advantages (Moss *et al*., 2018). However, under commercial feeding conditions, chickens are exposed to various challenges of infectious and non-infectious origin. For challenges of infectious origin, even without any clinical signs of disease, animals affected by chronic subclinical disease or intestinal parasites use nutrients less efficiently for production than healthy animals. Challenges of non-infectious origin such as heat stress, mycotoxins and other anti-nutritional factors also have impacts on nutrient digestibility. Therefore, it may be necessary to set the intestinal requirements of some amino acids higher than recommended in order to avoid compromising the immune system (Bortoluzzi *et al*., 2018). It is reported that reduced protein diets may result in amino acids being redistributed away from growth and production processes, toward intestinal cells involved in immune and inflammatory responses (Le Floc'h et al., 2004). Further, reduced-protein diets may change amino acid availability and promote negative interactions among amino acids (Nascimento *et al*., 2016). In addition, an unbalanced supply of amino acids in the diet can be deleterious to the immune system (Li *et al*., 2007). Thus, an ideal balance of AA is crucial for broiler chicken gut health in particular if birds are reared without antibiotics.

II. AMINO ACID NUTRITION AND DISTURBANCE OF INTESTINAL FUNCTION

Necrotic enteritis (NE) is a multifactorial, bacterial disease caused by *Clostridium perfringens* (CP) which produces a variety of extracellular toxins and invasive enzymes in the broiler chicken gut. It is widely believed that coccidiosis, wheat or barley-based diets with a high proportion of fish meal and removal of in-feed antibiotics are major disposing factors (Kaldusdal *et al*., 2016). Due to a damaged intestinal mucosa, subclinical NE usually results in poor body weight gain associated with reduced feed intake (Keerqin *et al*., 2017), and it is assumed that needs for some functional amino acids for immunity will be met by mobilizing skeletal muscle protein (Reeds and Jahoor, 2001). Compared to soybean meal, addition of fish meal might result in deficiency in arginine (Arg), leucine (Leu), isoleucine (Ile), aspartic acid (Asp), histine (His), phenylalanine (Phe) and glutamic acid (Glu) (Figure 1, adapted from Lemme *et al*., 2004). Compared to maize, use of wheat or barley might lead to a diet deficient

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in alanine (Ala), Leu and Asp (Figure 2, adapted from Lemme *et al*., 2004). Adding Arg has been demonstrated to reduce intestinal mucosal disruption during a coccidial challenge (Tan *et al*., 2014). Leu, Ile, Asp and Glu play vital roles in the metabolism and function of leucocytes and lymphocytes. Glutamine is important for maintaining the integrity of the gut barrier and the structure of the intestinal mucosa. Increasing total methionine levels from 0.35 to 1.2% in the diet of chickens infected with Newcastle Disease virus markedly enhanced immune responses: T-cell proliferation, plasma IgG levels, leucocyte migration and antibody titres (Li *et al*., 2007). Furthermore, threonine (Thr), Arg and Glu may help to minimize over-activation of the innate immune system and modulate the intestinal microbiota (Bortoluzzi *et al*., 2018). It is noteworthy that, although AA are required for the synthesis of a variety of specific proteins to sustain normal immunocompetence and protect the animal from a variety diseases, an imbalanced supply of AA in the diet can be deleterious to the immune system (Li *et al*., 2007). Recently, in an NE challenge trial conducted at the University of New England (UNE), challenged at the age of 9 days, birds fed diets formulated with an ideal AA profile completely recovered at the age of 35 days (Keerqin *et al*., 2017). Considering that the intestinal immune system is responsible for initiating and propagating responses to commensal and pathogenic microorganisms, ideal AA profile may play an important role in alleviating chicken gut disturbance.

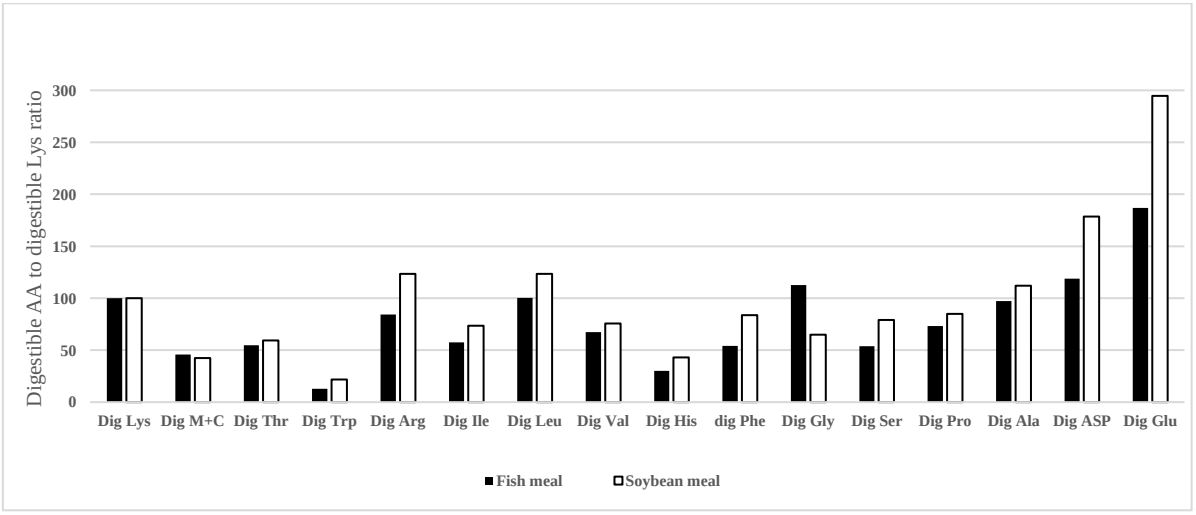

Figure 1 - Digestible AA profile in fish meal and soybean meal.

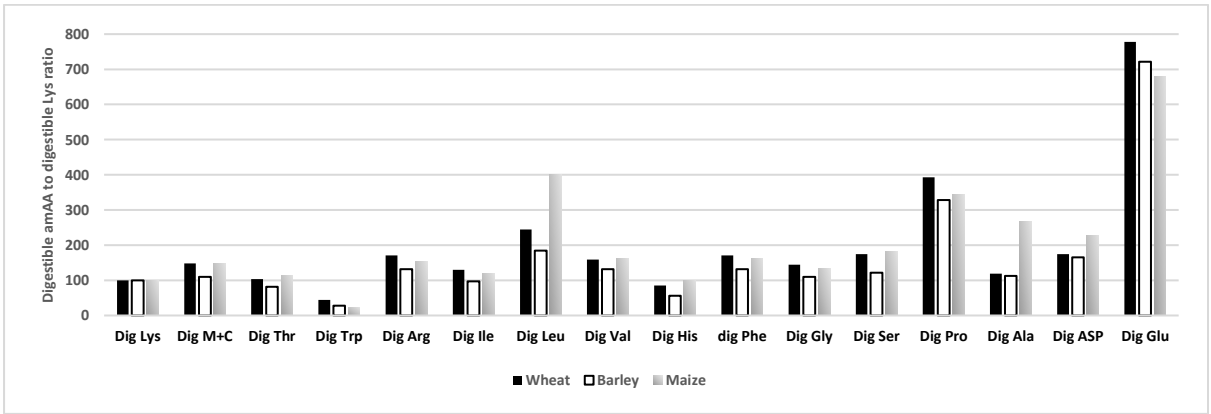

Figure 2 - Digestible AA profile in wheat, barley and maize.

