THE UNIVERSITY OF SYDNEY

Proceedings of the

AUSTRALIAN POULTRY SCIENCE SYMPOSIUM

Volume 34 2023

THE WORLD'S POULTRY SCIENCE ASSOCIATION

EDUCATION ORGANIZATION RESEARCH

34th ANNUAL AUSTRALIAN POULTRY SCIENCE SYMPOSIUM

SYDNEY, NEW SOUTH WALES

6TH - 8TH FEBRUARY 2023

Organised by

THE POULTRY RESEARCH FOUNDATION
(University of Sydney)

and

THE WORLD'S POULTRY SCIENCE ASSOCIATION
(Australian Branch)

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ISSN-1034-6260

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CONUNDRUMS FACING THE POULTRY INDUSTRY: HOW WILL WE ACHIEVE SUSTAINABILITY IN BOTH THE SHORT AND LONG TERM?

R. KLEYN[1] and M. CIACCIARIELLO[2]

Summary

Recent global events have given rise to volatility in feed ingredient supply and pricing. These issues are likely to remain in the medium term. Coupled with this concern is an increased demand for poultry products, driven both by population growth and socio-economic factors. Consumer perceptions and expectations will impact the manner of production of many poultry products. Ensuring poultry industry growth, all the while remaining sustainable, gives rise to a number of conundrums. All role players in the poultry supply chain must appreciate this point. This paper will explore several of the issues faced by the industry in the light of recent global events, and consider steps that can be taken to mitigate these issues. In addition, it examines the long-term impacts of these events on sustainability.

I. INTRODUCTION

A high degree of volatility with regard to the pricing and supply of inputs has arisen. This situation began with the COVID-19 pandemic and the negative effect it had on the global supply chain, and has been exacerbated by the Russian-Ukrainian war. This state of affairs will likely persist until at least the end of 2023 (Mulder, 2022). It has given rise to the intermittent non-availability of several essential feed ingredients. Coupled with these short- to medium-term stresses are several mega-trends and pressures. The global demand for eggs is expected to increase by 65% (Preisinger, 2018) and for poultry meat by 121% (Alexandratos & Bruinsma, 2012) by 2050. Poultry forms a critical component of food security, especially in communities that are deprived of nutrient-rich foods (UN, 2015). As citizens become more urbanised, providing protein at a price the urban poor can afford becomes an essential component of food security (Skinner & Haysom, 2016).

The overarching consideration for agriculture should be sustainability. The definition of sustainability is straightforward: *Sustainable systems should meet the needs of the current generation without compromising the ability of future generations to meet their own needs*. In practice, sustainability is a concept with four facets, namely environmental, ethical, economic and enactment or enforcement (the four Es of sustainability). Any scrutiny of sustainability should consider all aspects, not only the aspect that suits a particular narrative. Consumer demands are evolving, with more people wanting to eat ‘natural’ products, a trend supported by celebrity chefs and the retail and quick-service restaurant sectors. These trends have compelled the poultry industry to change production methods, forego many effective technologies, and implement exacting food safety measures along supply chains.

Regrettably, simple solutions to complex problems seldom exist. From a cynical perspective, many believe that anything ‘alternative’ is likely to address the issue of sustainability in animal production. This includes alternatives to meat, alternative production systems, alternatives to antibiotics, the use of alternative feed ingredients, and the development of alternatives to traditional retail chains as a route to market. A more realistic and pragmatic approach is required, which includes addressing many existing conundrums and the alternative mindset required of consumers, some of which this paper covers.

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II. SHORT-TERM CONUNDRUMS

a. Sustainable Poultry Production

Sustainability is important in both the short and the long term. Many food production systems are unsustainable and will continue to degrade the environment and compromise our ability to produce food. Mainstream agricultural development still concentrates on productivity and places limited focus on sustainability (Rockström et al., 2017). Under a 'business-as-usual' scenario, the harmful effects of agriculture on the environment will continue to increase. This will result in converting forests and savannahs into cropland, generating air and water pollution, increasing greenhouse gas (GHG), and threatening biodiversity (NAS, 2021). Attempts to improve welfare through alternative production systems, including organic and free-range, may harm the environment and sustainability (De Jong & Butterworth., 2016). Advances in environmental and ethical aspects will be restricted by divergent views on the economic characteristics of future agricultural systems (Wojtynia et al., 2021).

The poultry industry is probably more sustainable than other animal sectors (Pelletier et al., 2014; Fry et al., 2018), which places the industry in a strong position to buy scarce resources and convert these into edible protein. GHG from agricultural activities has contributed to climate change (Godfray et al., 2018; NAS, 2021). It is estimated that animal agriculture uses about 70–74% of all agricultural land and contributes about 15% of all GHG, but poultry is only responsible for about one-third of this amount (Steinfeld et al., 2006; Godfray et al., 2018). Changes to one aspect of sustainability often impact negatively on other areas of sustainability (EU, 2001). Food security will most likely be ensured by 'sustainable intensification' (Rockström et al., 2017), which entails producing more food on existing acreage. This is at odds with the current move to less intensive systems.

b. Ingredients

The demand for resources is relevant because most feed offered to poultry originates from commercial cropping, making ingredients a vital component of sustainability. Agriculture's extensive use of land, water and other resources is harmful to the environment and negatively affects biodiversity. Perhaps the largest impact of the current global turmoil is an erratic supply of feed ingredients, leading to price volatility. The use of alternative, preferably locally sourced, ingredients is often espoused as a means of enhancing the sustainability of animal production. The local supply of ingredients is characterised by so-called yield gaps. For example, the maize yield in the USA is 10 tons/Ha while, in Africa, it is one ton/Ha. This presents a short-term problem but offers huge scope for improvement in the longer term. The use of genetically modified (GM) technology brings new prospects in addressing food security problems (Muzhinji and Ntuli, 2020). GM crops facilitate no-tillage and conservation tillage practices that help to control soil erosion, conserve soil moisture, support carbon sequestration, decrease GHG emissions, reduce pesticide spraying, and increase crop yields by 16% (Van Acker et al., 2017). Despite the scientific consensus that GM crops are safe to eat, they are viewed with scepticism by many institutions and governments (Van Acker et al., 2017; Muzhinji & Ntuli 2020).

It is often expounded that the use of alternative ingredients in poultry diets is a sustainable option, but this presents a number of conundrums. First, there are only twelve or so major feed ingredients. Most commercial nutritionists spend a significant amount of their lives on the lookout for viable alternatives – and fail. Second, often the nutrient content is unknown, the quality is variable, and the quantities are constrained. In addition, many alternative ingredients are over-priced or of low nutrient density. It is essential that commercial

nutritionists evaluate the cost-effectiveness of alternative ingredients, not just in terms of formulated diets, but also bearing in mind return in the poultry production enterprise.

Ingredients vary in terms of their environmental impact. Production methods and land-use change (LUC), which describes practices such as deforestation or the re-deployment of 'set aside' land, impact on the carbon footprint associated with an ingredient (Cappelaere et al., 2021). Both the Global Feed LCA Institute (GFLI) (2022) database and the INRAE-CIRAD-AFZ (2022) feed tables carry data suitable for use in least-cost formulation systems. These data enable nutritionists to determine the environmental impact of diet formulation and animal production (Kleyn et al., 2021[a]). However, to do this effectively, it is essential to know the source and origin of each ingredient parcel.

c. Protein Usage

Modern broiler genotypes respond to protein (Naranjo and Lemme, 2017; Aviagen, 2022). Coupled with rising demand, this will result in a huge increase in protein requirements. Problematically, the protein levels in our major ingredients are declining, while yields of proteinaceous crops are lower. Most protein is derived from vegetable sources, with soya beans being the most important. Smaller amounts of rape, sunflower and other lupins are also used. The current turmoil in Ukraine has reduced the supply of sunflower meal, but it has not had any real impact on the global soybean supply, although the price of these beans remains volatile (high). It is unlikely that we will suffer a substantial shortage of soya beans in the short term.

Broiler diets high in essential amino acids (AA) are more expensive, but they lead to improved performance (Naranjo & Lemme 2017). Modern layers are lighter and lay smaller eggs than historic breeds. While they produce more eggs in their lifetime, they still lay a single egg daily. The utilisation of protein and energy has not changed over the past three decades (Kleyn et al., 2021[b]); thus, it is likely that the nutrient requirements of laying hens have declined. Pottgüter (2013) contends that modern genotypes perform adequately in any production system, provided that adequate feed intake is achieved. This may not always be the case in commercial production systems, which needs to be reflected in feed specifications.

The justification for reducing dietary crude protein (CP) is compelling (Greenhalgh et al., 2020[a]). Reduced CP levels can be fed applying enhanced ideal AA profiles and by utilising an ever-widening range of synthetic AA. Lower CP diets lead to an increase in performance, improved protein digestibility, a reduction in water intake, reduced manure nitrogen, and better bird welfare (Belloir et al., 2017; Chrystal et al., 2020). Lowering dietary CP by 1% reduces the carbon footprint of broiler production by 102 kg/ton of broilers produced (Martin, 2020). When reducing dietary CP levels, protein sources such as soya beans and fat are replaced by feed-grade AA and cereals (Chrystal et al., 2020; Cappelaere et al., 2021), giving rise to an increase in dietary starch and decreases in dietary lipid and true protein.

Three conundrums arise when considering protein usage. First, modern genotypes perform better when offered higher levels of essential AA. Second, minimum fat levels must be maintained in poultry diets. Significantly, however, it is e not appreciated that there is most likely a ceiling for the starch content of a diet (Greenhalgh et al., 2020[b]). Third, despite the overwhelming evidence that low CP diets support normal production levels, many countries have set minimum CP levels in their regulations, which has a negative impact on sustainability.

d. Dietary Energy

Energy is the most expensive component of the diet in both broilers and laying hens. Most energy is provided in the form of soluble carbohydrates (starch) and fat. While surplus protein can also be used as an energy source, it is utilised less efficiently in birds than in mammals (Cappelaere et al., 2021). Deciding on optimal energy levels is central to commercial poultry

nutrition. These levels should be determined by using the relative cost and availability of ingredients, together with the value of poultry meat or eggs produced. Broadly speaking, when fat is relatively cheap, high-energy diets tend to yield higher returns, while readily available, less expensive ingredients (cereal by-products, for example) make low-energy diets more attractive. Currently, all feed ingredients are expensive. The price of grain has increased by 60–70%, while feed oil prices have more than doubled. When cereal by-product supplies are adequate, they offer a cheaper alternative to commercial nutritionists. However, the moment supplies become constrained, prices shoot up and may even surpass those of grain.

It is at this point that one of the real conundrums of commercial nutrition begins. Clearly, if the price of fat trebles, all calculations regarding optimal dietary energy levels need revising. Logic dictates that dietary energy levels (nutrient density) should be reduced. However, our current practices might predicate any of the decisions made. For example, many millers add oil into the mixer to improve pellet throughput and then add more fat as a post-pelleting application to improve pellet quality. Unless millers are prepared to change this paradigm, they will be trapped using fat at $3.00/kg. Conversely, if the supply of milling by-products is constrained, the opportunities to reduce dietary energy levels may be limited. Remember, shipping low-density ingredients over distances negatively impacts sustainability. A final complication is commercial reality. On the whole, feed millers do not have the freedom to make changes to feed specifications or the selling prices of their products. Poultry producers in the main expect a certain feed efficiency (FCR) which cannot be guaranteed with reduced dietary energy. Alternatively, the likelihood exists of outpricing themselves if energy levels are increased.

e. Phosphorus

It takes about one ton of phosphate (P) to produce 130 tons of grain (Vaccari, 2009). In the long term, it is estimated that the supply of phosphate will fall below requirements by 2040 (Nedelciua et al., 2020). A large portion of phosphate originates from Russia, and the current turmoil has disrupted its supply. Morocco, the largest supplier of phosphate, has filled the void – but at a substantially higher price. The first issue is what to do if phosphate supplies are constrained. The rapid mineralisation of the skeleton of young chickens means that starter diets should take priority when supplies are limited. Second, the values published by the primary breeders (Aviagen, 2022; Cobb, 2022) for the grower and finisher phases exceed those published in the scientific literature (Angel, 2022) by some margin. In all likelihood, P levels can be reduced in the later stages of the broiler production cycle. The published requirements for laying hens (Lohman, 2020; Hy-Line, 2022) are also generous. Scientific studies to determine P requirements for laying hens show that far lower levels are adequate (Lambert et al., 2014).

III. LONG-TERM CONUNDRUMS

a. Consumers

Public opinion in developed countries is that ‘organic’ is natural, healthy and sustainable while intensive farming and antibiotic use are bad. This perception has led many consumers to assume, incorrectly, that alternative production systems are more sustainable. Unfortunately, a number of perceptions tarnish our industry: such as production occurring on factory farms; that animal welfare is flawed; poultry products contain hormones (erroneously so) and residual antibiotcs that may be harmful. Many of these beliefs are based on perception and misinformation, often created by the poultry industry itself which has used ‘Hormone-free’, ‘Drug-free’ and ‘Free-range’ as marketing slogans for decades. Consumer concerns, fueled by

food scares and the desire to eat healthier and safer food, influence food purchasing patterns (Magkos et al., 2006; Bray and Ankeny, 2018). Consumers want cheap, safe and sustainable products – at low prices. There is a lack of appreciation for what alternative production systems mean in terms of sustainability, or for how high product costs may negatively affect food security.

b. Antibiotic Use

The danger of people imbibing drug residues from consuming poultry products, and the notion that these drugs contribute to an increase in drug-resistant bacteria, are more perceptions than realities (Bywater & Casewell ., 2000; Cervantes, 2015). Evidence suggests that issues of anti-microbial resistance in human medicine are primarily due to the incorrect use of antibiotics by people rather than adverse effects derived from food animals (UK Government Office for Science, 2011). Regardless, antibiotics have been banned or voluntarily removed in many countries. Public perception is that antibiotic use must be handled effectively. Thus, the poultry industry needs to operate as responsible stewards of the limited compounds that we have at our disposal.

A conundrum with regard to antibiotic use arises in the developing world where the majority of smallholder farmers reside in the tropics. Not only are these farmers deprived, but also they inhabit areas where people and livestock live at high densities, frequently in close proximity. Biosecurity is often poor, and environmental conditions favour pathogen growth and year-round survival. These poverty-stricken people will be most impacted by a blanket withdrawal of antibiotics from animal agriculture (Robinson et al., 2017). Zoonosis is a real danger, and animal death represents a concurrent bank foreclosure and an empty pantry.

c. Alternative Production Systems

Poultry production systems that offer outdoor access to chickens (alternative systems) are potentially better for chicken welfare. However, these systems are associated with public health and food safety risks (Van Asselt, 2019). They have a direct bearing on resource usage and therefore on environmental sustainability (Williams et al., 2009). Alternative systems have a lower environmental burden when measured per unit of land use, but more land is required in total, increasing the burdens per bird or egg produced. Alternative systems are more ethically acceptable to consumers but, if welfare is measured in terms of flock mortality, then conventional systems are the more principled choice (Weeks et al., 2016). Production costs for alternative systems are higher, for instance; in fact, the cost of conventional systems is about one-third of the cost of organic production (Van Horne, 2020).

The latest global trend is the production of ‘slow-growing’ chickens. Widowski (2020) found that many indicators of welfare are directly related to growth rate, making slow-growing chickens an option on welfare grounds. Petersen (2017) estimated that if one-third of the US broiler industry switched to slow-growing systems, nearly 1.5 billion more broilers would be required annually. This would necessitate using an additional three million hectares of land for feed production and result in 12 million tons of additional manure. Conversion to cage-free egg production systems leads to an increased production cost of 14–28% due to higher feed intakes, increased mortality, more downgraded eggs, and greater space requirements (Preisinger, 2018).

An aspect that is often overlooked is the importance of subsistence (small-scale) poultry farming, which currently contributes 8% of egg production and 2% of global poultry meat production (Mottet and Tempio, 2017). It must be noted that 2.5 billion people rely on small farms for food (FAO, 2013). Poverty alleviation and sustainability targets will only be met by fostering small-scale, local production using local ingredients. This will require massive inputs from governments, NGOs and commercial companies. It would be unjust to expect these

producers to tackle this role as 'organic' farmers, as suggested by some authorities (UNEP-UNTAG, 2008). Small-scale farmers face structural and market-related challenges. It is unlikely that they will receive the premium prices required to overcome the higher input costs associated with organic production.

d. Precision Nutrition

Achieving 'precision nutrition' is a lofty goal that nutritionists continually strive for. Although we still use CP as our standard descriptor, it has been known since the 1930s that it is impossible to describe the actual protein content of ingredients by a single variable (Jones, 1931). Most, if not all, energy systems are based on the determination of apparent metabolisable energy (AME), yet it is still unclear how best to determine values for ingredients (Mateos et al., 2019). While energy continues to be construed as a property of the diet rather than a property of the bird consuming the diet, 'precision nutrition' is likely to remain elusive.

IV. DISCUSSION

In the medium term, high prices and ingredient shortages will probably be overcome, but long-term issues will only be solved using a holistic view. Many concerns of poultry producers and consumers are interwoven. A realistic approach will be required by all parties if the increased demands for animal products are to be met in a sustainable manner. In a perfect world, sustainability would be enhanced by the practice of precision nutrition. While improvements in our methods and procedures inch us towards this goal, there are still major gaps in its knowledge. Table 1 summarises some of the conundrums faced by the poultry industry. There are often more questions than answers.

Table 1 - A summary of some of the conundrums facing poultry producers and nutritionists.

Statement of the issue	Conundrum
Poultry producers are expected to meet the increased demand for poultry products.	a) Consumers expect the industrytry to fulfil its obligations safely, sustainably, and affordably. b) This will increase demand for all resources.
The better the feed efficiency and lifetime performance, the more sustainable production will be.	a) Consumers want 'alternative' products that have a larger carbon footprint.
Many production practices are used to improve performance.	b) The industry will be denieduse of such prectices (layer cages, antibiotic growth promoters (AGP), thinning in broilers).
The industry is striving to reduce CP levels/usage.	a) Broilers tend to be more profitable when fed high-protein diets. b) Low-protein diets lead to reduced fat and high dietary starch.
Reduced CP diets are advantageous in terms of performance, cost and environmental impact.	Low CP diets lead to: a) increased carcass fat and decreased breast muscle yield b) an increase in dietary starch levels.
Current grain and fat prices imply that a reduction in dietary energy levels may be cost-effective.	The low-density ingredients required to do so are in limited supply.
Alternative ingredients are espoused as a means of improving sustainability.	a) There are limited alternatives in the volumes that are required. b) There are risks associated with using unfamiliar ingredients.
Precision nutrition is a goal the industry strives to achieve.	Protein, energy and mineral availability is still measured in less than perfect ways.

Focusing on a single aspect, such as bird welfare, may not be sufficient to ensure sustainable poultry production. If there is an honest desire to become more sustainable, all role players in the poultry supply chain, and our consumers, need to be involved. Feed ingredients will need to be produced efficiently, as close to production sites as possible. Producers must continue to improve feed efficiencies and lifetime performance. More poultry products will need to be produced locally by small-scale farmers. Since organic or alternative production systems use more land (which may not exist) and have larger carbon footprints than conventional systems, consumers will have to make informed decisions about which products they purchase. The paradigm needs to shift from 'natural' to 'sustainable' products. As an industry, poultry producers need to market sustainable product ranges. The industry must ensure that consumers understand why this is being done, in order or them to make the correct choices themselves.

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INFLUENCE OF DIETARY CP, DIETARY SID LYS AND AMINO ACID PRODUCTION ORIGIN ON PERFORMANCE AND GLOBAL WARMING POTENTIAL OF BROILERS

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Summary

The objective of the present experiment was to investigate the influence of dietary crude protein (CP), standardised ileal digestible (SID) Lys level with two scenarios of AA production countries and their interactions in 0-35d male Ross 308 broilers. There was no interaction between SID Lys and dietary CP on any performance parameters. The decrease of dietary CP did not affect growth performance but decreased global warming potential (GWP). The increase in SID Lys improved growth performance but also increased GWP. There was an interaction between dietary CP and AA origin for GWP: GWP was reduced when reducing dietary CP by 2.9% when using AA produced in China and by 12.0% when using AA produced in EU. This study indicates that dietary CP can be drastically reduced in different nutritional contexts without negatively affecting growth performance, and with positive benefits on the environment.

I. INTRODUCTION

The nutritional strategy of reducing dietary crude protein (CP) in broiler diets to improve the sustainability of poultry production has been widely reviewed in the recent years (Hilliar and Swick, 2019; Selle et al., 2020). This strategy has proven benefits on global warming potential (GWP; Kebreab et al., 2016; Cappelaere et al., 2021) and litter quality (van Harn et al., 2018). In terms of growth performance, there is still a controversy on the ability of reduced-protein diets to maintain similar performance levels (Belloir et al., 2017; Chrystal et al., 2020; Maynard et al., 2021). To our knowledge, there has been no study so far on the influence of reducing dietary CP in broilers on GWP with two levels of dietary SID Lys and different origins of AA production. The objective of the present experiment was therefore to investigate the influence of dietary CP, AA density (SID Lys) and their interaction on global warming potential with two scenarios of AA origin.

II. METHOD

The six experimental treatments consisted in a 3 x 2 factorial design with 3 levels of dietary CP (High, Medium = -1%pt CP, Low = -2%pts CP) x 2 levels of dietary SID Lys (High, Low) with 0-35d male Ross 308 broilers (the grower diet composition is given as an example in Table 1). The reduction of dietary CP was applied by partially replacing SBM by cereals and feed-grade AA. The low SID Lys levels were formulated to match 90% of the Aviagen recommendations. Average daily gain, average daily feed intake and feed conversion ratio were monitored. Global warming potential was calculated kg of live weight broiler (GWPkg, kgCO2eq/kg of live weight (LW) broiler). GWP of the feeds was based on GFLI (Blonk consultants, 2019), except for the micro-ingredients (EcoAlim; Wilfart et al., 2016) and AA METEX NOOVISTAGO database, following FAO (2020) guidelines; available in Agribalyse (2022) V3.1). As GWP values of AA differ a lot between the countries of production, it was chosen to implement 2 scenarios: 1) AA are produced in Europe Union (EU) or 2) AA are

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produced in China (CN). The main difference between the two countries of production come from the raw material mix to produce feed-grade L-AA: EU = sugar beet, CN = glucose from corn. All data were submitted to a 2-way ANOVA statistical analysis. In addition, a 3-way ANOVA was performed for GWP with dietary SID Lys, dietary CP and AA sourcing strategy as main effects and their interactions. Significance was considered at $P < 0.05$. Statistical analysis was conducted using Minitab, version 21.

Table 1 - Ingredient and nutrient composition of the six experimental grower diets fed from 10 to 21d (grower feed) to broiler chickens.

SID Lys	Low			High		
Crude protein	High	Med	Low	High	Med	Low
Ingredient composition, %						
Corn	43.60	46.80	50.10	37.20	41.10	44.70
Soybean meal, 48%	27.20	24.00	20.70	32.90	29.20	25.60
Wheat	20.00	20.00	20.00	20.00	20.00	20.00
Soya oil	4.50	4.40	3.90	5.90	5.20	4.60
Monocalcium phosphate	1.30	1.30	1.30	1.30	1.30	1.30
Calcium bicarbonate	1.10	1.10	1.10	1.10	1.10	1.10
Sodium bicarbonate	0.17	0.35	0.52	0.13	0.13	0.33
Salt	0.30	0.30	0.30	0.30	0.30	0.30
DL-Met 99%	0.25	0.28	0.31	0.29	0.32	0.35
L-Lys HCl 99%	0.25	0.35	0.45	0.22	0.33	0.44
L-Thr 98.5%	0.19	0.23	0.27	0.20	0.25	0.30
L-Val 98%	0.07	0.13	0.18	0.07	0.13	0.19
L-Arg 98%	0.03	0.13	0.22	---	0.10	0.20
L-Trp 98%	---	---	0.01	---	---	0.01
L-Ile 93.4%	0.01	0.06	0.12	---	0.05	0.12
Premix	0.50	0.50	0.50	0.50	0.50	0.50
Formulated nutrient composition, %						
Crude protein	18.90	18.00	17.10	21.00	20.00	19.00
SID Lys	1.04	1.04	1.04	1.15	1.15	1.15
SID Lys/CP	5.50	5.78	6.08	5.48	5.75	6.05
AMEn broiler (kcal/kg)	3,100	3,100	3,100	3,100	3,100	3,100

III. RESULTS

Broilers outperformed the Aviagen (2018) performance objectives for Ross 308 male broilers by 385g of BW and by -11pts for FCR. No interaction between SID Lys and dietary CP was observed for any of the performance parameters ($P > 0.05$). Increasing dietary SID Lys significantly increased BW, ADG and reduced FCR from 0 to 14d, 14 to 21d, 21 to 35d and 0 to 35d ($P < 0.05$; Table 2). On the other hand, reducing dietary CP did not impact performance ($P > 0.05$) except for FCR before 21d. The reduction of dietary CP reduced GWPkg with both AA origin but the extent of reduction was much greater with EU AA as compared to CN AA (Figure 1). For instance, reducing dietary CP from High to Low dietary CP reduced GWPkg by 2.9% when using CN AA, and reduced GWPkg by 12% when using EU AA. In addition, switching the AA origin from CN to EU production reduced GWPkg by 2.4% in the High dietary CP treatments and reduced it by 10% in the Low dietary CP treatments.

Table 2 - Growth performance of broilers fed experimental diets differing in dietary CP and dietary SID lysine (10 replicates of 17 broilers per pen).

Treatments[1]		Body weight g/bird			Feed conversion ratio g/g		
SID Lys	CP	Day 14	Day 21	Day 35	Day 14-21	Day 21-35	Day 0-35
Low	High	563	1,141	2,530	1.294	1.591	1.385
Low	Med	557	1,128	2,520	1.299	1.592	1.391
Low	Low	570	1,142	2,520	1.330	1.599	1.397
High	High	570	1,195	2,705	1.190	1.506	1.313
High	Med	579	1,203	2,763	1.204	1.494	1.316
High	Low	576	1,193	2,687	1.221	1.520	1.332
	SEM	2.7	6.7	17.4	0.007	0.007	0.005
Main effects							
SID Lys							
	Low	564[b]	1,137[b]	2,523[b]	1.308[a]	1.594[a]	1.391[a]
	High	575[a]	1,197[a]	2,718[a]	1.205[b]	1.507[b]	1.320[b]
CP							
	High	567	1,168	2,617	1.242[b]	1.549	1.349
	Med	568	1,166	2,641	1.252[ab]	1.543	1.354
	Low	573	1,168	2,604	1.276[a]	1.560	1.364
P-value							
	SID Lys x CP	0.370	0.651	0.383	0.598	0.661	0.734
	SID Lys	0.035	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	CP	0.615	0.987	0.433	< 0.001	0.329	0.056

[a-c] Values in a column with no common superscripts differ significantly (P < 0.05) – Tukey test.

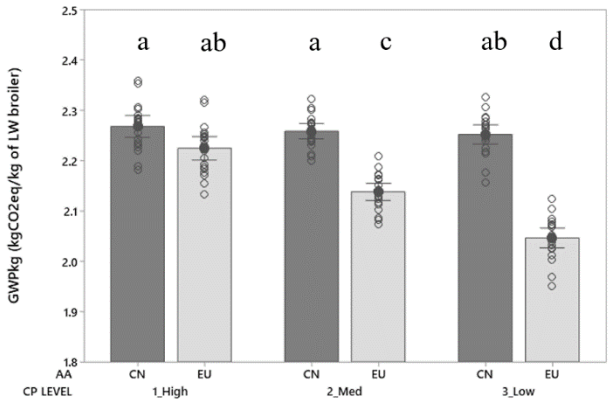

Figure 1 - Global warming potential of broilers in g CO2eq per kg of live weight broiler fed diets differing in dietary CP and AA sourcing strategy from 0 to 35d of age.

IV. DISCUSSION

The novelty of the study was to test dietary CP and SID Lys nutritional strategies in a factorial design. In this study, intermediate levels of SID Lys/CP were tested compared to recent publications (Van Harn et al., 2018, Chrystal et al., 2020; Maynard et al., 2021). There was no interaction between the two strategies, meaning that the reduction of dietary CP is achievable whatever if broilers are fed a low or a high level of AA density. Therefore, based on this experiment, the achievable level of dietary CP in practice could be as low as 17% in grower and 15.3% in finisher diets when formulating with dietary SID Lys at 90% of Aviagen (2018) recommendations. The effect of reducing dietary CP on GWP of broiler has already been evaluated by Mosnier et al. (2011), Kebreab et al. (2016) and reviewed by Cappelaere et al.

(2021). For example, Mosnier et al. (2011) evaluated that the reduction of dietary CP reduced GWP by 5.3% compared to control, but this result was dependent on regions of production of SBM and corn. The novelty of the present study is that 1) the latest published GWP databases (Wilfart et al., 2016; GFLI, 2019) were used to study the impacts of the nutritional interventions, 2) 2 scenarios of AA sourcing strategy were compared, and 3) basic nutritional strategies such as increased SID Lys or reduced dietary CP were tested. However, regarding the methodology of the study, it was not possible to integrate the farm characteristics and the manure management strategy in this study like in Mosnier et al. (2011).

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AMINO ACID IMBALANCES

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Summary

This paper considers amino acid imbalances in the context of reduced-crude protein diets, especially wheat-based diets. The likely genesis is differences in intestinal uptake rates of non-bound versus protein-bound amino acids results in their asynchronous parenteral appearances. Amino acid imbalances are more likely to occur in wheat-based diets because wheat typically has higher protein contents than other feed grains, which demands higher inclusions of non-bound amino acids.

I. INTRODUCTION

The term 'amino acid imbalances' was probably originated by Elvehjem and Krehl (1955) and the topic was addressed by Harper and Rogers (1965). Their conclusion was that imbalances retard growth by altering the normal pathways of amino acid metabolism. Thus, while the relevance of amino acid imbalances to efficient chicken-meat production is recognised, a precise definition has yet to be developed (Kurpad, 2018). Antagonisms between arginine and lysine (Austic and Scott, 1975) and the branched-chain amino acids, isoleucine, leucine and valine (Calvert et al., 1982) have been reported to depress feed intake in poultry and could be seen as amino acid imbalances. However, this paper considers amino acid imbalances generated by inclusions of non-bound (synthetic, crystalline) amino acids (NBAA) as opposed to protein-bound amino acids in diets for broiler chickens. These imbalances are increasingly declared in birds offered reduced-crude protein (CP) diets because of high NBAA inclusions to meet specifications. The lack of bioequivalence between non-bound versus protein-bound amino acids is fundamental to the genesis of amino acid imbalances in this context (Selle et al., 2022a).

Initially, synthetic *d,l*-methionine was made available for animals in 1953; however, intestinal uptakes of synthetic or non-bound methionine were subsequently shown to be more rapid than protein-bound methionine by Canolty and Nasset (1975). Cumulative plasma methionine concentrations in rats offered synthetic methionine at 15, 30, 60 and 120 minutes post-administration were 2.75 time higher (858 versus 312 μmol/L) than in rats receiving methionine only from intact protein. Moreover, that non-bound lysine and methionine are absorbed more rapidly than their protein-bound counterparts in broiler chickens was reported by Liu et al. (2013). It may be deduced from this study that the average digestion rate constants of non-bound lysine and methionine were approximately 3.7 times higher (8.64 versus 2.35 $10^{-2}min^{-1}$) than protein-bound amino acids.

II. AMINO ACID IMBALANCES IN BROILERS OFFERED REDUCED-CP DIETS

Instructively, Baker (2009) suggested that there are limits to the extent that intact protein can be replaced by NBAA in terms of achieving maximal weight gain and feed efficiency. This was illustrated by Macelline et al. (2022) in an equilateral triangle response surface design with diets formulated to 203 g/kg true protein but the three apical diets contained 6.75, 19.4 and 66.9 g/kg NBAA. The diet containing 13.1 g/kg NBAA supported maximum weight gain and minimum FCR observed and higher NBAA inclusions penalised growth performance.

Broiler chickens are intermittent, rather than continuous feeders (Aydin and Berckmans, 2016) and the likelihood is that this contributes to post-enteral imbalances between non-bound

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and protein-bound amino acids at sites of protein synthesis, stemming from differences in amino acid intestinal uptake rates. Amino acids may be captured by catabolic pathways as they transit enterocytes of the small intestinal mucosa and are denied entry into the portal circulation (Wu, 2008). However, the possibility is that non-bound amino acids are less likely to be catabolised given their proximal sites of absorption where starch/glucose is readily available as an alternative energy substrate (Fleming et al., 1997), which is supported by data in Moss et al. (2018). If so, this would exacerbate differences in amino acid intestinal uptake rates. Nevertheless, dietary amino acids that exceed requirements for protein synthesis are rapidly catabolized (Brosnan, 2003), which had been defined as post-prandial amino acid oxidation (Schreurs et al., 1997). Subsequently, Nolles et al. (2009) compared postprandial oxidation of egg white protein as the sole amino acid source with a corresponding blend of non-bound amino acids via [$^{13}CO_2$] breath tests in rats. Postprandial oxidative losses of non-bound leucine were significantly higher than protein-bound leucine by approximate factors of 1.52 (24.8 versus 16.3%) after a short adaptation period. It appears that NBAA are more likely to be lost to post-prandial oxidation because of their more rapid intestinal uptakes.

The moderation of amino acid catabolism would decrease amino acid requirements (Klasing, 2009); potentially, this holds importance and would be partially achieved if amino acid imbalances were to be diminished. Moreover, amino acid catabolism attracts metabolic costs in terms of both protein and energy. The catabolism of amino acids axiomatically generates a protein cost; however, the resultant synthesis and excretion of uric acid to void N in urine generates a minimal energy cost of 64.7 kJ/g N excreted as uric acid (Van Milgen, 2021). Uric acid concentrations in broiler excreta were determined in Selle et al. (2021) and the proportion of dietary energy intakes partitioned to uric acid synthesis and excretion was up to 2.26% of gross energy (17.21 MJ/kg GE) or 2.98% of metabolisable energy (13.06 MJ/kg AME) over the total excreta collection period to determine AME. Excreta uric acid concentrations were also determined in Brink et al. (2022) in a study involving wheat-based, grower and finisher diets with three CP levels. Mean excreta uric acid concentration was 66.4 mg/g (range: 47.9 to 80.5 mg/g), which represented 47.1% (range: 40.0 to 53.1%) of total N excreted. Thus, 47.1% of total N in excreta was derived from uric acid in urine and the balance of 52.9% was derived from undigested and microbial N in faeces. Interestingly, Brink et al. (2022) suggested that N derived from uric acid in litter is more readily volatilised into atmospheric NH_3 than other N forms in excreta.

There are indications that NH_3 is more toxic in poultry than mammalian species (Wilson et al., 1968). Under normal conditions, broiler chickens detoxify NH_3 via a reaction catalysed by glutamine synthetase in which NH_3 and glutamic acid are condensed into glutamine. Glutamine then enters the Krebs uric acid cycle which generates uric acid, which is voided in urine (Stern and Mozdziak, 2019). However, if NH_3 detoxification is inadequate, plasma NH_3 concentrations will be elevated and this has been associated with depressed growth performance in three broiler studies (Namroud et al., 2008; Ospina-Rojas et al., 2013, 2014). Adequate NH_3 detoxification could be challenged by excessive amino acid catabolism triggered by high dietary NBAA inclusions. Also, as glycine is a prerequisite for the Krebs uric acid cycle (Salway, 2018), it follows that any deficiency of glycine (and serine) would result in inadequate NH_3 detoxification. Selle et al. (2021) estimated that between 25.0% and 80.9% of dietary glycine entered the Krebs cycle for uric acid synthesis in the Chrystal et al. (2021) study, which does not account for endogenous glycine synthesis.

The concept of inadequate NH_3 detoxification or ‘ammonia overload’ is supported by the outcomes reported in Greenhalgh et al. (2022). The inclusion of 75 mg/kg L-carnitine in 160 g/kg CP, sorghum-based diets, containing 51.02 g/kg NBAA, improved weight gain by 15.0% (1580 versus 1374 g/bird) and FCR by 8.82% (1.521 versus 1.615) from 7 to 33 days post-hatch. However, it is recognised that L-carnitine is protective against NH_3 toxicity

(Kloiber et al., 1988). This raises the distinct possibility that the L-carnitine responses observed stemmed from its capacity to counteract the negative effects of ammonia overload. This is because L-carnitine inclusions in 220 and 190 g/kg CP diets, containing 15.19 and 29.26 g/kg NBAA, respectively, failed to generate growth performance responses. Also increasing NBAA inclusions were found to linearly ($r = 0.546$, $P = 0.019$) related to plasma NH_3 concentrations in an unpublished study. Both outcomes support the contention that high NBAA inclusions in reduced-CP diets could trigger 'ammonia overload'.

III. WHEAT-BASED, REDUCED-CP DIETS

Wheat is the dominant feed grain in Australian chicken-meat production. However, the capacity of broilers to accommodate CP reductions in wheat-based diets is highly variable as evidenced by several local studies. For example, 30 g/kg CP reductions in grower and finisher diets numerically depressed FCR by 2.19% (1.542 versus 1.509) from 10 to 35 days post-hatch in Hilliar et al. (2020). Alternatively, similar CP reductions significantly compromised FCR by 7.24% (1.452 versus 1.354) in broilers in Hilliar et al. (2019) and by 9.38% (1.609 versus 1.471) from 7 to 35 days post-hatch in Dao et al. (2021). In Yin et al. (2020), CP reductions from 215 to 190 g/kg CP depressed FCR by 1.42% (1.497 versus 1.476) and from 215 to 165 g/kg by 4.74% (1.497 versus 1.476) from 14 to 35 days post-hatch. In contrast, CP reductions from 197.5 to 180 g/kg CP compromised FCR by 11.5% (1.878 versus 1.684) and from 197.5 to 162.5 g/kg CP by 44.1% (2.426 versus 1.684) from 14 to 35 days post-hatch in Greenhalgh et al. (2020). Also, the CP reduction from 222 to 165 g/kg CP compromised FCR by 26.6% (1.840 versus 1.453) in Chrystal et al. (2021).

Maize was superior to wheat as the basis of reduced-CP diets in Chrystal et al. (2021). Moreover, there is a quadratic relationship ($r = 0.962$; $P = 0.0004$) between NBAA inclusions, which ranged from 7.23 to 49.39 g/kg, with mean FCR observed in birds offered nine dietary treatments. It may be deduced from the quadratic equation that the minimum FCR of 1.403 from 7 to 35 days post-hatch was realised with NBAA inclusions of 17.49 g/kg and FCR deteriorated in a quadratic manner when this inclusion level was exceeded. In a European study (Brink et al., 2022), 30.0 g/kg CP reductions in wheat-based grower and finisher diets numerically depressed FCR by 2.03% (1.50 versus 1.48) from 1 to 39 days post-hatch when fed as pellets. This promising outcome was probably facilitated by the relatively low average NBAA inclusions of 16.6 g/kg in the reduced-CP grower and finished diets. Curiously, when fed as mash, a significant improvement in FCR of 5.33% (1.60 versus 1.69) was observed. Presumably, mash diets had higher protein solubility and digestibility and it is probable that birds offered mash diets consumed feed more frequently (Fujita, 1974). Both factors may have reduced the magnitude of post-enteral amino acid imbalances to the benefit of FCR in birds offered reduced-CP, mash diets. Finally, the likelihood is that wheat-based, reduced-CP diets will be advantaged by limited NBAA inclusions, which can be facilitated by incorporating feedstuffs with lower protein contents than soybean meal into their formulations. This strategy should constrain the deleterious impacts of amino acid imbalances in birds offered reduced-CP, wheat-based diets, but the likelihood that there are additional inherent factors in wheat that will need to be addressed including soluble NSP, rapid starch digestion rates and possibly amylase-trypsin inhibitors and gluten (Selle 2022b).

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PHYSIOLOGICAL BASIS OF EARLY LIFE NUTRITION IN CHICKENS

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ABSTRACT

The transitions from late embryonic development through hatching and on through the first two weeks of post-hatch growth represent a period of tremendous changes in the gastrointestinal tract and nutrient metabolism in chickens. During late embryonic development, the principle metabolic pathways in the liver center around lipolytic and gluconeogenic metabolism. Immediately after hatching, hepatic metabolism shifts to glycolytic and lipogenic metabolism. This metabolic transition is evidenced by massive changes in expression of genes related to lipolysis, lipogenesis, glycolysis, and ketogenesis. Similar changes occur in other tissues, including adipose tissue and skeletal muscle. Concurrent with these dramatic changes in nutrient metabolism, the gastrointestinal tract undergoes substantial changes, including growth and functional maturation. One such example is growth of the small intestine, which increases three-fold in length during the final days of embryonic development and another seven- to ten-fold during the first two weeks after hatch. Similarly, villus height and number increase from late embryonic development through the first one to two weeks after hatch. These changes in the anatomy, physiology, and metabolism during early life development of the chick alter nutrient absorption and utilization. Similarly, feed and its formulation alter physiological systems related to feed intake, body growth, and nutrient metabolism. The transition from lipolytic to lipogenic metabolism at hatching is delayed when initial access to feed is delayed. Similarly, ontogenic profiles of pathways in skeletal muscle related to protein accretion and fiber growth are delayed in response to delayed feeding. Within the hypothalamus of the brain, pathways regulating appetite and satiety and whole-body metabolic rate are affected by delayed feeding. Most but not all of the gene expression patterns in these pathways revert to their normal level after the birds are provided feed. In other words, access to feed induces the remarkable changes in physiology and metabolism that occur at hatching. However, despite nearly two decades of research in this area, the specific components within feed responsible for functional changes in tissues as disparate as the liver, skeletal muscle, adipose tissue, and the hypothalamus remain unknown. A collaborative effort by nutritionists and physiologists at the molecular and genomics levels is necessary to elucidate the underlying mechanisms regulating the functional changes that occur in multiple organ systems from late embryonic development through the first two weeks of post-hatch growth. These functional changes in multiple organs affect early life nutrient absorption, metabolism, and utilization.

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A PROJECT DESIGNED TO PROMOTE ENVIRONMENTALLY VIABLE CHICKEN-MEAT PRODUCTION VIA ENHANCED NUTRITION, GUT INTEGRITY AND HOUSING

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Summary

This paper outlines the Project designed to promote environmentally viable chicken-meat production via enhanced nutrition, gut integrity and housing, and the Project is funded by AgriFutures Australia. Five Australasian institutions have formed a partnership to complete the necessary research and the objectives are briefly discussed.

I. BACKGROUND

The scale of the Australian chicken-meat industry has increased at an extraordinarily rapid rate so that now chicken-meat is clearly the first preference of consumers in comparison to pork, beef and lamb. In 1990/91, the Australian population was 17.2 million with a per capita chicken-meat consumption of 23.9 kg; however, this increased by 61.0% to 27.7 million people and consumption increased by 100% to 47.8 kg over the 30 years to 2020/21. As a direct consequence, the number of birds processed expanded by 138% (675.3 versus 283.7 million) and the volume of chicken-meat produced increased by 231% (1.285 versus 0.388 million tonnes) over the three decades (Australian Chicken Meat Federation, 2022). From a Treasury forecast, the Australian population is projected to increase to 35.9 million in 2050 and chicken-meat consumption may reach 52.5 kg. If these assumptions are valid, chicken meat production will need to increase, from an already high base, by a further 29.6% from 1.285 to 1.665 million tonnes over the next 30 years and if slaughter weights remain constant the number of birds processed will increase from 675 to 875 million. Global poultry production has been projected to increase by 72% from 105.6 million tonnes in 2020 to 181.3 million tonnes of chicken-meat by 2050 (Alexandratos and Bruinsma, 2012). This represents an average annual growth rate of 1.82%, but there are already indications that this is conservative. These forecasts emphasise the need for environmentally viable chicken-meat production to meet this demand in a responsible manner both in Australia and overseas.

Presently, broiler chickens have the best conversion rate of animal feed to meat for human consumption coupled with the smallest environmental footprint in terms of energy and water used for edible meat output across terrestrial animals (Vaarst et al., 2015). The estimated efficiency of protein deposition of 33.3% in broiler chickens clearly exceeds the estimates of 23.3% in pigs and 12.1% in feedlot cattle (Wu et al., 2014). Similarly, it was predicted that of greenhouse gas emissions (as CO_2 equivalents) generated from meat production in 2020 that 63.1% would arise from beef, 29.8% from pork, but only 7.1% from chicken-meat production. Australia imported 1.180 million tonnes of soybean meal in 2020 and the majority of these importations, perhaps 750,000 tonnes with an approximate landed cost of A$800 per tonne, would have been absorbed by the local chicken-meat industry. This reliance on imported soybean meal is incompatible with sustainable local production, which is only compounded by neotropical deforestation in South America to harvest soybeans (Gasparri et al., 2013). Therefore, a core objective of the project is to reduce Australia's dependence on imported soybean meal via an integrated, multi-faceted approach.

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The fundamental objective is to enhance feed efficiency, as any strategies that will improve feed conversion ratios (FCR) will axiomatically advantage sustainable chicken-meat production (Cowieson and Selle, 2011). Therefore, five Australasian research institutions have combined to form a partnership, in association with seven industry partners, to develop an integrated program to promote sustainable chicken-meat production. The genesis of this partnership was the successful bid to AgriFutures Australia for financial support of the extensive project envisaged and an indication of the scope and the participants involved in this project is provided in Table 1.

Table 1 - Research institutions, program descriptions and lead investigators involved in the AgriFutures Australia chicken meat project.

Program	Research institution	Description	Lead investigator
One	University of Queensland	Maternal programming and intergenerational amino acid nutrition	Professor Eugeni Roura
Two	University of Queensland	Carbohydrate and protein precision feeding of broiler chickens	Professor Mike Gidley
Three	The University of Sydney	Digestive dynamics and reduced-crude protein diets	Dr Sonia Yun Liu
	Massey University	Alternative protein sources	Dr Reza Abdollahi
Four	Central Queensland University	Early establishment of a healthy gut microbiota ecosystem	Dr Dana Stanley
Five	Department of Agriculture and Fisheries (Qld)	Enhanced environment in grow-out accommodation to improve litter quality and benefit bird welfare and performance	Dr Mark Dunlop
Six	The University of Sydney	Integration and extension to facilitate industry adoption	Professor Ruth Zadoks

II. PROJECT OUTLINE

Nutrition-based Program One will focus in developing transgenerational strategies. The overarching aim is to improve protein utilisation in meat chickens through maternal programming and *in ovo* paired with early-feeding programs. Nutritional constraints imposed on broiler breeders, such as a scarcity of dietary protein, trigger physiological adaptations that improve the growth rate and feed conversion under low dietary protein regimes in the progeny (Moraes et al., 2014; Lesuisse et al., 2018). Reducing dietary protein decreases nitrogen excretion in broiler breeders as well as the footprint of the broiler progeny (Meda et al., 2011). Broiler progeny hatched from feed-restricted breeders, as commonly practiced, has been shown to experience reduced growth and increased abdominal fat in comparison to more generous feeding regimes (van der Waaij et al. 2011). In addition, Program One will capitalise on the potential of *in ovo* interventions to influence embryonic development (Uni and Ferket, 2004; Kadam et al., 2013; Siwek et al., 2018). The *in ovo* nutrition practices will involve specific amino acids and other essential nutrients to target early gut and immune system developments during late stages of embryogenesis. The stimulation of embryonic development will liaise with early-feeding strategies in hatchlings. The anticipated result is a long-lasting impact on an improved feed efficiency and protein utilization during the life of the chicken.

