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Application of Low Protein Diet Concept for Sustainable Poultry Production

S. H. M. RAMOS and C. K. GIRISH

INTRODUCTION

With the continuous population growth, it is estimated that the food industry faces the challenge of producing food for at least 9 billion people in 2050 (Rabobank, 2017). Food and Agriculture Organization (2009) estimates that the animal protein demand will increase by more than 70% between 2005 and 2030. Livestock farmers are faced with the challenge of producing more with less resources taking into consideration factors such as global food security, animal welfare, greenhouse gas emissions, sustainable and efficient use of natural resources, among others.

Annually, about one third of the world food production is lost or wasted between field and fork (FAO, 2011). As part of the Sustainable Development Goals of United Nations in 2015 for responsible consumption and production states that to feed the world in a sustainable manner, producers need to grow more food while reducing the negative environmental impact such as soil, water and nutrient loss, greenhouse gas emissions, and degradation of ecosystems (FAO, 2015). They encourage consumers to shift to nutritious and safe diets with a low environmental footprint.

To address such environmental, production and sustainability goals, one of the strategies that are being put forward is the use of low protein diets in poultry nutrition.

In a typical corn-soy based diets for broilers, methionine (Met), lysine (Lys), and threonine (Thr) are the first three limiting amino acids. A specific supplementation with these limiting amino acids can easily compensate deficits in compound feed and lead to improved growth performance. Lemme et al. (2002), demonstrated this by showing that feed conversion improved as dietary methionine content increased. In addition to improving the efficiency of the feed to provide growth, fortification with these essential amino acids (EAA) also distinctly reduces the nitrogen emissions as well as the emissions of relevant greenhouse gases.

Nutrition and Care, Evonik (SEA) Pte. Ltd., Singapore

Several studies have shown a reduction in nitrogen excretion by about 10% with 1 percentage point reduction in dietary crude protein in broilers while balancing the amino acids via supplemental sources (Aletor et al., 2000; Bregendahl et al., 2002; Gomide et al., 2011). Furthermore, a low crude protein (CP) diet formulation might be a tool to minimize risk of disturbances of gut microbiome and proliferation of pathogen species causing dysbacteriosis or other challenges (Pan and Yu, 2014). Skin-burn lesions, foot pad dermatitis, hock burns and breast bristles can be related to uric acid excretions and increased litter moisture are also linked to protein levels in the diet. These injuries indicate animal welfare issues but also mean economic loss for the processors which can be avoided (Shephard and Fairchild, 2010).

Commercial Availability of Feeds Grade Amino Acids and Economic Feasibility

The question that is always put forward in low protein diet discussions is that how low we can go with CP levels. However, what we should also consider is that how far down the essential amino acids are kept in the feed formulations. The use and availability of feed grade amino acids (AA) has enabled nutritionists to formulate diets precisely to meet their nutrient requirements. The use of feed grade AA in feed formulation resulted in decreased crude protein content (CP) due to reduction in the levels of protein ingredients compared to a high CP diet without supplementation. Garland (2018), reported a study of van Harn and van Krimpen (2016) on broiler grower and finisher diets with reduced crude protein content by 1, 2 and 3% from control levels of 20.8 and 19.8% protein for the grower and finisher, respectively. The levels of digestible lysine were 1.05% and 0.99% for the control diets. Supplemental amino acids including L-Lysine.HCl, DL-Methionine, L-Threonine, L-Arginine, L-Valine, L- Isoleucine, L-Tryptophan and L-Glycine was added in the diet to maintain the ideal protein profile. The results for 0-35 day performance showed no effect on live weight but with significant improvement ($P < 0.05$) in FCR at 2% and 3% CP reduction. A reduction in water intake and foot pad scores were significant ($P < 0.05$) at all protein reductions and litter condition scores were also improved in terms of friability and dryness. A slight reduction in breast meat yield at -3% crude protein although carcass weight and yield were unaffected. However, the cost of adding amino acids gave lower margins per bird of between -€0.024 and -€0.061 per bird. In addition, the gCO2-eq/kg is estimated to have increased from 725 to 875 g CO2-eq/kg live weight.

This was due to the reliance on amino acids that has a negative impact on carbon foot print due to energy use in their production.

Dietary crude protein reduction in broiler diets resulted in reduced dietary cost, nitrogen excretion (Hernandez, et al., 2012), ammonia emissions (Ferguson et al., 1998) and the incidence of pododermatitis (Nagaraj et al., 2007). Kriseldi et al. (2018), reported that the use of supplemented AA (DL-Methionine, L-Lysine Hcl, and L-Threonine) in day 1 to 14 broilers to lower dietary CP content (23.9 vs. 21.7%) was also effective in decreasing nitrogen excretion (mg/bird/d) by 14.1% without compromising performance objectives. Belloir et al. (2017) reported that reducing the CP content of growing-finishing male broiler diets from 19% to 17% resulted in decrease nitrogen excretion and volatilization without negative consequence on animal performance or meat quality. On the contrary, several researches reported that reduction of dietary CP content of beyond 2% points may result in suboptimum body weight (BW) gains and feed conversion ratio (FCR) of broilers (Waldroup et al., 2005; Dean et al., 2006; Namroud et al., 2008; Hernandez et al., 2012). However, the approach of formulating low CP broiler diets supplemented with free AA to meet the level of essential AA requirement failed to achieve the growth performance as achieved with standard CP feed (Deschepper and de Groote, 1995; Bregendahl et al., 2002; Jiang et al., 2005). Previous studies have reported that reducing CP content in broiler diets can depress growth performance of broilers early in development (Si et al., 2004; Waldroup et al., 2005; Dean et al., 2006; Hernandez et al., 2012). Several strategies to mitigate the poor growth performance of broilers include potassium supplementation (Han et al., 1992), the inclusion of glutamine (Glu) or aspartic acid (ASP) as a source of nitrogen (Aletor et al. 2000), and increasing dietary energy (Hussein et al., 2001). However, the results obtained were all inconsistent. Aftab (2006), reported that these adverse growth responses may be attributed to sub-optimum EAA concentrations. Recent results show that in addition to meeting the requirements for EAA, broilers receiving reduced CP diets may require a total Glycine (Gly) + Serine (Ser) concentration in diet formulations to achieve similar growth performance to those fed higher CP diets (Corzo et al., 2004; Dean et al., 2006; Waguespack et al., 2009; Awad et al., 2015). A recent review by Evonik on the limitations of previous studies on CP reduction in 1-21 days old broilers show that they were fed only one diet (Lemme, 2017). The results indicate that along with N- reduction both weight gain and FCR was gradually impaired. However, if researchers chose a strategy with shorter feeding periods, indicate that performance can be maintained as exhibited by some trials. These trials indicate that the principle of phase feeding, which means adjusting the nutrient supply to closely meet the birds changing daily nutrient requirements, as a possible strategy to successfully implement low protein diets in 1- 21 day old broilers.

A recent review on CP reduction benchmarked with commercial or breeder performance standards showed that only a few examples can be considered as best practice (Lemme, 2017). Data of 14 trials showed that CP reduction in 22-37 day old broilers indicated positive results with production performance maintained at a reasonable high level showed some similarities in terms of the level of standardized ileal digestible (SID) Lys level of at least 92% with 0.70g/MJ ME and ideal protein ratios of methionine + cysteine (Met+Cys), Thr, arginine (Arg), valine (Val), isoleucine (Ile), Leucine (Leu) and glycine equivalent (Gly_{equiv}) of 75, 65, 112, 82, 72, 110, and 110%, respectively (Lemme, 2017). These SID Lys and ideal protein ratios paved the way for CP reduction to 17.5% or lower at this feeding phase.

The use of commercially available next limiting AAs like L-Isoleucine, L-Arginine and L-Tryptophan in reduced CP diets is dependent on the cost per unit of these amino acids as well as incentives, government initiative of environmental rules that warrants these. Dozier and Kriseldi (2017) cited an example in the US where L-Valine can be included in the corn-soybean meal diets with at an estimated rate of approximately 0.70 kg on a limited basis, which resulted to a savings of USD 1.50 per ton broiler feed. To increase further the L-Valine supplementation in broiler diets, the price of other next limiting AA such as L-Isoleucine, L-Arginine and L-Tryptophan should decrease first to be picked up in the formulations. It was also calculated that a change of 0.02 point increase in the digestible Val to Lys ratio has a large impact on the supplementation of L-Valine from 0.25 to 0.70 kg/MT.

Historical CP and Lys levels in selected countries

In every part of the world different feed ingredients are utilized to meet the broiler's nutritional requirements. There are different guidelines or standards of minimum levels used in feed formulation or feed production. Different broiler breeds have their own nutrient recommendations in order to maximize the broilers' full genetic potential. In South East Asia, the government bodies regulating the quality of broiler feeds that is being produced indicate minimums for CP. CP analysis is less complex and in-expensive than AA analysis, this serves as an indirect measure of ensuring the AA levels in the feeds.

Broiler feed samples, from selected major broiler producing countries such as Brazil, China, Germany, India and the USA, were analyzed at Evonik's AMINOLab® (Figure 1). All the broiler feed samples were analyzed by wet chemistry following the Dumas method of analysis for CP content. The analyzed CP content in the starter stage are still high as compared to the levels in LP studies reported. The average crude protein in the starter stage did not change much from 21.26% in 2005 to 21.56% in 2017. However, there is a general downward trend on the CP levels from 2005 to 2017 in the grower and finisher phase, 20.58% vs. 19.90% CP and 19.34 vs. 18.46% CP in 2005 and 2017, respectively. The lowest CP level in the all feeding phases was observed in the samples from China, this may be due to the commercial availability of other essential AA at affordable price that support low CP diet formulations.

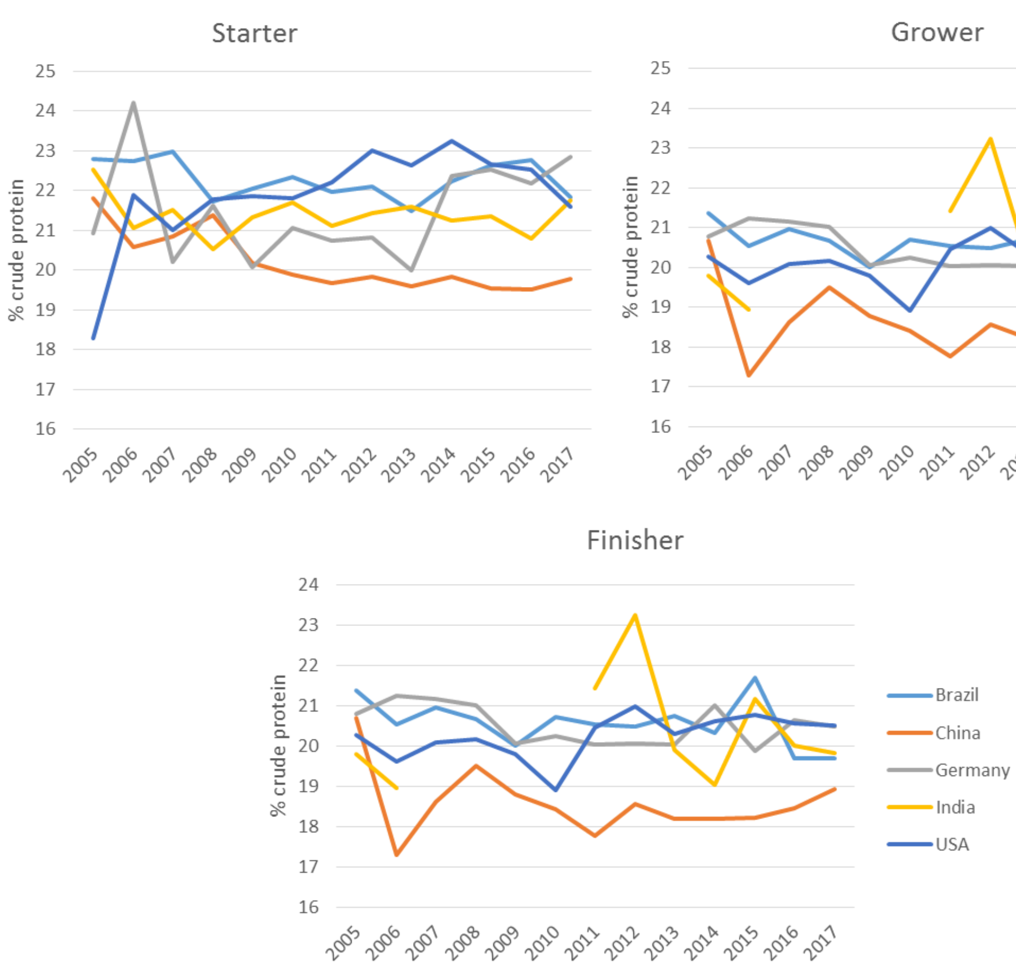

Figure 1. Average CP content of broiler feeds analyzed from 2005 to 2017 (Source: AMINOLab®)

In terms of the analyzed total AA content in all growth phases, countries with high CP levels also had higher analyzed total AA levels. (Figure 2). The level of total Lys increases as the level of CP increases. There was no clear trend in the total Lys levels used in all growth phases through the years from 2005 to 2017 but clearly the levels were decreasing when comparing the starter, grower and finisher phase. However, the ideal protein ratio in all phases is lower than Evonik's AMINOChick® 2.0 recommendations for Met+Cys while it is higher for Thr, Arg, Val, Ile and Leu. With these analyzed CP and AA levels, it clearly shows that formulating broiler diets with low CP diets are not yet fully put into practice especially in the starter stage in countries like Brazil, China, Germany, India and the USA. This may be due to the lack of access to digestible amino acid values for some raw materials and confounding published literature, high variability of raw materials thus the use of high safety margins in feed formulations and the cost of supplemented amino acids.

Figure 2. Historical average CP and total Lys (%) content of broiler feeds analyzed from 2005 to 2017 (Source: AMINOLab®)

Others factors to be considered in feeding low protein diets to broiler chickens Arginine (Arg)

The supplementation of L-Arg in low CP broiler diets depends on the availability and combination of raw materials in the diet. Arginine is the fifth limiting AA after Met, Lys, Thr and Val, in corn and soybean meal-based diets (Vieira & Berres 2007). It is more limiting in wheat based diets compared to a corn–soybean based diets. Arginine can be 'limiting' due its antagonism with Lys and also the requirements of Arg increases if Lys is added to a diet in excess (Jones et al. 1967; Austic and Scott 1975) and this will affect broiler performance (Balnave and Barke, 2002) and muscle development (Fernandes et al., 2009). Arg interacts with Lys as both share a few metabolic pathways (D'Mello, 2003). Excess Lys would stimulate the Arg-degrading enzyme arginase which lowers the Arg availability from the Arg pool. Thus, an oversupply of Lys should be avoided especially when Arg levels are low. Another function of Arg is in the creatine synthesis. With the supplementation of guanidine acetic acid (GAA) supports the energy metabolism as a creatine precursor as well as, sparing the metabolism of Arg. Dilger et al. (2013) reported that 1 kg of GAA, which is typically supplemented at 0.6-1.2 kg/MT, spares 770g of Arg. The use of GAA can be an option to address Arg deficiency in low CP diets.

Branched Chain AA (BCAA) – Val, Ile, Leu

BCAA share metabolic routes concerning their degradation similar to Lys and Arg. High Leu levels will trigger the BCAA degrading enzymes which degrades all BCAA (D'Mello, 2003). In 21-42 day old broilers showed that increasing the levels of Leu had less impact as compared to Val on weight gain and FCR (Ospina-Rojas, 2017). Furthermore, this trial showed that the optimal standardized ileal digestible (SID) Val and SID Leu levels is at 0.86% and 1.15-1.19% corresponding to SID Val:Lys and SID Leu:Lys of 83% and 110-114%, respectively. At the highest level Leu at 1.80%, breast meat yield was reduced indicating an imbalance in the diet which should be avoided. In another trial on BCAA, showed that graded levels of up to 140% of the recommendations did not significantly affect the broiler performance (Lemme et al., 2004). However, breast meat yield was reduced significantly at the highest level of BCAA. This suggests that Ile and Val requirements should be evaluated separately especially in low protein broiler diets. The optimal SID Val, Ile and Leu:Lys ratio for 22-35 days old broilers is at 80, 70 and 107%, respectively. Currently in some countries, feed grade Ile is not available and in countries where it is available the price is exorbitant so the requirement for such nutrient can be only addressed by a suitable ingredient combination. Feed ingredients like feather meal, fish meal, potato protein and soybean protein concentrate are high in Ile.

Glycine equivalents (Glyequiv)

Glycine (Gly) is the simplest amino acid in nature, without D or L configuration (Wu et al., 2013) and has been reported to be the first limiting non-essential AA (Ospina- Rojas et al., 2012). Traditionally, it was classified as nutritionally non-essential amino acid for mammals due to the presence of its endogenous synthesis in the body (Wu, 2009; 2010). Waguespack et al. (2009) described Gly as the fourth-limiting AA after Met, Lys and Thr in corn and soybean based diets for broilers from days 1 to 18 with 0.25% L-Lys HCl supplementation and 1.26% SID Lys, a level which previously had been shown to result in optimal growth performance of broilers as long as it was supplemented with Gly to maintain a minimum of 2.32% total Gly+Ser. The same researcher also reported that for 1 to 21 days old broilers both Val and Gly to be equally limiting after Met, Lys and Thr in corn- soybean meal based diets. It has been widely accepted that Gly in feed is one factor limiting CP reduction in broiler feeds since the publication of the trial of Dean et al. in 2006. Gly metabolism is complex (Akinde, 2014; Siegert et al., 2015) and can be a reversibly metabolized from serine (Ser) in a one-step reaction. It is generally assumed that the metabolic interconversion of Gly and Ser is not limiting as reported by Akrabawi and Kratzer, (1968), and Sugahara and Kandatsu, (1976). Both AA have the same effect on growth performance as long as the same molar quantity is taken into consideration. It is a common practice that most researchers take the analogue effect of Gly and Ser as the sum of the concentrations of both on an equimolar basis (Siegert and Rodehutscord, 2018). Thus, both AA are expressed as Gly_{equiv} (Gly_{equiv} (g/kg) = Gly (g/kg) + [0.7143 x Ser (g/kg)]) where 0.7143 is the ratio of the molar weight between Gly and Ser (Dean et al., 2006).

It should also be noted that Thr can be metabolized to Gly. In the successful low CP study of Belloir et al. (2017), Thr levels were increased to account for Gly. However, in a study by Siegert et al. (2015), it was not possible to correlate and quantify the effect between Gly_{equiv} and Thr intake. Based on molecular weights, however, 1 kg of Thr (119g/mol) can release 630 g of Gly (75g/mol). The degradation of 1 g choline (139.6 g/mol) or betaine (117.2 g/mol) will release 0.54 or 0.64g of Gly. This suggests that the presence of choline and/or betaine can interact with Gly metabolism at a low performance level with a reduced choline requirement by increasing the Gly_{equiv} requirements (Siegart et al., 2015).

Sulfur containing AA, Met can be converted to Cys but not the other way around. This is one of the reasons why Cys deficient diets can be easily addressed by Met supplementation (Siegert et al., 2015). However, the conversion of Met to Cys requires one molecule of Serine (Ser). Choline, with betaine and dimethylglycine as intermediate steps, is metabolized to Gly if homocysteine is available (Melendez-Hevia et. al., 2009). Gly is also needed in the formation of glutathione which is an important antioxidant coping with reactive oxygen species in the body. Indirectly, Gly plays a role in against oxidative stress (Lemme, 2017).

Another important function of Gly is in the nitrogen (N) excretion (D'Mello, 2003). A Gly molecule is required for every molecule of uric acid excreted. This means that the lower the dietary N surplus the lower is the Gly requirement. By improving the N-utilization by balancing the AA levels based on the ideal protein concept in low CP diets will have a major effect on the Gly requirement. However, it does not mean that by lowering the CP levels in the diet, N utilization is maximized or production performance is maintained.

Low Protein Diets: Experiences from Germany

In Europe, a European Directive on the reduction of national emissions (European Commission, 2013) focus on protein nutrition and nitrogen management are of increasing relevance concomitant with the Best Available Techniques reference document (BAT; Santonja et al., 2017) which set the frame for animal production. The latter document recommends to lower dietary crude protein in animal feed by adding and balancing with supplemental amino acids (Facts and Figures, 2018). In addition, there is also increasing pressure from consumers, retailers, food companies and national politics on animal production concerning environmental protection but also concerning animal welfare and animal health management – two aspects where protein nutrition plays a role as well. In Germany, the Ministry of Justice and Consumer Protection 2017 stipulate a revised fertilizing directive as well as a directive on the management of nitrogen and phosphorous at farm level. It is indicated in these documents the suggested values for the annual excretion of nitrogen per place and year which are more ambitious than in the above mentioned BAT paper and which set the basis for nitrogen (and phosphorous) balance calculations on one hand but on other hand also defining maximum excretions allowances. Based on rough calculations on key numbers from current broiler feeding practice imply that it will be difficult, if not impossible, to meet those threshold values. Farmers might be forced to run less cycles per year or reduce animals per sq. meter. The reduction of the dietary protein, as suggested in BAT document, with subsequent reduced nitrogen excretions can be an alternative appropriate strategy.

A broiler feeding trial with 4 treatments with decreasing dietary protein levels while maintaining amino acid balance over four feeding phases was conducted in Germany (Facts and Figures, 2018). The treatments were following recommendations by Hiller et al. (2014) recommendations where treatment 1 and 2 refer to protein levels of standard feed (22, 20.6, 20 and 19.5% calculated CP for the starter, grower I, grower II and finisher phase, respectively) or feed with reduced nitrogen content (21.0, 20, 19.9 and 18.9% calculated CP for the starter, grower I, grower II and finisher phase, respectively). In treatments 3 (Medium N-Reduction, 21, 19.5, 18.7 and 18% calculated CP for the starter, grower I, grower II and finisher phase, respectively) and treatment 4, nitrogen was reduced to higher degrees (Strong N-Reduction, 21, 19, 18 and 17% calculated CP for the starter, grower I, grower II and finisher phase, respectively). The CP levels in current German feeding practice are somewhere between treatment 1 and treatment 2. In the starter phase, treatment 1 had 21% CP and 22MJ metabolizable energy (AME) whereas, treatments 2, 3 and 4 had the same protein and energy levels of 21% and 11.5 MJ, respectively. The energy levels for the grower I (20, 19.5 and 19MJ), grower II (19.6, 18.7 and 18MJ) and finisher phase (12.6MJ for all treatments).

The results showed that there was only a marginal variation in body weight and feed conversion between treatments 1 to 3 while in treatment 4 performance impaired slightly resulting in 2.6 % lower final weight and 3 %-pts higher FCR (Table 2). A reduction of nitrogen intake by 8 % (medium N reduction) did not affect weight gain and feed conversion ratio while nitrogen excretion and litter weight were reduced by 19 % and 21 %, respectively (Table 3). The percentage of chicken foot pads with no lesions (score 0) almost doubled. In addition, the reduction of nitrogen intake by 13 % (Treatment 4 - strong N reduction) reduced nitrogen excretion and litter weight even by 26 % and 27 %, respectively. The percentage of chicken foot pads with no lesions were almost tripled compared to standard. However, final body weight and feed conversion impaired by 2.6 % and 1.8 %. Whereas, the carcass evaluation provided indifferent data for male and female birds but indicated that meat deposition might have slightly suffered with protein reduction. The dietary treatments with medium and strong N-reduction allowed for meeting legally requested target values for nitrogen excretion. In addition, economic calculations confirmed that at least medium N-reduction was competitive to the standard feeds but offered advantages concerning environmental and chicken welfare aspects.

Table 2. Performance of 40 day old birds with varying dietary nitrogen count.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
	DLG-standard	DLG-N-reduced	Medium N-reduced	Strong N-reduced
Body weight (g)	2808	2782	2792	2734
Feed intake (g)	4465	6680	6633	6623
Feed per gain* (g/g)	1.613	1.634	1.611	1.643
Mortality (%)	2.48	2.96	1.68	2.64
Feed per gain (g/g)	1.593	1.609	1.597	1.62
European efficiency factor (EEF)	424[a]	413[ab]	426[a]	405[b]
Gross margin (€/delivered bird)**	0.884	0.863	0.884	0.812
Net profit (€/delivered bird)***	0.22	0.198	0.231	0.154

Different superscripts (a,b) indicate differences at P < 0.05; * corrected for mortality by considering eight of lost birds; ** Income over feed cost assuming 82 cent/kg bird weight, no statistics *** considering cost of 10€/t litter and 287.5€/ other costs/1250 broilers (for all treatments), no statistics.

Table 3. Nitrogen consumption, N-retention, N-excretions and N-utilization of broilers with varying nitrogen intakes

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
	DLG-standard	DLG-N reduced	Medium N-reduced	Strong N-reduced
Average N-consumption (kg/pen)	35.19[a]	33.51[b]	32.52[c]	30.76[d]
relative	100	95	92	87
Average N-retention (kg/pen)	20.81[a]	20.56[ab]	20.77[a]	20.25[b]
relative	100	99	100	97
Average N-utilization (kg/pen)	50.84[d]	60.40[c]	63.3[b]	64.9[a]
relative	100	104	108	111
Average N-excretion (g/place/year)**	439[d]	399[c]	355[b]	324[a]

Different superscripts (a-d indicate differences at P < 0.05 * assuming 30 g N/kg body weight; inclusive bird losses; ** 7.3 cycles per year

Conclusions

Numerous research results demonstrate that protein reduction in broiler diets can be achieved. The additions of DL-Methionine, L-Lysine, L-Threonine, L-Valine, L-Tryptophan, L-Isoleucine, L-Arginine and L-Glycine, can be used to meet the requirements of broilers without compromising growth performance. This strategy together with the used of standardized ideal digestible AA and the use of the ideal protein ratios beyond essential amino acids can address issues on environmental regulations to reduce N excretions. To ensure sustainable broiler production, it is important to have a balance between N excretion and ammonia emissions relative to performance parameters and meat accretion.

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Role of Glycine and Serine for Poultry Production and Efficiency When Using Low Protein Diets

MARKUS RODEHUTSCORD and WOLFGANG SIEGERT

INTRODUCTION

Improvement of the efficiency of protein utilization and reduction of gaseous N emissions belong to the permanent aims of poultry production since many years. This led to extensive research on better characterization of feed raw materials on the basis of amino acid (AA) digestibility and AA requirements. It was possible to reduce crude protein (CP) levels in broiler diets based on results of all this work. However, it appears that with current broiler strains a threshold concentration of about 19 % CP in broiler diets is reached and further reduction of CP leads to reduced growth performance even when essential AA are added to the feed (Dean et al. 2006). Different reasons have been discussed to cause this reduction of growth performance in low-CP diets (Aftab et al. 2006) which do not need repetition herein. One possible explanation is a deficiency in supply of specific nonessential AA, especially glycine and serine. In protein sources of plant origin, the concentration of glycine in the protein is relatively constant and considerably lower than in most animal proteins. For this reason, the probability of a glycine deficient broiler diet is higher in regions of the world such as the EU where the use of animal proteins and free glycine is not approved. This initiated a series of experiments in our Institute to investigate responses of broilers to different glycine supply. Results from these investigations are in the focus of this contribution.

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Glycine equivalents

Glycine can be metabolized from serine and vice versa (Velíšek and Cejpek 2006). It is generally assumed that this metabolic interconversion of glycine and serine is not limited in poultry (Akrabawi and Kratzer 1968; Sugahara and Kandatsu 1976). The analogue effect of dietary glycine and serine often is considered in literature by using the sum of both AA (glycine + serine). However, doing so neglects that serine has the same effect as glycine only on equimolar basis. Hence, Dean et al. (2006) proposed to consider the physiological value by calculating the glycine equivalent (Gly_{equi}) as the sum of glycine and the molar equivalent of serine, which is 0.7143. This proposition will be adopted herein whenever possible.

Metabolic functions and precursor of glycine and serine

Glycine and serine are constituents of body proteins. In regard growth of broiler chickens, the concentration of glycine in accreted whole body protein has been analyzed in the range of 7.8 - 11.4 g/16 g N and that of serine in the range of 4.2 - 5.5 g/16 g N (Fatufe et al. 2004; Fatufe and Rodehutscord 2005). Collagen and elastin belong to those proteins richest in glycine, bearing a risk of low skin strength during the slaughter process as a consequence of low dietary glycine supply (Christensen et al. 1994). Keratin is rich in both glycine and serine, and glycine deficiency affected feather development (Robel 1977). Mucin proteins are rich in serine and as endogenous secretions they interact in different ways with digestive enzymes, epithelial structures, and the microbiota. Intestinal mucin secretion of broilers increased with increased glycine and serine in the diet (Ospina-Rojas et al. 2013).

Functions of glycine other than in protein synthesis are also important, such as glycine involvement in metabolic ammonia detoxification. Formation of each molecule uric acid requires one molecule glycine to build the purine ring. Glycine also is an integral part of creatine along with arginine. Creatine can either be supplied by animal proteins or is endogenously synthesized via guanidinoacetic acid formation from arginine and glycine and subsequent methylation to creatinine. Serine is needed when cystathionine is formed from homocysteine and cystathionine reacts to cysteine and ammonia (Velíšek and Cejpek 2006). Hence, serine is important for cysteine formation from methionine.

Variation of responses to graded glycine supply

Since the importance of glycine and serine in poultry nutrition has been recognised, the number of published studies in this field has largely increased. Most of these studies focused on the first three weeks post-hatch. Figure 1 is a summary of Gly_{equi} concentrations needed in the diet to achieve 95 % of the maximum gain:feed response in several studies. These estimates varied widely, which makes it impossible at this time to recommend a specific Gly_{equi} concentration for general application in the broiler industry. Instead, the variation shown in Figure 1 indicates the need for a better understanding of what the reasons of variation are and whether the variation can be related to the functions of glycine and serine that were mentioned before.

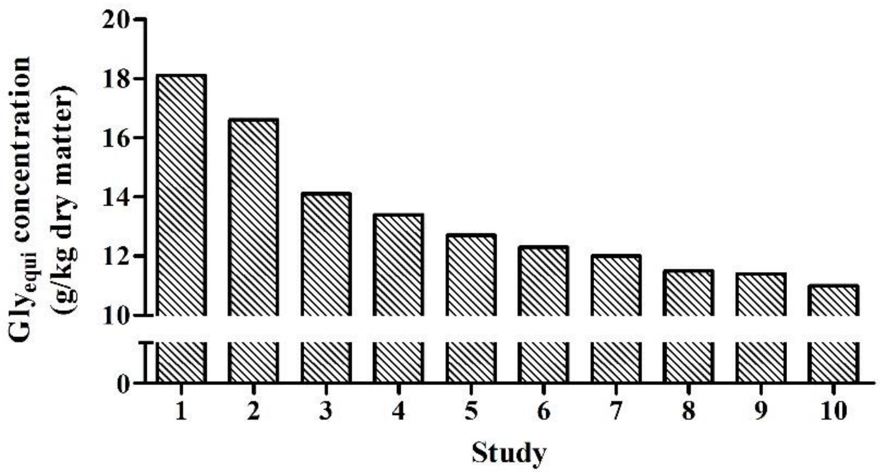

Figure 1: Summary of Gly_{equi} concentrations needed to achieve 95 % of the maximum gain:feed response in different dose-response studies with broiler chickens (Siegert et al. 2015b)

Interactions with sulphur-containing amino acids

It has been shown that the effect of glycine and serine on gain:feed is related to the conversion of methionine to cysteine, for which serine is required (Powell et al. 2011). In their study, gain:feed increased upon addition of glycine when the diet was adequate in total sulphur-containing AA (TSAA) but deficient in cysteine. Additional methionine supply did not increase growth, but additional cysteine above the requirement reduced the growth-increasing effect of glycine supplementation. Because DL-methionine or analogues but not L-cysteine is commonly added to poultry feed, the ratio between methionine and TSAA (Met/TSAA ratio) in the feed is changed, disregarding a specific requirement of cysteine. This means that the need for metabolic conversion of methionine to cysteine including the relevance of Gly_{equi} is changed. Figure 2 depicts results from a study that demonstrated the impact methionine and cysteine supplements can have on the response of broiler chickens to Gly_{equi} supplements. When the requirement of both methionine and cysteine is met, the need for metabolic conversion of methionine to cysteine and for Gly_{equi} is reduced.