III. IDEAL AA PROFILE FOR BROILER CHICKENS

The ideal AA profile, with AA in ratio to lysine, was first proposed and tested in broiler chickens by Baker and Han (1994). Because the standardized ileal digestibility (SID) values of AAs are more likely to be additive in mixed diets, most ideal AA profiles for broiler chickens are provided based on SID values. Table 1 summarizes several ideal AA profiles based on essential AA SID values. Although there are no significant differences in AA ratios to SID lys among those ideal AA profiles, dietary SID Lys concentration will have strong impact on the order of dietary limiting AA.

Table 1 - Ideal AA profiles from different sources.

Phase	Lys	Met	M+C	Thr	Trp	Arg	Ile	leu	Val
Cobb 500									
0- 10 d	1.18	0.45	0.88	0.77	0.18	1.24	0.79		0.89
11-22 d	1.05	0.42	0.80	0.69	0.17	1.10	0.70		0.80
23- d	0.95	0.39	0.74	0.65	0.17	1.03	0.65		0.73
Ross 308									
0- 10 d	1.28	0.51	0.95	0.86	0.20	1.37	0.86	1.41	0.96
11-22 d	1.15	0.47	0.87	0.77	0.18	1.23	0.78	1.27	0.87
23- d	1.03	0.43	0.80	0.69	0.17	1.10	0.71	1.13	0.78
Rostagno *et al.*, 2011 (in Nascimento *et al.*, 2016)									
0- 10 d	1.31		0.94	0.85	0.25	1.42	0.88	1.71	1.01
11-22 d	1.18		0.85	0.76	0.22	1.27	0.79	1.61	0.90
23- d	1.04		0.76	0.68	0.20	1.12	0.71	1.50	0.82
Ratio to Digestible Lys									
Cobb 500	100	38	75	65	16	105	67		75
	100	40	76	66	16	105	67		76
	100	41	78	68	16	108	68		77
Ross 308	100	40	74	67	16	107	67	110	75
	100	41	76	67	16	107	68	110	76
	100	42	78	67	16	107	69	110	76
Rostagno *et al.*, 2011	100		72	65	19	108	67	130	77
	100		72	65	19	108	67	130	77
	100		72	65	19	108	68	130	77

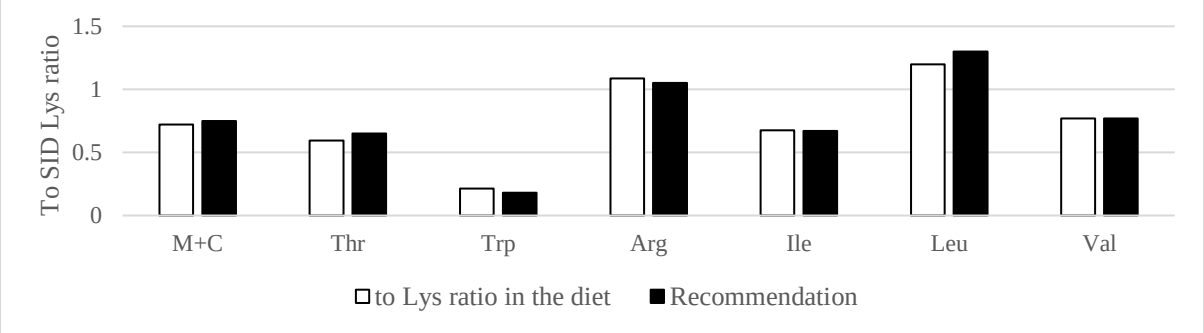

Figure 3 - The dietary AA profile in Table 2 compared with Cobb 500 recommendation.

Table 2 - The feed formulation of a typical wheat-soybean meal-based diet (Wu *et al*., 2015).

Ingredients	Starter	Ingredients	Starter
Wheat	60.3%	Lysine.HCL	0.27%
Soybean meal	24.2%	Methionine	0.21%
Canola meal (solvent)	9.6%	Threonine	0.03%
Meat meal	1.7%	Choline Cl 70%	0.11%
Canola oil	1.8%	Mineral premix	0.075
Limestone	1.1%	Vitamin Premix	0.05%
Salt	0.31%	Xylanase	0.005%
Sodium bicarbonate	0.20%		

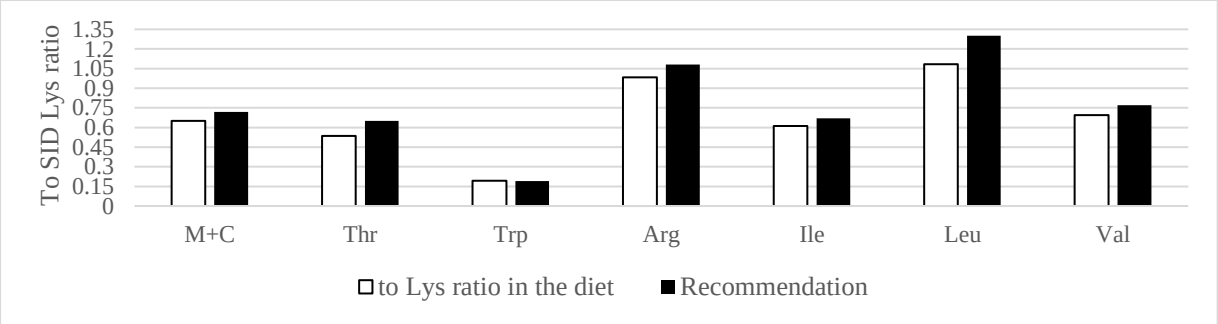

Figure 4 - The dietary AA profile compared with Rostagno's recommendation.

For a typical wheat-soybean meal-based diet shown in Table 2, 1.20% SID Lys was used to formulate the starter diet. Obviously, this diet perfectly matched the ideal AA profile recommended for Cobb 500 chickens (Figure 3). However, compared with the ideal AA profile recommended by Rostagno (Nascimento *et al.*, 2016) (Figure 4), almost all essential amino acids were deficient in this diet, potentially disturbing gut health of the bird.

In conclusion, an ideal AA profile in broiler chicken diets may alleviate gut disturbance. When dietary Lys concentration is increased, other essential AA concentrations also need to be increased accordingly.

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HYDROXY-SELENOMETHIONINE CAN IMPROVE PRODUCTIVE PERFORMANCE AND EGG QUALITY OF LAYING HENS IN THE LATE PHASE OF PRODUCTION

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Due to the world's population increase, egg production is expected to develop rapidly and, to increase egg production, the goal is to enhance laying persistency while maintaining egg quality (Bain et al., 2016). As they age, laying hens gradually decrease productivity and eggshell quality (Dunn, 2013). Oxidative stress is an important factor of ageing and selenium (Se), being an essential mineral involved in several antioxidant processes, can play an important role influencing both laying performance and egg quality (Surai et al., 2018). Hydroxy-selenomethionie (OH-SeMet) has been proven to be a more efficient Se source than sodium selenite (SS) to improve poultry performance particularly in critical periods of their production cycle (Surai et al., 2018). The aim of this trial was to evaluate the effects of OH-SeMet on productive performance and egg quality of laying hens from 50 to 70 weeks of age.

A total of 384 Dekalb Brown laying hens (average weight 1.86 kg) was randomly assigned to two treatments (12 replicates; 16 hens each). The two experimental diets were supplemented at 0.3 mg Se/kg feed supplied by either SS or OH-SeMet. Standard production parameters were recorded. Egg quality traits analyzed included weight and percentage of albumen, yolk and eggshell, eggshell thickness, eggshell strength, Haugh unit and yolk color. Data were analyzed by Student t-test for independent samples ($P < 0.05$).

Table 1 - Performance and egg quality results of layer fed sodium selenite or OH-SeMet.