Improving the utilisation of dietary carbohydrates, especially starch, and protein via precision feeding is another cornerstone to be addressed in Program Two. The digestion rate of wheat starch is more rapid than that of maize and sorghum (Giuberti et al., 2012); however, some slowly digestible starch has been shown to advantage broiler performance (Herwig et al., 2019). Moreover, dietary starch:protein ratios hold relevance for the growth performance of broiler chickens as demonstrated by Sydenham et al. (2017). Interactions between these two macro-nutrients are pivotal as maize-based diets with high starch levels have been shown to

compromise apparent amino acid digestibility coefficients, particularly in the proximal and distal ileum of broiler chickens (Moss et al., 2018).

Decreasing our dependence on imported soybean meal via the development and adoption of reduced-crude protein (CP) diets and/or the identification of alternative protein-rich feedstuffs is the objective of Program Three. Reduced-CP diets have the potential to halve soybean meal importations via dietary inclusions of non-bound (synthetic, crystalline, feed-grade) amino acids to meet amino acid requirements at the expense of soy protein (Selle et al., 2020). This is a real challenge, especially for wheat-based diets (Selle et al., 2022), which demands further investigations into the digestive dynamics of starch/glucose in alignment with protein/amino acids (Liu and Selle, 2017). Obviously, alternatives to soybean meal are not confined to non-bound amino acids and extend to Canola meal (Ajao et al., 2022). This is of extreme relevance to Australia and there is scope to develop improved Canola meal production processes and the possibility of producing Canola protein isolates and/or concentrates. Additional alternatives consist of a range of legumes including field peas (Wang and Daun, 2004) and novel protein sources including insect protein (Khan SH, 2018) and krill meal (Ryś and Koreleski, 1979).

The relationships between the gut microbiota, dietary nutritional inputs and the host will be explored in Program Four as the gut microbiota can influence both broiler growth performance and flock health (Stanley et al., 2014). As one example, Ndotono et al. (2022) reported that inclusions of black soldier fly larvae as a protein source reshaped the gut microbiota to the advantage of gut health, immune response and food safety. Also, elucidating the mechanisms whereby probiotics positively impact the gut microbiota to the benefit of the avian host (Stanley et al., 2016) will constitute part of Program 4.

The issues of litter quality specifically (Dunlop et al., 2016) and, more generally, on better housing or accommodation for broiler chickens during the grow-out phases will be the focus of Program Five. Bird welfare is becoming an increasingly important issue (Bessei, 2006; Tahamtani et al., 2020) and, as discussed by Garland (2018), the incidence of foot-pad lesions is a sensitive barometer in this respect under practical conditions. There is accumulating evidence that reducing dietary CP results in enhanced litter quality and, consequently, in reduced incidences of foot-pad lesions (Van Harn et al., 2019). In fact, this is just one example of how the different programs overlap in the overall project.

Finally, and arguably most importantly, the role for Program Six is to integrate the outcomes generated by the project and communicate them to the chicken-meat industry so that their adoption will be facilitated. In addition, there will be a strong focus on mentoring students with the provision of scholarships, exchange programs between research institutions and support for industry placements. Annual stakeholder meetings will be held to showcase the progressive outcomes from each of the six programs. It is confidently anticipated that the integrated and coordinated inputs from the participating research institutions to this AgriFutures Australia project will be of real benefit to local chicken-meat production.

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β -MANNANASE GENERATED PROMISING OUTCOMES IN BROILER CHICKENS OFFERED STANDARD AND LOW ENERGY DENSITY DIETS

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Summary

This study reports the outcomes in growth performance and carcass traits in broiler chickens offered either standard or low energy density diets with four β-mannanase inclusions (0, 100, 200, 300 mg/kg) from 1 to 35 days post-hatch as a 4 × 2 factorial array of dietary treatments. A treatment interaction (P = 0.030) was observed for weight gain as 100 and 200 mg/kg β-mannanase in low energy diets improved weight gain relative to the standard energy, control diet from 1 to 35 days post-hatch. As a main effect, dietary energy reduction increased feed intake (P = 0.037) but did not impact weight gain and FCR. β-mannanase (300 mg/kg) in the low energy diet supported best breast (*Pectoralis major*) yields (P = 0.012) and lowest relative abdominal fat-pad weights (P < 0.001).

I. INTRODUCTION

Mannan is a plant based-NSP derived from polymerisation of mannose sugars, it is categorised as galactomannan or glucomannan based on the presence of galactose and glucose side chains (Aspinall, 1973). Generally, galactomannan is the dominant form in legumes (Sundu et al., 2012). The ratio between mannose to galactose dictates the water solubility of galactomannan where galactose has a strong capacity to bind water and increase gut viscosity. The β-mannan in soybean meal is considered insoluble as it has galactose to mannose ratio of 1:1.8 (Whistler and Smart, 1953; Whistler and Saarnio, 1957); ß-mannan concentrations in soybean meal ranged from 10.2 to 21.2 g/kg (Hsiao et al., 2006). Therefore, ß-mannan is one of the main antinutritive factors in conventional corn-soybean meal broiler diets as it increases gut viscosity, encapsulates dietary nutrients, suppresses energy utilisation, facilitates pathogenic bacterial growth and provokes innate immune responses (Shastak et al., 2015). The feed enzyme, ß-mannanase, degrades ß-mannan and counteracts the negative impacts of ß-mannans in broiler chickens. Therefore, the present study was designed to test the hypothesis that ß-mannanase will improve growth performance and carcass traits in broiler chickens with energy-spearing effects.

II. MATERIAL AND METHODS

This feeding study was conducted in compliance with the guidelines of the Animal Ethics Committee of The University of Sydney. A total of 720 off-sex, male Ross 308 broilers were randomly distributed into 48 floor pens with 15 birds per pen and 6 replicates for each treatment from 1 to 35 days post-hatch. The trial design was a 4 x 2 factorial array of dietary treatments with four inclusions (0, 100, 200, 300 mg/kg) of β-mannanase (VTR Bio-Tech Co., Ltd. Guangdong 519060, China), coupled with standard or low energy densities. Energy densities were reduced by 0.18 MJ in the starter phase and by 0.21 MJ in the grower and finisher phases. The composition and nutrient specifications of the dietary treatments are shown in Table 1. Exogenous phytase (1000 FTU/kg) was included across all dietary treatments. Growth performance from 1 to 35 days post-hatch was monitored and relative abdominal fat-pad weights and carcass traits were determined at 35 days post-hatch. The starter diets were cold-pelleted at approximately 65ºC, whereas the grower and finisher diets were steam-pelleted at a conditioning temperature of 80ºC. Analyses of variance of the experimental data was completed with the JMP Pro 16 program, a 5% level of probability was considered statistically significant and pair-wise comparisons were completed when relevant.

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Table 1 - Composition and specifications of standard and low energy density diets in starter, grower and finisher phases.

Item (g/kg)	Starter (1 to 8 days post-hatch)		Grower (9 to 18 days post-hatch)		Finisher (18 to 35 days post-hatch)	
	Standard	Low	Standard	Low	Standard	Low
Maize	622	630	632	643	650	662
Soybean meal	310	308	308	307	282	280
d,*l*-methionine	4.44	4.42	3.45	3.43	3.28	3.27
Glycine	2.07	2.05	0.30	0.29	-	-
l-arginine	2.70	2.71	1.05	1.06	0.84	0.86
l-histidine	0.47	0.46	-	-	-	-
l-isoleucine	1.35	1.34	0.39	0.38	0.33	0.33
l-lysine HCl	4.86	4.88	2.98	3.00	2.66	2.69
l-threonine	2.53	2.53	1.48	1.47	1.24	1.24
l-tryptophan	-	0.01	-	-	-	-
l-valine	1.64	1.63	0.56	0.54	0.40	0.40
Soy oil	17.4	10.0	22.9	13.6	34.9	25.5
Limestone	14.5	14.5	12.9	12.9	12.0	12.0
Monocalcium phosphate	9.22	9.21	7.17	7.16	6.00	5.99
Sodium chloride	3.87	3.87	3.10	3.10	2.27	2.25
Sodium bicarbonate	-	-	-	-	1.23	1.23
Vitamin-mineral premix	2.50	2.50	2.00	2.00	2.00	2.00
Choline chloride	1.00	1.00	0.80	0.80	0.60	0.60
VTR phytase	0.10	0.10	0.10	0.10	0.10	0.10
Inert filler (sand)[1]	0.30	0.30	0.30	0.30	0.30	0.30
Nutrient specifications						
AME (Kcal/kg)	3050	3010	3098	3048	3198	3148
GE (Kcal/kg) analysed	4534	4468	4476	4422	4528	4497
Crude protein	216	216	206	206	193	194
Calcium	9.60	9.60	8.70	8.70	8.10	8.10
Total phosphorus	5.15	5.16	4.70	4.71	4.32	4.33

III. RESULTS

The effects of dietary treatments on broiler feed intake, FCR and growth performance are shown in Table 2. As a main effect, 200 and 300 mg/kg β-mannanase improved FCR (P = 0.015) in starter phase and three levels of β-mannanase improved FCR (P = 0.008) in grower phase relative to β-mannanase free diet. Based on pair-wise comparisons, 200 mg/kg β-mannanase improved FCR in standard energy diets by 2.01% (0.977 versus 0.997; P = 0.010) and by 2.61% (0.970 versus 0.996; P = 0.042) in the starter phase. In the grower phase, the corresponding improvements were 2.17% (1.218 versus 1.245; P = 0.033) and 2.36% (1.202 versus 1.231; P = 0.021). There was a significant treatment interaction on body weight gain and feed intake but not for the FCR in the finisher period.

Table 3 shows the effect of dietary treatments on growth performance for 1 to 35 days post-hatch and carcass traits at 35 days post-hatch. A treatment interaction (P=0.030) was observed for weight gain where 100 or 200 mg/kg β-mannanase added to low energy density diets obtained best body weight gain across all treatments. As a main effect, dietary energy reductions increased feed intake by 3.28% (3784 versus 3664 g/bird; P = 0.027). On average, inclusions of β-mannanase at 100, 200, and 300 mg/kg supported better breast (*pectoralis major*) yield by 2.85% (192 versus 187 g/kg; P = 0.012), regardless of dietary energy densities. As a main effect, low energy diets supported better *pectoralis major* yields than standard energy diets (P < 0.001). As a main effect, β-mannanase inclusions reduced relative abdominal fat-pad weights (P < 0.001) where 300 mg/kg β-mannanase reduced fat-pad weights by 24.9% (9.79 versus 13.03 g/kg). Low energy diets reduced relative abdominal fat-pad weights than standard energy diets by 8.05% (10.96 versus 11.92 g/kg; P = 0.002)) as an energy density main effect.

Table 2 - Effect of treatments on growth performance in each feeding phase.

Energy	Mannanase (mg/kg)	Starter			Grower			Finisher		
		Weight gain (g/bird)	Feed intake (g/bird)	FCR	Weight gain (g/bird)	Feed intake (g/bird)	FCR	Weight gain (g/bird)	Feed intake (g/bird)	FCR
Standard	0	191	191	0.997	546	680	1.245	1995^{ab}	2681^{b}	1.345
	100	188	186	0.992	583	712	1.221	2019^{ab}	2742^{ab}	1.358
	200	194	190	0.977	589	717	1.218	2041^{ab}	2735^{ab}	1.340
	300	198	192	0.971	615	744	1.209	2064^{a}	2813^{ab}	1.364
Low	0	192	191	0.996	572	703	1.231	2062^{ab}	2816^{ab}	1.367
	100	199	196	0.984	620	752	1.213	2071^{a}	2861^{a}	1.382
	200	207	201	0.970	644	774	1.202	2043^{ab}	2863^{a}	1.402
	300	194	189	0.977	603	731	1.213	1977^{b}	2692^{b}	1.362
SEM		4.8	4.2	0.0080	24.6	28.6	0.0087	28.3	48.9	0.0218
Main effects										
Energy density										
Standard		193	190	0.984	583	713	1.223	2033	2748	1.352
Low		198	194	0.982	610	741	1.215	2041	2817	1.381
β-Mannanase										
0		192	191	0.997^{a}	559	692	1.238^{a}	2029	2749	1.356
100		193	191	0.988^{ab}	602	732	1.217^{b}	2043	2796	1.369
200		201	195	0.973^{b}	617	746	1.210^{b}	2042	2799	1.371
300		196	191	0.974^{b}	610	738	1.211^{b}	2029	2765	1.363
Significance (P=)										
Energy density (E)		0.134	0.134	0.644	0.137	0.189	0.180	0.665	0.068	0.097
β-Mannanase (M)		0.267	0.648	0.015	0.098	0.247	0.008	0.794	0.574	0.895
E × M		0.222	0.243	0.836	0.600	0.668	0.679	0.049	0.038	0.495

Table 3 - Effect of treatments on growth performance from 1 to 35 days post-hatch and carcass traits at 35 days post-hatch.

Energy	β-Mannanase (mg/kg)	Weight gain (g/bird)	Feed intake (g/bird)	FCR	Breast major (g/kg)	Breast minor (g/kg)	Leg quarters (g/kg)	Abdominal fat-pads (g/kg)
Standard	0	2773^{c}	3605	1.300	184	34.5	209	14.30
	100	2790^{c}	3639	1.305	190	35.7	208	11.57
	200	2828^{abc}	3644	1.288	188	33.8	208	11.77
	300	2877^{abc}	3749	1.303	186	34.8	207	10.39
Low	0	2882^{abc}	3770	1.309	189	34.3	209	11.97
	100	2931^{a}	3854	1.315	196	33.8	210	9.59
	200	2894^{ab}	3838	1.326	195	34.4	203	12.06
	300	2792^{bc}	3629	1.300	201	35.5	208	9.06
SEM		38.1	69.1	0.0145	2.2	0.72	2.6	0.570
Main effects								
Energy density								
Standard		2821	3664^{b}	1.299	187^{b}	34.7	208	11.92^{a}
Low		2890	3784^{a}	1.314	195^{a}	34.5	207	10.96^{b}
β-Mannanase								
0		2827	3688	1.304	187^{a}	34.4	209	13.03^{a}
100		2854	3737	1.309	193^{b}	34.8	209	10.58^{c}
200		2861	3741	1.307	191^{b}	34.1	205	11.92^{b}
300		2843	3701	1.302	193^{b}	35.2	207	9.79^{c}
Significance (P=)								
Energy density (E)		0.040	0.027	0.201	<0.001	0.731	0.810	0.002
β-Mannanase (M)		0.750	0.737	0.948	0.012	0.489	0.451	<0.001
E × M		0.030	0.071	0.478	0.111	0.275	0.508	0.107

IV. DISCUSSION

β-mannanase significantly improved FCR in starter and grower phases, but not in the finisher phase. Interestingly, a similar pattern in responses was reported by Latham et al. (2018) where β-mannanase improved FCR from 1 to 28 days ($P < 0.001$) but not from 1 to 42 days post-hatch ($P > 0.35$). This may indicate that anti-nutritive effects of β-mannans are more potent in younger birds. In the present study, energy intakes in low- and standard-energy, control diets (0% β-mannanase) were not significantly different based on pair-wise comparison (standard: 46.79 MJ versus low: 46.04 MJ; $P = 0.655$) from 1 to 35 days post-hatch, suggesting that broilers in low energy diets achieved their energy requirement by increasing their feed intake. Interestingly, β-mannanase quadratically affected feed intake in low-energy diets ($r = 0.596$, $P = 0.030$) where 300 mg/kg β-mannanase inclusion reduced the feed intake, obtaining similar body weight gain and FCR to the control diet with high energy density for overall experimental period. This supports the concept that β-mannnanse has energy-sparing effects in broiler chickens. Nevertheless, there was no significant correlation between β-mannanase inclusion levels and feed intake in standard energy diets.

The significant and positive impact of β-mannanase on relative breast yields is an important outcome for chicken-meat producers. Similarly, Cho and Kim (2013) reported that a β-mannanase increased breast-meat yields in 28-day-old birds offered maize-soy diets. The increased yields were more evident in birds offered lower energy density diets in the present study, where a numerical increase of 6.35% (201 versus 189 g/kg) was observed with 300 mg/kg β-mannanase in low energy diets; whereas, only an increment of 1.09% (186 versus 184 g/kg) was observed in standard energy diets. Moreover, it is deduced from the data that standard energy, control diet generated 1 kg of breast muscle yield by spending 76.7 MJ of dietary ME whereas low-energy diets with 300 mg/kg β-mannanase spent 70.4 MJ dietary ME to generate 1 kg of breast muscle yield.

Curiously, β-mannanase inclusions substantially reduced abdominal fat-pad weights, regardless of dietary energy densities. Higher dietary energy intakes usually generate heavier abdominal fat-pad weights via *de novo* lipogenesis. Based on the pair-wise comparison, 300 mg/kg β-mannanase increased energy intake by 8.03% (49.74 versus 46.04 MJ; $P = 0.035$) but reduced abdominal fat-pad weights by 27.3% in standard energy diets. This may indicate a positive impact of β-mannanase on carbohydrate metabolism such that more glucose was directly oxidized, and less glucose converted into fat via *de novo* lipogenesis.

V. CONCLUSIONS

Inclusion of β-mannanase to maize-soybean based diets showed promise in the present study as it improved FCR from 1 to 18 days, tended to increase weight gain, increased breast meat yield at 35 days post-hatch, while decreasing abdominal fat-pat weight. The increased breast meat yield was more evident in birds offered low energy diets, showing that β-Mannanase could spare 40 to 50 kcal /kg AME.

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USE OF A NON-IONIC SURFACTANT TO FACILITATE CARVACROL DIFFUSION THROUGH EMBRYONIC STRUCTURES AFTER 'IN OVO' INJECTION

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Enteric diseases can severely affect the health and welfare of broiler chicks, often requiring antibiotic treatment. A key focus of livestock research has been the development of preventive measures to avoid the need for antibiotic products. Within this context, the use of dietary essential oils (EO) has been widely studied, showing consistent positive effects on gut health (Brenes & Roura, 2010). However, little is known regarding in ovo applications of EOs, and if they improve intestinal development in broiler chicks. Transdermal absorption of carvacrol, the main component of oregano EO (OEO), increased when emulsified using a non-ionic surfactant (polysorbate 80) (Laothaweerungsawat et al., 2020). Further understanding of EO diffusion patterns into egg compartments and dispersion into embryonic structures following in ovo injection is required. The main objective of the current experiment was to determine the effects of a non-ionic surfactant on the diffusion of carvacrol through, or into different embryonic structures after in ovo injection at day 17.5 of incubation (E17.5). It was hypothesised that in ovo injection of OEO emulsified with a surfactant would increase the diffusion of carvacrol to embryonic structures, such as amniotic fluid, blood, and yolk.

Three treatments were injected in two sites (air cell or amnion); 1) 0.9% saline control, 2) OEO (1.75% v/v) in 0.9% saline with (1:1) v/v polysorbate 80 (Tween®80, Sigma-Aldrich, St. Louis, USA) or 3) OEO (1.75% v/v) in 0.9% saline without (0:1) polysorbate 80. Eggs were incubated, and at E17.5 the blunt side of the egg was injected with 0.5mL of each solution with a 23G 1¼" needle. For injection in the air cell a guard was used. Amniotic fluid, blood and yolk were collected at 3, 6 and 9h after injection. Samples were analysed ($n \geq 3$ per treatment) for carvacrol concentration using gas-chromatography mass-spectrometry (GC-MS). Differences in carvacrol concentration and mortality were analysed using PROC MIXED in SAS 9.4.

Polysorbate 80 administered with OEO (1:1) through amnion or air cell resulted in a significant increase in carvacrol diffusion into amniotic fluid ($P = 0.004$), blood ($P = 0.049$), and yolk ($P < 0.0001$). In contrast, no carvacrol was detected in amniotic fluid or blood without the surfactant, and only trace amounts were detected in yolk. In addition, the OEO treatment with polysorbate 80 in air cell was associated with 100% embryonic mortality ($P < 0.0001$), indicating a potential toxic effect of the surfactant when injected in air cell. Detection of carvacrol in the yolk, which is not directly on the transmission route of OEO injection, could be due to the lipophilic nature of carvacrol emulsified with the surfactant, favouring diffusion into lipophilic environments such as yolk. Yolk is absorbed into the embryonic GIT during late incubation. This indicates that carvacrol, when administered with a non-ionic surfactant, may reach the GIT through this route in sufficient amounts to improve gut health and development.

In conclusion, the use of polysorbate 80 facilitates carvacrol diffusion to embryonic structures after injection of OEO but has a potential toxic effect when injected in the air cell.

ACKNOWLEDGEMENTS: This work was supported by AgriFutures and Delacon Biotechnik.

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pH CHANGES IN EGG COMPARTMENTS DURING EMBRYO DEVELOPMENT IN BROILER CHICKENS

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Broiler chickens spend one third of their lives developing inside an egg. Hence, understanding and optimising the in ovo environment is fundamental. The pH of body fluids is tightly regulated to maintain critical physiological functions such as gas exchanges. Enzymes equally have an optimum pH range that determines their efficiency. Changes to these conditions, may impact on the efficiency of O_2 transport or enzyme activity. Embryonic development requires a continuous flow of O_2 delivery and supply of amino acids and energy through the activity of digestive enzymes. Albumen and yolk provide most nutrients to the embryo during incubation, the amnion provides protection, and the allantois serves as an excreta reservoir (Moran, 2007). It is anticipated that each structure will have a tightly controlled pH during incubation. The current study was developed with the objective to better understand the dynamics of pH changes in egg compartments. We hypothesized that the high respiration rate during embryonic development would be associated with CO_2 release and partial depletion of bicarbonate, which is thought to be the main buffer in the egg. Thus, we expected metabolic alkalosis (a decrease in pH) to occur in all main water-rich compartments (albumen, amnion, and allantois).

The pH changes in albumen and yolk, were measured using a laboratory pH meter (HANNA instruments, Model HI98163) on embryonic days (E) 0 (T1), E3 (T2), E6 (T3), E9 (T4), E12 (T5), E15 (T6), and E17.5 (T7). The pH changes in amnion and allantois were studied on E9 (T4), E12 (T5) and E15 (T6). Data was analysed using ANOVA in R Studio. Significance level was set at $P<0.05$.

The results showed higher albumen pH on E0 (9.21, $P<0.05$) than in successive days with values in continuous decline to 8.94, 8.31, 8.10, 7.39, and 7.18 on E6, E9, E12, E15 and E17.5, respectively. Albumen pH was negatively correlated with incubation day ($P<0.05$) showing maximal negative increments ($P<0.05$) between E7 to 9 and E13 to 15. In contrast, yolk pH increased from E0 to 15 with values of 6.67, 7.03, 7.56, 7.73, and 8.046 on E0, E6, E9, E12, and E15, respectively. However, by E17.5 the pH decreased back to 7.734 ($P<0.05$). Yolk pH was positively correlated with incubation day ($P<0.05$). The pH of amnion decreased over time, being higher (7.64) on E9 than on E12 (7.36) and E15 (6.83) ($P<0.05$). Amnion pH showed a negative correlation with incubation day ($P<0.05$). Similarly, allantois which had a higher pH at E9 (8.83), decreased over time to 8.16 on E12 and 6.54 on E15. The yolk pH was negatively correlated with the pH of the albumen, amnion, and allantois ($P<0.05$), while the pH of amnion, albumen, albumen, and allantois pH were positively correlated ($P<0.05$).

During the course of incubation, the pH of water-rich compartments (albumen, allantois, and amnion) decreased, while the pH of the fat-rich compartment (yolk) increased, thus confirming our hypothesis. Evidently, a dynamic homeostasis of metabolic acid-base balance is critical for embryonic respiration and development.

ACKNOWLEDGEMENTS: This study was supported by AgriFutures and Delacon.

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INVESTIGATION OF THE IMPACT OF A BLEND OF A TRIPLE STRAIN PROBIOTIC ON PERFORMANCE AND GUT-BRAIN AXIS

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Summary

A total of 1600 male broilers (Aviagen AP95) were randomly assigned to one of four dietary treatment groups (50 birds per replicate, 8 replicates per treatment). Four treatments evaluated were: a) antibiotic growth promoter (Halquinol; HAL), b) negative control without antibiotic growth promoter, probiotics, essential oils or organic acids (NC), c) mix of probiotic strains consisting of two *B. subtilis* strains and one *B. amyloliquefaciens* (PRO) d) blend of organic acids and essential oils (OA&EO). The dietary treatments were fed from bird age day 0 to 42. Feed intake, body weight, feed conversion ratio (FCR) and mortality (%) were determined weekly. The effects of the dietary treatments on bird well-being parameters were examined using a latency to lie (LTL) test, gait scoring and approximation test at 42 days of age. Plasma concentrations of serotonin were measured at 40 days of age. Daily feeding of PRO and OA&EO resulted in significant improvements in final body weight and European Production Efficiency Factor (EPEF) corrected for mortality ($P < 0.05$) compared to feeding the NC and HAL treatments. FCR was lower and LTL time was higher in birds fed the PRO treatment compared to those fed other treatments ($P = 0.001$ and $P = 0.001$, respectively). Birds receiving probiotic (PRO) exhibited a significantly higher level of circulating blood serotonin ($P < 0.05$). In this trial, feed supplementation of PRO significantly positively impacted broiler performance and behaviour related parameters.

I. INTRODUCTION

To ensure productivity of poultry farming, the intestinal health of chickens, among other factors, must be considered to achieve maximum nutrient absorption and utilisation for the animals to develop properly (Oleforuh-Okoleh et al., 2015). Nonetheless, with the reduction or ban of the use of performance-enhancing antimicrobials on a worldwide basis, the quest for products able to modulate the intestinal microbiota of birds has intensified. Enzymes, organic acids, prebiotics, symbiotics and probiotics stand out among additives that enhance performance by acting on the intestinal microbiota (Bozkurt et al., 2014). In poultry farming, *Lactobacillus*, *Bacillus*, *Bifidobacterium* and *Enterococcus* bacteria are commonly used as probiotics to support intestinal health and performance (Zaghari, et al., 2015). An ever-present concern under these intensive conditions is the behaviour and welfare of birds. In human health, the study of the microbiota-gut-brain axis has advanced greatly in recent years as investigators seek to understand the interaction of probiotic strains and cognitive functions (Bested et al., 2013; Carabotti et al., 2015). Therefore, for poultry production, evaluations of probiotics have been investigated to determine potential beneficial impact for both performance indexes and broiler welfare. Welfare parameters such as LTL, approximation test and serotonin plasma concentrations were described to be relevant bird behaviour and well-being indicators (Almeida et al., 2019). Serotonin (5-hydroxytryptamine; 5-HT) is a known neurotransmitter critical for the development function of the central nervous system and a good indicator of well-being status (Ezenwa et al., 2012).

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II. MATERIAL AND METHODS

1600 male broilers (Aviagen AP95) were randomly assigned to one of four treatment groups (50 birds per replicate, 8 reps). Four treatments were applied, HAL (Halquinol at 75mg/kg feed), NC (negative control group with no AGP, no probiotic, no blend of essential oils or organic acids, PRO (mix of probiotic strains at 500g/T feed), consisting of *B. subtil*is (DSM 32325), *B. subtilis* (DSM 32324) and *B. amyloliquefaciens* (DSM 25840), and OA&EO (blend of benzoic acid and thymol, eugenol, piperine at 300 g/T feed). The same nutritional profile was used for the dietary treatments during the different feeding phases. Broilers were fed from age day 0 to 42. Feed intake, body weight, feed conversion ratio and mortality (%), were collected on a weekly basis. At 42 days of age the birds were submitted to 2 well-being tests using an LTL test and approximation test. For the LTL test, a plastic container measuring 75 × 50 × 20 cm was filled with 3 cm of water at room temperature, where 6 chickens were placed at a time. A digital stopwatch recorded the time the birds took until the first attempt to lie down. The test was interrupted if the bird was still standing after 370 s. For the approximation test, an assessor entered the pen and, after 3 min, extended his or her arms and counted how many animals could be touched. At 42 days of age, gait scoring was measured using a three-category system (0: No obvious signs, 1: Obvious signs, 2: Severe signs). Plasma concentrations of serotonin were measured at 40 days of age using a Serotonin/hydroxytryptamine 5-HT Elisa kit. Samples were collected from 8% of broilers in each treatment. Data were analysed using SAS 9.2 program, using ANOVA and Tukey's test ($p < 0.05$) for parametric data and Chi-Square or Fisher's exact test ($p < 0.05$) for non-parametric data.

III. RESULTS AND DISCUSSION

As presented in Table 1, daily feeding of PRO and OA&EO resulted in significant improvements in final body weight and EPEF corrected for mortality ($P<0.05$) compared to the NC and HAL groups. FCR was significantly lower in birds fed the PRO treatment compared to those fed any other treatment. Treatment had no significant impact on mortality rate (%). Latency to lie for both gait scores 0 and 1, representing 90% of the birds, is shown in Figure 1. The group fed PRO demonstrated a significantly longer standing time in the LTL test compared to those fed NC, HAL and OA&EO, suggesting these birds had a better resilience to stress and better leg health status.

Table 1 - Body Weight (g), FCR, Mortality (%) and EPEF results by treatment group at 42 days of age.

Treatment	Body Weight (g)	Feed Conversion Ratio (FCR)	Mortality (%)	EPEF
HAL	3103 [c]	1.52 [a]	1.71	478 [c]
NC	3086 [c]	1.51 [a]	1.79	478 [c]
PRO	3209 [a]	1.48 [b]	2.57	503 [a]
OA & EO	3168 [b]	1.51 [a]	2.00	489 [b]
P-values	*0.001*	*0.001*	*0.522*	*0.023*

a,b,c means within column not sharing a common suffix are significantly different at $P < 0.05$.

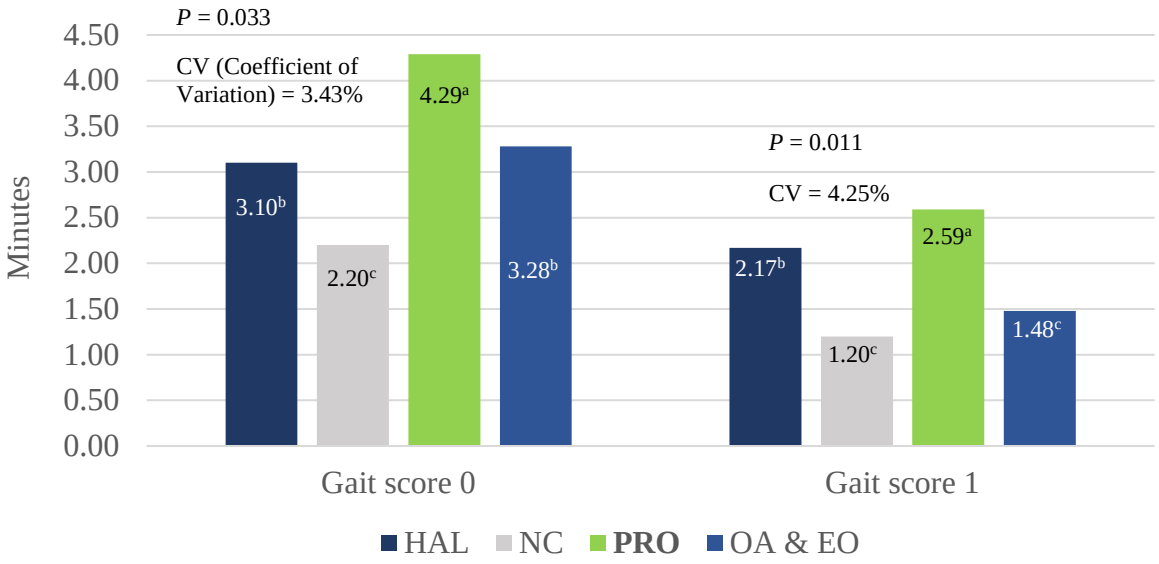

Figure 1 - Results for latency to lie (LTL) test (in minutes and seconds) by treatment group for broilers with Gait 0 and 1 scores.

Figure 2 shows that, in the approximation test evaluation performed on birds with Gait 0 score, after 3 min in pens, the % of birds touched by the assessor was significantly higher for broilers fed with PRO than for the groups fed with HAL, NC and OA&EO treatments which exhibited lower % of birds touched by the assessor.

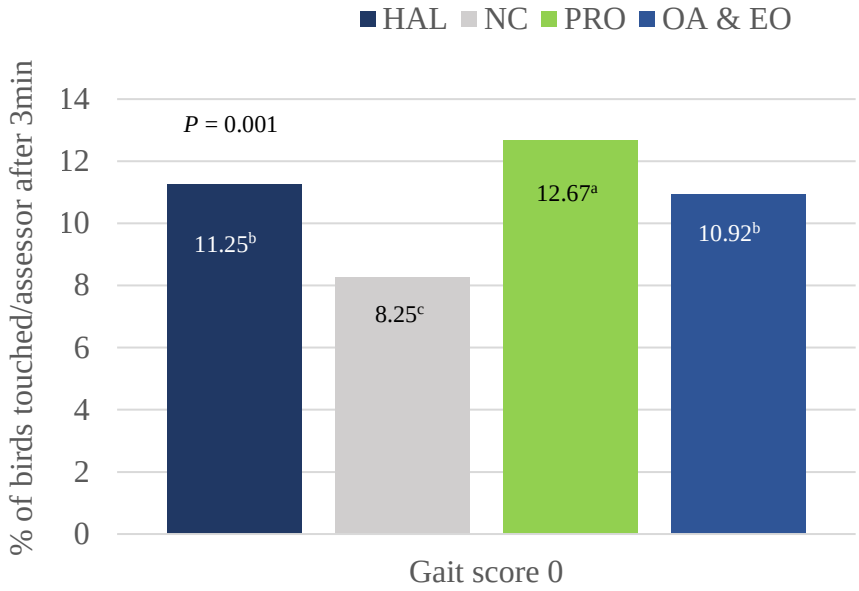

Figure 2 - Results of approximation test results expressed in % of birds touched by the assessor after 3 minutes in a pen (for broilers with score Gait 0) per treatment group.

In parallel to the LTL and approximation tests, plasma serotonin levels measured at 40 days of age and results are presented in Table 2. Birds receiving the mix of triple strains probiotic (PRO) exhibited a significantly higher level of plasma serotonin (P = 0.001) in comparison to HAL, NC and OA&EO treatments.

Table 2 - Measurements of plasma serotonin (5-HT) levels in broilers per treatment group at 40 days of age.

Treatment	Serotonin (5-HT) µg/ml
HAL	91 [c]
NC	100 [c]
PRO	402 [a]
OA & EO	316 [b]
P-value	*0.001*
CV(%)	1.86

a,b,c means within column not sharing a common suffix are significantly different at $P < 0.05$.

In conclusion, the birds fed daily with treatment PRO had better feed conversion, better growth and consequently, better production efficiency factor as compared with birds receiving treatments HAL, NC and OA&EO. Concurrently, the birds' well-being significantly improved when the PRO diet was provided. Significantly improved scores in the LTL and approximation tests indicates that those birds tended to be more calm and resilient to stress. Significantly higher levels of plasma serotonin (µg/ml) at 40 days of age for broilers fed the PRO treatment, support the positive impact of the PRO treatment on performance and well-being parameters of broilers.

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COULD PHOSPHORUS AND CALCIUM BE THE MISSING LINKS IN UNLOCKING RADICALLY LOW PROTEIN DIETS FOR COMMERCIAL BROILER PRODUCTION?

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Summary

Reducing the crude protein (CP) concentration of broiler diets without compromising live production end points is an aspirational goal for most poultry producers. Economic and environmental benefits can be considerable and improvements in animal welfare have also been reported. However, feeding radically low CP diets to broilers results in unpredictable animal performance outcomes despite best efforts to balance digestible amino acids, metabolisable energy and electrolytes. Historically, research intended to optimise digestible amino acid supply has been disconnected from work on digestible macro-minerals such as calcium (Ca) and phosphorus (P). This separation is logical as nutrient requirement studies that simultaneously explore amino acids, energy, Ca, and P would be cumbersome and statistically vulnerable. It is also somewhat counterintuitive that digestible amino acid, Ca and P requirements may interact. However, recent evidence from rodents and broiler chickens suggests that requirement for P and Ca may increase and decrease respectively when dietary CP is reduced. Conclusions are tentative and a lot more research is required to fully explore the mechanisms and optimize nutritional ratios. It is the purpose of this short review article to describe some of the recent research in this space and where opportunities may exist for future exploration.

I. INTRODUCTION

Stephen Jay Gould famously introduced the term 'non-overlapping magisteria' in a *Natural History* article in March 1997, to describe the separation between scientific and religious lines of enquiry (Gould, 1997). From a broiler nutrition perspective, it would be accurate to represent digestible amino acid and metabolisable energy research, and digestible P and Ca research, using the same vocabulary. Despite these distinct research domains, considerable value has been created in both spheres. For example, before the advent of synthetic amino acids, broiler diets were formulated to contain up to 700 g/kg soybean meal and 350 g/kg CP in order to meet the birds' requirement for methionine (Pesti, 2009). This contrasts with contemporary broiler diets that include several 'unbound' or 'free' amino acids and can satisfy the animals amino acid requirement with diet CP concentrations as low as 160-210 g/kg, depending on the age of the chick. Similarly, the commercial introduction of exogenous phytase in 1991 and more recent work on phytase dose optimisation and digestible Ca formulation systems has led to substantial decreases in the use of inorganic phosphate to the point where many broiler grower and finisher diets are entirely denuded of inorganic P sources (Moss et al., 2018; Walk et al., 2021). Despite these 'localised' successes, there are very few published papers that explore the potential interaction between dietary CP and diet Ca and P concentrations and these magisteria remain largely non-overlapping. It is the purpose of this short review article to describe some of the relevant literature in this space, offer mechanistic insights that may be of importance and suggest opportunities for further research that may increase the precision of nutrient delivery to the bird without compromising live production metrics.

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II. RODENTS

As far as the author is aware, the first studies that were designed to explore the potential for an interaction between diet CP and P (and by association, Ca) were conducted using rodent models. Hammoud et al. (2017) fed rats a low CP diet (100g/kg CP relative to a standard diet of 200g/kg CP) and titrated P from 0.15g/kg to 3.0g/kg. The authors observed significant increases in weight gain, feed intake, energy efficiency and plasma glucose concentration. In addition, plasma urea nitrogen (N) was reduced from around 6.5 mM/l to 4 mM/l as diet P was increased. Rats that received the low CP diet with the highest concentration of P returned growth performance that was equivalent to those fed the standard CP diet. These observations were confirmed more recently in a rodent model when the addition of digestible lysine and/or P to a low protein diet (100g/kg) resulted in synergistic effects on growth rate and energy efficiency and P alone had a positive effect on protein metabolism and significantly reduced plasma urea N (Ragi et al., 2019).

III. PUTATIVE MECHANISMS

The role of P in determining digestible amino acid requirements and animal response to dietary CP is not fully established. However, there are three potentially valuable lines of enquiry. First, protein intake has a calciuretic effect (Margen et al., 1974; Zemel, 1988) whereby N and Ca compete for resorption at the renal level. A high protein intake results in excess urinary Ca losses and vice versa. Thus, as broiler diet CP is reduced it is possible that Ca retention is increased, a disequilibrium that may be partially mitigated via supplementation of diets with additional P. This possibility was recently supported by Dao et al. (2022a) where a reduced crude protein diet fed to Ross 308 broiler chickens generated an increase in serum Ca but had no effect on serum P. Importantly, this was associated with a numerical decrease in d28 tibia ash in the chicks that received the low protein diet. Second, low CP broiler diets are compositionally distinct from diets formulated at standard CP concentrations. For example, low CP broiler diets typically have a higher concentration of cereals and lower concentrations of protein meals such as soybean meal or canola. It may be relevant that the concentration of phytate-bound P in cereals is lower than in protein meals (Eeckhout and De Paepe, 1994) and the *per se* availability of phytate-bound P in protein meals may be higher than for cereals (Weremko et al., 1997). The influence of diet CP *per se* on P digestibility is likely to be dependent on a number of additional factors such as dietary cation concentration, strategies used in feed formulation to achieve the reduction in crude protein and phytase dosing. Indeed, Dao et al. (2022b) observed an increase in ileal P digestibility in broilers fed a low protein diet, which contradicts earlier observations, so further work to explore the role of low protein diets on mineral digestibility is warranted. Finally, protein synthesis requires appreciable quantities of P for manufacture of ATP (Shariatmadari and Forbes, 1993) and it is possible that additional dietary P may reduce protein catabolism and promote protein accretion via provision of P for ATP synthesis. In short, a low CP diet may simultaneously provide lower concentrations of available phytate-P, promote Ca retention and increase the demand for ATP to drive protein synthesis. Increasing digestible P supply in low CP diets may be an effective strategy to mitigate these influences. This could be done by elevating phytase dosing, addition of inorganic P or reductions in total dietary Ca. Optimal strategies require further research.

IV. VALIDATION IN BROILERS

In attempt to extend the principals described above to commercial broiler production a study was conducted to specifically explore the interaction between diet CP and P (Cowieson et al.,

2020). Ross 308 male broiler chickens were offered diets with low, medium or standard CP concentrations and either low, standard or high available P (for context the grower diets were formulated at 215, 195 or 175g/kg CP and offered at either 4.8, 4.3 or 3.8 g/kg available P). All amino acids were balanced using non-protein bound amino acids and energy, macro- and micro-minerals and dietary electrolyte balanced were equivalent across all dietary treatments. Response of broiler chicks to supplemental available P in terms of body weight corrected feed conversion ratio (FCRc) was more pronounced in the low CP diet than in the standard CP diet, resulting in a significant interaction. Specifically, increasing available P from 3.8 to 4.8g/kg in the diet with standard CP concentration had no effect on FCRc over the full experimental duration (d8-35) whereas the same increase in available P in the diet with the lowest CP concentration resulted in a decrease in FCRc of 7 points. Interestingly, increasing available P concentration resulted in a decrease in plasma uric acid concentration and this was particularly marked at the moderate level of dietary CP resulting in an interaction between diet CP and available P. This confirmed previous research in rodents that dietary P may influence post-absorptive N metabolism and may influence deamination and ammonia detoxification processes. The potential for available P to influence N retention and ammonia management is an area of interest for future study.

V. CONCLUSIONS

The role of P (or more accurately digestible or available P) in amino acid metabolism, nitrogen cycling, ammonia detoxification and nitrogen emissions is not clear. The possibly exists, however, that reducing dietary CP may influence the requirement of the bird for both P and Ca. This may occur via direct mechanisms such as the importance of P in ATP synthesis and protein accretion and the competition between Ca and nitrogen in renal tubules and also indirectly via subtle changes in the soluble phytate concentration of low CP diets. As the global poultry industry moves toward increasingly low CP diets, and in concert with important ongoing work to optimize the deployment of non-protein bound amino acids, some attention to the dietary supply of Ca and P is warranted. Given the complexity of these interactions and the constraints in multi-factor experimental designs a mechanistic modelling approach may be justified.

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REQUIREMENT OF DIGESTIBLE CALCIUM AT DIFFERENT DIETARY CONCENTRATIONS OF DIGESTIBLE PHOSPHORUS FOR BROILER FINISHERS (DAYS 25-35 POSTHATCH)

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Summary

An experiment was conducted to determine the digestible calcium (Ca) and digestible phosphorous (P) requirements of 25-35-day old broiler chickens. Fifteen experimental broiler finisher diets based on maize-soybean meal were formulated in a 5 × 3 factorial arrangement with diets containing five concentrations of standardised ileal digestible (SID) Ca (2.0, 2.5, 3.0, 3.5 and 4.0 g/kg) and three concentrations of SID P (2.5, 3.5 and 4.5 g/kg) and were fed to broilers from day 25 to 35. Each experimental diet was randomly allocated to six replicate cages (eight birds per cage). Body weight was recorded on day 25 and 35. On day 35, the birds were euthanised to collect tibia for the determination of the tibia ash concentrations. Fixed effects of the experiment were dietary concentrations of SID Ca and SID P and their interaction. If the interaction or main effects were significant ($P < 0.05$), the parameter estimates for second-order response surface model were determined using General Linear Model procedure of SAS (2019). The prediction for the maximum response was not made for weight gain and tibia ash as the Ca effect was linear, which indicates that the estimated requirement of dietary SID Ca for the maximisation of these parameters depends on the dietary SID P concentration in finisher broilers. However, based on factorial analysis, the higher weight gain was observed at 3.5 g/kg SID P concentrations and 3.5 g/kg SID Ca concentrations. The recommended SID P and SID Ca requirements for optimum tibia ash are 3.5 and 3.0-3.5 g/kg, respectively. The estimated SID Ca requirements (at 3.5 g/kg SID P) for higher weight gain and optimum tibia ash are lower than the current Ca recommendation (7.80 g/kg total Ca equivalent to 4.25 g/kg SID Ca; Ross, 2019) for broiler finishers.

I. INTRODUCTION

Calcium (Ca) and phosphorous (P) are two essential minerals for the skeletal growth and other biological functions in poultry. Maintaining an appropriate balance between Ca and P is necessary as these minerals are closely associated in their absorption and post absorptive utilisation. Because of the recent works on the measurement of digestible Ca in feed ingredients, initiative has been taken to determine the standardised ileal digestible (SID) Ca and SID P requirements in broiler starters (day 1-10 post-hatch; David et al., 2021) and broiler growers (day 11-24 post-hatch; David et al., 2022) for growth, bone mineralisation and mineral utilisation. These results showed that the recommended SID P for the maximum growth performance, bone mineralisation, and Ca and P utilisation of broiler starters and growers were 5.0 and 3.5 g/kg, respectively. At respective SID P, the requirement of SID Ca for the maximum weight gain of broiler starters and growers was 3.32 and 3.05 g/kg, respectively, and for the maximum tibia ash was 4.51 and 3.69 g/kg, respectively. Corresponding SID Ca to SID P ratio that maximised the weight gain in broiler starters and growers was 0.66 and 0.87, respectively, and that maximised the tibia ash was 0.90 and 1.05, respectively. Therefore, the objective of the current follow-up study was to determine the requirements of digestible Ca and digestible P for the maximisation of weight gain and tibia ash in broiler finishers (days 25-35 post-hatch).