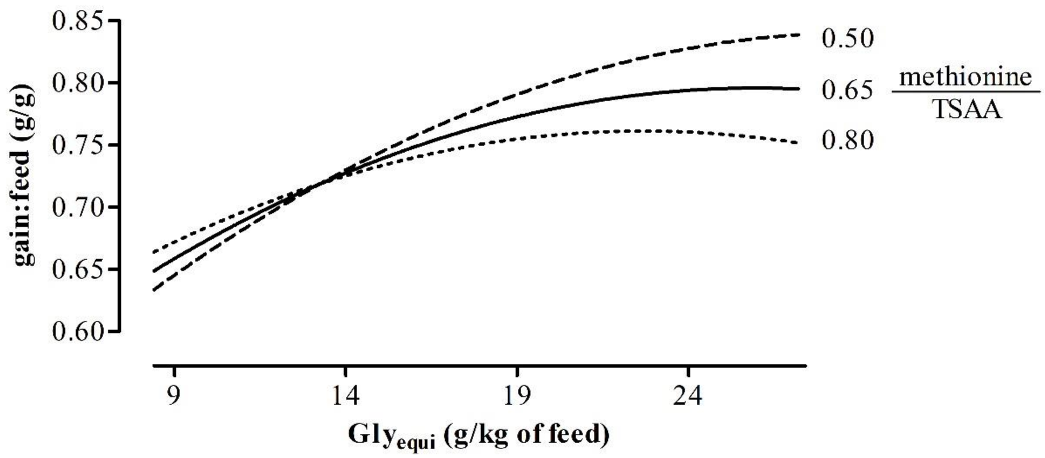

Figure 2: Effect of the methionine to total sulphur amino acid (TSAA) ratio on the gain:feed response measured in 10 different studies with broiler chickens up to 21 days old (Siegert et al. 2015b)

Interactions with endogenous precursors of glycine

Threonine can be metabolized to glycine (Meléndez-Hevia et al. 2009). Interactions between dietary threonine and Glyequi have been reported (Corzo et al. 2009; Ospina-Rojas et al. 2013) and later quantified (Siegert et al. 2015a; Lambert et al. 2015). An increase in threonine concentration reduced the Gly_{equi} concentration needed to achieve target response levels (Figure 3). One molecule of threonine can be converted to one molecule of glycine. Taken the molar weights of both molecules, the replacement value due to the endogenous conversion of one mass unit of threonine cannot exceed 0.63 mass units of glycine. However, a much higher replacement can be derived from studies that were conducted (Siegert et al. 2015a; Lambert et al. 2015; Ospina-Rojas et al. 2013). This apparent discrepancy probably is the consequence of relative excess of AA in threonine-limited diets. Increasing threonine probably reduced catabolism of AA other than threonine, thereby reducing the need for Gly_{equi} in uric acid formation.

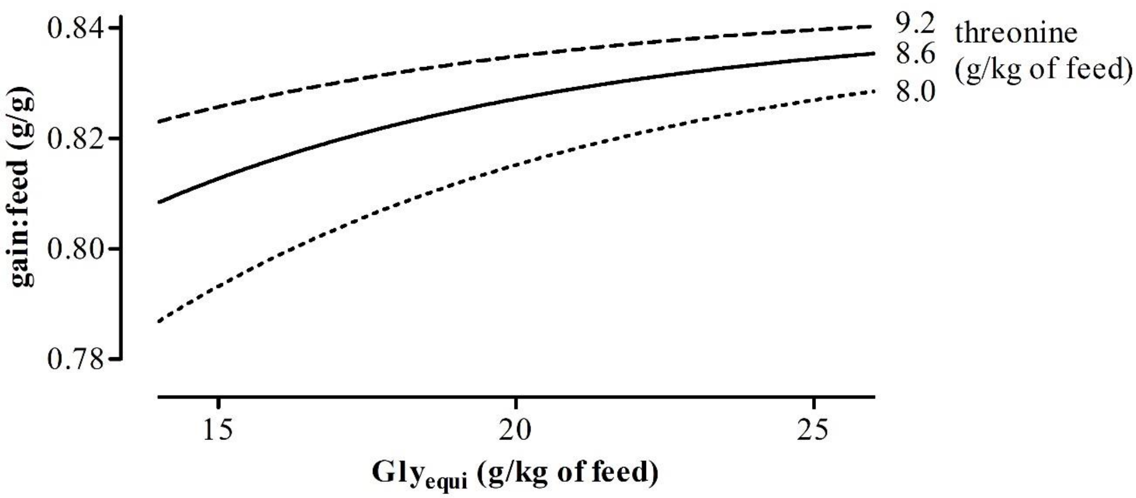

Figure 3: Effect of different threonine concentrations on the gain:feed response of broiler chickens to dietary Gly_{equi} (Siegert et al. 2015a)

Choline also is a precursor which can be metabolized to glycine via betaine if homocysteine is available. Choline supplements can affect the growth response of broiler chickens to Gly_{equi} (Siegert et al. 2015a). The effect of choline on the response to Gly_{equi} was lower compared to the effect of threonine.

Arginine and glycine are precursors of guanidinoacetic acid, which further reacts to form creatine (Akinde 2014). Significant interaction effects of creatine, guanidinoacetic acid, arginine, and glycine on the growth response were reported for broiler chickens (Dilger et al. 2013). However, we are not aware of studies that investigated combinations of Gly_{equi}, arginine, and guanidinoacetic acid in the feed simultaneously.

Interactions with the dietary CP level

Recent studies indicated that growth performance of broilers is not reduced at CP concentrations below 19 % in industry-type diets when combined with glycine supplements (van Harn et al. 2017; Hilliar et al. 2017). However, how far the CP concentration in Gly_{equi} adequate diets can be reduced is not well understood. Broiler growth performance from 7 to 21 days was similar when using 16.7 % or more than 20 % CP in Gly_{equi} adequate feed (Siegert et al. 2015c). In a follow-up study, a similar level of growth performance was achieved using a corn-soybean meal-based diet with 16.3 % CP and adequate concentrations of essential AA and Gly_{equi} (Hofmann et al. 2018). However, when CP was reduced to 14.7 %, growth performance was reduced although concentrations of essential AA and Gly_{equi} were adequate (Figure 4). It is not clear what the limitation was that caused this reduction. We hypothesize that it was another nonessential AA. Glycine and serine are the only nonessential AA for that requirement values were estimated. Probably, a further reduction of CP in broiler diets is possible once the role of other nonessential AA is better understood and their possible requirement better estimated. Of note, the reduction of CP in the diet down to 13.2 % in the study of Hofmann et al. (2018) allowed for an efficiency of N utilization of the birds of 75 % (N accretion/N intake).

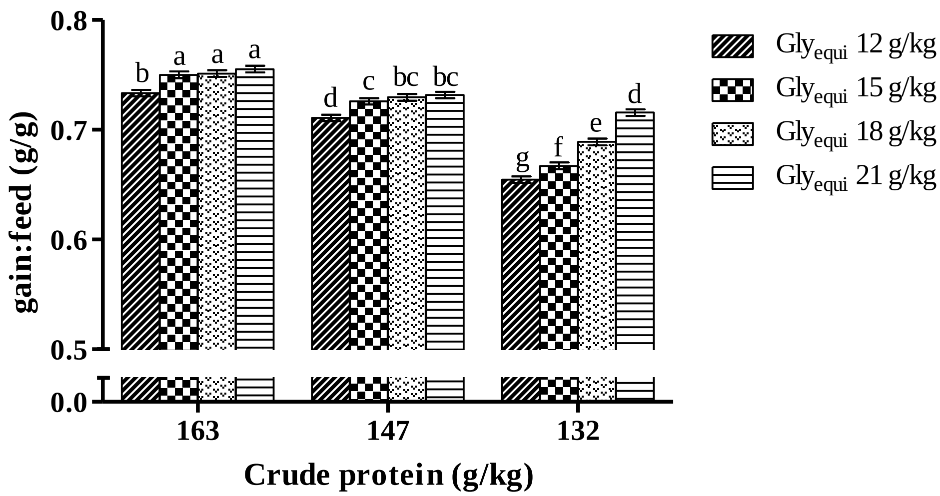

Figure 4: Responses in gain:feed ratio of broiler chickens from 7-21 days fed essential AA adequate diets with different combinations of CP and Gly_{equi} (Hofmann et al. 2018)

In older broilers (growing from 21 to 35 days), Gly_{equi} limited growth when the feed contained less than 16-17 % CP (Belloir et al. 2017). As in younger broilers, the response to dietary Gly_{equi} at this age differed between studies (Ospina-Rojas et al. 2014; Vasconcellos et al. 2011). We are not aware of studies that investigated the effect of the earlier mentioned influencing factors on the response to Glyequi with broilers of different age. However, the interactions mentioned before to be relevant in up to 3 weeks-old broilers are likewise similar in older broilers.

Concluding remarks and perspectives

Increasingly restrictive legislation in regard to emissions caused by animal industries and expected price increases for protein feeds will lead to further reduction of CP in poultry feed. This goes along with an optimization of the AA composition based on updated requirement estimates. Considering Glyequi in feed formulation is necessary to reduce the crude protein concentration in poultry feeds below the current standard. Glycine supplements can become useful especially when animal proteins are not used as feed raw materials. However, the Gly_{equi} requirement of broiler chickens is variable depending on other constituents of the diet. Therefore, a single value for the 'requirement of Gly_{equi}' cannot be provided. As a rough estimate, 16 g Gly_{equi}/kg should cover the requirement of broilers in the first three weeks of age in most conditions. Needed Gly_{equi} concentrations in the feed, however, can vary from below 12 g/kg to above 21 g/kg depending on other characteristics of the feed.

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Gut Health and Barrier Function in Broilers Fed Reduced Protein Diets

REZA BAREKATAIN

ABSTRACT

Maintaining and improving gut health is fundamentally important as the gut supports optimal digestion and therefore performance and profitability of production. Managing gut health through barrier function is regarded as a new frontier for disease prevention across different species but little is known for poultry. While there has been interest to feed broilers with low protein diets supplemented with amino acids, some recent data have shown that such practice may not necessarily improve gut barrier function of broilers compared with a diet containing higher concentration of amino acids. A series of studies are underway to investigate the role some key amino acids in low protein diets with respect to gut barrier function and in particular intestinal permeability.

Intestinal barrier function

A single layer of epithelial cells cover villi in the small intestine. These cells, on one hand, act as a barrier to prevent entry of harmful organisms and toxins into the body and on the other hand, facilitate the selective absorption of nutrients, water and electrolytes (Groschwitz and Hogan, 2009). This selective absorption is mediated through complex pathways of paracellular or transcellular permeability. The enterocytes are connected with each other through complex proteins known as adherens junctions (AJ), tight junctions (TJ) and desmosomes. Adherens junctions and desmosomes create the mechanical link between enterocytes e.g. Zonula Adherens.

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Tight junctions including Claudin, Occludens and Jams play an important role in paracellular permeability as reviewed by Groschwitz and Hogan (2009). Intestinal permeability (IP) can be regulated or altered by several factors including but not limited to diet, intestinal microflora, stress, disease, infections, toxins and drugs (Bischoff, et al., 2014). Increased IP is often associated with compromised health, performance, bacterial translocation, immune activation and lameness (Gilani, et al., 2016). Therefore, there is an increasing interest on IP as a new frontier for disease prevention and therapy across several species. IP can be assessed or investigated using several *in vitro* or *in vivo* biomarkers or assays. The term "permeability" has most likely originated from electrophysiological assays using Ussing chambers that later led to *in vivo* sugar tests including lactulose, rhamnose, mannitol and fluorescein isothiocyanate dextran (FITC-d) (Bischoff, et al., 2014). In poultry, FITC-d has been increasing utilised as a biomarker of IP in *in vivo* studies. Several models including fasting (Gilani, et al., 2018a), rye-based diets (Tellez, et al., 2014), synthetic glucocorticoids (Barekatain, et al., 2018), lipopolysaccharides (Gilani, et al., 2016) and dextran sodium sulphate (Gilani, et al., 2017) have been studied to increase IP in poultry, each representing particular limitation or conflicting results. Extensive studies are therefore required to investigate both modulation of IP and barrier function through nutrition and effective models.

Dietary protein and amino acids

Protein content and in particular amino acid composition of the diet is remarkably important to support animal performance and health. It is now evident that amino acids are one of the main driver of feed intake and major contributors to several metabolic pathways in poultry (Cho, 2012). As a result of extensive breeding programs, modern broiler chickens are now around 400% more efficient than breeds grown in 1956 (Zuidhof, et al., 2014). This has resulted in increased nutrient specifications which unavoidably increased the total crude protein of the diets in order to provide the desired digestible amino acids in a broiler diet. Increased environmental impact, wet litter issues, and ever increasing reliance on quality protein sources are amongst the main drawbacks of a high protein diet. Therefore, in the recent years, there is more interest to feed birds with low protein diets supplemented with amino acids. Supplementation of amino acids have been extensively researched in relation to reduced protein diets and the order of limiting amino acids are very much known (Ospina-Rojas, et al., 2012). As for the performance of broiler chickens, this practice has often resulted in contradictory results but mostly impaired performance and unbalanced supply of amino acids.

There has not been a comprehensive investigation of the effect of low protein diets or simply even dietary protein level on gut health and intestinal barrier function in broiler chickens. It is a perception that by reducing the protein of the diet, the amount of undigestible protein decreases and therefore there is a potential for gut health improvement through reducing the harmful organisms such as *clostridium perfringens* (C.p). However, for poultry, there seems to be no real data backing this hypothesis particularly for the intestinal barrier function or permeability. In a study by Drew, et al. (2004) birds fed diets containing 40% protein from fish meal had a higher intestinal C.p. However in the same group of birds, a higher body weight was observed. As noted, the level of dietary protein was clearly excessive and no other gut health related measurements were investigated. In the concept of intestinal barrier function, the role of specific amino acids and their relationship together for intestinal integrity and development are still not fully understood when dietary protein is reduced. Chen, et al. (2016) showed that reducing dietary protein exacerbated the effect of aflatoxin in broiler performance and nutrient utilisation along with a numeric tendency to increase IP. These researchers found that increasing dietary protein to 26% completely restored the adverse effect of aflatoxin on bird performance.

Highlights of a current SARDI project

Lack of data in poultry prompted a project supported by AgriFutures Australia. As part of this project, an experiment was conducted to investigate both performance and IP of broiler chickens fed a low (LP) protein, a standard (SP) and a high protein (HP) diet (Barekatain, et al., 2018). The LP diets (17 for grower and 15% CP for finisher) were fortified with essential amino acids. The LP and SP (20.2/19%) were formulated to meet the Ross 308 specifications while HP (22/21%) contained 10% above the recommended Ross specifications. The non-essential amino acids (NEAA) were not taken into consideration in order to investigate whether supplementation of EAA could produce a similar result compared with SP or HP diets for IP and barrier function.

As shown in Table 1, the concentration of FITC-d in serum was similar between birds fed LP and SP. However, when LP was compared with HP, a significantly higher ($P<0.05$) FITC-d concentration was observed. A higher FITC-d concentration in blood indicates a more permeable intestine. Therefore this study does not support the perception that increasing dietary protein could have an adverse effect on IP. Birds were also subjected to a leaky gut model induced by dexamethasone (DEX), a synthetic glucocorticoid at 0.5mg/kg body weight. With no significant interaction, DEX also caused a higher IP as shown by a higher concentration of FITC-d in serum of DEX injected birds.

As a major highlight, while the results of this particular trial support that a same level of gut permeability can be maintained with supplementation of EAA to a low protein diet, the common perception of improving gut health by feeding LP diet could not be substantiated. In fact, some positive signs of reducing IP was evident in birds fed HP diets.

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

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

[1]Barekatain et al. (2018)

Role of specific amino acids

For low protein diets, specific amino acids have been extensively studied with most promising candidates being glycine (Gly) and serine that are often become limiting when protein of the diets are reduced (Siegert, et al., 2016). Despite abundant data on the performance parameters associated with supplementation of AA to the low protein diets, less studies have been conducted to investigate their underlying roles for intestinal barrier function and permeability. For instance, there are no data to show whether the intestinal function is dependent on dietary Gly in low protein diets. A report has shown the effect of Gly for permeability in enterocytes in piglets but without consideration of dietary protein (Li, et al., 2016). With regard to the IP, glutamine (Gln), Arginine (Arg) and Threonine (Thr) have been documented to alter intestinal barrier function in other species and more recently in poultry. Their roles are briefly discussed in this paper.

Several studies have confirmed that Gln is vital in maintaining the functional integrity of the gut as it plays a nourishing role for rapidly dividing intestinal epithelial cells in particular enterocytes and lymphocytes (Soares, et al., 2014). This maintenance role is directly related to tight junctions, mucosal cell proliferation and differentiation (Soares, et al., 2014). Gln is involved in mucin synthesis. N-acetylglucosamine is a glycoprotein and a component of the mucin that protects mucosal surfaces and its formation is fully dependent on Gln (Coster, et al., 2004). Gln may be considered conditionally essential amino acid when animal suffers from stress, injury or malnutrition. Under such circumstances the requirement may exceed the capacity for endogenous synthesis required to maintain gut integrity and reduce inflammation because during an immune response a marked increase in the uptake of plasma Gln by immunocytes occurs (Coster, et al., 2004). Previous studies have demonstrated some positive response to Gln in terms of growth performance and gut development in broiler chickens (Bartell and Batal, 2007; Murakami, et al., 2007). However, our recent studies have failed to show any positive response on broiler chickens to Gln in terms of gut permeability or performance (Gilani, et al., 2018b). Gilani et al. (2018b) found that providing 1% Gln in drinking water to the newly hatched broilers did not reduce IP in delayed fed birds. In another study, Gilani, et al. (2018a) supplemented 1% Gln to a wheat-based diet and found no effect on IP assessed by FITC-d test or lactulose, mannitol and rhamnose (LMR) sugar assays. It should be noted that in both trials (Gilani, et al., 2018a; Gilani, et al., 2018b), the Gln was given alongside a normal commercial diet not a low protein one. It may be possible that Gln could be more effective when the dietary protein is reduced or animal is under a serious stressor or disease challenge. Current research is in progress to investigate the role of Gln in low protein diets for intestinal barrier function. Nevertheless, it has been revealed that Glu is superior to glucose alone in promoting net absorption of water, sodium and potassium in rabbit ileum (Islam, et al., 1997). Gln is also widely known as a treatment for diarrhoea in humans (Yalçin, et al., 2004). For poultry, Gln potential for intestinal function, reabsorption of water and thereby reducing excreta moisture has been overlooked and needs to be investigated.

As an essential AA for chickens, mounting evidence is emerging on various fundamental roles that Arg plays in various metabolic pathways and regulation of intestinal function and ultimately protein synthesis and performance. The synthesis of nitric oxide and polyamines is dependent on Arg (Wu and Knabe, 1995). Production of NO along with enterocyte migration is crucial for restoration of epithelial continuity (Jacobi and Odle, 2012). Therefore Arg can modulate immune response, inflammation and process of recovery from injury or stress. Recent data with poultry suggest that there is a potential for increasing feed specification for Arg beyond current industry practices to support optimum growth and intestinal function (Tan, et al., 2014). Supplementation of Arg can therefore improve the gut barrier function as shown by reducing the ileal permeability measured in Ussing chambers by Zhang, et al. (2017). The specific role of Arg in low protein diet for intestinal barrier function is currently being investigated.

Threonine is another key essential AA with potential to impact gut barrier function in poultry. Deficiency of Thr impairs the mucosal integrity evidenced mainly by differences observed in intestinal morphology and mucin production. Zhang, et al. (2016) showed that the Thr deficiency can increase IP and impair the intestinal morphology and worsen the effect of coccidial challenge and feed withdrawal. Research in piglets has also shown that a moderate deficiency of Thr (30% reduction) increased paracellular permeability in the ileum assessed by Ussing chambers (Hamard, et al., 2010). Expression of genes related to ZO1, cingulin and MLCK controlling the paracellular permeability were also modified by low Thr in the diet of piglets (Hamard, et al., 2010). It is under investigation that when protein of the diet is reduced, additional Thr may provide benefit for maintaining intestinal function.

To conclude, the research on intestinal barrier function of poultry is still in an early stage and much attention should be made on ways to optimise the intestinal function and permeability as we embark on various feeding strategies for modern broiler chickens. While feeding birds with LP protein diets have resulted in some contradictory data, more research is warranted on the impact of reducing protein with respect to specific role of individual amino acids.

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The Challenge of Reducing Crude Protein in Meat-Type Chicken Diets

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INTRODUCTION

Amino acids (AA's), the building blocks of protein have received considerable attention in avian nutrition and, retained nitrogen, in the form of lean tissue, in growing meat-type chickens is the most abundant component after water. There are a plethora of research papers, theses and books on protein and amino acid (AA) nutrition and subsequent dietary responses to changing AA and crude protein (CP) contents of diets offered to meat-type chickens. Almquist and Grau (1944) appear to be the first investigators to use crystalline AA's to completely replace the CP in meat-type, semi-purified chicken diets. However, these researchers were unable to maintain both maximum bird growth or feed conversion efficiency (FCE) using reduced CP diets. Many researchers have attempted, since this early research, to either theorise why these types of diets are not successful or have attempted to solve the conundrum through many iterations of AA combinations. However, reducing CP in broiler diets and maintaining performance is possible, providing the decrease is not too excessive. For example, in van Harn and van Krimpen (2016) reducing CP by 2% and 3% for 0-35 days in Ross 308 male broilers, had no effect on live weight and a significant improvement ($P < 0.05$) in FCE.

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The reduction of CP in broiler feeds reduces water intake, improves litter condition and leads to an improvement in associated welfare issues, such as foot pad dermatitis. In addition, nitrogen excretion is reduced and less soyabean meal is used providing strong incentives to successfully reducing CP in broiler feeds (Garland, 2018).

Much focus has taken place on determining the “requirements” of individual amino acids and the development of an “ideal protein” concept where the nominally “essential amino acids” (EAA’s) are expressed in a ratio to the reference amino acid, which is usually lysine (Baker *et al.*, 1996; Lemme *et al.*, 2003; Tillman and Dozier, 2013; Wu, 2014 and Rostagno *et al.*, 2017). More recently, the reduction of nitrogen excretion from poultry and other intensively farmed livestock has re-ignited research into reducing CP content of modern meat-type diets by using crystalline AA’s to meet the determined requirements to sustain an objective function, which maximises specific performance parameters in the animal, or minimizes costs associated with meat production. The research that has been done with reduced CP diets has had variable outcomes but the most common thread is that carcass lipid increases and bird performance deteriorates as CP is decreased from standard practical diets. Whilst many researchers hypothesize, logically, that reducing CP in diets may result in insufficient nitrogen for the animal to metabolise AA’s that have been determined to be “non-essential” (NEAA), there are many other dietary factors that change when crude protein is reduced in diets and these are often ignored. Limiting the provision of amino nitrogen may cause a shortage of precursors (Wu, 2014). In addition, there is a likelihood that some metabolic processes are too slow to provide the modern broiler with sufficient amounts of NEAA’s (Alhotan and Pesti, 2016). Somewhat surprisingly, the vast majority of published data does not try to distinguish between true protein and non-protein nitrogen and this may be of relevance since a reduction in CP with the addition of crystalline AA’s leads to a concomitant decline in the dietary non-protein nitrogen (NPN) content. The importance of the NPN compounds in feed that include nucleotides, nucleic acids, amines, amides, urea, nitrates, nitrites, ammonia, phospholipids, nitrogenous glycosides and even some vitamins (Mariotti *et al.*, 2008) are largely beyond the scope of this paper. However cognisance of their role in nutrition should be considered and, whilst data on many of these compounds in the context of reduced CP diets is not well documented, they cannot be ignored.

The determination of all the factors, including AA’s, that change as CP reduces in practical meat-type chicken diets should be considered and the effects of these either ruled out or taken into account when formulating these diets. AA nutrition, particularly with respect to the branched chain amino acids (isoleucine, valine and leucine) due to their role in muscle synthesis, as well as NEAA such as glycine and serine have received considerable attention recently. In addition, as CP is reduced in diets and additional crystalline AA's are added, there are changes in dietary lipid and starch content, dietary electrolyte balance (DEB), fibre and the possible partial destruction of AA's through Maillard reactions in pelleted (heat treated) feed. The reduction of CP content of feed also reduces the phytic acid content of the feed and this may reduce the response to phytase enzymes, which are now common in practical, modern meat-type chicken diets. Therefore the reduction of the phytic acid substrate, on which phytase works when CP is reduced, cannot be ignored. The effects of access to feed (or feed restriction) and addition of biogenic amines have received some attention but are beyond the scope of this paper. More recent research on the influence of digestive dynamics in reduced CP diets will form an integral part of the implications of reducing CP in practical diets and, identifying where the nutrient "gaps" exist, in both current and past research; will be crucial to applying an effective reduction in CP in practical meat-type chicken diets. The effect of fibre and whole grain feeding in conjunction with reduced CP diets, with respect to gizzard functionality, reverse peristalsis and digestive dynamics, warrants further research (Selle, 2018).

Finally, the practical, commercial implementation of reduced CP diets within the feedmill does not receive attention normally, but should also be considered. By reducing CP, the number of ingredients to be weighed per batch of feed increases and an extra 4 crystalline AA's can represent approximately 20% additional ingredients (by number) within a batch. Depending on the feedmill design, such as scale and bin configuration, the added ingredients might add significant time to batching and decrease feedmill capacity. Providing the capacity is available, this would represent only an added cost although, if the mill is already running at capacity, it may be impossible to implement these diets without major capital investment. Most feedmills are old in New Zealand and Australia and do not have the available extra bins to either add these extra amino acids or the space within the mill to add more bins. Weighing and subsequent hand addition of the additional AA's is a possibility but, it is a very costly option in both New Zealand and Australia. In addition, relying on a person to weigh these ingredients introduces the added risks of human error in batching.

"Crude" versus "true" protein

CP has been used for over 150 years and is simply the nitrogen content of the feed multiplied by 6.25. It assumes that, on average, feed ingredients contain 160 g/kg protein. As a measure of the nitrogen content of feed, the use of CP may be justified in practical diet formulation since most commercial broiler diets are formulated to digestible AA's. However, true protein (TP), as reflected by the total of EAA's and NEAA's, may hold more relevance in reduced CP diets, with the liberal use of supplemental crystalline AA's. Indeed, based on nitrogen content alone, the CP of some crystalline AA's exceed 1,000 g/kg. For example, L-glycine has a CP value of 1,154 g/kg and L-arginine a CP value of 1,996 g/kg. Clearly, the TP content of the relevant AA used in feed formulation cannot exceed the concentration of the AA itself. Dietary nitrogen from NPN sources decline as CP is reduced with a concomitant increase in AA nitrogen as ever increasing amounts of crystalline AA's are added to feed. Moreover, much of the research into reduced CP diets doesn't define what values of CP have been used for the crystalline AA's, whilst several researchers do not assign CP values to the crystalline AA's (Aftab *et al.*, 2006).

For feed ingredients, Mariotti *et al.*, (2008) in a literature review have proposed an average factor of 5.60 as more appropriate than 6.25. These authors have summarised data from a number of different sources and some of the main ingredients used in feed formulation are shown (Table 1).

Branched-chain amino acids

The importance of the branched-chain amino acids (BCAA); leucine (Leu), isoleucine (Ile) and valine (Val) in reduced CP diets is significant since these make up nearly 50% of the supply of EAA's in typical meat-type poultry diets and constitute 33% of all the amino acids in the bird (Cole, 2015). Some unique features of the catabolism of the BCAA have implications for improving the understanding of inter-organ relationships in nitrogen and carbon metabolism. The initial enzymes for catabolism of the BCAA's are regulated differently from other amino acid degrading enzymes; one or more of the BCAA's appear to exert specific regulatory effects on tissue protein degradation and synthesis and BCAA's, through competition with other AA's in blood and appear to play a role in controlling brain AA concentrations and thereby in the synthesis of AA-derived neurotransmitters (Harper *et al.*, 1984)

Table 1. Review of conversion factors for nitrogen to TP of some major ingredients used in feed formulation (after Mariotti *et al.*, 2008)

Ingredient	Mossé (1990)	Sosulski & Imafidon (1990)	Tkachuk (1969)	Other Data	Mean Value
Maize	5.65	5.61		5.59	5.62
Wheat	5.33	5.66	5.49		5.49
Barley	5.50		5.40		5.45
Triticale	5.36		5.62		5.49
Oats	5.36		5.32		5.34
Pearl Millet	5.30		5.63		5.47
Sorghum	5.65	5.68			5.67
Rice	5.17	5.37		5.47	5.34
Wheat Bran			4.71	5.20	4.96
Soyabean Meal	5.52		5.44	5.64	5.50
Peas	5.44	5.24		5.40	5.36
Lupins	5.40			5.47	5.44
Canola			5.29	5.41	5.34
Sunflower (hulled)			5.29		5.29

Whilst the antagonistic effect of high levels of Leu on the absorption of Ile and Val have been known for many years (Harper, 1955; Smith and Austic, 1978), the influence of low Leu (and low BCAA's) concentrations in reduced CP diets is not well documented. Recommendations of dietary BCAA inclusions differ markedly between researchers and, in starter diets, suggested ratios to Lys (100%) vary between 92% and 139% for Leu (Rhône-Poulenc Animal Nutrition, 1993; Austic, 1994), 65% and 84% for Ile (Austic, 1994; Sklan and Noy, 2005), and 67% and 96% for Val (Coon, 2004), respectively. More recently, Pastor *et al*., (2013) reviewed ratios of the BCAA's to lysine (Lys) that differ from accepted published figures and suggested that higher ratios to Lys are required in the grower period compared with the starter period. These authors also suggested standardised ileal digestible (SID) Leu ratios to Lys of 106% in grower diets and 94% in starter diets; 72% Val in grower diets and 65% in starter diets. In addition, SID Ile to Lys ratio remained similar in starter and grower phases (56% in grower vs. 55% in starter). In contrast, Wu (2014) reviewed various ideal AA ratios and, in the Texas A&M University data, the SID Leu to Lys ratio was 109%, for birds of all ages; SID Val to Lys was 77% in birds younger than 21 days of age and 80% for birds that were older. The SID Ile ratio to Lys was 67% in birds younger than 21 days of age and 69% for older birds. Wu (2018) pointed out that the Texas A&M recommendation for leucine is 21.1% higher for pigs than poultry - despite the fact that the leucine contribution to whole body protein is identical in the two species. The ideal corresponding ratio of SID Leu to Lys in pigs is 132% whilst SID Ile and Val are similar to poultry at 67% and 77% respectively. It is therefore plausible that nutritionists have underestimated the Leu requirements of poultry, particularly when CP is reduced, since normal, practical diets would have ratios that exceed 120%. The published, recommended ratios of BCAA to Lys have been included in typical meat-type chicken diet formulation; however the enormous range of BCAA ratios to Lys for optimal broiler performance requires further investigation, particularly in the context of reduced CP diets.

Glycine and serine

Siegert et al., (2016) suggested that the NEAA's, Glycine (Gly) and serine (Ser) may limit growth of broiler chickens if enough metabolic precursors are not available or, if metabolic processes are not rapid enough. Consequently, supplementing Gly in reduced CP diets has been shown to enhance the growth performance of meat-type chickens (Jiang *et al*., 2005; Dean *et al*., 2006). Gly equivalents (Gly_{equi}) has been used in the literature to describe the dietary requirement of Gly plus Ser in broiler chickens. Gly_{equi} is calculated by adding Ser (multiplied by 0.7143, the ratio of Ser molar weight) to Gly (Siegert *et al.*, 2016).

Siegert *et al.* (2016) investigated the influence of Gly_{equi} in broiler diets that were either supplemented with "intact" protein bound AA's as soya protein isolate or a mixture of free crystalline AA's in reduced CP diets and they reported raising the Gly_{equi} increased the average daily gain (ADG) in broiler chickens offered diets containing both AA sources. However, for the soya protein isolate treatment, average daily feed intake (ADFI) increased and consequently, gain to feed (FCE) was not influenced, whilst treatment using the crystalline AA sources, ADFI was not influenced and therefore, FCE was improved.

More recently, Hilliar *et al.*, (2017) investigated the supplementation of Gly to diets based on wheat, sorghum and soyabean meal. The control treatment contained 217/198 g/kg CP for the grower and finisher phases respectively, and the remaining treatments contained either 200/180 g/kg CP, 185/165 g/kg CP or 170/150 g/kg CP. Gly was supplemented to ensure equal levels of Gly_{equi}

in the experimental diets and all the diets were balanced with EAA's. The addition of Gly improved FCE (g gain/g feed) from day 10 to day 35 by 4.71% (695 versus 663; $P < 0.001$) in birds fed the diet with 200/180 g/kg CP and by 4.78% (679 versus 646; $P < 0.05$) in birds fed the diet with 185/165 g/kg CP, but had no significant effect in birds fed the diet with 170/150 g/kg CP. Gly addition also improved BWG from day 10 to day 35 in birds fed the diet with 200/190 g/kg CP by 10.8% (2,234 versus 2,017 g/bird; $P < 0.05$) and by 13.9% (2,076 versus 1,822 g/bird; $P < 0.05$) with the 185/165 g/kg CP diet. However, no significant effect on BWG was observed at 170/150 g/kg CP. These results suggest that the supplementation of Gly could significantly enhance performance in broilers offered diets with moderate CP reductions until other dietary factors become limiting.