	Sodium Selenite	OH-SeMet	SEM	P-value
Egg production (%)	88.80	90.41	1.71	0.038
Egg weight (g/egg)	62.50	63.63	1.05	0.014
Egg mass (g/d)	55.50	57.54	1.56	0.004
Egg mass conversion (g/g)	2.05	1.95	0.09	0.005
Number of eggs *per* hen housed	124	126	2.40	0.030
Eggshell thickness (mm)	0.458	0.470	0.008	0.002
Strength (kg/f)	3.092	3.335	0.264	0.034

OH-SeMet increased several production parameters compared with SS (Table 1). Concerning egg quality traits, OH-SeMet improved eggshell thickness and strength as compared with SS. It can be speculated that improved eggshell quality could be linked with the higher Se concentration achievable with OH-SeMet in the shell and shell membrane. Overall, dietary supplementation of OH-SeMet, by improving both production and egg quality parameters in laying hens from 50 to 70 weeks of age more efficiently as compared to SS, appears to be an effective potential solution to prolonging the production cycle of laying hens.

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ASSOCIATION OF FEED TO EGG EFFICIENCY WITH BODY WEIGHT AND DIGESTIVE ORGAN CHARACTERISTICS IN LAYING HENS

Y. AKTER[1], P.J. GROVES[1], S.Y. LIU[1], A.F. MOSS[1], D. ANENE[1,2] and C.J. O'SHEA[1,2]

Summary

The objective of this study was to characterise the individual feed conversion ratio (FCR) of a cohort of laying hens and investigate the relationship of body weight (BW) with feed intake (FI), FCR, and digestive organ parameters. From an initial screening phase (6 weeks) using 450 Isa Brown layers (28-week-old), 50 high feed efficiency (HFE, FCR < 1.8), 50 medium FE (MFE, FCR < 2.0) and 50 low FE (LFE, FCR > 2.3) hens were identified. Individual BW, FI, egg production (EP) and egg mass (EM) were determined in 150 ISA brown (35 weeks of age) laying hens that were given a wheat-soybean meal-based mash diet for 6 weeks (41 weeks of age). To investigate the association of BW with FI and FCR, the data from the 150 birds were collected and digestive organs measurements undertaken on 10 birds per group (n = 30) randomly selected from 150 laying hens. The birds were euthanised and the weights of abdominal fat pad, liver, gizzard, total intestinal tract and pancreas measured. Both FI and FCR had significant positive correlations with the final BW of birds (r = 0.63, P = 0. 002; r = 0.44, P < 0. 01). The percentage of abdominal fat pad weight (P < 0.001) and liver weight (P < 0.01) were lowest for the HFE group followed by the MFE and then LFE groups. A lower gizzard weight in proportion to body weight was noticed in LFE group (P < 0.01) when compared with HFE group of hens. There was a strong positive association between final BW and percent abdominal fat pad weight (r = 0.95, P < 0. 001). A moderate positive relationship was noticed between final BW and percent liver weight (r = 0.44, P < 0.01) while a slight negative relationship was found between final BW and percent gizzard weight (r = - 0.31, P = 0.09) of the birds.

I. INTRODUCTION

The feed efficiency (FE) of commercial laying hens has improved steadily since genetic selection began in earnest, with modern hybrids converting approximately 2.1 kg of feed into 1 kg of eggs. Recent research suggests considerable variation in FI and FE between hens kept under common management and dietary conditions (Akter et al., 2018). Research also indicates that many factors affect FE, including body composition, the digestion and metabolism of nutrients, energy output, body activity and body temperature regulation (Herd and Arthur, 2009). However, there is little information describing the BW and digestive organ of individual hens and how these contribute to variation in flock FE. The aim of this study was to determine the associations of BW with FI, FCR and digestive organ parameters in ISA brown commercial laying hens.

II. MATERIALS AND METHODS

This work was conducted at the Poultry Research Facility, University of Sydney, Camden. All procedures used in this study were approved by the Animal Ethics Committee of the University of Sydney. All birds were housed individually in 25 × 50 × 50 cm cages for 6 weeks to facilitate individual weekly FI, daily EP and EM. After the initial screening phase, 150 hens (35 weeks

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of age; HFE, MFE and LFE; 50 hens/group) were selected from a total 450 individually housed hens. The experimental diet (wheat and soybean meal-based mash) consisted of 16.3% CP, 2,750 kcal / kg ME. Body weight for all individual bird was recorded at the start and end of the study period (35 to 41 weeks of age). FCR was calculated from weekly EP and FI over 6 weeks to verify the FE of each group. Ten birds from each of the HFE, MFE and LFE groups (n = 30) were randomly selected from a total 150 birds and euthanised by intravenous injection of Na pentobarbitone. The abdominal fat pad, liver, pancreas, gizzard and whole intestine were excised and weighed accordingly. Data were analysed using the generalised linear model procedure of SAS (SAS Institute) with FE group as the main effect. All data are presented as least square means ± standard error of the mean (SEM). Means were separated using the Tukey-Kramer method Pearson or Spearman correlation coefficients output for production traits and egg quality measurements was generated using the proc corr procedure SAS Institute Inc. The probability value which denotes statistical significance was $P < 0.05$.

III. RESULTS AND DISCUSSION

The results of this study indicate that final BW of hens was moderately correlated with FI (r = 0.63, P = 0.002; Figure 1) and FCR (r = 0.44, P < 0.01; Figure 2). This clearly supports the proposition that the more feed hens eat, the heavier they become and the higher their inefficiency to convert feed to eggs. This finding agrees with the findings reported in laying hens divergently selected for HFE and LFE (Nolan et al., 2018).

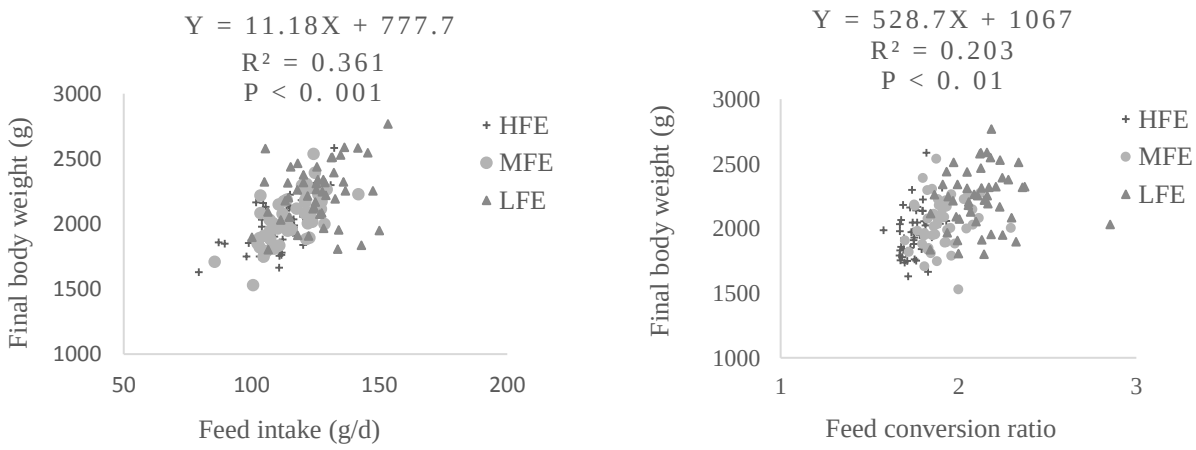

Figure 1 - Correlation of final body weight (BW) with feed intake (FI) and FCR.

The final BW and weight of the digestive organs is presented in Table 1. The final BW (P < 0.001) and both abdominal fat pad weight (P < 0.001) and percent weight of abdominal fat pad (P < 0.001) were higher in LFE. The weight of the liver and percent liver weight were lower (P = 0.002; P = 0.04) in HFE group while the percent weight of gizzard was higher (P < 0.001) in the HFE than in the LFE group (Table 1).

Table 1 - Body weights and digestive organs weights of high feed efficiency (HFE), medium feed efficiency (MFE) and Low feed efficiency (LFE) groups of hens (45 weeks old).