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II. MATERIALS AND METHOD

The experimental protocol was approved by the Massey University Animal Ethics Committee. Fifteen experimental finisher diets based on maize-soybean meal were formulated in a 5 × 3 factorial arrangement with diets containing five concentrations of digestible Ca and three concentrations of digestible P. Diets were formulated to contain 2.0, 2.5, 3.0, 3.5 and 4.0 g/kg SID Ca (corresponding to 3.7, 4.6, 5.5, 6.4 and 7.3 g/kg total Ca, respectively) and 2.5, 3.5 and 4.5 g/kg SID P (corresponding to 3.4, 4.9 and 6.4 g/kg total P, respectively). A total of 720, day-old male broilers (Ross 308) were fed broiler starter crumbles (4.4 g/kg SID Ca; 5.0 g/kg SID P) from day 1 to 10 and the broiler grower pelleted diet (3.69 g/kg SID Ca; 3.5 g/kg SID P) from day 11 to 24 post-hatch. The concentration of SID Ca for broiler starter and grower diets were based on the previous findings (David et al., 2021; 2022) and the concentration of other nutrients were based on Ross (2019). The concentration of total Ca ranged from 0.47 to 0.94 times the requirement for total Ca (Ross, 2019). All experimental diets were isoenergetic and isonitrogenous. Each diet was separately mixed and pelleted. On day 25, the birds were weighed and allocated (mean ± SD, 1.31 ± 0.02 kg) to 90 grower cages (eight birds per cage). The experimental diets were offered *ad libitum* to six replicate cages of broilers from day 25 to 35 post-hatch. The birds had free access to water. Body weight was recorded on a cage basis at the start and end of the experimental period and the weight gain was calculated. On day 35, right tibia from six birds per replicate was removed and processed as described by David *et al.* (2021). The tibia ash concentration was determined using AOAC (2016) procedures. Data were analysed using the General Linear Model (GLM) procedure of SAS (2019), with cage serving as the experimental unit. Two sets of analyses were conducted. First, as a factorial arrangement of treatments examining the effects of dietary concentrations of SID Ca and SID P and their interaction. The effects were considered significant at $P < 0.05$. Second, if the interaction or main effects were significant, then the estimates for the second-order response surface model were determined using the GLM procedure of SAS (2019) and these estimates were used to calculate the maximum response and the SID Ca concentration required for maximum response.

III. RESULTS AND DISCUSSION

All birds remained healthy during the experiment. Table 1 and Figure 1 present the body weight gain and the concentrations of tibia ash of birds fed the diets containing different SID Ca and SID P from day 25 to 35 post-hatch. There was an interaction ($P < 0.001$) between SID Ca and SID P for weight gain. At the lowest SID Ca (2.0 g/kg) concentration, increasing concentration of SID P reduced weight gain. In contrast, increasing SID P concentrations increased the weight at the highest SID Ca concentration (4.0 g/kg), but had no effect on the weight gain at 2.5 and 3.0 g/kg SID Ca concentrations. At 3.5 g/kg SID P concentration, the weight gain was higher ($P < 0.05$) at 3.5 g/kg SID Ca. Predictions for SID Ca at maximum response was not calculated for weight gain due to the linear Ca response. The findings suggest that the requirement of SID Ca for the weight gain depends on the dietary SID P concentration. Accordingly, the SID Ca concentration of 2.0, 3.5 and 3.0-4.0 g/kg can be recommended for broiler finishers when the dietary SID P is 2.5, 3.5 and 4.5 g/kg, respectively. However, based on the factorial analysis, a concentration of 3.5 g/kg SID Ca and 3.5 g/kg SID P can be recommended for broiler finishers as the weight gain was higher at this combination. The equivalent total Ca (6.4 g/kg) value of the current estimate is lower than the current Ross (2019) Ca recommendation (7.8 g/kg total Ca) for 25-39 day old birds.

Based on factorial analysis, there was no interaction ($P > 0.05$) between SID Ca and SID P for the tibia ash. Tibia ash increased ($P < 0.05$) with increasing concentration of SID P. Similar to weight gain, the predictions for SID Ca at maximum response was not calculated for tibia ash due to the linear Ca response and therefore the findings suggest that the requirement of SID Ca for the tibia ash depends on the dietary SID P concentration.

Table 1 - Body weight gain (g/bird) and tibia ash concentration (g/kg dried defatted matter) in broiler chickens fed diets containing different concentrations (g/kg) of standardised ileal digestible (SID) Ca and SID P from day 25 to 35[1].

SID Ca	SID P	Body weight gain	Tibia ash
2.0	2.5	1134^{bcde}	393
	3.5	1122^{cdef}	395
	4.5	1064^{f}	404
2.5	2.5	1102^{def}	391
	3.5	1161^{abcd}	399
	4.5	1114^{def}	409
3.0	2.5	1113^{def}	395
	3.5	1144^{bcde}	411
	4.5	1148^{abcd}	417
3.5	2.5	1117^{def}	389
	3.5	1212^{a}	412
	4.5	1143^{bcde}	409
4.0	2.5	1079^{ef}	384
	3.5	1187^{abc}	406
	4.5	1198^{ab}	425
SEM[2]		23.7	5.7
SID Ca			
2.0		1107	397
2.5		1126	400
3.0		1135	408
3.5		1157	403
4.0		1155	405
SEM[2]		13.7	3.3
SID P			
2.5		1109	391^{c}
3.5		1165	404^{b}
4.5		1134	413^{a}
SEM[2]		10.6	2.5
Probability, P ≤			
SID Ca		0.059	0.184
SID P		0.002	0.001
SID Ca × SID P		0.020	0.211

[1]Each value represents the mean of six replicates (eight birds per replicate for body weight gain and six birds per replicate for tibia ash).
[a-f] Means having different superscripts within the column are significantly different ($P < 0.05$).
[2]Pooled standard error of mean.

Based on factorial analysis, dietary concentrations of 3.0-3.5 g/kg SID Ca and 3.5 g/kg SID P can be recommended for the optimum tibia ash in broiler finishers. The estimated SID Ca (3.0-3.5 g/kg) requirement for tibia ash is lower than the current Ross (2019) total Ca recommendation for finishers (7.80 g/kg total Ca equivalent to 4.25 g/kg SID Ca) and that reported for broiler starters (4.51 g/kg SID Ca) and growers (3.69 g/kg SID Ca), demonstrating a reduction of 22-33% and 5-19%, in broiler finishers compared to starters and growers, respectively. Unlike our previous

studies on broiler starters (David *et al*., 2021) and growers (David *et al*., 2022), the digestible Ca requirement for maximum tibia ash was not greater than that for maximum weight gain in broiler finishers, suggesting the low Ca demand for bone development in broiler finishers.

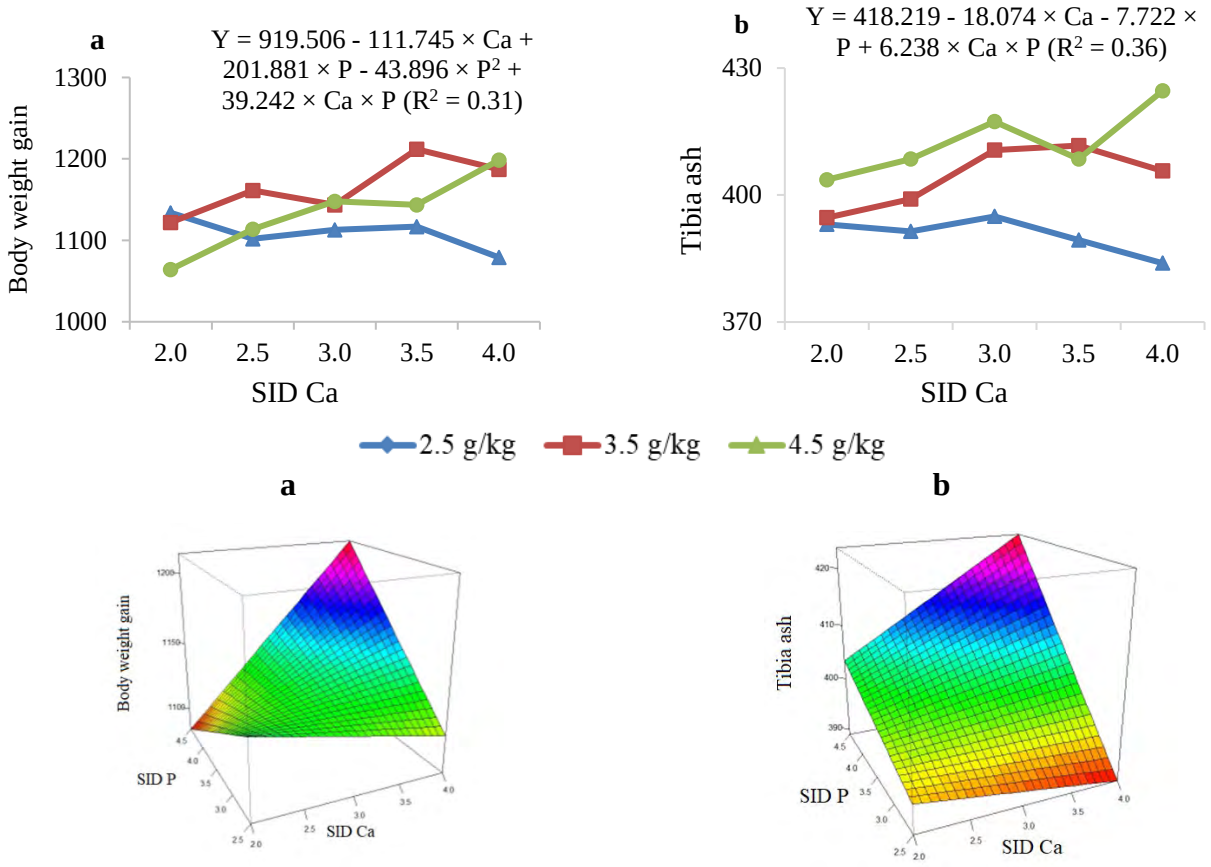

Figure 1 - Interaction and response surface plots for (a) body weight gain (g/bird) and (b) tibia ash concentration (g/kg dried defatted matter) of broiler chickens fed different standardised ileal digestible (SID) calcium (Ca) and SID phosphorous (P) concentrations (2.5, 3.5 and 4.5 g/kg) from day 25 to 35 post-hatch

In conclusion, the requirements of SID P for the weight gain and tibia ash of 25-35 day old broilers is 3.5 g/kg. At 3.5 g/kg SID P, weight gain and tibia ash are maximised at 3.5 and 3.0-3.5 g/kg SID Ca concentrations, respectively.

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SUMMARY OF THE STANDARDISED ILEAL DIGESTIBLE CALCIUM REQUIREMENTS OF BROILERS FROM HATCH TO DAY 42 POST-HATCH WHEN CONSIDERING BREED AND GENDER

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Summary

A meta-analysis was conducted to estimate the standardised ileal digestible calcium requirement of broilers from hatch to day 42 post-hatch. The analysis included 12 studies (n = 143) conducted from 2020 to 2022 and utilised predominantly males from Ross 308 (74%), Cobb 500 (15%), and Arbor Acres Plus (11%) genetic lines. The standardised ileal digestible calcium requirements were estimated using quadratic regressions. The response variables included body weight gain, feed intake, feed conversion ratio, tibia ash percent and weight, apparent total tract retention of calcium and phosphorus and apparent ileal digested calcium and phosphorus. There was a significant effect of broiler breed on almost all parameters, but no standardised ileal digestible calcium by breed interaction. Therefore, the standardised ileal digestible calcium requirement was the same for all three genetic lines and estimated at 0.488 or 0.452% for the starter and grower phase, respectively. There were limited effects of dietary calcium level on the response variables in the finisher phase and therefore the requirement was not estimated using the current meta-analysis.

I. INTRODUCTION

Calcium (Ca) is an important nutrient for skeletal development, muscle contraction, nerve impulses, acid-base balance, and a cofactor for blood clotting and endogenous enzymes. Due to the involvement of Ca in numerous functions in the body, accurate supply of dietary Ca is essential for optimal growth, feed efficiency and nutrient utilisation. Oversupply of dietary Ca or an imbalance between dietary Ca and phosphorus (P) has been linked to significant reductions in growth performance and nutrient digestibility (Amerah et al., 2014; Mutucumarana et al., 2014). Accurate supplementation of both dietary Ca and P may provide further improvements in growth and efficiency as well as responsible use of resources and reduce nutrient excretion into the environment.

Recently, a series of studies were conducted to estimate the standardised ileal digestibility (SID) or apparent ileal digestibility (AID) of Ca in various feed ingredients for broilers (as reviewed by Walk et al., 2021a). With this information it is now possible to formulate broiler diets using SID Ca coefficients in feed ingredients. Over the last two years studies have been conducted to estimate the SID Ca requirements of broilers from hatch to day 42 post-hatch (David et al., 2021, 2022; Walk et al., 2021b, 2022a, 2022b). The objective of this work was to combine the results from studies designed to estimate the SID Ca requirements of broilers and provide an updated SID Ca requirement for the starter, grower, and finisher phases of broiler production.

II. METHOD

A series of studies (n = 12) were conducted between 2020 and 2022 to determine the SID Ca requirement of broilers from hatch to day 42 post-hatch. In all studies, four to six graded concentrations of SID Ca were fed (Table 1) to broilers using P-adequate (77%) diets or diets formulated with graded levels of SID P (23%). The studies were conducted from hatch to day

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10/17 (starter, n = 54), day 11/17 to 21/31 (grower, n = 57), or day 22/32 to 42/44 (finisher, n = 32). This resulted in a total of 143 data points in the experimental model.

Data from all studies were combined into one excel file. This included an arbitrary study number, animal husbandry and diet information, experimental treatments, formulated and analysed nutrient concentrations, particularly total Ca and P, and the mean results from growth performance, AID of Ca and P, tibia ash percent and weight, and apparent total tract retention (ATTR) of Ca and P. The analysed total Ca concentration in the experimental diets was used to estimate the SID Ca within each experiment. The studies included genetics from Ross 308 (74%), Arbor Acres Plus (11%), or Cobb 500 (15%) broilers and used predominantly males (85%) or straight-run birds.

Table 1 - Experimental design, mean, minimum, and maximum value of key variables.

Item	Starter phase (n = 54)		Grower phase (n = 57)		Finisher phase (n = 32)	
	Mean	Range	Mean	Range	Mean	Range
Study start, day	1	-	13	11 - 17	26	21 - 29
Study end, day	13	10 - 17	26	21 - 31	42	42 - 44
Formulated SID Ca, %	0.43	0.18 - 0.67	0.37	0.16 - 0.62	0.34	0.10 - 0.70
Formulated total Ca, %	0.87	0.37 - 1.39	0.73	0.34 - 1.27	0.65	0.18 - 1.42
Digestible P, %	0.46	0.40 - 0.60	0.41	0.35 - 0.55	0.34	0.32 - 0.38
Body weight start, g/bird	44	39 - 50	467	282 - 692	1538	839 - 2172
Body weight end, g/bird	480	274 - 692	1518	839 - 2172	3146	2775-3681
Body weight gain, g/bird	381	204 - 649	1081	488 - 1480	1608	1249-2012
Feed intake, g/bird	431	220 - 681	1503	646 - 2340	2869	2439-3284
Feed conversion ratio, g:g	1.12	1.02 - 1.30	1.38	1.24 - 1.73	1.82	1.58 - 2.03
Mortality, %	1.8	0 - 15	2.7	0 - 13	2.3	0 - 6
Apparent ileal digested Ca, %	0.39	0.10 - 0.60	0.33	0.21 - 0.61	0.22	0.06 - 0.34
Apparent ileal digested P, %	0.36	0.10 - 0.60	0.36	0.21 - 0.72	0.28	0.12 - 0.39
Tibia ash, %	45	32 - 56	43	36 - 49	45	39 - 49
Tibia ash weight, mg/bone	479	383 - 534	1291	783 - 1660	3169	2881-3623

Data were analysed using JMP Pro v. 16. Prior to statistical analyses, data were checked for outliers using the explore outliers platform. Means were analysed using the fit model platform and separated by feeding phase. The full statistical model included the linear and quadratic effects of SID Ca (as a continuous variable), broiler breed, and the interactions. Non-significant model effects, that were not part of a significant interaction, were removed and the parameter estimates recalculated using the reduced model. The SID Ca requirements were determined using the maximum response values from the linear or quadratic equations. Significance was accepted at $P < 0.05$ and trends discussed at $P < 0.10$.

III. RESULTS

The formulated and analyzed total Ca concentration in the experimental diets was in good agreement ($R^2 = 0.91$) and this meant the formulated and estimated SID Ca concentrations were in good agreement as well ($R^2 = 0.90$). There was a significant effect of trial site on all response variables, and it accounted for a large proportion of the variation in the statistical model. Therefore, the statistical model was updated to include location (random variable) nested within broiler breed.

During the starter, grower and finisher phases, there was no significant ($P > 0.10$) SID Ca × breed effect on body weight gain, feed intake, feed conversion ratio (FCR), tibia ash

weight or percent, ATTR of P, or apparent ileal digested P. However, there was a main effect of breed on almost all parameters measured. This means there are differences in performance, bone ash, and P digestibility or P retention among the broiler breeds tested, but the estimated SID Ca requirement is the same, regardless of the broiler breed. However, the apparent ileal digested Ca or ATTR of Ca was influenced by a significant SID Ca × breed interaction. In the starter phase, the apparent ileal digested Ca was greater in Ross 308 birds compared with Arbor Acres birds and increased to a greater extent (linear, $P < 0.05$) in Ross 308 birds (38 vs 45%) as the SID Ca concentration in the diet increased (SID Ca × breed, $P < 0.05$). In the grower phase, the ATTR of Ca was greater in Arbor Acres birds and decreased (linear, $P < 0.05$) to a greater extent compared with Ross 308 birds (SID Ca × breed, $P < 0.05$).

The linear and quadratic effect of graded levels of SID Ca within each feeding phase are presented in Table 2. The estimated SID Ca requirement, based on growth performance, apparent ileal digested Ca or P, tibia ash percent and weight, and ATTR of Ca or P, within each feeding phase, is presented in Table 3. There were limited effects of the graded levels of SID Ca during the finisher phase and therefore the estimated SID Ca requirement was not determined (data not shown).

Table 2 - Summary of the statistical analyses (P-values) used to estimate the standardized ileal digestible Ca requirements of broilers from hatch to day 42 post-hatch.

Item	Starter phase (n = 54)		Grower phase (n = 57)		Finisher phase (n = 32)	
	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic
Body weight gain, g/bird	0.037	0.039	0.001	0.002	0.064	0.119
Feed intake, g/bird	0.574	0.519	0.555	0.640	0.568	0.961
Feed conversion ratio, g:g	0.001	0.002	0.131	0.143	0.003	0.015
Apparent ileal digested Ca, %	0.001*	0.862	0.001	0.647	0.010	0.025
Apparent ileal digested P, %	0.016	0.861	0.003	0.608	0.001	0.426
Tibia ash, %	0.314	0.366	0.001	0.001	0.011	0.107
Tibia ash weight, mg/bone	0.007	0.049	0.027	0.187	0.573	0.651
ATTR[1] Ca, %	0.001	0.006	0.001*	0.160	-	-
ATTR[1] P, %	0.187	0.192	0.652	0.940	-	-

* Significant SID Ca × breed interaction ($P < 0.05$).
[1] ATTR, apparent total tract retention.

IV. DISCUSSION

The results from the current meta-analysis were highly variable and significantly influenced by study location and broiler breed. These factors were included in the statistical model to account for variation between the studies, but this may have resulted in an over-fit model and reduced the applicability of these results outside of this dataset. The impact of breed or lack of a significant interaction between the SID Ca requirement and breed also requires careful consideration as the majority (74%) of the birds in the dataset were of Ross 308 genetics. Additional data will improve the model and predictability in the future.

The influence of dietary Ca on growth performance and bone ash appears less consistent compared with the impact of dietary P. This is reflected in the lower R^2 in the published SID Ca studies (David et al., 2021, 2022; Walk et al., 2021b, 2022a, 2022b) compared with those evaluating non-phytate P responses on growth or tibia ash (Vieira et al., 2015; Yi et al., 1996). This phenomenon is most likely due to the birds' ability to adapt to excess or deficiencies of dietary Ca, especially in long-term performance studies, through hormonal regulation of calcitonin, parathyroid hormone, and vitamin D via the kidneys and the bones (Shafey, 1993). The combination of these factors and the birds' own ability to

adapt to dietary Ca may have all contributed to the variation noted in the meta-analysis. This was particularly true for the finisher phase, in which very few response variables were influenced by the graded levels of SID Ca in the diets; and this limited the ability to estimate the SID Ca requirement using the meta-analysis.

However, for the starter or grower phase, body weight gain, FCR, or tibia ash were responsive to the graded levels of SID Ca in the diets. In this regard, the SID Ca requirement was estimated using the maximum response from quadratic regressions. The quadratic response may over-estimate the requirement compared with a broken-line or a quadratic broken-line (Walk et al., 2022a), but is a good model to estimate toxic or negative effects of both an over- or an under- supply of dietary nutrients (Pesti et al., 2009). In conclusion, a meta-analysis of the SID Ca requirement studies was conducted and the estimated SID Ca requirement for broilers was 0.488 or 0.452% in the starter or grower phase, respectively.

Table 3 - Summary of the estimated standardized ileal digestible (SID) Ca requirements of broilers from hatch to day 42 post-hatch using the maximum response from quadratic regressions.

Item	Starter phase (n = 54)	Grower phase (n = 57)
	Estimated SID Ca	Estimated SID Ca
Body weight gain, g/bird	0.450	0.405
Feed intake, g/bird	0.391*	0.488*
Feed conversion ratio, g:g	0.477	0.400*
Tibia ash, %	0.481*	0.515
Tibia ash weight, mg/bone	0.619	-
ATTR[1] Ca, %	0.562	-
ATTR[1] P, %	0.439	-
Average	0.488	0.452

* Non-significant quadratic effect of SID Ca.
[1] ATTR, apparent total tract retention was estimated using the minimum response for ATTR Ca or ATTR P.

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LIMESTONE QUALITY: IMPLICATIONS FOR POULTRY HEALTH AND PERFORMANCE

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Summary

Limestone is the predominant source of calcium (Ca) used in poultry diets and is most often formulated using estimated Ca content and assumed quality characteristics. However, limestone quality, traditionally determined by its mineral composition and particle size, varies considerably. Beyond these two factors, the rate of solubility has more recently been shown to also contribute to limestone quality. While it is known that limestone composition and particle size vary, less is understood about the rate of limestone solubility and how this may impact Ca and phosphorus (P) digestibility as well as bird performance. Formulating poultry diets using a general book value composition of limestone may result in over or under estimating Ca content and overall nutrient digestibility which may manifest as physiological and performance issues in production. As the poultry industry moves towards adopting digestible Ca values when formulating, it is important to understand the variability in limestone quality and the influence this may play on poultry health and performance.

I. INTRODUCTION

Calcium (Ca) is a dietary essential mineral for poultry and the requirements for poultry have been investigated extensively for more than 75 years (Driver et al., 2005). Calcium is the most prevalent mineral in the body and is important for many physiological processes such as enzyme activation, intracellular signalling, acid base balance, eggshell formation and bone mineralisation (Li et al., 2017). Almost 99% of Ca is stored in the skeleton as hydroxyapatite (Proszkowiec-Weglarz and Angel, 2013); hence the importance of providing sufficient dietary Ca to meet this requirement has dominated much of poultry nutrition's focus. However, the objective of providing adequate Ca for skeletal integrity has often led to an oversupply of Ca in poultry diets which may have unintended antinutritive effects. High dietary Ca concentrations have been reported to impede the availability of minerals such as P, Mg, Mn, and Zn, reduce the efficacy of phytase through the formation of Ca-phytate complexes (Wilkinson et al., 2014).

Demonstrating the influence of dietary Ca concentration on endogenous and exogenous phytase efficacy, Tamim et al. (2004) showed in broilers that were fed diets without supplemental Ca (0.2% total Ca) or exogenous phytase that 69% of phytate-P was hydrolysed (at the ileal level) from corn-soy based diets. However, when birds were fed diets containing 0.7% total Ca, the birds' capacity to hydrolyse phytate-P was reduced to 25%, representing a 44% reduction in endogenous phytase efficacy. Moreover, when diets containing 0.2% total Ca were supplemented with a 6-phytase (500 FTU/kg), phytate-P disappearance increased to be greater than 75%. However, when diets with 0.7% total Ca were supplemented with phytase, phytate-P digestibility only increased to 59%, a result that was considerably lower than for the low Ca diet with no supplemental phytase. It should be noted that the antinutritive effects of 0.7% total Ca observed are lower than the total Ca recommendations for broiler starter and grower diets (Aviagen, 2022). It is therefore important to manage the total amount of Ca in poultry diets.

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II. LIMESTONE QUALITY

Limestone ($CaCO_3$) is primarily added to poultry diets as a cost-effective source of Ca. Limestone in feed can contribute greater than 50% of total Ca consumed and in all-vegetable diets, this value may be as high as 70% (Gilani et al., 2022). Concomitant to the limestone added at the feed mill, limestone may also be used as a carrier in vitamin-mineral premixes and may also be used as a flow agent such as in soybean meal. This may result in significantly more limestone and therefore Ca being added to the ration than intended. The quality of these other sources of limestone is much less likely to be understood yet the same considerations should apply. The utilisation of dietary Ca and P depends on their concentration and availability from the feed that is modulated via intestinal, renal and skeletal mechanisms (Li et al, 2017). Calcium digestibility and metabolism are influenced by several factors, including phosphorus and vitamin D so that any deficiency or excess of one may significantly change the metabolism of the other (Gilani et al., 2022). Recent research has shown that limestone quality (beyond the traditional quality characteristics) can influence Ca and P digestibility and is an important determinant in poultry nutrition (Kim et al., 2019).

a) Mineral composition

Limestone has a maximum Ca concentration of 40.04% based on chemical composition and molecular weight (Gilani et al., 2022), yet it is rare to see commercial limestone this pure. Typically, the Ca concentration of limestone used in animal feeds varies from 38-39% for high quality limestone but it is also possible to see examples with much lower Ca concentrations. Plumstead et al. (2020) reported that the analysed Ca content of limestone ranged from 30.4-40.0%. Lower quality limestone (~34% Ca) with higher levels of impurities such as magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) may also be encountered and potentially bring more to consider when formulating. As mineral nutrition advances and the effect of excess minerals, for example Fe, are better understood, the amount of Fe from limestone may need to be considered to minimise the proliferation of pathogenic microbes in the gastrointestinal tract (Nairz et al., 2018).

b) Particle size and solubility

Other components contributing to the quality of limestone are particle size and solubility. Two broad categories of particle size, coarse (> 1000 μm) and fine (< 1000 μm), are ubiquitously used in poultry nutrition. However, these classifications are not authoritative. *In vitro* limestone solubility has been linked to *in vivo* solubility and Ca digestibility in layers. Broiler studies have shown increased particle size results in increased Ca digestibility (Kim et al., 2019). Rapidly soluble limestone is thought to be less beneficial for broilers as more ionisable Ca is present in the gizzard and proventriculus, presenting greater opportunity for chelation with phytic acid (Kim et al., 2019). More recently, research has shown that limestone particle size is not in and of itself a reliable predictor of solubility rates, and that the geology of the limestone rock also contributes to the rate of solubility. So, while limestone at first glance may appear relatively innocuous, due to the inherent differences in purity, particle size and solubility between limestone sources, inclusion of limestone (from all sources) in poultry diets should be managed closely.

c) Australian context

Recently, 29 limestone samples were collected from Australian sources and analysed for mineral concentration (Table 1), particle size (Tables 2 and 3) and solubility (Figures 1 and 2)

according to the methods of Kim et al. (2019). While limestone is generally thought to contain 38% Ca, the results of this survey show there is substantial variation in Ca concentration and should be accounted for when formulating poultry diets. Of the limestone samples analysed, the highest Ca concentration reported was 38.7% and the lowest was 32.0 %. This represents a difference of 6.7% and may lead to inadequate Ca being provided in the diets if book values are used.

Table 1 - Mineral concentration of samples of limestone used in Australian poultry diets.

Source	Form	Ca (%)	P (%)	Mg (%)	K %)	Na (%)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
NSW	Fine	37.19	0.01	0.49	0.04	0.02	671.2	291.7	15.8	0.0
NSW	Fine	36.89	0.01	0.52	0.03	0.01	631.5	320.2	73.2	0.9
QLD	Fine	37.59	0.01	0.17	0.04	0.02	1422.9	347.7	11.0	2.3
TAS	Fine	33.87	0.01	0.53	0.04	0.02	4131.7	93.2	20.0	5.8
SA	Fine	37.16	0.01	0.32	0.03	0.02	2704.8	385.1	5.8	3.2
QLD	Fine	38.51	0.01	0.17	0.03	0.02	1676.8	351.2	9.3	0.9
SA	Fine	36.17	0.01	0.30	0.03	0.02	2532.3	341.8	11.9	7.6
NSW	Fine	34.74	0.01	1.58	0.03	0.02	1403.5	1596.7	8.9	0.1
NSW	Coarse	36.22	0.01	0.34	0.04	0.02	3729.9	101.3	14.8	1.1
NSW	Fine	38.50	0.01	0.07	0.02	0.02	989.4	44.6	9.2	1.4
NSW	Coarse	38.39	0.01	0.08	0.02	0.01	1032.9	52.1	7.3	0.0
QLD	Fine	38.22	0.01	0.21	0.02	0.02	363.9	93.2	19.1	0.0
QLD	Coarse	36.82	0.01	0.20	0.03	0.02	2458.2	387.1	10.7	3.1
VIC	Fine	37.74	0.01	0.27	0.03	0.02	869.4	124.4	17.1	11.8
VIC	Coarse	37.91	0.01	0.24	0.03	0.02	668.4	111.2	11.8	0.4
VIC	Fine	34.70	0.01	0.40	0.05	0.03	6812.2	177.8	18.7	0.0
QLD	Fine	38.48	0.01	0.22	0.01	0.01	104.3	10.8	12.9	0.0
QLD	Fine	38.48	0.01	0.19	0.02	0.01	106.8	8.5	12.9	0.0
QLD	Coarse	38.31	0.01	0.24	0.02	0.01	81.2	11.5	14.2	0.0
QLD	Fine	38.15	0.01	0.23	0.01	0.01	55.8	7.1	13.1	0.0
NSW	Fine	38.30	0.01	0.08	0.02	0.01	893.8	78.3	5.1	0.0
SA	Coarse	36.90	0.01	0.36	0.05	0.02	2841.6	275.5	4.2	0.9
SA	Coarse	37.70	0.01	0.31	0.02	0.01	1668.3	248.8	3.6	6.9
SA	Fine	37.95	0.01	0.29	0.02	0.02	2027.9	294.6	3.9	0.5
SA	Coarse	37.92	0.01	0.26	0.02	0.01	1891.6	248.0	3.9	0.0
QLD	Fine	38.74	0.01	0.22	0.02	0.01	258.9	64.3	13.9	0.0
QLD	Fine	38.71	0.01	0.22	0.01	0.01	205.4	61.7	12.3	0.0
QLD	Coarse	38.69	0.01	0.20	0.02	0.01	485.3	85.2	15.9	0.0
TAS	Coarse	32.01	0.03	1.25	0.04	0.01	5000.6	262.9	239.1	0.4
Mean		37.27	0.01	0.34	0.03	0.02	1645.5	223.3	21.4	1.6
Max		38.74	0.03	1.58	0.05	0.03	6812.2	1596.7	239.1	11.8
Min		32.01	0.01	0.07	0.01	0.01	55.8	7.1	3.6	0.0
Range		6.73	0.02	1.51	0.04	0.02	6756.5	1589.6	235.5	11.8

Table 2 - Mean particle size of Australian coarse limestone samples used in poultry diets.

Source	Particle Size (Dgw)	Std dev (Sgw)
NSW	2663.94	1301.82
NSW	1488.79	547.42
QLD	1821.03	640.04
VIC	1548.48	801.94
QLD	1868.67	769.1
SA	902.61	1108.9
SA	2587.02	835.29
SA	760.08	814.2
QLD	2109.35	895.03
TAS	821.5	1643.72
Mean	1657.15	935.75
Max	2663.94	1643.72
Min	760.08	547.42
Range	1903.86	1096.30

Table 3 - Mean particle size of Australian fine limestone samples used in poultry diets.

Source	Particle Size (Dgw)	Std dev (Sgw)
NSW	333.39	261.43
NSW	354.15	254.86
QLD	451.98	219.4
TAS	426.24	641.77
SA	203.61	229.72
QLD	507.63	225.54
SA	312.1	413.49
NSW	355.05	243.59
NSW	168.59	145.53
QLD	128.72	60.44
VIC	298.57	296.01
VIC	213.84	309.36
QLD	719.53	422.05
QLD	61.28	39.31
QLD	680.69	458.19
NSW	186.58	157.7
SA	124.2	98.73
QLD	424.14	216.53
QLD	458.2	221.35
Mean	337.29	258.68
Max	719.53	641.77
Min	61.28	39.31
Range	658.25	602.46

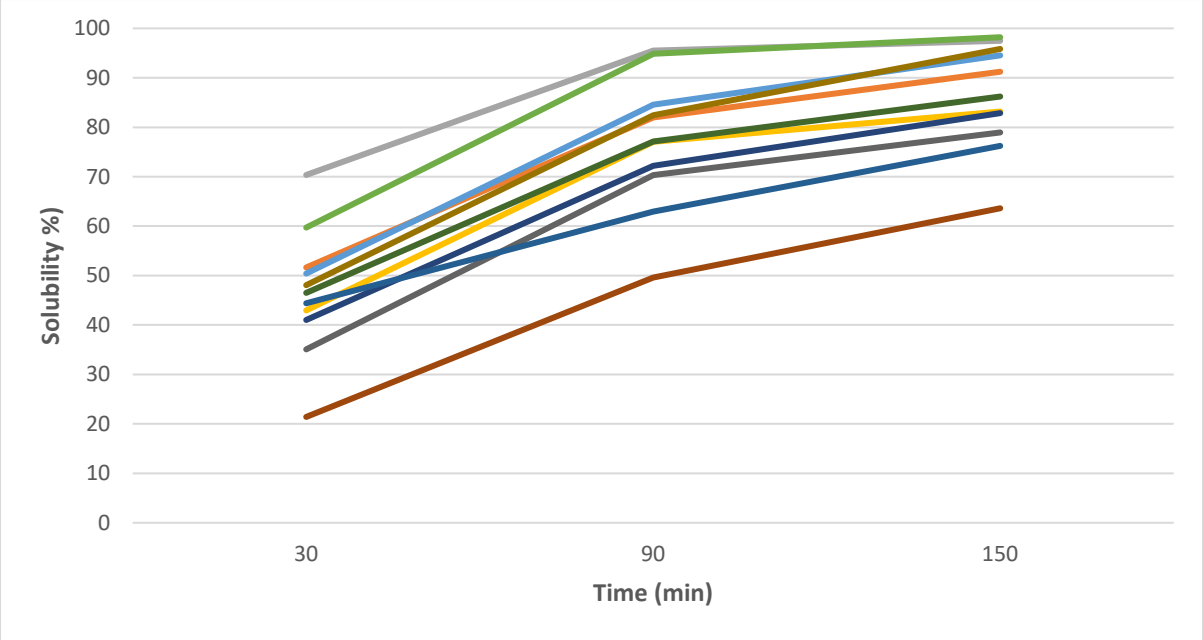

Figure 1 - Solubility (%) over time of coarse limestone samples used in Australian poultry diets.

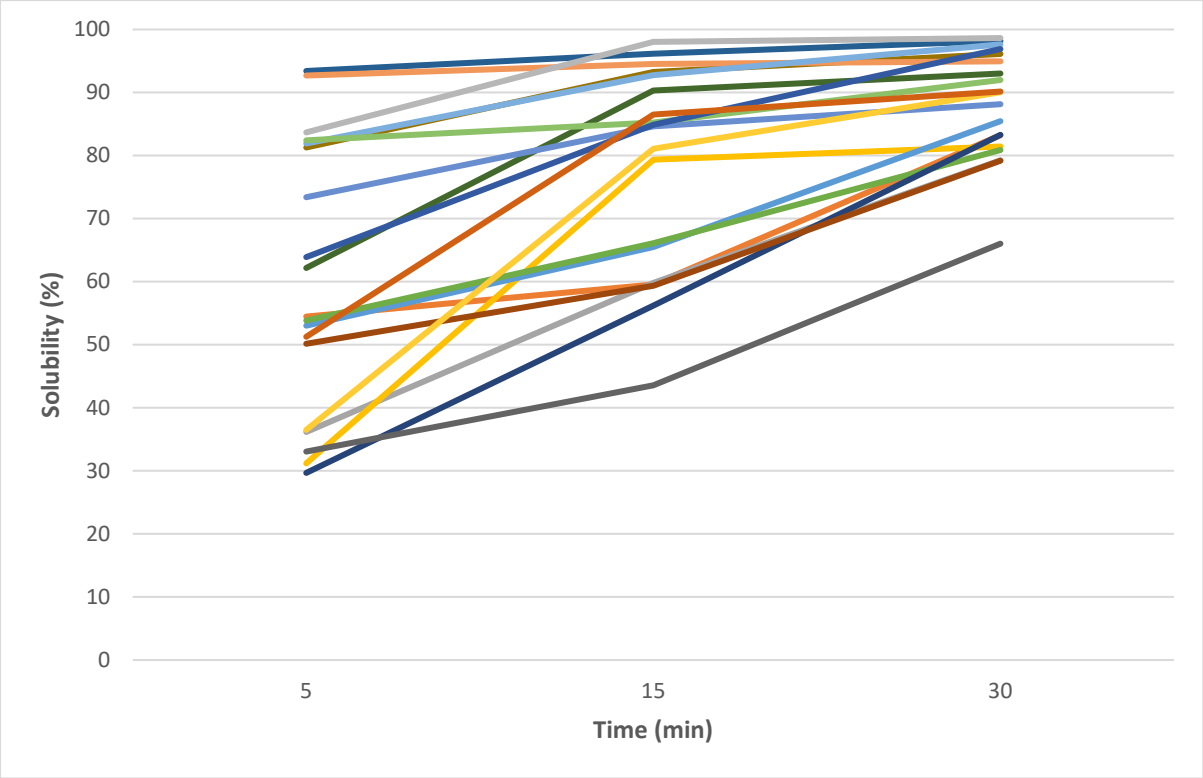

Figure 2 - Solubility (%) over time of fine limestone samples used in Australian poultry diets.

Of the other analysed minerals, variable amounts of Fe, Mn and Mg were also detected. Concentrations of Fe ranged from 55.8-6812 mg/kg, Mn ranged from 7-1596 mg/kg and Mg ranged from 0.07-1.58%. The negative effects of high amounts of divalent cations in diets on phytate destruction and phytase efficacy are well established. However, the effects of excess Fe and its role in the proliferation of pathogenic microbes are perhaps less widely known. The mineral composition results presented herein support the recommendation to closely manage limestone inclusion and account for variations in mineral composition when formulating.

The mean particle size distribution of the Australian limestone samples surveyed are shown in Tables 2 and 3. As expected, there is considerable variation in mean particle size and distribution. Particle size alone is thought to be highly correlated with in vitro solubility and generally, may be useful for this purpose. However, mean particle size does not entirely explain differences in limestone solubility. For example, of the coarse limestone samples, instances of lower solubility at 30 minutes were not correlated with particle size. In fact, the limestone sample with the highest particle size was more soluble at 30 minutes than other samples with lower particle sizes. Comparing the rates of solubility between coarse and fine limestone samples also revealed that some fine limestone samples had lower overall solubility at 30 min compared to some coarse samples. These findings concur with those reported by Kim et al., (2019).

d) Limestone quality: Digestible Ca recommendations

Despite the voluminous amounts of research published that have investigated dietary Ca concentrations required for optimum growth and performance, there has been no general agreement on what the appropriate concentrations of dietary Ca should be and, importantly, little information exists on digestible Ca recommendations. The concept of formulating poultry diets using available or digestible Ca values is not a new concept (Walk et al., 2021). Yet somewhat quizzically, Ca stands alone in poultry nutrition for it remains one of, if not the only, nutrients formulated using total dietary values. Even calcium's nutritional partner, P, is formulated to digestible or available values. For Ca to remain being formulated to a total dietary concentration belies the importance of calcium to the bird as well as the potential for dietary Ca to have antinutritive effects.

Several reasons exist that have undoubtedly contributed to the lack of digestible Ca recommendations, perhaps most notably the inextricable link with P, which makes it difficult to accurately determine digestible Ca requirements. Other reasons cited include the lack of digestible Ca values for common raw materials used in poultry feeds, as well as discrepancies in analysed Ca values of (Walk et al., 2021). These same authors also reference the challenges in interpreting digestible Ca value between research groups

The need to formulate diets close to birds' nutrient requirements is not a new concept; however, as the poultry industry moves toward refining formulations to more accurately reflect their requirements, more information pertaining limestone and Ca are required. Historically, Ca has been over supplied in broiler diets. This is perhaps a product of its relatively inexpensive cost (relative to other raw materials) as well as the objective to provide adequate Ca for bone mineralisation. However, this view only considers the benefits of Ca and does not account for the antinutrient effects of excess Ca. The data presented herein supports the findings of other published work that shows considerable variation in limestone mineral composition and solubility profiles. These may have an impact on Ca digestibility and may need to be considered when formulating. Anwar et al. (2016) showed in birds fed diets containing 0.9% total Ca from one of three different limestone sources had differing Ca digestibility coefficients (0.54-0.61) and in the review by Walk et al. (2021) it is suggested that the variability in Ca digestibility from limestone may be influenced by particle size, solubility as well as the in vivo assay methodology. Formulating to digestible Ca will need to account for these considerations.

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EFFICACY OF A NOVEL PHYTASE IN RESPONSE TO LOW AND HIGH PHYTATE DIETS WITH BROILER CHICKENS AT PRE-STARTER OR STARTER PHASE

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An increase of dietary phytate concentration may limit the availability of both phosphorus (P) and calcium (Ca) as a result of the progressive formation of Ca-phytate complexes along the gastrointestinal tract (Selle et al., 2009). However, inconsistent results were reported regarding the impact of phytate on P and Ca release in the presence of phytase with broilers, presumably due to the differences in phytase characteristics, bird age and feeding duration of P-deficient diets (Angel, 2017). To maintain the digestive and absorptive capacity of birds and prevent adaptation to low Ca and P diets, a 36-h feeding period was applied in the current study, which aimed to evaluate the efficacy of a novel phytase on phytate hydrolysis and apparent ileal digestibility (AID) of P and Ca, in response to low and high phytate diets with broilers during the pre-starter or starter phase, which was at day 9 to 11 or day 19 to 21 post-hatch, respectively.

The experimental diets were in a 2 × 5 factorial arrangement with 2 dietary phytate P concentrations (0.24 or 0.34% at pre-starter, 0.22 or 0.32% at starter) and 5 doses of the novel phytase (HiPhorius™, DSM Nutritional Products, Switzerland) supplemented at 0, 500, 1,000, 2,000 FYT/kg or extradose (3,000 or 4,000 FYT/kg for low or high phytate diet, respectively). The novel phytase was encoded by a 6-phytase gene from *Citrobacter braakii* with great improvement in intrinsic temperature (approximately 20˚C increase for thermal unfolding) and pH stability (retains > 90% activity down to pH 2 in the presence of pepsin, Thorsen et al., 2021). The extradosing of phytase was supplemented for nearly complete phytate hydrolysis when considering the phytate levels in the diets. Ten treatments with 10 replicate cages each were fed to birds for 36-hours at 9 to 11 days of age (12 birds per replicate) or 19 to 21 days of age (8 birds per replicate). Body weight gain (BWG) and feed conversion ratio (FCR) were improved ($P < 0.05$) in birds fed diets supplemented with phytase or diets high in phytate; an interaction between phytase and phytate was observed for 9-11 d FCR ($P < 0.05$), with a lower dose of phytase being able to minimize 9-11 d FCR for birds fed the high phytate diet. Phytase supplementation improved phytate P degradation, the AID of P and Ca, the AID of P for non-phytate P fraction and the apparent ileal digestible P and Ca ($P < 0.05$). However, the degree of improvement was influenced by phytate and related to bird age. Briefly, phytase increased digestible P to a greater extent in high phytate diet compared with low phytate diet, regardless of bird age. For birds at pre-starter phase, the ileal phytate P degradation, AID of P and ileal digestible P of low or high phytate diets achieved the maximal values with phytase at 2,000 or 4,000 FYT/kg, respectively, whereas this phytate and phytase interaction was not detected for birds at starter phase. The responses of AID of Ca and ileal digestible Ca were similar for the two phases, with a lower dosage of phytase at 1,000 or 500 FYT/kg (pre-starter or starter) achieving the maximal responses for high phytate diet compared to the 2,000 or 1,000 FYT/kg inclusion (pre-starter or starter) for low phytate diet. In conclusion, phytase effectively hydrolyzed phytate, increased digestible P and Ca, and improved growth performance of broilers. The impact of dietary phytate on phytase efficacy differed depending on bird age.

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EFFECTS OF COMMERCIAL PROCESSING CONDITIONS OF EXTRUDED SOYBEAN ON GROWTH PERFORMANCE AND AMINO ACIDS DIGESTIBILITY OF BROILER CHICKENS

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Summary

Expeller Soybean (SB) is commonly used as the main protein source in broiler diets due to its high amino acid digestibility. Optimal SB processing is required to ensure that antinutritional components that negatively interfere with digestion, absorption, and metabolism of nutrients leading to lower growth performance, are deactivated. In this study, we investigated the effect of 3 commercial expeller SB batches processed at different temperatures, on growth performance, intestinal integrity and amino acid digestibility. A total of 1,860 male Cobb 500 broiler chicks were randomly allocated to the 3 different treatments with 10 replicate floor pens (62 birds/pen) from 0 to 35 d of age. The 3 expeller SB batches were processed with different extruder temperatures of 182, 199, and 154 °C for normal-control (NC), overcooked (OC), and undercooked (UC) SB, respectively. Performance parameters, body weight gain (BWG), feed intake (FI), and feed-to-gain ratio (FCR) were recorded on d 0, 14, 28, and 35. Intestinal integrity was assessed in one bird per pen by determining serum fluorescein isothiocyanate-dextran (FITC-d; 4 kD) concentrations for gut permeability at d 16. The relative weights of the right pectoralis major (RPM) were determined at 35d of age. Intestinal permeability increased in birds fed the OC SBM ($P < 0.05$). On d 14, 28, and 35, birds given the OC SBM diet had the lowest BWG and FI and the highest FCR, as well as the smallest RPM ($P < 0.05$). Both OC and UC expeller SB reduced the mean apparent ileal digestibility (AID) of all non-essential and essential amino acids at d 14 ($P < 0.05$) by 3.8% and 3.2%, respectively. The AID of Lys was 7% lower ($P < 0.05$) in birds fed the OC SBM batch compared to the NC treatment at d14 and 28. The adverse effects of OC expeller SB on BWG were driven by digestible amino acid (AA) intake, which was lower ($P<0.05$) for Lys, Met+Cys, and Thr in the OC treatment compared to the NC and the UC treatment groups. In conclusion, these results showed that inappropriate SB processing can lead to a lower AA digestibility resulting in lower growth performance and economic losses. Extruded soybean results from mechanical extraction of oil from soybeans (SB) (Ravindran et al., 2014). During processing, soybeans are exposed to heat treatment to remove antinutritional factors and to increase the nutritional value of extruder SB for optimal bird growth performance. Variations in SB processing conditions, such as moisture, drying time, and toasting and drying temperature, lead to variability in the trypsin inhibitors(TI) amount ingested by poultry between SB batches (Karr-Lilienthal et al., 2004).