Lipid, starch and digestive dynamics

As dietary CP reduces, without the use of unusual or "novel" ingredients, the grain content of the diet increases and added lipid tends to decrease in diets that are iso-energetic in terms of apparent metabolisable energy (AME). The proportion of energy derived from dietary starch will increase whilst the dietary energy contribution from lipid and, to a lesser extent, from protein, decrease (van Harn and van Krimpen, 2016). In van Harn and van Krimpen (2016) CP in the control grower diet was reduced from 208 g/kg by increments of 10 g/kg to create 3 additional trial diets of 198, 188 and 178 g/kg CP, respectively. Similarly, the control finisher diet of 198 g/kg was decreased by the same 10 g/kg increments and resulted in 3 additional trial diets of 188, 178 and 168 g/kg CP, respectively. All birds were on a standard starter feed to 10 days of age and the grower diets were offered from 11 to 28 days post-hatch and the finisher diets were offered from 29 to 35 days post-hatch. All EAA's were maintained at the same levels within the grower and finisher phases and the SID Gly + SID Ser ratio to SID Lys was maintained at 142% in the grower diets and 138% in the finisher diets. Experimental diets were based on wheat and maize which increased as CP (and soyabean meal) decreased. Added dietary lipid also decreased and the variation is shown in Table 2.

Table 2. Proximate analyses of CP, lipid and starch concentrations in experimental diets. (after van Harn and van Krimpen, 2016).

	Grower Diets				Finisher Diets			
Nutrient (g/kg)	Control	Tmnt 1	Tmnt 2	Tmnt 3	Control	Tmnt 1	Tmnt 2	Tmnt 3
Crude Protein	200	194	186	178	191	184	176	168
Crude Lipid	81	76	70	66	75	71	66	60
Brunt Starch[1]	350	364	385	404	359	379	407	432

[1]Brunt *et al*., (1998) starch analysis method.

Many examples of similar dietary changes that occur when CP is reduced are available in the literature but the example of van Harn and van Krimpen (2016) was selected, since the diets are commercially feasible as commercial diets and overall bird performance in birds offered reduced CP diets was comparable to Ross 308 2014 performance objectives (Table 3).

Table 3. Overall performance of male Ross 308 broilers on reduced CP diets (after van Harn and van Krimpen, 2016).

	Control	Tmnt 1	Tmnt 2	Tmnt 3
Liveweight (g)	2,416	2,433	2,450	2,444
Mortality (%)	5.4	5.9	2.7	6.1
FCE (g lwt./g feed)	660.9[a]	664.0[ab]	672.0[b]	671.6[b]
Feed Intake (g/d)	107.3	106.4	105.0	106.0
Water Intake (ml/d)	199.7	193.0	179.6	188.9
FPS[1]	143[a]	110[b]	79[c]	39[d]

[a,b,c,d] Values within a row without a common superscript are significantly different ($P<0.05$)

[1] FPS (foot pad score) = [(n score 0 * 0) + (n score 1 * 0.5) + (n score 2 * 2)]/n total *100

In practice, reduction of supplemented dietary lipid increases feed dust in the feedmill and reduces throughput within the pellet press, because added lipid acts as a lubricant during the pelleting process. The influence of dietary lipid concentrations on pellet quality (Kleyn, 2015), and/or gastric emptying (Martinez *et al.,* 1995) also needs to be considered in the context of reduced CP diets because of its potential to alter feed intake and feed passage rate in the GIT.

In diets where "novel" ingredients such as maize starch are used to replace maize grain in reducing dietary CP, in iso-energetic (AME) diets with equal EAA contents, the direct impact of starch can then be considered. Selle *et al.,* (2018) compared a standard diet (219 g/kg protein, 269 g/kg starch) and a low-protein diet (190 g/kg protein, 439 g/kg starch) offered to male Ross 308 broilers from 7 to 28 days of age post-hatch. The transition to the low-protein diet significantly increased ileal starch digestibility by 10.8% but decreased digestibilities of EAA's and CP by 6.1%. In this study, replacing maize with maize starch also compromised GIT development shown by a higher pH in gizzard contents and a decreased relative pancreas weight compared to the control. These authors suggested that there was interaction between the digestion of starch and protein and/or absorption of glucose and AA's. Furthermore, paradoxically, the reduced CP diet significantly increased free concentrations of Lys, Met, Thr and Val in plasma taken from the anterior mesenteric vein. Selle *et al.,* (2018) suggested that several Na+-dependent (and independent) transport systems may be involved with the absorption of AA's and conclude that Arg, Ile and Phe appeared to be the AA's most likely to have their digestibility compromised by a large dietary concentration of readily digestible starch and competition with glucose for intestinal uptakes. Clearly there are dynamics of digestion that need to be considered when reducing CP in meat-type chicken diets, particularly as dietary starch increases and large volumes of free crystalline AA's are supplemented.

Digestive dynamics may be considered as a three tier process: digestion of protein and starch in the gut lumen, absorption of amino acids and glucose along the small intestine and their transition across the gut mucosa into the portal circulation (Liu *et al.*, 2017) and it may also hold relevance when investigating reduced CP diets. Liu and Selle, (2015) suggested glucose and amino acids should be made available in appropriately balanced quantities at the sites of protein synthesis for efficient protein deposition and growth performance and that the rate of protein digestion is more important to feed conversion efficiency and nutrient utilisation than starch. Crystalline AA's are a source of rapidly available protein because they do not require digestion. However, the asynchrony between availabilities of AA's (protein-bound and crystalline) may potentially compromise protein utilisation and FCE in meat-type chickens.

In Liu *et al.,* (2017) both weight gain and feed conversion ratio (FCR) were improved with increased protein to starch disappearance rate ratios (Figure 1). The maximum weight gain 1,731 g/bird corresponded to a protein:starch disappearance rate ratio of 0.637 g/g ($r^2 = 0.714$, $P < 0.03$). This is consistent with Truong *et al.,* (2015) who found that there was a significant linear regression between weight gain and protein:starch disappearance rate ratios in the proximal ileum. This suggests that increases in protein disappearance rates advantage weight gain; whereas, increases in starch disappearance rates are disadvantageous. These authors also conclude that protein is a more important determinant on growth performance and nutrient utilisation than starch.

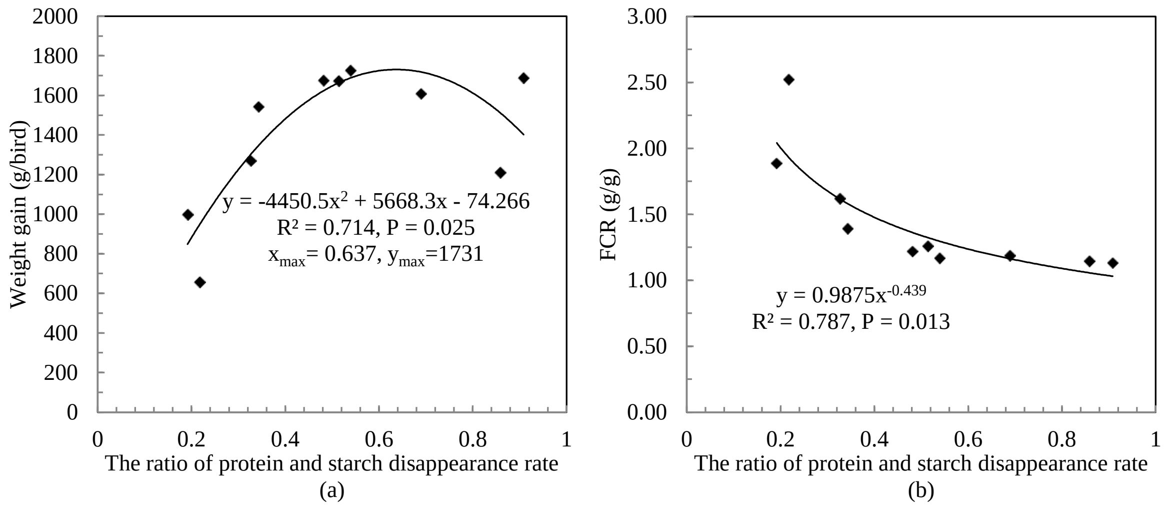

Figure 1. The influence of the ratio of protein and starch disappearance rates in the distal ileum on weight gain (a) and FCR (b), (after Liu *et al.,* 2017).

Liu *et al*., (2017) concluded that the balance between protein and starch digestive dynamics is important to growth performance and nutrient utilisation and that future investigations into digestive dynamics should also consider the post-enteral availability of amino acids and glucose in broiler chickens in order to advance our understanding of the interactions between protein and energy utilisation in chicken-meat production. The difference in the digestion and absorption rates of free crystalline AA's in reduced CP diets compared to AA's in the bound protein of conventional diets coupled with the AA profiles in the portal blood system of chickens' warrants further research.

Dietary electrolyte balance

The interrelationship the electrolytes of sodium (Na+), potassium (K+) and chloride (Cl-) is known as dietary electrolyte balance (DEB = Na+K-Cl; mEq/kg) and it plays an influential role in homeostasis of the body fluids. However, DEB is often overlooked in the investigation of reduced CP diets. Teeter and Belay, (1996) suggested that the maintenance of blood pH and CO_2 levels were critical to growth rate. Furthermore, a reduction of CP in diets based on maize (or wheat) and soyabean meal, reduced the dietary levels of K^+ concentrations due to a reduction of soyabean meal inclusion and this may also increase Cl level from synthetic sources of AA's (Teeter and Belay, 1996; Borges *et al.*, 2011; Lambert and Corrent, 2018). The buffering systems within the bird ensure that pH is usually maintained at optimal physiological levels. However, 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 can predispose to tibial dyschondroplasia and it is known to affect the metabolism of a number of the EAA's, particularly Arg and Lys (Kim *et al.*, 1989; Riley and Austic, 1989). A DEB of around 250 mEq/kg of feed would appear to be the optimum although Borges *et al.*, (2011) have 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.

Most of the research into reduced CP diets have ignored DEB and the combination of lower CP and lower K^+ reduces water intake by 1.4%, decreases the water to feed intake ratio and leads 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, 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 FCE ($P < 0.01$). Nevertheless, these authors were able to conclude that a reduction of the dietary CP content by several CP percentage points is possible in growing-finishing broilers with positive implications for the sustainability of broiler production.

Maillard reactions

The Maillard reaction is a chemical reaction that occurs between amino acids and reducing sugars whereby the reactive carbonyl group of the sugar reacts with the nucleophilic amino group of the amino acid forming a complex mixture of poorly characterized molecules. Named after Louis-Camille Maillard, who first described the reaction in 1912, while attempting to reproduce biological protein synthesis, it was Hodge (1953) who published a paper that established a mechanism for the Maillard reaction. The Maillard reaction is an integral and unavoidable part of feed manufacturing and its intensity increases with temperature during the conditioning and pelleting process. Thermal over-processing of high-protein materials reduces lysine bioavailability, depending on temperature levels and the duration of heat application. From a nutritional perspective, the Maillard reaction reduces the availability of both the amino acids and the reducing sugars involved in the reaction. In the case of lysine, for example, destruction is accomplished in two ways. First, lysine is bound to sugars, forming early-Maillard reaction products. Although at this stage lysine is still detectable in chemical assays, it is no longer bioavailable. Second, the formation of late-Maillard reaction products, such as melanoidins, reduce the amount of chemically analysed lysine (Kleyn, 2013).

Somewhat surprisingly, Maillard reactions have not been considered in reduced CP diets that are steam pelleted. Establishing whether free crystalline AA's are binding to sugars, or other compounds and becoming unavailable may partly explain why performance is often worse in most of the reduced CP diets. However, further research is warranted in this area.

Phytate (phytic acid)

Moss et al., (2018) indicated that the use of exogenous phytase by the global chicken-meat industry has won broad and almost complete acceptance for inclusion in poultry diets. In the review by these authors, it is further noted that diets for broiler chickens contain in the order of 2.7 g/kg phytate-P or 9.6 g/kg phytate. The phytate molecule is an anti-nutrient and its presence in the GIT may result in significant losses of endogenous nutrients (mainly AA's) and energy in the form of mucins, intestinal cells and pancreatic enzymes. This means that expected gains in bird performance from the hydrolysis of phytate go beyond the supply of phosphorus. Many of the anti-nutritive effects of phytate have been well defined in the literature (Selle *et al.*, 2012; Selle and Ravindran 2007). Cowieson *et al.*, (2017) reported on a meta-analysis conducted specifically to explore the way phytase impacts on the digestibility of AA's in broilers and found that phytase enhanced AA digestibility by between 2 and 6%. This improvement was directly linked to phytate degradation and the responses of AA digestibilities were negatively correlated with the inherent digestibility of different AA's. In reduced CP diets, the crystalline AA's are usually given a digestibility of 100% which is probably not entirely correct since the efficiency of the digestion process is not perfect and GIT micro-organisms would also compete for nutrients including free dietary AA's. However, what is clear is that phytase would have a much lower impact on these free amino acids compared to the phytate-bound AA's and protein.

When CP is reduced in meat-type chicken diets, particularly with the use of semi-purified ingredients and crystalline AA's, the phytate levels are reduced and this leaves researchers in a quandary when designing trial diets. Since phytase is used in virtually all broiler feeds in Australia, New Zealand (and many other parts of the world) it should be included in trial diets but, as the phytate substrate reduces, the amount of nutrients liberated by phytase declines. The nutritional contribution of phytase therefore changes as dietary phytate content changes and as the digestibility of individual AA's improves due to the increasing addition of crystalline AA's. If phytase is included without its nutritional contributions in feed formulation, this would skew the treatments in favour of the standard positive control diets so most research into reduced CP diets excludes the use of phytase to avoid these confounding factors. However, it is important for phytase to be included in basal diets for experiments evaluating reduced CP diets because of its broad acceptance in commercial broiler diets. Similarly, xylanase needs to be included when experimental diets are based on wheat.

Despite phytate concentrations decreasing, due to the reduction of protein-bound AA's and the increases of crystalline AA's, the negative impact of phytate on digestive dynamics and nutrient absorptions may still make the presence of phytase important in modern broiler diets. The likelihood is that exogenous phytase will have a positive bearing on the post-enteral bioavailability of glucose and AA's, perhaps largely stemming from its influence on Na^+-dependent intestinal uptake of these nutrients. Dilworth *et al.,* (2005) reported that phytate depressed Na^+ pump activity and reduced blood glucose levels in rats and phytase has been shown to increase concentrations of Na^+ pumps and glucose in duodenal and jejunal enterocytes in chickens (Liu *et al.,* 2008). Therefore, there is a distinct possibility that phytate and phytase have reciprocal impacts on Na^+, K^+-ATPase activity requiring clarification and, ideally, identification of the underlying mechanisms (Moss *et al.,* 2018). The balance between availabilities of glucose and AA's, protein-bound AA's and crystalline AA's may be more important in reduced CP diets than conventional broiler diets because crystalline AA's are rapidly absorbed. Therefore, the negative impact of phytate on nutrient absorption may be more pronounced in reduced CP diets and further investigations are required to verify this hypothesis.

Conclusions

The complex metabolic pathways of carbohydrate, protein and lipid metabolism and the rates at which the various components are digested and absorbed, when CP is reduced and large amounts of crystalline AA's are added to broiler diets, needs to be considered when implementing these commercially. Notwithstanding limitations within the feedmill, the effect of conditioning and pelleting temperatures on the possible reduction of the availability of AA's to the broiler should receive some attention.

It is prudent to maintain the DEB at a level, consistent with optimal broiler performance in the bird, when reducing CP to exclude any possible confounding effects of changes to Na^+, K^+ and Cl^-. Ensuring that there is enough NEAA content in the feed is also of importance although it is still not entirely clear what these levels should be and this warrants further research.

The use of phytase enzyme is likely to continue, even with a reduction in CP of broiler feeds. However, phytate content of the feed should determine the nutrient contribution assigned to phytase.

Finally, the concept of using ideal amino acid profiles in feed formulation has been widely adopted although there are differences between various authors. It is probable that the ideal ratio of AA's to Lys is altered when CP is reduced substantially in meat-type chicken diets and the nominally conditionally EAA's as well as sufficient NEAA's would need to be considered in future research.

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Calcium in Poultry - A New Look at an Age-Old Nutrient

DAVID ISAAC[1] and BENJAMIN POLLET[2]

INTRODUCTION

Calcium (Ca) has a key role in poultry nutrition. This macromineral is intricately involved in many metabolic processes. Most of the body calcium is found as calcium hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$) in bones. Calcium in the circulatory system, extracellular fluid, muscle, and other tissues is critical for mediating vascular contraction and vasodilatation, muscle function, nerve transmission, intracellular signalling, and hormonal secretion. Bone tissue serves as a reservoir for and source of calcium for these critical metabolic needs through the process of bone remodelling *(A. Catharine Ross et al 2011*). There is a constant exchange of calcium ions between serum and bone.

Calcium Metabolism and Absorption

Calcium metabolism is regulated in large part by the parathyroid hormone (PTH)– Vitamin D endocrine system, which is characterized by a series of homeostatic feedback loops. The rapid release of mineral from the bone is essential to maintain adequate levels of ionized calcium in serum. During Vitamin D deficiency state, bone metabolism is significantly affected because of reduced active calcium absorption. This leads to increased PTH secretion as the calcium sensing receptor in the parathyroid gland senses changes in circulating ionic calcium. Increased PTH levels induce enzyme activity (1α-hydroxylase) in the kidney, which converts Vitamin D to its active hormonal form, calcitriol. In turn, calcitriol stimulates enhanced calcium absorption from the gut (*A. Catharine Ross et al 2011*). As such, there is a strong interplay between calcium and Vitamin D.

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Nutritional factors can increase or reduce calcium absorption. Calcium availability from raw materials and its solubility in the intestinal tract are major factors affecting absorption. Further to this, the ability of the intestinal cell to transport the calcium across the enterocyte and into the blood is a key determinant in calcium absorption.

Calcium is absorbed by both a passive, paracellular route and by an active, transcellular mechanism. Transcellular calcium absorption involves three steps:

- entry into the cell
- diffusion across the cell, and
- extrusion from the cell.

The first step, entry through the apical brush border, occurs via a member of the vanilloid (TRPV) superfamily of channels, namely TRPV6. After calcium has entered the cell, it must be transported through the cytoplasm and extruded from the cell against a steep gradient. The mechanism by which calcium moves through the cell involves the small cytosolic protein Calcium Binding Protein (CaBP). Calcium entering the cell via the apical TRPV6 channel becomes tightly associated with the CaBP, which buffers the relatively large mass of entering calcium and minimizes its impact upon cytosolic free calcium concentrations. The CaBP /calcium complex then diffuses across the cytosol to the basolateral membrane. Free calcium then dissociates into the low-cytosolic calcium environment maintained immediately subjacent to the basolateral membrane by high-affinity membrane Ca^{+2}-ATPases located there. Finally, these calcium ATPases actively extrude calcium out of the cell. Indeed, the importance of this buffered diffusional process predicts that enterocyte CaBP content is likely to be a major determinant, along with TRPV6 activity, of the overall rate of enterocyte calcium transport (Rajesh V Thakker et al, 2016).

Calcium Carbonate

Calcium carbonate is the most commonly used calcium source in poultry nutrition due to its economic advantage. Calcium carbonate is comprised of a carbonate ion (CO3 -2) combined with a calcium ion (Ca+2). On average, it contains 38 – 39% of calcium. The main source is limestone rock. The nature of the rock it originates from not only determines its purity but also its physico-chemical properties, ionization solubility and hence bioavailability.

The major digestive feature affecting digestibility of $CaCO_3$ is the ability of the digestive organs to produce acids to dissolve the calcium and the ability to maintain the most favourable pH to ionize the calcium. Only ionized Ca can be absorbed into the enterocytes.

In avian species, gastric secretion only occurs in the proventriculus, with the main original feature being the secretion of hydrochloric acid and pepsinogen. The hydrochloric acid can dissolve 7 to 8 grams of calcium carbonate per day, hence the non-negligible role of the proventriculus in controlling calcium metabolism, especially in laying hens. The pH of the gastric acid is between 1 and 2 (Beghoul S, 2006). At this pH, calcium carbonate reaches its maximum solubility.

Calcium in Laying Birds

Layer and breeder hens have higher calcium requirement for egg formation. Egg formation takes about 12 hours. During this period, calcium is deposited continuously at a rate of 170 mg of calcium per hour (+0.5g of shell/hour) *(Nau et al., 2010*). Hen's blood only contains 25 mg/dL of calcium, thus requiring regular renewal of blood calcium while an egg is being produced. As such, calcium need to be constantly absorbed from the digestive tract and for this to happen, the calcium source need to be retained in the gizzard. Solubility and particle size are the two parameters to be taken into account to promote retention of calcium in the gizzard. A study by *Zhang and Coon (1997)* highlighted the importance of the source of calcium, the particle size, in vitro solubility and the level of calcium in the feed on retention in the gizzard.

	Solubility (%)					
Carbonate size (mm)	In vitro		In vivo		Retention in the gizzard	
	A	B	A	B	A	B
3.3 – 4.7	29.8	36.3	84.8	82.5	15.4	3.4
2.0 – 2.8	45.8	54.8	79.0	84.0	11.8	4.3
1.0 – 2.0	49.3	57.7	77.8	74.4	5.5	4.7
0.5 – 0.8	63.1	67.6	76.5	69.4	0.7	1.6

A = slightly soluble form
B = highly soluble form

Zhang et al.,1997

Table 14: Influence of particle size and "in vitro" and "in vivo" solubility of calcium and retention in the gizzard

Zhang and Coon, 1997 in the Technical Bulletin, ISA 2010

The recent recommendations promote 50 % or more of larger limestone grits (particle size >2mm) to be incorporated in the laying stage.

Calcium Particle Size

PARTICLE SIZE	STARTER, GROWER, DEVELOPER	PRE-LAY	WEEKS 17–35	WEEKS 36–55	WEEKS 56–74	WEEKS 75–90	POST-MOLT
Fine (0–2 mm)	100%	50%	50%	40%	35%	35%	35%
Coarse (2–4 mm)	–	50%	50%	60%	65%	65%	65%

Hyline Brown Commercial Layer Management Guide 2016

Calcium in Broilers

In broilers, there is a much lower requirement for calcium. However, the absorption of calcium in young broiler chicks is not effective. Young birds have an immature digestive tract which produces low level of acids for calcium ionisation. This, paired with fast transit time, limits the amount of calcium that enters the blood circulation. A strategy to assist the calcium absorption is to provide limestone in fine particles, as shown by the Guinotte et al in 1995.

Particulate size of calcium carbonate	Weight gain (g/cage)	Retention of Ca (%)	Tibia resistance (N)
< 0-5	198	48,2	56,2
> 1-2	174	21,5	49,9

Table 16: Effect of calcium particulate size on calcium retention and bone resistance in chicks

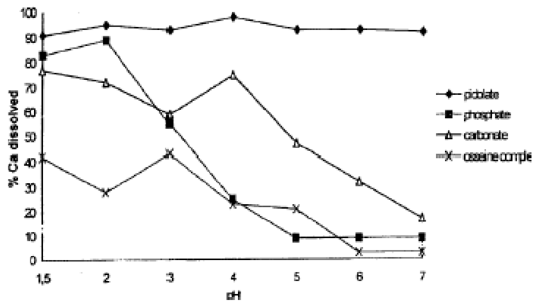
Guinotte et al., 1995

The measures above have provided reasonable success with calcium absorption in layers and broilers. However, with the increasing demand for better performance with improved genetics, nutrition and health, we need to find alternate sources to supplement these strategies. One such product is Calcium pidolate.

Calcium Pidolate

Calcium pidolate or calcium pyrrolidone carboxylate is a calcium salt with formula $C_{10}H_{12}O_6N_2Ca$. It is comprised of 13% calcium and 87% pidolic acid (dry matter basis). The solubility of calcium pidolate in water is high (250 g/l), unlike that of calcium carbonate (15 to 20 mg/l, at 25°C). The dissolution constant in solution of pidolate is Kd = $1.3.10^{-3}$. This is higher than that of calcium acetate or calcium propionate (EFSA, 2007).

The solubility of calcium carbonate is very much controlled by environmental ph. As such, the maximum absorption is limited to the front part of the small intestines. Calcium Pidolate has high solubility throughout the different pH range of the chicken gut and as such is able to be absorbed throughout the digestive tract.



pH	% of Ca dissolved Pidolate	Phosphate	Carbonate	Ossein complex
1,5	91	83	77	42
2	95	89	72	28
3	93	55	59	43
4	98	25	75	23
5	93	9	47	21
6	93	9	32	3
7	92	9	17	3

Figure 11: Solubility of calcium pidolate, calcium phosphate, calcium carbonate and the ossein complex at different pHs, in aqueous solution

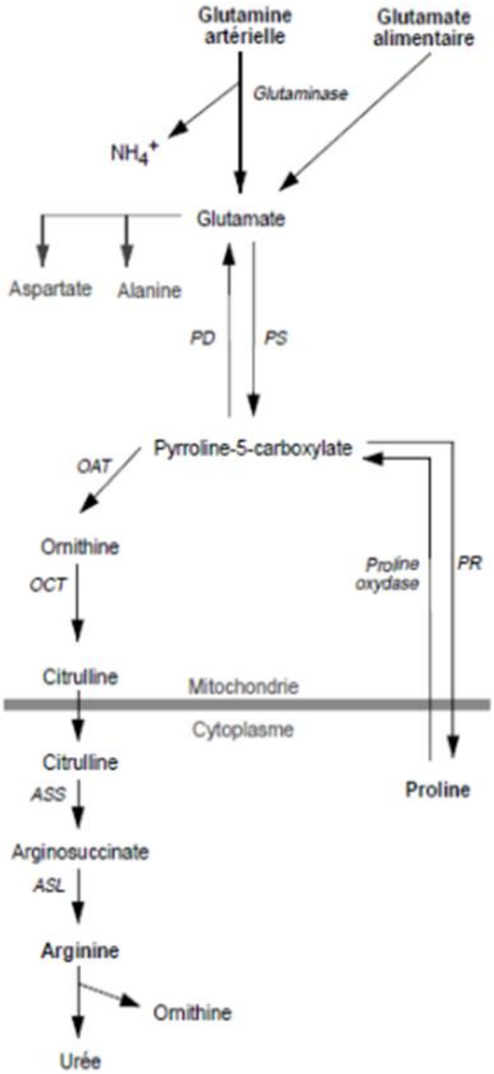
Cayon and Roquer, 1997

The absorption of calcium, provided in the form of pidolate, has been studied in humans and animals.

In rats, providing 10 mg/kg of calcium pidolate increased the serum calcium level by 65% in 40 minutes and by 100% after 3 hours *(Terafeed, Internal report: Marmo).*

In humans, 50 subjects received 405 mg of calcium in the form of calcium pidolate or 2500 mg in the form of reference calcium (5 pills of 500mg of calcium in carbonate and gluconate form). Pidolate enabled serum calcium to be increased by 14.5%, compared to an increase of 7.9% with the other calcium source- which was 6 times richer in calcium *(Terafeed Internal report: Prof. P. Benoît, Prof. Ag. F.X. Michelet and Coll. - Institute of Stomatology – Bordeaux).*

Calcium pidolate not only provides a highly bioavailable calcium, it also assists in the absorption and formation of calcium complex in the body. Following dissociation, the Pidolic acid becomes a precursor of Arginine. As one of the main components of CaBP is arginine, calcium pidolate assist in synthesis of CaBP to transfer calcium ions (from all sources) across the enterocyte for more efficient absorption.

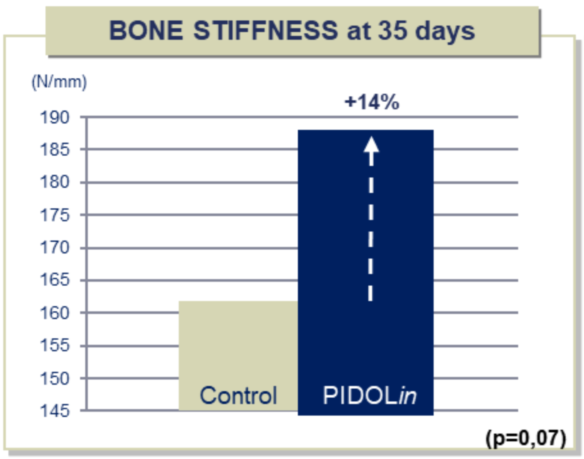


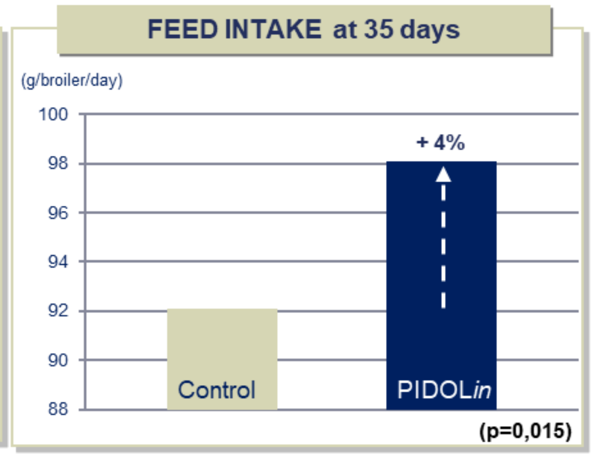
Jones M.E., 1985. Conversion of glutamate to ornithine and proline : pyrroline-5-carboxylate, a possible modulator of arginine requirements. J. Nutr., 115, 509-515.

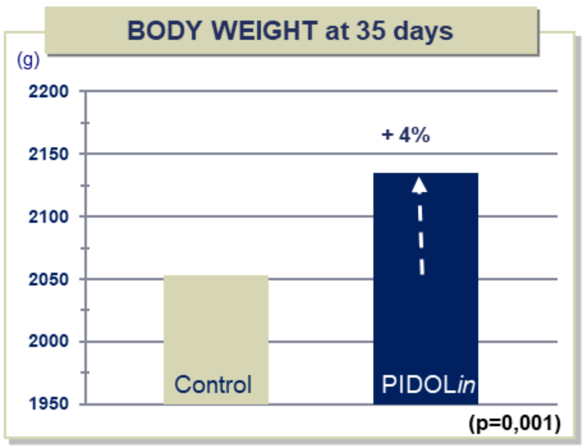
Calcium pidolate is also involved in the synthesis of collagen. Collagen constitutes of mainly proline and hydroxyproline. As Pidolic acid is also a precursor of Proline, calcium pidolate assist in formation of collagen. The increased level of collagen can lead to stronger bones as 30% of bone matrix is made of collagen. In laying birds, the egg shell quality improves due to higher collagen deposition on the shell membrane as well as improved calcium absorption.

Trials in broilers, pullets and layers have shown significant improvement in performance.

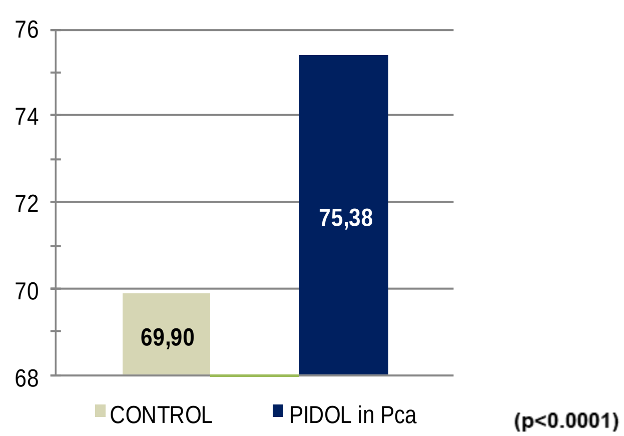
In a 2013 broiler trials in France, supplementation of Calcium pidolate at 300g/mt for the first 21 days of life, gave a significant improvement in performance and bone stiffness at 35 days of age. The flock uniformity improved by1.8% and the mortality and culling reduced by 21% (*Roulleau et al*, *2015*)







In a 60 000-layer pullet trial, 50% of the Hyline pullets were fed with Calcium Pidolate from day 1-28 at 500g/mt and then again at week 17-18 at 300g/mt. The tibial length significantly improved (P<0.0001) with control group tibia at 69.9mm and the calcium pidolate treated group at 75.38 mm.



These pullets were followed through the production cycle. At 71 weeks of age, the egg breaking strength and egg size was significantly higher than control group as well as the Hyline standard.

In a recent trial presented at APSS 2018 (Bain et al, 2018), it was shown that providing commercial free-range layer hens with a diet supplemented with calcium pidolate from 50 weeks of age can improve egg quality: the % seconds decreased, and eggshell strength and shell colour were also improved.