Measurements	HFE	MFE	LFE	*P*-value
Final body weight (g)	1852 ± 0.03[c]	2190 ± 0.07[b]	2371 ± 0.06[a]	<0.001
Abdominal fat pad weight (g)	76 ± 3.0[c]	141 ± 16.3[b]	184 ± 15.2[a]	0.001
Abdominal fat pad weight (%)	4.1 ± 0.15[c]	6.3 ± 0.54[b]	7.7 ± 0.48[a]	0.001
Liver weight (g)	42 ± 2.3[b]	65 ± 5.2[a]	67 ± 6.4[a]	0.002
Liver weight (%)	2.3 ± 0.09[b]	3.0 ± 0.25[a]	2.8 ± 0.20[a]	0.04
Intestine weight (g)	110 ± 3.4[c]	128 ± 6.5[b]	144 ± 3.4[a]	0.002
Intestine weight (%)	5.9 ± 0.16	5.8 ± 0.19	6.1 ± 0.14	0.50
Gizzard weight (g)	37 ± 1.0	40 ± 2.1	40 ± 2.6	0.46
Gizzard weight (%)	2.0 ± 0.05[a]	1.9 ± 0.06[ab]	1.7 ± 0.09[b]	0.01
Pancreas weight (g)	3.2 ± 0.19	3.2 ± 0.27	3.7 ± 0.29	0.26
Pancreas weight (%)	0.17 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.25

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

The results of correlation analysis indicated that BW showed a strong positive correlation with the percent weight of abdominal fat pad (r = 0.95, P < 0. 001; Figure 3). A moderate correlation was found between final BW and percent liver weight (r = 0.44, P < 0.01) of birds while a slight negative correlation was noticed between final BW and percent gizzard weight (r = - 0.31, P = 0.09; Figure 5) and percent pancreas weight (r = - 0.24, P = 0.2; Figure 6). Body weight differences are largely a reflection of differing amounts of fat. In agreement with the current study, Carre et al. (2008) reported that LFE birds had greater fat deposition in the body and were 13% more inefficient than efficient birds. Research indicated that the size of internal organs is related to FI (Johnson et al, 1990), as the energy expenditure of these organs increases after feeding and is dependent on FI (Seal and Reynolds, 1993).

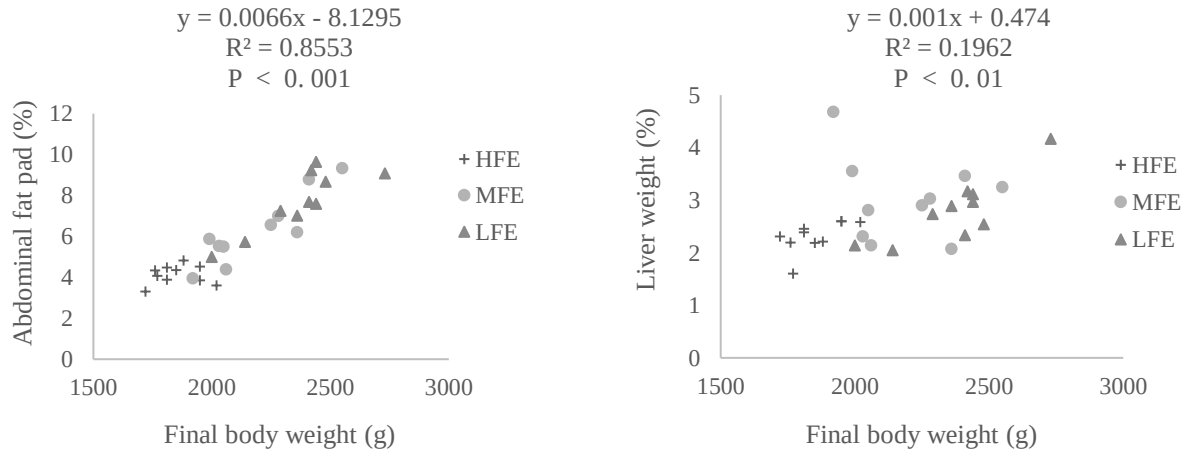

Figure 2 - Correlation between final body weight (BW) and percent weight of abdominal fat pad and liver; HFE – High feed efficiency, MFE – Medium feed efficiency and LFF – Low feed efficiency groups.

In this study, higher gizzard weight proportion to body weight in the HFE group suggests that the birds which have been specifically selected for HFE are genetically predisposed to growing a large gizzard.

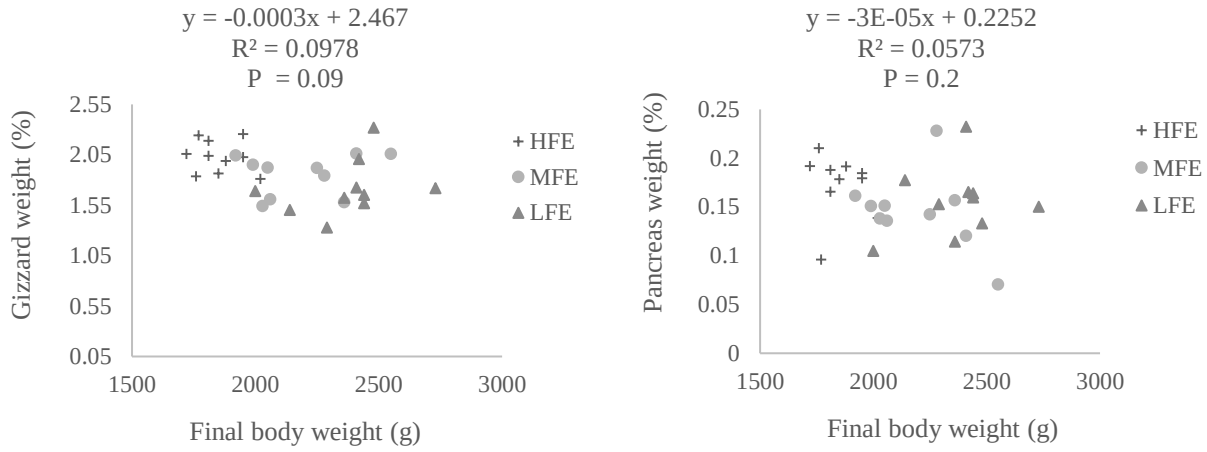

Figure 3 - Correlation between final body weight (BW) and percent gizzard weight and percent pancreas weight; HFE – High feed efficiency, MFE – Medium feed efficiency and LFE – Low feed efficiency groups.

IV. CONCLUSION

The present study indicates that HFE hens clustered in the body weight range of 1750 to 2000 g are leaner, have smaller liver and heavier gizzard in proportion to their BW. Smaller liver could be related to lower energy consumption and slower metabolic rate while bigger gizzard probably leads to greater nutrients availability in the small intestine which seems related to greater FE in the HFE hens. Clearly hens in the upper end of the BW distribution are predictably inefficient and managerial efforts must be made to reduce the proportions of these birds within flocks to improve economic performance.

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MONITORING INTAKE PATTERNS OF LAYER HENS: A LINK BETWEEN BEHAVIOUR AND FEED CONVERSION RATIO?

Y. AKTER[1], A. HUNGERFORD[2], C.E.F. CLARK[2], P. THOMSON[2], M.R. ISLAM[2] and C.J. O'SHEA[3]

Summary

Feed accounts for approximately 70% of the total cost of laying hen egg production and there is substantial variation in feed conversion efficiency between individual hens. Despite this understanding, there is a paucity of information regarding layer hen feeding behaviour and its impact on feed efficiency. We determined 49-week-old Isa Brown layer hen intake of an *ad-libitum* mash diet at 2 minute time intervals, 24 h a day, for 1 week for each of 35 high and 35 low feed conversion efficiency birds as screened from an initial flock of 450. Our findings indicate a distinct intake pattern for layer hens with intake rate increasing from 0300 to 1700 h followed by a sharp decline to 2100 h. However, this intake pattern was similar between high and low feed efficiency birds. Our work is now focused on individual hen diet selection from the mix of feeds in the mash diet and the association with feed efficiency.