I. INTRODUCTION

Both overheating and underheating affect the nutritional value and quality of SB (Karr-Lilienthal et al., 2004). Undercooked SB results in an excessive concentration of antinutritional factors, such as TI, decreasing the intestinal activity of pancreatic proteases. In contrast, excessive heat treatment results in a Maillard reaction that reduces nutrient digestibility (Araba and Dale, 1990). Thus, the objective of this study was to investigate the contributions of poorly processed expeller SB on bird growth performance, amino acid digestibility and intestinal integrity of broiler chickens.

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II. METHOD

Male Cobb 500 chicks (1,860) were obtained from the Cobb-Vantress® hatchery (Cleveland, GA, US). Before placing, birds were vaccinated with a commercial coccidia vaccine Coccivac®-B52 (Merck Animal Health, Kenilworth, NJ, U.S.) at The University of Georgia's Poultry Diagnostic and Research Center (Athens, GA). After vaccination, birds were randomly distributed into 3 treatments (10 replicate pens per treatment; 62 birds per pen) such that mean BW was not different between pens. The treatments from d1 to d35 consisted of a expeller soybean-maize diet with SB processed with different extruder temperatures of 182, 199, and 154 °C for normal-control, overcooked, and undercooked SB, respectively (Perdue Agribusiness LLC). Extruder SB was fed as a fixed inclusion in the diet, which notably varied by CP (dry matter basis) by 50.7, 49.1, and 48.8 % amongst the control, overcooked, and undercooked, respectively. Basal diets consisted of a expeller-SB concentration of 35, 30, and 25% for the starter (1-14d), grower (15-28d), and finisher (29 to 35d) with 0.5% inclusion of titanium dioxide as an indigestible marker. The fixed inclusion of extruded SB during the dietary phases was aimed to reflect processing variations seen when producing commercial diets.

Bird body weight (BW) and feed intake by pen were recorded on d 0, 14, 28, and 35 to evaluate body weight gain (BW gain) and mortality-adjusted feed conversion ratio (FCR). On d 35, five birds per pen were randomly selected and humanely euthanised by carbon dioxide. The weight of the right pectoralis major was recorded after excision, and the relative weights within a pen were determined at 35 d of age. Data were analyzed as one-way ANOVA using JMP software (JMP Software, Cary, NC, U.S.), with the pen as an experimental unit. Tukey's multiple comparison test was used to compare the significant means ($P < 0.05$).

III. RESULTS

Intestinal permeability increased in birds fed the OC SBM (P <0.05). On d 35, birds given the OC SBM diet had the lowest BWG and FI and the highest FCR, as well as the smallest RPM (P <0.05) (Table 1). Both OC and UC expeller SB reduced the mean apparent ileal digestibility (AID) of all non-essential and essential amino acids at d 14 (P <0.05) by 3.8% and 3.2%, respectively. The AID of Lys was 7% lower (P <0.05) in birds fed the OC SBM batch compared to the NC treatment at d14 and 28 (Table 2). The adverse effects of OC expeller SB on BWG were driven by digestible amino acid (A.A) intake, which was lower (P<0.05) for Lys, Met+Cys, and Thr in the OC treatment compared to the NC and the UC treatment groups (Table 3).

Table 1 - Effects of commercial processing conditions of extruder SB on growth performance and intestinal permeability of broilers.

Treatments	BWG(g)	FI(g)	FCR	FITC-d translocation (ng/mL)	Right pectoralis major (RPM)	
					Weight (g)	Relative (%)
Normal	2207a	3995a	1.719b	562.4b	168a	7.32a
Overcooked	1391c	3580b	2.330a	699.0a	88b	5.77b
Undercooked	2083b	3957a	1.815b	593.0b	162a	7.37a
SEM	77	63	0.061	19.1	8	0.24
P-value	<.0001	0.01	<.0001	<0.01	<.0001	0.01

[abc]Means in column with no common superscripts are significantly different (P<0.05).
[1]Means represent 10 pens of broiler chickens.
IAA: Indispensable amino acids, DAA: Dispensable amino acids.

Table 2 - Effects of commercial processing conditions of extruder SB on apparent ileal digestibility coeficients of amino acids at 14 and 28 days.

					Day 14	
Treatments	Lys	Met	M+C	Thr	IAA	DAA
Normal	88.7a	92.7	85.2	79.4a	86.9a	83.3a
Overcooked	81.5b	91.5	83.1	74.4b	83.6b	78.4b
Undercooked	87.1a	91.9	83.7	75.9b	84.2b	80.3b
SEM	0.644	0.223	0.370	0.583	0.402	0.515
P-value	<.0001	0.081	0.052	<.001	<.001	<.0001
					Day 28	
Treatments	Lys	Met	M+C	Thr	IAA	DAA
Normal	83.5a	92.3a	84.3a	76.5a	84.7a	81.0a
Overcooked	76.2b	89.3b	82.0b	72.0b	82.4ab	76.7b
Undercooked	83.2a	90.4ab	81.4b	72.9b	82.0b	79.2a
SEM	0.890	0.447	0.411	0.639	0.449	0.509
P-value	<.001	0.010	0.006	0.005	0.030	<.001

[ab]Means in column with no common superscripts are significantly different (P<0.05).
[1]Means represent 10 pens of broiler chickens.
IAA: Indispensable amino acids, DAA: Dispensable amino acids

Table 3 - Effects of commercial processing conditions of extruder SB on digestible amino acid intake from 0 to 35 days.

			Amino acid intake 0-35d (g)			
Treatments	BWG(g)	FI(g)	Lys	Met	M+C	Thr
Normal	2207a	3995a	13.7a	5.7a	9.5a	9.1a
Overcooked	1391c	3580b	10.8b	5.1b	7.9b	7.7b
Undercooked	2083b	3957a	13.1a	5.6a	9.2a	8.6a
SEM	77	63	0.311	0.407	0.193	0.164
P-value	<.0001	0.01	<.0001	0.010	<.0001	<.001
			Amino acid intake 0-35d (g)			
Pearson correlation			Lys	Met	M+C	Thr
BWG(g)			0.86	0.66	0.82	0.74
P-value			<.0001	0.001	<.0001	<.0001

[ab]Means in column with no common superscripts are significantly different (P<0.05).
[1]Means represent 10 pens of broiler chickens.

IV. DISCUSSION

Varying the extruder temperature of SB batches during processing had a negative impact on protein quality in terms of nutrient density and antinutritional components. Such physicochemical and structural differences observed amongst the three SB batches may disrupt the digestion and absorption of nutrients within the intestine, affecting growth performance, muscle accretion, and intestinal integrity.

Increased serum FITC-d concentration is indicative of paracellular permeability due to inflammation and disruption of the intestinal mucosa barrier (Liu et al., 2021). In this study, birds consuming overcooked SB showed a greater serum FITC-d concentration at d 16. The Maillard reaction products includes glycation end-products, and they are digestion resistant and accumulate in the intestinal mucosa resulting in gut barrier damage and intestinal inflammation (Webster et al., 2005). Thus, the greater FITC-d concentration observed in birds consuming overcooked SB may be related to Maillard reaction products with pro-inflammatory capacity impairing the intestinal barrier function (Oh et al., 2017).

The reduced BW at d35 observed in birds consuming the overcooked SB was significantly correlated with the decreased cumulative amino acid intake observed from d 0 to 35, particularly for Lys, Met, M+C, and Thr. Differences in BWG, FCR, and muscle accretion were likely due to decreased amino acid intake and Maillard reaction, with the lowest bird performance shown in birds consuming overcooked SB (Aburto et al., 1998; Parsons et al., 1992). These findings suggest that both differences in nutritional composition and physical components of expeller SB have robust effects on bird performance, particularly when SB has been overheated.

In conclusion, these findings stress the importance of optimizing a safe extrusion temperature range without penalty of the SB nutrient value and preventing processing and live production costs in field operations. It is necessary to have quality assurance programs on CP quality with appropriate methods to determine poor SB processing conditions in an effective manner.

ACKNOWLEDGEMENTS: We acknowledge Perdue AgriBusiness LLC for providing the commercial expeller SB for this project.

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INCREASING THE DOSE OF A NOVEL CONSENSUS BACTERIAL 6-PHYTASE VARIANT REDUCED P AND N EXCRETION IN BROILERS FED DIETS WITHOUT INORGANIC PHOSPHATE FROM 10 DAYS OF AGE

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Summary

In broilers fed a low inorganic phosphorus (P) starter and inorganic P-free grower and finisher diet, increasing the dose of a novel consensus bacterial 6-phytase variant (PhyG) exponentially reduced P and nitrogen (N) excretion. The data demonstrated that increasing the phytase dose to further replace added inorganic P is a potential strategy to decrease nutrient excretion and improve sustainability in broiler production.

I. INTRODUCTION

Phytase is commonly used in broiler diets at a typical dose level of 1000 FTU/kg feed to increase the P availability from phytate and reduce the need for inorganic phosphate inclusion. Increasing the phytase dose above this level is expected to further reduce the need to add inorganic phosphate and further reduce P excretion. In addition, if a phytase can break down phytate quickly and completely in the upper gastrointestinal tract, it will mitigate the anti-nutritional effect of phytate, resulting in improved amino acid (AA) digestibility and reduced N excretion. Recent studies have reported that a novel consensus bacterial 6-phytase variant (PhyG) at 1000, 2000 and 4000 FTU/kg feed can break down 88, 97 and 100% of inositol hexaphosphate (IP_6), respectively, in a mixed grain diet containing 3 g/kg phytate-P (Dersjant-Li et al, 2022a) as well as improve P and AA digestibility with increasing phytase dose (Babatunde et al., 2021, 2022; Dersjant-Li et al., 2022b). This study evaluated the effect of increasing the dose of PhyG on P and N excretion and bone ash in broilers, compared to control diets with increasing level of digestible P from monocalcium phosphate (MCP).

II. METHOD

A basal diet (negative control, NC) was formulated based on wheat, corn and soybean meal (SBM), with inclusion of 5–7% rapeseed meal and 1.4–2.2% rice bran, meeting nutrient and energy requirements (CVB, 2019) except for P and calcium (Ca). The basal diet contained 1.2 g/kg MCP (providing 0.23g/kg digestible P) in starter (0–10 d) and was inorganic phosphate-free in grower (10–21 d) and finisher (21–35 d). The NC diets were supplemented with MCP to provide 0.6 g/kg (PC1), 1.2 g/kg (PC2), 1.8 g/kg (PC3) or 2.4 g/kg (PC4) digestible P from MCP; PC4 was considered a commercially relevant control diet containing 4.2, 3.9 and 3.8 g/kg digestible P in starter, grower and finisher phases, respectively. Levels of Ca were adjusted in the PC diets to maintain $Ca:P_{total}$ between 1.23 and 1.3. The NC was supplemented with 250, 500, 1000, 2000, 3000 or 4000 FTU/kg feed of PhyG (Axtra® PHY GOLD, Danisco Animal Nutrition & Health, IFF). No additional digestible AA or energy matrix was applied in order to maintain a consistent feed composition of the major ingredients in all diets with the only variables being MCP and limestone content. A filler was used to maintain dietary composition at 100%. Ross 308 male broilers were used with 6 replications of 20 birds/pen at the trial start.

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Celite was used as a digestibility marker. Diets were fed as pelleted diets *ad lib* and water was freely available. The analysed phytate-P content across control diets was 3.3, 3.1 and 2.8g/kg in starter, grower and finisher diets respectively. Table 1 summarizes the formulated digestible P, analysed total P, Ca and crude protein in the 5 control diets, in each feeding phase. Total excreta samples were collected from 6– 9, 17–20 and 31–34 d of age for P and N excretion measurements. Pooled toe samples from 4 birds/pen at day 10, 21 and 35 were collected for ash analysis. Linear and quadratic, or exponential curve fitting was performed to model the effect of increasing MCP level and of increasing phytase dose on toe ash, Ca, P retention, P and N excretion, respectively.

Table 1 - Formulated (Form) digestible P, analysed (Ana) total P, Ca and crude protein (CP) in control diets (g/kg as fed).

		NC		PC1		PC2		PC3		PC4	
		Form	Ana	Form	Ana	Form	Ana	Form	Ana	Form	Ana
Starter	Total P	5.1	4.1	5.8	4.8	6.5	5.1	7.2	6	7.9	6.5
	Digestible P	1.8		2.4		3		3.6		4.2	
	Ca	6.7	7.3	7.4	7.4	8.2	8.3	8.9	10	9.7	10
	CP	209	209	209	209	209	206	209	212	209	210
Grower	Total P	4.6	3.7	5.3	4.2	6	5.2	6.7	5.4	7.4	6.2
	Digestible P	1.5		2.1		2.7		3.3		3.9	
	Ca	6	6.5	6.7	7.3	7.5	8.3	8.2	9.1	9	9.9
	CP	197	199	197	200	197	198	197	196	197	199
Finisher	Total P	4.3	3.7	5	4.1	5.7	4.9	6.4	5.4	7.1	5.6
	Digestible P	1.4		2		2.6		3.2		3.8	
	Ca	5.7	6.1	6.5	6.2	7.2	750	8	8.6	8.7	8.5
	CP	185	189	185	189	185	187	185	190	185	183

III. RESULTS

Bone ash results are presented in Table 2. Increasing MCP inclusion linearly or quadratically increased toe ash percentage. Increasing phytase dose exponentially increased toe ash percentage at all three timepoints. At all three timepoints, toe ash in treatment PhyG2000 FTU/kg was maintained equivalent to PC4; at d 35, toe ash in treatment PhyG3000 FTU/kg feed was numerically improved vs. PC4.

The effect of MCP inclusion and phytase dose on P and Ca retention, retainable P and total tract P excretion is presented in Table 3. Increasing phytase dose improved P and Ca retention and reduced P excretion in each phase. However, the reduction in P excretion was greater in starter and grower than in finisher phase. Phytase at 1000 FTU/kg reduced P excretion by 71 (starter), 71 (grower) and 65% (finisher) compared to PC4. Increasing the phytase dose from 1000 FTU to 2000 or 3000 FTU/kg further reduced P excretion (by 11 and 16%, respectively, in grower phase). Similarly, the improvement in Ca retention with increasing phytase dose was greater in starter and grower than in finisher phase (Table 3). In all phases, increasing the digestible P-from-MCP content of the diet linearly increased retainable P and P excretion, whilst in finisher phase the response to P retention was quadratic.

Across all three timepoints, increasing the phytase dose decreased total tract N excretion (Figure 1 left) and exponentially decreased P excretion (Figure 1 right). At dose level of 1000 and 2000 FTU/kg feed the phytase reduced N excretion by 5.3 and 6.0%, and reduced P excretion by 66.4 and 69.3%, respectively, vs PC4.

Table 2 - Effect of increasing MCP concentration or phytase dose on toe ash (%).

Digestible P from MCP above NC, g/kg	Phytase (PhyG), FTU/kg feed	d10	d21	d35
2.4 (PC4)		14.66	12.73	12.60
1.8 (PC3)		14.18	12.11	11.91
1.2 (PC2)		12.87	11.48	11.40
0.6 (PC1)		11.04	10.21	10.90
0.0 (NC)	0	8.94	9.50	9.94
	250	11.97	10.65	10.92
	500	11.76	10.83	11.53
	1000	14.19	12.06	12.02
	2000	14.52	12.86	12.45
	3000	14.91	12.91	13.08
	4000	14.71	12.94	13.20
SEM		0.225	0.177	0.287
P linear MCP[1]		<.0001	<.0001	<.0001
P quadratic MCP[1]		<.0001	0.1471	0.6478
P linear phytase[2]		<.0001	<.0001	<.0001
P exponential phytase[2]		<.0001	<.0001	0.0046

[1] Linear or quadratic response with increasing digestible P level from supplemented MCP above NC.
[2] Linear or exponential response with increasing phytase dose.

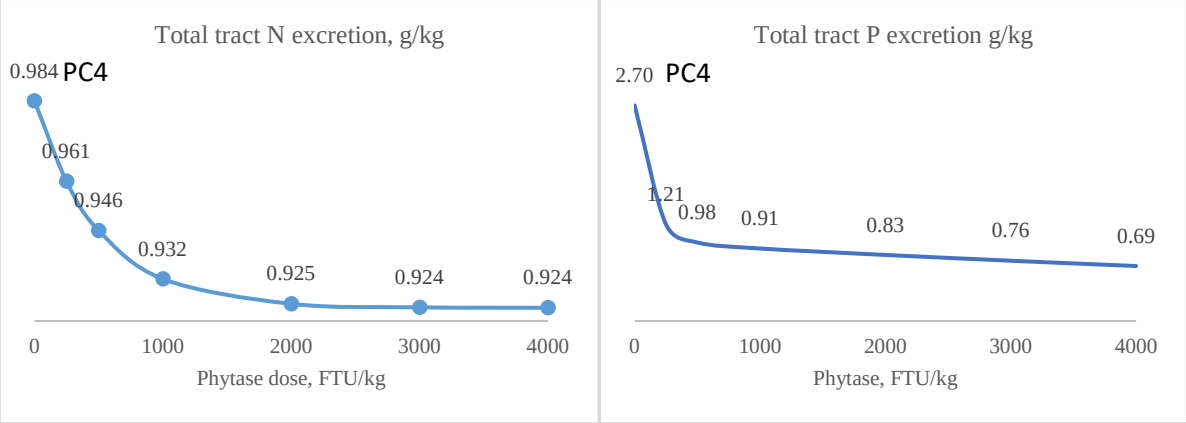

Figure 1 - Effect of increasing phytase dose on total tract P and N excretion, data analysed across all three timepoints).

IV. DISCUSSION

The retainable P in PC4 was 4.2, 3.4 and 2.6 g/kg respectively in starter, grower, and finisher phase, representing, respectively, 64, 55 and 46% of P intake. This indicated that the starter diet met the expected target of 4.2 g/kg digestible P. However, in grower and finisher phase, the formulated P exceeded the requirement and led to greater P excretion, despite the lower analysed total P content of the diet. Clearly, for maximizing P release, the optimal phytase dose level should be linked to both the dietary P requirement and phytate substrate level of the diet. However, increasing the phytase dose up to 2000 FTU/kg can further reduce N excretion due to extra-phosphoric effects. Recent studies have reported that when PhyG is applied in a phased dosing strategy to plant-based diets containing sufficient phytate substrate, inorganic P can be totally removed from day 1; bone ash, BW and FCR were maintained comparable to a nutritionally adequate control diet and to breeder's performance objectives (Bello et al., 2022; Dersjant-Li et al, 2022c; Marchal et al, 2021). In conclusion, these data indicate that by increasing the dose of a highly effective phytase, it is feasible to further replace inorganic P in the diet, further reduce P and N excretion and enhance sustainable chicken meat production.

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Table 3 - MCP inclusion and phytase dose effect on P and Ca retention (Ret, %), retainable P (Ret P, g/kg) and total tract P excretion (exc, g/kg).

Sampling age		6–9 d					17–20 d					30–34 d				
dig P from MCP above NC	Phytase	Ca ret	P ret	Ret P	P exc	% P exc	Ca ret	P ret	Ret P	P exc	% P exc	Ca ret	P ret	Ret P	P exc	% P exc
g/kg	FTU/kg	%	%	g/kg	g/kg	vs PC4	%	%	g/kg	g/kg	vs PC4	%	%	g/kg	g/kg	vs PC4
2.4 (PC4)		61.0	64.3	4.16	2.30		49.9	54.7	3.39	2.81		47.4	46.4	2.60	3.01	
1.8 (PC3)		61.4	63.9	3.82	2.15		55.4	57.4	3.09	2.29		50.8	52.2	2.83	2.59	
1.2 (PC2)		57.9	64.2	3.26	1.82		48.6	58.4	3.02	2.15		52.2	58.3	2.90	2.07	
0.6 (PC1)		53.5	65.8	3.21	1.67		44.1	54.8	2.28	1.88		49.2	58.7	2.41	1.70	
0 (NC)		59.0	65.0	2.69	1.45		46.5	58.8	2.20	1.54		53.5	63.8	2.40	1.36	
	250	66.9	74.6	3.09	1.05	-54	45.1	64.6	2.42	1.33	-53	59.7	68.1	2.55	1.20	-60
	500	66.3	75.8	3.14	1.00	-57	56.6	71.4	2.67	1.07	-62	61.0	70.4	2.64	1.11	-63
	1000	73.6	83.7	3.47	0.67	-71	63.1	78.1	2.93	0.82	-71	64.8	72.2	2.71	1.04	-65
	2000	74.4	85.1	3.52	0.62	-73	66.6	80.6	3.02	0.73	-74	67.3	70.4	2.64	1.11	-63
	3000	74.1	85.7	3.55	0.59	-74	68.2	81.7	3.06	0.69	-76	68.2	72.6	2.72	1.03	-66
	4000	75.6	86.8	3.60	0.55	-76	70.4	85.4	3.20	0.55	-81	69.9	73.5	2.76	0.99	-67
SEM		0.99	0.98	0.05	0.05		2.37	1.34	0.06	0.06		1.39	1.04	0.04	0.04	
P linear MCP[1]		0.014	0.428	<.0001	<.0001		0.028	0.311	<.0001	<.0001		0.022	<.0001	0.002	<.0001	
P quadratic MCP[1]		0.090	0.900	0.606	0.863		0.715	0.746	0.499	0.481		0.022	0.062	<.0001	0.069	
P linear phytase[2]		<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	
P exponential phytase[2]		<0.0001	<0.0001	<0.0001	<0.0001		0.0013	<0.0001	<0.0001	<0.0001		0.0002	0.0034	0.003	<0.0001	

[1] Linear or quadratic response with increasing digestible P level from supplemented MCP, above NC.
[2] Linear or exponential response with increasing phytase dose.

PRECISION FEEDING OF EGG- AND MEAT-TYPE PULLETS

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Summary

Precision feeding is the practice of providing the right amount of the right feed to the right bird at the right time. Sensors detect the state of the animal and/or the environment, and an optimal strategy is chosen and implemented in real-time. This strategy may look different depending on the type of poultry being fed. In meat-type pullets, feed restriction must be practiced to prevent obesity- and body weight-related declines in welfare and chick production. Thus, the right amount of feed is a much lower amount than each bird would choose to eat *ad libitum*. In layer pullets, where feed intake is often limiting, it may involve providing different formulations to stimulate growth and development. In group housed birds, these scenarios require different specialized precision feeding equipment. Precision feeding systems were used to evaluate the impact of pullet rearing strategies on the reproductive efficiency of both laying hens and broiler breeders.

I. INTRODUCTION

Modern broilers grow quickly because they have tremendous genetic potential due to intensive selection for increased growth rate and efficiency (Zuidhof et al., 2014). The parents, broiler breeders, carry this genetic potential and when fed *ad libitum*, easily become overweight (Heck et al., 2004), compromising reproductive performance (Renema and Robinson, 2004; Chen et al., 2006) and reducing welfare (Mench, 2002). Thus, broiler breeder hens are typically feed restricted during rearing, and to a lesser extent during the lay period. However, despite increased genetic potential, breeder-recommended body weights (BW) have remained virtually unchanged over the last 40 years (Renema et al., 2007). Thus, the severity of feed restriction has increased. Severe feed restriction decreases nutrient availability in the body, like fat, which may affect body composition and might delay the onset of lay in modern broiler breeder hens. When fed precisely to a breeder-recommended BW by providing multiple meals per day, broiler breeder pullets partitioned energy to lean rather than adipose tissue. Thus, they were very lean, and under suboptimal lighting conditions, in particular, many did not undergo sexual maturation in a timely manner (van der Klein et al., 2018). Thus, it is likely that BW recommendations are becoming too severe, and new optimal growth trajectories are needed.

Laying hens have the opposite feed intake problem compared with broiler breeders. For many layer lines, it is challenging to get the birds to eat enough to sustain their health, welfare and productivity. It is well known that feed composition and feed intake affect pullet growth and development (Kwakkel et al., 1995; Traineau et al., 2015). As with broiler breeders, it is equally important to ensure that the pullet is in optimal metabolic and physiological condition around the time of sexual maturation. However, rather than restricting feed intake, optimal diet formulation is likely a more desirable approach to achieve optimal condition prior to the laying period. This feeding strategy is more complex and requires more a specialized system.

At the University of Alberta, recent precision feeding studies with broiler breeders and layers have been conducted with the objective of managing nutrient intake such that the pullets achieve optimal body condition to enter into lay and sustain production of eggs of the highest quality. This paper describes different precision feeding approaches for broiler breeders and

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layer pullets. Both had the same goal of achieving optimal body condition around the time of sexual maturation to maximize reproductive efficiency. The broiler breeder studies focussed on optimizing the growth trajectory, while the layer study examined mild growth restriction in combination with dietary energy levels during rearing. The objective of these studies was to evaluate the impact of pullet growing strategies on sexual maturation and reproductive efficiency.

II. RESEARCH WITH MEAT-TYPE PULLETS

a. Study methodology

Two concurrent broiler breeder precision feeding experiments were conducted. Both consisted of a set of 12 growth trajectories in 2 x 6 factorial arrangements. A total of 576 day-old Ross 308 broiler breeder females were randomly assigned to various BW trajectories, with 24 birds per growth trajectory. Birds on all treatments were housed together in 3 large floor pens, and the various treatments were applied to each individual bird (experimental unit). Birds in both experiments were managed using a precision feeding system (Zuidhof et al., 2017), which enables the implementation of different growth curves in free-run birds. In both studies, the BW trajectories were designed using a 3-phase Gompertz model (Zuidhof, 2020) to fit the breeder-recommended target (Aviagen, 2021) as follows:

$$BW_t = \sum_{i=1}^{i=3} g_i \, exp^{(-\exp(-b_i(t-I_i)))}$$

where BW_t was body weight (kg) at time t (wk); g_i was the amount of gain (kg) occurring in phase i; b_i was the rate of maturing of phase i; and I_i was the inflection point of phase i (wk).
In experiment 1 (Exp1), growth trajectories had 2 levels of early growth: standard, where g_1 was equivalent to phase 1 growth estimated from the breeder-recommended target; or a 20% increase, 20% shift from g_2 (pubertal growth) to g_1 (pre-pubertal growth); and six I_2 (timing of pubertal growth spurt; PGS) ranging from 15 to 23 wk, 21 wk being the breeder-recommended target I_2 (*Figure 1*). Experiment 2 (Exp2) growth trajectories had two rates of b_2 (pubertal rate of growth; PR): standard or 50% faster; and six early growth levels, shifts of growth from g_2 to g_1 ranging from -10 to 40% of breeder-recommended g_2 (*Figure 2*).

After a training period, individual feeding in the precision feeding system started at 14 d of age. Feed intake and BW were recorded, and feed conversion ratio (FCR) was calculated for the rearing period (0 to 21 wk of age). Photostimulation occurred at 21 wk of age with 11L:13D increasing one hour of light per week until 13L:11D (30 lux). Dissections were done at 21 wk of age, with one bird per growth curve being dissected. Five birds per growth trajectory were dissected at the sexual maturation time point, the day of the first egg being laid. In both dissections, abdominal fat pad weight was recorded.

Analysis of variance was performed for FCR (two-way ANOVA for both trials) and analysis of covariance for abdominal fat pad and total egg production variables with early growth as a discrete source of variation and pubertal growth spurt as a continuous variable (Exp1). For Exp2, pubertal growth rate was a discrete source of variation and shifts to earlier growth were continuous. The MIXED procedure of SAS (Version 9.4, SAS institute Inc., Cary, NC) was used.

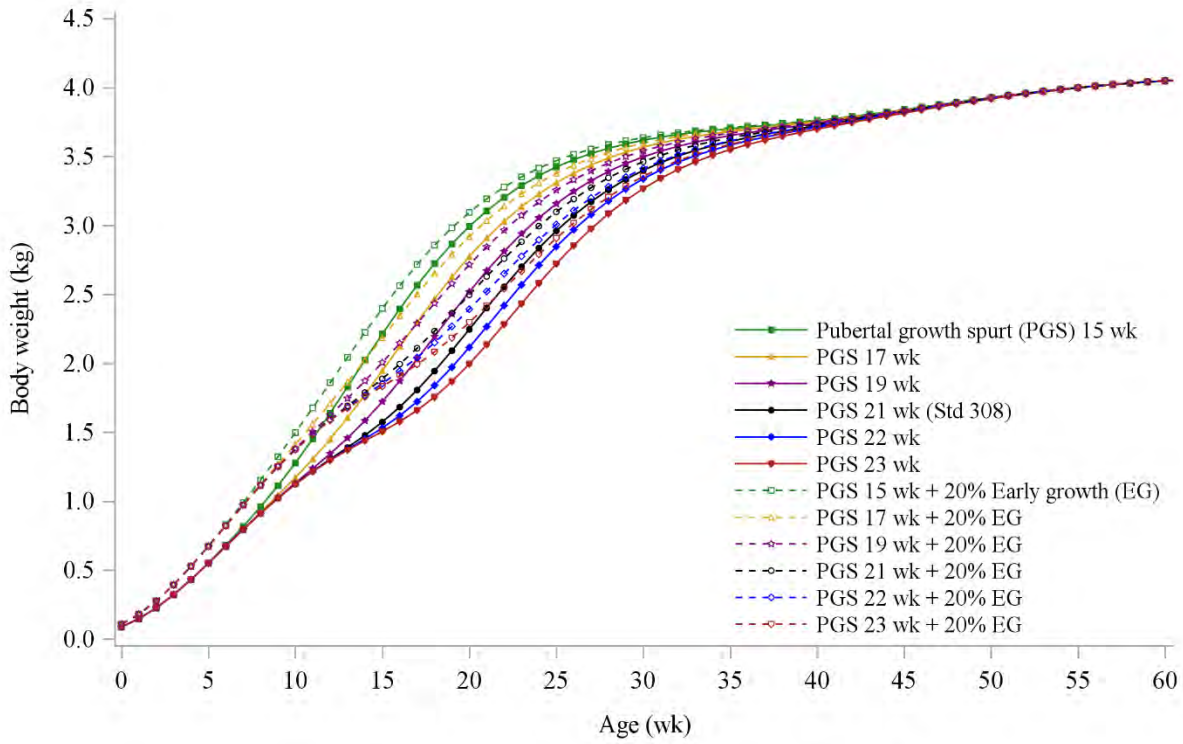

Figure 1 - Growth trajectories designed for broiler breeders (Experiment 1).

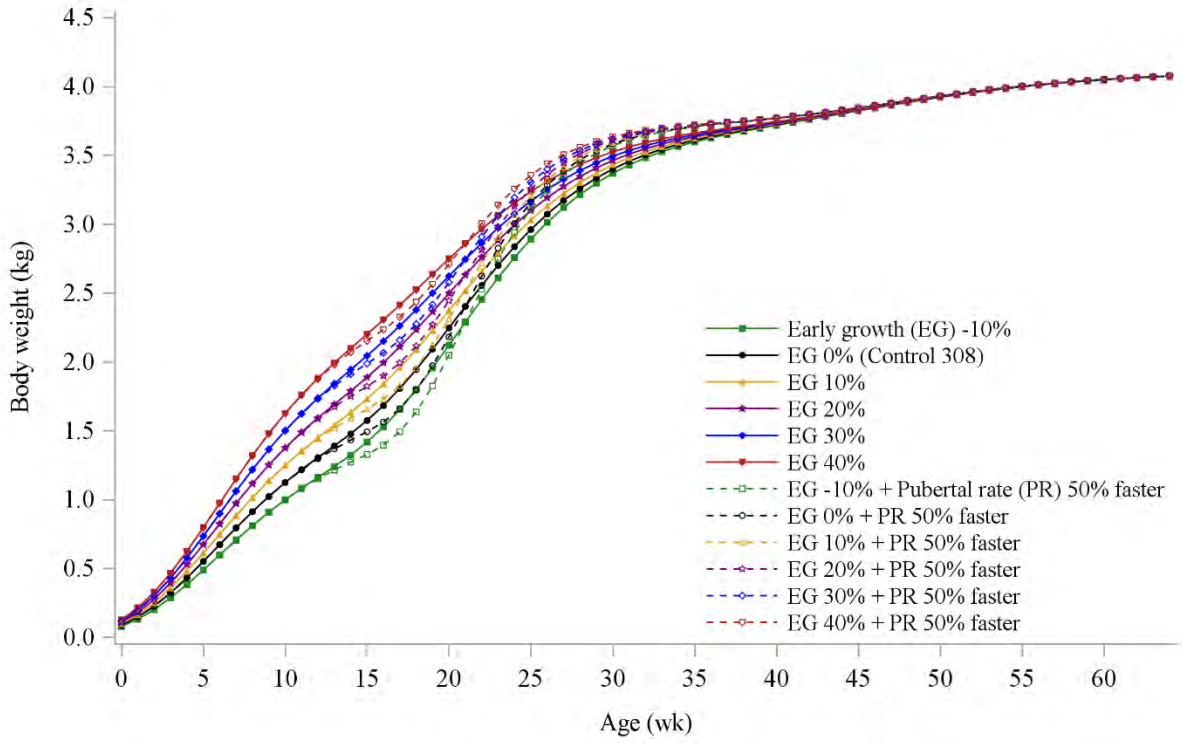

Figure 2 - Growth trajectories designed for broiler breeders (Experiment 2).

b. Results

Feed conversion ratio (0 to 21 wk of age) was not affected by the timing of the pubertal growth spurt nor pubertal growth rate during the rearing phase. However, in both experiments FCR increased as second phase growth was shifted to the first phase ($P < 0.05$), reflecting the greater cost of maintenance of a larger body through the rearing period. At 21 wk of age, abdominal fat pad weight increased by 0.22%, and at first egg 0.09%, per week that the pubertal growth spurt was advanced ($P < 0.05$; Exp1). In Exp2, the abdominal fat pad at 21 wk of age was not affected by the growth trajectories, but was affected at first egg; the 50% faster pubertal phase growth rate (I_2) yielded greater abdominal fat content (1.87 vs. 1.37%; $P < 0.05$). Egg production to 58 wk of age increased as the timing of the pubertal growth spurt was advanced in Exp1. For every week that the pubertal growth spurt was advanced, the number of eggs increased by 3.2 ($P < 0.05$). No difference in total egg production was observed in Exp2 to 58 wk of age.

c. Implications for commercial broiler hatching egg producers

Feed conversion ratio of pullets during the rearing period increased mainly by increased early growth (shifted from the pubertal to the prepubertal phase). The earliest growth is typically the leanest growth. Stimulating growth in this manner increased maintenance energy costs, but did not increase egg production; this approach is not recommended.

When feed restriction was relaxed during the pubertal phase, specifically when the pubertal growth spurt was advanced, birds increased fat deposition, and also increased total egg production. During the pubertal phase, the reproductive system develops and extra fat deposition during this time appears to be a key to increasing egg production. Further analysis will be conducted to determine if the increased egg production response is based on physiological signalling molecules originating from fat tissue (e.g. adipokines) or nutrient supply (e.g. lipoproteins) for egg production. In addition, an economic analysis will be conducted to determine the revenue for producers, but also on the supply chain level. As an initial conclusion, advancing the timing of the pubertal growth phase of broiler breeders is recommended because it did not affect FCR, but increased egg production to 58 wk of age.

III. EGG-TYPE PULLETS

a. Study methodology

Lohmann Brown-Lite pullets were randomly assigned to one of eight treatments in a completely randomized 2 feed restriction (FR) × 4 dietary metabolizable energy (ME) factorial arrangement. The two FR levels were Meal Every Visit (MEV) and Restricted feeding (RES). Birds assigned MEV were permitted to consume feed *ad libitum* when they entered the station, while RES birds were subjected to the lower range of the breeder-recommended target BW trajectory and were only permitted to eat from the feeder if their real-time BW was less than the target BW. There were three levels of dietary ME: Low (10.89 MJ/kg; 2,600 kcal/kg), Standard (Std; 11.72 MJ/kg; 2,800 kcal/kg), or High (12.56 MJ/kg; 3,000 kcal/kg). A fourth treatment (Choice) allowed birds to choose from amongst the diets. Diets were isonitrogenous. Birds from all treatments were comingled in two floor pens from 0 to 50 wk of age. A multi-feeder precision feeding station (Zuidhof et al., 2022) was used which permitted appropriate feeding of comingled treatments. Each bird received its allocated ration individually, allowing each bird to be its own experimental unit.

Photostimulation occurred at 18 wk of age. Data collection from the multi-feeder stations included the identity of each bird, and time-stamped BW, diet provided, and feed intake.

Based on this data, flock uniformity and energy intake were calculated. Flock uniformity was measured using the coefficient of BW variation (CV). Egg production data was collected using a nest box system which was equipped with radio frequency identification readers which identified the nest occupant, the egg weight, and the time of lay. All data were analyzed as a 3-way ANOVA using the MIXED Procedure in SAS (Version 9.4. SAS Institute Inc., Cary, NC, 2012), with FR, dietary ME, and age as main effects. Tukey's multiple range test was used to compare treatment means. Differences were reported where $P \leq 0.05$, and trends noted where $0.05 < P \leq 0.10$.

b. Results

There was an interaction between FR and dietary ME on body weight. Birds on the MEV treatment had greater BW than RES birds. MEV birds on Low and Std diets had greater BW than MEV birds on High and Choice diets (1,437 and 1,427 vs 1,399 and 1,400 g, respectively, $P < 0.001$). RES birds on the Std diet had the lowest BW (1,341 g). Overall, MEV birds had a greater CV (lower uniformity) than RES BIRDS. However, the effect of FR on CV depended on dietary ME. MEV birds fed the Choice diet had the greatest CV (least uniform) and RES birds on the High ME diet had the lowest CV (10.32 vs 3.68 %, respectively, $P < 0.001$). In terms of energy intake, in general, MEV birds had greater energy intake than RES birds (0.94 vs 0.91 MJ/d, respectively, $P=0.009$). However, the effect of dietary ME level on ME intake depended on age. At 20 wk of age, birds fed the Low diet had greater energy intake compared to birds fed the High and Choice diets (1.12 vs 0.95 and 0.97 MJ/d, respectively, $P < 0.001$). There was also an interaction between FR and age. At 20 and 21 wk of age, birds fed MEV had greater energy intake than birds fed RES (1.09 vs 0.94 MJ/d, respectively, $P < 0.001$). Total body fat was lower in RES birds ($P < 0.001$).

There was no effect of dietary ME on total number of eggs laid to 50 wk of age. There was a trend for birds fed MEV to lay more eggs than birds fed RES (194 vs 188 eggs, respectively, $P=0.08$). For egg weight, there was an interaction between FR and dietary ME. RES birds fed Std diets had the greatest egg weight, while MEV birds fed the Std diets had the lowest egg weight (58.1 vs 55.5 g, respectively, $P < 0.001$). RES birds fed the Low diet had a greater egg weight than MEV birds fed the Low diet (57.6 vs 56.1 g, $P < 0.001$). MEV and RES birds assigned Choice had greater egg weights than MEV and RES birds fed the High diets (58.0 and 57.4 vs 56.4 and 55.9 g, respectively, $P < 0.001$).

c. Implications for commercial egg farmers

Precision feeding stations successfully reduced the BW CV, which could facilitate flock management. However, feed restricted birds with lower BW tended to have lower egg production. However, feed restricted Std birds produced heavier eggs than unrestricted Std birds. Further economic analyses considering weight-specific egg prices will determine ultimate profit routes for producers.

IV. CONCLUSIONS

In both broiler breeders and laying hens, there was a correlation between total body fat reserves around the time of sexual maturation and reproductive efficiency. In broiler breeders, advancing weight gains too early decreased efficiency, while strategically advancing the timing of the pubertal growth phase increased body fat and reproductive success. In laying hens, feed restriction of pullets decreased body fat reserves and tended to reduce egg production, while pullet phase dietary ME had little impact on reproductive efficiency. Giving laying hens a choice of dietary ME led to the poorest egg production outcome. Therefore, full feeding of

layer pullets on a standard diet is recommended. Further analysis of underlying physiological mechanisms and the economics of production will add further insights on the sustainability of these recommendations.

ACKNOWLEDGEMENT: Funding for this research was provided by Results Driven Agricultural Research (Edmonton, Alberta, Canada), Aviagen (Huntsville, Alabama, USA), Canadian Poultry Research Council (Ottawa, Ontario, Canada), Canadian Broiler Hatching Egg Producers Association (Ottawa, Ontario, Canada), Canadian Hatching Egg Producers (Ottawa, Ontario, Canada), Egg Farmers of Canada (Ottawa, Ontario, Canada), Egg Farmers of Alberta (Calgary, Alberta, Canada), Trouw Nutrition (Eindhoven, NL), and TopSector Consortium, led by Laura star (Aeres University, NL). Special thanks to Xanantec Technologies, Inc. (Edmonton, Alberta, Canada) for providing in-kind support for the design, manufacture, and maintenance of the precision feeding systems used in these studies.

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MACHINE VISION DETECTION OF RANGE USE BY FREE-RANGE MEAT CHICKEN FLOCKS

C. MCCARTHY[1], P. TAYLOR[2] and C. DEKONING[3]

Quantification of range use in commercial meat chicken flocks is desirable so that farmers can objectively assess meaningful range access, including number of chickens using the range and how far chickens roam from the shed. Currently there is no cost-effective technology for quantifying range use in commercial conditions, and farm staff perform assessments based on visual inspection. A proof-of-concept machine vision system was developed for automated range use assessment, using simple and low-cost camera hardware with visibility of the length of the range area. A colour camera with solar panel and power bank was used to monitor a free-range site in South Australia in March 2022. The camera was placed at one end of the range adjoining a meat chicken shed, midway across the 12 m width of the range, and oriented towards the far end of the range. The camera was positioned at a height of 1.8 m and programmed to capture one image per minute during daylight hours. Image resolution was 1600 × 1200 pixels. Images were captured for 14 days on which pop holes were open, spanning flock age of 21 to 42 days.

Captured images were a one-point perspective scene, with the edge of the shed and the boundary fence of the range receding to a vanishing point at the middle top of the image. Plant ground coverage was 85%, comprising green kikuyu grass, dry annual grasses, and isolated broadleaf weeds. The shed was oriented east-west such that shadows cast by the shed onto the range were relatively short. However, there were silos behind the camera on the eastern end of the range which cast a long shadow onto the range in the morning. Hence, image analysis was required to detect chicken pixels for sunlit, shaded and overcast conditions. After chicken pixels were detected, number of chickens and their distance from the shed were automatically calculated taking image perspective into account.

Image analysis counts of chickens achieved R^2 of 0.85 to 0.94 when compared with manual counts of 120 images across four days with variable natural lighting. Reduced image analysis accuracy occurred within 1 m of the shed where chickens were seated closest together. Image analysis and visual inspection both indicated that range use was highest at approximately 0900 - 0930 h for the observed flock, with range use declining after about 1030 h for sunny days. Based on image analysis, it was common for chickens to range up to 4 m from the shed. Chickens appeared to prefer ranging in the shadow cast by the silos, as chickens that were the greatest distance from the shed (i.e. more than 4 m) were always seated or standing in the shadow cast by the silos, where that same shadow also reached within 3 m of a pop hole.

It was concluded that a single low-cost colour camera with a viewpoint along the range was suitable for image analysis algorithms to quantify range use. Automated counts were accurate compared to manual counts from images. The algorithms could distinguish chickens under the different lighting conditions of overcast, sunlit and shaded, indicating potential for further research of meteorological and environmental conditions and ranging behaviour. Further work should evaluate and refine algorithms for segmenting chickens from the background for various ground covers, sunlight directions and flock ranging routines, through trials for a greater number of farms, shed orientations and times of year.

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REVEALING THE SECRET LIFE OF HENS ON THE RANGE USING CAMERA TRAPS

C. DE KONING[1]

Summary

The objective of a 10-week pilot study was to assess the frequency of hens visiting the outer range areas (> 50 m from the shed) on a free-range layer farm by using camera traps. Additional information collected from the cameras was hen behaviour and wild bird species. Distance from the shed had a strong influence on the frequency of visits by hens, with a large reduction in the number of visits at 120 m (10 visits) compared to 70 m (26 visits). Whilst on the outer range hens were walking (Frequency = 23), foraging in the open (Frequency = 33), foraging at saltbush (Frequency = 27); resting behaviour was minimal (Frequency = 3, $P < 0.001$). Six species of wild birds were identified on the range with the Australian raven the most common and wedgetail eagles the least frequent (Frequency = 36 and 2 respectively, $P < 0.001$). Information gained from camera traps could be used to design more attractive outer range areas for hens, specifically the need for shelter, and gauging biosecurity risks from wild birds.

I. INTRODUCTION

Determination of range usage by hens can be difficult for researchers and free-range egg farmers, particularly usage on the outer sections of the range (i.e., > 50 m from the hen shed). Live counts only give a snapshot of numbers of hens on the range at a defined time point and do not show the frequency of range usage. Camera traps, also referred to as trail cameras and wildlife cameras, are relatively cheap and useful tools that could produce valuable information on the level of range visitation by hens, especially the distant areas on the range. Camera traps have primarily been used for ecological wildlife surveys (e.g. Rovero et al. 2013) and more recently for assessing biosecurity risks on poultry farms by identifying/quantifying the types of wild bird species visiting free-range poultry farms (Scott et al. 2018, Atzeni et al. 2020, Elbers & Gonzales, 2020).

This paper reports on a pilot study that used camera traps to assess the frequency of hens visiting the range areas greater than 50 m (outer range) from the shed on a free-range layer farm in southern Australia. Camera traps were also used to determine the times of the day hens visited the outer range along with hen behaviour and other bird species seen on the range.

II. METHOD

Animal ethics approval for the study was from the Department of Primary Industries and Regions, South Australia (PIRSA 13-17). The free-range layer farm was located within the Mediterranean climatic zone of southern Australia. Two flocks (A and B) were studied with camera traps placed on their range areas. The flocks were not adjacent but separated by two other flocks. The hens were Hyline Brown (beak trimmed at hatchery). At the start of camera placement, flock A was 47 weeks of age and flock B was 57 weeks of age. Individual flock sizes on the farm were 30,000 hens with an outdoor stocking density of 10,000 birds/ha.