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

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

Conclusion

Calcium is a key mineral in poultry nutrition and plays a major role in multiple physiological processes. Calcium carbonate has been the major source of this nutrient due to availability and economic advantage. However, due to limitations in solubility at different ph levels and variable quality of this ingredient, it is pertinent that we employ the right strategy for different production stages. The right particle size for the corresponding production stage ensures optimum digestion and absorption of calcium from calcium carbonate.

The unravelling of the absorption mechanisms of calcium at the enterocyte level has provided opportunity to supplement with highly bioavailable calcium sources such as calcium pidolate.

This molecule not only has high solubility throughout the gastrointestinal tract, it also supports the absorption of calcium from all sources via its role in arginine synthesis. Pidolate's ability to form proline gives it the added advantage to form collagen, that improves bone integrity and shell strength.

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Effects of Beta Glucans in Poultry Health and Nutrition

GEOFF HORST

Increasing Role of Immune Modulators in Poultry Production

The poultry production industry has seen astonishing improvements over the past several decades as evidenced by increased growth rates and improved feed conversion efficiency. We can now produce bigger and faster growing birds with far less feed than imagined even 20 years ago. These improvements can be attributed to a combination of genetics, nutrition, farm management and disease management. Genetics ultimately limit the maximum potential growth performance of the birds, while nutrition, farm management and disease management can each impact how close to the genetic potential the birds can achieve on a given farm. This paper addresses the rapidly evolving field of disease management and the new tools that producers can use to improve the health and thus performance of their animals.

Antibiotics and specifically antibiotic growth promoters (antibiotics used prophylactically for disease prevention and modest growth enhancement) have made a significant contribution to some of the performance gains in recent decades. While the exact mode of action of these drugs is still not completely understood, the potential impacts on promoting antibiotic resistance is becoming more apparent and thus their usage is being phased out across many regions of the world.

As some of these antibiotics are being removed from the market, producers are now taking a more comprehensive approach to disease management including improved biosecurity at the farm, expanded vaccination programs and feed additives that improve gut health and immunity. This last group of products, loosely called immune modulators, include probiotics, peptides, and various carbohydrate molecules that interact with the animal's immune system to help it fight infections and/or manage inflammation caused by infections.

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Background on Beta Glucans

Beta glucans, and specifically, beta-1,3-glucan, are polysaccharides that are widely recognized as potent immune modulators (Barsanti et al. 2011). When animals are exposed to beta glucans either orally, through the skin or even intravenously, the immune system detects the specific pattern of glucose monomers connected through beta-1,3 linkages (Fig. 1) as a pathogen associated molecular pattern (PAMP), which triggers a cascade of immune system responses. Evolutionarily, this makes sense because many pathogenic organisms including fungi, yeasts and bacteria have beta-1,3-glucan incorporated into their cell walls/membranes.

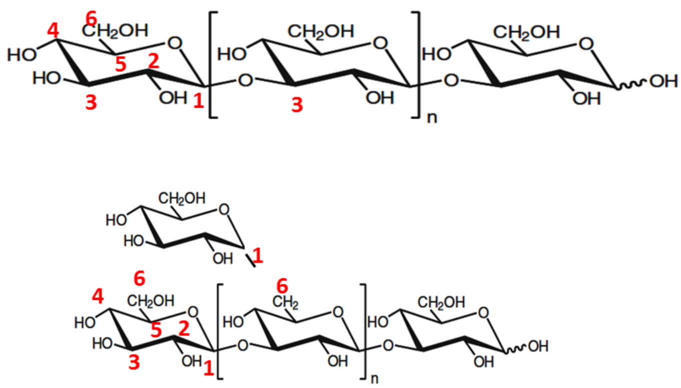

Figure 1: Linear beta-(1,3)-D-glucan (top) and side-branched beta-(1,3;1,6)-D-glucan (bottom)

Researchers have taken advantage of this known immune response to beta glucans by attempting to "prime" the immune system of animals before challenging them with a specific pathogen diseases and/or stressful environmental conditions. Notable positive results in poultry studies include:

- Increased immune cell activation: Chae et al. (2005); Guo et al. (2003); Lowry et al. (2005)
- Increased vaccine antibody titers: An et al. (2007)
- Reduced impact of bacterial infection by *Salmonella* and *E. coli*: Chen et al. (2008); Lowry et al. (2005); Huff et al. (2006)
- Improvements in intestinal health following coccidiosis infection: Cox et al. (2010)

- Improved growth rate and feed conversion efficiency: An et al. (2007); Chae et al. (2005); Cox et al. (2010); Moon et al (2016); Morales et al (2009); Zhang et al. (2008)

A complicating aspect of the prior art is that beta glucans are a ubiquitous class of molecules produced by a wide array of organisms ranging from bacteria, yeast, fungi, seaweeds, plants and algae. Each class of organisms produces vastly different forms of beta glucan with different linkage patterns (e.g. linear beta-1,3 linkages with or without beta-1,6 side branches, Fig. 1). Furthermore, commercially-available beta glucans range in purity from a few percent up to over 95% purity. Most of the research on beta glucans in animals has been conducted with yeast beta glucans, which have been commercially available for over a decade. However, most of these products are still a mixture of beta glucans and other yeast cell wall components so it is difficult to parse out the effects of the beta glucan alone.

Some species of *Euglenoids* (protists) produce beta glucan granules (paramylon) to store excess energy similar to the way some plants store energy in starch granules. These small (1-3 micron) granules are composed of nearly pure linear beta-1,3-glucan strands arranged in a triple-helix crystalline format similar to a tightly bound ball of yarn (Fig. 2).

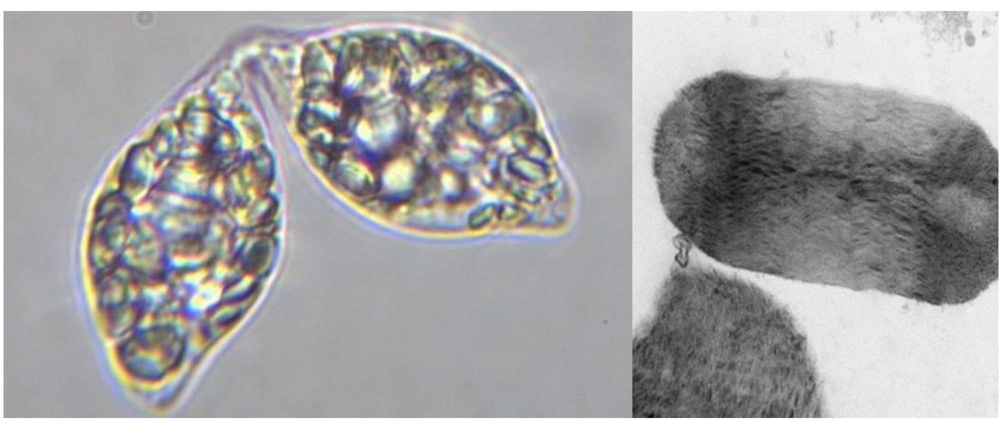

Figure 2: Two Euglena gracilis cells packed with beta glucan (paramylon) granules (left), SEM image of a paramylon granule.

Paramylon Mode of Action

Paramylon appears to modulate the innate (cellular) immune system of animals in the same fashion as other forms of beta glucan containing linear beta-1,3-glucan as reviewed extensively by Chan et al. (2009) and Barsanti et al. (2011) (Fig. 3). Paramylon granules transit into the small intestine where immune cells (dendritic and macrophage cells) actively capture and phagocytose the granules (Fig. 4). These immune cells then start to "prime" the immune system by releasing both pro and anti-inflammatory cytokines (Fig. 5) as well as chemokines that help recruit more immune cells to the gut (Fig. 6). After priming, many facets of the innate immune response are enhanced including increased natural killer cell activity, foreign particle phagocytosis rates and oxidative burst potential. As a result, with the innate immune system "primed", the animal should be better prepared for fighting potential pathogen infections.

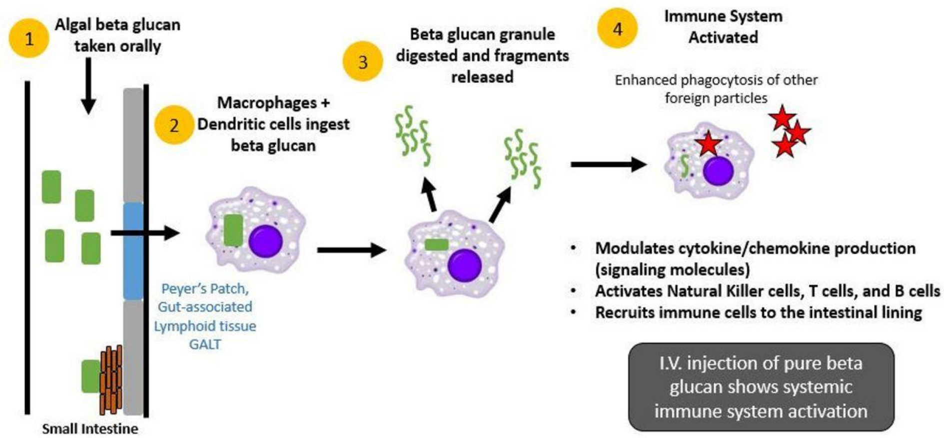

Figure 3. Simplified mode of action for immune modulation by beta glucans.

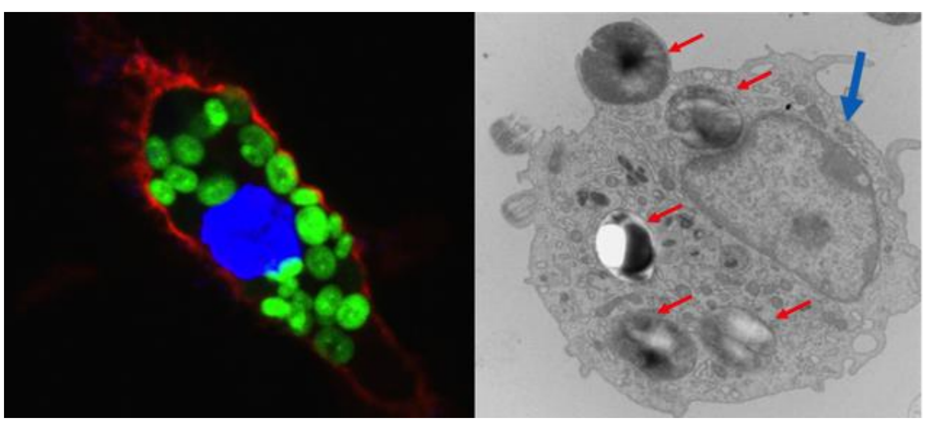

Figure 4. Mouse macrophage cell (stained red) after phagocytosing fluorescent stained Euglena beta glucan granules, SEM of macrophage cell (blue arrow) actively phagocytosing beta glucan granules (red arrows).

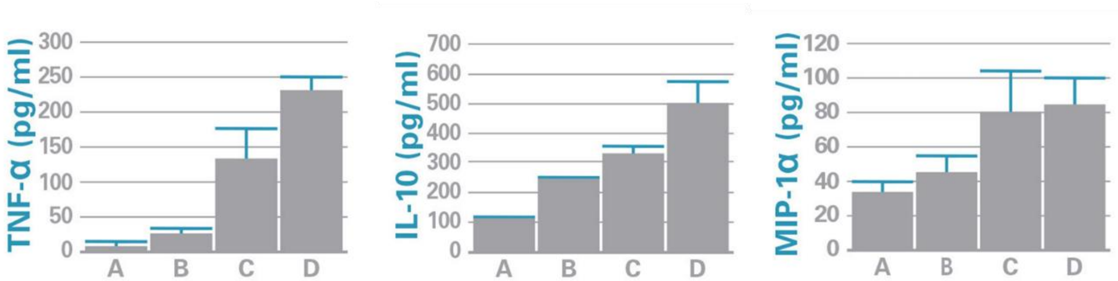

Figure 5. Increasing doses of euglena beta glucan (A=control; B=25 ppm; C=100 ppm; D=1000 ppm) in the diets of mice leads to increases in pro-inflammatory cytokines (TNFa), anti-inflammatory cytokines (IL-10) and chemokines (MIP1a).

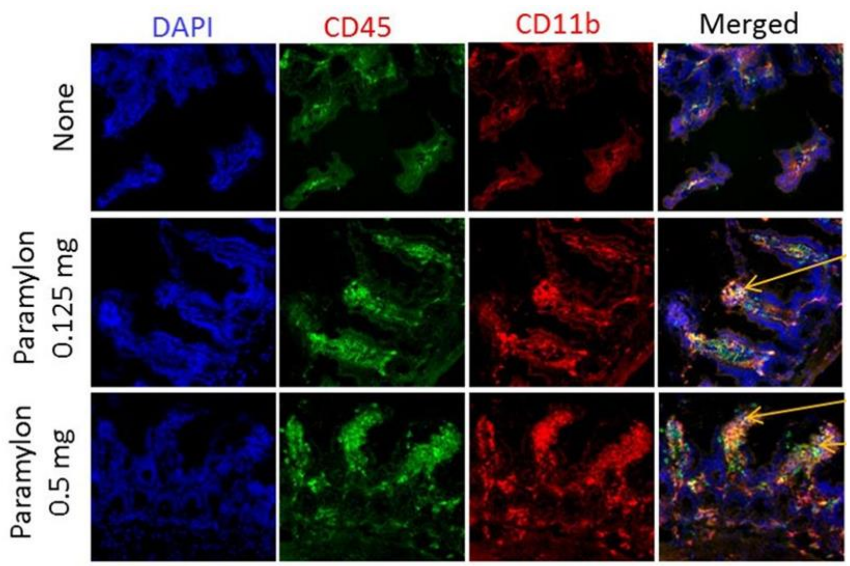

Figure 6. Mouse intestinal sections after 7 days of oral dosing of paramylon. Increased green and red color indicates higher density of immune cells recruited into the villi.

Applications to Poultry Production

Birds on a farm are exposed to a nearly constant threat of disease from a wide range of viral, bacterial, fungal and parasitic organisms. Bio-security is a key strategy for reducing some of the disease pressure but it is not a fail-safe system. Vaccines are also a great strategy for controlling many common diseases but we still do not have vaccines for many of the remaining commercially-important diseases, especially many bacterial diseases. Antibiotics can also be highly effective for controlling an outbreak but there is a global shift away from using antibiotics prophylactically. Therefore, immune modulators like beta glucan, that prop up an animal's own innate immune system, can be an important tool for helping to manage disease, especially for very young birds that do not have a fully-matured immune system and during times of immune suppression caused by environmental stresses (e.g. heat, cold, ammonia) or biological stress (e.g. live vaccines).

Example 1: Coccidiosis challenge

Levine et al. (2018) conducted a series of experiments to test whether dosing *Euglena* beta glucan into the diets of broilers could help mitigate the effects of a severe coccidiosis challenge. Briefly birds received diets that were amended with a range of euglena beta glucan levels (25-100 ppm). On day 14, the birds were challenged with an oral gavage containing a mixture of three *Eimeria* species (*E. acervulina, E. maxima and E. tenella*) which are known to cause coccidiosis in broilers. Lesion scores were assessed on day 20 in addition to gut immune histology and performance metrics (growth rate, feed conversion, mortality).

In the first experiment, the birds that received feeds supplemented with beta glucan showed a significant increase in immune cell density in the gut, which was also correlated with reduced lesion scores. This is a particularly interesting finding since it is known that the *Eimeria* parasite can actually trick the host into perceiving that there is no infection and thus reduce immune cell density in the gut which allows secondary infections to take hold. In the second experiment, the beta glucan treated birds showed significantly improved feed conversion efficiency during the challenge period (day 14-20). Together, these results suggest that modulating the bird's own immune system can have a drastic impact on a commercially-important gut health disease like coccidiosis.

Example 2: Dirty litter challenge (multiple disease stressor)

Since birds are exposed to many potential pathogens simultaneously, we tested whether using a beta glucan immune modulator could reduce the impacts of a severe, multi-pathogen stressor on broilers in a 42-day pen trial. Briefly, the birds were fed a control diet (antibiotic free), or the control diet amended with 50 ppm of *Euglena* beta glucan for either the first 28 days or over the full 42 days of the trial. On day five, all of the birds were challenged by introducing untreated dirty litter into the pens from the last production cycle which contained *E. coli*, *Clostridium* and *Eimeria* spp. In both of the beta glucan treated groups, the birds had significantly improved growth rates and feed conversion efficiencies (Fig.7), presumably because the birds were able to fight the infections more efficaciously when primed with the treated feeds. The fact that the birds given beta glucan for only the first 28 days performed similarly to the birds dosed for the full term of the trial suggests that, in this case, there was no extra benefit to continued usage of the immune modulator during the final two weeks.

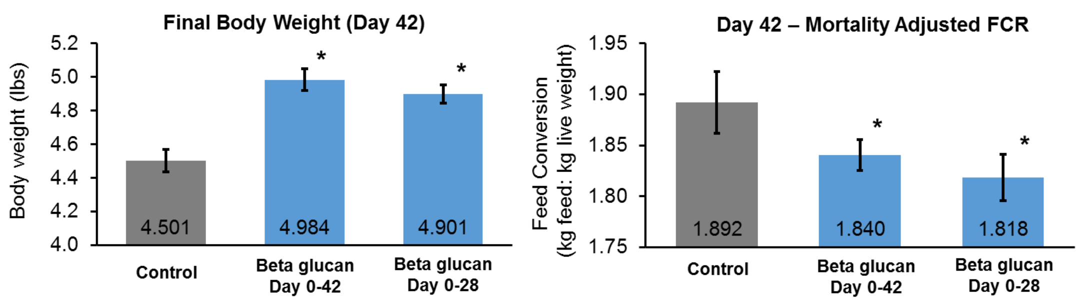

Figure 7: Growth and feed conversion efficiency for broilers challenged with multiple diseases in recycled litter. Beta glucan dose level was 50 ppm.

Conclusions

Beta glucans and other immune modulators have begun to show promise as another useful tool for livestock producers to improve the health of their flock. While we are still in the early stages of fully understanding how to best use immune modulators like beta glucans, there are a few general patterns developing:

1) Young animals are especially in need of innate immune support since their immune system is still developing.
2) Immune modulators can only help improve performance metrics if the flock is underperforming due to wasting energy or suffering mortality/morbidity from disease pressure. If a flock is already performing very well, then the scope for improvement by an immune modulator will be small.
3) Immune modulators may be helpful during vaccination periods by both helping prime immune cells that are also active in the humoral (antibody) immune system as well as helping to mitigate some of the setback that can occur from live vaccines.

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An Overview of the Duck Industry in New Zealand and Australia

JOHN HOUSTON

Australian and New Zealand Duck Industry

- Valued at $165 million per annum

- Production is around 13.5 million ducks per annum
  - 29,250 tonnes of processed product
  - Over the last 5 years sales have increased by approximately 40% in Australia

- Approximately 30% of duck meat is supplied to:
  - Fine dining restaurants
  - Delicatessens
  - Major retail outlets
  - Export markets

Woolworth

Foodstuffs NZ

- Remaining 70% is supplied to Chinese BBQ Shops for "Peking Ducks"

Pepe's Ducks, Australia

There are three major vertically integrated duck producers in Australia and New Zealand – Quack'a'Duck, Pepe's Ducks and Luv-a-Duck

Vertical Integration

- Breeder farms - breeder duck and fertile egg production
- Hatchery – day old ducklings
- Broiler farms – meat duck production
- Processing facility
- Sales, marketing and distribution

The Pekin duck is the choice of breed for production

- Breeders
  - 260 eggs every 46 weeks
  - Sexual maturity - 24 weeks
  - Fertility - 92%
- Broilers
  - Standard growth - 42 days
  - Average Weight - 3.2kg
  - FCR - 1.9
  - Growing density – 18kg / m^2

Genetic stock is sourced from overseas

- Grimaud stock from France by Pepe's Ducks (Quack 'a' Duck – NZ)

- Cherry Valley stock from the United Kingdom by Luv a Duck
- Fertile egg importations come through an accredited quarantine station

Duck Products

A wide range of products are available including:

- Whole ducks (head on, head off)
- Duck pieces (maryland, breast, fillet)
- Offal (liver, hearts, giblets)
- Valued added (sausages, smoked fillet, fat, ready to go meals)

The versatility of the industry extends to products outside the food industry and includes manchester and bedding.

Markets

- **P**rinciple Market – Asian BBQ shops, restaurants and butchers
- Western market – restaurants, cafes, catering sectors
- Approx. 10% retail, balance food service

Biosecurity, Welfare and Food Safety

- Duck industry has developed the Farm Biosecurity Manual for the Duck Meat Industry in association with Animal Health Australia and has been implemented on farms
- Duck industry operates under the model code of practice for the welfare of domestic poultry
- Duck industry has to comply with Poultry Standard 4.2.2 which is a FSANZ (Food Standards Australia New Zealand) initiative for food safety

There is a strong commitment to biosecurity, animal welfare and food safety within the industry which has been recognised with awards such as:

- *Australian Farmer of the Year - Biosecurity Farmer of the Year*
- *Australian Farmer of the Year - Livestock Producer of the Year (finalist)*
- *Bayle Asia - Pacific Customer Excellence Award*

Research and Development

- Research work has been conducted through partnerships with the Rural Industries Research and Development Corporation (RIRDC), Poultry Hub and the University of Sydney
- Trials have been conducted to establish the strain effect on growth rate, feed consumption, water usage, processing and cooking
- Trials have been conducted to investigate the effects of stocking density, transportation, heat stress and behavioural traits

Australian Duck Meat Association

- The Australian Duck Meat Association (ADMA) was formed and incorporated in 2008 as an initiative of the two major duck producers to represent the duck industry at a national level
- The Association (ADMA) became a member of Animal Health Australia (AHA) in 2008

ADMA
australian duck meat association incorporated

Future

- The industry has a bright future with production expected to continue to increase to meet domestic and export market demand
- Asia is also predicted to continue to increase its consumption of duck meat and the New Zealand and Australian industry is well placed to export to this region based on:
    - Modern growing and processing facilities
    - World class genetic stock

- High standards of biosecurity, welfare and food safety
- Proximity to the region

Practical Aspects of Managing Poultry Health in NZ – Monitoring the Vaccination Programme

CAROL BATES

ABSTRACT

Vaccination plays a key role in the health management of the poultry flock and is especially important for breeders as it protects not only vaccinated birds but also their progeny.

In spite of the availability of high quality vaccines and explicit directions on their applications, there are vaccination failures. Most of these can be attributed to mishandling and poor administration. Regular audits of vaccine handling and administration techniques are critical to control challenges and improve performance.

Reviews of vaccination audits including wing web stab, water, spray, intramuscular and subcutaneous routes highlight the importance of providing adequate labour resources and on-going training. Poor vaccination technique leads to lousy serology results. Monitoring of vaccination responses by blood collection and subsequent serology testing (most commonly by ELISA) is recommended. Each laboratory will have a preference for its ELISA monitoring system. Titres from each system are not directly comparable so it is important to know which one is used and that protective titres are established for each system. A titre can only be interpreted when the ELISA that was used is known. Routine schedule examples for timing of serologic testing alongside guidance on interpretation of serology data will be illustrated.

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Drought, Dumping and Disease – A review of the South African Poultry Industry

MICHAEL PRETORIUS

The South African poultry industry is the largest contributor to total gross agricultural production in the South African agriculture sector, constituting 18% of all agricultural production in 2016 and 39% of all animal product gross value, estimated at R47.0 billion. Its long, complex and highly developed value chain has a massive multiplier effect. An example of this would be the fact that nearly 21% of chicken produced go to fast food companies. It is estimated that the industry directly employs 47,000 people and another 59,000 indirectly. The industry is one of the most developed on the continent and is competitive on a global scale with regards to production efficiencies.

Chicken remains the most affordable animal protein source to the South African public, which consumes approximately 39kg of poultry meat and almost 8kg of eggs per capita. In 2016 the average producer price for broiler meat was R18.65/kg and R18.29/kg of eggs respectively. South Africa has a population of approximately 55 million people.

Because of its popularity there have been calls to have poultry meat tax exempt, as is the case with brown bread & maize meal, to increase affordability. Government would be hesitant to entertain this, as it would reduce tax revenue by approximately R5 billion. Approximately 19 million birds are slaughtered every week with an additional, meat equivalent, of 8 million birds imported to satisfy South Africans consumption of about half a bird per person per week.

The majority of broiler meat and layer eggs are produced by large commercial (and often listed on the South African Stock Exchange) companies, for whom scale and competitiveness in the global context has become paramount since entering a free market system post 1994. The South African Poultry Association has 73 registered members as broiler producers and 87 (including co-operative members) registered layer producer members.

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Five major broiler producers dominate the market and supply close to 70% (main two making up close to 50%). Small-scale broiler producers only contribute approximately 3% of the national poultry product. Subsistence farming contributes an additional 3%, with culled/spent breeder and layer hens making up 3-4% of chicken sold for meat. Imports make up approximately 20% (2015) but has recently increased dramatically.

At the end of 2016 importation of broiler meat rocketed to 24.9%, bringing into question the global competitiveness of local poultry producers, and their inability to meet local demand. Key amongst reasons for the upward trend in imports had to do with the industry unable to absorb rising feed prices due to an unprecedented drought in 2015/2016 throughout much of the country.

The total rainfall between January and December 2015 was the lowest (403mm, compared to a long-standing average of 608mm) since records began (1903). The maize crop was estimated to be 22% less than the previous year (7.8 vs. 9.9 million tons). South Africa is traditionally a net exporter of yellow maize (main ingredient in animal feeds) but at the time was expected to import 1.9-2.2 million tons of yellow maize from Argentina to make up the shortfall, at a cost of R12 billion. Yellow maize futures reached 20-year record highs at R3954/t.

Despite increasing the local production of Soya bean meal (45% of requirement), South Africa remains a net importer. Which results in having to trade on protein meal prices based on import parity levels, sensitive to exchange rate fluctuations. Political and Economic instability was and remains a big influencer of the exchange rate. Despite the Rand appreciating by 9% between 1 Jan and 15 Dec 2016 after "Nene-gate", it was still 22% lower than levels of 1 Jan 2015 (R11.56: $1.00).

Layer and boiler feeds increased by 19% (average price R4096/ton) and 13.5% (average price R5602/t) respectively. The Bureau for Food and Agricultural policy's "Baseline (2017)" report suggests that during the 2015/16 drought the U.S. and Argentina enjoyed production costs 4% lower than those of South African producers. Brazilian and Ukrainian producers were able to produce chicken for 17.5% and 12.5% less than their South African counterparts.

Increases in feed prices are not matched by increases in local finished products. Indeed, the South African Poultry Association (SAPA) estimates that feed prices increased by 87% between 2010-2016, and that the average producer price increased by only 52% during the same time.

It must be said that under better maize harvesting conditions South Africa's finished feed prices and cost of productions are usually below those of most of European producers. Also, because South Africans prefer a lighter bird, it allows producers to optimize feed efficiency through greater feed conversion in a shorter production cycle. Something that the industry took full advantage off, when a dramatic turnaround in rainfall figures for maize and soya producing regions during the 2016/17 season, produced the largest ever maize crop of over 14.5 million tons (89% up from the previous year).

Why is it then that imports into South Africa, mostly from the Americas and Europe increased by 179% between 2005 and 2016?

It comes down to customers tastes and preferences. In South Africa portions of the bird described as "brown meat" (bone-in portions, leg quarters, drum sticks, wings, thighs) are preferred over "white meat" (bone less, breast meat) which is preferred in European and U.S. markets.

If the premium earned for breast meat is sufficiently high, an exporting country could sell off the rest of the carcass to a receptive market at reduced prices. Given that the cost of production of the whole bird is covered by the price received for the breast meat, the "waste" cuts of the bird can be sold below the cost of production per kg of the whole bird. Local producers then must compete with these reduced prices without receiving a premium for their own locally produced product.

IQF (individually quick frozen) pieces, dominates the local marketing mix of poultry meat products (60%), with fierce competition and pricing strategies amongst the top 3 producers already pushing prices down. Whole Fresh and Fresh cuts make up about 15% with Whole Frozen, Frozen Sundries and Frozen cuts making up the remaining 25%.

Importing countries selling below the cost of local production constitutes "dumping" and as such some regulations in the form of import tariffs must be in place to protect the local industry. The proportion of whole frozen birds (82 % tariff) or boneless chicken portions (12 % tariff) has decreased imports of these products in recent years; whereas the proportion of bone-in portions (37 % tariff; except for EU) is steadily increasing and exceeded 40 % of total imports from 2012 to 2016.

Brazil remains the largest exporter of MDM (Mechanically deboned meat) into South Africa and was responsible for 42% of total imports in 2016 (down 3%).

The EU currently enjoys duty-free access to the South African poultry market, under the Trade, Development and Co-operation Agreement (TDCA). And this is where the increase in imports stem from. The EU was responsible for 48.1% of total imports. The International Trade Administration

Commission (ITAC) has accepted that imports of frozen bone-in portions from the UK, Germany and the Netherlands are causing downward pressure on domestic prices and that these imports are essentially being dumped.

The Commission determined that the local industry has been unable to pass-on increases in input costs (feed and electricity) to consumers because of competition from dumped imports. Anti-dumping measures against these three countries have been legislated with a safe guard tariff of 13.9% suggested.

The AGOA (African Growth and Opportunities Act) trade agreement between African countries (including South Africa) and the U.S. was renewed in 2015. The new agreement sees the U.S. able to supply bone-in chicken portions to South Africa, at an anti-dumping tariff of R9.40/kg, however the first 65000 tons of meat is exempted from this tariff. U.S imports in 2016 constituted almost 5% of total imports (22,000 tons).

At the end of 2016, several large, integrated poultry businesses announced downscaling of their operations and associated retrenchments. Some producers divested their interests or diversified into other sectors (food service, export) to spread risk. Poultry imports contributed a quarter of the poultry consumption in South Africa in 2016. It is debatable whether these tariffs and legislations will result in any appreciable reduction in importation.

While imports over the first half of 2017 were 5% lower than for the same period in 2016, they were still 24% higher than the 5-year average for the period of January to July. Ironically outbreaks of AI in many European countries resulted in trade bans which reduced overall importation by approximately 4.5%.

Due to a record maize harvest (2017), poultry feed prices eased off, and with some indication that imports were slowing down, producers had reason to feel bullish. That was until early winter 2017 when the first outbreak of HPAI (Highly Pathogenic Avian Influenza) strain H5N8, in Mpumalanga was recorded on a broiler breeder farm near Villiers. All farms were placed under quarantine and all 260,000 breeders were culled.

Up until January 2018, there have been 107 reported cases, with at least 21 of these outbreaks from commercial operations. Outbreaks occurred in the Western Cape, Mpumalanga, Gauteng, KZN, Eastern Cape and the Free State.

The layer industry has been hit hardest with an estimated 4.7 million birds culled, 86,500 of which were parent breeder stock. In the Western Cape layer populations were reduced by 70% (3.3

million birds), as a result egg prices in the province increased by 20%. It is estimated that the biological value of birds lost is approximately R234 million with a further loss of R15.6 million in destroyed eggs.

AI infections in broilers were all in breeder flocks. A total of 712,377 broilers breeders were culled. This represents approximately 11% of the domestic breeding stock, of which the value was estimated to be R68 million. Total biological loss (layer and broiler) is calculated to be R318 million.

Income forgone because of the depleted breeder stocks (pullets, point of lay, day-old chicks) and loss of revenue from egg and broiler meat sales was calculated to be R1.5 billion. Which is a reduction in gross value of 15% for layer and 3.9% for broiler production compared to 2016. Layer feed sales dropped by at least 81,000 tons. If one also considers that approximately 42 million broilers (from the 700,000 culled broiler breeders @ 60 chicks per breeder) never made it to market that is another 117,000 tons of lost broiler feed. Basically, the production capacity of two average sized feed mills.

Globally, two different strategies have been employed to curb AI. Some countries, such as the USA and Europe have followed a culling strategy, which is typically accompanied by compensation to producers that have to cull. Others employ a vaccination strategy.

Layer producers in the Western Cape are unwilling to restock unless a vaccination regime is approved. However, if employed it would change the current “live bird” market that exists for spent/culled breeder and layer hens as these birds would need to be slaughtered before being sold.

Considerations of changing trade patterns should HPAI become endemic should also be considered before vaccination regime is approved. This could influence broiler export strategies and opportunities in the future. Improved biosecurity measures and controls could be an alternative to consider before embarking on a vaccination strategy. The risk of AI will return during the winter months of 2018.

There is no doubt that the South African poultry industry operates in an extremely volatile and challenging environment. Despite all these challenges some producers, have recently been able to return to profitability as poultry prices remained stable from December 2017 throughout the first quarter of 2018. Feed prices remain relatively low and lower unprotected imports are expected. Consumer confidence however remains low. Food safety has become a key issue, because of a

Listeria outbreak, resulting in some 180 deaths. The source of contamination was traced to processed meat (mainly form pork industry).