I. INTRODUCTION

Feed efficiency (FE) is an important production trait in poultry. A commonly used measure of efficiency is feed conversion ratio (FCR), which is defined as feed intake (FI) per unit of egg mass (EM) in laying hens. It is widely recognised that behaviour is an important aspect of the physiological status of animals (Pennisi, 2005). Feeding behaviour may reflect animal meal habit as a potential predictor of FE (Schwartzkopf-Genswein, 2002). With the help of electronic feeders, individual feeding information can be collected automatically and measured accurately (Basso, 2014). In broilers, feeding behaviours were found to be related with FE in different selected lines (Howie, 2011). However, how feeding behaviour changes over time and how it affects FE is not clear in commercial laying hens. The objectives of this study were to investigate the association between feeding behaviour and FE in Isa Brown layer hens.

II. MATERIALS AND METHODS

This work was conducted at the University of Sydney, Poultry Research Facility, Camden, NSW using 450 Isa Brown birds (25-week-old), randomly selected and housed individually in 25 × 50 × 50 cm cages for an initial screening period of 6 weeks with a 14 h lighting program from 0600 to 2000 and 10 h of darkness. All birds were housed individually and offered *ad libitum* feed (wheat-soybean meal-based mash) as the common experimental diet and water.

The experimental diet consisted of 16.3% CP, 2,750 kcal / kg ME, 0.82% total lysine, 0.42% methionine, 4.0% Ca and 0.4% available P. At the end of the initial screening phase, 150 birds were ranked and grouped based on their overall mean FCR. The top 35 high feed efficiency (LFE) and bottom 35 low feed efficiency (LFE) birds (49-week-old) were selected for a feeding behaviour study of 10 weeks duration. Using a hanging scale system (G7 wireless analogue sensor range 0-5kg; ease mind technology ltd., Hong Kong), 14 birds (7 HFE and 7 LFE) were monitored at one time for intake every 2 minutes of 24 h for 1 week following a 1

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week adaptation period with data outputs automatically recorded (Figure 1). After this period, a new group of 14 birds was monitored for intake behaviour in the same way until all birds had been monitored over 5 periods. Weekly individual FI, daily egg production (EP) and daily egg weight (EW) were manually recorded to determine average daily FI, daily EM and FCR.

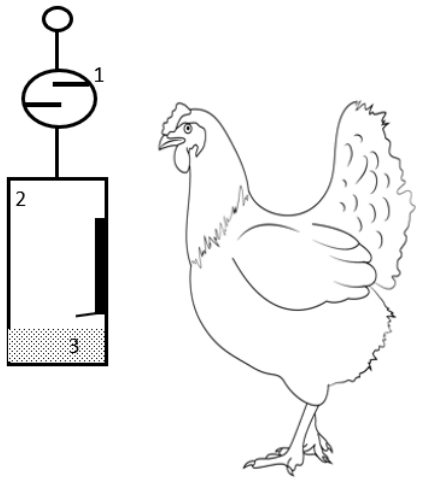

Figure 1 - Scale system with layer hen; 1 – wireless analogue 'pull' load cell sensor; 2 - bottle with opening and lip to prevent feed spillage; 3 - feed

Differences between consecutive weight observations every 2 minutes were calculated as an estimate of the amount of feed consumed over that interval by the bird (n = 324,119 weight differences). However, the addition of feed resulted in extreme weight differences, hence any weight differences more than five standard deviations from the mean were excluded from analysis. This process was repeated four times resulting in a dataset of 320,837 differences. Difference data were then binned into consecutive 1h intervals, and the mean and standard deviation over each interval for each bird calculated. Mean values were multiplied by 30 to obtain total amounts of feed consumed over each 1h period. The mean data and standard deviations were used in subsequent analyses (n = 10,933 and n = 11,024 SDs) after further extreme-value filtering.

For the total and standard deviations, the following linear mixed model was fitted to the data:

$$Y = \text{constant} + \text{Group} + \text{Day} + \text{Hour} + \text{Group.Day} + \text{Group.Hour} + \text{Bird} + \varepsilon$$

where Y is the trait being analysed (total or SD); Group, Day, and Hour are fixed effects, with fitted interactions Group.Day and Group.Hour, and Bird as a random effect. The random errors ε were modelled using an ARMA (P = 1, q = 1) structure to allow for serial correlation between consecutive observations. The 'lme' function from the 'nlme' package in R was used for model fitting, and all analyses were undertaken using R.

III. RESULTS

Daily FI (g/d), daily EW (g/d) and FCR for HFE and LFE birds are provided in Table 1.

Table 1 - Feed intake (g/d), egg weight (g/d) and feed conversion ratio for 35 high feed efficiency (HFE) and 35 low feed efficiency (LFE) Isa Brown layer hens.

Measurement	HFE	LFE	SED	P-Value
Feed intake (g/d)	120	136	2.5	<0.001
Egg weight (g/d)	65	63	1.5	0.42
Feed Conversion Ratio	1.8	2.2	0.03	<0.001

SED – Standard error of the difference between means

The group designated as HFE had a lower FCR when compared with the LFE group of hens ($P < 0.001$). There was no effect of FE group or day of study on intake patterns. However, there was an impact ($P<0.001$) of time of day on intake rate (Figure 2) with both HFE and LFE birds steadily increasing feeding activity (g/h) from 0300 h to 1700 h, after which intake rate linearly decrease to zero by 2100 h. There was an association ($P<0.001$) between time of day and standard deviation of intake rate. Both HFE and LFE birds showed a rapid increase in intake rate standard deviation from 0300 h reaching a peak at 0800 h which was maintained until 1700 h, after which time this intake variability decreased to night time levels by 2100 h.

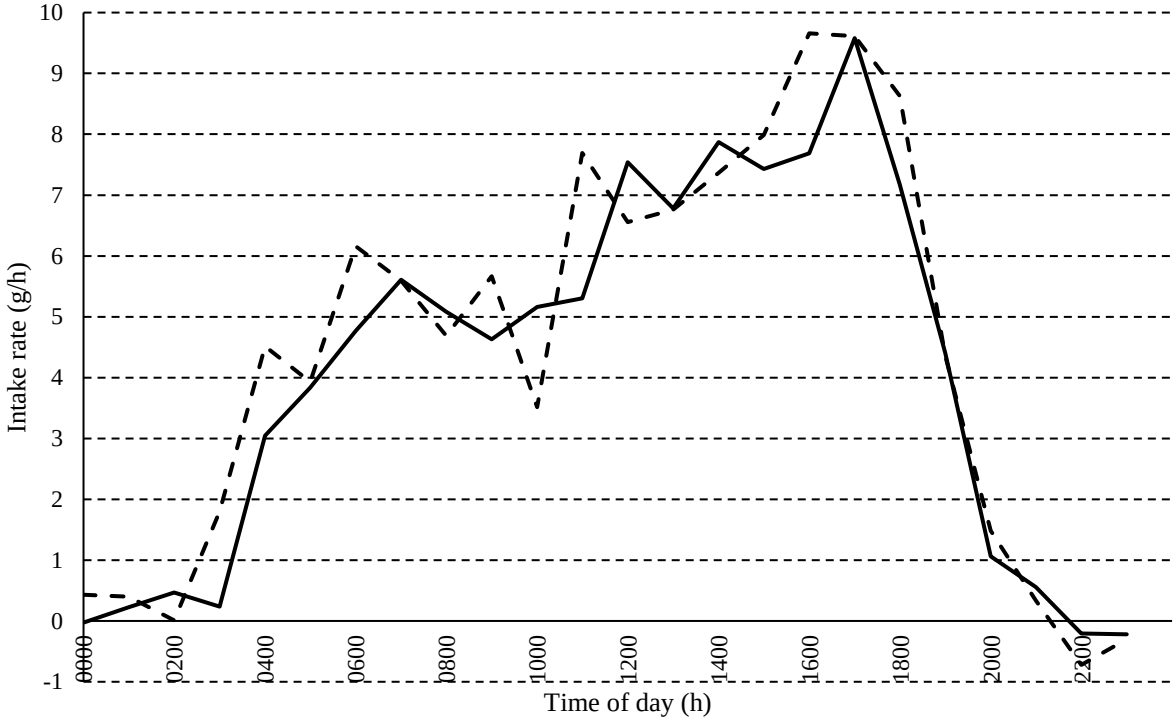

Figure 2 - Predicted mean hourly intake rate (g/h) 24 h a day for 1 week for 35 HFE (solid line) and 35 LFE (dashed line) Isa Brown birds.