Sheds (16.5 x 132 m) were orientated East - West with pop holes along both long sides of the shed (north and south facing). The corresponding range area was 4 hectares also orientated East – West in a rectangular shape with the shed located centrally at the Eastern end. Each range area for flocks A and B had four twin rows of oldman saltbush (*Atriplex nummularia*) variety ‘De Kock’ planted on the range in 2017 and 2018. Rows were 16 m apart

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commencing 50 m from the western end of the sheds and were 250 m long. Saltbush was arranged in twin rows, 4 m apart, and 4 m separation within rows. Saltbush height ranged from 0.5m to 1 m tall at the time of camera placement.

Cameras (*SIGNIFY*®, Model Number EA1427, Silverwater, NSW, Australia) with PIR (Passive Infrared) sensors were used. Trigger time was 0.5 sec, trigger distance up to 25 m and the lens with a 100-degree angle. Each camera was loaded with 8 batteries (alkaline AA 1.5v) and a 16 GB SanDisc memory card. Placement of cameras was halfway (8 m from saltbush) between the northern most saltbush twin row and the next saltbush twin row. All cameras were positioned to face south to avoid bright sunrise and sunset effects on the camera lenses. Two cameras were placed on the range of each shed (flocks A and B) at 70 m and 120 m from the west end of the sheds (Total 4 cameras). Cameras were mounted on steel droppers 1m high from the ground using cable ties and were set for medium sensitivity. When triggered, they would take three photographs (5 MB each) in rapid succession followed by a 20-sec video (1280 x 720p).

Photos and videos from three of the four cameras were used. One camera (flock B, 70 m from the west end of shed) had reached storage capacity within 30 days after placement. This was due to excessive bird activity (117 visits by ravens and hens) following the wedge tailed eagle attacks at that location. Therefore, the data from this camera were not included, nevertheless it was used to observe hen behaviour during and following eagle attacks. Hen behaviour was determined from the videos. The following definitions were used; 'walking' - to or from the shed with no ground pecking; 'foraging in open' - actively pecking and scratching at open ground between saltbush rows; 'foraging at saltbush' - pecking and scratching the ground under saltbush; and 'resting' - sitting or standing under saltbush without movement. The frequency of hens visiting the area was determined by counting how many times hens appeared during the period the cameras where on the ranges, but only during the times of the day when pop holes were open (10 00 to 18 00 h). Operating time for the cameras was from the 2 August 2019 to 9 October 2019. This covered seasonal weather conditions from late winter to mid spring. A comparison between the number of hens in the photos at 70 m and 120 m was made for flock A using a T-test, unequal variances. Wild bird species were identified from photos and videos. Photos were also used to assess how often hens appeared on the range without wild birds, wild birds without hens, and both hens and wild birds in the same photo. Pearson's X^2 was used to analyse hen behaviour on the range, species of wild birds, and wild birds on the range with and without hens.

III. RESULTS

The total number of events while popholes were open was N = 102 (based on 3 cameras). False positives represented 11.7 % of the total event numbers. The majority of false positives were on windy days (67%).

At 70 m from the shed, hens from flock A appeared 26 times while at 120 m the hens only appeared 10 times. Yet, there were no significant differences in the average number of hens shown in the photos at 70 m compared to 120 m (3.42 ± 0.81 hens v. 4.37 ± 1.47 hens respectively, P = 0.577, two tailed T-test). Hens ranged away from the shed throughout the day with peak numbers between 10 00 to 11 00 h (shortly after pop holes were opened) (Figure 1). Hens were actively foraging in the open (Frequency = 33), foraging at saltbush (Frequency = 27) or walking on the outer range (Frequency = 23), and resting behaviour was minimal (Frequency = 3) (Pearson's $X^2 = 23.58$ with 3 d.f., $P < 0.001$). When resting behaviour was removed from the analysis, the difference between walking and foraging was not significant (Pearson's $X^2 = 1.83$ with 2 d.f., P = 0.400).

Hens mostly appeared on the range without wild birds (Frequency = 49) (Pearson's X^2 = 27.93 with 2 d.f., P < 0.001), followed by wild birds seen without hens (Frequency = 28), and very few occasions where both hens and wild birds were seen in the same photo (Frequency = 9) (except following the eagle attack and these data were not included). The most commonplace wild birds found on the range were Australian ravens (*Corvus coronoides*), Figure 2, Pearson's X^2 = 80.38, with 6 d.f., P < 0.001. Unidentified birds were the next biggest category. These were small brown birds, half the size of the common starling, probably European house sparrows (*Passer domesticus*).

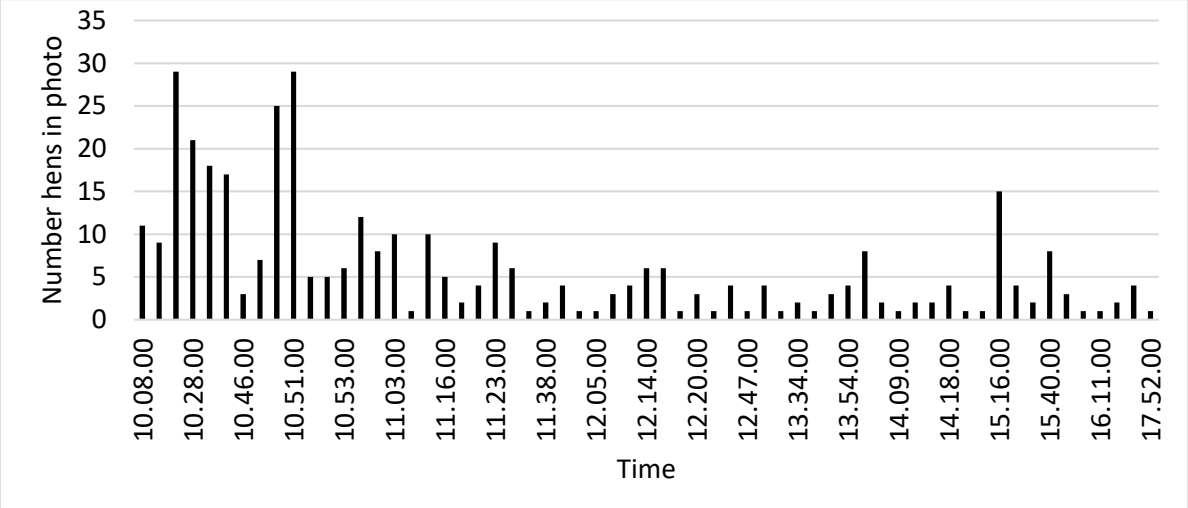

Figure 1 - The number of hens in the photo frame and the time of day hens were on the outer range (50 m < from the shed) on a free-range layer farm in southern Australia during 2 August 2019 to 9 October 2019, while pop holes open.

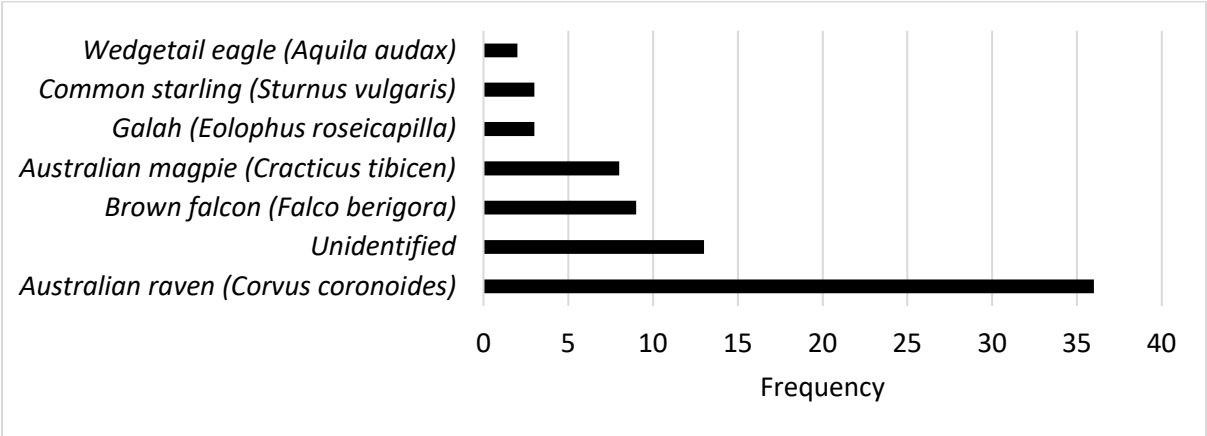

Figure 2 - The frequency of wild bird species on a free-range layer farm in southern Australia over a 10 week period from 2 August 2019 to 9 October 2019, while pop holes were open.

IV. DISCUSSION

The false positives seen on windy days may have been due to moving vegetation triggering camera traps (Rovero et al. 2013). Other false positives may have been the result of fast flying wild birds or insects triggering cameras. There is a compromise between camera sensitivity settings; set too high would result in more false positives and set too low may not trigger the camera when birds/animals are present. Another issue with camera traps is that it takes time to interpret the photos and videos. Despite these issues there is much to be gained from camera traps by showing the behaviour of hens and how often they are on the outer range. As such,

very little resting behaviour was seen on the outer range, with hens actively foraging and walking (Campbell et al. 2020). However, hen behaviour performed while on the outer range maybe season dependent. For instance, dust bathing behaviour was observed by the researcher (no cameras were in place at the time) on the outer range during late spring when soil conditions were dry, with most hens dust bathing under saltbush and blue bush (*Maireana brevifolia*). Therefore, camera traps are needed at different times of the year to capture seasonal effects on visitation levels to the outer range and the variety of hen behaviour. Distance from the shed had a strong influence on the frequency of visits by hens, with a large reduction in the number of visits at 120 m compared to 70 m. More cameras are required beyond 120 m from the shed to determine whether the number of visits reduce even further.

Even though wedge tailed eagles were among the least frequent visitors to the range their impact was great. One camera recorded a pair of wedgetail eagles simultaneously attacking a hen each, with the two hens trying to reach the cover of saltbush. The other hens nearby initially moved toward the eagles and their attacked flock mates, but soon retreated. Regardless of the eagle attacks, hens returned to the area within 30 minutes and were seen only 3 – 4 metres from the eagles feeding on the hen carcasses. This is contrary to the perception that hens are frightened by birds of prey. Similar behaviour by hens in response to birds of prey was observed in a Dutch study (Bestman & Bikker-Ouwejan 2020). Generally, other wild bird species were not seen often in the same photo or video with hens, and maybe avoiding the hens as suggested by Scott et al. (2018) and Elbers and Gonzales (2020). They were mostly found on the range before pop holes opened and after they had closed, also implying that hens detered wild birds. While hens are on the range there was minimal direct contact with wild birds (except eagle attack), however the biosecurity threat would most likely result from hens contacting wild bird excreta on the range (Scott et al. 2018 and Elbers & Gonzales 2020). Nevertheless, photos and videos would be useful to assess biosecurity risks based on the types of wild birds seen, but field observations of wild birds are also needed (Atzeni et al. 2020). As this study was for only 10 weeks, further camera trapping throughout the year is warranted to cover different climatic seasons, thereby potentially revealing more species of wild birds.

In conclusion, camera traps are a simple and cost-effective way of assessing how often hens go to the outer range areas and what they do while out there. This information would assist with the design of outer range areas to make them more attractive for hens, specifically the need for shelter (e.g. trees and shrubs). Photos and videos from camera traps also provide evidence of the wild bird species that visit the range and the implications for biosecurity.

ACKNOWLEDGEMENTS: Many thanks to Australian Eggs for funding this research and the participation by the farm collaborator.

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HYPERSPECTRAL IMAGING IS A PROMISING TECHNOLOGY FOR REAL-TIME MONITORING OF FEED AND LITTER QUALITY, AND MYCOTOXIN DETECTION

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Summary

Monitoring the quality of feed and litter moisture in the poultry industries is necessary to maintain/increase production and improve chicken health and welfare. Wet chemistry methods are time consuming and near infrared spectroscopy (NIR) depends on sampling methods (same as wet chemistry) that introduce uncertainties. Hyperspectral imaging (HSI) scans and analyses a large portion of a load, which minimises sampling error. In contrast to wet chemistry and NIR, HSI can display the variability within materials (feed and litter) and perform qualitative (identifying and sorting) and quantitative measurements simultaneously, which can be used in automatic quality monitoring systems using machine vision. In this study, we used wheat samples (ground and kernels) to investigate the possibility of using HSI combined with machine learning for quantifying carbon and nitrogen concentrations, identifying impurities and mouldy grains and quantifying deoxynivalenol (DON) infection (artificially added). We also investigated the possibility of using HSI for quantifying moisture contents in three poultry litter types (pine shavings, hardwood and re-used hardwood litter). The results showed that HSI was able to accurately quantify wheat carbon and nitrogen concentrations; identify impurities and moist/mouldy grains; identify, quantify and visualise mycotoxins in grains; and quantify/visualise moisture contents in different types of poultry litter. These capabilities, while partially dependent on future development and adoption of AgTech, could be applied to reduce the variabilities and impurities in feed ingredients, and contribute to improving the in-shed environment by accurately monitoring litter moisture.

I. INTRODUCTION

The quality of poultry feed and litter are important for maintaining and improving poultry production. Poultry feed (raw materials and formulated diet) is highly variable and large safety margins are needed to buffer the nutritional variability in feed ingredients when formulating diets (Moss et al., 2021). Feed ingredients delivered to feed mills are not comprehensively analysed using current methods (i.e., wet-chemistry and NIR). This is because the large quantities of feed being processed daily and the current methods rely on sampling and making assumptions that the ingredients are homogenised, which may not be the case. HSI can analyse a large quantity of material or an entire load, quantify the targeted properties, and display outputs in real-time. This allows automatic identification and quantification of impurities, moulds and mycotoxins, moist grains and nutritional values, while illustrating the variations for better management practices.

Litter moisture content (LMC) is another critical factor associated with concerns regarding environmental impacts, animal welfare, flock health, food safety and reductions in production efficiency (Carpenter et al., 1986; Dunlop et al., 2016; Lai et al., 2009). LMC varies in the shed due to many factors. Regular monitoring of LMC using the conventional methods in grower sheds is time-consuming and may produce in-consistent values. HSI can quantify

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and visualise LMC on a regular basis and potentially be combined with machine vision equipment in poultry sheds.

HSI cameras measure the light reflected from samples at different wavelengths in the visible to near infrared spectrum and uses artificial intelligence algorithms to correlate the targeted properties with the spectral information. HSI identifies different materials by using their spectral signatures (their reflectance/absorbance pattern), regardless of their colour and shape, and finds the relevant wavelength to quantify the concentrations.

In this study, we investigated the possibility of using HSI for quantifying the nutritional values of wheat as a feed ingredient, identifying impurities and wet grains in wheat, quantifying mycotoxins in wheat and quantifying LMC in different types of litter collected from a meat chicken shed during growing period.

II. METHOD

Wheat C and N concentrations 69 samples of wheat were collected from Queensland, New South Wales, Victoria, Western Australia, Tasmania and South Australia between 2016 and 2019. The samples were ground and analysed for carbon (C) and nitrogen (N) concentrations using a combustion analyser. The samples were then scanned using a visible-near infrared (VNIR) HSI camera (Resonon model Pika XC2, 400–1000 nm) and a shortwave infrared (SWIR) HSI camera (Hyspex model SWIR-384, 1000–2500 nm). Partial least square regression models (PLSR) were trained to correlate the concentrations of C and N with their spectral reflectance. Multiple PLSR models were trained to identify the most important spectral regions for predicting C and N concentrations using HSI (Tahmasbian et al., 2021). Models were tested using external (independent) test samples and evaluated using coefficient of determination (R^2) and root mean square error (RMSE).

Wheat impurities: wheat samples from the previous stage were mixed with impurities including metal nuts, metal shavings, rubber, wood sticks and mouldy/moist grains. Samples were scanned using a SWIR HSI camera and partial least square discriminant analysis (PLS-DA) was trained to identify the spectral signatures. An independent test sample was used to evaluate the model and visualise the impurities.

Wheat mycotoxins: Wheat samples were artificially contaminated with different concentrations (0 to 10000 µg kg^{-1}, added at 500 µg kg^{-1} intervals) of deoxynivalenol (DON), which is the most common mycotoxin in wheat. The samples were scanned using a SWIR HSI camera. The HSI data were correlated with the reference concentrations of DON using PLSR model. The model was tested using independent test samples.

Litter moisture content (LMC): Three types of chicken litter samples, including pine shavings, hardwood shavings and re-used hardwood were collected on multiple occasions during the growing period. Samples were dried and re-moistened to make moisture contents of 10%, 20%, 30%, 40%, 50% and saturated. Samples were scanned using the SWIR HSI camera inside plastic bags to maintain the LMC. The images of each sample varying in type, age and LMC were halved. The first half was used for training PLSR models to correlate the actual LMC with their spectral reflectance, while the other halves were used to evaluate the performance of the models. The models were initially trained by the entire spectra (950–2500 nm) to investigate and prove the possibilities of using HSI for litter moisture predictions and then a smaller spectral range (950–1000 nm) was used to investigate whether the prediction is possible using more affordable multispectral cameras, measuring 950–1000 nm only.

III. RESULTS

Wheat C and N concentrations: HSI combined with PLSR modelling predicted the concentrations of C and N in the ground wheat samples. The R^2 of C prediction in the external

samples were 0.89 when the full VNIR spectral range (400–1000 nm) was used. The best spectral region for predicting C was 400–550 nm, being able to predict C concentrations with the R^2 of 0.86.

HSI was also able to predict N concentrations in the samples with the R^2 of 0.99 when using the full SWIR spectra (1000–2500 nm). VNIR HSI (full spectrum) was also able to predict the N concentrations with R^2 of 0.91. While narrower spectral region in VNIR range could not predict the N concentrations, the best spectral regions for predicting N concentrations was 1451-1600 nm with R^2 of 0.99.

Feed impurities: the PLS-DA model recognised and classified the pixels related to the normal wheat, metal nuts and shavings, rubber, wood sticks and mouldy/moist grains and displayed them in the images using different colours (Figure 1). The accuracy of the classification was > 90%.

Figure 1 - Identification of impurities and moist/mouldy grains in wheat using hyperspectral imaging

Feed mycotoxins: HSI was able to predict the concentrations of DON (0 to 10000 µg kg^{-1}) in the external test samples (R^2 of 0.97). The HSI was also able to display the concentration gradient of DON in the contaminated samples (Figure 2-left).

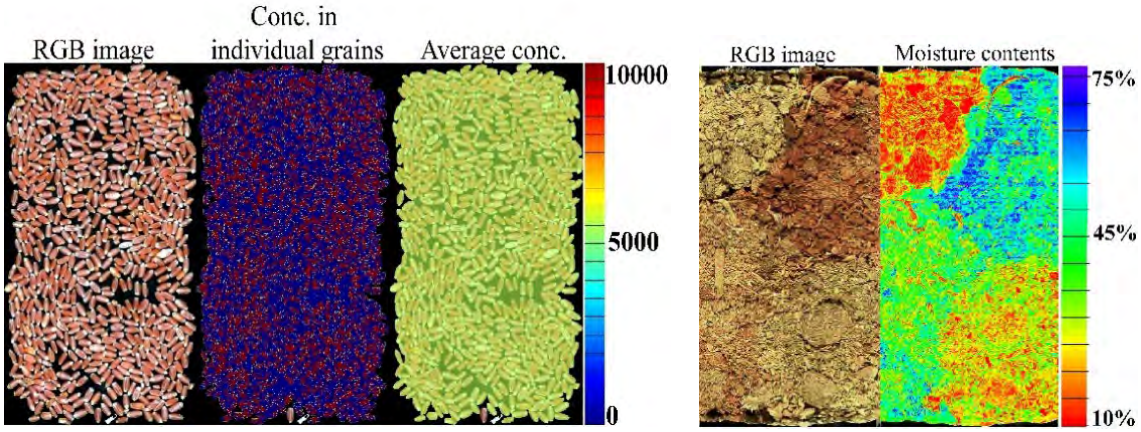

Figure 2 - Quantification of deoxynivalenol (µg/kg) in wheat samples (*left*) and moisture content of an array of four samples of chicken litter (re-used hardwood) ranging from 10-75% (*right*) using hyperspectral imaging (HSI, 950-2500 nm).

Litter moisture contents: HSI (950–2500 nm) combined with artificial intelligence predicted LMC in all litter type and age with R^2 of 0.98 for the independent test samples. Reducing the spectral range to 950–1000 nm, reduced the R^2 of the general model to 0.89 (Figure 3). Separating the samples based on litter type (pine, hardwood and re-used hardwood) increased the 950–1000 nm prediction R^2 to over 0.91 for each litter type (Figure 3). The prediction algorithms were applied to a re-used hardwood litter sample to produce a gradient map of moisture content (Figure 2-right).

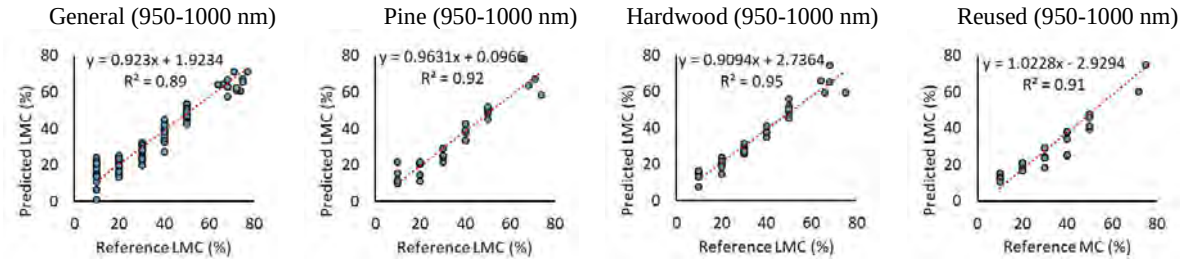

Figure 3 - Reference litter moisture content (LMC) versus the LCM predicted using general and separate partial least square models developed using 950-1000 nm wavelengths.

IV. DISCUSSION

HSI was successfully used to measure wheat C and N concentrations, impurities, mould and mycotoxins and litter moisture content in this study. Our results are consistent with and complementary to previous studies that used HSI for quantifying nutrients including calcium, magnesium, molybdenum, zinc and protein in wheat kernels and flour and detecting diseases including *Fusarium* infection (Bauriegel et al., 2011; Caporaso et al., 2018; Hu et al., 2021). HSI has potential to improve the accuracy of feed formulating by minimising or completely removing random/systematic sampling errors (a major source of inaccuracy) by scanning a large portion or the entire load of raw materials or formulated diet. This study was the first using HSI for predicting litter moisture content and we were unable to compare our results with previous studies.

HSI cameras are currently used mainly for research due to the high cost of the equipment (currently $80–250k depending on the spectral range and quality of the equipment). However, recognizing and using the important wavelengths only, such as what has been done in the present study, enables using cheaper multispectral cameras that can be used for different purposes (e.g., online monitoring of birds and measuring litter moisture) simultaneously.

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VISION-BASED DYNAMIC DENSITY ESTIMATION OF LAYING HENS FOR PILING-UP PREVENTION

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Summary

Piling-up is a behaviour that can be common in laying hens on commercial egg farms, which can result in smothering and mass mortality events. We propose to provide early warning of pileups by automated video monitoring of bird density and activity in real-time. This will allow farmers to receive early warnings and take necessary measures to prevent negative impacts. We have designed an auto-monitoring system using computer vision and machine learning to allow automated and dynamic estimates of local flock densities under both indoor and outdoor environments suitable for use on commercial egg farms. This automated monitoring system provides a low-cost intervention to pile-ups, and potentially other behavioural problems on egg farms, with egg production and animal welfare benefits and the added benefit of more efficient labour utilization.

I. INTRODUCTION

The welfare of all farmed livestock is of importance to the consuming public. Australian consumers especially have some concerns about welfare within more intensive animal production systems, such as commercial egg farms (Rachel et al., 2017). Most consider cage-free and free-range production systems to be more `natural` and associate this with `good` animal welfare (Buddle et al., 2021). Commercial egg farms experiencing smothering and mortality events as a result of pile-ups experience reduced egg production and risk social licence from compromised animal welfare. Pile-ups occur when a group of hens increasingly cluster together within a small space, which can progress to smothering and death. Recurring pile-ups seem more prevalent on some farms and in some sheds. Birds that do not die can suffer in other ways including heat stress, injury and increase fear responses (Gray et al., 2020). Risk factors for pile-ups include several environmental factors (including light and temperature), shed infrastructure, genetics and sudden disturbances resulting in the mass movement and/or attraction (Gray et al., 2020).

The monitoring of bird welfare is a challenging task on large egg farms that have tens of thousands of birds per shed. For this reason, an increasing number of Precision Livestock Farming methods have recently been developed (Dawkins, et al., 2013). Specifically, density estimation technologies provide the potential for non-intrusive and continuous monitoring and management support on egg farms by providing effective auto-analysis focused on animal welfare and egg production.

In this paper, we present our applied computer vision and machine learning techniques that operate within a video-based monitoring system to enable dynamic estimates of the local density of birds at any time and place. Using low-cost equipment, the system can work continuously giving real-time information. This means that high-density ‘problem’ areas can be effectively detected without labour, and mitigating steps taken in response in a timely fashion.

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II. METHOD

Our system is based on crowd counting and automated region analysis from video streams. To effectively estimate the flock density, a top-view video camera is mounted to capture the video footage, as illustrated in Figure 1. Such a view setting can avoid occlusion and enlarge the observation area. The video camera is connected to a desktop, which provides backend computation and communication. The live video feed is analysed in real-time, providing the essential density estimates and monitoring to prevent pile-ups.

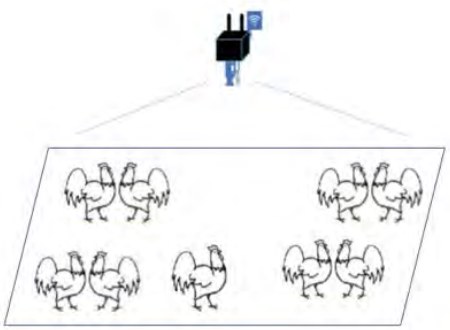

Figure 1 - The camera setting.

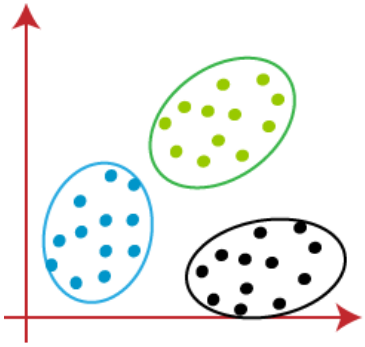

Figure 2 - An illustration of k-means on 2D coordinates.

The backend computational model is data-driven, which requires image data collection and manual annotation. To do this, we set up two cameras on a commercial egg farm in Windsor, NSW to collect video data. One camera was mounted in the shed, and the other was mounted on a pole in the open area outside the shed with the cameras covering 15m^2 and 40m^2 for indoor and outdoor environments respectively. After annotation of each observable bird on sampled images, we built a simple crowd-counting model as described by Wang (Wang et al.). Given an image, the model outputs a density map of the same size, where the value of each pixel represents the probability of an object instance, i.e., a bird in our case. The summation of the values across the whole density map is the total number of observable instances. However, alone, it cannot identify the specific region within the field of vision where maximal density occurred; this is the key indicator of risk for potential pile-ups (the ratio of count and area). Hence, we applied the k-means cluster algorithm on the density map, and then counted the birds in each cluster to calculate the bird density separately. Note that the k-means requires several iterations to identify and calculate clusters, which does not support the real-time computation of frame streams. Also, when the seeds of the randomly selected centroids are initialized differently, the final clusters are different. However, given there are only minor motion differences between any two consecutive frames, we only need to update the cluster centroids once within each frame and the next iteration uses the current cluster centroid as the starting point. In the 2D clustering, each bird is allocated to a cluster, which is represented as a circle. So, the density can be estimated by the ratio between the number of observable birds within the circle and its area. When there is an observable pile-up event, the bird density becomes very high in the specific area within camera coverage. In our camera setting, the top-down camera has spatial distortions, i.e., the observation area is not an exact rectangle. We used a scale mask for each cluster, that adjusts the image according to the relative position and angle of the camera, to control spatial distortions and thereby ensure the same area is used to give more accurate density estimations.

III. RESULTS

Based on the design and development of the system, we conducted a case study for bird density estimation. Figure 3 shows the visualization of the density measured in both indoor and outdoor environments. In the two cases, the cameras are 2.5 and 3.5 metres high, covering about 15m^2 and 40m^2 areas, respectively. In our setting, we set the number of clusters to 5, and the real-time calculation only returns the cluster with the maximal density. The maximal densities are 5 and 7 birds per square metre for indoor and outdoor environments respectively.

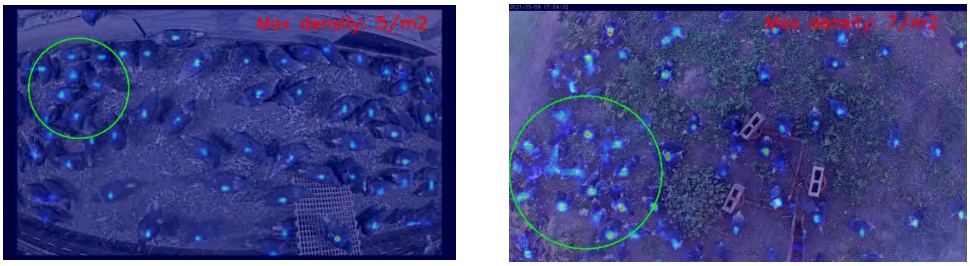

Figure 3 - The visualization of density estimations in both indoor (left) and outdoor (right) environments.

The system has a very high computation performance using low-cost hardware; it can process video streams with 30 frames per second (FPS), thereby enabling real-time monitoring. In Figure 4, we show the statistics for dynamic density estimation for a given day from the sampled video streams. We did not observe any pile-ups during the video recording, however, the real-time density monitoring capability identified potential for pile-ups by flagging densities above a user-defined threshold density. If the observed density is above the threshold, the system can give an early warning to help prevent piling behaviours.

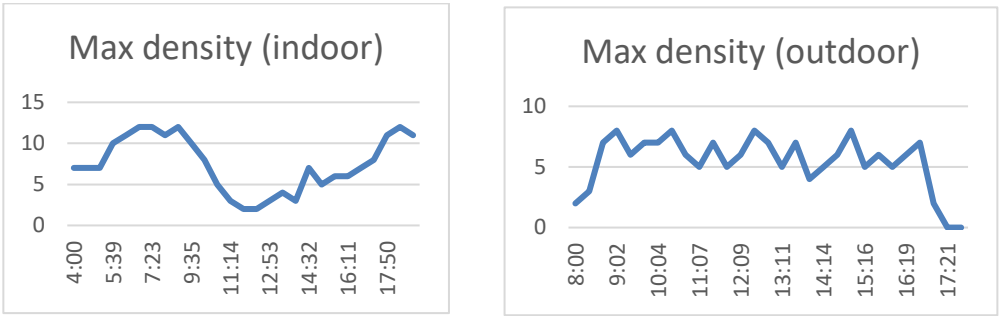

Figure 4 - The density estimation statistics in both indoor (left) and outdoor (right) environments, where the horizontal axis represents the recorded time, and the vertical axis represent the maximal density (number of birds/m^2) within the observable area.

IV. DISCUSSION AND CONCLUSION

In this paper, we have proposed applying advanced computer vision and machine learning techniques to monitor bird densities in commercial egg farms. With automated observation and analysis, the system has the potential to help monitor animal welfare and improve the productivity and hen welfare on commercial egg farms in Australia.

ACKNOWLEDGEMENT: The authors are grateful to Australian Eggs for their financial support of this study.

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VISUAL ACCESS TO AN OUTDOOR RANGE DURING REARING BOOSTS RANGING BEHAVIOUR OF MEAT CHICKENS

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Frequent visits to an outdoor range has been associated with good meat chicken welfare but not all chickens will access the outdoor range when provided with the opportunity (Taylor et al., 2018). On average, it takes an average of three to four days for most meat chickens in commercial conditions to access the range after the pop-holes first open (Taylor et al., 2017a). Yet chickens that access the range soon after the pop-holes first open, range more frequently throughout their lives compared to chickens that take a longer time to first access the range (Taylor et al., 2017b). Therefore, reducing the latency to first access an outdoor range may be key to optimising ranging behaviour and subsequently improving bird welfare. A critical step in reducing the latency to range may be 'preparing' the birds to range. The indoor housing environment prior to range access is typically simple and predictable until pop-hole doors open, usually at 21 days of age. Providing more environmental complexity and novelty during rearing may allow meat chickens to better adapt to the abrupt change in environment when range access is available, reducing the time taken for chickens to access the range and improving life time ranging behaviour. We provided complexity and novelty during rearing with the aim to optimise meat chicken ranging behaviour, specifically to reduce latency to range and increase the number of birds that ranged, the time spent on the range and the distance ranged.

Day old, mixed sex Cobb500 (n = 450) were housed in groups of 25 across 18 pens and were randomly allocated to one of three treatments. Controlled rearing (CON) was reflective of industry standard conditions and included wood shaving litter, feed and water. Visual access rearing treatment (VA) was the same as the control with addition of visual access to the range via transparent pop-hole covers. The complex rearing treatment (COMP) was the same as control with the addition of visual barriers, artificial haybales and fans with streamers. Range access was provided daily from 21 days of age and individual range use was tracked via RFID until 42 days of age. The proportion of birds that accessed the range was analysed with a GLMM with a binomial distribution. Latency to access the range was analysed with a Cox Regression and time spent on the range and number of days the range was accessed was analysed with a GLMM with a Gaussian distribution. Sex was included in all models.

VA birds accessed the range earlier (3.1 ± 0.5 days) than CON (6.3 ± 0.7 days) and COMP (9.1 ± 0.8 days) birds ($F_{(2,187)}12.2$, $P < 0.001$). A greater proportion of VA birds accessed the range (94.8%) compared to COMP birds (78.9%; $F_{(2,270)}11.5$, $p = 0.003$) but CON (89.5%) did not differ to any group. Few birds ventured further than 5 m from the shed (5.9% of all birds), but more CON (8.1%) and VA (9.3%) birds accessed the range area further from the shed (> 5m) compared to the COMP group (0.0%; $\chi^2_{(2,273)}8.5$, $p = 0.015$). There was a trend for VA birds to spend more time on the range ($F_{(2,16)}3.4$, $p = 0.058$). Birds from the VA group accessed the range on more days (13.7 ± 0.7 days) than CON (9.9 ± 0.7 days, $p = 0.016$) and COMP birds (7.6 ± 0.7 days, $p = 0.002$).

These results demonstrate that providing meat chickens with visual access to the outdoor range during rearing resulted in earlier range use and more days on the range than birds reared in typical industry conditions or when provided with environmental complexity. Therefore, visual access to an outdoor range is an effective method to improve ranging behaviour of meat chickens.

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A REVIEW OF LIPID DIGESTION, METABOLISM AND NUTRITIVE VALUE IN BROILER DIETS

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Summary

Whilst lipid digestion, metabolism and nutritive value have been well documented in avian species, the dramatic increase in dietary energy content costs, accompanied by unprecedented market volatility, has resulted in renewed interest in dietary fats and oils (lipids). This brief review considers the physiological role, metabolism, absorption and nutritive value of dietary lipids by bird age. Advances in broiler genotype through genetic selection have reduced the relative energy requirement compared with decades ago. Nevertheless, energy remains the costliest component of modern broiler feeds. Furthermore, the formulation of broiler feed with lipids needs to be considered, since the calorific value of added oil declines quadratically as dietary inclusion levels increase. The dietary essential fatty acid, linoleic acid recommendations may increase feed costs and may not be required at published levels. The addition of emulsifiers, or biosurfactants, such as lysolecithin, enhance lipid digestion, particularly in young broilers and lipids provide a source of essential fatty acids (EFA). Additionally, lipids are needed for fat soluble vitamin storage in the bird and required in the formation of bilayer cell membranes. Lipid digestion is accompanied by a low heat increment of digestion compared with other dietary energy sources and mobilisation of lipid reserves from adipose tissue provides an important source of metabolizable energy in avian species.

I. INTRODUCTION

In Australia, the cost of tallow and vegetable oil has more than doubled over the past two years (www.finvis.com), prompting broiler nutritionists to re-evaluate dietary energy content for meat-type chickens. Globally, a wide range of lipids with diverse chemical composition, are routinely included in poultry feed including soapstocks or acid oil, crude vegetable oil, hydrogenated material, recycled vegetable oil, restaurant grease, animal tallows and even products from the refining of oils such as bleaching earths, which are sometimes available as “dry fat” products (Wiseman, 1984, 1990; Krogdahl, 1985).

Fatty acids (FA) have four major physiological roles (Berg et al., 2019). Primarily, these are fuel molecules for the bird and are stored as triacylglycerols (also called neutral fats or triglycerides) which are uncharged esters of FA with glycerol. FA mobilised from stored triacylglycerols are oxidised to meet the energy needs of the bird. This is particularly important in migratory birds that cover vast distances across terrain where food is unavailable. Triacylglycerols are highly concentrated stores of metabolic energy since they are reduced and anhydrous. The energy yield from the complete oxidation of triacylglycerols is approximately 38 kJ/g in contrast with about 17 kJ/g for carbohydrates and protein (Berg et al., 2019). Carbohydrates are polar whilst lipids are non-polar and one gram of glycogen, for example, binds about two grams of water. Consequently, a gram of anhydrous lipid stores 6.75 times as much energy as a gram of hydrated glycogen. Secondly, FA are the building blocks of amphipathic molecules, namely phospholipids and glycolipids that are important components of cell bilayer membranes. Thirdly, the covalent attachment of FA to many proteins modifies and targets these to various membrane locations. Lastly, fatty acid derivatives serve as hormones and intracellular messengers.

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II. DIGESTION, ABSORPTION AND LIPID ENERGY

Physiologically, the mechanism of fat digestion and absorption in non-ruminants has been well documented (Freeman, 1984). Lipid digestion occurs primarily in the duodenum and jejunum and consists of emulsification of dietary lipid by conjugated bile acids, followed by hydrolysis of triglycerides by endogenous pancreatic lipase. The resultant mixture consists essentially of 2-monoglycerides and free FA (FFA) whilst the subsequent absorbability of these is dependent upon their solubility in bile salt micelles. Lipids pose a special problem compared with carbohydrates and protein since they are not soluble in water. Bile acids coat ingested lipids and the ester bond of the lipids are orientated towards the surface of the bile salt-coated particle, rendering the bond more accessible to digestion by lipases in aqueous solution. Pancreatic colipase is required to bind lipase to the bile salt-coated particle to permit lipid degradation and the resulting product is transported across the plasma membrane in micelles (Berg et al., 2019; Rodriguez-Sanchez et al., 2021). Triacylglycerols are resynthesised from FA and monoacylglycerols in the intestinal mucosal cells and packaged into stable lipoprotein transport systems, known as chylomicrons, and transported to adipose tissue for storage. Tissues gain access to stored lipid through three stages of processing. Firstly, lipids are mobilised by degrading triacylglycerols into FA and glycerol and transported to the required site. Secondly, the FA are activated and transported into the cell mitochondria and thirdly, these are broken down in a step-by-step process into acetyl CoA which is then processed within the citric acid cycle (Berg et al., 2019).

Polar solutes are more readily incorporated into micelles which explains the superior absorbability and higher digestibility of unsaturated compared with saturated fats in broilers (Renner and Hill, 1961). Accordingly, oils have a higher dietary energy than hydrogenated or even partially hydrogenated fats (Wiseman, 1990). Instructively, a quantitative measurement of the degree of saturation described by Stahly (1984) and Wiseman (1990) is important and increasing the ratio of unsaturated to saturated dietary lipid was shown to be associated with a non-linear improvement in dietary energy. Additionally, an intact triglyceride is superior to hydrolysed lipid in terms of dietary energy (Young, 1961; Sklan, 1979) and increasing the proportion of FFA is associated with a linear reduction in lipid digestibility (Freeman, 1968). Based on the degree of saturation, proportion of FFA and, to a lesser extent, fatty acid chain length, in seminal work, Wiseman et al. (1998) were able to calculate the energy content of dietary lipids for both pigs and poultry. However, the Wiseman equation had a matrix assumption that lipid moisture content, impurities and unsaponifiable matter (MIU) totalled 20 g/kg. More recently, a correction factor was applied to the Wiseman equation, by extending the equation using a MIU (%) dilution constant (Wealleans et al., 2021). These authors calculated the constant as one minus MIU/100 and concluded that lipid apparent metabolizable energy (AME) variation was exaggerated by including MIU in the Wiseman equation. Interestingly, of the 724 commercially available fats and oils samples analysed in Wealleans et al., (2021), linseed oil had the largest range followed by poultry fat and beef tallow (Table 1). Unsaponifiable matter forms the largest component of MIU and is positively correlated to the levels of FFA that are known to have a pro-oxidant effect. Higher FFA increase sensitivity and rate of lipid oxidation, accompanied by a decline in the levels of poly-unsaturated fatty acids (Frega et al., 1999; Chen et al., 2011; Wealleans et al., 2021). In contrast to impurities, non-elutable material (NEM) represents the portion that cannot be used as an energy source by the animal. This is of particular importance for heat-damaged oils that are estimated to contain three to four times the levels of NEM than refined vegetable oil (Wiseman, 2017). Additionally, this has implications for recycled vegetable oils and oils that are recovered from further processing, for use in poultry feed, which is common practice in Australia. The NEM fraction is determined by exclusion gel chromatography and is considered by the European

Parliament to be the best indicator of cooking oil degradation, particularly with respect to unsaturated fatty acids. In turn, the percentage of degraded fatty matter is calculated as the percentage of NEM minus the percentage of unsaponifiable matter (Boatella Riera and Codony, 2000). Other commonly used lipid quality measurements include colour, fatty acid profile, degree of unsaturation, or saturation, measured using iodine value (IV; titre) and measures of oxidative rancidity (Shurson et al., 2015).

III. OXIDATION AND RANCIDITY

Oxidative rancidity may occur prior to, or post-feed production and leads to the destruction of other fat-soluble nutrients such as vitamins A, D, E and K. Also, the greater the degree of unsaturation of lipid, the more prone to oxidation whilst some body lipid is required for fat-soluble vitamin storage (Kleyn and Chrystal, 2020; Wealleans et al., 2021). The complete oxidation of lipids, described as peroxidation, takes place over three phases; initialisation, propagation and termination with each step degrading and producing many different lipid peroxide compounds (Belitz et al., 2009). Primary oxidation results in lipid hydroperoxides, reducing the quality of the feed lipid and forming both secondary and tertiary peroxidation products (alcohols, ketones, aldehydes, hydrocarbons, epoxy compounds and volatile organic acids) that have a detrimental impact on broiler growth performance (Hung et al, 2017; Lindblom et al., 2019). At least 19 volatile compounds have been identified during linoleic acid peroxidation and the initial peroxides and aldehydes formed during primary oxidation are ultimately degraded as peroxidation continues (Belitz et al., 2009; Shurson et al., 2015). Dual evaluation of lipid peroxidation via firstly, indicative analyses that measure specific chemical compounds at time of sampling or secondly, predictive stability methods that test the ability of the lipid to withstand peroxidation, when exposed to standardised accelerated conditions, to induce peroxidation (Shurson et al., 2015).

Common indicators of peroxidation in feed lipids have been peroxide value (PV), *p*-anisidine value (AV) (deRouchey et al. 2004; Danowska-Oziewicz et al., 2005) and thiobarbituric acid reactive substances (TBARS) (Liu et al., 2014). However, other measures such as the total oxidation value (TOTOX = AV + 2 × PV), conjugated dienes, triacylglycerol dimers and polymers, total carbonyls, hexanal value and oxirane value, have been occasionally used to assess lipid peroxidation (Seppanen, 2005) as well as assays that measure specific peroxidation compounds such as 2,4-decadienal (DDE) and 4-hydroxynonenal (HNE). In Wealleans et al., (2021) a PV for non-fish oils below five and TBARS below 0.5 would suggest no oxidation whilst 5 to 10 and 0.5 to 1, respectively, suggest first signs of oxidation. Oxidation has occurred at a PV of 10 to 20 (TBARS one to two) and strong oxidation above 20 and two TBARS respectively. Peroxidation compounds measured by PV, AV, TBARS, conjugated dienes, total carbonyls and hexanal are produced and subsequently degraded at various stages of the peroxidation process, making interpretation of results difficult and misleading (Shurson et al., 2015). Instructively, Gray (1978) concluded that there is no single chemical method that can entirely predict or explain changes in organoleptic properties of oxidised lipids, highlighting the importance of using multiple measures to assess the oxidative status of a lipid sample.

IV. FEED FORMULATION

Feed formulation assumes the apparent metabolizable energy (AME) assigned to individual feed ingredients is both linear and additive and it is unlikely that this is true. For example, in Kleyn and Chrystal (2020) composite data was used to calculate an “extra-calorific” effect of low levels of added dietary lipid (below 20 g/kg) and a non-linear decline in AME with increasing dietary lipid above 20 g/kg (Figure 1.). This is further complicated by the change

in fat digestibility as broilers age and in Ravindran and Abdollahi (2021) the average of three lipid sources during the first seven days post-hatch was only 52.0% increasing to 79.9% by day 14 and reaching a maximum of 87.6% at day 21. Also, FFA may react with other nutrients to form soaps and compounds with minerals that may or may not be soluble. If insoluble salts are formed, both the mineral and the FA become unavailable to the bird and this is associated with reduced bone ash and calcium content (Kleyn and Chrystal, 2020). In high-yielding, modern broiler genotypes, the primary breeder recommendations for dietary AME have declined whilst digestible amino acid profiles have increased (Aviagen, 2022; Cobb-Vantress, 2022). This is due largely to the increased growth rate whereby the tangible amount of proportional dietary energy required for maintenance has declined. Also, selection for improved feed conversion has reduced the amount of total body lipid at maturity, and lipid has a worse feed conversion compared with lean gain. Furthermore, the heat increment of digestion of dietary lipid is lower than that of protein or carbohydrate and this has implications for formulating feed to net energy (NE), where NE is equivalent to the AME minus the heat increment of digestion (Wu et al., 2019). In Wu et al., (2019) an adjustment was made to AME, whereby dietary crude protein and crude fibre reduced NE whilst ether extract, used to measure dietary lipid, increased NE utilising metabolic closed-circuit calorimetry chambers, housing male Ross 308 broilers.

Interestingly, both primary breeders stipulate a minimum dietary linoleic acid for high yielding broilers; namely 12 g/kg from zero to 28 days post-hatch then 10 g/kg (Cobb-Vantress, 2022) and 12.5 g/kg from zero to 10 days post-hatch, then 12.0 g/kg from 11 to 24 days followed by 10 g/kg above 25 days post-hatch (Aviagen 2022). It would appear that the requirement is based on liver triene:tetraene ratio that changes at about 10 g/kg of dietary linoleic acid rather than broiler growth performance (Zornig et al., 2001). Instructively, in a series of 3 experiments with male Ross 208 chicks to 21 days post-hatch over two decades ago, Zornig et al., (2001) were able to demonstrate that broiler growth performance was very good at levels of 2.0 g/kg dietary linoleic acid.