2019 is an election year in South Africa, with increased political instability and low economic growth forecast (1.5%) likely to put pressure on labor relations between companies and their employees. It is unlikely that the threat of imports will go away, and industry would do well to come up with a sustainable alternative to competing against bone-in imports. The future of the South African poultry industry remains uncertain and producers need to be adaptable and innovative to be able to overcome challenges and explore new opportunities.

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Options for Control of Mite Infestations in Chickens

TOM GRIMES

SUMMARY

Mites, particularly Red Mites (*Dermanyssus gallinae*), are economically important ectoparasites of poultry. Red Mites and Northern Fowl Mites (*Ornithonyssus sylviarum*) are the most common mites infesting poultry. Infestation of poultry with Red Mites is detrimental to bird performance, bird welfare, bird health and human welfare. As the mite life cycle can be completed in seven days and each mite can produce many offspring, infestation of poultry houses can occur within a very short time. Red Mites are particularly difficult to control because they spend most of their life hiding in house equipment, only feed on birds at night time and reproduce rapidly and in large numbers. Fluralaner is a recently developed, novel, efficacious and safe drinking water medication for control of poultry mites. Hens in lay can be treated without discarding eggs, as a zero withdrawal period for eggs has been established. Appropriate biosecurity procedures need to be implemented to prevent re-infestation. Mite traps can be used to diagnose and monitor mite infestations.

Chicken Mites

- Red Mite (Dermanyssus gallinae) and the Northern Fowl Mite (Ornithonyssus sylviarum) are the mites that most frequently infect poultry.
- Mites are obligate parasites needing blood to survive but can survive for up to 10 months without a blood meal
- Mites retreat to the environment when not feeding on the bird.

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Red Mite Life Cycle

- Red Mites are the most economically important of the poultry mites
- There are five stages of the Red Mite life cycle – egg, larvae, protonymphs, deutonymphs and adults
- Protonymphs, deutonymphs and particularly adults need a blood meal to survive
- Under optimal conditions, it takes seven days for the Red Mite to complete its life cycle
- Most of the time the Red Mites hide in house structures and equipment, infesting poultry only during a dark period (night) to drink blood
- Depending on temperature and humidity, each Red Mite can lay 32-40 eggs in one life cycle
- In a 6-week period, there can be exponential multiplication with one Red Mite producing up to 3125 adults which can result in 25,000 offspring in that period.

Impact of Red Mite Infestation

<u>Bird Performance</u>

- Fewer eggs in a laying cycle
- Increase of downgraded eggs due to mites or blood stains on egg shells
- Anaemia and mortality in severe cases of infestation

<u>Bird Welfare</u>

- Birds are stressed due to restlessness
- Irritation due to mites biting skin
- Feather picking due to irritation
- Pale birds due to anaemia in severe infestations

<u>Human Significance</u>

- Human infestation known as Gamasoidosis
- Mite bites cause severe itching and mild skin necrosis
- Spread of bacterial diseases such as salmonella infections and erysipelosis and viral diseases such as avian influenza
- Staff industrial consequences

Fluralaner

- Fluralaner is the first ecto-parasiticide to be developed for drinking water administration to poultry. Drinking water administration is less stressful to birds and safer for staff than insecticide spraying.
- Fluralaner is highly effective with up to 99% of mites being killed by 14 days after the commencement of treatment
- Safety has been confirmed by no adverse effects occurring when adult commercial egg layers were treated with 6 administrations of fluralaner at 5 times the recommended dose
- There is a zero withdrawal period for eggs which means that poultry in lay can be treated without discarding eggs. The withdrawal period for poultry meat and offal is 14 days.
- The treatment program is two doses of 0.5mg fluralaner per kg of bodyweight seven days apart in drinking water
- All infested houses on a farm should be treated concurrently
- Appropriate biosecurity procedures are required to prevent re-infestation of houses
- Mites can be diagnosed or monitored after fluralaner treatment by visual examination of birds, houses and equipment or by strategic placement of mite traps

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Relevance of Gastrointestinal Phytate Degradation for Phosphorus Utilisation in Poultry

MARKUS RODEHUTSCORD

INTRODUCTION

Phosphorus (**P**) utilisation along the entire food chain is an important issue of agricultural research because global rock phosphate stores are finite and unequally distributed, fertilizer and feed phosphates are expensive, and soil phosphate enrichment can be harmful to the environment. For the animal industries, achieving the optimum of P utilisation depends on knowing both the requirement of the animal and the value that different P sources have when used in animal feeding. The latter is subject of this contribution.

Different approaches have been developed and are applied to characterise P availability in poultry, which made it difficult to develop a generally applicable P evaluation system (Shastak and Rodehutscord, 2013). Aiming for harmonization, a working group of the World's Poultry Science Association in Europe has suggested a standard trial protocol for the determination of P availability based on precaecal digestible P (**pcdP**) (WPSA, 2013). The aim was to reduce the very high variation that was seen in data sets of P availability compiled from the literature (Shastak and Rodehutscord, 2015), and to make results from different institutions better comparable by using a standard protocol. While this seems to be achieved to some extent, variation between institutions remains to exist even when a standard protocol is used. In an international ring test, pcdP values of soybean meal varied from 19 % to 51 % between 16 collaborating institutions although the broiler trial protocol was standardised and the same diets were used in all institutions (Rodehutscord et al., 2017). Reasons for the remaining variation in this ring test are unclear, but the authors concluded that they likewise were related to the gastrointestinal degradation of phytic acid (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate; **$InsP_6$**) and their salts (phytate).

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Potential of phytate degradation in broilers

In plant seeds, InsP6 is the primary stored form of P (Eeckhout and De Paepe, 1994; Ravindran et al., 1994). This, together with the dominant role of plant seeds and their processing products (e.g., oilseed meals) in animal feeding, makes $InsP_6$ the most important source of organic P in diets for poultry. Diets contain approximately 0.2 % - 0.3 % of $InsP_6$-P, depending on what raw materials are used and their growing and processing conditions. Substantial variation exists in $InsP_6$ concentration between different batches of the same feed raw material (Rodehutscord et al., 2016). Inositol pentakisphosphates ($InsP_5$) and other $InsP_x$ were found to be contained in cereal grains only at marginal levels, except barley. However, in oilseed meals, especially rapeseed meal, up to one third of the total InsP-P can be contained as $InsP_5$-P (Haese et al., 2017; Pontoppidan et al., 2007).

In contrast to textbook knowledge, recent studies consistently showed that the biological potential of broilers to degrade $InsP_6$ from phytase-free diets is very high. Rodehutscord and Rosenfelder (2016) summarised different studies that found precaecal $InsP_6$ disappearance in broilers to range between 62 % and 89 % when diets that were mainly based on maize and soybean meal were fed. Of note, all these studies did not contain a mineral P supplement and were low in Ca content. Apparently, conditions of marginal P supply trigger the 'system' to make most out of the plant P sources. The interesting question is where the phytases and other enzymes needed for $InsP_6$ hydrolysis come from when not contained in the feed.

Studies conducted with broiler chickens and laying hens revealed some phytase activity when using purified brush border membrane vesicles from different sections of the small intestine (Huber et al., 2015; Maenz and Classen, 1998; Onyango et al., 2006). Epithelial phytase was found highest in preparations of the duodenum and it decreased in the more posterior parts of the small intestine (Maenz and Classen, 1998). There was also some indication that epithelial phytase activity is reduced at higher inorganic phosphate concentration in the intestinal lumen (Huber et al., 2015). Epithelial phytase secretion was also reduced when diets containing 0.9 % Ca compared to 0.4 % Ca were fed (Applegate et al., 2003). However, it was stated that the regulation of endogenous phytase secretion is not well examined, and that the quantitative contribution of endogenous phytase to intestinal $InsP_6$ breakdown cannot be assessed at this time (Huber et al., 2015). Because of their localization, brush border membrane-associated phytase may only have a restricted relevance for hydrolysis occurring in the gut lumen.

Another potential source of luminal phytase activity is the microbiota colonizing the digestive tract. Gnotobiotic broiler chickens compared with non-gnotobiotic chickens had much higher $InsP_6$ levels in the caecal content (Kerr et al., 2000). This indicated an involvement of microbes in $InsP_6$ breakdown in the intestine. Lactic acid producing bacteria isolated from the chicken intestine were identified as possible $InsP_6$ degrading candidates (Raghavendra and Halami, 2009). Among the bacteria in the small intestine of broiler chickens, lactobacilli are the most common ones (Rehman et al., 2007). Interestingly, broiler chickens fed a diet supplemented with *Lactobacillus* species had increased P retention (Angel et al., 2005). When using deep sequencing techniques, Witzig et al. (2015) confirmed lactobacilli to be the dominating bacteria in the crop and small intestine of broiler chickens, but the phylotypes of lactobacilli differed between the crop and the small intestine. In addition to the dominant phylotypes, genes for $InsP_6$ phosphatases were identified in *Bacteroides* spp., *Burkholderia* spp. and three species of the genus *Bifidobacterium*, which are all also present in the digestive tract of chickens (Stentz et al., 2014; Tamayo-Ramos et al., 2012). This supports the hypothesis of the chicken's gut microbiome contributing to $InsP_6$ breakdown. Probably the crop microbiota is important for $InsP_6$ breakdown further down the digestive tract. Lactobacilli colonize there and produce enzymes. Hydration and temperature favour bacterial growth and enzyme activity (Svihus et al., 2002). Short retention time of digesta in the crop may cause enzymes produced there to be active mainly in the subsequent sections, especially in the gizzard. Later in the small intestine, additional phytase and other phosphatases from the epithelium get involved, all together contributing to the development of $InsP_6$ disappearance shown in Figure 1.

Interactions of phytate breakdown with P and Ca supplements

While the previous chapter was about the biological potential of InsP6 degradation at low dietary P and Ca, industry-type diets usually contain both, P and Ca supplements. InsP6 hydrolysis in the digestive tract is subject to phytate solubility. Dietary factors influencing phytate solubility may therefore have an effect on $InsP_6$ disappearance. The level of pc $InsP_6$ disappearance decreased from about 70 to about 20 % with increasing Ca from 0.2 to 0.7 % (Tamim and Angel, 2003; Tamim et al., 2004). The pcdP of maize was increased from 25 to 57 % when dietary Ca was reduced from 0.95 to 0.13 % (Perryman et al., 2016), which was perhaps caused by Ca effects on $InsP_6$ degradation. In another study, pc $InsP_6$ disappearance was about 25 % and not significantly different when 0.7 and 1.0 % Ca were used in the diet (Li et al., 2016). This is an indication that Ca does not exhibit negative effects once a certain threshold Ca level is exceeded. When comparing results of the aforementioned studies it should be kept in mind that the diets used by Li et al. (2016) included P from meat and bone meal or monocalcium phosphate, meaning that Ca was included together with mineral P and raising the question whether mineral P can have an effect as well.

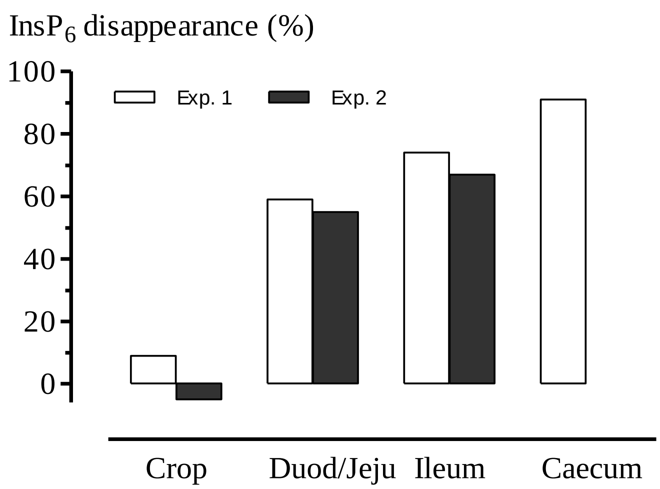

Figure 1: $InsP_6$ disappearance in sections of the digestive tract of broiler chickens fed maize-soybean meal-based diets without a phytase supplement (Zeller et al., 2015a,b; Zeller et al., 2016)

P addition to the medium in an *in vitro* assay decreased synthesis of phosphatases including phytase by *Aspergillus ficuum* (Shieh et al., 1969). Such inhibition of enzymes by mineral P may also happen in the digestive tract, with the consequence of reduced $InsP_6$ hydrolysis. When mineral supplements containing both Ca and P were included in the diet, $InsP_6$ disappearance was repeatedly found to be significantly reduced (Figure 2). However, a diminishing effect on $InsP_6$ disappearance was also found when P was supplemented without Ca, by monosodium phosphate inclusion. If the end product inhibition shown *in vitro* by Shieh et al. (1969) also happens in the digestive tract, then this would partially explain why pc $InsP_6$ breakdown is reduced upon supplementation of a (Ca-free) mineral phosphate.

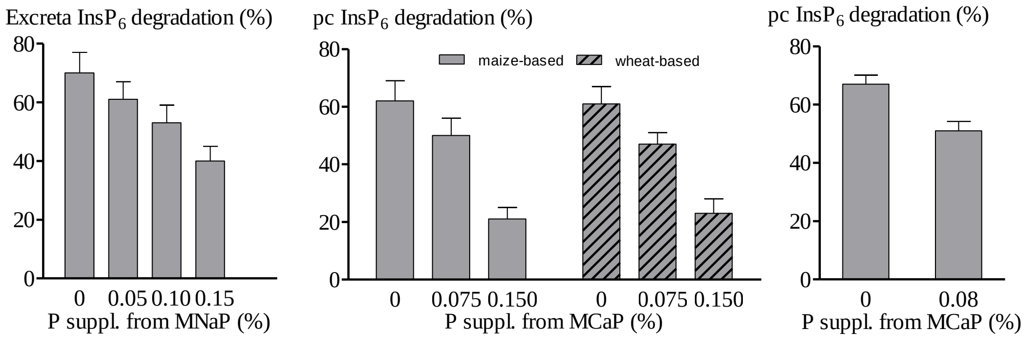

Figure 2: $InsP_6$ disappearance in broiler chickens fed maize- or wheat-based diets supplemented or not with anhydrous monosodium phosphate (MNaP) and monocalcium phosphate monohydrate (MCaP) (Rodehutscord, 2016)

In a recent experiment, again a mineral P supplement reduced pc $InsP_6$ disappearance when no phytase was contained in the diet, and this reduction was even more pronounced when P and Ca were supplemented together (Sommerfeld et al., 2018). When diets contained a phytase supplement, the negative effect of the P supplement was not found, but Ca still tended to reduce $InsP_6$ disappearance. Perhaps, the modulating effects of P and Ca are different because phytases of different origin (endogenous mucosal, endogenous microbial, exogenous) or their production are targeted by the minerals in a different way.

All these interactions reveal a dilemma: Naturally, broiler chickens are able to digest about two thirds of $InsP_6$-P when they are challenged by feeding diets with marginal P and Ca concentrations. But when mineral P and Ca sources are added to meet the requirements, less $InsP_6$-P is available with the consequence that either mineral P must be further increased or exogenous phytase added. With the increasing pressure to reduce P excretion in the poultry industry it belongs to the great challenges to solve the dilemma in finding the right balance between the mineral and enzyme supplements used in the different growth phases of the birds.

Consequences for P evaluation

Determination of P digestibility as well as determination of relative bioavailability implies that graded levels of a test P source are used and the response to any test P source is calculated by regression analysis. This assumes interactions between the test P source and other ingredients of the diet not to exist. As explained in the previous sections this assumption was found to be wrong. The interactions probably have the highest relevance when mineral and animal P sources are studied because P supplementation will reduce the degradation of $InsP_6$ contained in the basal diet and hence digestibility of the supplemented P source is underestimated. The pc disappearance of $InsP_6$-P is reduced by approximately 0.4 - 0.5 g/kg with each gram of mineral P supplemented to the diet (Rodehutscord, 2016). This effect remains unconsidered when experiments are only based on total P determination. However, it can have a great influence on the calculated digestibility or bioavailability of the supplemented P source. The influence is demonstrated in Figure 3 as an example. The digestibility value of monocalcium phosphate (MCP), calculated without correction for impaired $InsP_6$ disappearance, was about 54 % with only marginal effects of the basal diet used (Shastak et al., 2014). However, when the data were corrected for the reduction in $InsP_6$ degradation simultaneously measured, the calculated pcdP of MCP is much higher. The conclusion from this model calculation is that total P analysis in P digestibility trials should be extended by $InsP_6$ analysis of diet and digesta samples so that $InsP_6$-P disappearance is traceable.

Interactive effects can be avoided if the basal diet is free of $InsP_6$. Thus, an alternative approach is to use a phytate-free semi-purified basal diet, such as done for testing pcdP of meat and bone meal (Mutucumarana et al., 2015). Nevertheless, it should be kept in mind that semi-purified diets might not allow for feed intake and growth as typical in the industry, and might change digesta passage rate and microbiota composition; hence effects on the determined pcdP are possible.

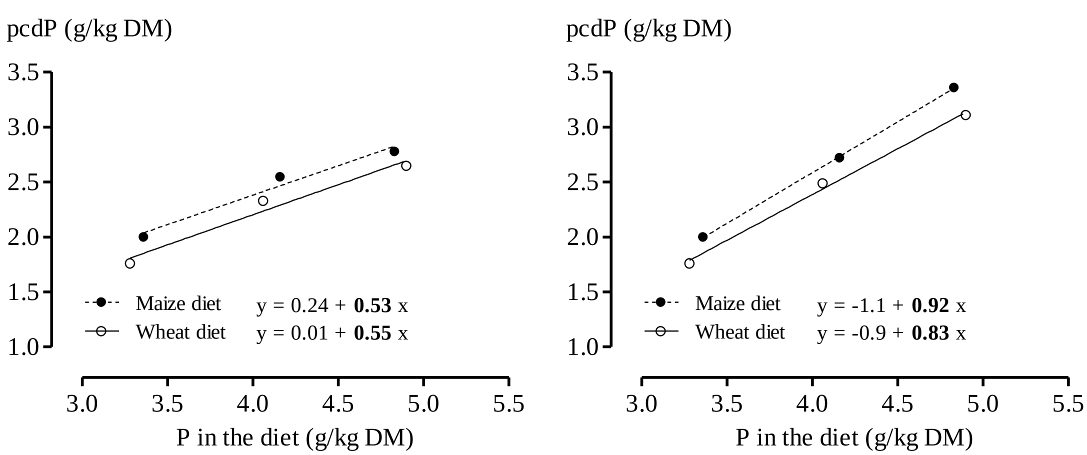

Figure 3: Example for the calculation of pcdP of monocalcium phosphate in broilers by linear regression using either a maize-based or wheat-based diet. Values were without (left panel) or with (right panel) correction for $InsP_6$-P disappearance (Rodehutscord, 2016).

Genetic effects on P utilization

In regard to epithelial phytase expression, effects of age, species, strain and gender of poultry can become relevant (Abudabos, 2012; Applegate et al., 2003; Maenz and Classen, 1998). Differences in P utilization potential between broiler strains are known for a long time (Edwards Jr., 1983). It has been hypothesized that epithelial phytase expression is affected by the bird's genetic background (Beck et al., 2014). Indeed, genomic studies showed significant heritability in the range of 0.10 - 0.22 for P utilization, P excretion rate, and phytate-P bioavailability in broilers and Japanese quail (Beck et al., 2016; de Verdal et al., 2011; Zhang et al., 2003). However, underlying physiological mechanism still need to be unraveled. Genome effects on epithelial phytase expression are only one potential reason for differences in P utilization. The animal's genes may also affect the abundance of phytase-producing bacteria in the gut microbiota or the expression of phosphate transporters in the intestine, or both.

Conclusions

Broiler chickens are able to digest about two thirds of $InsP_6$-P when they are challenged by feeding low-P and low-Ca diets. The high potential for gastrointestinal hydrolysis of $InsP_6$ in broilers is suppressed by feed phosphate and calcium supplementations. Excess in calcium is detrimental to P utilisation of broilers. Studies on the digestibility of non-plant P sources need additional determination of $InsP_6$ disappearance.

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The Phosphorus Sparing Effect of Phytase: Extracting the Most Value

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SUMMARY

The extent of phytate hydrolysis, and the resultant release of phosphorus (P), is affected by several factors. These include the main and interactive effects of dietary level of inorganic phosphate, calcium (Ca), and the dose and type of phytase. With the correct level of dietary P and Ca, and the correct dose of a high efficacy phytase, it appears possible to achieve optimal growth performance and bone mineralization of broiler fed all-vegetable diets largely devoid of inorganic phosphate. This opens up new opportunities for significantly reducing reliance on inorganic phosphates and P excretion, while improving cost efficiency of broiler production.

INTRODUCTION

The effect of supplementary microbial phytase in improving the availability of phytate-bound phosphorus (PP) is well recognized in monogastric nutrition. Improved utilization of PP in the diet reduces reliance on supplemental inorganic phosphates. The P-sparing effect, or P-equivalency of a phytase, is established by assays involving feeding graded level of a reference source of inorganic phosphate and different doses of phytase, to compare the relative growth performance and bone mineralization [1, 2, 3]. The relationship of phytase dose and available phosphorus (AP) sparing typically follows a log-linear curve, and offer the flexibility to choose the most cost-effective dosage under given conditions.

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It has become apparent that the potential of phytases to replace inorganic phosphates, and thus reduce feed costs, is far from being completely exploited – a common approach is to use phytases to replace 0.10-0.15% AP, despite the evidence that modern phytases can potentially offer more complete phytate destruction and higher P-release [3, 4, 5, 6]. Higher doses of phytase can potentially offer, 1) direct savings in feed cost through replacing more inorganic phosphates, and 2) improved animal performance associated with more complete phytate destruction [5, 7, 8], 3) reduced reliance on non-renewable inorganic phosphate reserves [9], and 4) a further reduction in manure P [10].

The current communication reports findings from three experiments of similar design aimed at studying the effect of incremental doses of an enhanced *E. coli* phytase on the growth performance and bone mineralization of broilers fed diets gradually reduced on available phosphorus (AP) and calcium (Ca). The main objective was to test the hypothesis that a higher dose (1500 FTU per kg) of an enhanced *E. coli* phytase should enable an all-vegetable, corn-soy diet devoid of supplemental inorganic phosphate to be fed without negatively affecting the growth performance and bone mineralization of broilers.

MATERIALS AND METHODS

With a few minor exceptions, the following paragraph provides a summary design of all three trials. An all-vegetable diet mainly based on corn-soybean meal, with AP/Ca levels of 0.40/0.80%, 0.35/0.70% and 0.30/0.60%, respectively for 0-10, 10-25 and 26-42 days post-hatch served as experimental control (PC). No phytase was added to the PC hence the level of AP and Ca was met primarily by inorganic phosphate, and limestone. A series of test diets was formulated with 500, 1000, and 1500 FTU per kg (Exp 1 and 3) and an addition dose of 250 FTU per kg (Exp 2) of an *Escherichia coli*-derived phytase. Each test dose of phytase had been assigned an AP matrix based on the log-linear curve of the phytase understudy (Figure 1). In addition to AP, each dose of phytase was also assigned a Ca matrix that was 110% the respective AP matrix (Exp 1 and 2) or kept fixed at 0.15% Ca for all doses (Exp 3).

Pen feed intake (FI) and body weight gain (BWG) were recorded at the end of every feeding phase, and feed conversion ratio (FCR) corrected for mortality was calculated. Two birds (one male and one female) close to the pen average weight from each pen were chosen for the collection of tibia at 21 d of age (Exp 1 and 2) or toe at 25 d of age (Exp 3), to determine the bone mineralization.

Body weight gain, feed intake, feed conversion ratio, livability and bone data were subjected to analysis of variance as a randomized complete block design using PROC MIXED procedure of the statistical software JMP Pro 13 (SAS). When P-values from ANOVA where ≤ 0.05, then LS means were separated using Tukey's test.

RESULTS AND DISCUSSION

Formulating diets with increasing doses of phytase caused a stepwise reduction in the supplemental inorganic phosphate, to the point where all inorganic phosphate was removed and hence no further reduction in the calculated AP was possible. As a result, the finisher diet in Exp 1 and grower and finisher diets in Exp 2 and 3 had no added inorganic phosphate.

The overall gain and FCR in these trials surpassed breed standards for age, suggesting the nutrients set in PC were sufficient to meet or exceed the requirement for the optimal growth. In general, diets containing 1500 FTU/kg phytase had er ($P<0.05$) BWG and FCR compared with the PC during the starter- and grower-phases. This trend continued through to the market age e.g. 1500 FTU/kg treatment maintained ($P=0.03$, Exp 2) or tended to maintain ($P=0.09$, Exp 1) better BW and feed efficiency ($P=0.09$, Exp 2) compared with PC at 42 d of age. Improved BWG at higher doses of phytase may relate to the release of nutrients and provision of inositol associated with an extensive phytate hydrolysis [8, 11]. Measurement of the bone ash did not reveal any differences across treatments.

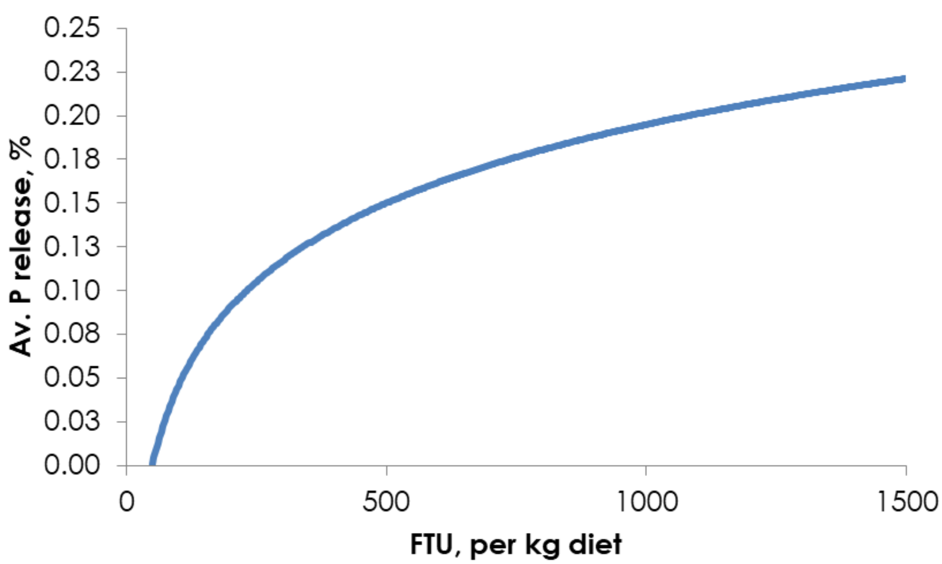

Figure 1 The log-linear curve used as the basis for available phosphorus matrix for phytase dose

Combined together, the data from these three experiments suggest that 1500 FTU/kg of enhanced *E. coli* phytase can support optimal growth performance and bone mineralization of broilers fed all-vegetable diets practically devoid of inorganic phosphates. This suggests extensive phytate hydrolysis, and utilization of PP in the gut. It is important to highlight that nutritional factors, including dietary level of Ca [12] and AP [13], can influence *in-vivo* phytate hydrolysis and hence these factors need to be considered in addition to the proper dose of a high efficiency phytase when targeting a complete elimination of supplemental inorganic phosphates in broiler diets.

Conclusions

1. High doses of phytase enables greater reduction of dietary supplemental inorganic phosphates
2. The results of the current series of studies demonstrated that 1500 FTU/kg of an enhanced *E. coli* phytase supported optimal growth performance and bone mineralization of broilers fed corn-soy diet practically devoid of inorganic phosphate

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Ezyme Application Links to Livestock Gut Health

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INTRODUCTION

The application of enzymes in feed formulas for single-stomached animal production have been well recognized to improve the utilization of nutrients of costly feed ingredients, directly reducing unwanted effluents like nitrogen (N) and phosphorous (P) excretion derived from livestock manure and improving on animal welfare (Oxenboll *et al*., 2011; Angel *et al*., 2011; Kiarie *et al*., 2013; Perez-Maldonado, 2014;). During the 1950s, pioneering work showed the addition of amylases to the diets of livestock resulted beneficial in animal production linked to productivity (Adeola and Cowieson, 2011). Since then, the use of exogenous feed enzymes has been one of the most widely studied and reported disciplines in animal science achieving a tremendous growth and acceptance worldwide in the animal industry. In in terms of value, the global feed enzyme expanded from $550 million, in 2011 to 750 million in 2013 (Bao *et al*; 2013), saving the global feed market an estimated $5-7 billion/year. Since mid80s there has been an improved understanding on the chemistry of nutrients in feed ingredients, microbiology technologies coupled with further development in the area of submerged fermentations and solid-state fermentations (Kiarie et al., 2013). Thus, in most poultry producing markets the use of exogenous enzymes like phytases, carbohydrases (xylanases, cellulases, α-amylases, β-mannanases, and pectinases) and recently the proteases are commonly accepted in poultry and more commonplace in swine feed (Cowieson and Roos, 2014).

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On the other hand, during 2000s, the pressure from legislation, retailers, and consumers on the use of prophylactic antibiotic in livestock diets, has promoted a switch for less antibiotics usage in livestock feed formulas. This now has consolidated a clear need for the control of enteric disruptions that were previously contained with the use of antibiotics growth promotant (Mateos, *et al*., 2012; Bedford and Cowieson, 2012; Cowieson and Kluenter 2018). There is not a "silver bullet" non-antibiotic product or management practice that alone can fully support productivity in poultry as cost-effective as antimicrobial agents (Cowieson and Kluenter, 2018). However, to effectively sustain the use of antibiotic free diets (ABFD) there is a need for a holistic combination of several feed additives and practices, including a better understanding on how feed enzymes are influencing gut health (Kiarie *et al*, 2013). In this context, enzymes have demonstrated the potential benefits to be gained that enhances gut health with nutrient delivery to the host. These includes the hydrolysis of specific chemical bonds in raw materials (Phytic acid- P complex, NSP), de-caging encapsulating nutrients of cell wall polysaccharides, breaking down antinutritional factors (ANF) present in the various ingredients (protease inhibitors in soybean), solubilization of water insoluble NSP to deliver fermentable oligosaccharides derived from large inert fibrous material, the destruction of antinutritional phytic acid, the reduction of protein waste, the curve of unwanted pathogenic bacteria and parasites (protease) and the reduction of bacterial cell wall debris in the gut (muramidases). All the above, in the context ABFD, seems to convey to improve livestock health, better gut functionality and promoting growth and efficiency of nutrient utilization (Kiarie *et al* 2013; Cowieson and Kluenter, 2018; Cowieson and Roos, 2014; Cowieson *et al*., 2017). It is the scope of this publication to briefly highlight some of the work that might contribute to the knowledge on the use of enzymes as a key component in ABFD and some relationship between feed, microbiota, and host by using enzymes and how to link it to optimal gastrointestinal functionality.

Optimal Gastrointestinal Functionality

For a sustainable animal production, without in-feed antibiotics, optimal gastrointestinal functionality is pivotal to maintain an effective efficiency of the gastrointestinal tract (GIT) and its health (Celi, et al., 2017). The definition of gut health initiated by Conway, (1994), proposes three main components; the diet, the mucosa, and the microbiota. The mucosa is composed of digestive epithelium with its specific structure, the gut-associated lymphoid tissue (GALT) and the mucus overlying the epithelium. The GALT, microbiota, mucus layer and the host epithelium interact, forming a complex and dynamic equilibrium within the GIT for an efficient functioning of the digestive system. In this respect Celi *et al*., (2017) propose an enhanced definition of gut health as *"a steady state where the microbiome & the GIT exist in symbiotic equilibrium & where the welfare & performance of the animal is not constrained by intestinal dysfunction".* This definition covers the principal domains and its interaction involved for gastrointestinal functionality. They include, the diet that provides nutrients for efficient growth, the effective gut for digestion and absorption of nutrients, an effective structure and function of the GIT barrier; the normal and stable microbiota and the mucosa and an effective immune status. All these components play a critical role in GIT functionality, animal health welfare and for the environment. We will highlight some aspects in the relationship between feed, microbiota, and host by using enzymes.