IV. DISCUSSION AND CONCLUSION

The main objective of this experiment was to determine the association between FE and intake pattern. Overall, the FI of LFE was greater when compared with HFE hens; however, intake pattern was similar between HFE and LFE layer hens. Our findings revealed a distinct intake pattern at an hourly level for layer hens, with intake rate increasing from 0300 to 1700 h and a rapid decrease in intake rate to 2100 h. Birds started eating approximately 3 h before the lights came on at 0600 h and reached an initial intake rate peak between 0600 and 0700 h. This initial peak occurred 1-2 h before peak oviposition at 0800 – 0900 h (data not presented). In line with our findings, Savory (1977) and Kadono et al. (1981) showed eating activity to decrease for 1-2 h before oviposition with intake rate increasing after this. The high intake rate after oviposition may firstly compensate for low intakes during oviposition, an increased demand for nutrients that occurs due to ovulation 30 minutes after oviposition and the birds' demand for calcium which is greatest from early afternoon until late evening. In line with our findings, Duncan and Hughes (1975) showed feeding activity to decrease at the time of luteinizing hormone release at ovulation, when the egg enters the shell gland, before oviposition and then increases following oviposition. In this study, intake decreased to 2100 h when lights were turned off at 2000 h suggesting anticipation of darkness by the hens as per Khalil et al. (2010)

an implication of this anticipatory behaviour is the importance of enough feed supply to meet this increased FI before lights go off.

Our results show that factors other than intake pattern impact FE in layer hens. Preliminary data (data not presented) indicate that there are differences in diet selection from components of the same mash between birds of divergent FE and this will be the focus of ongoing work.

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UNDERSTANDING THE PERCEPTIONS AND KNOWLEDGE OF LAYING HEN WELFARE: INDUSTRY AND COMMUNITY STAKEHOLDER FOCUS GROUPS

J. POWER-GEARY[1], H.R.J. NOLAN[1], L. HEMSWORTH[2] and P.S. TAYLOR[1]

Decisions that impact hen welfare may be influenced by the expectations of the community, such as development of regulations (Coleman et al., 2018). Although some reaserch has been conducted on Australian consumer attitudes (Bray and Ankeny, 2017), wider community perceptions (for example, vegans) towards laying hen welfare remain largely unknown.

This research was approved by the University of New England's Human research ethics committee (HE18-235). Focus groups of industry stakeholders (ISG) and community stakeholders (CSG), were held to establish an understanding of Australian poultry stakeholder knowledge of, and perceptions towards, hen housing and welfare. The CSG was held in Tamworth, NSW, (n = 7; 6 female, 1 male). The ISG was held in Brisbane, QLD (n = 6; 2 female, 4 male). Each focus group included a mixture of open- and closed-ended questions in a semi-structured discussion. Focus groups were audio recorded and later transcribed and analysed. Participants were asked what is important for hen welfare; a word count was performed (excluding irrelevant words such as 'the', 'I') and key words were grouped into themes (for example, 'disease' and 'mortality' were grouped into the theme 'health') and are presented as a percentage of the total words used for each group.

When asked what is important for hen welfare, ISG utilized more frequently than the CSG terms specific to health (ISG 40.0%, CSG 22.2%) and biological needs (ISG 26.7%, CSG 7.4%), but did not mention housing (ISG 0%, CSG 33.3%), behaviour (ISG 0%, CSG 12.9%), or psychological needs (ISG 0%, CSG 5.5%).

Discussions with the CSG highlighted misconceptions within the community regarding the egg industry, including the belief that beak trimming is illegal and that hens are housed under 24 light schedules to increase production. Furthermore, there was a lack of understanding of the scale of egg production in Australia evident by CSG discussion about rehoming spent hens and manual collection of eggs. Stakeholder groups were asked if they would support furnished cage housing systems (FCHS). No CSG members were aware of FCHS. However, after a briefing of the nature of the system, 100% of the CSG indicated they would support, and believed the rest of the community would support, a FCHS. However, 100% of ISG believed the adoption of FCHS is unlikely. The ISG believes that Australian consumers who do not support cage housing systems will similarly not support FCHS due to the notion that "a cage is a cage".

The data gathered from these focus groups highlight differences between the industry and community stakeholders' perceptions toward hen welfare. Furthermore, they highlight knowledge deficits and the potential impact of language on perceptions of hen welfare within the Australian community. This study will inform a national survey to investigate the impact of language during education on hen welfare.

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IS RANGE USAGE AT THE ONSET OF EGG PRODUCTION ASSOSCIATED WITH TIBIAL BONE MINERAL DENSITY AT THE END OF LAY?

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Osteoporosis in laying hens is of health, welfare, and economic concern. During the onset of lay, hens rapidly build up the amount of medullary bone which has less structural integrity instead of structural bone formation (Whitehead, 2004). However, freedom of movement in cage free systems can stimulate structural bone formation to avoid mechanical failure which may improve bone health at the end of laying period. The aim of this study was to compare the ultrastructure features in laying hen tibia and to compare these parameters in hens with different range usage.

A total of 1,875 Lohmann brown hens were housed on a commercial farm amongst 40,000 other hens in an aviary system. These 1,875 hens were individually monitored for their range usage from 18 to 72 weeks. At week 22, hens were grouped into "stayers" (hens that had ranged 2.10 ± 0.7 days), "roamers" (hens that had ranged 8.20 ± 0.7 days), and "rangers" (hens that ranged 15.30 ± 1.0 days) and placed in separate pens (treatment groups) based on the previous 4 weeks of range use. At 72 weeks, 176 left tibiae of the hens were randomly collected and individually scanned using a GE-Phoenix V|tome|xs 240 micro CT scanner (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany) to determine various parameters (Table 1). Parameters were compared using a one-way ANOVA.

Table 1 - Comparison of volumetric bone measurements between the stayers, roamers and rangers.