In feed formulation, nutritional emulsifiers and biosurfactants such as lysophospholipids mimic the effect of natural bile salts and have their main effect on saturated fatty acids (C16:0 and C18:0). Lysophospholipids are more hydrophilic than phospholipids because they have a single FA residue per molecule and form spherical micelles in aqueous solution, leading to enhanced emulsification in the gastrointestinal tract. They may also play an important role in young broilers where bile production and recirculation are low (Ravindran, 2014). Studies have shown that lysophospholipids (lysolecithin) is more effective than bile and lecithin (Zhang et al., 2011; Zaefarian et al., 2015; Wealleans et al., 2020). In New Zealand studies, the addition of lysolecithin at 250 g/t to tallow and soyabean oil improved AME by 3.62% on average (from 13.54 to 14.03 MJ/kg, $P < 0.05$) in Ross 308 male broilers from one to 35 days post-hatch suggesting a raw material matrix value of 1960 MJ/kg for lysolecithin (Ravindran, 2014; Zaefarian et al., 2015). Lysolecithin has also been shown to enhance collagen expression and increase villus length in the jejunum of broiler chickens (Brautigan et al., 2017). Other commercially available emulsifiers include non-ionic liquids and powders and a novel glycolipid identified as sophorolipid (Kwak et al., 2022).

V. CONCLUSIONS

Dietary lipids cover a wide range of compounds and, a brief review is only able to cover some aspects of lipid nutrition without delving into detail. In least-cost linear feed formulation, energy remains the costliest component and, whilst it is treated as a “nutrient”, it comprises the energy released through the chemical oxidation of the feed. Both bird factors and feed factors play a role in the amount of energy available for broiler growth and maintenance as illustrated by Wu et al., (2019). Lipid digestion, absorption and metabolism are affected by numerous

factors inherent in the quality of the dietary lipid used, level of saturation and the age of the broiler. The degree of oxidation, lipids damaged through heat, MIU and other NEM compounds are difficult to quantify but, they are vitally important in assessing the quality of the lipid used. The quality of dietary lipids therefore needs to be assessed using several measures. Furthermore, in feed formulation, response to added dietary lipid is quadratic, whilst bird age has an influence on lipid digestibility and resulting AME. Dietary lipid also has an influence on the NE, due to reduced HI of digestion, increasing the energy available for growth and maintenance in broilers, thus affecting its relative worth to other dietary energy components, particularly in hot climates. However, selected nutritional emulsifiers and biosurfactants may enhance the digestibility of dietary lipids, particularly with respect to young broilers.

Due to the primary breeder recommendations for dietary AME declining, added lipid has also declined in many broiler feeds raising implications for the EFA, linoleic acid. For maize/soyabean meal-based diets, it is unlikely the linoleic acid would be below 10 g/kg, but low-energy wheat/soyabean meal-based broiler diets regularly formulate to below this level and, may result in increased feed costs. For broiler feeds, removing the minimum constraint for linoleic acid in feed formulation should be considered. Also, some dietary lipid and adipose tissue in the bird are required for metabolism of the fat-soluble vitamins, A, D, E and K.

In conclusion, adjusting accepted Wiseman calculations for dietary energy by including adjustments made for the portion of dietary lipid that does not contain an inherent AME value is prudent. However, further research is required to determine the levels of these diluents in commercially available feed lipids used in Australia, and establish the true energy values of these lipids more accurately.

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Table 1 - Calculated minimum (Min.) and maximum (Max.) apparent metabolizable energy (AME) values based on Wiseman (1997) equation and analysed moisture, impurities and unsaponifiable matter (MIU) in broilers, at 10 and 53 days post-hatch (adapted from Wealleans et al., 2021).

Parameter	Soya oil		Canola oil		Sunflower oil		Maize oil		Linseed oil		Palm oil		Beef tallow		Poultry tallow	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
FFA[1] (g/kg)	0.0	20.9	0.0	14.0	0.3	15.3	107.7	143.5	0.0	60.3	0.0	69.7	3.8	160.7	7.2	23.29
U:S[2] ratio	3.63	5.94	11.49	16.15	6.71	9.76	4.33	6.12	1.00	9.39	0.77	1.09	0.26	1.13	1.54	2.77
MIU (g/kg)	3.9	13.0	8.3	13.9	4.5	15.2	0.3	40.7	4.4	12.5	3.3	7.2	2.7	49.5	2.9	51.8
AME (MJ/kg)																
10 days	35.92	37.61	37.86	38.10	37.50	37.90	35.23	37.17	28.34	37.94	27.49	28.95	26.44	29.77	29.19	34.30
53 days	37.22	38.41	38.74	39.01	38.39	38.81	36.77	38.12	32.99	38.82	32.23	33.38	31.97	33.62	33.13	36.20
10 days + MIU	35.26	37.34	37.41	37.77	36.95	37.62	34.04	36.64	28.21	37.61	26.54	28.82	24.22	29.35	28.17	35.90
53 days + MIU	36.59	38.22	38.28	38.65	37.81	38.50	35.23	37.55	32.84	38.47	32.15	33.23	30.02	33.44	31.48	36.24

[1]Free fatty acids.
[2]Ratio of unsaturated to saturated fatty acids.

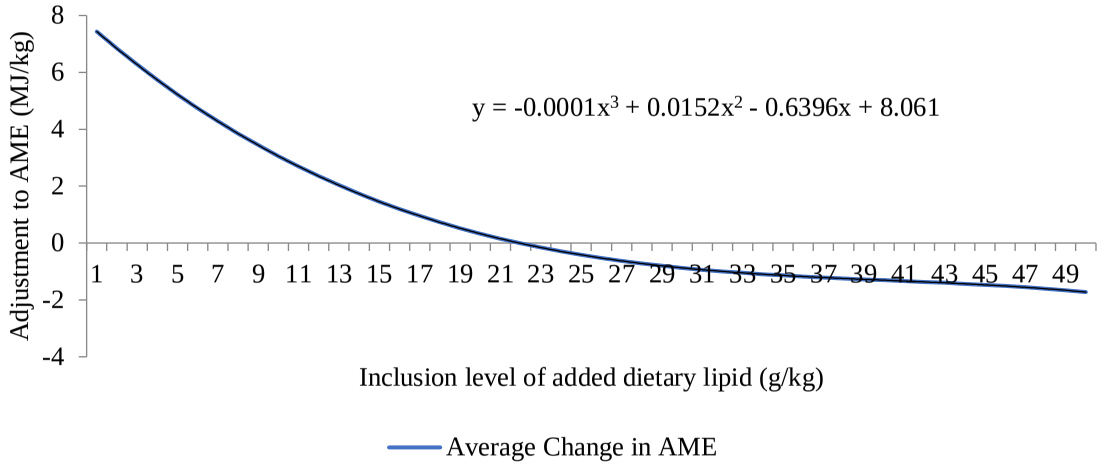

Figure 1 - Average adjustment to lipid AME (MJ/kg) based on added dietary lipid in broiler diets from 1 to 42 days post-hatch.

RECYCLED FOOD WASTE AS A FEED FOR POULTRY

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Summary

Feeding recycled food waste-based feed to poultry promotes sustainability, the circular economy and improves food security. A study was conducted to examine the nutrient composition, chemical residue and efficacy of processed and sterilized recycled food waste (RFW) as a feed for laying hens. Performance, egg quality and nutrient digestibility were examined from 24 to 63 wk of age. Hy-Line Brown hens (n = 150) were randomly distributed to three dietary treatments with 50 replicate single bird cages per treatment. Treatments were: control feed based on wheat, sorghum, and soybean meal (CON); RFW based-feed; and a 50:50 blend of CON and RFW. Hens fed RFW had similar egg weight, hen day egg production and egg mass, but lower feed intake ($P < 0.001$) and higher feed efficiency ($P < 0.001$) as compared to those fed CON. The 50:50 blend treatment showed an intermediate response for most measurements. Dietary treatment had no effect on albumen height, Haugh units, yolk colour, shell thickness, shell breaking strength or shell reflectivity of eggs ($P > 0.05$) at wk 63. Lower dry matter digestibility ($P < 0.05$) but higher fat digestibility ($P < 0.001$) was observed in hens fed the RFW compared to CON at wk 43. Detected pesticide, antibiotic and mycotoxin contamination was below maximum reportable limits for human food in all of the individual waste streams and finished feeds. The results demonstrate the viability of RFW as a feed for laying hens to improve the sustainability of poultry production.

I. INTRODUCTION

Feeding the world's projected burgeoning human population increase of 2.2 billion people by 2050 will place tremendous pressure on food security. The poultry industry is innovative and well positioned to address this problem through increased efficiency and waste reduction. Globally, 32% (by weight) of produced food − equivalent to 1.3 billion tons − is lost or wasted every year. Conversion of food waste into raw materials for production of poultry feed offers a potential solution to address both waste management and food security challenges. However, using food waste as a nutrient source for poultry is not without challenges. Issues associated with the logistics of collection, spoilage, chemical residues, pathogens, processing and digestibility need to be addressed. Feeding waste to livestock is not a new concept. Raw swill has been fed to pigs for centuries, but has been banned in developed countries due to presence of residues and pathogens.

Typical kitchen and restaurant waste contains around 50% to 85% moisture that must be removed if a stable product is to be produced (Myer *et al.*, 1999). Dewatering followed by short-time, high temperature drying is an effective drying method that minimises spoilage organisms and potential pathogens. The results of feeding dry waste streams to pigs have been reported (Kwak and Kang, 2005; Westendorf et al. 2000; Myer et al. 1999). Cho et al. (2000) reported on feeding dried leftover food waste to laying hens, where an increased protein level was required to maintain performance of hens fed the higher levels (200 and 300 g/kg) of food waste. Another study reported by Kojima (2005), found that feeding diets with 500 g/kg dehydrated kitchen waste had no effect on FCR or egg production but resulted in smaller eggs. A comprehensive review by Torok et al. (2021) concluded that food waste from retail and food service can be effectively and safety utilized in commercial production systems, as long as the

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correct processing and safety measures are implemented. A study is reported here that aimed to investigate the efficacy of recycled food waste from various streams formulated into a complete feed and compare it to a commercial type diet. Residue analysis, laying performance, egg quality and nutrient digestibility are reported. It was hypothesised that hens offered a food waste-based diet would perform similarly to those offered a commercial diet.

II. METHODS

Food waste streams were collected from breweries, pubs and restaurants, abattoirs, bakeries, vegetable and fruit markets, hospitals, nursing homes and fish processing facilities. The waste streams and finished feeds listed in Table 1 were analysed for nutrient content (AOAC, 2019), pesticide, antimicrobial and mycotoxin residues (Queensland Department of Agriculture and Fisheries Chemical Residue Laboratory, Coopers Plains). Amino acids and gross energy were assumed to be 65% digestible based on previous reports with pigs (Chae et al. 2000) and laying hens (Kojima 2005; Cho et al. 2004). Minerals were assumed to be 95% digestible. Each food waste stream was processed by steaming, maceration, dewatering, direct fired drum drying and particle size reduction as described in Dao et al. (2019). The patented process is designed to ensure inactivation of pathogenic and spoilage organisms with steam heating to exceed 100 deg C for 30 min (Boyle, 2018).

Treatments were: control (CON, commercial feed based on cereal grain and oilseed meal), 100% recycled food waste (RFW), and a 50:50 blend of CON and RFW. All diets contained additions of xylanase, phytase, L-lysine HCl, D,L-methionine, L-threonine, red and yellow pigments, antioxidant and vitamin-mineral premix. Table 2 shows the diet composition and Table 3 the nutrient composition from 24 to 37 wk. Feeds were mixed an additional three times from 37 to 44 wk, 44 to 58 wk and 58 to 64 wk, using fresh dried waste streams and conventional feed ingredients after analysis. Feed formulations required slight adjustments (not shown) as per assay results to keep the nutrient composition of each feed as constant as possible throughout the duration of the study.

Experimental procedures were approved by University of New England Animal Ethics committee. A total of 150 Hy-Line Brown 15-wk old pullets were fed a pre-lay diet from 15 to 19 wk of age, a commercial layer diet from 19 to 22 wk of age and were randomly allocated to the treatments at wk 23. Experimental feed was slowly increased during a 10-day adaptation period then the experimental diets were fed from wk 24. There were 50 replicate cages (30 cm wide × 50 cm deep × 45 cm high), each containing a single hen, per treatment. Experimental feed and water (one nipple drinker) were offered *ad libitum*. Feeds were formulated to meet minimum nutrient standards per the Hy-Line Brown nutrient recommendations (Hy-line Genetics, 2020). Hen day production, feed intake, egg weight, egg mass and FCR were measured wkly. Hens were weighed every 5 wk. Egg quality was measured monthly. Excreta was collected daily over a 3-day period to monitor moisture content and nutrient digestibility on 43 and 63 wk of age.

III. RESULTS

The average indoor shed temperature and relative humidity during the study ranged from 10.0 to 19.7°C and 49.4% to 76.1% respectively. Maximum daily temperature ranged from 13.0 to 29.0°C (average 20.4°C), while minimum daily temperature ranged from 4.0 to 14.0°C (average 10.4°C). The analyzed composition of various waste streams is given in Table 1. The fish offal and spent brewers grain blend (FBG), pub and restaurant (PR) and meat and bone (MB) wastes contained higher levels of crude protein (CP), crude fat, and total phosphorus relative to grains and oilseed meals (P and fat). Sodium (Na) content of the PR waste was higher than other commercially available ingredients and the Na requirement for hens. The

final diets formulated with waste streams met the formulation objectives in terms of meeting the minimum nutritional requirements of Hy-Line Brown hens. The analysed nutrients of CON were similar to calculated values. In the recycled food waste (RFW) diets, the analysed CP, crude fat, calcium (Ca) and Na levels were lower, while crude fiber level was higher than calculated values (Tables 1 and 3).

The particle size distribution test showed that certain amounts of over-size particles (≥ 4 mm) were still observed in BK, MB, PR, FBG, HN and OS after processing. High percentages of fine particles (≤ 0.5 mm) were detected in BG (72.8%) and VF (72.3%).

The residue levels for pesticides and mycotoxins are given in Table 4. All detected levels were below the maximum reportable limits (MRL) for food products, feed ingredients and finished feeds as per the Australian Agricultural and Veterinary Chemicals Code F2022C00839.

Over the entire study, birds in all treatments were visibly in good health. The mortality rates of the control, food waste and 50:50 blend treatments from 24 to 43 wk of age were 0%, 0% and 2%, respectively. There was only one mortality recorded in the 50:50 blend treatment and the mortality was not related to dietary treatment. No additional mortalities were observed from 43 to 63 wk of age.

Table 1 - Analyzed nutrient values of food waste materials from 24 to 44 wk (as-is basis, g/kg, otherwise as indicated).[1]

	Waste streams								Feed	
Nutrient	BG	FBG	PR	MB	BK	VF	HN	OS	CON	RFW
Dry matter	981	975	898	947	992	895	894	973	910	912
Crude protein	256	374	277	353	179	163	234	6	174	196
GE, kcal/kg	5155	5414	4251	4009	4571	3298	4469	3728	3523	4175
Crude fat	98	248	233	217	56	29	196	1	51.9	67.6
Crude fiber	173	79	41	32	26	148	24	134	90.0	95.4
Ash	35	128	249	328	34	158	207	942	152	113
Calcium	2.3	47.8	86.7	135.7	5.9	35.3	82.0	381.3	57.1	40.4
Phosphorus	5.3	23.5	34.1	62.9	2.7	4.6	30.0	0.22	5.8	9.2
Sodium	0.2	3.2	22.9	5.9	6.7	8.9	-	4.8	1.4	3.2
Potassium	0.6	3.8	4.3	3.5	2.8	30.8	5.2	0.00	6.6	4.3
Lysine	11.1	20.4	17.1	18.2	5.2	5.2	13.0	0.6	8.3	7.9
Methionine	2.7	7.0	5.2	5.5	2.8	1.4	3.7	0.1	3.9	4.1
Threonine	9.8	14.1	10.3	10.9	5.6	4.9	-	-	6.2	6.4
Arginine	12.5	22.7	17.3	24.9	7.3	7.3	150.0	0.45	9.7	9.9
Isoleucine	11.3	13.8	10.2	9.6	6.8	4.6	7.90	0.27	6.8	7.0
Leucine	20.1	23.1	18.8	19.6	12.0	7.1	16.00	0.46	13.1	12.7
Valine	14.8	17.0	13.2	14.0	7.8	6.9	-	-	8.1	8.7

[1]Values of all the amino acids presented were total amino acids (measured on an as-is basis).
Waste streams: BG= brewers grains, FBG = fish waste and brewers grains blend 50:50, PR= Pub and restaurant waste, MB = meat and bone abattoir waste, BK = bakery waste, VF = vegetable and fruit waste, HN = hospital and nursing home waste, OS = oyster shell. Feed: CON = control (commercial feed), RFW = 100% recycled food waste feed.

Table 2 - Ingredient composition of diets (as-is basis, g/kg), 24 to 37 wk.

Dietary treatment		
	CON	RFW
Wheat	407.5	-
Sorghum	200.0	-
Soybean meal	135.1	-
Canola meal	100.0	-
Commercial meat and bone meal	20.0	-
Canola oil	29.6	-
Limestone (50:50 coarse and fine)	96.0	-
Di-calcium phosphate	4.8	-
Salt	2.4	-
Vegetable and fruit meal (VF)	-	50.0
Spent brewers grain (BG)	-	349.0
Fish offal and spent brewers grain blend (FBG)	-	150.0
Hospital and nursing home meal (HN)	-	150.0
Pub and restaurant meal (PR)	-	31.1
Recycled meat and bone meal (MB)	-	83.0
Bakery meal (BK)	-	1.8.4
Oyster shell meal (OS)	-	12.8
Choline Cl 70%	0.61	2.68
L-lysine HCl	0.73	-
DL-methionine	1.69	1.66
L-threonine	0.16	-
Xylanase[1]	0.05	0.05
Phytase[2]	0.06	0.06
Pigment Jabiru red	0.04	0.04
Pigment Jabiru yellow	0.03	0.03
Antioxidant[3]	0.25	0.25
Vitamin-mineral premix[4]	1.00	1.00

CON = Control diet; RFW = 100% recycled food waste diet.
[1]Econase XT, 25, AB Vista , [2]Quantum Blue 5G Layers, AB Vista.
[3]Feedox, FeedWorks, Australia.
[4]Vitamin-mineral premix provided the following per kilogram diet: vitamin A, 10000 IU; vitamin D, 3000 IU; vitamin E, 20 mg; vitamin K, 3 mg; nicotinic acid (niacin), 35 mg; pantothenic acid, 12 mg; folic acid, 1 mg; riboflavin (B2), 6 mg; cyanocobalamin (B12), 0.02 mg; biotin, 0.1 mg; pyridoxine (B6), 5 mg; thiamine (B1), 2 mg; copper, 8 mg as copper sulphate pentahydrate; cobalt, 0.2 mg as cobalt sulphate 21%; molybdenum, 0.5 mg as sodium molybdate; iodine, 1 mg as potassium iodide 68%; selenium, 0.3 mg as selenium 2%; iron, 60 mg as iron sulphate 30%; zinc, 60 mg as zinc sulphate 35%; manganese, 90 mg as manganous oxide 60%; antioxidant, 20 mg.

Hen weights and weight gain showed lower body weight in hens fed RFW compared to CON at 29, 39 and 43 wk. The laying performance from wk 24 to 63 is given in Table 5. Hens offered the RFW diets had similar egg weight, hen day egg production and egg mass, but lower feed intake ($P < 0.001$) resulting in a lower FCR ($P < 0.001$) compared to those fed the CON diets. Hens fed the RFW diets had approximately 22 points lower FCR compared to CON from 24 to 63 wk of age. Hens fed the 50:50 blend had intermediary FCR.

No treatment differences in internal or external egg quality were detected as shown in Table 6. However, the proportion of yolk as a percent of total egg was slightly higher in the RFW as compared to CON. Shell thickness and shell reflectivity (darkness of colour) were not affected by the dietary treatments ($P > 0.05$).

Table 3 - Calculated nutrient composition of diets, as-is basis, g/kg unless otherwise indicated, 24 to 37 wk.

	CON	RFW	50:50
AMEn[1], kcal/kg (MJ/kg)	2800 (11.72)	2800 (11.72)	2800 (11.72)
Crude protein	178	256	217
Crude fat	53	134	94
Crude fiber	28	89	59
SID[2] arginine	9.5	9.6	9.5
SID lysine	7.8	8.1	8.0
SID methionine	4.2	4.5	4.4
SID methionine + cysteine	7.2	6.7	6.9
SID tryptophan	2.1	1.9	2.0
SID leucine	12.0	13.3	12.7
SID isoleucine	6.3	7.4	6.9
SID threonine	5.6	6.1	5.9
SID valine	7.3	9.6	8.5
Calcium	42.0	42.0	42.0
Available phosphorus	4.5	10.2	7.4
Sodium	1.8	4.5	3.2
Potassium	7.0	-	-
Chloride	2.2	-	-
Choline, mg/kg	1400	1400	1400
Linoleic acid	15.8	-	-

CON = control; RFW = recycled food waste.

[1]AMEn: N corrected apparent metabolizable energy. Metabolizable energy levels of food waste materials were estimated at 65% of gross energy on an average.

[2]Standardised ileal digestible amino acid coefficients of conventional feed ingredients were determined by Near-Infra Red spectroscopy (Bruker MPA, USA) using Evonik AMINONIR Advanced calibration. Digestible amino acid coefficients of food waste materials were estimated at 0.65 (65%).

Table 4 - Analyzed pesticide and mycotoxin residues of food waste materials and feed, as-is basis after processing, mg/kg, otherwise as indicated[1].

	Food waste streams								Feed	
Residue	BG	FBG	PR	MB	BK	VF	HN	OS	CON	RFW
Azoxystrobin	*	*	*	*	*	0.03	*	*	*	*
Boscalid	*	*	*	0.02	*	*	*	*	*	0.03
Chlorpyrifos	*	*	*	*		0.01	0.01	*	*	0.01
Chlorpyrifos CH3	1.3		0.04		0.03	*	0.05	*	0.25	0.16
Cypermethrin	*	*	*	*	*	0.17	*	*	*	*
Fenitothron	*	*	*	*	*	*	*	*	*	0.04
Fludioxonil	*	*	*	*	*	0.03	*	*	*	*
Flubendiamide	*	*	*	*	*	0.41	*	*	*	*
Piperonyl butoxide	*	*	0.11	*	*	0.10	0.25	*	0.36	0.70
Indoxacarb	*	*	*	*	*	0.08	*	*	*	*
Iprodione	*	*	*	*	*	0.11	*	*	*	*
Thibendazole	*	*	*	*	*	0.02	*	*	*	*
Chlortetracycline	*	*	trace	trace	*	*	*	*	*	*
Mycotoxins	*	*	*	*	*	*	*	*	*	*

* = limit of reporting. Waste streams: BG= brewers grains, FBG = fish waste and brewers grains blend 50:50, PR= Pub and restaurant waste, MB = meat and bone abattoir waste, BK = bakery waste, VF = vegetable and fruit waste, HN = hospital and nursing home waste, OS = oyster shell. Feed: CON = control (commercial feed), RFW = 100% recycled food waste feed.

Tested number of analytes (if not listed above, not detected). Pesticides: fungicides 50, herbicides 10; insecticides and other 39, organophosphate 38, organochlorine 23. Antimicrobials: tetracyclines 4, aminoglycosides 5, cephaloporins 3, sulfonamides 14, macroides/lincosamides.triamilides 8. Mycotoxins: aflatoxin G1 G2 B1 B2, fumonison B1 B2, 3 acetoxynivalenole, deoxynivalenole, nivalenole, zearalenone. All detected levels are below the maximum reportable limits (MRL) for food products, feed ingredients and finished feeds.

Table 5 - Performance of hens fed dietary treatments from 24 to 63 wk.

Variable	Control	Food waste	50:50 blend	SEM	P-value
Egg weight, g	61.7	61.2	61.6	0.29	0.747
Hen day egg production,%	95.9	96.2	96.6	0.34	0.534
Egg mass, g/day	59.1	58.8	59.6	0.35	0.709
Feed intake, g/day	128[c]	114[a]	121[b]	0.84	< 0.001
FCR, kg feed/kg egg	2.172[c]	1.948[a]	2.044[b]	0.015	< 0.001

[a,b,c] Means in rows with different superscripts are significantly different (P < 0.05).

Table 6 - Egg quality at 63 wk.

Variable	Control	Food waste	50:50 blend	SEM	P-value
External egg quality					
Shell breaking strength (Kgf)	3.71	3.68	3.74	0.07	0.961
Shell thickness (mm)	0.448	0.424	0.429	0.005	0.157
Reflectivity (%)	27.9	28.3	28.1	0.31	0.856
Internal egg quality					
Albumen height (mm)	9.05	8.95	8.87	0.11	0.800
Yolk colour	12.1	11.9	12.1	0.10	0.592
Haugh unit	94.1	93.9	93.7	0.55	0.956
Egg proportion					
Albumen (%)	63.6	62.8	62.9	0.18	0.181
Yolk (%)	26.6[a]	27.5[b]	27.2[ab]	0.16	0.044
Shell (%)	9.8	9.7	9.6	0.07	0.619

[a,b] Means in rows with different superscripts are significantly different (P < 0.05).

The results shown in Table 7 indicate a higher level of moisture in excreta from birds fed RFW as compared to CON (P < 0.01). The 50:50 treatment was intermediate. Dry matter digestibility was lower in hens fed the DFW compared to the CON (P < 0.05). Fat digestibility was higher in hens fed the RFW and 50:50 diets compared to those fed CON (P < 0.05).

Table 7 - Percent excreta moisture and apparent nutrient digestibility at 43 wk.

Variable	CON	RFW	50:50 blend	SEM	P-value
Excreta moisture	75.4[a]	78.2[b]	76.3[ab]	0.39	0.005
Dry matter	69.3[b]	66.6[a]	68.0[ab]	0.47	0.049
Energy	74.2	72.1	72.9	0.38	0.064
Fat	67.2[a]	81.7[c]	76.2[b]	1.35	<0.001
Protein	44.8	47.3	44.1	0.79	0.240

CON = control; RFW = recycled food waste
[a,b,c] Means in rows with different superscripts are significantly different (P < 0.05)
Total collection method

IV. DISCUSSION

When formulated to meet minimum nutrient requirements of the Hy-Line Brown, RFW diets contained higher concentrations of CP, crude fat, crude fibre, total P and Na compared to the CON diet. As the study progressed, and new batches of food waste were utilised, closer nutritional levels between the CON and RFW diets were observed in the second period of the study from wk 38 to 43 compared to the initial period (wk 24 to 37).

The 100% RFW diet had 912 g/kg dry matter (DM), 196 g/kg CP and 67.6 g/kg crude fat and 95.4 g/kg crude fibre. This was similar to the food waste collected by Cho et al (2000) with 937 g/kg DM, 206 g/kg CP, 99 g/kg crude fat and 89 g/kg crude fibre.

No mycotoxins were detected in waste streams or finished feeds in the current study indicating no issues with mould growth between collection of waste streams and processing

and drying. The analytical screen for insecticides, herbicides, fungicides and antibiotics detected trace amounts of mainly insecticides in the dried vegetable and fruit waste stream. The highest was flubendiamide, a ryanoid insecticide, detected at 0.41 mg/kg DM basis. The MRL in Australia for this chemical in food vegetables (as is basis) ranges from 2 to 20 g/kg depending on the individual vegetable. Piperonyl butoxide, a common synergist agent used with pyrethrin based insecticides was detected at low levels of 0.11 mg/kg in PR, 0.10 mg/kg in VF and 0.25 in HN waste streams after processing. The MRL for this chemical is 8 mg/kg in vegetables and 20 mg/kg cereal grains for human consumption. The insecticide chlorpyrifos-methyl was detected at 1.3, 0.04, 0.03, 0.05, 0.25 and 0.16 mg/kg in BG, PR, BK, HN, CON and RFW respectively. The MRL for this chemical in cereal grains for human consumption is 10 mg/kg. The pesticide results all indicate that levels detected for food waste streams and finished feeds were below the MRL limits for human consumption as stated in Table 1 of the Australian Agricultural and Veterinary Chemicals Code F2022C00839.

In the current study, no differences were detected between CON and RFW for hen day production, egg size and egg mass. However, feed intake and FCR were lower in hens fed RFW as compared to CON with birds fed the 50:50 blend being intermediate. Cho et al. (2000) reported on feeding food waste at 100, 200 and 300 g/kg in normal and 10% higher protein levels against a control diet in isoenergetic diets. Food waste was dried in a fluidised bed drying apparatus (temperature and time not reported). Overall seven-wk results through peak production showed higher FCR, lower hen day production and no different in egg size with the 200 and 300 kg/t at normal protein. Increasing the protein by 10% improved performance of the 200 and 300 kg/t food waste treatments to that of control suggesting lower digestibility of the amino acid fraction of the food waste as compared to conventional ingredients.

Kojima (2005) reported on dehydrated kitchen food waste as an ingredient for laying hens at 125, 250 and 500 kg/t of feed. Food waste was dried at 65 deg C to 878 g/kg DM and contained 151 g/kg CP, 53 g/kg crude fat, 23 g/kg crude fibre and 3.1 g/kg Na. Hens fed the diet with 50.0% food waste had decreased egg weight but no differences in hen day production or FCR.

In the current study, relative yolk size was larger in eggs from hens fed the RFW as compared to those fed the CON. This was likely due to higher total fat in the diet and possibly higher levels of linoleic acid, although not analysed or reported. Eggshell breaking strength and shell reflectivity were not affected by the dietary treatments. In the study of Kojima (2005), eggshell strength tended to weaken with increased food waste in the diet and eggshell reflectivity was higher (lighter) with increasing food waste. The differences between the current study and those reported by Kojima (2005) may be due to differences in formulation and Ca content and Ca particle size.

In the current study, Na levels in the RFW diet exceeded nutrient recommendations for Hy-Line hens. The higher excreta moisture levels in the RFW fed hens was likely due to the higher levels of Na and salt causing increased water consumption. This could be an issue in areas of high humidity and may require lower use of the high salt waste streams or some other methods to remove the salt during processing.

The higher digestibility of crude fat and higher protein content in the RFW diet compared to the CON may explain the lower FCR observed in hens fed the RFW.

V. CONCLUSION

This study has clearly demonstrated the viability of using RFW as a feedstuff for laying hens. Feed conversion was improved as a result of feeding RFW with egg production and quality unchanged. No issues with pesticide, antibiotic or mycotoxins residues were detected. The food waste diets met or exceeded nutrient specification for laying hens and would provide both

economic and environmental advantages by diverting waste from landfill. This study demonstrates that RFW is a highly feasible feed for poultry and represents an opportunity for sustainable and profitable poultry production.

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EXTENDING LAYER HEN LIFESPAN: STUDIES IN THE AUSTRALIAN CONTEXT

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Summary

Extending the lifespan of egg laying hens would contribute to decreasing the size of the national flock and the use of limited resources, increasing the sustainability of the Australian egg industry. For extension of flock life to be economically viable, aspects of hen management including hen feed efficiency, eggshell quality, and hen health need consideration. To this end some recent Australian studies of brown egg producing hens in longer laying cycles have been undertaken. Overall, smaller sized hens at point of lay that gain small increments of weight through to mid-lay consume less feed, are more feed efficient with good bone health and more favourable liver function throughout a longer laying cycle compared to larger sized hens. Eggshell quality in older hens benefitted when a more nutrient dense diet was fed in the early laying period. The transferability of these outcomes into cage free egg production systems in Australia should be evaluated.

I. INTRODUCTION

With ongoing genetic selection for improved persistency of lay and feed efficiency (FE) in laying hens, the egg industry is pursuing the opportunity to extend layer hen lifespan until they are 100 weeks of age (WOA), with an aim for each hen to produce up to 500 eggs. This presents some challenges for the hen as during this longer laying cycle she will be producing more than 31 kg of egg product (Muir et al., 2022c) and using approximately 1 kg of calcium (Ca) to produce up to 3 kg of eggshell (Nys, 2017). Continuous egg production places high demand on the organs and tissues involved in producing eggs. This includes the liver, which generates yolk lipid and, the oviduct which produces the egg white, shell membranes and eggshell. Further, the continuous demand for Ca for eggshell formation may impact bone integrity, especially the risk of bone fractures and osteoporosis (Whitehead and Fleming, 2000). However, recent reports have not found a direct relationship between continuous egg production and bone quality (Dunn et al., 2021). Eggshell quality is central to the production of first grade eggs and is a key determinant for the flock to continue through a longer laying cycle. Optimal liver and skeletal health are also critical for the successful extension of flock life. Recent Australian studies that have explored hen characteristics, productivity, FE, egg quality, liver health and bone health in longer laying cycles will also be discussed.

II. EGG PRODUCTION AND FEED EFFICIENCY

The transition of a pullet from rearing into egg production presents many challenges. This includes the stressor of transportation from the rearing to the laying facility (Kolnik, 2021), and acclimatisation to the layer shed. During this transition it is ideal that the hen continues to grow and then starts to lay eggs (Bain et al., 2016). In Australia, layer pullets are typically reared to above breed standard weight (BSW) at point of lay (Parkinson et al., 2015). This is practiced as larger pullets appear to manage the transition to the laying shed more readily than lighter sized hens and they start to lay eggs of a larger size at an earlier age (Summers and Leeson, 1991). However, heavier hens have higher nutritional needs and hence higher feed intake (FI).

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There is also a greater risk of a drop in egg production during peak lay if their nutritional needs are not met (Pottguter, 2016). The ongoing measurement of FI, egg production and egg mass throughout the laying cycle allows hen FE to be calculated (O'Shea et al., 2020).

III. EGG WEIGHT AND EGG QUALITY

Egg characteristics, including the appearance of the yolk, albumen and eggshell, are a priority for the consumer. Eggs classified as Extra-Large, where a carton of 12 eggs weighs 700g, with average egg weight (EW) 60 g, is the target for most Australian producers. Maintaining EW below 70 g avoids increased egg losses due to cracked eggshells (Parkinson et al., 2015). An EW of 60-65g (Parkinson et al., 2008) accommodates market demand and eggshell quality.
Egg size typically increases with hen age (Leeson and Summers, 1987; dePersio et al., 2015). Larger eggs contain a larger yolk and less albumen (Jiang and Sim, 1991). In Australia, a more golden coloured yolk, i.e. ≥11 on the Roche colour scale, is preferred (Roberts 2004). Albumen viscosity and quality calculated as Haugh unit (HU) also tends to decrease with hen age (Marzec et al., 2019). A HU of 82 is recommended throughout an 18–100 week egg laying cycle (ISA Brown Product Guide, Cage Production System, 2017).

The shell of each egg requires approximately 2.2 g of Ca (Bouvarel et al., 2010). To meet this requirement, Ca is sourced from both the hen's diet and her skeletal system. There are several comprehensive reports on nutritional management for eggshell quality (Roberts, 2004; Nys, 2017) and specifically within the longer laying cycle (Pottguter, 2016; Korver, 2020). As hen age and EW both increase, eggshell quality tends to decline, becoming thinner and more liable to cracks and fractures (An et al., 2016). Concurrently there is a reduction in intestinal uptake of Ca by older laying hens (An et al., 2016). Eggs with ≤9.5% eggshell weight are more susceptible to breakage (Abdallah et al., 1993) whereas ≥10% eggshell weight will minimise cracks. Typically, increased variability in eggshell quality with flock ages will determine when the flock will be terminated (Dunn, 2013).

V. LIVER AND BONE HEALTH

The liver processes starch, carbohydrate, and fat to form yolk lipid, which also involves the production of hepatic fat (Squires and Leeson, 1988). When this process is disrupted or experiences an imbalance, fatty liver haemorrhagic syndrome (FLHS) may ensue (Yang et al., 2017). Hens housed in cages and receiving high energy diets are most susceptible to FLHS (Shini et al., 2019). Environmental and bird genetics may also predispose birds to FLHS (Squires and Leeson, 1988). In addition to abdominal and hepatic fat accumulation, severe or extensive acute liver haemorrhage can cause sudden death (Shini et al., 2020). The impact of less severe chronic FLHS is more poorly understood (Bryden et al., 2021).

Korver (2020) concisely described the continual recruitment and re-deposition of labile Ca in the medullary bone deposits of the skeletal system for the formation of eggshell. At night, when low dietary Ca intake coincides with eggshell formation, Ca may also be mobilised from structural bone. This can occur even when there is ample medullary bone but, unlike medullary bone, structural bone is not replaced while the hen is in lay (Korver, 2020). Loss of structural bone increases the risk of bone fractures and osteoporosis (Whitehead and Fleming, 2000). As hens age, bone density declines and bone porosity increases (Yamada et al., 2021), which may be exacerbated with longer laying cycles. Interestingly, recent studies have not found a direct relationship between high egg production, eggshell quality, and bone integrity (Alfonso-Carrillo et al., 2021, Dunn et al., 2021) suggesting that eggshell and bone quality may be managed independently through optimum nutrition and genetic selection.

Continuous high egg production has also been attributed to keel bone fractures (KBF) (Toscano et al., 2020). These are most frequently seen in cage free systems but also occur in

caged flocks (Baker et al., 2020). The incidence of KFB has been reported to peak around 50 WOA (Petrik et al., 2015; Toscano et al., 2020), but KBF have been observed in more than 50% of hens in older flocks (Käppeli et al., 2011). In addition to high egg production, age of first egg and hen inactivity have also been implicated with KBF (Toscano et al., 2020).

VI. AUSTRALIAN STUDIES

As Australian egg farmers are interested in extending layer hen lifespan, there is a need to identify ways of extending the egg laying cycle together with the production of saleable eggs. This has been the objective of some recent studies funded by Australian Eggs.

a) Study 1

This study was designed to compare the performance of pullets of either above or below BSW when 18 weeks of age (WOA) (former heavier weight; HW and latter lighter weight; LW) in a laying cycle that extended from 18 to 89 WOA (Muir et al., 2022a,b,c). As lighter sized hens have innately lower FI (Harms et al., 1982), the experiment also included two early-lay dietary treatments. This entailed a diet of higher nutrient density (HND), as a potential mechanism for the LW birds of lower FI to receive adequate nutrition as they entered lay, and a more common diet of lower nutrient density (LND). The HND diet may also be a primer for hens destined for an extended laying cycle (de Persio et al., 2015). Given the higher cost of HND diets, it was provided for 7 weeks only, rather than the more common duration of production (dePersio et al., 2015; Perez-Bonilla et al., 2012; Scappaticcio et al., 2021).

Individually housed LW and HW ISA Brown hens were allocated to one of the two early-lay dietary treatments. These were a LND diet, formulated to 110g FI/day, (2726 kcal/kg, 16.4% crude protein (CP) and 0.74% standardised ilea digestible Lysine (SID), 2.54% crude fat (CF)) or the HND formulated to 90 g FI/day (2901 kcal/kg, 17.63 % CP, 0.83% SID and 4.92% CF) which birds received from 18–24 WOA inclusive. From 25-39 WOA all birds were fed the early-lay LND diet, followed by a mid-lay LND diet formulated to >110g FI/day (2724 kcal/kg, 16.0% CP, 0.70% SID, 2.53% CF) from 40-77 WOA. Finally, when 78 WOA all birds were moved onto a late-lay LND diet formulated to 110g FI/day (2753 kcal/kg, 16.2 CP, 0.73% SID, 2.5% CF). This was fed until hens were 90 WOA, when the experiment concluded. Observations when hens were 69-70 WOA and 89-90 WOA are presented.

The HW pullets (at 18 WOA) remained comparatively heavier with higher FI throughout the study (Table 1). All birds gained weight between 18–70 WOA (Muir et al., 2022b) with comparatively smaller increases in BW until 90 WOA. Despite this, HW birds continued to be notably heavier than BSW for age. Lighter weight hens achieved BSW for age around 62 WOA and BW remained relatively stable to 90 WOA. There is agreement that small increments of weight gain for LW hens are beneficial (Perez-Bonilla et al., 2012, O'Shea et al., 2020), with the latter recommending LW hens attain BSW for age during mid-lay. Further, the gradual increase in weight of smaller sized hens allows them to reach full maturity, with sustained egg production and FE.

Hen-day egg production (EP) was similar for birds of both BW groups, averaging 89% at 69 and 81% at 89 WOA, above breed standard rate for age of 84% and 74.4% respectively (ISA Brown Product Guide, Cage Production System, 2017). The total number of eggs produced were similar for all birds, averaging approximately 465 eggs/hen at 89 WOA (Table 1).

Cumulatively, LW hens had lower FI from 18-89 WOA, generating lower egg mass and lower, but not statistically significant cumulative FCR compared to HW hens (Table 1; Muir et al., 2022c). This is in contrast with the significantly lower cumulative FCR of LW hens earlier in production at 18-24 and 18-69 WOA (Table 1; Muir et al., 2022b) and 18-50 (Muir

et al., 2022a), Hence characteristics of FE with hen size observed during early lay were maintained until 69 WOA but became more variable later in lay.

Table 1 - ISA Brown hen bodyweight at 70 and 90 weeks of age and, cumulative feed intake, number of eggs laid/hen and feed conversion ratio from 18-69 and 18-89 WOA (Muir et al., 2022b,c).

Treatment	BW 70 wk (Kg)^	BW 90 wk (Kg)	Cum. FI 18-69 wk (Kg)	Cum. FI 18-89 wk (Kg)	Cum. eggs/hen 18- 69 wks	Cum. eggs/hen 18-89 wk	Cum. FCR 18-69 wks	Cum. FCR 18-89 wks
BW (18woa)								
HW	2.20	2.23	42.7	58.4	348	470	2.09	2.14
LW	1.99	2.01	39.7	53.5	343	463	2.03	2.10
DND								
HND[#]	2.10	2.12	41.1	55.4	346	465	2.04	2.11
LND	2.09	2.11	41.4	56.5	346	468	2.08	2.12
Interaction								
HW*HND	2.22	2.25	42.5	57.9	348	465	2.08	2.16
HW*LND	2.18	2.20	43.0	58.9	349	475	2.09	2.12
LW*HND	1.98	1.99	39.6	52.9	344	465	2.01	2.07
LW*LND	2.00	2.02	39.7	54.2	343	460	2.06	2.13
P- Values								
BW	< 0.001	<0.001	<0.01	<0.001	0.07	0.29	0.053	0.33
DND	0.63	0.78	0.48	0.29	0.98	0.70	0.18	0.85
BW*DND	0.42	0.31	0.65	0.86	0.82	0.31	0.47	0.21

BW(18woa): 18 weeks of age body weight; HW: Heavier weight; LW: Lighter weight; DND: Diet nutrient density; HND: Higher nutrient density diet (formulated on 90g FI/day; 2900 kcal/kg, 0.83% SID.Lys); LND: Lower nutrient density diet (formulated to 110g FI/day; 2725 kcal/kg; 0.74% SID.Lys); BW (Kg)^: body weight; Cum FI: cumulative feed intake; Cum eggs/hen: total number of eggs produced per hen;; Cum FCR: Cumulative feed conversion ratio as kg feed/kg egg mass.

O'Shea et al., (2020) also identified that, compared to LW hens, heavier ISA Brown hens had higher FI and higher FE at peak and late lay. Earlier studies in White layers also calculated superior FE in LW hens through to 84 WOA (Lacin et al., 2008). Comparisons of hen BW with FI by Leeson and Summers, (1987) and Parkinson et al. (2015) drew similar conclusions, determining that for each additional 100 g BW a further 3.5 g FI/day was needed. The former also estimated a concurrent increase of 1.2 g EW.

As continuous FI and egg production data was collected (Table 1; Muir et al., 2022c), a simple cost-benefit comparison of cumulative FI with cumulative egg production across this extended 18-89 WOA laying period was possible. Compared to LW hens, HW hens consumed an extra 4.85 kg feed to produce an additional 7 eggs. Feed costs and returns on eggs will vary but at estimated cost of $512 AUD /ton layer feed and $0.14 AUD return/first grade egg, the extra cost is approximately $1.48/HW hen. In a 50,000 flock this is an additional $74,000 from 18-89 WOA. Alternatively, to break even, each HW hen needed to produce an additional 11 eggs, or to consume only 1.91 kg extra feed.

Diet nutrient density did not affect EW in older hens. At 69 WOA, EW for all hens was above 60 g and HW hens produced the largest eggs (Muir et al, 2022b). At 89 WOA, the heaviest eggs were being produced by HW hens that had received the LND (average 63.4 g) and lightest eggs were from LW hens on early-lay LND diet (average 60.8 g). LW hens on HND early-lay diet generated an intermediate EW of 62.3g (Muir et al., 2022c).

Egg quality was assessed on a focal group of hens at 66-70 and 86-90 WOA (Muir et al., 2022b,c). Yolk colour score decreased with age from 11 at 70 WOA to 9 by 90 WOA (Muir et al., 2022b,c). Haugh units were generally high, including average 90 HU at 90 WOA (Muir et al., 2022c). Hens that had received the LND diet during early-lay had higher HU between 86-90 WOA. Several studies have assessed internal egg quality in relation to hen BW

and diet nutrient density, with varying results (Muir et al., 2022b). Interestingly hens of higher FE and lower BW produced eggs with higher HU and higher amino acid concentration in the albumen, compared to less efficient, heavier hens (Akter et al., 2019; Anene et al., 2021). Neither hen size nor diet nutrient density altered eggshell weight. At 66-70 WOA, shell weight was > 10% and at 86-90 WOA > 9.5% EW. However, at both 70 and 90 WOA, hens fed the early-lay HND diet produced thicker and stronger eggshells than hens fed the LND diet (Muir et al., 2022b,c). Neither shell weight %, shell ash nor shell mineral levels provided an insight into the reason for the thicker and stronger shells.

At 70 and 90 WOA, FLHS scores were similar for all treatment groups (Muir et al., 2022b,c). However, at 50 WOA, FLHS scores and hepatic lipid peroxidase were lower in LW hens and hens that had received the HND diet during early-lay (Muir et al., 2022a). O'Shea et al. (2020) also reported lower FLHS scores in LW, more FE hens at 45 WOA. Liver lipid peroxidase did not differ at 70 WOA, but at 90 WOA it was lowest in HW hens that received the LND diet and in LW birds that received the HND diet during early lay. Keel curvature and bone breaking strength were similar across all treatments at 70 and 90 WOA (Muir et al., 2022b,c). However, higher levels of zinc (Zn) and manganese (Mn) were found in the bones of 90-week-old LW compared to HW hens (Muir et al., 2022c). Lower serum levels of both Zn (Mutlu et al., 2007) and Mn (Rondanelli et al., 2021) have been observed in osteoporotic female patients, and hence their higher levels in LW hens are indicative of a lower likelihood of developing osteoporosis.