Diet and bird age affecting nutrient digestibility

Diet composition and quality can influence the development and function of the digestive system, including the immune system and the microbiota (Conway, 1994). There are many factors through the diet that negatively affect the health of the GIT (Klasing, 1998) these includes fibers, trypsin inhibitors, phytate, lectins, undigested protein in the distal GIT, mycotoxins, pathogenic and putrefactive microorganisms, unbalanced diets, temperature during manufacturing, poor water quality, feed particle size, pelleting, certain vaccination programs and many others (Celi, *et al*., 2017). Systematic feed and ingredient processing and the addition of feed additives/supplements that contain functional properties can improve on the negative factors that affect correct gut functionality and maintain animal production performance including health and welfare. Early work has shown that endogenous enzymes, particularly in young poultry (0 -7 d), are insufficient to fully optimize feed protein utilization since valuable amounts of CP and AAs passes through the GIT without being completely digested (Batal and Parson; 2002; Lemme *et al*., 2004; Angel *et al*., 2011; Kiarie *et al*, 2013). Thus, the interest in administering amylase, protease, lipase in young animal's diets to cover their immature digestive system, which have shown positive outcomes. On the other hand, it has been a traditional acceptance that older poultry do not need as much enzyme supplementation as they are more capable to digest feed since their endogenous enzyme activity system is superior compared to young birds. However, when considering the development of the intestinal developmental changes relative to body weight gain there is an interesting observation. In young chicks that have clearly a limited capacity to produce endogenous enzymes, the gut size and weight development accounts for a more substantial proportion of its body mass when compared to a grower/finisher broiler (Cowieson and Kluenter, 2018; Cowieson and Kluenter, 2018). In young birds from d1-10, the GIT develops from around 0.1% to about 0.5% to increase enzyme activity in terms of units of activity per gram of pancreatic or intestinal tissue. But in older birds d21-56, the absolute amount of digestive enzyme per unit of body weight, is reduced from 0.5% to around 0.15% by d56 (Krogdahl and Sell, 1989; Cowieson and Kluenter, 2018). Thus, it may be appropriate to also supplement enzymes in grower/finisher broilers where the intestine and pancreatic tissue become gradually reduced in proportion of the bird metabolic body weight. In addition, when considering other various factors affecting diet quality and bird age, it has been estimated that in swine and poultry around 25-30% of ingested feed protein escapes and is wasted in the faeces (Bao *et al*; 2013; Glitso *et al* 2012; Mingan, 2014).

Regarding antinutrients in the feed, soybean meal (SBM) is by far the most commonly used meal protein ingredient worldwide (Ravindran *et al;* 2014). But, the nutritional value of SBM and the health status of non-ruminant can be compromised as soybean seeds contain various ANFs for poultry and swine, the most problematic being the Kunitz and the Bowman–Birk trypsin inhibitors (TI). These ANFs are present on unproperly processed SBM negatively affecting normal digestion/absorption of nutrients, altering digesta flow which has been correlated to high feed passage rate creating a condition in which broiler droppings lose their normal shape and consistency and feed conversion is negatively affected and body weights are lower (Ruiz, and Belalcazar, 2005; Ruiz, 2012). The *in vitro* work by Pettersson and Pontoppidan, (2013) using sodium dodecyl sulfate polyacrylamide gel electrophoresis techniques clearly demonstrated the tested exogenous protease was highly effective against soybean TI and was more effective than animal pancreatic trypsin enzyme. Recent publications have also shown the improve physicochemical properties and enzymatic *in vitro* nutrient digestibility when microbial protease was tested on full-fat soybean meal (Erdaw *et al*., 2016). The ability of *Nocardiopsis prasina* feed protease to degrade ANFs offer the possibility to alleviate the negative impacts of including raw soybean or under processed SBM in poultry or swine diets. To confirm this work Mayorga *et al.*, (2009; 2012) using *in vivo* trails with various corn/soybean diets containing various TI levels showed that birds consuming diets containing protease supplementation proved to be effective to improve bird performance and yield. Erdaw *et al*., (2017) have also shown during *in vivo* work acceptable chick responses on diets containing a proportion of raw, full-fat soybean substituting SBM testing the ability of microbial protease to overcome some of the negative TI effect. Therefore, it is now clear that the use of microbial protease can protect young livestock from unwanted no-well processed soybean in their diets.

More recent Pedersen, (2017) was able to visualize using novel coated gold with ion chromatography and electron microscopy techniques mechanisms by which protease by forming pin wholes was able to penetrate and degrade the protein storage within soybean vacuoles. This action by the enzyme influenced on the solubility of phytic acid located within soybean protein storage vacuoles. This was a significant novel observation as microbial protease shows a high potential to increase phytic acid to be hydrolyzed by feed phytases which in turn would improve on the destruction of this phytic acid that is considered a major ANF affecting AA digestibility in poultry and swine (Cowieson, et al., 2017; Pedersen *et al*., 2017).

Dietary Fiber affecting microbiota responses on gastro intestinal functionality

Dietary fiber encompasses a group of very diverse polymers fractions with large differences in physicochemical properties that, when included in the diet, results in differences in digestive viscosity, ion exchange capacity, fermentation capability, and bulking effect within the GIT. The composition and the various classifications of plant cell walls including the water soluble and water insoluble non-starch polysaccharides (NSP) and other cell wall components that provides distinct negative or positive effect on gastro intestinal functionality are given in the various publications found in the literature (Annison, *et al*., 1997 Hetland *et al.*, 2004; Mateos, *et al.*, 2012; Celli, *et al*., 2017). The human nutrition has focused on the fecal bulking effect of fibre and the cholesterol-reducing effect of soluble dietary fibre (DF). Therefore, DF is now well documented for its prebiotic beneficial effect. However, since in animal nutrition, birds do not possess endogenous enzymes capable of cleaving and digesting the β(α) linked NSP, the focus of research has been on the antinutritional effect of NSP particularly the soluble NSP (Annison, *et al*., 1997). Soluble fibers in the small intestine are considered antinutritional as they can produce high viscosity inhibiting digestion and absorption that can affect feed intake due to slower passage, which in turn causes microbial proliferation and fermentation in the intestine (Adeola and Cowieson, 2011; Annison, *et al*., 1997). The processing of important raw materials like soybean to obtain SBM or canola meal for example does not alter the structure of indigestible carbohydrates fractions (Ravn *et al*., 2015). If digestion is compromised the flow of undigested/unabsorbed nutrients in the small intestine increases and the dynamic of the bacterial population will change dramatically. This proliferation of fermentative organisms in the small intestine of chickens is considered as an energy – inefficient outcome and detrimental to the bird (Choct, 1999) and discussions for this inefficient effect is well described in Bedford and Cowieson, (2012). Another antinutritive role of soluble NSP in broilers diets is encapsulation (cage effect) in which inhibits the access of digestive enzymes to starch, fat, and protein molecules (Annison *et al*., 1997). Other more complex fibers to break down by poultry, are pectin, xyloglucan, and mannan that are present in dicotyledonous plants. Thus, canola or rapeseed (*Brassica napus*), cottonseed (*Gossypium)*, sunflower (*Helianthus annuus*) and soya (*Glycine max*), that are considered, the most useful vegetable protein meal ingredients that compose non-ruminant diets, will entrap nutrients, reducing greatly their energy value. These mentioned fibers increase water holding capacity, swell and consequently increasing luminal viscosity, reducing animal feed intake (Pedersen et al., 2017). The work by Ravn *et al*., (2015) demonstrated the substantial beneficial reduction of viscosity by enzymes product form of *Aspergillus aculeatus* for solubilizing galactomannan, xyloglucan and acid pectin cell walls from SBM. The total sum of all NSP also showed high solubilization with a total disappearance of about 31% from the total NSP. Toghyani, *et al*., (2017) in two broiler trials using canola meal replacing SBM and supplemented with a carbohydrase and a microbial protease enzyme evaluated broiler performance and partitioning of energy contribution to the bird. Both enzymes alone or combined improved the final body weight and FCR; with the combination having a larger improvement. The net energy that was previously affected by high fiber content derived particularly by canola meal was substantially improved by these enzyme supplementations. Exogenous enzymes have been proposed as key compounds to produce ABFD in animal production as they can produce beneficial effect like the production of prebiotics that enable fermentation for the proliferation of specific beneficial population of bacteria through their action in the substrate. (Celi, *et al*., 2017; Pedersen, et al., 2017; Cowieson and Kluenter, 2018). It is now getting more accepted that, the stimulation for microbial fermentation in the caecum for producing specific short-chain fatty acids, is desirable, which is accompanied in a reduction of intestinal pH (den Besten *et al*., 2013). Therefore, stimulation of beneficial bacteria in this section of the gut is being described as a positive outcome, like for example the production of *lactobacillus* that can deliver GIT health and can protect the host from infection (Celli *et al*., 2017). Ravn *et al*., (2017) has shown the successful production of a prebiotic derived from a commercial feed GH11 xylanase when using wheat bran cell walls to generate arabonoxylooligoshaccharides (AXOS) patterns by the fermentation with the mentioned enzyme. The generated AXOS significantly increased butyrate-producing bacterial genera *Faecalibacterioum* and *Intestinimonas* while the levels of *Bacteroidetes* which are considered non-beneficial microbes were significantly reduced. The fermentation reactions were also providing epithelial layer resistance loses. Therefore, The Xylanase GH11 type was able to solubilize and degrade wheat bran cell walls to yield low polymerized AXOS that can be fermented by caecal microbiota, resulting in microbiota shifts and beneficial effects on trans-epithelial resistance *in vitro*. Thus, fibre plays a crucial role in the complex interaction between the diet, endogenous enzymes and hence digestion and absorption, also with the host and the GIT microbiota, all of which are considered key elements for optimal gastrointestinal functionality. But too much or too little fibre can have an imbalance with negative effects, therefore moderation and the type of fiber added is key for success on improving GIT functionality and animal performance (Celli *et al*., 2017 2012; Mateos *et al*., 2012).

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Fiction is Fact: The Influence of Public Perceptions on Poultry Production Systems in Australia

VIVIEN KITE

INTRODUCTION

There has been much spoken – but less documented – about the disconnect between the average Australian and agriculture. According to the National Farmers' Federation, a nation-wide poll[1] commissioned by the organisation in 2017 showed that 83% of Australians would describe their connection with farming as 'distant' or 'non-existent'. The corollary of this is that the average Australian knows little, and understands less, of how we farm and why agricultural industries adopt the practices that they do. In the absence of this understanding, misunderstandings and perceptions can proliferate. Many of these perceptions are negative, with members of the public, often led by single-issue advocacy groups, increasingly challenging how our industries operate and demanding change. The extension to this is that agricultural industries risk losing their social license to operate in the manner they deem most appropriate, if these perceptions are not addressed.

Traditionally, the poultry industries in Australia have used science and logic to make the case to regulators regarding the desirability and 'need' for their producers to farm in the manner of their choice – generally the most practical and efficient production systems available, taking into consideration a range of other, sometimes conflicting, imperatives, such as bird welfare and food safety.

Industry has similarly used science-based evidence, other factual data and logic to attempt to describe and explain to the community its practices and to justify why it does things the way it does. It has assumed and expected community acceptance based on this. This has been the approach adopted in many areas where the general public has demonstrated some level of scepticism of or unwillingness to fully accept and embrace industry practices, but in no area more so than in the area of animal welfare.

Executive Director, Australian Chicken Meat Federation Inc.

A plethora of science and other evidence has therefore been produced to show that there are pros and cons that need to be weighed up in reaching a conclusion about the welfare outcomes for birds kept in different housing systems, or conclusions as to whether it is better to preventatively treat an entire flock by beak trimming than to risk the substantial welfare consequences for a variable proportion of the flock due to cannibalism later on in life, as examples.

It has been widely assumed that, armed with these facts, we will be able to go out and convince the public that our practices are not only sound, but in the best interests of all, and we have believed that the approach to achieving this is to go forth and 'educate' the increasingly disconnected consumer about industry practices.

Has this approach worked?

While this approach has met with some success in the past, the more recent history in Australia suggests that this strategy may not be tenable in the longer term, at least in some areas of intense community scrutiny and concern. Primary reasons for this derive from two fundamental flaws in our assumptions, and these flaws are that (a) the industry's and the community's understanding of what terms such as 'good welfare' mean are aligned, and (b) if people know and understand why we do things the way we do, they will accept the way we do things.

(a) What constitutes good welfare?

It is not so long ago that our animal industries more generally defined animal welfare simplistically as the absence of cruelty and suffering. Few in our poultry industries today would argue that simply surviving equates to 'good welfare'. Indeed, this broader acceptance that welfare constitutes more than the absence of premature death and acute suffering has been progressively reflected in animal protection legislation in Australia, with many jurisdictions enacting animal welfare or animal care legislation over and above or in place of the older 'prevention of cruelty to animals' legislation over the past two decades. Current animal protection and welfare legislation in place in Australia is listed in Table 1 below.

Table 1. Current animal welfare legislation in Australia

ACT	Animal Welfare Act 1992
NSW	Prevention of Cruelty to Animals Act 1979
NT	Animal Welfare Act
Qld	Animal Care and Protection Act 2001
SA	Animal Welfare Act 1985 (previously titled the Prevention of Cruelty to Animals Act 1985, up to 2008)
Tas	Animal Welfare Act 1993
Vic	Prevention of Cruelty to Animals Act 1986 Livestock Management Act, 2010
WA	Animal Welfare Act 2002

It is still often assumed by many in our industries that it is sufficient for the birds we use to not only survive, but to be healthy and grow well to meet the requirements of 'good welfare'. This assumption incorrectly equates low mortality and good flock performance to good welfare outcomes. Certainly, elevated flock mortality and poor performance is likely to be reflective of poor welfare, and it would be difficult to substantiate a claim of having achieved a good welfare outcome if mortality is elevated and/or performance poor, but low mortality and good performance doesn't necessarily equate to good welfare, at least not at the individual bird level.

Over the past 30 years, industry has gradually come to accept the internationally well-known and generally well understood and accepted principles of the 'Five Freedoms' as defining what constitutes 'good welfare'. These are:

- Freedom from thirst and hunger
- Freedom from discomfort
- Freedom from pain, injury, and disease
- Freedom to express most normal behaviour
- Freedom from fear and distress

While the 'five freedoms' have proven to be a useful concept to achieve better alignment of community and industry expectations with respect to appropriate animal welfare standards, and they possibly continue to satisfy the majority of the community's expectations with respect to achieving adequate (if not good) welfare, there remain problems with their use, as explained by Mellor (2016[2]). The major problem is that their major focus is on removing negative experiences, and this has in more recent times been challenged.

Today, a growing part of the broader community adheres to the belief that it is not sufficient that animals we use not be exposed to negative experiences, but that should have experienced a range of positive experiences and states during their lifetime – that they should have lived a 'good life' or a 'life worth living'. This is not a concept that has been broadly by the industry – not through lack of caring, but because it is not clearly understood what it means in practice.

While the above highlights the lack of alignment between what industry and the community understands to be appropriate specifically in the context of animal welfare, potential issues of lack of alignment on what is appropriate in other areas exist also e.g. antibiotic use, genetic modification.

(b) 'Education' is the answer!

There is a widely held assumption that if the community is given the facts, then people will understand how and why industry does things the way it does, and they will accept industry practices. This common school of thought goes that by 'educating' the consuming (and non-consuming) public in this way, that they will actually feel better about the way things are done having been 'educated'.

But what if the public doesn't actually *want* to know or doesn't care about the facts? What if:

- they don't want or have the time to be 'educated'
- they aren't interested in the facts or the science
- they don't believe the science or the industry's interpretation of it
- they prefer to believe someone else's take on the science, or someone else's opinion over industry's (someone they trust more than industry)
- even if they accept the facts, they don't care, because their own instinctive reactions to what they see and believe tell them something else?

I would contend that the majority of the community doesn't want to be 'educated'.

A good illustration of a combination of the above forces at play can be seen in the response to the public consultation on the proposed new Australian Poultry Welfare Standards and Guidelines (S&Gs), which went to public consultation on 28 November 2017 for a 90 public consultation period. The draft S&Gs were made available on the Animal Health Australia's animal welfare standards website[3], where they were accompanied by instructions for how submissions could be made, a Regulatory Impact Statement (RIS) and associated other supporting materials. Interestingly:

- Over 167,000 submissions to the public consultation were received.
- The "Poultry" page on this website received 19,713 visits during the consultation period;
- Only 11,238 of these resulted in visits to the Poultry Public Consultation page[4], from which the actual draft S&Gs and RIS were accessible (and this was the only place from which they were available or any information on what was proposed was available).
  - unfortunately, the website analytics do not allow statistics to be provided on how many times these two documents may have been accessed.
- Therefore, it's fairly safe to assume* that less than 7% of people or organisations who made submissions bothered to visit the site to view what was actually being proposed in the draft standards, or the analysis of what the implications were of adopting the various options proposed.

  *NB Both the documents in question were large (2.5MB and 8.8MB) and no major organisations which publicly enlisted support for submissions to be made by their supporters and the public more generally made these documents directly available as PDFs in their online electronic communications.
- Out of the 167,000 submissions received, only 209 submissions were assessed as being substantive or major submissions in that they addressed the RIS and/or the draft S&G (a list of these is available from the consultation report[5]) i.e. less than 0.13% of submissions actually addressed either the actual draft S&Gs or RIS.

A reasonable conclusion that could be drawn from the above points is that the vast majority of submissions from people who felt strongly enough about the issue of poultry welfare to be bothered to make a submission at all, reflected and were driven by opinion, not necessarily fact. We also know that the opinions expressed were largely shaped by others, not by the industry.

Would 'better' or more education, or presentation of the facts in an easier to assimilate or more relatable way have changed the outcome of the consultation? We don't know, but we do know that our message is not always straightforward or simple to communicate and that we will never dominate the mainstream or social media, at least not with factual information anyway.

The reality is that our industries are now operating in an environment in which opinion trumps facts.

Perception is the New Reality

How Has This Trend Impacted Australian Poultry Production Systems?

To date, the most obvious example of perceptions driving change is the development of the free-range sector, now representing more than 36% of all eggs produced and approximately 20% of all meat chickens produced.

However, we have also seen the emergence of production systems specifically designed to meet the demands for poultry products produced to standards that satisfy a range of consumer expectations around attributes such as animal welfare, more ‘ethical’, more ‘natural’ and ‘safer’ production practices. In the chicken industry, consumer demand for chicken meat produced without the use of any antibiotics (including ionophores) saw Hazeldene’s launching its “Bare Bird”[6] label in October 2017. This offering not only ticks off the antibiotic free, but also guarantees that the birds were raised on a vegetarian diet and have outdoors access as well.

The above example illustrates the fact that community perceptions that drive consumer demand can provide opportunities, as producers develop niche products to take advantage of new, emerging markets. However, the problem for industry is when niche becomes mainstream, and this is particularly problematic if this arises from regulatory intervention, rather than being driven by broad consumer popularity.

Evidence of community perceptions and opinions driving government policy making to the extent that regulatory changes are imposed on production systems have to date been largely ‘around the edges’, such as changes in cage dimensions for laying hens and standards for what constitutes ‘free range’ in the case of egg production. However, the current Australian poultry welfare standards and guidelines process could well see the imposition of more significant changes imposed under Australian law.

What will the outcome from this process be? That’s yet to be seen, however it is notable that, in acknowledgement of the widespread community concern regarding conventional cages for laying hens expressed through the consultation process, Egg Farmers of Australia in its own submission supported a regulated cap being placed on current conventional cage farming in Australia. This was offered as a temporary solution until the industry can expand its engagement program and show that it has community support for the way it farms. In effect this means that any cage installations made while the cap is in place would need to be enriched with the addition of nest and perches.

While the chicken industry is largely supportive of the changes proposed in the original draft S&Gs that apply to its sector, there is growing concern that its future operating environment – not just in the area of animal welfare, but a range of other fields including management of animal health - will increasingly be defined by the views and perceptions of an increasingly disconnected community, innately suspicious of industry's motives and sceptical of existing industry practices.

Is There a Better Way to Obtain Community Support?

The answer to this question is 'yes'….maybe! However, to be successful it will require industry to take some steps that it has previously been somewhat reluctant to fully commit to.

Ideally, our industries would like the community's opinions to be shaped by industry, not by other groups with their own agendas. But in order to achieve this it needs the community's trust.

This is not a new idea – it is a concept that has been promulgated by the Centre for Food Integrity in the US since 2007 (see http://www.foodintegrity.org/; subsequently taken up in Canada see http://www.foodintegrity.ca/) and adopted in a range of programs around the world.

But what are the practical steps that can be taken to build trust?

Building Trust

There are a range of elements that are necessary to build trust, some of which are described below.

Understand what the community's opinions and perceptions are

It is impossible to build trust without understanding what the community's current opinions and perceptions are and what the underlying concerns and beliefs are that underpin these.

While simple attitudinal surveys are useful in helping to determine what the broader community – and not just what some vocal sub-section of it - really thinks about our industry, its practices and issues, some serious social science is required in order to get to the bottom of what the fundamental issue is in people's belief system that drives their opinions.

There may be some issues and practices that are more easily addressed than others. It is clear from a recent consumer survey undertaken by the ACMF in February 2018 that some opinions are more entrenched than others.

For example, when participants were asked whether chickens raised for meat in Australia are genetically modified in any way, the most common answer given was that the respondents really didn't know (see Figure 1 below).

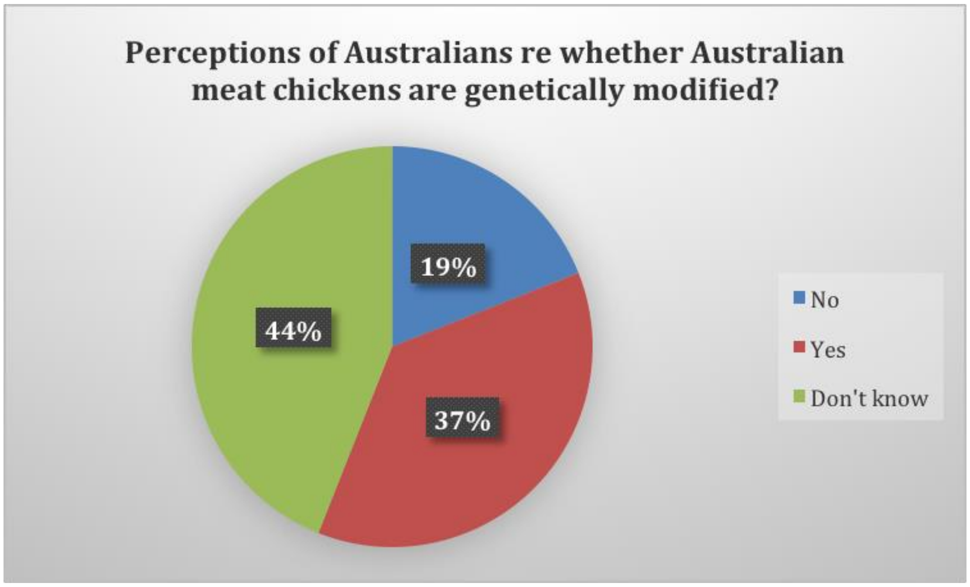

Figure 1. Australian consumers' views on whether chickens raised for meat are genetically modified

When asked what circumstances it would be appropriate for chickens to be genetically modified, more than half agreed that it would be acceptable for one or more of a range of reasons:

- 32% agreed it would be acceptable if used to improve the overall health and welfare of chickens;
- 27% said it would be acceptable to help build resistance to avian flu strains;
- 23% said to build resistance to food safety risks;
- 20% said to improve environmental sustainability;
- 19% if it would improve the nutritional value of the chicken meat; and
- 15% even believed it would be acceptable if directed towards improving chicken meat production efficiency and reducing chicken meat cost.

While 46% of respondents did not know of a reason when genetic modification of chickens would be acceptable, the above data shows that opinions are not firmly entrenched in this area, and there is some willingness in the community to consider a new technology.

On the other hand, there are some perceptions (often factually incorrect) of our industries that are deeply entrenched.

When asked what proportion of chickens in Australia that are raised to produce meat (as opposed to chickens that are raised to produce eggs) are kept in cages, the vast majority (84%) of Australians responded to the effect that 50% or more were (Figure 2).

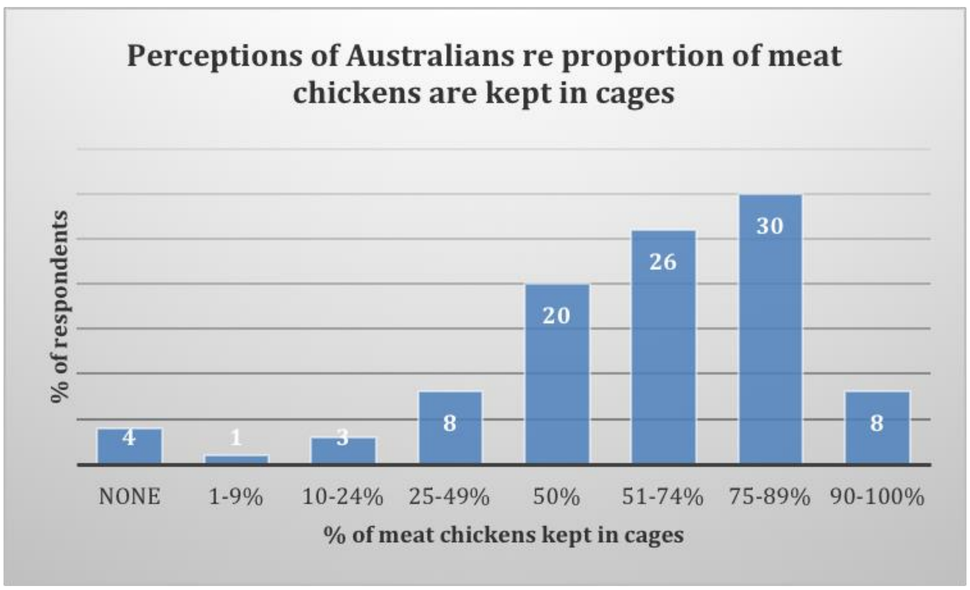

Figure 2. Perceptions of Australians regarding proportion of meat chickens kept in cages

Interestingly, only 4% of those surveyed correctly identified that cages are not used for chicken meat production in Australia, an outcome which is unchanged since a similar survey was last undertaken in 2012 (Figure 3), illustrating how largely ineffective industry's substantial efforts have been in communicating the reality of meat chicken housing practices in Australia to the community.

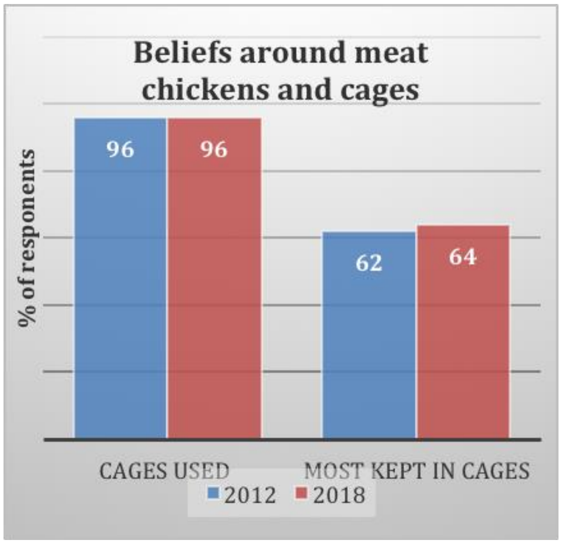

Figure 3. Perceptions of Australians regarding the use of cages for keeping meat chickens (2012 vs 2016)

Identify the common ground

It's easier to start a conversation when you have a common interest. Understanding where the shared interests and shared values lie can provide the ideal starting point for a discussion around industry practices.

For example, somewhat surprisingly, the recent ACMF consumer survey found that the majority of Australian consumer (over 18 years of age) were open to the idea of antibiotics being used in meat chickens if used to protect their health and welfare:

- 56% said it was appropriate to use antibiotics to protect meat chicken health and welfare;
- of the 44% who initially said it was not appropriate, 48% of these said it was appropriate if the use posed no risk to human health;
- when asked 'in what circumstances would it be appropriate to use antibiotics in chickens produced for meat' 47% said 'to treat infections' and 44% said 'to prevent disease and infections', with views mixed on whether 'prevention is better than cure' or use in both circumstances is appropriate;
- 24% said that 'antibiotics should never be used in animals produced for food'.

From the above statistics, it is possible to see that industry's and the community's views are actually largely aligned, particularly if the issues around human safety are addressed. They also reveal that there may be a part of the community whose beliefs and opinions may not easily be moved.

Engagement

To build trust requires a dialogue to be had with the community around the issues that concern them. A good starting point is the common ground.

Engagement is not about 'educating' the other party; it's about sharing and discussing the issues and concerns each party has, respecting each other's views and agreeing where there is common interest, and perhaps even agreeing upon areas in which we cannot reconcile our respective positions.

However, compromise is also important. Compromise demonstrates respect for another's views and beliefs which is an essential component in building trust. In entering into an engagement with the community, industry will need to accept that there will need to be some things that are done differently. That however is all part of evolution.

How this dialogue can be initiated and conducted will require some serious consideration and investment. Some models do exist, such as a successful animal welfare initiative undertaken by the German Poultry Association through 2012 and 2013 in which members of the public were publicly invited to attend open house days on a number of farms across Lower Saxony to see and discuss poultry production practices on German poultry farms. Thousands of members of the German public, including many families, took up this offer and most visitors said they had changed their mind about poultry production as a result of their experience, now believing that the birds are kept in good conditions and are well cared for by their owners.

Demonstrate that industry deserves the community's trust

This may be the most challenging hurdle for industry to come to terms with because it requires transparency and a commitment to being honest about our performance in areas that the community is acutely concerned about and to "doing what we say we do".

(a) Transparency

One of the greatest impediments to the development and maintenance of trust is when one party appears to be hiding something. How can industry expect the community to trust it if it isn't prepared to be open up and show its practices? This can be challenging to achieve at a practical level, because of the physical and biosecurity challenges of permitting the public access to farms, but innovative solutions to overcoming these are available, including webcam monitoring inside poultry farms and observation windows and systems for visitors.

(b) *Do what we say we do*

Building trust is not a PR exercise; it requires changing practices and improving performance. To do this requires industry and participants in it to examine how well they do what they say they do, and ask themselves "are we really doing the right thing 100% (or at least the majority) of the time?"

Confronting and addressing the issue of performance against issues that matter to the community is perhaps one of the most challenging things for any industry to come to terms with in its engagement with the community.

Industry can't hope to build and maintain the community's trust by claiming that everything is perfect, if it isn't. If things aren't quite right, it's better to admit that there is room for improvement and commit to improvement. There are plenty of people and groups out there looking for opportunities to document any failures in performance against stated achievements.

Robust systems for verifying that we actually do what we say we do can also help to demonstrate that the community's trust is well placed.

Can the above proposed strategy for building trust and gaining community support actually work actually work?

Concrete examples of the strategy outlined above resulting in successful alignment of industry and community views on key issues and improved community acceptance of industry practices are difficult to find, noting that the adoption of such an approach has often only come out of a position of desperation, when all other approaches have failed and the industries affected are already teetering on the brink of losing their license to operate.

Nevertheless, examples in Australian agriculture do exist, perhaps the most successful example being the cotton industry's engagement with the community around its environmental practices and sustainability, which largely followed the principles described above and which resulted in a significant change in the public's mindset about the cotton industry. A key element of this program involved the adoption of an ambitious environmental stewardship program, underpinned by a comprehensive Best Management Practices program which was adopted across the majority of the industry, which helped to demonstrate to the community the Australian cotton industry's improved farming practices and careful management of natural resources. Through the adoption of this program, the industry was able to achieve and demonstrate an 89% reduction in pesticide use and a 40% improvement in the efficiency of water use across the industry[7]. Despite the success of this program, participants in that industry will be the first to point out just how easily community trust can be eroded and how tenuous its hold on social license to operate can be, with significant damage flowing from the exposition of the failures of just one or two farmers.

Whether this approach will work in the Australian poultry industry is yet to seen, particularly as animal welfare is a whole other level of community concern and opinion. It will be interesting therefore to observe the outcomes of the implementation of the Australian egg industry's new community engagement strategy.

Is There a Role for Science?

Science still has and always will have a role to play in helping to establish key benchmarks for both appropriate and best practice. Science should underpin what industry aims to achieve in its dialogue with the community and may help us decide what issues we should be prepared to compromise on and what we are prepared to defend at all costs.

Conclusion

Those of us involved in the poultry industries have traditionally understood fact to equate to what is proven by hard scientific data or other 'evidence', and a fact to be something that is 'real'.
However, facts are no longer the reality we operate in. Perception is the new reality and, yes, in many areas of industry's operations, therefore, fiction has indeed become fact.

In order to successfully adapt to this new reality, we have to first accept that we are in a new operating environment - one in which we can no longer expect community acceptance; one in which we need to earn community trust, rather than assume that we will always achieve understanding and acceptance through 'education'.

Building trust requires a deep understanding of what the community's opinions and perceptions are and the underlying reasons for these, identifying common ground, shared interests and shared values, engaging with the community on the issues that concern them - even compromising in some cases, and demonstrating that the community's trust is deserved through transparency and doing what we say we do. This is not an easy path for industry to follow, but it may be the most productive way for industry to maintain some control over the way it operates in the future.

We shouldn't think of this as 'giving in'; it's more productively thought of as 'getting on with it'.