Parameters	Stayers (n=51)	Roamers (n=61)	Rangers (n=64)	P Values
Number of days at range (22 weeks)	2.10 ± 1.0^{a}	8.20 ± 1.0^{b}	15.30 ± 1.0^{c}	<0.001
Number of days at range (72 weeks)	144.5 ± 1.0^{a}	186 ± 8.0^{b}	191.5 ± 8.0^{b}	<0.001
Body weight at 22 weeks (g)	1684 ± 3.0	1733 ± 2.0	1749 ± 3.0	ns
Body weight at 72 weeks (g)	1863 ± 2.0	1830 ± 2.0	1866 ± 2.0	ns
Length (mm)	122.1 ± 0.5	121.4 ± 0.5	121.5 ± 0.45	ns
Diaphyseal diameter (mm)	8.23 ± 0.1	8.31 ± 0.1	8.25 ± 0.1	ns
Bone breaking strength (N)	157.3 ±7.9	141.8 ± 8.6	150.1 ± 7.8	ns
Total bone volume (cm^3)	8.41 ± 0.1	8.46 ± 0.1	8.42 ± 0.1	ns
Relative proportion of cortical bone (%)	54.2 ± 1.0	54.1 ± 1	53.9. ± 1.1	ns
Relative proportion of blood vessels (%)	2.6 ± 0.1	2.4 ± 0.1	2.8 ± 0.1	ns
Relative proportion of the marrow (%)	41.1 ± 1.0	40.1 ± 1.0	41.8 ± 1.0	ns
Cortical bone mineral density (mg/cm^3)	488.2 ± 8.0	491.2 ± 7.0	491.9 ± 7.0	ns
Eggshell breaking strength (kgf)	4.06 ± 0.19	3.81 ± 0.29	3.99 ± 0.23	ns

All values in the table are represented as Mean ± SEM. P< 0.05 was considered significant.

Ranging at the onset of lay did not affect any volumetric measures of bone quality. Further investigation about the possible contribution of hen movement *within* the aviary system, the impact of range usage on egg shell quality, as well as on other mineralisation parameters such as keel bone damage should be investigated.

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EGG CORTICOSTERONE CONCENTRATIONS AFTER ACUTE STRESS EXPOSURE IN FREE RANGE HENS WITH DIFFERENT RANGE USAGE

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Free range hens are exposed to various potential stressors including weather conditions and risk of predation (Gilani et al., 2014). Distress can result in impaired biological functions including reduced reproduction, immunity and growth (Palme, 2012). In order to investigate the impact of early range usage, corticosterone concentrations in egg albumen were measured in response to a stressor (manual handling and relocation) in free range hens.

Two groups (n = 625) of commercial free range hens were selected based on their individual range usage between 18 and 22 weeks of age: "Stayers (S)" accessed the range for 3.51±0.3 days while "rangers (R)" accessed the range for 14.9±0.2 days on an average. At 22 weeks of age, these hens were exposed to "stressors" (being caught, confined for ~10 h, weighed, and rehomed into a new partition within the same area and the same bird numbers). Ninety eggs of each group were randomly collected immediately after the release (baseline– representing pre-stress corticosterone concentrations), 7 days, and 20 weeks later. Ninety eggs were also randomly collected from the other 36,875 hens located in the same shed (negative control group, NC, no experimental stressors, calculated avg. range use 10.73±0.12). Corticosterone concentrations of the albumen were analysed using a Radio Immune Assay (Downing and Bryden, 2008). Data analysis was performed using a one way ANOVA (SPSS v.24, IBM Corp., Armonk, NY, USA).

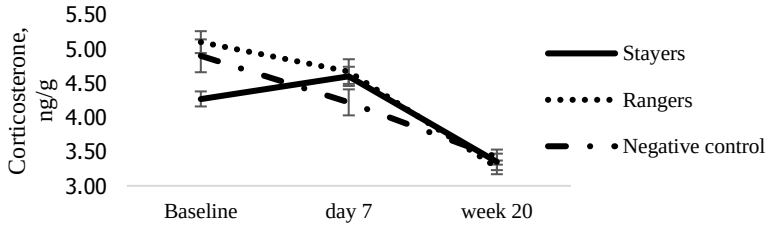

Figure 1 - Comparison of egg albumen corticosterone levels in stayers, rangers and the negative control group.

Initially, egg corticosterone concentrations of S were lower compared to both R and the NC group (4.32±0.1, 5.12±0.2, and 4.90±0.2ng/g respectively; P=0.03; figure 1). While corticosterone concentrations of R and NC hens decreased within the first 7 days to 4.67±0.2ng/g; P=0.04 and 4.22±0.2; P=0.01, the values of S increased (4.60±0.1; P=0.06). In all groups, corticosterone concentrations decreased over time and reached their lowest point at week 20 (S: 3.35±0.1, R: 3.3±0.1, NC: 3.4±0.1ng/g; P=0.627). The reasons for the low initial corticosterone concentrations in S require further investigation.

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EVALUATION OF A NOVEL SLOW-GROWING STRAIN FOR CHICKEN MEAT

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Summary

The slow-growing broiler (SGB) strain offers an alternative chicken meat choice, while appealing to the 'niche' market and attracting a premium price. SGBs have also been associated with improved leg health and lower mortality, making them a welfare-oriented alternate strain. The aim of this study was to compare the SGBs with the conventional chicken breed Cobb 500 (Cobb), for their performance, immune response, leg health, digestibility of nutrients, productivity and meat quality, in an Australian context. Results showed that the SGB had longer standing times, higher thigh-drumstick and wing yields as well as darker and redder meat in comparison to the Cobb. However, the Cobb had better feed conversion ratios (FCR), higher antibody (IgM) production, higher apparent metabolizable energy (AME) and heavier breast yield.

I. INTRODUCTION

Since the 20th century when commercial poultry breeding first started, the growth rate of meat chickens has quadrupled, and their body structure has also changed dramatically especially with the enlarged size of the breast muscles (Muir and Aggrey, 2003). This is the result of a combination of selective breeding, efficient production systems, improved diets and veterinary care (Fanatico et al., 2007). It now takes about 38 days for day old fast-growing broilers to reach market live weights (~ 2.0-2.5kg) in comparison to the year 1976 when it took 63 days (Quentin et al., 2003). However, the fast growth rate of broilers has been associated with metabolic and skeletal disorders such as tibial dyschondroplasia, twisted legs, sudden death syndrome and ascites. (Blagojević et al., 2009, Baghbanzadeh et al., 2008; Shim et al., 2012). In contrast to some countries, the only meat chickens available commercially in Australia are the fast-growing strains. A slower-growing strain which, although it may only appeal to a specific market and attract a premium price owing to its higher production costs, environmental footprint and reduced carcass yield (Elanco Animal Health, 2016), will have the advantage of improved leg health and lower mortality rates (Fanatico et al., 2008; Sirri et al., 2011; Cömert et al., 2016). Moreover, there is a need to develop an SGB strain in an Australian context that would provide an alternative choice for chicken meat consumers. This study provides a better understanding of the productivity, feed efficiency, and meat quality of a candidate SGB strain.

II. METHODS

Eggs were obtained from a Cobb X New Hampshire cross and incubated. Hatched chicks (116) and 120 one-day-old Cobbs obtained from a commercial hatchery were vaccinated for Marek's disease, infectious bronchitis and Newcastle disease. The chicks were allocated into four mixed-sex pens for each breed, consisting of either 30 Cobbs or 29 SGBs, and were grown to a final weight of 2-2.2kg live weight. Both breeds were reared on the same diet (starter: ME 12.10 MJ/kg, CP 20.2%, grower: ME 12.70MJ/kg, CP 18.7% finisher: ME 13.05MJ/kg, CP 16.8%) based on the nutrient specifications for Cobb 500. Mortality was recorded daily, and post-mortem analysis was conducted accordingly. The birds and feed were weighed on a weekly basis. At 21 days of age, 2mL of blood was drawn from 2 SGBs and 2 Cobbs from each pen which were wing tagged, and subsequently injected subcutaneously with 0.25mL of a 2% suspension of sheep red blood cells (SRBC) for an antibody response test (Haghighi et al. 2005). According to the procedures of Groves and Muir (2014), latency-to-lie (LTL) test was performed on 30 male birds for each breed when they reached the final weight, as an objective measure used to assess broiler leg strength. At the

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respective final weights for each breed, 6 male birds were randomly selected from each pen and housed in four metabolism pens for 48hrs collection of excreta to calculate the AME of the diet (Ravindran et al. 2000). A total of 40 broilers (20 female and 20 male) of each breed were randomly selected at their final weights for carcass analysis. A Konica Minolta chroma meter 400 was used to determined L* (lightness), a* (redness) and b* (yellowness) values for breast meat. The ultimate pH was measured by placing the electrode of a portable pH meter on the outer side of the cranial breast muscle (Quentin et al. 2003). Drip loss is a measure of the water-holding capacity (WHC) of the meat product. The breast muscle used for drip loss evaluation was stored on a suspended net and removed, wiped and weighed on days 1, 3, 5 and 10 post-slaughter. Data were analysed with a one-way analysis of variance (ANOVA) using Genstat 18th edition, with breed as the main effect. A t-test was used to compare the means and considered to be significantly different if $P < 0.05$.