Overview of study 1: Lighter weight hens demonstrated persistency of lay comparable to HW hens, together with more favourable liver health in mid-lay and bone mineral composition in very late lay. They also maintained a lower FCR until late lay. Feeding a HND diet in early lay increased eggshell strength in late and very late lay for all hens.

b) Study 2

As Study 1 illustrated that hen size trajectory is established by point of lay, Study 2 was designed to grow pullets to three different BW at 16 WOA. Their egg production and egg quality are to be followed through to 100 WOA. Using two lighting and three feeding programs during rearing either BSW or BW above and BW below BSW were attained.

Hy-line Brown chicks were grown in floorpens under two lighting and three feeding programs. Lighting was either standard (SL) lighting of 10h/day from 7–16 WOA, or rapid step-down (RSD) lighting of 9 h light/day from 4-16 WOA. From 4 WOA pullets were either fed *ab libitum*, or to achieve either BSW or 88% BSW, identified as Managed feeding, at 16 WOA. At 16 WOA the pullets were transferred to the Poultry Research Unit Camden and housed in individual pens in the layer shed. Here they received a pre-lay, early, mid and late lay diet *ad lib*. Lighting was stepped up from 11h /day at 16 WOA to 16 h/day at 32 WOA, where it remained until hens were 100 WOA and the study concluded. Pullet FI, BW at 16 WOA, age of first egg and weight of first three eggs was measured. Hen performance, egg quality and hen health will be assessed through to 100 WOA, with data to 92 WOA being reported here.

The outcomes of the rearing phase are presented in these proceedings (Muir et al., 2023). In brief, *ad lib* feeding under SL lighting resulted in the heaviest pullets at 16 WOA. There was an interaction between feeding and lighting on age of first egg. *Ad lib* fed pullets under RSD lighting were the first to lay, and pullets on Managed feeding under SL lighting were the last to start producing eggs. Weight of the first three eggs was independently affected by lighting and feeding where heaviest eggs were from SL lighting, BSW and Managed feeding.

At 92 WOA, birds reared under SL lighting were heavier than those reared with RSD lighting. Pullets fed both *ad lib* and to achieve BSW at 16 WOA, were also heavier than Managed fed pullets. The average BW of all treatment groups at 92 WOA was >2.2kg, above 1.92-2.04 kg BSW for age (Hy-Line Brown Commercial Layers Management Guide, 2019). This may be due to the individual hen housing with ready access to feed and water. Average daily FI at 92 WOA ranged from 110-115g/d, whereas breed recommendation is 105-111 g/d. At 92 WOA there were no differences in rate of lay (ROL), EW, FI, egg mass nor FCR due to treatments during rearing. When reviewing the data, a range of hen BW within each treatment group was noted. This indicates that managing feeding during rearing may not have an ongoing impact on FI and BW once the hens have *ad libitum* feeding. Based on individual hen BW at 92 WOA the flock was divided into quartiles (Q), ranging from lightest to heaviest BW (Q1–4 respectively). Production data of the Quartiles is presented in Table 2.

Table 2 - Hy-Line Brown hen body weight, feed intake, rate of lay, egg weight and feed conversion ratio at 92 weeks of age and, cumulative feed intake, cumulative eggs per hen surviving and cumulative feed conversion ratio from 17.4 to 92 weeks of age.

Body weight quartile at 92 wk	BW 92 wks (Kg)	FI 92 wks (g/d)	Rate of lay 92 wks (%)	Egg weight 92 wks (g)	FCR 92 wks (kg/kg)	Cum FI 17.4^- 92 wks (Kg)	Cum. eggs/h 17.4^-92 wks	Cum. FCR 17.4^-92 wks
Quartile 1	1.99^{d}	106^{c}	76^{a,b}	61.9^{b}	2.22^{b}	57.6^{c}	468^{a,c}	2.17^{b}
Quartile 2	2.20^{c}	111^{b,}	83^{a}	62.8^{b}	2.22^{b}	59.9^{b}	474^{a,c}	2.18^{b}
Quartile 3	2.35^{b}	115^{a,b}	78^{a,b}	63.1^{b}	2.25^{b}	60.9^{b}	463^{b,c}	2.38^{a,b}
Quartile 4	2.60^{a}	119^{a,}	70^{b}	66.6^{a}	2.50^{a}	64.0^{a}	448^{b}	2.49^{a}
P- Value	<0.001	<0.001	0.04	<0.001	<0.001	<0.001	<0.007	<0.002

BW (Kg)^: body weight; FI: feed intake; FCR: feed conversion ration as kg feed/kg egg mass; Cum FI: cumulative feed intake from 17.4 to 92 wks; Cum eggs/h 17.4- 92 wks: total number of eggs produced per hen surviving from 17.4 to 92 wks; Cum FCR: Cumulative feed conversion ratio from 17.4-92 wks calculated as kg feed/kg egg mass.
^ cumulative data is calculated from when pullets were placed on the early lay diet at 17.4 wks and before egg production started.

Quartile 1 had the lowest BW which corresponded with Hy-Line Brown BSW for age. Average BW increased with each quartile groups (Table 2). Daily FI was lowest in Q1, and birds in Q3 and Q4 consumed significantly more feed/day. Quartile 2 had the highest ROL (83%) which was significantly higher than Q4 (70%) and above 92 WOA breed recommended 71-73% ROL. Quartile 4 birds produced the heaviest eggs (66.6 g) (Table 2), above recommended 60-65 g EW range. Quartiles 1,2 and 3 had significantly lower 92 WOA FCR than Q4. Cumulatively 17.4-92 WOA FI was highest in Q4, lowest in Q1, while Q4 hens produced the least number of eggs. Quartiles 1 and 2 had the lowest cumulative FCR, compared to Q4 (Table 2).

As in Study 1, a simple cost-benefit analysis based on BW quartile rankings in Study 2 was completed using the same estimated feed costs and return/first grade egg. Comparing Q1 (lightest hens) with Q4 (heaviest hens) the latter consumed an additional 6.4 kg feed to produce 20 fewer eggs to 92 WOA. Quartile 4 hens had additional feed costs of $3.28/hen and lower return on eggs (-$2.80/hen) totalling an extra cost of $6.08 /hen compared to Q1. Quartiles 1 and 2 had similar and the lowest FCR. Compared to Q1, Q2 hens produced 6 more eggs, an additional return of 0.84c/hen, for an extra 2.3 kg feed, an additional cost of $1.18/hen. Therefore, each Q2 hen cost an extra 0.34c to 92 WOA when compared to Q1 hens. In a 50,000-hen flock this is an additional cost of $17,000 from 17.4-92 WOA.

These findings to 92 WOA generally concur with the outcomes of Study 1 (to 89 WOA), and O'Shea et al. (2020) between 70-75 WOA, in that LW hens are capable of sustained egg production but with a lower FCR compared to HW hens.

VII. CONCLUSION

In Australian studies, lighter sized hens at the start of lay have demonstrated strong persistency of lay, with lower FI and FCR through an extended production lifespan compared to larger sized hens. Gradual weight gain to mid-lay allows LW hens to reach mature body size, without being overly fat and with more favourable liver function and bone integrity. Further, an early-lay diet of HND can improve eggshell quality, especially shell thickness and breaking strength in older hens. These findings require further evaluation in cage free systems in Australia.

ACKNOWLEDGEMENTS: Thank you to Australian Eggs for funding *Studies 1* and *2*.

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AN EVALUATION OF THE PROTEIN AND ENERGY REQUIREMENTS OF LONG-LIFE LAYING HENS

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Summary

Work on the protein and energy requirements of long-life layers has been sparsely reported, and as a result, is poorly understood. This study measured the response of individually housed Hy-Line Silver Brown hens aged 80 to 90 weeks to three levels of dietary AME_n, and four levels of standardised ileal digestible lysine (SID Lys). The hens manipulated feed intake such that calorie consumption remained constant across all levels of AME_n. Birds with a higher daily egg output (g egg per day) consumed more SID Lys daily, but dietary SID Lys levels had a small but significant negative impact on egg output. The current understanding of the energy and protein requirements of laying hens is applicable to the feeding of long-life layers, although care should be taken not to overfeed protein

I. INTRODUCTION

Improved production efficacy in laying hens has been achieved by selecting individual birds that lay longer clutches of eggs (Dunn, 2013; Bain et al., 2016; Preisinger, 2018). In addition, the length of the productive life of hens has been extended to one hundred weeks of age or more. These 'long-life' layers were predicted to produce 500 eggs by 100 weeks of age (Bain et al., 2016; Hy-Line, 2020), and this is now being achieved commercially (Gautron et al., 2021). Primary breeding companies have used genomic tools to address several issues, including clutch lengths, control of egg size, reducing the decline in egg quality, improving shell breaking strength and bone quality and improving the oviduct's weight and efficiency (Bain et al., 2016; Preisinger, 2018).

Little research has been carried out into the nutrition and metabolism of the long-life hen, principally because the stock has not been available. Most of the information available is based on supposition and anecdotal evidence. In an attempt to better understand the nutrient requirements of long-life hens, an experiment was carried out using individually housed Hy-Line Silver Brown hens aged 80 to 90 weeks post-hatch. An evaluation of the protein and energy requirements and their impact on daily egg output and quality was conducted.

II. METHOD

This study was approved by the Animal Ethics Committee of the University of KwaZulu Natal (AREC/044/017), and investigated three levels of dietary AME_n and four levels of SID Lys on production parameters in 192 individual Hy-Line Silver Brown layers, from 80 to 90 weeks of age. Data for the period 87 to 90 weeks of age were used for analytical and modelling purposes. The birds were housed individually in wire cages (500 mm depth × 450 mm height × 350 mm width) in an open-sided convection house. A completely randomised 3 × 4 factorial block design was used, with 16 replicates per treatment. Feed and water were supplied ad libitum, and the photoperiod was maintained at a constant 16 hours/d by artificial lighting.

Eggs were collected daily, and hen day production (% eggs produced per hen per day) was calculated. Egg weight (g) was measured three times per week, and the mean was

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determined. Feed intake (FI) was determined weekly, while body weight (BW) was determined before and after the four-week data collection period. The change in BW (g/d) was calculated for this period. Daily egg output was calculated as the product of egg weight × hen day production (g egg/hen day-1), and feed conversion ratio (FCR) was calculated as the ratio of average daily FI (g) to daily egg output (g/day). The daily intakes of AME_n and SID Lys were calculated as the product of FI and dietary level.

Diets were formulated using similar ingredients to provide three levels of AME_n (11.0, 11.75, 12.5 MJ/kg) and four levels of dietary SID Lys (6.0, 7.0, 8.0, 9.0 g/kg) (Kleyn et al., 2021). SID Lys was used as the reference amino acid (AA) but contained the same ideal AA profile. Feed analysis was undertaken by Evonik Africa (Pty) Ltd. Data were analyzed by full factorial ANOVA using JMP® Pro 14.2.0. (SAS Institute Inc., 2018). Each hen represented a single data point. Linear regression prediction equations were used to model FI, FCR, egg output and nutrient requirements. Any hen that fell outside the specified FCR range of 1.5 to 2.4 was excluded (Spek, 2018) to reduce the effect of body protein and energy on deposition or mobilisation and minimise the impact on SID Lys and AME_n utilisation.

III. RESULTS

The main effects for the period 87 to 90 weeks of age are shown in Table 1. There were no significant interactions between the protein and energy levels of any diets used. The transition from 11.0 to 12.5 MJ/kg dietary AME_n decreased daily FI by 15.7% (96.7 versus 111.9 g/day; $p < 0.01$), but there was no significant difference in AME_n intake. The FCR differed by 13.3% (2.72 versus 2.05; $p < 0.001$). The increase in dietary SID Lys from 6.0 to 9.0 g/kg increased daily SID Lys intake by 33.8% (683 versus 914 mg/day; $p < 0.01$), but this had no significant impact on egg weight or egg number. The SID Lys level of the diet had no significant impact on AME_n intake, FI, egg weight, egg mass or FCR, and no interactions were observed. Individual hens were able to adjust FI to meet their requirements (Eq. 1). Dietary SID Lys content was not significant and excluded from the model. AME_n intake was predicted with high accuracy ($r2 = 0.796$; $p < 0.001$) (Eq. 2), with body weight ($p < .001$), and daily egg output ($p < .001$) being the only factors that impacted energy intake. The prediction of daily egg output as determined by AME_n intake (kJ/d) is both significant ($r2 = 0.780$; $p < 0.001$) and linear (Eq. 3), whereas the impact of dietary energy level on egg out was NS (Eq. 4.) The energy requirement for maintenance was 193 kJ/kg body weight, and that of egg output was 18.6 kJ/g.

The response of daily egg output to SID Lys intake (mg/d) was significant ($r2 = 0.274$; $p < 0.001$), with the only variable having an impact being daily egg output ($p < 0.001$). Body weight had an NS impact and was excluded from the model (Eq. 5). Increasing the levels of dietary SID Lys from 6 to 9 g/kg, had a significant, linear but negative impact on egg output ($r2 = 0.020$; $p < 0.040$) (Eq. 6). Those hens consuming higher levels of AME_n consumed less SID Lys, but this had an NS effect on egg output. Egg weight remained the same regardless of the SID Lys intake achieved, which is at odds with the results in younger hens (Bouvarel et al., 2011; Spek, 2018; Kleyn et al., 2021).

IV. DISCUSSION

The advantage of housing and measuring individual hens is that outcomes are not blurred by averaging the measurements from two or more individuals, giving rise to a more accurate measurement of underlying biological factors (O'Shea, 2019). Conversely, the social and spatial constraints between hens living in a colony of cohorts are absent. This social interaction likely limits FI under commercial, particularly cage-free conditions.

The finding that long-life hens can adjust their FI to match their requirements is contrary to the findings of other workers (Bouvarel et al., 2011; Classen, 2016), who suggested that

modern genotypes may have lost the ability to regulate energy intake in this manner. These differences are likely explained by the fact that there is nothing to impede FI in individually housed hens, whereas this may not be the case when hens are housed in colonies, as found by Scappaticcio et al. (2022) The energy requirement for maintenance declined from 352 kJ/kg of body weight to 193 kJ/kg, while in the case of daily egg output, this value increased from 9.16 kJ/g to 18.6 kJ/g when compared to younger hens (Kleyn et al., 2021). This result may not be an accurate reflection of energy partitioning in long-life hens, as many of the individual birds had either gone out of production or were in the process of doing so, suggesting that body reserves were utilized for egg production.

The hens lost body weight. Thus, it was likely that some endogenous protein was available for egg production. Increasing the levels of dietary SID Lys from 6 to 9 g/kg, had little impact on long-life strain hens in terms of hen day production, even though those hens consuming higher levels of AME_n consumed less SID Lys. T those diets formulated to provide 6 g/kg of SID Lys may have exceeded the hen's requirements. This would suggest that SID Lys was not deficient in any of the diets offered in this experiment. Contrary to expectation, higher dietary SID Lys levels lead to reduced performance. The protein requirement of long-life hens may be considerably lower than that of younger hens.

V. CONCLUSIONS

Long-life hens can meet their energy requirements by adjusting FI, much the same as hens of any age or genotype. This implies that nutritionists can make decisions about dietary energy levels in the same manner for hens of all ages and genotypes. Hens with a higher daily egg output consumed more SID Lys, but incremental increases in dietary SID Lys did not result in responses in daily egg output, while surplus dietary protein may well have a negative impact on performance. Thus, the protein requirements of the long-life layer differ from those of younger birds. It is likely that the current understanding of the energy and protein requirements of laying hens is applicable to the feeding of long-life layers, although care should be taken not to overfeed protein.

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Table 1 - Main effects of dietary treatments on production parameters, body weight, feed intake, FCR nitrogen-corrected AME_n and SID Lys intake of Hy-Line Silver Brown layers from 87 to 90 weeks post-hatch.

	Hen day (%/day)	Egg weight (g/egg)	Egg output (g/day)	Feed intake (g/day)	Body weight (g)	FCR (g feed/g egg day^{-1})	AME_n intake (KJ/day)	SID Lys intake (mg/day)
AME_n (MJ/kg)								
11.00	70.96	60.65	43.11	111.9[c]	1984	2.719	1226	837.5[c]
11.75	72.20	59.95	43.00	102.6[b]	1953	2.567	1199	766.5[b]
12.50	70.00	60.17	42.19	96.72[a]	1956	2.406	1201	721.8[a]
SID Lys (g/kg)								
6.0	74.66	59.13	44.33	108.6	2010	2.652	1264	652.1[a]
7.0	73.54	60.35	44.14	104.2	1960	2.444	1211	728.1[b]
8.0	67.12	60.97	41.00	101.4	1940	2.697	1177	808.8[c]
9.0	68.90	60.57	41.64	101.3	1948	2.467	1182	912.0[d]
Significance (*p* =)								
Dietary energy (AME_n)	0.814	0.664	0.871	< 0.001	0.469	0.243	0.716	< 0.001
Digestible lysine (Lys)	0.176	0.157	0.370	0.155	0.130	0.520	0.132	< 0.001
AME_n x Lys interaction	0.631	0.150	0.554	0.861	0.965	0.436	0.865	0.759

[abc] Means within columns not sharing a common superscript are significantly different at the 5% level of probability.
Mean performance; Hen day 71.07%, Egg weight 60.26 g, Egg production 42.77, Feed intake 103.88, Bodyweight 1964 g, FCR 2.563.

Table 2 - Prediction of AME_n intake (kJ/d) egg output response to AME_n intake and dietary level, SID Lys intake (mg/d), egg output response to SID Lys intake and dietary level and feed intake (g/d) using linear regression (n= 125 measurements with 12 diets).

Equation	Dependent variable	Independent variable	Parameter estimate	Standard error	*p-value*
1	Feed intake, g/d (r^2 = 0.834)	Intercept	75.198	14.568	< 0.001
		Body weight, g	0.016	0.004	< 0.001
		Egg output, g/d	1.621	0.098	< 0.001
2	AME_n intake, kJ/d (r^2 = 0.796)	AME_n, MJ/kg	-6.674	1.018	< 0.001
		Body weight, g	0.193	0.059	< 0.001
		Egg output, g/d	18.609	1.159	< 0.001
3	Egg output, g/d (r^2 = 0.780)	Intercept	0.659	2.240	0.769
		AME_n intake, kJ/d	0.038	0.002	< 0.001
4	Egg output, g/d (r^2 = 0.020)	Intercept	73.602	13.939	<.001
		AME_n MJ/kg	-2.240	1.184	0.061
5	SID Lys intake, mg/d (r^2 = 0.274)	Intercept	245.524	80.1901	0.003
		Egg output, g/d	11.597	1.676	< 0.001
6	Egg output, g/d (r^2 = 0.325)	Intercept	-8.232	11.650	0.481
		SID Lys intake,mg/d	0.119	0.030	< 0.001
		SID Lys $intake^2$, mg/d	-6.0×10^{-5}	1.9×10^{-6}	< 0.001
7	Egg output, g/d (r^2 = 0.026)	Intercept	56.200	4.358	<0.001
		SID Lys g/kg	-1.188	0.573	0.040

PREDICTING EGG PRODUCTION LOSSES USING PEAK ANALYSIS AND A RANDOM FOREST MODEL

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Early warning systems and decision-making tools have the potential to forecast and simulate egg production losses, allowing producers to implement corrective actions pre-emptively. The aim of this study was to create an egg loss forecasting model for free-range egg producers. Commercial farm records comprising of 281 Australian free-range flocks dating from January 2010 until November 2021 were used. Flocks were located in 4 states, with sizes ranging from 10,000 - 40,000 laying hens. Relevant data used in the model included hen-day production, mortality, feed intake, in-house temperatures, and weather conditions. The egg production drops within the egg production curves were identified using a change detection algorithm or peak analysis. The approach outlined in figure 1 was used to train a random forest model designed to forecast egg production losses using a window size of 7,14, 21 and 28 days before egg production loss onset. The forecasting periods ranged from 0 - 5 days. The performance of each model was evaluated using the Area Under Curve (AUC), sensitivity, specificity and positive predictive values.

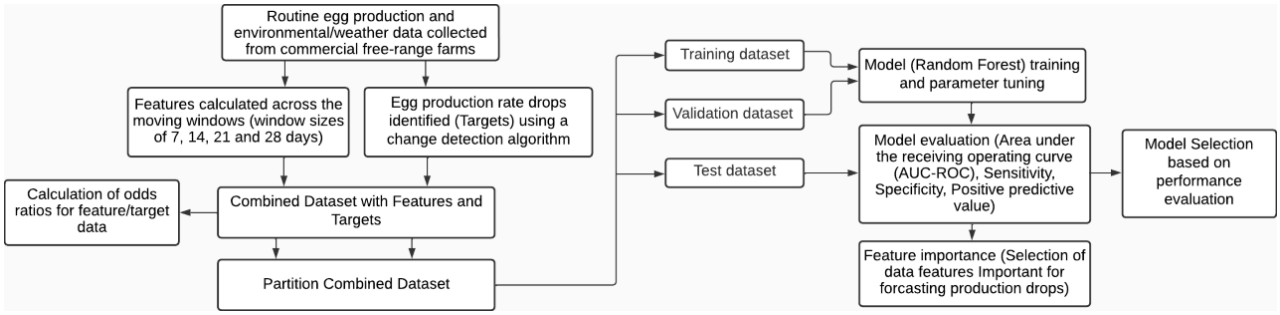

Figure 1 - Machine learning workflow for identifying the egg drops and forecasting the egg drops using random forest classification.

While the 7-day window size produced the least predictive results (77% accuracy) across all performance metrics, 14, 21 and 28 windows predicted comparable outcomes (87%, 88%, and 90% accuracies, respectively). The sensitivity, specificity, and positive predictive values showed a likewise pattern. Using the 28-day data to further train the algorithm, the mean laying rate, hen age and the laying rate change over two consecutive days were the best predictors with a mean decrease accuracy of 0.75, 0.6 and 0.6 respectively. When analysing the odds ratio, the low daily laying rate standard deviation which is an indicator of good consistent flock performance was negatively correlated to the lower risk of egg production drop. We conclude that the AUC value of the random forest model was 0.90 using a 28-day window, indicating the random forest model had an outstanding classification performance. These prediction outputs which indicate the likelihood of egg production losses may be used to implement corrective action to avoid egg production losses. Further research is required on the application of machine learning models in the development of egg production loss alert system and diagnostic tool.

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HEAT STRESS AND THE QUALITY OF WHEAT GRAINS

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Summary

Increasing temperature and consequent changes in climate adversely influence plant growth and development, resulting in loss of wheat productivity. High environmental temperature may influence crops in different ways and such effect varies depending on the crop's genotype and phenological stages. The aim of the present research was to study the contributions of genotypes, and year of sowing and their interaction on the physico-chemical quality of wheat cultivars. Four wheat cultivars, EGA Gregory (E), Lancer (L), Trojan (T) and Borlaug 100 (B) were sourced from Narrabri during the 2019 and 2020 growing seasons. Starch, protein, viscosity, and mineral contents (P, Ca, Na and K) were analysed. The data showed that starch, and mineral contents and viscosity were significantly impacted by environmental factor (year) and genotype. However, the interaction of year and genotype did not show any significant changes on the quality of tested grains.

I. INTRODUCTION

Wheat is the predominant feed grain in Australia and other dry regions in the world. Typical broiler diets in Australia contain 60-70% wheat. Climate-induced factors, including elevated temperature, have been reported as depressing crop production around the globe during the last few decades (Fernie et al., 2022) and there is limited understanding of how climate-induced factors may influence the nutritional quality of wheat.

The sowing time of wheat is the most important factor for temperature-sensitive cereals. Late sowing normally depresses grain yield because of the low temperature during germination and the high-temperature stress during the reproductive stage (Hussain et al., 2021). Moreover, several authors have reported that climate factors such as high temperature or extreme rainfall have a significant impact on the physical and nutritional quality of wheat grains such as grain number, size, grain weight, hardness and the composition of protein and carbohydrate content (Buttar et al., 2020, Djanaguiraman et al., 2020, Choct et al., 1999).

The chemical quality of wheat grain is mainly explored by measuring the content and composition of protein and carbohydrates in response to heat stress. The protein content is increased by heat stress, particularly during the grain-filling period (Zhang et al., 2019, Yang et al., 2018).

Carbohydrate concentration and composition are critical measures of grain chemical quality and have important implications for the functionality of wheat flour. Starch is the main carbon reserve in wheat, which comprises up to 75% of total grain dry weight (Barutcular et al., 2016) and heat can inhibit the efficiency of starch biosynthetic enzymes, affecting starch deposition in developing grains. Post-anthesis heat also alters the amylose and amylopectin ratio in grains, which may influence the pasting properties of wheat flour (Poudel and Poudel, 2020; Wang et al., 2017). The aim of the present study was to evaluate the effects of the

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environment (year), genotype and their interaction on the quality of Australian wheat grain cultivars.

II. METHOD

Four wheat grains cultivars (EGA Gregory (E), Lancer (L), Trojan (T) and Borlaug 100 (B)) were sourced from crops grown in Narrabri during 2019 and 2020. The 2019 harvest experienced higher temperatures compared to the grains harvested in 2020. Wheat grains were milled using a Cyclone Sample Mill (UD Corporation Boulder Colorado USA) to a particle size of 500 µm and transferred to a small zip-lock bag, stored in the refrigerator at 4 °C until analysis.

Total starch was measured using an assay kit from Megazyme (K-TSTA).

The total protein content of milled wheat flour was determined using a nitrogen/protein Vario MACRO Cube analyser (Frankfurt, Germany). Thirty mg of samples were prepared in 12 x 6 mm pressed aluminium capsules. Samples were combusted with oxygen and nitrogen oxide. The measured nitrogen content in this method was converted to total protein content by applying a conversion factor of 6.05 (Yamaguchi, 1992).

Pasting property was measured using a Rapid Visco Analyser (RVA) (Newport Scientific). 3.5 g of the ground wheat sample was added to 24.5 g of water and ran using 50 °C as base temperature to 95 °C and then back to 50 °C within 13 min cycle.

Mineral content (Ca, K, P, and Na) was analysed using a Perkin Elmer Avio Inductively Coupled Plasma Optical Emission Spectrometer (ICPOES). The results are analysed by two-way ANOVA to check the impact of genotype, year of harvest and G xY interaction.

III. RESULTS

The starch, protein, viscosity, and mineral contents were determined to check the impact of the year of harvest, genotypes and their interaction (G x Y) (Table 1). The impact of year or genotype was significant on quality attributes of all tested samples. However, the obtained results showed no significant difference in the protein content among different genotypes (P=0.071). The results also indicated that the year of harvest had no impact on the final viscosity of the tested grains (P=0.314).

The starch content increased in grains harvested in 2020 while significantly higher protein content was observed in 2019. The highest level of starch and the lowest level of protein were for Trojan.

The results showed that the genotype remarkably impacted the RVA final viscosity (P=0.002). The greatest viscosity belonged to the Trojan.

The results obtained in the experiments showed that the mineral content level was significantly impacted by all of the factors including, genotype, year and G x Y interaction. Overall, the Ca, Na and P contents increased in the hotter year (2019) while the K content decreased.

Table 1 - Physicochemical parameters of wheat grain cultivars.

Variety	Year	Ca (mg/g)	K (mg/kg)	P (mg/kg)	Na (mg/kg)	Protein (%)	Starch (%)	Final Viscosity (cps)
Borlaug	2019	582	2370	3819	118^{bc}	15.8	60.8	1779
Borlaug	2020	493	2384	3522	111^{c}	13.4	65.0	1610
EGA Gregory	2019	632	2528	3764	137^{a}	15.4	61.5	1914
EGA Gregory	2020	607	2872	3637	45^{d}	12.9	64.2	2016
Lancer	2019	575	2446	4131	139^{a}	16.4	59.4	1750
Lancer	2020	497	2790	3902	127^{ab}	14.0	62.5	1938
Trojan	2019	641	2992	3544	134^{a}	15.3	63.3	1943
Trojan	2020	586	3489	3294	131^{a}	12.6	65.2	2033
SEM		*24.8*	*140.7*	*133.4*	*4.1*	*0.49*	*0.96*	*72.9*
Main effect: variety								
Borlaug		537^{b}	2377^{c}	3670^{bc}	114	14.6	62.9^{ab}	1695^{b}
EGA Gregory		620^{a}	2700^{b}	3700^{b}	91	14.1	62.8^{ab}	1965^{a}
Lancer		536^{b}	2618^{bc}	4017^{a}	133	15.2	61.0^{b}	1844^{ab}
Trojan		613^{a}	3241^{a}	3419^{c}	133	13.9	64.2^{a}	1988^{a}
Year								
2019		607^{a}	2584^{b}	3814^{a}	132	15.7^{a}	61.3^{b}	1847
2020		546^{b}	2884^{a}	3589^{b}	103	13.2^{b}	64.2^{a}	1900
P-Value								
Genotype (V)		0.002	<0.0001	0.002	<0.0001	0.071	0.022	0.002
Year (Y)		0.002	0.006	0.025	<0.0001	<0.0001	<0.001	0.314
G x Y		0.587	0.389	0.933	<0.0001	0.977	0.710	0.109

IV. DISCUSSION

Regardless of genotype, the average protein content increased significantly in harvested samples in 2019 compared to 2020 samples. The trend is supported by Singh et al. (2021). The starch content was numerically changed among EGA Gregory, Lancer and Trojan. But the starch content was significantly different between the years. The samples from 2019 (hotter year) had the lowest level of starch.

Plant responses to environmental stress (heat) during grain filing led to an increase in total protein content and decrease in starch biosynthesis and a lower grain weight (Rakszegi et al., 2019; Matsuki et al., 2003). These changes in the amount and quality of wheat's major components could be directly linked to different genotypes with different genetic backgrounds (Matsuki et al., 2003)

The exposure of the wheat to heat might impact the protein composition as gliadin production continues at bigger rate than glutenin. This leads to an increase in the level of gliadin and decreases the glutenin content (Phakela et al., 2021). The heat stress also influences the amylopectin branch chain length and reduces the production of short chain branched amylopectin (Matsuki et al., 2003). The heat stress conditions might also influence the amount of soluble NSP which can affect the viscosity. These changes in the values could later influence the wheat dough pasting property and strength (Ferreira et al., 2012; Flagella et al., 2010; Guzmán et al., 2016) by increasing or decreasing the final viscosity.

Mineral content significantly increased in 2019 with some exceptions. Helal et al. (2022) reported that an increase in mineral content in hotter environment is a mechanism

of heat tolerance in wheat. In general, the data shows that heat stress in wheat reduced starch content and increased protein and mineral content.

ACKNOWLEDGEMENT: We would like to thank the School of Life and Environmental Science at the University of Sydney for providing the fund and support to run the project.

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ASSESING FOUR DIFFERENT IDEAL AMINO ACID RATIOS IN ISA BROWN LAYER HENS' DIET DURING THE PEAK AND POST PEAK PRODUCTION

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In previous assays (Macelline et al., 2022), data on egg production rate (EP) and egg mass (EM) of Isa Brown layers from 27 to 33 weeks of age (peak production -PP) and from 42 to 48 weeks of age (Post PP) were used to predict amino acid (AA) requirements by fitting linear broken-line (LBL), and quadratic broken-line (QBL) models. Subsequently, the ideal ratios of Ile, Met + Cys, Thr, Trp, and Val relative to Lys for maximal EM and EP were determined.

The current feeding study was designed to validate the two ideal AA ratios established based on LBL and QBL models against existing Evonik (AMINOHen®) and breeder recommendations. The four ratios tested are given in Table 1. A minimum ratio of 105 was set for Arg across all the four diets, and the rest of the AA were left to float in the formulations. A total of 224 Isa Brown pullet hens were allocated to individual battery cages with individual nipple drinker and trough feeder. At week 20 of age, birds were weighed and randomly allocated to their respective diets. Each of the four diets were replicated eight times with seven birds per replicate. The diets were offered ad-libitum from 20 to 44 weeks (PP) and from 44 to 75 weeks of age (Post PP). Egg production was recorded daily, and individual egg weights were measured at the end of each week. Feed consumption was measured at the end of each period on a replicate basis. During the PP and post PP, the egg production rate was not significantly affected by the diets and remained at around 98.0 and 95.0 %, respectively. Egg weight was consistently lower (3.5 %) during both phases in birds offered the diets based on LBL models, resulting in a lower egg mass (59.8 *vs* 62.0 PP, and 60.3 *vs* 63 Post PP; $P < 0.05$). Feed consumption and bodyweights were not affected ($P > 0.05$), but birds in LBL treatment tended to have the highest FCR during the peak ($P = 0.067$) and post peak ($P < 0.05$) of production. Feed cost per kg of egg was not statistically affected by diets but remained the lowest in LBL and the highest in QBL groups. In conclusion, all the four ideal AA ratios tested in this study adequately support egg production rate. However, ratios based on LBL models may potentially decrease the input feed cost per kg of eggs but are not set to maximize egg mass.

Table 1 - Amino acids requirements and ideal AA ratios during the peak and post peak of production.

mg/hen/day (ratios)	Peak production (20 -44 weeks)					
	Lys	M+C	Thr	Val	Ile	Trp
LBL Models	713 (100)	606 (85)	512 (72)	587 (82)	654 (92)	150 (21)
QBL Models	891 (100)	820 (92)	572 (64)	692 (78)	757 (85)	193 (22)
Evonik	831 (100)	756 (91)	582 (70)	731 (88)	665 (80)	174 (21)
Isa Brown	850 (100)	740 (87)	595 (70)	750 (88)	680 (80)	191 (22)
	Post peak production (44- 75 weeks)					
LBL Models	688 (100)	562 (82)	459 (67)	603 (88)	567 (82)	153 (22)
QBL Models	861 (100)	691 (80)	607 (70)	726 (84)	751 (87)	191 (22)
Evonik	810 (100)	740 (91)	570 (70)	710 (88)	650 (80)	170 (21)
Isa Brown	815 (100)	710 (87)	570 (70)	715 (88)	650 (80)	180 (22)

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REACTIVE LYSINE; A BETTER DETERMINANT FOR SOYBEAN MEAL QUALITY?

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Summary

In the 1990s researchers from the American Soybean Association (ASA) showed that soybean meals with similar proximate analysis can give inconsistent animal performances. Since then, there has been a significant amount of research to find a determinant or determinants that can differentiate between soybean meals that can give different performances in animal feeding. Currently, the general consensus in the feed industry is that soybean meals with high protein solubility in potassium hydroxide solution (KOHPS) and low trypsin inhibitor (TIA) / urease activity will perform satisfactorily in animal feeding. The ideal standards for determining soybean meal quality should be apparent metabolisable energy (AME) and digestible lysine (Hirai et al., 2020). However, these tests have to be done with live animals and therefore are not practical for day to day quality control. There are some commercial NIR calibrations for AME and digestible lysine but there are some concerns about their accuracy as they are based on certain equations which are currently being disputed. We collected 98 samples of soybean meal from our crushing plants and the domestic market and measured their reactive lysine content. These included 70 samples of soybean meal produced from US, Brazilian and Argentinian origins at our crushing plants and 28 samples were collected from the Malaysian domestic market. We correlated the reactive lysine of these samples with their Protein Dispersibility Index (PDI), KOHPS, TIA, Minolta colour L and a. In general, there is reasonable correlation of reactive lysine with KOHPS ($R^2 = 0.70$; $P < 0.001$), PDI ($R^2 = 0.60$; $P < 0.001$), colour L ($R^2 = 0.55$; $P < 0.001$) but not with TIA or colour a. The reactive lysine of the soybean meal samples differed by up to 28% ranging from 2.5 to 3.2%. We intend to carry out broiler feeding trials using soybean meals with reactive lysine levels of 2.6, 2.7, 2.8, 2.9, 3.0, 3.1 and 3.2% in the near future to correlate reactive lysine with feed conversion ratio (FCR) and body weight gain (BWG). We have also undertaken NIR calibrations using the reactive lysine data from the soybean meals and managed to obtain a good result with $R^2 = 0.956$, SEC = 0.030, SECV = 0.046 and SEP = 0.052. This has enabled us to calibrate our online NIR for real time control of reactive lysine in our crushing plants.

I. INTRODUCTION

In the 1990s, researchers from the American Soybean Association (ASA) showed that soybean meal with similar proximate analysis can perform very differently in poultry and swine feeding. Subsequently, Creswell and Swick (2008) carried out feeding trials, AME and digestible amino acid analysis of 4 soybean meals. They found that there was a large difference between the soybean meals in BWG and FCR from the broiler feeding trials as well as in AME and digestible lysine. They also quantified the financial value of each soybean meal which exceeded USD70/MT between certain soybean meals. Our analysis of the data from that trial showed that there is excellent correlation between digestible lysine of the soybean meals with BWG and FCR of broilers (Neoh and Ng, 2006). Interestingly, the digestible lysine in soybean meal was almost perfectly correlated with AME of soybean meal. This suggests that processing conditions that give high digestible lysine will also increase the AME of the soybean meal.

In recent years, there has been much research done to find a more accurate but simple to measure determinant or determinants that can differentiate between soybean meals that can give different performances in animal feeding. The KOHPS method was suggested by Araba and Dale (1990) to be a good determinant for protein digestibility of soybean meal. Generally, it is accepted by the industry that soybean meal with KOHPS over 75% and with TIA less than 4 mg/g can be considered to be of good quality and will perform satisfactory for animal feeding. Unfortunately,

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Araba and Dale (1990) used an autoclave to simulate processing condition in the solvent extraction plant. Their conclusion was that soybean meals with KOHPS exceeding 85% will be under toasted. However, in our opinion this is not applicable for soybean meal samples from solvent extraction plants due to the presence of hexane during desolventising and toasting which has a big effect on protein solubility. Regrettably, most of the research done on soybean meal processing conditions and protein solubility was done with autoclaves in the laboratory. These results do not truly reflect the processing conditions in the solvent extraction plants.

There are numerous research studies on reactive lysine of soybean meal using homoarginine, FDNB and furosine methods. In our opinion, the furosine method is suitable for soybean meal as it has normally undergone only early Maillard reactions producing mainly Amadori compounds. In general, it is accepted that reactive lysine is correlated with digestible lysine in animal nutrition. Kim et al. (2015) demonstrated there was an excellent correlation between reactive lysine with apparent ileal digestible (AID), standardised ileal digestible (SID) and true ileal digestible (TID) lysine with $R^2 = 0.99$ in pigs. Kim and Mullan (2013) also managed to produce a workable NIR calibration for reactive lysine using a mixture of soybean meals obtained from the market as well as samples produced in an autoclave at their laboratory.

For this study, we collected 98 samples of soybean meal from our 2 crushing plants together with some soybean meals imported from Argentina. These samples were not subjected to further heat treatment and were directly analysed for reactive lysine, KOHPS, PDI, TIA, colour L and a. The reactive lysine contents were then correlated with their corresponding KOHPS, PDI, TIA, colour L and a results. The reactive lysine data were also used for calibrating our online NIR with the intention of using it for online real time control of reactive lysine content in our soybean meal.

II. METHOD

The soybean meal samples were analyzed for TIA (AOCS Ba 12-75), PDI (AOCS Ba 10-65) and KOHPS (Arada and Dale, 1990). These samples were also analyzed for color L and a using a Konica Minolta Chroma Meter CR-400/ DP-400.

Amino acids were determined by using Waters AccQ•Fluor™ reagent for post column derivatization and L-α-Aminobutyric acid as the internal standard. The derivatives are analyzed by HPLC with FLR detector. Before analysis, samples were hydrolyzed with 6 *N* HCl for 24 h at 110°C (AOAC 982.30 E). Methionine and Cys were analyzed as methionine sulfone and cysteic acid, respectively, after cold performic acid oxidation overnight before hydrolysis. Tryptophan was determined after NaOH hydrolysis for 16 h at 120°C.

For quantification of furosine content (Dong et al., 2019; Pahm et al., 2008), samples were hydrolyzed with 10.6 *N* HCl for 24 h at 110 °C and further processed using reversed-phase solid-phase extraction (SPE). 0.5 mL of the filtrate was eluted with 3 mL of 3 *N* HCl and evaporated by nitrogen stream. The dried samples were reconstituted in 3 mL of a mixture of water, acetonitrile, and formic acid and filtered through a 0.45-μm filter before HPLC injection. Mobile phases gradient (0.1% trifluoroacetic acid in deionised water for mobile phase A and Methanol for mobile phase B), and samples were detected by HPLC with UV/VIS detector.

III. RESULTS AND DISCUSSION

The reactive lysine of the soybean meals ranged from 2.5 to 3.2% which is a variation of 28%. This is considered to be a very large variation and would have a very big impact on the growth performances of animals. The KOHPS of these samples ranged from 53 to 90% and there is a reasonable correlation with reactive lysine of $R^2 = 0.70$; $P < 0.001$ (Figure 1). The PDI of soybean meal samples from our plant ranged from 7.5 to 49% and is correlated with reactive lysine of $R^2 = 0.60$; $P < 0.001$ (Figure 2). This PDI measurement was done within 3 days of production as we have previously published showing that PDI will deteriorate quickly with time and is not a reliable measurement unless we know the storage time and condition of the soybean meal (Neoh, 2008). Therefore, we excluded all the imported soybean meal samples from the PDI correlation study with

the reactive lysine. There was also reasonable correlation between reactive lysine and colour L of $R^2 = 0.55$; $P < 0.001$ (Figure 4). However, there was no correlation between TIA and colour a with reactive lysine as shown in Figure 3 and Figure 5.

From these results, we can propose that reactive lysine may be a good determinant of soybean meal quality. The correlations between reactive lysine and KOHPS as well as PDI would suggest that reactive lysine content is able to differentiate between soybean meals with different protein digestibility over a wide range. However, in order to establish that reactive lysine content of feed ingredients can be a good predictor of animal performance we need to correlate reactive lysine content of feed ingredients with growth rate and FCR of animals. In the near future, we will be carrying out broiler feeding trials using our soybean meals with reactive lysine content of 2.6, 2.7, 2.8, 2.9, 3.0, 3.1 and 3.2% to determine if there is a correlation between reactive lysine, BWG and FCR.

We are also able to calibrate our NIR online using the reactive lysine data to obtain a robust calibration with $R^2 = 0.956$, SEC = 0.030, SECV = 0.046 and SEP = 0.052 (Figure 6). This will enable us to use real time online NIR to optimize the level of reactive lysine in soybean meal produced from our crushing plants.

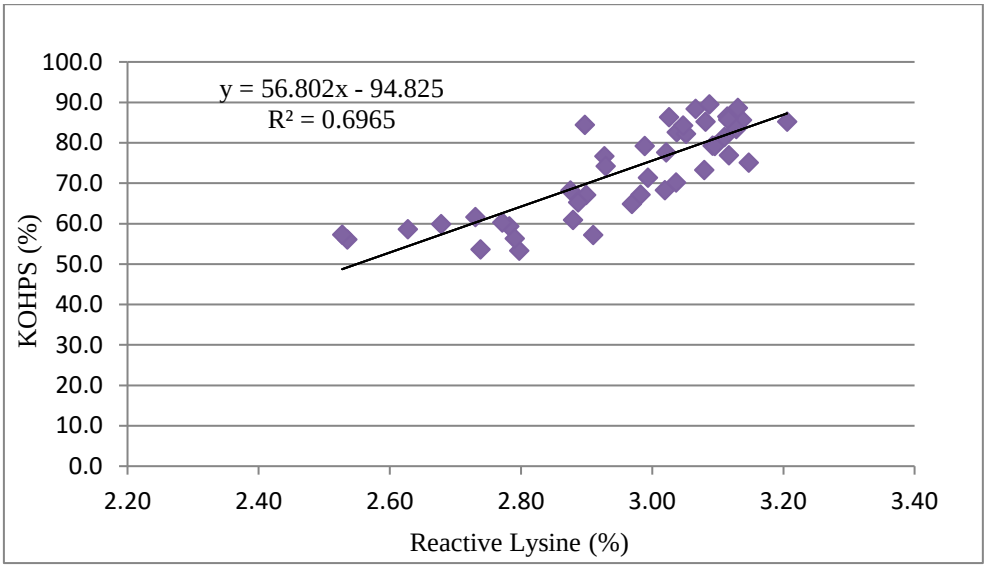

Figure 1 - Correlation between KOHPS and reactive lysine.

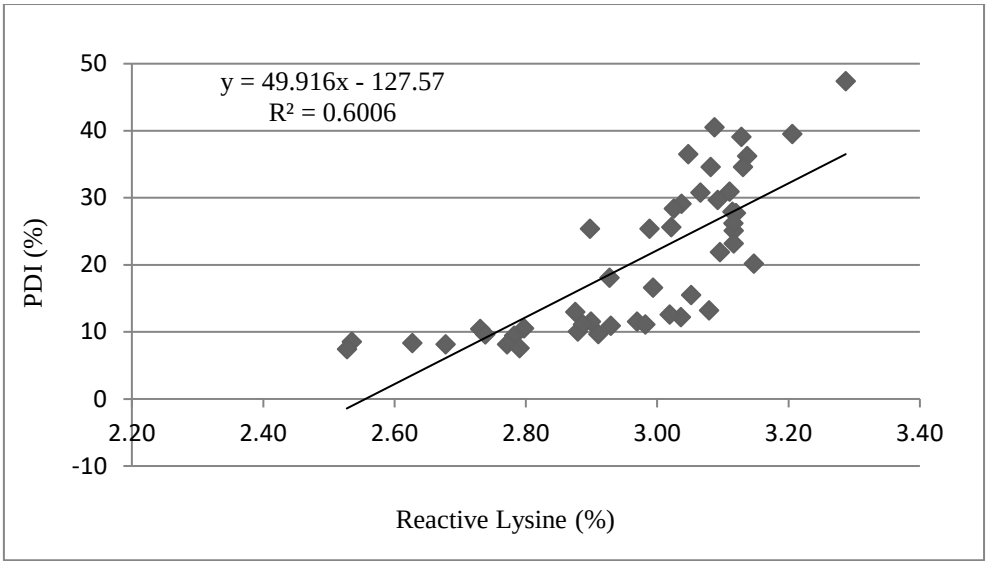

Figure 2 - Correlation between PDI and reactive lysine.

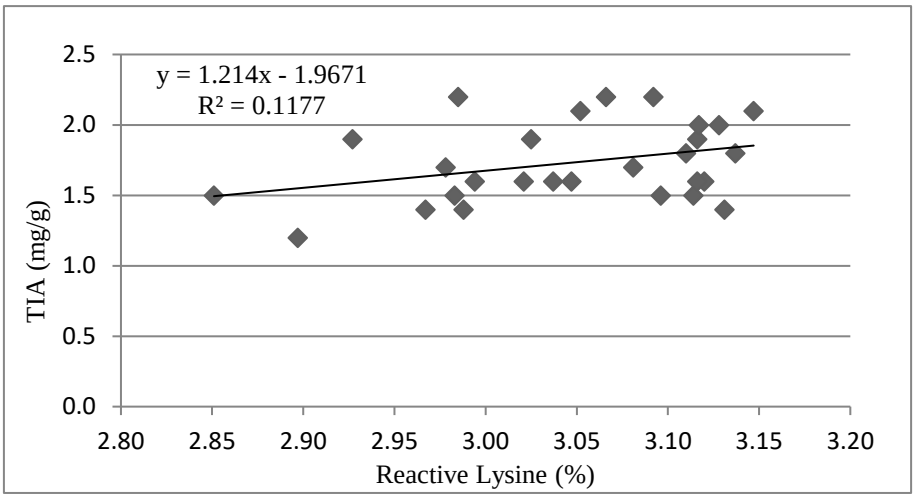

Figure 3 - Correlation between TIA and reactive lysine.