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The Social Media Conversation about Animal Agriculture: Global Insights

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INTRODUCTION

A wide range of stakeholders are engaged in online conversation about animal agriculture. With so many voices, it can be difficult to pinpoint what is truly being said by whom and how these voices are shaping the concerns and expectations of consumers, investors and policy makers. Elanco uses social listening software to track conversations from social media, blogs, forums, and online news and publications. These are then analysed to determine key learnings, trends and other insights. Elanco has been following the global social media conversation about animal agriculture since 2014 with access to a rolling 27 months of data for historical perspective. Insights are collated related to key species (beef, poultry, swine and dairy) as well as sustainability, animal welfare and antibiotics. Insights are shared with stakeholders and customers, help shape how Elanco engages in online conversation about animal agriculture, and contribute to Elanco's understanding of the social and political environment in which it operates.

Summaries of the online conversations related to poultry, sustainability, animal welfare and antibiotics during April – June 2018 are detailed below. The metrics for analysis include volume, sentiment, channel distribution, geography and passion intensity. Key learnings include understanding animal agriculture's share of the overall online conversation, understanding what consumers (really) care about in terms of animal source foods, identifying the agriculture sector's share of voice in the online conversation about production issues, and identifying opportunities for positive engagement in online discussions about animal agriculture.

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Poultry[1]

During April to June 2018, there were over 43 million (English language) mentions of poultry keywords across all geographies. The conversation was generally consistent with volume across previous quarters, and nearly identical to the volume of posts as the same quarter in 2017. In general, the poultry conversation, along with the dairy conversation (56 million mentions), was particularly large compared to the swine (14 million mentions), and beef conversations (11 million mentions). Eggs were mentioned two out of three times when discussing poultry, whereas chicken and turkey pulled in 20 percent and 12 percent of the overall conversation respectively.

Discussion of poultry in the context of industry issues (sustainability, animal welfare and antibiotics) increased, whereas discussion about food safety in conversations about poultry showed a significant drop in volume. On the other hand, consumer-related topics (price, taste, cooking/ recipes, nutrition, clean labels and meat alternatives) were more consistent with the previous quarter, with most of them just slightly decreasing. The one exception was meat alternatives, which saw an increase as consumers discussed the pros and cons of quorn and seitan (for example) as alternatives to chicken and eggs. These conversations focused on taste differences, and whether these alternatives were still as tender and flavorful. KFC's move to begin testing a vegetarian alternative to chicken (in the UK) drove a significant part of that conversation.

Cooking and recipes made up most of the conversation for eggs and chicken, followed by discussion around taste and price. Food safety as a topic of discussion about poultry focused on recalls, as more than 200 million eggs were being recalled because of potential contamination of salmonella across nine states in the U.S. Nutrition conversation often entailed eggs being considered as staple in a healthy diet, and the fact that many different meals can be created with them. Many others also highlighted the fact they are an excellent source of good fats, and a great way to get extra protein. Some others warned about the high presence of cholesterol, while others actually said this is a myth, and studies have shown this is not the case.

Within the sub-topic of animal welfare, a post from Peter Singer (advocate of animal rights and Professor of Bioethics at Princeton University) sparked attention. He was quoting Professor John Webster, a vet and animal welfare expert, saying that our treatment of chickens is "the single most severe, systematic example of man's inhumanity to another sentient animal." The post sparked hundreds of engagements. Sustainability-related content saw some references to unhealthy factory farm chickens, as well as polluted environments, although the conversation was usually high-level as no big threads of conversation occurred during the quarter.

The top posts mentioning poultry were dominated by taste, price, cooking and recipes, including a fried chicken recipe video that attracted 22 million views, and a story on AOL about 24-carat gold-coated chicken wings offered by a restaurant in New York for $1000, which attracted over 15,000 engagements (likes or shares) and 25 million views. A New York Times article about the eggs recall attracted over 2,000 engagements.

Some of the top media news stories related to agriculture and farming within the poultry topic included a National Geographic article discussing Marian McKenna's book Big Chicken, an article in Poultry World about the use of data to manage bird health, an article in Farming UK about the reduction of antibiotic use by poultry farmers since 2012, and an article in Food Navigator-USA about a petition for eggs to be able to carry health claims. Although the number of engagements for these stories were extremely modest compared with consumer topics such as taste, cooking and recipes, they trended above other content on similar topics.

Sustainability[2]

During April to June 2018, the overall sustainability conversation reached 3.2 million mentions during the second quarter. Of those mentions, agriculture-related content drove approximately 1 million mentions. A video published on the 'NowThis' Facebook Page (which has more than 13 million fans) captured attention, highlighting the consequence of pollution in oceans by showing plastic bags and trash pulled from a tiger shark's stomach. The World Economic Forum reported on a similar issue, showing how Guatemala is cleverly tackling the problem with 'biofences' across rivers. Greenpeace Africa's Facebook page (which has more than 200,000 fans) published video footage taken at the Nairobi River to report on the problem of plastic in bodies of water, sparking debate and general apprehension.

The very popular Facebook Page "I f***ing love science" (which has more than 25 million fans) covered an article from International Animal Rescue, showing the dramatic impact of deforestation for orangutans. A story of the Japanese village of Kamikatsu, which deployed the Zero Waste Declaration, (with the goal of being completely waste-free by 2020) went viral. In the agriculture-related sustainability conversation, Iowa Farm Babe had a Facebook post about a visit to her hometown university by Anti-GMO activist Vandana Shiva. The post had about 2,000 engagements.

The top sustainability news stories during this period included a CNBC story about Trump officials' concern about a 'public relations nightmare' over contaminated drinking water near military bases, a BBC article about a UK Environment Agency warning about the risk of water shortages, a National Geographic article about plastics in the environment, and a Guardian article about Brazilian villagers turning plastic pollution into profit. News stories from the New York Times about 'how to reduce your carbon footprint' and The Guardian about air pollution in the UK also featured among the top media news stories in the social media conversation about sustainability.

The top sustainability news stories related to agriculture and farming included an article about a new financial growth plan for organic agriculture in Denmark, worth 1.1 billion kroner (147 million euros), an article about the UK dairy sector's significantly reduced environmental footprint over the last 10 years, and an article about efforts in California to fight global warming.

Animal Welfare[3]

During April – June 2018, the animal welfare conversation saw a large decrease in volume compared to January - March, from 520,000 posts to 350,000 posts, although sentiment was relatively consistent, with 6 percent positivity (5 percent in Q1), and 12 percent negativity (same as Q2). PETA's social handles drove the overall conversation, with many posts from its Facebook page sparking thousands of interactions. Some of the most shared PETA posts included content about violence toward animals in circuses, jokes with funny images of cats and other pets, and a turtle in pain as a plastic drinking straw is pulled from its nose. Overall, posts were usually tied to very graphic pictures and videos of animals in bad conditions and/or in pain, which typically prompted a strong emotional response by commentators.

A video from the 'Young Turks' YouTube channel (3.9 million subscribers) published a report about how some activists are facing "years in prison for showing turkey farm abuse". The video received more than 3,000 likes, 2,000 comments and 85,000 views. In April some sources (including CNN) reported on the court ruling that monkeys don't own the copyright to selfie photos after the idea was challenged in a lawsuit brought by PETA.

The top animal welfare news stories during the April – June period included an article in The Guardian about "the true cost of eating meat", a Huffington Post article about the monkey selfie copyright legal ruling, a Huffington Post article about the potential impact of proposed amendments to the US Farm Bill that could be detrimental to animals, a Bloomberg article about South Africa's Mohair Industry, a Times of India article about a local PETA activist promoting veganism, a Brietbart article about pit bull dogs no longer allowed as service animals on Delta Airlines, and an article on AgWeb about the proposal to prevent the term 'meat' being used to promote non-meat alternatives.

Within the animal welfare topic, the top media news stories related to farming and agriculture were an article about smart collars for cows, an article about a proposal to change branding requirements for cattle from Mexico, and an article about how changes to poultry barn lighting can reduce stress for chickens.

Antibiotics[4]

Resistance to antibiotics, as well as treatments, were the most important topics for the overall antibiotics conversation during the second quarter of 2018. Totaling 500,000 mentions, the overall antibiotics conversation saw a significant increase since the first quarter (459,000 mentions). Compared to the previous quarter, agriculture antibiotics conversations slightly decreased, dropping from 30,000 mentions to 28,000 mentions. The environmentalist Paul Nicklen reported how antibiotics and pesticides have dramatic impacts for salmon farming, launching the hashtag #GetFishFarmsOut. A video by Dr Eric Berg (29,000 views) recommends to not consume farm raised fish, partly because of the use of antibiotics. In mid-June, Pizza Hut announced its intention to remove all antibiotics considered important to human medicine from its chicken products in the U.S. by 2022. The social media conversation around the announcement attracted less than 1,800 posts, mainly from news sources. This is compared to a similar announcement by McDonalds in 2014 that attracted over 30,000 mentions in the first three days.

The top news stories about antibiotics included an article in the New York Times about drug-resistant typhoid in Pakistan, an article in The Economist about increasing antibiotic use in developing countries, an article on NBC News about 'why some bacteria eat antibiotics', an article in The Guardian about guidance for consumers to make better meat choices, and an article in USA Today about KFC plans to test plant-based chicken.

The top antibiotics news stories related to agriculture and farming included the story about Pizza Hut's pledge to drop some chicken antibiotics by 2022, an article by Health for Animals about the delicate balance required in addressing drug resistance, an article about the US FDA's expansion of on-farm antibiotic monitoring, articles about efforts by pig farmers and the beef industry to address antimicrobial resistance, and an article about antibiotic residues in waste milk fed to calves.

Insights summary

The overall poultry conversation (including chicken, eggs and turkey) was second only to the dairy conversation in terms of volume during April – June 2018 with the largest proportion of that related to cooking and recipes. Despite some popular posts by activists and others critical of animal agriculture, the proportion of conversations focused on these sub-topics is relatively small. Even stories about 'meat alternatives' are discussed mostly in relation to how they taste, and even with the large egg recall in mid-April, the monthly average mentions of the food-safety sub-topic in poultry were lower than monthly average mentions in 2017. The most popular activist content (the most views, 'likes' and 'shares') usually includes graphic visual content, such as the tiger shark video (1 million views) in the sustainability conversation, though this is still significantly smaller than a video about a fried chicken recipe in the poultry conversation that attracted 22 million views. News from food brands such as KFC, Pizza Hut and McDonald's can drive higher spikes in conversation about food production issues such as policies related to antibiotics. Analysis of the antibiotics conversation since 2014 has identified the agriculture sector's share of voice as less than 0.04% of the overall conversation about antibiotics in agriculture, though it has grown in the last three years from 0.02% of the overall conversation about antibiotics in agriculture. Increasing the share of voice for animal agriculture will help to balance the overall conversation about the links between food, farm practices, sustainability and animal welfare.

Acknowledgements

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Feed Resources for Poultry Feeding in the South Pacific: Dietary Recommendations, Issues and Prospects

ASHIKA DEVI and SIAKA SERIBA DIARRA

INTRODUCTION

The South Pacific region is consists of 22 small islands with an estimated population of 11 million (Diarra, 2017). There is huge demand for poultry products in the Pacific Island Countries (PICs) and this is expected to increase due to rapid population growth, taste, and health benefits of poultry products and changing lifestyle. According to Diarra (2017) poultry meat imports in Fiji increased by 69% between 2010 and 2011 and during the same period egg production also increased. In Samoa, poultry meat import was valued at SAT$ 45 million from July 2012 to June 2013 or 87% of total cost of meat import in the country (APHD, 2014 cited in Diarra, 2017). Major hindrance to expansion of the poultry industry in PICs is exorbitant feed cost due to unavailability and high cost of traditional ingredients for diet formulation (FAO, 2012; Diarra, 2015a; Diarra, 2017). Even in countries where there are feed mills, these mainly mix imported ingredients. Fiji was reported to have imported up to 15,000 tonnes of cereals for the feed industry in 2011 (Diarra, 2017). Ayalew (2011) reported a 56 to 100 % increase in retail prices of commercial pig and poultry feeds in Papua New Guinea (PNG) from 2003 to 2011. This massive importation has caused loss of foreign exchange, income and employment opportunities to the region.

There are however, several materials locally available in the region which could be used to replace part of the conventional ones and reduce poultry feed cost (ALFID, 2002; FAO, 2012; Diarra, 2015a). This paper reviews availability, composition, dietary recommendations and prospects of locally available feed ingredients for poultry in the South Pacific.

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Availability, composition and dietary recommendations of selected alternative feed resources

There are several raw materials available in the region which could be used to reduce the quantity of traditional ingredients and feed cost. However, it is difficult to qualify these resources in the region due to lack of statistics of their production in most countries. Estimates of the availability and composition of selected alternative resources are discussed.

Energy sources

Roots/ tubers and by-products

Cassava (*Manihot esculenta*) grows well in the South Pacific region (Ochetim, 1991) but with the exception of Fiji, statistics of production are limited in most countries. In Fiji, cassava production was estimated at 74,028 metric tonnes in 2016 (FAOSTAT, 2018). Currently, there are over 30 cultivars of sweet cassava produced in Fiji mainly for human consumption (Dayal et al., 2018). This high demand for food makes cassava root unavailable for poultry feeding in most countries of the region. During cassava harvesting and processing for food, by-products such as peels are readily available and may pose disposal problem. Cassava peel is reported to make up to 10-13% of the tuber weight (Oladunjoye et al., 2010). Based on the production data of FAOSTAT (2018), this will represent between 7,402.8-9,623.64 tonnes of cassava peel produced annually in Fiji. The crude protein content of cassava peel is reported to be 31-52g/kg, metabolisable energy 10.7-12.89 MJ/kg, crude fibre 39-127g/kg, ether extract 14g/kg and ash 87g/kg (Kortei et al., 2014; Diarra, 2015a; Dayal et al., 2018).

The recommendation of cassava peel meal in poultry diet has been variable ranging from 10-75% (Tewe, 1983; Salami (2000); Babatunde, 2013; Diarra, 2015a; Dayal et al., 2018). Several factors including feed composition, source of cassava peel, method of peeling and processing, age of birds and enzyme supplementation may affect the utilisation of cassava peel by poultry. Dayal et al. (2018) reported reduced performance of broiler starter and finisher fed 116g/kg and 154g/kg of cassava peel per kg diet respectively, but this was overcome by enzyme and tallow supplementation. These results suggest that both low energy and complex structure limit the utilisation of cassava peel by poultry.

Giant swamp taro (*Alocasia macrorrhiza*) is a fast growing aroid which is becoming invasive in most countries of South Pacific (Foliaki et al., 1980; Diarra, 2018). Giant taro has little food value on account of its high calcium oxalate content (Diarra, 2018) which is responsible for the acrid taste (Ravindran et al., 1996). A variety in Samoa was reported to contain 127.7g/kg crude protein, 12.3 MJ/kg metabolisable energy, 71.3g/kg crude fibre, 4.8g/kg calcium oxalate (Diarra, 2018).

Dietary inclusion of giant taro root meal at 100g/kg diet maintained performance of broilers and laying hens but performances were deteriorated when inclusion was increased to 200g/kg (Diarra (2018). This author observed that supplementation of the diet with coconut oil slurry improved utilization of 200g/kg by laying hens but not in broilers probably due to age difference in fat digestion by poultry (Sell et al., 1986).

Fruit by-products

There are fruit processing companies in Fiji which produce concentrated juices from several fruits including pineapple (*Ananas comosus*), orange (*Citrus sinensis*), guava (*Psidium guajava*) and mango (*Mangifera indica*) (Fiji Islands Trade and Investment Board, 2009). The wastes from these processing are currently underutilised and mostly dumped. In Samoa, spoilt and rejected papaya (*Carica papaya*) and breadfruit (*Arocarpus altilis*) are reported to be dumped (FAO, 2009). Orange peel/pulp contains 72.8, 107 and 615.5g/kg crude protein, crude fibre and nitrogen free extract respectively (Siyal et al., 2016). Gross energy, carbohydrate, crude protein, crude fibre, ash and fat contents of 13.6-17.4 MJ/kg, 630-692g/kg, 32-58g/kg, 50-79 g/kg, 31-116 g/kg and 9-50g/kg respectively have been reported in dehydrated breadfruit (Aregheore, 2005; Oladunjoye et al., 2010). In addition to the nutritive composition, several functional properties including antioxidant, antimicrobial and cholesteromic activities have been identified in most fruit and their wastes (Halvorsen et al., 2006; Ahn, 2011; Dahunsi et al., 2016) which could be used for the production of functional poultry products.

Phenolics, flavonoids, tannins and stilbenes are the major anti-nutritional factors in fruit peels which limit their utilization in animal feed (Ahn, 2011). Total phenolic compound contents of 5.74, 2.15, 7.39 and 9.16 µ/mol have been observed in orange, pineapple, mango and papaya peels/pulp respectively (Sun et al., 2002; Harris and Brannan, 2009; Isabelle et al., 2010; Ahn, 2011).

The utilization of fruit peels in poultry feeding is well documented. Dalkilic et al. (2015) found that orange peel essential oil supplementation improved weight gain, feed conversion ratio and relative weight of breast of Japanese quails. Inclusion of citrus pulp meal at 10-15% in layers and broilers had no adverse effects on growth, egg quality and FCR but above this level performance was deteriorated (Mourao et al., 2008; Nazok et al., 2010). These adverse effects could be attributed to anti-nutritional factors as citrus seed, a component of the pulp, is known to contain limonin which is toxic to monogastric animals (Göhl, 1982). This suggests the need to separate the seed and research into processing methods that would reduce the content of limonin. Banana peel meal, citrus and breadfruit waste meals at 30-100g/kg of the diet improved performance of broilers and layers (Adekunle et al., 2006; Oladunjoye et al., 2010; Syial et al., 2016). Inclusion of raw mango kernel meal 5-10% in broilers (Diarra et al., 2008) and 5% in layers (Odunsi, 2005) maintained performances comparable to the control diet. Guava waste meal has been successfully included at 5-10% in broiler diet (Lira et al., 2009; Adityo et al., 2013).

Brewers' spent grain

Brewers' spent grain (BSG), a by-product of brewing is readily available at cheaper cost in most countries of the region. Spent grain contains 19.7-20.6 MJ/kg metabolisable energy, 200-292g/kg crude protein and 158-178g/kg crude fibre (Mussatto et al., 2006; Swain et al., 2012; Heuzé et al. (2017). The feeding of BSG to poultry has been well studied. Inclusion of BSG at 10-20% and 30% maintained performance of broiler starter and finisher respectively (Denstadli et al., 2010; Ademosun, 1973 cited in Heuzé et al., 2017). According to Mussatto et al. (2006) and Swain et al. (2012) feeding BSG to dual purpose Vanaraja birds at 20% improved carcass yield, maintained performance and attracted more profit.

The composition and recommendations of selected locally available energy sources for poultry are summarised in Tables 1 and 2.

Table 1: Summarised composition of selected locally available energy sources for poultry (g/kg)

Constituents	Cassava peels	Giant swamp taro	Orange peel	Breadfruit	Pineapple peel	Mango peel	Papaya pulp	Brewers spent grain	Citrus pulp/peel
ME (MJ/kg)	10.7-19.8	11.3-12.3	17.2	13.6-17.4	17.0	17.8	17-18.4	17.7-22.4	17.3
Carbohydrates	-	-	-	630-692	-	-	-	23-145	18-124
Crude Protein	29-82	127.7	72.8	32-58	35-58	36-56	179-184	200-292	59-93
Crude Fibre	39-127	71.3	107	50-79	161-211	93-160	239-296	158-178	109-161
Crude Fat	7-30	1.37	11-40	9-50	5-1.7	12-53	-	-	15-39
Ash	47-87	6.4	50-94	31-116	51-141	39-83	-	-	55-86
NFE	-	66.28	615.5	-	-	-	-	-	-
Phenolic µ/mol	-	-	5.74	-	2.15	7.39	9.16	-	-
Calcium oxalate	-	4.83	-	-	-	-	-	-	-
Sources	Kortei et al. (2014); Diarra (2015a); Heuzé et al. (2016); Dayal et al. (2018)	Diarra (2018)	Siyal et al. (2016); Ahn (2011); Heuzé et al. (2018)	Aregheore (2005); Oladunjoye et al. (2010); Heuzé et al. (2017)	Sun et al. (2002); Harris and Brannan et al. (2009); Isabelle et al. (2010); Ahn (2011); Heuzé et al. (2015); Heuzé and Trans (2015)			Mussatto et al. (2006); Swain et al. (2012); Heuzé et al. (2017)	Heuzé et al. (2018)

Table 2: Recommendations of selected locally available energy sources for poultry

By- product	Recommendations (%)	Sources
Cassava peel meal	10-75 (broilers and layers)	Tewe (1983); Salami (2000); Babatunde (2013); Diarra (2015a); Dayal et al. (2018)
Giant swamp taro	10 (broilers)	Diarra (2018)
	20 plus coconut oil slurry (layers)	
Banana peel meal	3-10 (broilers and layers)	Siyal et al. (2016)
Citrus pulp meal	10-15 (broilers and layers)	Mourao et al. (2008); Nazok et al. (2010)
Breadfruit waste meal	10-20 (broilers and layers)	Adekunle et al. (2006); Oladunjoye et al. (2010)
Mango kernel meal	5-10 (broilers)	Diarra et al. (2008)
	5 (layers)	Odunsi (2005)
Guava waste meal	5-10 (broilers and layers)	Lira et al. (2009); Adityo et al. (2013)
Brewers spent grain	10-30 (broilers)	Denstadli et al. (2010); Ademosun, 1973 cited in Heuzé et al. (2017)
	20 (layers)	Mussatto et al. (2006); Swain et al. (2012)

Protein sources

Copra cake

Copra cake (CC), is readily available from coconut oil processing industries and small scale processors in many countries of the region. Copra Millers Fiji Limited produces about 900 metric tonnes of copra waste annually (Deo, 2018; personal communication). Samoa and Vanuatu are reported to produce about 800 tonnes (Devi and Diarra, 2017) and 6,000 tonnes (Quigley, 2018; personal communication) of copra meal respectively per annum.

The crude protein, crude fibre, gross energy, ash and fat contents of CC range from 150-250g/kg, 70-189g/kg, 17.8-24.6 MJ/kg, 49-80g/kg and 35-120g/kg respectively (Sundu et al., 2009; Diarra et al., 2014; Heuzé et al., 2015; Devi and Diarra, 2017; Iji et al., 2017). The complex structure, low nutrient density and poor essential amino acid profile limit utilisation of CC by poultry.

Dietary recommendations of CC for poultry range from 2-30% (Diarra et al., 2014; Heuzé et al., 2015; Devi and Diarra, 2017). Several factors including, cultivar and age of the plant, methods of oil extraction, diet composition, enzyme and amino acid supplementation are all known to affect the utilization of CC by poultry (Low, 1993; Sundu et al., 2004; Devi and Diarra, 2017).

Palm Kernel cake

Palm kernel cake (PKC) is readily available in some countries of the region. Solomon islands was reported to have produced about 32,000 tonnes of palm kernel oil in 2015 (Quarterly Report, 2015). After oil extraction, PKC is readily available and can be harnessed for poultry feeding. Palm kernel meal contains 140-200g/kg crude protein, 130-210g/kg crude fibre, 18.8-21 MJ/kg gross energy, 30-150g/kg fat and 30-120g/kg ash (Alimon, 2004; Dairo and Fasuyi, 2007; Sundu et al., 2008; Abdollahi et al., 2016; Iji et al., 2017). PKC has a good level of lysine but low in cysteine, methionine and tryptophen. Like copra meal, high fibre, low nutrient density and high water holding capacity limit the utilisation of PKC in poultry diets (Sundu et al., 2008; Iji et al., 2017). Dietary inclusion of 20-30, 25-35 and up to 40% PKC have been recommended for broilers, laying hens and laying ducks respectively (Dairo and Fasuyi, 2008; Chong et al., 2008; Bello et al., 2011; Sundu, 2011; Samsudin et al., 2016).

Slaughter by-products

Fiji and Vanuatu have established beef and broiler slaughter houses but the by-products from these processing are not yet fully utilised. Diarra (2015, personal communication) estimated the by-products from broiler slaughter at Goodman Fielder limited in Fiji at about 20 tons. An average of 736 (FMIB, 2015) and 1,375 (PHAMA, 2012) heads of cattle were reported to be slaughtered monthly in Fiji and Vanuatu respectively. The by-products from these slaughters, which could be processed into high quality protein concentrates, are currently underutilised in the region due to lack of functional processing plants and poor knowledge of the nutritive value (Diarra, 2017). Poultry offal meal contains 600.2g/kg crude protein, 117.9g/kg crude fibre, 111.1g/kg minerals (Caires et al., 2010) which could replace fishmeal and soybean meal in poultry diets (Silva et al., 2000; Mutucumarana et al., 2011; Asafa et al., 2012). Poultry offal meal has been recommended between 3-10% and 5-7.5% for broilers and layers respectively (Kirkpinar et al., 2004; Mutucumarana et al., 2010; Hosseinzadeh et al., 2010).

Snails

Giant African snail, currently a major agricultural pest in some countries of the region, could be used to reduce spending on expensive protein ingredients. The crude protein, crude fibre, total ash and metabolisable energy content of snail meal ranges from 505-830g/kg, 4-43g/kg, 190-230g/kg, 12-14 MJ/kg respectively (Diarra et al., 2015; Heuzé et al., 2016). Snail meal protein has a good balance of polyunsaturated fatty acids (linoleic, linolenic and arachidonic) and amino acids but lower in methionine comparable to that of fishmeal (Diarra, 2015b). Complete replacement of fish meal with snail meal had no adverse effect on performance of boiler starter, grower, laying hens and ducks (Ulep and Buanefe, 1991; Arockian et al., 1992; Diomandé et al., 2008; Diarra, 2015b).

Leaf meals

Root crops such as cassava, sweet potato and taro are staple food sources in the South Pacific region (Waramboi et al, 2011; Khan, 2017) and their leaves, by-products of the harvest, are readily available. Leaf meals (LM) are average sources of proteins with a good profile of sulphur amino acids (Ravindran et al., 1991; Diarra and Devi, 2015). Leaf meals are also high in carotenoid content (Ravindran et al., 1991) which could reduce the use of commercial colorings in the feed.

Cassava leaf meal contains 172-399g/kg crude protein and 103-248g/kg crude fibre (Ravindran, 1991; Salvador et al., 2014; Heuzé et al., 2017). Crude protein and crude fibre contents of sweet potato leaf have been reported at 40-250g/kg and 77.4-303g/kg respectively (Bovell-Benjamin, 2007; Heuzé et al., 2017; Khan, 2017).

Major factors affecting the utilisation of LM by poultry include high fibre content and likely presence of toxic factors such as cyanogenic glucosides (Ravindran, 1993; Morgan and Choct, 2016). The high content of sulphur amino acid especially methionine and cysteine profile of LM may however, be an advantage in view of the role of sulphur in detoxification (Ravindran et al, 1991; Khan, 2017). Source of leaf, processing method and diet composition may all affect the recommendation of leaf meals in poultry diets. Cassava and sweet potato leaf meals have been recommended between 5-20% (Tamir and Tsega, 2010; Diarra and Devi, 2015; Morgan and Choct; 2016; Heuzé et al., 2017; Khan, 2017) in broiler and layer diets.

The composition and dietary recommendations of selected locally available protein sources are presented in Tables 3 and 4.

Table 3: Composition of selected locally available protein sources for poultry (g/kg)

Constituents	Copra cake	Palm kernel cake	Snail meal	Poultry offal meal	Cassava leaf meal	Sweet potato leaf meal
ME (MJ/kg)	17.8-24.6	18.8-21	12-14	-	12.6-209	16.8-18.9
Crude protein	150-250	140-200	505-630	600.2	172-399	40-250
Crude fibre	70-189	130-210	59	-	103-248	122-303
Crude fat	35-120	30-150	4-43	117.9	36-106	10-54
Ash	49-80	30-120	190-230	-	57-125	56-166
Sources	Sundu et al. (2009); Diarra et al. (2014); Devi and Diarra (2017); Iji et al. (2017)	Alimon (2004); Dairo and Fasuyi (2007); Sundu et al. (2008); Abdollahi et al. (2016); Iji et al. (2017)	Diarra et al. (2015); Heuzé et al. (2016)	Caires et al. (2010)	Ravindran (1991); Salvador et al. (2014); Heuzé and Trans (2016)	Bovell-Benjamin (2007); Khan (2017); Heuzé et al. (2017)

Table 4: Dietary recommendations of selected locally available protein sources for poultry

By-products	Recommendations (%)	Sources
Copra cake	2-30 (broilers and laying hens)	Diarra et al. (2014); Heuzé et al. (2015); Devi and Diarra (2017)
Palm kernel cake	20-30 (broilers) 25-35 (laying hens) 40 (laying ducks)	Dairo and Fasuyi (2008); Chong et al. (2008); Bello et al. (2011); Sundu (2011); Samsudin et al. (2016)
Poultry offal meal	3-10 (broilers) 5-7.5 (laying hens)	Kirkpinar et al. (2004); Silva et al. (2000); Asafa et al. (2012) Hosseinzadeh et al. (2010)
Snail meal	Complete replacement of fishmeal	Ulep and Buanefe (1991); Arockian et al. (1992); Diomandé et al. (2008); Diarra (2015b)
Cassava leaf meal	5-20 (broilers and laying hens)	Tamir and Tsega (2009); Diarra and Devi (2015); Morgan and Choct (2016)
Sweet potato leaf meal	5-20 (broilers and laying hens)	Heuzé et al. (2017); Khan (2017)

Issues with the utilisation of local resources for poultry feeding in the PICs

Problems of collection

In most countries of the region there are no reliable statistics of the feed resources. This coupled with the scattered island nature of the countries makes the efficient collection of these resources difficult. Most available resources are generally high in moisture, low in dry matter contents and this does not justify their transportation at economic cost.

Inadequate Feed processing facilities

Local feed resources need to be processed adequately to improve their utilisation by poultry. Unfortunately, basic processing equipment are lacking in most countries of the region. In countries where they may be basic feed processing facilities, these are mainly found in urban centres far away from production areas. In view of the variability in nutrient composition and anti-nutritional factor contents these feed resources always need to be analysed before inclusion in the diet. Currently however, many of such analyses cannot be done in the region.

Future opportunities

The low dry matter content, problems of collection make efficient utilisation of locally available feed resources for large scale feed production difficult in the region. However, there are opportunities to improve their utilisation for on-farm feed production. These opportunities include:

Feed processing

Basic feed processing facilities can be owned by government, small scale farmers or communities to reduce cost. Several processing technologies such as sun-drying, pelleting, boiling, ensiling/fermentation and fat addition are reported to reduce anti-nutritional factors and improve utilisation of plant products by poultry (Diarra and Devi, 2015; Ravindran and Abdollahi, 2017; Iji et al., 2017). These could be used to increase inclusion levels of available raw materials in poultry diets in the region.

Feed additives

The presence of several commercial feed additives in the market today is an opportunity for improved utilisation of local feed resources in the region. Feed additives such as synthetic amino acids, feed enzymes, probiotics and essential oils have been reported to beneficially improve nutrient utilization by poultry (Windisch et al., 2007; Ravindran and Abdollahi, 2017; Alagawany et al., 2018).

Research/ Training

Several varieties of crops such as cassava and sweet potato are grown in the region and this calls for more research into varietal difference in their utilisation by poultry. Processing methods that will reduce toxicity and maintain maximum nutrient profile of these materials also need to be researched. There is need for regular training to update farmers and extension staff on the opportunities and strategies in the utilisation of local resources in the diet.

Conclusion

Feed cost remains the most important factor affecting domestic production of poultry products despite their high demand in the PICs. There are several by-products which have potential as energy and protein sources, for poultry feeding in the South Pacific region but these remain underutilised due to anti-nutritional factors, inadequate processing facilities and poor knowledge of their nutritive value. There is need for regular training and establishment of small to medium scale community feed processing plants for maximum utilisation of locally available resources and reduce poultry feed cost in the region.

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From Antimicrobial Effect to Host Mediated Response- A Shift in Paradigm of the Use of Plant Extracts

RAUL FELIPE CORTES CORONADO

The world of birds is changing. This has come from the incredible process of evolution that led megasaurus to become modern birds, to the use of genetic selection that has taken the birds to levels of productivity not seen before and turned them into "small dinosaurs". In addition to this, the production systems have changed with birds raised in conditions of higher density per square meter, which creates a series of challenges not only from an environmental management point of view, but also in nutritional and health management of the flocks.

Referring more precisely to the broiler, it turns out that the speed of growth imposed by modern genetics implies that the animals are very voracious. It is this ability to eat and convert these nutrients that drives the impressive body growth development that is essential to maintain the economic returns of rearing broiler birds.