III. RESULTS

On a weekly basis, body weight gain between the two breeds was significantly different ($P < 0.001$) with the final weight of 2-2.2 kg achieved by Cobbs on day 32 and SGBs on day 55. The cumulative feed consumption on a weekly basis was also significantly different between the two breeds, with Cobbs consuming 76% more feed as compared to SGBs on day 32 ($P < 0.001$) although, SGBs overall consumed 24% more feed than Cobbs to reach the final weight. SGBs, therefore, had a higher FCR at processing of 1.977 which was 0.536 points higher than the Cobbs (1.441). Cobbs had a significantly higher total anti-SRBC antibody titre of 13 in comparison to the SGB titre of 6 ($P = 0.05$), and an IgM titre of 11, compared to 4 for the SGBs. The IgG titre of 2 for both breeds meant that neither produced anti-SRBC IgG. During the LTL test, Cobbs had a significantly shorter mean standing time (177.3 secs) as compared to SGBs (242.7 secs; $P < 0.01$). The AME:GE of diets for Cobbs (0.79) was 2.60 % higher than the SGBs (0.77; $P < 0.05$).

Table 1 - The effect of genotype on the carcass characteristics of broilers.

	Cobb	SGB	SEM	P-Value
Carcass Weight, CW (g)	2025	1892	40.25	0.0219
Length of body (cm)	23.5	30.3	0.3447	<.0001
Width of body (cm)	18.8	17.6	0.2130	0.0001
Length of breast (cm)	17.9	18.8	0.1963	0.003
Width of breast (cm)	15.2	13.7	0.1446	<.0001
Length of shank (cm)	5.8	7.2	0.1180	<.0001
Length of thigh (cm)	9.8	12.3	0.1521	<.0001
Length of drumstick (cm)	9.4	12.9	0.1905	<.0001
Length of wing (cm)	23.1	29.0	0.3344	<.0001
Breast weight (% CW) (g)	21.7	13.9	0.1734	<.0001
2 thighs (% CW) (g)	10.9	11.5	0.1527	0.0081
2 Drumsticks (% CW) (g)	9.6	12.0	0.1105	<.0001
2 Wings (% CW) (g)	8.3	9.7	0.0844	<.0001
Heads (% CW) (g)	2.5	3.0	0.0465	<.0001

The carcass characteristics of Cobbs and SGBs are presented in Table 1. SGBs had significantly longer bodies and breast, whereas Cobbs had wider bodies and breast ($P < 0.01$). Significantly higher shank, thigh, drumstick and wing length were observed in SGBs as compared to the Cobbs ($P < 0.001$). Relative to the carcass weight, breast weight of Cobbs was significantly higher than SGBs, while the thighs, drumsticks, wings, and head were significantly heavier in SGBs ($P < 0.01$). The crop, proventriculus, jejunum and ileum as well as the liver and abdominal fat pad were significantly heavier in Cobbs ($P < 0.05$) as compared to the SGBs, which had significantly heavier gizzards, duodenum and caeca ($P < 0.001$).

Cobb breast meat was paler as compared to the SGBs (L* = 51.93 and 45.39 respectively) ($P < 0.001$). In contrast, the breast meat of SGBs was significantly redder (a* = 2.64) and less yellow (b*= 3.84) than the Cobbs (a* = 2.08; b* = 4.97) ($P < 0.05$). The ultimate pH of the breast for SGBs (5.74) was significantly lower than the Cobbs (5.88) ($P < 0.001$), while the pectoralis major of SGBs had higher drip loss than the Cobbs ($P < 0.05$).

IV. DISCUSSION

Weight gain, feed intake and FCR were found to be significantly different between Cobbs and SGBs as supported by previous research (Fanatico et al., 2008). Cobbs have been genetically bred for increased body weight and breast muscle yield, whereas the SGBs in the current study had a layer strain as one of its parents, thus diluting this genetic effect. The difference in feed intake between the two breeds was related to the duration of rearing (Fanatico et al., 2005). In the current study, the Cobbs had a lower cumulative feed intake (3.04 kg per bird) as compared to the SGBs (4.03kg per bird) ($P<0.05$). However, the daily feed intake at day 32 of Cobbs (0.790 kg/bird) was higher than the SGBs (0.726kg/bird). The fast growth rate of Cobbs leads to the rapid conversion of feed to meat. Further, SGBs have a slower growth but higher maintenance requirements due to their higher mobility, as seen in the longer LTL times reported in this study, thus affecting the feed efficiency (Fanatico et al., 2008).

The fast-growing Cobb strains had a stronger immune response (IgM) to the SRBC antigen as compared to SGBs. This is in agreement with earlier studies in turkeys (Li et al., 2000; Cheema et al., 2007) where selection for fast-growing strains altered the T cell subpopulations and the response of the humoral immunity to the SRBC antigen while also showing a decline in IgG antibody production. Genetic difference also affects the broilers' immune response, with higher IgM titers to SRBC produced by meat chickens than layers (Koenen et al., 2002).

SGBs have stronger legs than the Cobbs as fast growth rates negatively impact the leg strength (Tuyttens et al., 2008) and results in greater pressure being exerted on their immature bones (Webster, 1995). According to de Verdal et al. (2010), chickens which possessed larger gastric compartments had more effective nutrient utilization. In the current study, Cobbs had higher proventriculus weight in comparison to the SGBs, possibly resulting in higher enzyme production and gastric secretions, subsequently improving AME utilization. Intensive selection for increased breast muscle yield in fast-growing Cobb leads to the reduction in the relative yield of other parts (Fanatico et al., 2008; Fanatico et al., 2005). The higher wing and leg-quarter yield identified with SGBs can be explained by their increased activity and greater utilisation of their wings, promoting bone mass and supporting muscle mass (Abdullah and Buchtova, 2016). The heavier fat pad in Cobbs could be a consequence of faster growth as proposed by Havenstein et al. (1994) where the selection for heavier broilers concomitantly promoted accumulation of fat.

The varying degree of redness of the breast meat seen between strains can be associated with the difference in slaughter ages with increase in the myoglobin content reported with age (Berri et al. 2001; Gordon and Charles 2002). The selection for increased growth rates and breast meat yield of Cobb broilers leads to diminished post-mortem glycolysis and higher ultimate pH (Berri et al., 2001). This may be due to the lowered glycogen content of Cobb breast muscle, thus, explaining the differences in breast muscle pH between the breeds. Berri et al. (2001) suggested a strong negative correlation between breast muscle pH 24 hours post-mortem and drip loss, thus, accounting for the higher drip loss in SGBs. A poor WHC leads to lack of juiciness in whole meat and further-processed products.

V. CONCLUSION

The main advantage of SGBs is the provision of an alternate chicken meat that is also welfare-oriented, as indicated by their longer standing time during the LTL, and would likely attract a 'niche' market at a premium price. However, with regards to their performance, carcass

characteristics and meat quality, further research and refinement would be necessary in terms of breeding objectives and nutrient requirements to improve this strain.

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