In this way the main problem we face is to maintain the feed intake of birds in the best way, which obviously implies the facilities, but above all that, that the bird is willing to eat. Voluntary feed intake depends primarily on the search for energy and other limiting nutrients, which is achieved with the proper formulation of the diet. However, there are numerous factors that depress this feed consumption and a primary driver of this is the health condition of the gut.

The interaction of the microbiota with the immune system in the gut determines primarily the conditions of the gut morphology, but also the degree or lack of inflammation. Biological inflammation is characterized by several changes, but mainly by the presence of pro-inflammatory cytokines released by several of the immune cells, including enterocytes.

MVZ , DrCPA

These cytokines have not only local effects but can also travel through the blood to reach other organs and modify their metabolism. Furthermore, they reach the brain and effect the centers that are linked to food intake. For this reason, the first consequence of gut inflammation is the reduction of feed intake, which in turn reduces the growth rate of the bird.

Antibiotics (that we know today as antibiotic growth promoters or AGP's), have been used to increase the growth of the birds and reduce food conversion. The most accepted theory today about its activity in the organism, implies that these AGP's causes a change in the microflora that leads to an inflammatory state lower than that of animals without these products in their diet.

Considering this situation, we are not really talking about replacing AGP's, but replacing actions that reduce gut inflammation. There are several ways to achieve it based on what we currently know about the interaction of different variables in the gut.

First of all, as we mentioned in the *Nature* Journal, we consider that the concept of **Intelligent Gut** exists to refer to the interaction between enterocytes (different classes), the complex immune system linked to the gut, the neurons that it has (the second Brain) and the microflora that interacts with them. For this reason, it is important to consider that we can interact with this system in different ways through the use of different strategies.

The bioactive molecules derived from plants can be used in a differentiated way to interact with this complex intestinal system and allow to fulfill the objective of reducing intestinal inflammation. We develop a concept that we can define as the **Gut Effect.**

The mucosa of the gut (enterocytes, Goblets cells, Enteroendocrine cells, Paneth Cells) are involved in forming a barrier to the microbiota, but also in charge of secretion processes (enzymes, IgA, antimicrobial Peptides, mucus) and absorption of nutrients. Furthermore, they are also in complete interaction with the immune system that is directly included (intraepithelial lymphocytes) and those that are in intimate contact (as in Peyer's plates and those of the own lamina). The ability of the mucosa to manage the nutrients allows it to achieve the highest energy recovery (digestible and metabolizable energy) ensuring to meet the needs for the growth of the birds.

Is it possible to influence the secretion of enterocytes? What would that effect be? The action of certain molecules (such as Capsaicin) that can act on the TRPV1 receptor located in the neurons of the gut and dendritic cells allows to increase the response in the production of mucus (Muc2). This controlled increase in mucus production implies changes in the bacterial population. On the one hand it stands as a barrier to avoid contact of some bacterial strains, but on the other it is a food for bacteria such as the bifidobacterium and lactobacilli genera, which can be demonstrated by the effect on the growth of these colonies and their consequent implication in the formation of biofilms that reduce on the other hand the presence of bacteria like E. coli in the gut. An additional effect of the presence of the beneficial bacteria is the increase in strength of the bonds between the enterocytes. That is, if we know the specific effect of a component of the bioactives, it is possible to achieve fundamental changes in the quality of the health of the gut and the consequent reduction of the inflammation that allows the normal growth of the birds.

One of the advantages that allows the use of bioactives on other strategies that have been used to replace the anti-inflammatory effect of the AGP's, is that we can combine different effects on the complex structure of the gut.

Bioactives such as Curcuma in the oleoresin of certain plants allow changes in the capacity of the lymphocytes associated with the gut. The lymphocytes in the gut arrive from the first week of life, and another surge in the second week in the case of broilers. These lymphocytes are "naive" so they must be "trained" in the gut not only to expand their ability to recognize antigens (which can also be improved) but also to modify their resistance and the ability with which they interact with antigens.

The interaction of the lymphocyte with an antigen development depends on the line. The antigen-presenting cells (dendritic, macrophages) will take the antigen, process it and present it to the cells that will carry the immune reaction. This process is key to achieve success in vaccinations and the protection of birds from different diseases. The process of presentation of antigens involves internal reactions of intracellular oxidation and the activation of other genes among those that produce the lymphokines that have to do with the quality and quantity of the inflammatory process in the gut.

Molecules such as turmeric, thiosulfonates and others interact with different enzymes that characterize this process, regulating its action and allowing the reaction to take the direction that causes lower degrees of active inflammation.

On the other hand, these same molecules interact with other lymphocyte lines extending their resistance capabilities and the ability to resolve the presence of antigens, which limits the number of these cells in the gut, decreases the inflammatory reaction and controls the problem caused by the microbiota.

That is, the response of the immune system and the secretory capacity of the gut can be modulated in a beneficial way for the bird with the use of certain bioactive molecules that come to precisely replace the effects of the AGP's. This is the concept of HOST MEDIATED

RESPONSE where by applying knowledge of chemistry, receptors, cells, microbiota we can improve the productivity of birds within intensive production environments while maintaining the economic benefits for producers.

It is clear that these programs require the proper selection of molecules, that these are precisely dosed in the diet (the same as we did with the AGP's) and in the amounts necessary to cause effects. It is the responsibility of the industry to present these products and the farmers to use them based on the scientific evidence of the mechanisms of action.

The use of Antibiotics will obviously suffer downward changes as we carry out correct actions in the additive use programs in birds, with which we will also comply with social responsibility: ONE HEALTH

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β-mannanase Supplementation Reduced Signs of Intestinal Inflammation in Broilers

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INTRODUCTION

β-mannans (β 1-4 galactomannans) are present in many feed ingredients. Among the protein sources frequently used in feed rations, β-mannan in soybean meal (SBM) is relatively high and β -mannan concentration in wheat is more than in corn (Ferrel et al., 2014). Broiler diets with high concentrations of β-mannans demonstrated poor growth performance however, the performance was restored by dietary β-mannanase supplementation (Lee et al., 2005). The reduction in digesta viscosity contributed to the improved performance of birds fed diets with high concentration of β-mannan and supplemented with β-mannanase (Lee et al., 2003). However, the impact of viscosity maybe too small to explain the improved growth performance of birds fed typical diets such as diets based on corn and SBM (Jackson et al., 2004) or diets based on corn, wheat and SBM with supplemental xylanase (Van Eerden et al. 2014). β-mannans responded as potent activators of immune cells involved in innate inflammatory reactions (Tizard et al., 1989). These unnecessary responses are wasteful because the process impacts digestive and absorptive intestinal function (Peuhkuri et al., 2010). Therefore, the removal of β-mannans in the diet would be of benefit to the birds. The hypothesis that breaking dietary β-mannans to low molecular weight β-mannan fragments removes the ability of β-mannans to cause inflammation was supported by the results of Geneic et al. (2015) where reduced expressions of pro-inflammatory cytokines, increased expression of anti-inflammatory cytokines, and less inflamed intestines were observed in broilers fed supplemental β-mannanase.

Elanco Animal Health, Philippines[1], Elanco Animal Health, Australia[2], Elanco Animal Health Belgium[3], Elanco Animal Health, USA[4], Elanco Animal Health, Poland[5]

A reduction in the number of activated metabolic pathways involved in immune signaling and an increase in the number of activated anabolic pathways was observed when β-mannanase was supplemented in diets fed to birds (Arsenault et al., 2018). Collectively, these studies indicate that the degradation of dietary β-mannans reduced β-mannan-induced intestinal inflammation and may consequently improve intestinal health. The objective of this study was to evaluate the impact of β-manannase supplementation on intestinal integrity of birds raised mostly under commercial conditions.

MATERIALS AND METHODS

Twenty experiences were selected from the Elanco Health Tracking Sytem (HTS) database. These 20 commercial experiences were selected based on the following selection criteria: (1) Diets without and with supplemental β-manannase (Hemicell®) were used at the same time, (2) all diets were similar with the exception of the absence or presence of a β-mannanase, (3) All diets contained an anti-coccidia medication but not antibiotic growth promotants, and (4) completeness of data information in the database. The selection process did not differentiate for broiler genetic line and the manner by which the β-mannanase enzyme was used (on-top or with a matrix value). Lesion scoring was conducted by a trained Elanco employee or an independent veterinarian using methods described in the Elanco Broiler Disease Reference Guide. Briefly, the HTS requires the necropsy of 5-10 apparently healthy birds per house. In most cases, necropsy was done once during the whole growing period. In cases where necropsy was done twice, the necropsy was conducted when the birds where between 16 and 39 d. Fifteen intestinal conditions associated with 23 lesion scores were used to evaluate the intestinal health of the birds and to calculate for the intestinal integrity (I^2) scores. Chi-square analysis of categorical score proportions was used to compare treatment means of data. Analysis of variance and Tukey-Kramer HSD were used to compare treatment means of I^2 scores.

RESULTS AND DISCUSSION

The Elanco HTS is a data management system that monitors the health status of poultry flocks in a farm over time. It is a tool which enables broiler producers to anticipate health outcomes based on historical performance and provide them with the ability to adjust management or medication programs accordingly. The 20 experiences obtained from this global database were selected from 13 different countries in 19 different locations within

Europe and the Middle East (Table 1). All diets were formulated to provide adequate energy and nutrients to the birds in accordance with commercial practices. Soybean meal, rapeseed meal, and sunflower meal were the protein sources predominantly used in these 20 experiences with corn, wheat, or a mixture of corn and wheat as the cereal sources. All diets contained both phytase and xylanase. When β-manannase was not added on top, the calorie credit given to β-mannannase was between 23 to 90 kcal/kg.

Birds fed diets supplemented with β-manannase had 14% less birds (*P* <0.001) experiencing severe (Score 2) pododermatitis or burned feet (Fig. 1).

Table 1. Summary information of the 20 experiences selected for evaluation.

Metric	Diet	
	Without β-mannanase	With β-mannanase
Number of countries	13	13
Number of locations	19	19
Number of flocks	204	216
Number of birds	1535	1583
Average age at necropsy, d	26.8	26.7

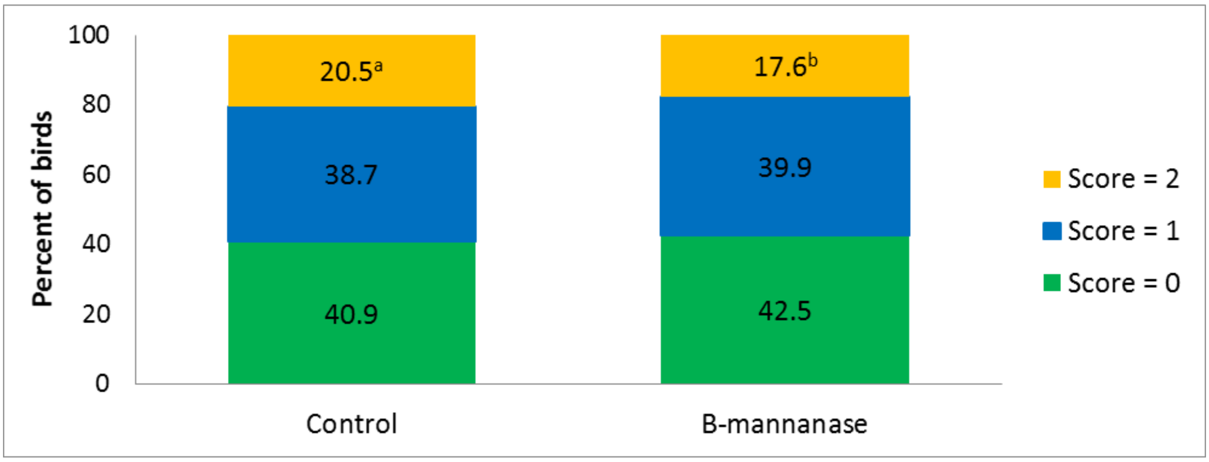

Fig. 1. Incidence (%) and severity of Pododermatitis in birds fed diet without and with β-mannanase supplementation. (Score = 0 No or very small, superficial lesions, slight discoloration on a limited area, mild hyperkeratinosis; Score = 1 Mild lesion. Discoloration of the foot pad, superficial lesions, dark papillae; and Score = 2 Severe lesion. Ulcers or scabs, signs of hemorrhages or swollen foot pads).

Pododermatitis is an increasingly important consideration from an animal welfare perspective and a reduction in its incidence is suggestive of better litter quality (Dunlop et al., 2016). The results of this evaluation is consistent to the results of Grieve et al. (2016) where birds fed diets with β-mannanase had less severe pododermatitis lesions compared to birds fed diets without β-mannanase.

The I^2 score is an index of the birds' intestinal health. A bird with an I^2 score of 100 indicates that no lesions or abnormal intestinal conditions are observed which is suggestive of a healthy gut. Birds fed diets supplemented with β-manannase had better I^2 scores compared with birds fed diets without supplemental β-mannanase (94.0 vs 93.1; P <0.001). The improvement in I^2 scores was contributed by the reduction of several intestinal conditions including reduced incidence of excessive cellular sloughing (P <0.01), reduced incidence of excessive fluid (P <0.05), and a tendency for reduced incidence of excessive mucus (P = 0.054) in the intestines (Fig. 2). A reduction in the severity of coccidiosis lesion scores was also observed in birds fed β-manannase than without β-manannase (Fig. 3). This is an interesting outcome despite the use of in-feed coccidiostats in all 20 experiences. However, the results of this study supported the results of a previous study where coccidia lesions where also reduced when β-mannanase was added to the diets of chicks challenged with necrotic enteritis and coccidiosis (Jackson et al., 2003).

(A) Intestinal cellular sloughing (B) Intestinal fluid (C) Intestinal mucus

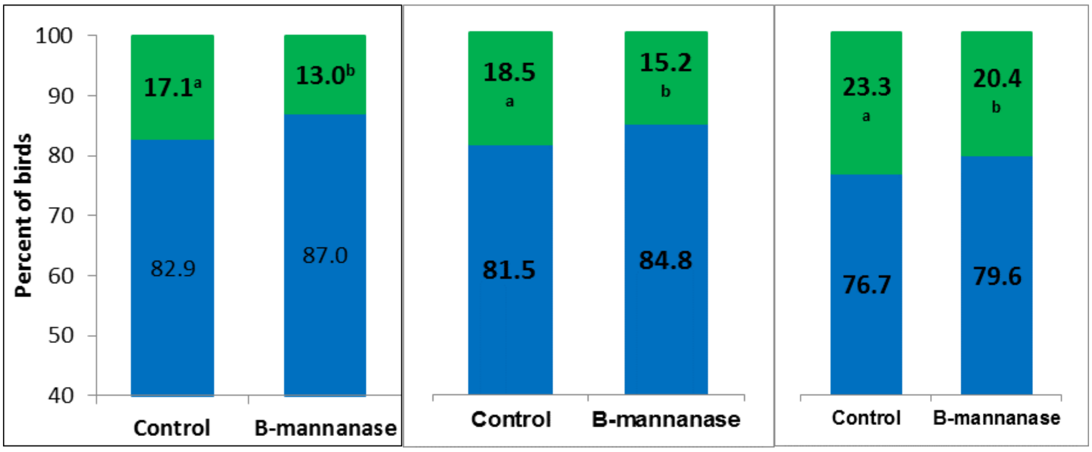

Fig. 2. Incidence of birds with normal (Blue; score 0), and excessive (Red; score 1) intestinal cellular sloughing (A), intestinal fluid (B), and intestinal mucus (C) fed without and with β-mannanase supplementation.

A number of experiments have demonstrated that β-mannans are ligands that activates immune cells (Zhang et al., 1996; Ross et al., 2002). Under conditions of intestinal leakage, β-mannans may cross the intestinal mucosa and bind with the immune cells in the gut resulting to cytokine production and possible inflammation. Considering that β-mannans are present in so many feed ingredients, it is believed that they can cause intestinal irritation and may even exacerbate an existing inflammatory process (Jackson et al., 2003). The intestines respond to inflammation by initially increasing mucus production (Sicard et al., 2017).

Inflammation also increases the rate of cell death and detachment of epithelial cells in the lumen (Piquet et al., 1998). The reduction in the incidence of excessive mucus and excessive cellular sloughing when β-mannanase was added in the diet suggests that the degradation of immunogenic β-mannans potentially diminished inflammatory reactions in the intestines. The reduction in the severity of coccidia lesions observed with β-mannanse supplementation in this study is consistent to the belief that β-mannans, as potent immune stimulators, may exacerbate an ongoing inflammation, increasing the severity of a pathogenic condition. Therefore, the degradation of β-mannans and the subsequent removal of its inflammatory impact may have mitigated the inflammatory process such that the severity of the coccidia lesions observed was reduced.

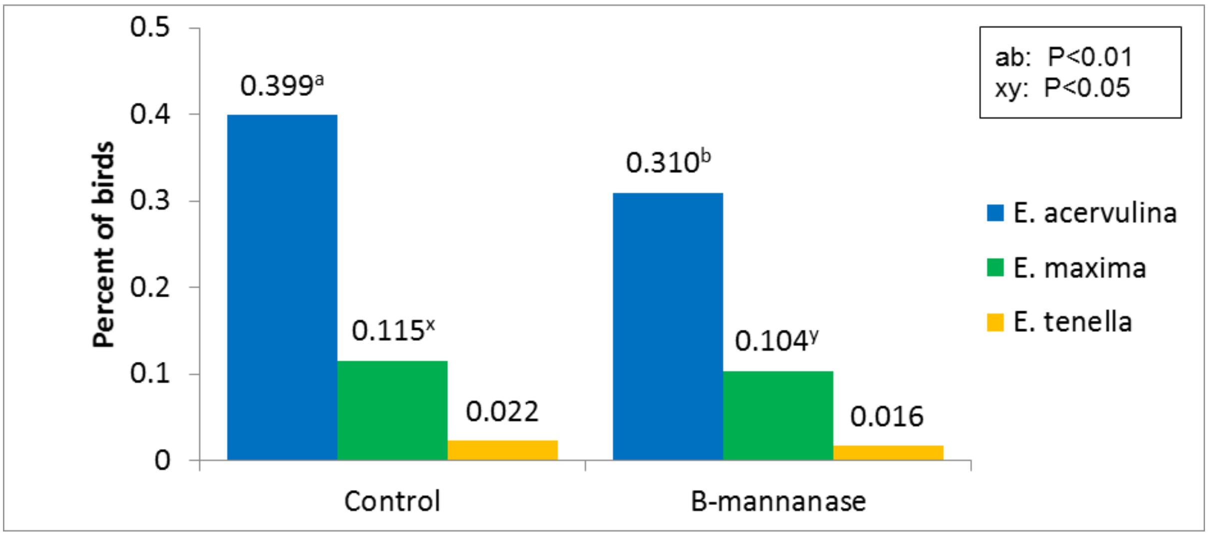

Fig. 3. Coccidia lesion scores of birds fed diets without and with β-mannanase. Gross coccidiosis lesions by Eimeria acervulina, E. maxima and E. tenella were all scored according to the 5-step scale. Score 0 = no lesions can be seen with the naked eye, Score 4 = very severe lesions.

Overall, β-mannanase supplementation improved the intestinal integrity of birds by reducing the incidence and severity of some intestinal conditions associated with inflammation. This is consistent to the idea that a reduction in β-mannan induced inflammation is the mechanism for the improved intestinal health of birds fed a β-mannanase.

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Overview and Challenges of Poultry Production in India

YASHPAL SINGH

INTRODUCTION

Poultry industry is one of the fastest growing segments of the agricultural sector with around eight to ten percent growth rate per annum. In earlier years broiler farms had produced on average a few hundred birds (200-500 chicks) per cycle. Today units with fewer than 5,000 birds are becoming rare, and units with 10,000 to 50,000 birds per week cycle are common. Similarly, in layer farms, units with a flock size of 20,000 to 50,000 birds have become common. Small units or backyard poultry are probably finding themselves at a disadvantage because of high feed and transport costs, expensive vaccines, and veterinary care services and the non-availability of credit. However the structure of India's poultry industry varies from region to region. While independent and relatively small-scale producers account for the bulk of production, integrated large-scale producers account for a growing share of output in some regions. Integrators include large regional farms that incorporate all aspects of production, including the raising of grandparent and parent flocks, rearing DOCs, contracting production, compounding feed, providing veterinary services, and wholesaling. The southern region account for about 57 percent of the country's egg production, the eastern and central regions of India account for about 17 percent, the northern and western regions contribute 26 percent of egg production.

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In addition to modern hybrid layer and broilers, India is having a huge (300-500 millions) population of indigenous chicken in the backyard or free range; which is a source pin money to the house wives. The Japanese quail framing is also growing rapidly as alternative to chicken. Native Ducks and their cross with Khaki Campbell are reared in north eastern state and in Kerala mostly by nomadic tribes in the free range.

Historical Background

Backyard poultry keeping dates pre-historic period though the modern commercial poultry production in India is barely 50 years old. 1955 to 1965 , some Christian missionaries have imported while leghorn, RIR and White rock breeds to upgrade the local chicken, having highly brooding characteristic and high disease resistance power. Later hybrid broiler (Arbor Acres) and layer (Babcock) strains were brought to India in early seventies to start the era of modern poultry. Amongst the two, the layer took wings early and showed a spectacular growth rate between 1970 to 1985. Other hybrids like Bovans, Hisex , Hyline, Keystoine and Lohmann were introduced into the Indian Market which are laying just white shell egg only. On the contrary, the native chicken and crossbred developed by Agricultural and Veterinary universities, produces brown shell egg; which constitute around 18% of the total egg produced in India The broiler industry came to existence around 1974 with the import of arbor Acres and Cobb broiler G.P stock and later on other hybrids like Hubbard, Hybro, Marshall and Ross hybrid G.P stock were introduced. Around 18% of broiler market share in India is with indigenously developed cross-bred colour broiler and local free range birds.

Native chicken breeds of India

A total of sixteen native chicken breeds have been recognized and registered as indigenous breeds of chicken in India (Table 1). Among them, most popular breeds are Aseel, Kadaknath, Nicobari etc

Some of the native chicken breeds of India are under threat of extinction due to various factors including incidence of diseases and other re-emerging diseases. Furthermore, introduction of exotic/improved germplasm is diluting or eroding the genetic base of native chickens. So conservation of native is on top priority of Indian government to have sustainable mean of livelihood under rural (backyard) poultry production.

Table 1. Registered native chicken breeds of India

Sl. No.	Breed	Home Tract
1	Ankaleshwar	Gujarat
2	Aseel	Andhra Pradesh, Orissa and Chhattisgarh
3	Busra	Gujarat and Maharashtra
4	Chittagong	Meghalaya and Tripura
5	Danki	Andhra Pradesh
6	Daothigir	Assam
7	Ghagus	Andhra Pradesh and Karnataka
8	Harringhata	Black West Bengal
9	Kadaknath	Madhya Pradesh
10	Kalasthi	Andhra Pradesh
11	Kashmir Favorolla	Jammu and Kashmir
12	Miri	Assam
13	Nicobari	Andaman & Nicobar
14	Punjab Brown	Punjab and Haryana
15	Tellichery	Kerala
16	Mewari	Rajasthan

(Source: http://www.nbagr.res.in/regchi.html)

Current scenario

The recent data of the year states that India ranks 3rd in egg production and 5th in chicken meat production in the world (Tables 2 and 3). The growth rate of layer market is 6-7 percent per annum and broiler market is 8-10 percent per annum. Approx. 75 percent of egg production is contributed by commercial poultry farms, remaining comes from household/backyard poultry. The Indian poultry sector is valued at INR 1 lakh cr or USD 15.38 bn and providing **em**ployment to more than 4 million people either directly or indirectly.

Non chicken poultry framing in India

Japanese quail and Emu are reared in India on a commercial scale following the modern scientific method. Other species of poultry like duck, geese, turkey and pigeon are still reared by traditional method in a small scale to supply the local market.

Japanese quails are reared for both egg and meat purpose in cage or deep litter. Average quail farm has capacity of 10,000 and above with its own hatchery and breeding stock. Quails are ready for market at 3 to 4 weeks with a body weight of 125-180g. They start laying eggs from the age of 6 week and hatching egg are collected from 8 weeks onwards. The egg weight is 10g and sold at US $ 2.50/100egg for table purpose and US $ 4.50to 5 /1000 hatching egg. They attain the average hatchability of 80% however mortality rate is around 6 and 8 % in cage and deep litter respectively. The major cause of mortality during growing period re chilling, drowning, stampeding, huddling and starvation; which needs to be controlled by using right brooding temperature, right size feeder and waterer combined with proper management.

Emu can be seen in Zoological park only prior to 1990. The commercial farming was first started in 1992 in Andhra Pradesh. The number of Emu in farms range from four to 10,000 with an average of 60 birds per farm. Now the few big corporate entrepreneurs are coming into this business in particular for their valuable oil and skin. Ducks are mostly reared in free range system by nomadic tribes in north-eastern and Sothern state of Kerala for eggs. Ducks will be maintained for 2- 3 years with a lay of 120-180 egg (65-80g), which are sold at a premium price of 50-100% more than the cost of chicken egg but duck meat is only a by-product of egg industry. Turkey available in India are not hybrid and are not as commercialised as that of other poultry species and there is no commercial Gosse production for meat or egg.

Overall Meat production (from all species) is estimated at 4. million tones, standing eighth in rank in the world's meat production. Buffalo in India contributes about 30% of total meat production. The contribution by **cattle**, sheep, goats and poultry is 30%, 5%, 10%, 10.2% and 11.5%, respectively.

Poultry Associations in India

There are several poultry associations in India like

- Poultry Federation of India (PFI),
- Compound Livestock Feed Manufacturers Association (CLFMA),
- National Egg Coordination Committee (NECC),
- Indian National Federation of Animal Health (INFAH),
- Broiler Coordination Committee (BCC) and likewise.

The associations also play an important role by regularly guiding the farmers, creating awareness among consumers and presenting industry requirements to the government, promoting egg consumption on various occasions etc.

Challenges

In recent times, in spite of rapid growth, the poultry industry suffered many setbacks due to rising cost of feed, emergence of new or re-emerging of existing diseases, fluctuating market price of egg and broilers, etc. which need to be addressed to make the poultry sector as a sustainable enterprise like.

- ✓ The lack of basic infrastructure such as storage and transportation, including cold chain.
- ✓ An inefficient marketing system
- ✓ The price and availability of feed resources
- ✓ Emerging and re-emerging diseases of poultry-Mutations in viral genomes leading to new variants in viruses and developing resistance to vaccines and antibiotics.
- ✓ Issues relating to animal welfare and environmental pollution by poultry units have been of increasing concern recently.

India is focussing on "Development" i.e. Good Food, Better Health & Living conditions for his 1.25 billion people and poultry production and consumption pattern in India foresees its further expansion and industrialization. Adoption of small scale poultry farming in backyards of rural households will enhance the nutritional and economic status of the rural people. Large commercial layer and broiler industries with the advent of knowledge, sustainability and profitability sees a bright future for poultry industries.

Table 2:Production and per capita availability of egg in India 1950-51 and 2016-2017

Years	**Eggs Production (In Million Nos.)**	**Human Population (In Million Nos.)**	**Per Capita Availability (In Nos./Annum)**
1950-1951	1832	359	5
1955-1956	1908	393	5
1960-1961	2881	434	7
1968-1969	5300	518	10
1973-1974	7755	580	13
1979-1980	9523	664	14
1980-1981	10060	679	15
1981-1982	10876	692	16
1982-1983	11454	708	16
1983-1984	12792	723	18
1984-1985	14252	739	19
1985-1986	16128	755	21
1986-1987	17310	771	22
1987-1988	17795	788	23
1988-1989	18980	805	24
1989-1990	20204	822	25
1990-1991	21101	839	25
1991-1992	21983	856	26
1992-1993	22929	872	26
1993-1994	24167	892	27
1994-1995	25975	910	29
1995-1996	27198	928	29
1996-1997	27496	946	29
1997-1998	28689	964	30
1998-1999	29476	983	30
1999-2000	30447	1001	30
2000-2001	36632	1019	36
2001-2002	38729	1040	37
2002-2003	39823	1056	38
2003-2004	40403	1072	38
2004-2005	45201	1089	42
2005-2006	46235	1106	42
2006-2007	50663	1122	45
2007-2008	53583	1138	47
2008-2009	55562	1154	48
2009-2010	60267	1170	51
2010-2011	63024	1186	53
2011-2012	66450	1210	55
2012-2013	69731	1212	58
2013-2014	74752	1228	61
2014-2015	78484	1244	63
2015-2016	82929	1260	66
2016-2017	88139	1275	69

Source. : State/UT Animal Husbandry Departments.
Ministry of Agriculture, Govt. of India. (ON1300), (1328) & (ON1617)

Table 3: Broiler meat production and growth rate in India 1990- 2018

Market Year	Production	Unit of Measure	Growth Rate
1990	190	(1000 MT)	NA
1991	420	(1000 MT)	121.05 %
1992	520	(1000 MT)	23.81 %
1993	560	(1000 MT)	7.69 %
1994	507	(1000 MT)	-9.46 %
1995	578	(1000 MT)	14.00 %
1996	665	(1000 MT)	15.05 %
1997	596	(1000 MT)	-10.38 %
1998	710	(1000 MT)	19.13 %
1999	820	(1000 MT)	15.49 %
2000	1080	(1000 MT)	31.71 %
2001	1250	(1000 MT)	15.74 %
2002	1400	(1000 MT)	12.00 %
2003	1500	(1000 MT)	7.14 %
2004	1650	(1000 MT)	10.00 %
2005	1900	(1000 MT)	15.15 %
2006	2000	(1000 MT)	5.26 %
2007	2240	(1000 MT)	12.00 %
2008	2490	(1000 MT)	11.16 %
2009	2550	(1000 MT)	2.41 %
2010	2650	(1000 MT)	3.92 %
2011	2900	(1000 MT)	9.43 %
2012	3160	(1000 MT)	8.97 %
2013	3450	(1000 MT)	9.18 %
2014	3725	(1000 MT)	7.97 %
2015	3900	(1000 MT)	4.70 %
2016	4200	(1000 MT)	7.69 %
2017	4400	(1000 MT)	4.76 %
2018	4600	(1000 MT)	4.55 %

Source: United States Department of Agriculture

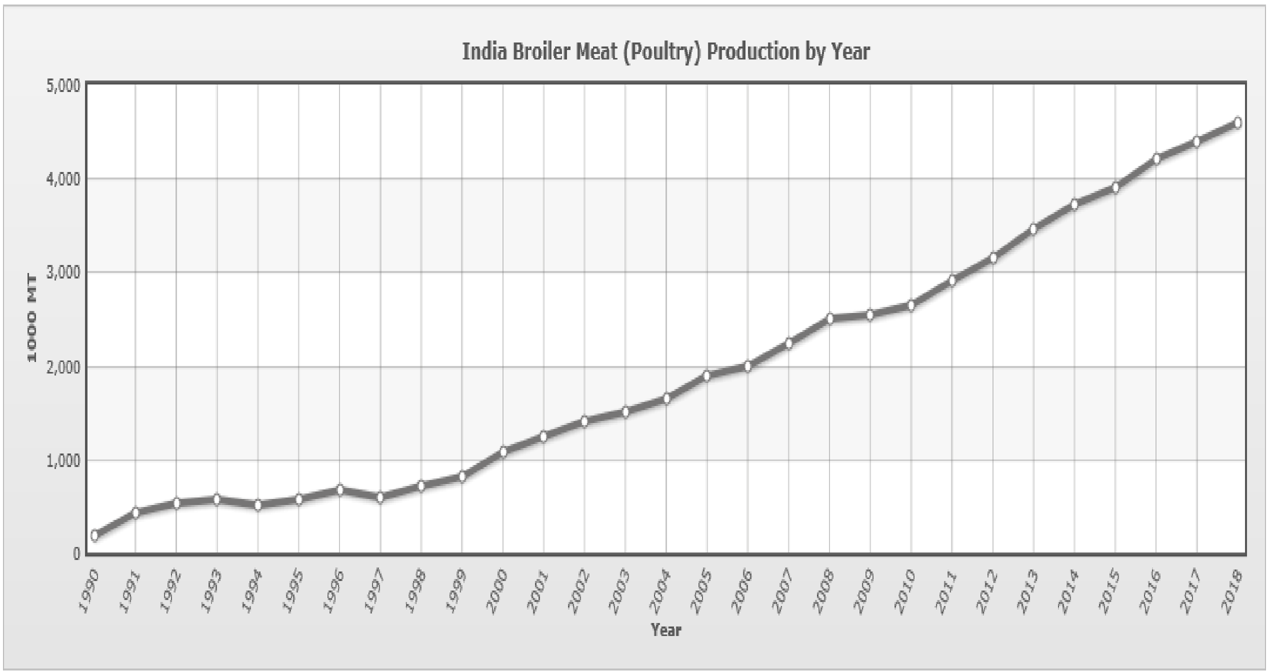

Fig 1 : Trend of broiler meat production in India

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