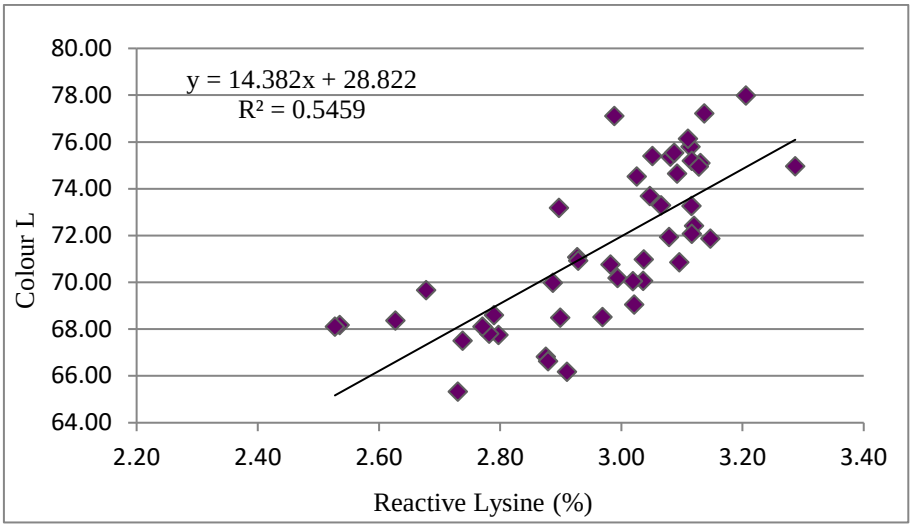

Figure 4 - Correlation between colour L and reactive lysine.

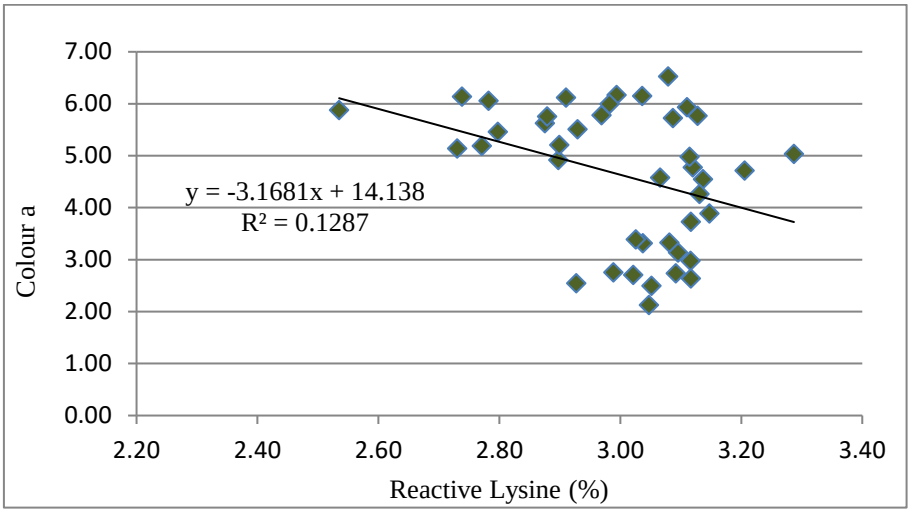

Figure 5 - Correlation between colour a and reactive lysine.

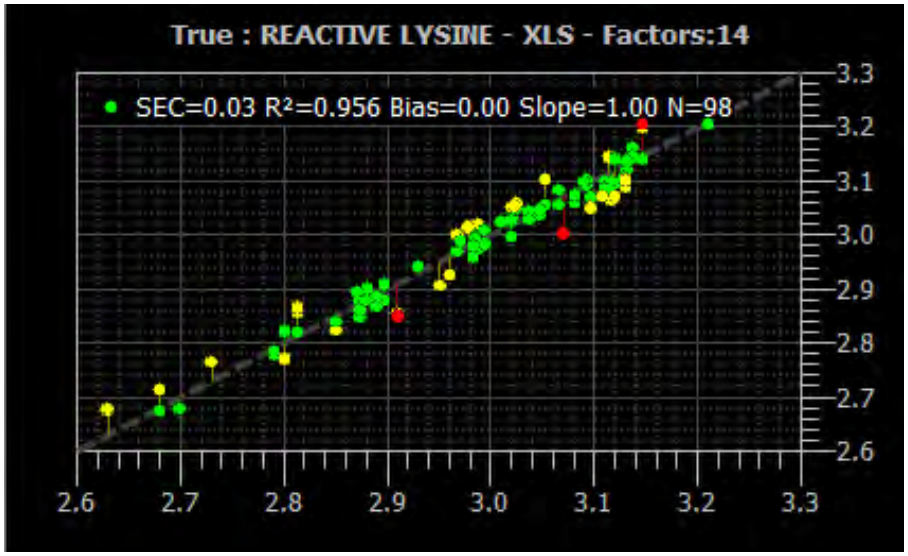

Figure 6 - NIR calibration of reactive lysine.

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OPTIMIZING DIETARY NUTRIENT DENSITY FOR IMPROVED ECONOMIC RETURNS

U. AFTAB[1]

Summary

A case is presented to suggest reduced nutrient density can offer significant feed cost savings. Depending upon the prices of major feed ingredients, and the choice of alternative ingredients, the optimum nutrient density appears to range from 94 to 99% of the current industry standard. Low nutrient density feeding programs appear commercially viable and will allow more efficient utilization of the local-, alternative-feed ingredients, perhaps resulting in lowered pressures on across continent imports.

I. INTRODUCTION

Nutrient requirements are established by means of dose-response studies. Data is subjected to appropriate regression analysis and the level needed to maximise a given response (body weight gain, feed efficiency, carcass yield, etc.,) is defined as 'requirement'. The estimates are compiled as 'tables of nutrient requirement' e.g., NRC, CVB, Brazilian Tables, nutrition guides by the genetic suppliers, and are used by field nutritionists as reference. The classical estimates of nutrient requirement are established based on maximum growth performance and may not necessarily reflect the levels needed for maximum economic returns.

Growth is the function of nutrient intake. This implies that, given a nutritional balance, increasing nutrient density would improve feed efficiency (FE), more accurately feed efficiency corrected for body weight (BWFE), in a linear fashion, theoretically, to unity. This hypothetical maximum is constrained by the highest nutrient density achievable in a practical-type diet. Likewise, reducing nutrient density would precipitate proportionate reduction in BWFE, at least to the point where bulk-density of diet begins to limit daily nutrient intake. The economic optimum nutrient density – the one resulting in lowest feed cost per unit live or product gain - is a moving target between these two extremes and is dictated by the price, availability, and choice of feed ingredients. This schematic, however, contrasts with reality in that most feed markets, especially those functioning as stand-alone-, non-integrated-models, are driven by a targeted certain fixed level of technical performance e.g., xyz BWFE or production index, rather than the optimum feed cost per gain. Consequently, diet composition hardly sees an 'adjustment' for changes in the price of major feed ingredients. It seems logical to argue that increasing prices of energy and protein, as for example experienced by the industry in recent times, will shift the economic equation in favor of lower nutrient density diets. The focal point of this communication is the idea that there could have been situations where a few points reduction in BWFE, carefully imposed through reduced nutrient density, may increase net monetary returns.

II. METHOD

a. Performance trials

Growth performance studies were conducted to establish the relationship between diet nutrient density and technical performance of broiler chickens. The studies were conducted at the R&D facility of Jadeed Feed and Farms Pvt Ltd. Pakistan during May-August 2020. Commercial-

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type broiler diets based on maize-soybean meal (Trial 1) and maize-soybean meal-canola meal (Trial 2) were formulated to supply energy and nutrients as per industry practice (100% nutrient density). A series of test diets representing 90, 95, 100, and 105% nutrient density (Trial 1) and 90, 100, and 110% nutrient density (Trial 2) were assigned to 10 replicated pens of 20 straight-run, Ross 308 broilers from 1 to 35 days. A constant SID lysine to ME ratio was maintained across diets. A two-phase feeding program was employed in these trials with feed offered as crumbles (1 to 14 and 1 to 18 days post-hatch, respectively for trials 1 and 2) and pellets (15 to 35 and 19 to 35 days post-hatch, respectively for trials 1 and 2). The research facility was based on an environmentally-controlled house fitted with adjustable floor pens. Each floor pen was equipped with a feeder and a drinker, rice husk was used as bedding and space was adjusted to provide 0.75 square feet floor area per bird. Feed samples were analysed for proximate, total P, Ca, and Na by Association of Official Agricultural Chemists (AOAC, 2015) methods. Pen feed intake (FI) and body weight (BW) were recorded at the end of every feeding phase, and feed efficiency (FE) corrected for mortality was calculated. BW, FI, FE, and mortality was subjected to analysis of variance as a randomized complete block design using SPSS. Means were compared using Tukey's test using $P < 0.05$ as conventional minimum level of significance.

b. Economic analysis

The economic model was based on the premise that BWFE was a linear function of nutrient density. With a single change that the energy (AME) was reduced by 100 kcal (0.418 MJ) in each phase, nutrient recommendations for mixed-sex Ross 308 (Aviagen, 2019) were set as 100% nutrient density (the 'input') while FE at 35 d of age was selected as the corresponding benchmark for performance (the 'output'; Aviagen, 2021). Reduction of 100 kcal from Ross 308 AME in the control (100% nutrient density treatments) was employed to reflect more closely the commercial practice. A series of example diets ranging from 94 to 102% nutrient densities were formulated using the list of ingredients and prices described for three selected markets (Table 1). Prices of minor ingredients i.e., crystalline amino acids, salt, inorganic phosphate, phytase, vitamin and mineral premixes, were assumed constant across regions.

Table 1 - Feed ingredients, prices, and limits used in formulation exercise.

Ingredients	Thailand/Malaysia	Pakistan	Brazil
	USD per MT (limit)		
Corn	397	274	277
Rice bran, full-fat	347 (2% max.)	199 (4% max.)	260 (2% max.)
Rice bran, extracted	286 (4% max.)		210 (4% max.)
Cassava	263 (3% max.)		
Soybean meal	643	697	407
Canola meal		473 (8% max.)	
Rapeseed meal		299 (2% max.)	
Sunflower meal		299 (3% max.)	
Palm kernel meal	273 (6% max.)		
Soy hulls		174 (2% max.)	100 (2% max.)
Meat & bone meal			400 (4% max.)
Soybean oil		1741	1100
Palm oil	1548		

The numbers in parenthesis represent maximum limits on ingredient usage placed in formulation exercise.

The corresponding BWFE (out-put term) for unit change in nutrient density was calculated following the trend in Figure 1. Cost per unit of live gain was calculated by dividing

the cost of each diet with corresponding BWFE (Figure 2). Following the recommended phase-feeding plan, each treatment group was composed of three diets i.e., starter (1-10 d), grower (11-24 d) and finisher (25-35 d). Translating to performance objectives outlined by Ross 308, the economic calculations assumed feed intake distribution in the order of 10, 45, and 45%, respectively for starter, grower, and finisher diets.

III. RESULTS

With average BW of 2250 g per bird and FCR of 1.42 (Trial 1) and BW of 2260 g per bird and FCR of 1.44 (Trial 2), the growth performance corresponding to 100% nutrient density treatments in these studies was comparable to the published performance objectives of straight-run Ross 308 (Aviagen, 2021). Liveability was > 97% in both studies. Figure 1 summarizes the results of these studies which confirmed the hypothesis that BWFE was a linear function of diet nutrient density. In trial 1, increasing nutrient density by 17% (105 vs. 90%) resulted in a 17% improvement in BWFE (R^2 = 0.998), while in trial 2, an increase in nutrient density by 22% (110 vs. 90%) resulted in 20% improvement in BWFE (R^2 = 0.987). At or above 95% nutrient density, the diet had no effect on BW (P > 0.05).

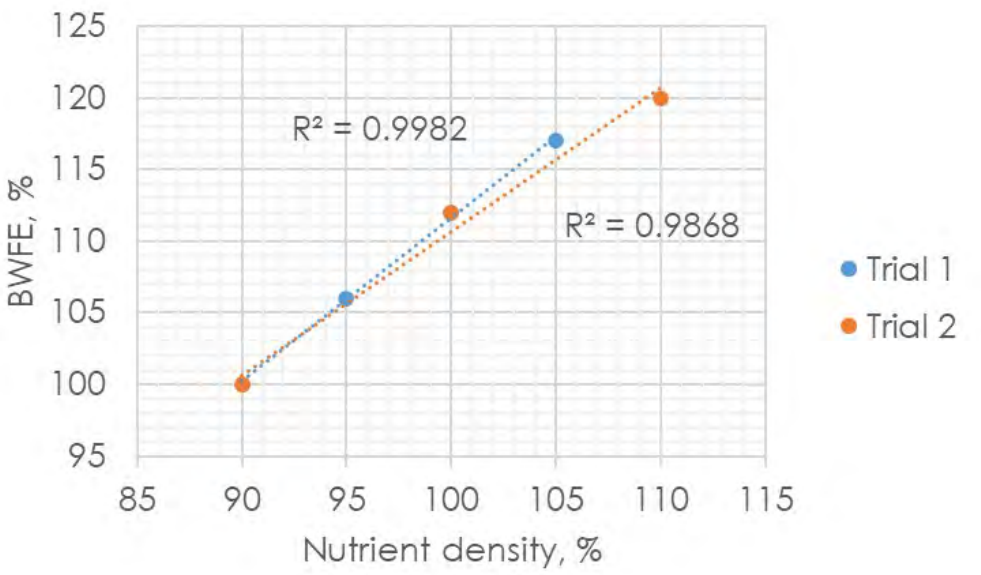

Figure 1 - BW-corrected FCR as a function of diet nutrient density

IV. DISCUSSION

Broiler chickens adjusted their FI to maintain constant BW to 95% nutrient density. This adjustment however was not precise beyond this point and the final BW (35 d) differed by ~100 g across lowest (90% nutrient density) vs. highest (105 and 110% nutrient density treatments, respectively, in trials 1 and 2). This means the lowest nutrient density groups (90%) would require an additional ~1 to 1.5 days to reach target slaughter weight. This observation was contrary to Leeson's work in 1990's in which they observed no differences in BW-for-age of broiler chickens even when nutrient density of the diets was reduced to 50% (Leeson et al. 1996). The results of these studies confirmed that BWFE was a sensitive predictor of nutrient density of diets and could be used to model economic optimum nutrient density under a varying set of ingredient prices and choices.

Nutrient requirement and performance objectives of Ross 308 (Aviagen, 2019, 2021) were used to calculate optimum nutrient density under current prices (May 2022) of feed

ingredients in three markets: Thailand/Malaysia, Pakistan, and Brazil. Thailand and Malaysia had a comparable set of prices for key feed ingredients and hence were combined as one market. The selection of these markets provides a good description of the range of available feed ingredients and variability in relative prices of energy (cereals and vegetable oils) vs. protein meals vs. cereal and other by-products which potentially exist in different regions (Table 1). The economic analysis suggested the optimum nutrient density was 98.8, 96.4, and 94.8%, respectively for Brazil, Thailand/Malaysia, and Pakistan markets (Figure 2).

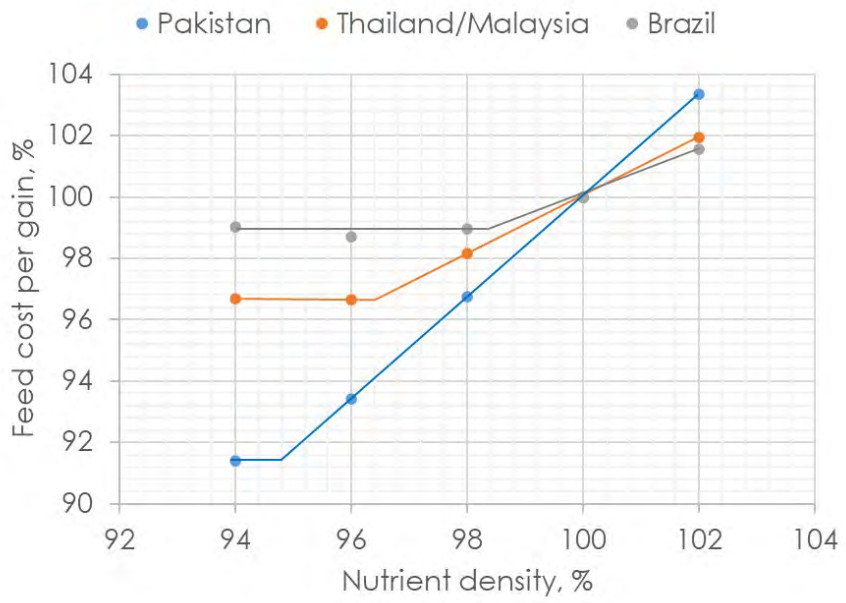

Figure 2 - Summary economic analysis

As expected, the extent and the benefit of lowering nutrient density was dictated by the price of the major ingredients i.e., corn, soybean meal, and added oil, as well as on the choice of the alternative feed ingredients. Therefore, Pakistan, which has a relatively high price of vegetable oil and soybean meal, benefitted most (i.e., 9% reduction in feed cost per unit live gain) from lowering nutrient density down to 94.8% which was made possible by the range of alternative ingredients available at hand. On the other hand, Brazil, with the lowest prices of corn, soybean meal, and soy oil did not see the benefit of lowering nutrient density below ~99%. Thailand/Malaysia, despite having high prices of energy and protein, only saw a modest benefit i.e., 3% reduction in feed cost per gain, maximized at 96.4% nutrient density, likely due to a rather narrow choice of alternative ingredients. In all selected example markets, reducing nutrient density offered commercial benefit with the magnitude varying from 1 to 9% reduction in feed cost per unit live gain.

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HYBRID FEED - NEW APPROACH IN LAYER HEN FEED AT START OF PRODUCTION

A. AKPULAT[1] and X. ARBE UGALDE[1]

Summary

The actual genetic improvements in layer hens are providing the market with a layer hen producing saleable size eggs quicker than before and extending the production life. These two parameters are related to the start of the production. A good feed intake at the start of production assures a good egg size at the beginning of production and a good eggshell quality at the end. At the start of production, a second bone development happens that will load the bones of the layer hens with calcium, that is critical in the calcification of the egg during production. The hybrid feed is a recommendation of how to approach the layer hen feed at the start of production just after the developer rearing feed phase is finished. This hybrid feed is a mix of concepts of rearing developer feed and production feed; it will stimulate feed intake development and will have enough nutrients to support the body and bone development happening when the first egg is produced and it can be used in any layer hen, brown or white. This feed is meant to be safer and cheaper solution than other traditional approaches of feeding layer hens and it supports the genetic potential of the birds.

I. INTRODUCTION

The continuous genetic improvements are increasing the first commercial egg size produced and extending the production life of the layer hens worldwide. These improvements are related to what happens at rearing and at the start of the production. Early studies showed that while approximately 60 to 75% of the calcium destined for the shell comes directly from intestinal absorption (Driggers and Comar, 1949), up to 36% could be traced to bones (Mueller et al., 1964). This bone source is critical since shell calcification occurs during the dark period, when the hen is not eating. Proper skeletal development is integral during pullet growth, which has a first period of growth during the first 6 weeks of rearing and a second period of growth with long bones reaching their maximum length and cortical bone reaching optimal thickness by week 22 of age (Whitehead and Fleming, 2000). Upon activation of the reproductive axis and in preparation for the laying cycle, increasing estradiol 17-beta (E2) concentrations shift the activity of osteoblasts toward the formation of medullary bone (Miller, 1992), with cortical bone accumulation terminated before lay (Hudson et al., 1993). This medullary bone is deposited on the interior, endosteal surface of the cortex in the marrow cavities of long bones (Mccoy and Reilly, 1996), with the largest reserve in the femur, followed by the tibia (Clunies et al., 1992). This second phase of calcium deposition is very dependent of the feed intake at the start of production. Nutritionists have different approaches when the start of production happens in layer hens and today there is a new option suggested by H&N International called Hybrid Feed that aims to support the start of production better than current and traditional approaches.

II. CONCEPT OF HYBRID FEED

The feed intake development at the beginning of production will allow the bird to have the correct nutrient intake. This feed aims to keep developing the feed intake as it happens at the end of rearing of the pullet and supply enough nutrients to support the bone development and the production of the first egg. The numbers recommended in the Hybrid Feed should be adapted to the different raw material information sources and to the available raw materials in the market. Here below a description of the principals and recommendations when developing a Hybrid Feed:

- Low energy diet: the feed intake of the layer hens is regulated by calories intake (A.S. Hussein et al 1996). During the developer phase of the pullet the feed should focus in developing the feed intake capacity of the bird, the metabolizable energy content of the Hybrid Feed should be like the developer feed, around 2650-2750 kcal/kg, depending on the source/s of information used

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to evaluate the energy of the raw materials. This approach stimulates the feed intake at the start of the production, and it will not hurt the start of the production because the production needs are still low, and the feed intake and composition of the Hybrid Feed will provide the necessary nutrients.

- Fibre content of the diet: low density diets will be completed by fibre. There are different ways we can classify the type of fibre (M. Chot 2015); it was shown that with the commonly used raw materials in layer hen diet, the increase of the crude fibre has a positive effect in gut development and therefore the feed intake capacity of the birds (Kondra et al 1974).
- Amino acids level: the amino acids level is like the layer phase 1 recommendation. There will be enough amino acids for body weigh growth and egg production. The amino acids requirements aren't maximum until week 22-23 of age as the flock is in full production (Table 1). However, due to the low feed intake it is necessary to have a high concentration of the amino acids to be sure that there will be an enough supply.

Table 1 - Requirements of digestible Lysine at the start of egg production, H&N International 2019.

Week of age	Maintenance (Mg D lys / bird)	Growth (Mg D lys / bird)	Egg mass (Mg D lys / bird)
17	122	209	0
18	129	217	0
19	136	229	52
20	142	226	246
21	149	214	389
22	154	171	486

- Calcium level: the average hen requires 2.2 g of calcium for each eggshell formation (Bouvarel et al., 2011). At the start of production, we will have a growth of the bones and we will have some early productive hens. As per the bone development with 2-2.5% of calcium level would be enough, however the early producing bird need more. Therefore, the recommendation is to formulate to 3.8% Ca in browns and 4% in whites layer hens. What is important is the source of calcium, to avoid possible feed refusal the coarse calcium carbonate should be the 60-70% of the total calcium carbonate in the diet. The particle size of the coarse calcium carbonate should be 2-4 mm.
- Phosphorus level: the phosphorus level in poultry is related to the feed intake and the growth of the birds, critical in this stage and in layer hens it is related to the calcium deposition in the bones (J.D. Summers et al 1976). Therefore, the level of available phosphorus should be as high as in the layer phase 1 recommendation, 0.44 % in browns and 0.46 % in whites.
- High level of salt: the salt is a raw material related to the feed intake in birds. It has been shown that salt stimulates the water intake and feed intake. Therefore, it is recommended to include salt at the level of 0.28%. It shouldn't be replaced with a level of sodium in the diet. The use of sodium bicarbonate as raw material in the layer diets could complete the needs of sodium requirement in the diet but it doesn't have the same feed intake stimulation effect as the salt. Therefore, it will be important to be sure that we have the level of salt in the diet, and we don't exceed the maximum of 0.34% of chloride as nutrient.

All this parameter will make the diet, but it is also about the use at the farm.

III. USE OF HYBRID FEED

There are challenges at the farm when getting accurate data at the start of production. The long transfer period from rearing to production house and the stress of the arrival make it difficult to calculate the feed intake at the start of production. In addition, the low production of the flock delays the egg collection of the flock, therefore we don't have an accurate data of the daily production.

The use of Hybrid feed meant to be simple and easy to apply, therefore this is the recommended program:

1. House the pullets in production with Rearing Development feed, as long as there wasn't a previous light stimulation of the flock at rearing.

2. Hybrid feed should be available to the hens as soon as the light stimulation is given to the pullets.
3. Hybrid feed will be provided until 70% of production is achieved. This will happen around the week 21-22 of age and it will be easy to have an accurate data of the % of production as the egg collection is regularly done at the farm at this age. The feed intake by then will be around 95-100 grams/hen and even higher if the pullet had a good feed development.
4. The next feed should be a 60-58 egg mass target feed as the egg production will reach the peak.

IV. ADVANTAGES OF HYBRID FEED

There are other approaches during the onset period like using a pre-lay feed and/or a super-starter feed. Both approaches have shortcomings that Hybrid Feed doesn't and has all the advantages of a pre-lay or super-starter feed.

The pre-lay feed is meant to be a transition feed between pullet and production, however the application at the farm level is very difficult and dangerous. The pre-lay feed use is recommended to do by two ways, and both are difficult to apply:

- Use of pre-lay until 2-5% of production: this recommendation doesn't consider that many farms don't collect the eggs daily when the flock is starting in production. The egg collection is decided by the workload at the farm and how the egg collection belts look like; therefore, a lot of mistakes can happen. The pre-lay diet can be extended too long and early producer hens can become decalcified, stop the production and in the peak of production we will have peaks of production 2-5% lower than the standard.
- Use of pre-lay 0.5-1 kilogram per bird: this recommendation forgets that there is variation of the feed intake at the pullets due to effect of weather changes and feed development challenges during rearing. This recommendation will have same result as the previous one and even we can have problems of eggshell quality late in production.

Another approach is using a super starter of lay after the developer feed or after the use of the pre-lay. This super starter of lay is highly concentrated feed in nutrients and very expensive; it is designed for supplying all the nutrients needed at this critical moment. However, it creates new challenges: the high energy of this feed restricts the feed intake development of the layers, therefore, many times the birds don't have enough nutrient intake at the peak of the production; it will lead to production drop at the peak of the production when changing to a layer 1 diet because of lack of feed intake; or it will push the farmer to use an expensive feed until 40 weeks of age because the layer hen doesn't eat enough nutrients.

The hybrid is a proven alternative that skips the disadvantages of the other recommendations and has all the advantages (Table 2). The use is very friendly for the farmers and it gives a more cost effective solution for the start and peak of the production.

Table 2 - Comparison of the different effects of the three options at the start of lay.

	Pre -Lay	Super Starter	Hybrid Feed
Application	-	+	+
Feed intake development	+	-	++
Calcification	+	+	+
Egg production	-	+	++
Cost of feed	+	-	+

V. EXPERIENCE OF HYBRID FEED

The use of Hybrid Feed started in 2019 and it keeps increasing the acceptance as it is tested by the egg producers. This feed was tested in Australian conditions in caged brown birds and cage free brown birds; at that time, we could see a good feed intake development, performance of the birds was on standards (Graph 1) with good eggshell quality. There are more positive experiences when using Hybrid feed in brown layer markets like Colombia or Ecuador and in white markets like in USA and Canada.

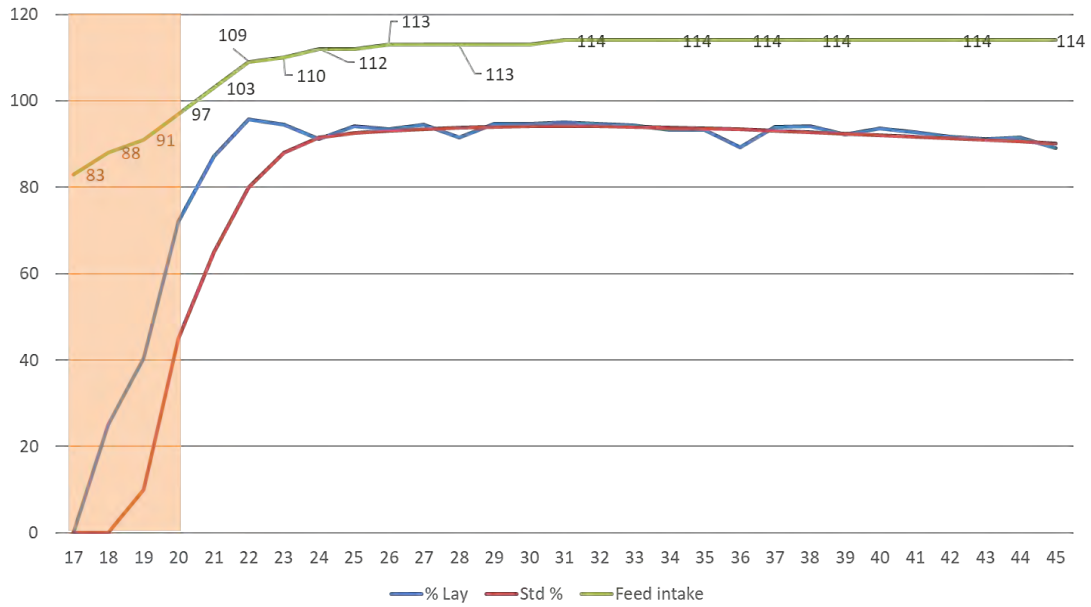

Figure 1 - Production performance and feed intake when used Hybrid Feed from 17 to 20 weeks.

In all these experiences using the Hybrid feed we have seen an improvement of the feed intake development and a faster egg size development without affecting the performance of the birds.

VI. CONCLUSIONS

The use of the Hybrid Feed is a new approach to the start of production where different challenges happen. This new approach can replace the previous practices that are difficult to apply at farm level or are too costly. The use of the Hybrid Feed should be part of the feed intake development program where first step starts at the develop phase of the pullet. At this stage there should be a feed intake capacity development and later at start the production Hybrid Feed will be the perfect match for having a bone development, sexual maturity growth and first egg production. The Hybrid Feed brings solutions to a critical part of the egg production, the start of production, no matter the breed.

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EFFECT OF MULTIENZYME ON LATE-PHASE HENS FED WITH CORN-SOY-BASED DIET

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Summary

Eggs are the most affordable animal protein; thus, there is a surging increase in the number of laying flocks, especially in developing countries. The current trend in the Philippines is extending flock age beyond recommended egg production for economic purposes but decreasing egg quality. To save costs, farmers use low-quality ingredients, which negatively impact the performance and health of laying hens. Therefore, this study aims to evaluate a multienzyme product in terms of hen performance, egg quality, gut morphology, and bone strength. Three hundred twenty-four (n= 324) Dekalb hens of 96 weeks of age were randomly allotted to one of the 3 dietary treatments in a completely randomized design (CRD). The diets were: T1 is the basal diet, T2 is the reduced energy (-90 Metabolizable Energy, ME) diet, and T3 is reduced energy plus multienzyme (350g/tonne). Each treatment had 27 cage replicates with 4 active laying hens in each cage. After 16 weeks, body weight gain had no changes among dietary groups. Feed intake was higher in T1 ($P = 0.0102$), which may result from lower Ether Extract (EE) and Crude Fiber (CF) proximate value results. T3 was also able to be at par with T1 regarding Hen Day Egg Production, Hen Housed Egg Production, egg weight, egg mass, and FCR ($P > 0.05$). T3 demonstrated superior albumen height and Haugh unit compared to T1 and T2 ($P = 0.0033$) and ($P = 0.0464$), respectively. The other parameters with favorable results in T3 include eggshell width, length, and egg shape index ($P < 0.05$). The gut morphology demonstrated that T1 was not significantly different from T3 regarding villi height and goblet cell count ($P = 0.7933$ and $P = 0.6924$, respectively). T3 showed the best result in bone-breaking strength ($P = 0.0116$). Thus, it was found that adding the multienzyme product (350g/tonne) to layer diets improved production parameters despite the decreased ME, indicating cost-effectiveness for farmers wanting to extend their flock age.

I. INTRODUCTION

With the recent COVID-19 pandemic disrupting the regular supply chain in agriculture and threatening food security, it is imperative to look for innovative ideas to meet the population's food demand (FAO, 2021). The agriculture sector is pressured to offer sustainable, economical, and ethically-produced animal protein. Egg, a cheap and relatively easy-to-produce commodity, is one of the best candidates for such protein. Thus, there is a surging increase in layer population, especially in developing countries where food security is still an issue. The Philippine Statistics Authority (2021) reported that a record high of 605,786.16 metric tons or 12.721 billion pieces of eggs were produced in 2020, a 4% higher compared to 2019.

To sustain the egg production requirement and to lower the cost of farming, the current tactic in the Philippines is to extend the flock age beyond breed recommendations. However, due to physiological limitations, aged birds produce low-quality eggs and have bone problems like osteoporosis (Alfonso-Carrillo et al., 2021). With this premise, it is not economical for the farmers to use expensive raw materials for the feeds since expectations of performance at this stage of the hen are different from when it is at peak production.

In nutrition, various approaches can be implemented to decrease cost, which includes using cheaper alternative feed sources and altering nutrient specifications to reduce expensive

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inputs. With cheaper source materials, higher ANFs are expected while alteration of specs needs formulation adjustment that will still meet nutrient standards. Both can be addressed via the addition of feed additives. Feed additives are a well-studied aspect of nutrition for improving hens' production (Świątkiewicz et al., 2018). Enzymes, and enzyme cocktails, are among the most prevalent nutritional feed additives in poultry for the last decades. It is widely accepted that the enzymes' nutrient-liberating ability can help birds enhance performances and lower production costs. Indirectly, it can be one of the actions to support our growing population's food demand by assisting the farmers in producing eggs efficiently and economically. This study investigates the effect of multienzyme supplementation on reduced energy diets on late-phase hens' egg production, quality, gut morphology, and bone strength.

II. METHODS

Three hundred and twenty-four (n = 324) Dekalb hens 96 weeks of age were randomly allotted to one of the 3 dietary treatments in a completely randomized design (CRD). Each treatment had 27 replicates with 4 hens/cage. The trial was 16 weeks, with feed and water provided ad libitum. The cage specifications were: 46 cm x 47 cm x 33 cm (L x W x H). All cages were in a well-shaded and naturally-ventilated facility experiencing a temperature of 25.3-26.6º C and humidity of 85-86% (Philippine Atmospheric, Geophysical and Astronomical Services Administration, no date). The 3 dietary treatments were a. Treatment 1(T1), positive control b. Treatment 2 (T2), 90 kcal ME reduction, and c. Treatment 3 (T3), T2 plus multienzyme (350g/tonne). Formulations among the treatments were isonitrogenous with the same level of EE, CF, and Ca, and P. The diets were based on the Dekalb guide manual and in mash form (0.5 to 3.0mm). Nutrient specs standards are as follows: ME 2750 kcal/kg, CP 17.5 g/kg, EE 3.8 g/kg, CF 2.8g/kg, Ca 4.3 g/kg, and P 0.6 g/kg. The multienzyme product Natuzyme® (BIOPROTON) contains the following at varying concentrations and activities: phytase (1,876,000 u/kg), xylanase (11,539,000 u/kg), alpha-amylase (760,000 u/kg), beta-glucanase (742,000 u/kg), cellulase (6,924,000 u/kg), protease (271,000 u/kg) and pectinase (74,000 u/kg). Initial and final body weight (BW) and average daily feed intake (ADFI) were recorded. All eggs produced were also recorded for Hen Day egg production (HDEP), Hen-housed egg production (HHEP), Average Egg Weight (Ave. EW), egg mass (EM), and FCR. Twenty-seven (27) eggs per treatment were randomly selected to be measured for Shell width (SWd) and Shell length (SL) and eventually Eggshell Shape Index (SWt/SL x 100). The eggs were broken and subjected to Egg Analyzer (ORKA Food Technology LLC, Utah, USA) to determine the Haugh unit (HU) and albumen height (AH). At the end, twelve (12) hens from each treatment were sacrificed for gut morphology and bone-breaking strength assessment (BBS). Cross sections (4um) of the gut's ileum were stained by H&E (villi and crypt measurements) and Periodic-Acid Schiff (goblet cell counting) The left tibia was excised and subjected to strength determination using the Universal Testing Machine (4411 Instron Co., Canton, MA, USA). All data were analyzed using the General Linear Model (GLM) procedure of Statistical Analysis System software version 9.2 (SAS Inst. Inc. Cary, NC). The model assigned cages as a random effect, while dietary treatments were the fixed effect.

III. RESULTS AND DISCUSSION

In this study, there were no changes in body weight gain between treatments (P = 0.9313). The current research aligns with the previous study in multienzymes that body weight is not affected regardless of decreased ME kcal (Lee et al., 2014). In this study (16 weeks) and that of Lee et al (2014), the trial period may be too short to elucidate the long-term effect of reduced energy on BW. Feed intake showed a different pattern as the T1 gave the best results among the 3 diets (P = 0.0102), which may be related to lower dietary crude fat and crude fiber as per proximate

analysis (not shown). This translates to lower heat increment; thus, T1's hens would eat more to compensate for the heat needed. Also, the results did not conform to the theoretical idea that hens with reduced ME (T2) would tend to eat higher amounts of feed to compensate for energy requirements. The HDEP, HHED, egg weight, egg mass, and eventually, the FCR (T3) were all at par with the standard diet (T1) (P > 0.05), even with decreased energy. T2, as expected, was reduced in all parameters except higher FCR.

Table 1 - Parameters for hen performance, egg quality, gut morphology, and bone breaking strength.

Parameters	Unit	T1	T2	T3	SEM	*P* Value
Ave Daily Gain (ADG)	g	85.03	49.60	176.73	1.04	0.93
Hen Day Egg Production (HDEP)	%	74.45^{a}	64.80^{b}	72.78^{a}	6.66	<0.001
Hen Housed Egg Production (HHEP)	%	69.42^{a}	62.23^{b}	70.90^{a}	5.64	<0.001
Ave.Egg Weight (Ave EW)	g	65.31^{a}	62.76^{b}	65.83^{a}	1.20	<0.001
Egg Mass (EM)	g	48.63^{a}	40.84^{b}	47.67^{a}	4.59	<0.001
Ave Daily Feed Intake (ADFI)	g	132.70^{a}	127.23^{bc}	123.00^{c}	11.46	0.0102
FCR egg mass		2.80^{b}	3.25^{a}	2.66^{b}	0.28	<0.001
Haugh Unit (HU)		67.65^{b}	67.49^{b}	69.22^{a}	2.77	0.05
Albumen Height (AH)	mm	5.42^{b}	5.34^{b}	5.62^{a}	0.31	0.0033
Shell Width (SWd)	mm	44.87^{a}	44.10^{b}	45.06^{a}	0.45	<.0001
Shell Length (SL)	mm	58.85^{a}	58.17^{b}	59.11^{a}	0.59	<.0001
Albumen Weight (AW)	g	47.42^{a}	45.32^{b}	48.07^{a}	1.38	<.0001
Egg Shape Index (ESI)	%	76.21	75.99	76.23	1.04	0.71
Bone Breaking Strength (BBS)	N	0.20^{c}	0.20^{b}	0.26^{a}	0.05	0.0116
Villus Height (VH)	um	566.34^{a}	419.87^{b}	553.90^{a}	86.54	0.01
Crypt Depth (CD)	um	119.53	110.76	133.94	15.76	0.06
VH/CD ratio	um	4.84	3.83	4.21	0.98	0.18
Goblet Cell Count (GCC)	#goblet/umVilli	0.13^{a}	0.07^{b}	0.13^{a}	0.03	0.01

In egg quality, T3's Haugh unit (P = 0.0464) and albumen height (P = 0.0033) were of superior results. Factors affecting albumen height were not fully understood, but Scott et al. (2001) suggested that it may be due to the involvement of the multienzyme's phytase-releasing P. This could be the reason why T3 showed better results than T1 and T2 as there were no reduction in P inclusion rate in the formulation of the diets. Thus, multienzyme's phytase in T3 diet would further increase P availability. In eggshell width (SWd) (P = < 0.0001) and eggshell length (SL) (P = <.0001), all gave good results for multienzyme-supplemented (T3) as it was consistent with the T1. The T2's egg sizes decreased, and so did the width and length. Nevertheless, the eggshell shape index (ESI), a ratio of SWd and SL, was uniform among the groups (P = 0.6406). The result implies that even if width and length is reduced in T2, the hens produced an egg shape as proportioned as possible regardless of treatment conditions.

In gut morphology, the villi mean (553.90um) of T3 was not statistically different from T1 (566.34um), and both were superior over T2 (419.87um) at P = 0.0134. In opposition, both crypt depth and VH/CD ratio were not significantly different among diets (P > 0.05). The

enzyme's action of enhancing nutrient availability in the gut lumen causes the response of increased villi length. A longer villus result is good indicator of a healthy and well-absorbing gut. Previous studies showed similar results regarding ileal crypt depth (Madigan-Stretton et al., 2021, de Souza et al., 2014), where there was no change. In contrast, a high crypt depth value is unwanted as crypts are 'villi factory' where an increase in length indicates a faster turnover rate of cells to produce new villi. Thus, this translates to more unhealthy cells needed of immediate replacement and is pathologic in nature. In goblet cell counting (GCC), the T3 result was at par with T1, and both were statistically significant over the T2 ($P = 0.0093$). The consistent result of T3 with T1 is the ideal and implies that there were no gut problems. A high density of goblet cells is unwanted as it also suggests an increase in goblet cell proliferation and consequently decreases rate of enterocyte formation, thus, lower absorptive capability.

In bone breaking strength (BBS), T3 showed superior results compared to T1 and T2 at $P = 0.01$. As mentioned, all diets followed Ca and P standard, but when egg production varies, the bone reserves utilization will also vary among treatments thereby affecting BBS. In T2, it was statistically higher than T1 at $P = 0.021$ because T2 hens did not need reserves as production also decreased in frequency. Thus, more Ca and P are still present in the bones of T2, making it 'harder'. Meanwhile, in T1, the group is still in good production and giving decent egg sizes which still demands high Ca and P. Thus, T3 needs bone reserves as the standard Ca and P feed inclusion rate may be inadequate. This explains better BBS for the enzyme-supplemented diet (T3), even if T1 and T3 had the same egg production and sizes. Minerals liberated by multienzyme's phytase are adequate or even excess in T3 that it did not need the bone reserves thereby addressing production demand while maintaining bone strength. Thus, supplementing late phase hen's diet with a multienzyme at 350g/tonne and a 90 ME reduction was able to maintain or outperform the positive control in terms of hen performance, egg quality, gut morphology, and bone health. Furthermore, no adverse effects on feed intake and body weight gain occurred. Therefore, the product is advantageous for farmers planning to extend flock age without sacrificing egg production and quality and promoting animal welfare through improved bone strength.

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MATERNAL FEEDING CALL PLAYBACKS REDUCED ANXIETY AND DEPRESSION-LIKE STATES IN MEAT CHICKEN CHICKS

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Summary

Mother hens can teach chicks important life skills resulting in lifelong benefits. Providing chicks with mother hens in industry is not practical but there is evidence that providing part of the maternal call artificially can influence chick behavior and improve welfare. We provided recordings of mother hen feeding calls to chicks from hatching with the aim to improve meat chicken welfare. Chicks were either played maternal call playbacks in their home pens throughout life, or white noise (control group). Negative affective states were assessed at 5 or 6 days of age using a validated chick anxiety/depression model which demonstrates that anxious chicks emit more distress vocalizations when isolated for 60-minutes and chicks in a depressive like state will rapidly reduce the number of calls over time. In the first 10 minutes of isolation, control chicks vocalized nearly twice as much as chicks that received the maternal call playbacks throughout life. Furthermore, distress vocalizations emitted by control chicks declined rapidly in the first 30 minutes of the test followed by a relatively stable number of distress calls, yet the reduction was more gradual for maternal call chicks. These results suggest that chicks played maternal feed calls during early life showed fewer indicators of anxiety and depression-like states. As such, we provide evidence that maternal call playbacks can improve the welfare of meat chickens.

I. INTRODUCTION

Brooding chicks with maternal care can improve the welfare of chicks; specifically, by improving prosocial behavior and reducing feather pecking (Edgar et al., 2016). Yet providing chicks mother hens in industry is impractical and a biosecurity risk. Artificial components of maternal care may provide some benefits to chicks and improve welfare. Indeed, there is some evidence that lighting that mimics the maternal environment, synthetic maternal olfactory cues and mother hen vocalizations can affect chick behavior and welfare (Edgar et al., 2016). Playback vocalizations are a promising method to provide part of the maternal environment to chicks, due to the low cost of installation and maintenance of technology and the multifunctionality of speaker systems that could emit various calls for different contexts and desired outcomes. Furthermore, playbacks of maternal calls have been shown reducing stress responses of layer hen chicks (Edgar et al., 2015) and increase growth of meat chickens by 25% (Woodcock et al., 2004). However, the effect of maternal call playbacks on meat chicken welfare is largely unknown. We aimed to improve meat chicken welfare through the use of a maternal feed call playback by improving stress resilience.

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II. METHOD

Mixed sex Cobb 500 birds (n = 224) were housed in groups of 14 across 16 pens (3.2m × 2m) with wood shaving flooring, a perch, one round feeder and two nipple drinkers. Treatment groups were audibly isolated across separate rooms. Chicks in pens within the same room/treatment were visually isolated. Playback recording of either a maternal feeding call (maternal call treatment group; MC) or white noise (control group; CON) were played through speakers mounted above the center of each pen (Flex 15, Australian Monitor Integration Intelligence, Silverwater, NSW, Australia). Maternal call playbacks were 5 minutes in duration and contained 30 seconds of a feed call followed by 30 seconds of silence. The playback contained recordings from two hens when their brood was 3 or 4 days of age. The playback was played each hour in a 24-hour cycle from the first day of life. At either 5 or 6 days of age, chicks (n = 14 MC; n = 12 CON) were assessed for behavioral indicators of anxiety and depression using the validated domestic fowl anxiety-depression chicken model (Sufka et al., 2006). Briefly, each chick was isolated in a test arena (1.7m × 1.7m) with 10cm wood shaving flooring, located in an adjacent room for 60 minutes. Vocalizations were recorded during the test (BAR recorder, Frontier Labs, Salisbury, Qld). The number of distress calls emitted during the test were quantified using Raven Sound Analysis software (The Cornell Lab of Ornithology, New York, USA) and summed into 5-minute intervals. The number of vocalizations for each interval was analyzed with a Generalized Estimating Equation to account for non-parametric data and repeated measures.

III. RESULTS

Chicks that had been given maternal feeding call playbacks in their home pen vocalized less relative to control chicks during the social isolation test. However, control chicks showed a steeper progressive decline in distress calls overtime compared to chicks that had received the maternal call (interaction time × treatment: $\chi^2_{(11,299)}$=21.6, P = 0.028; Figure 1).

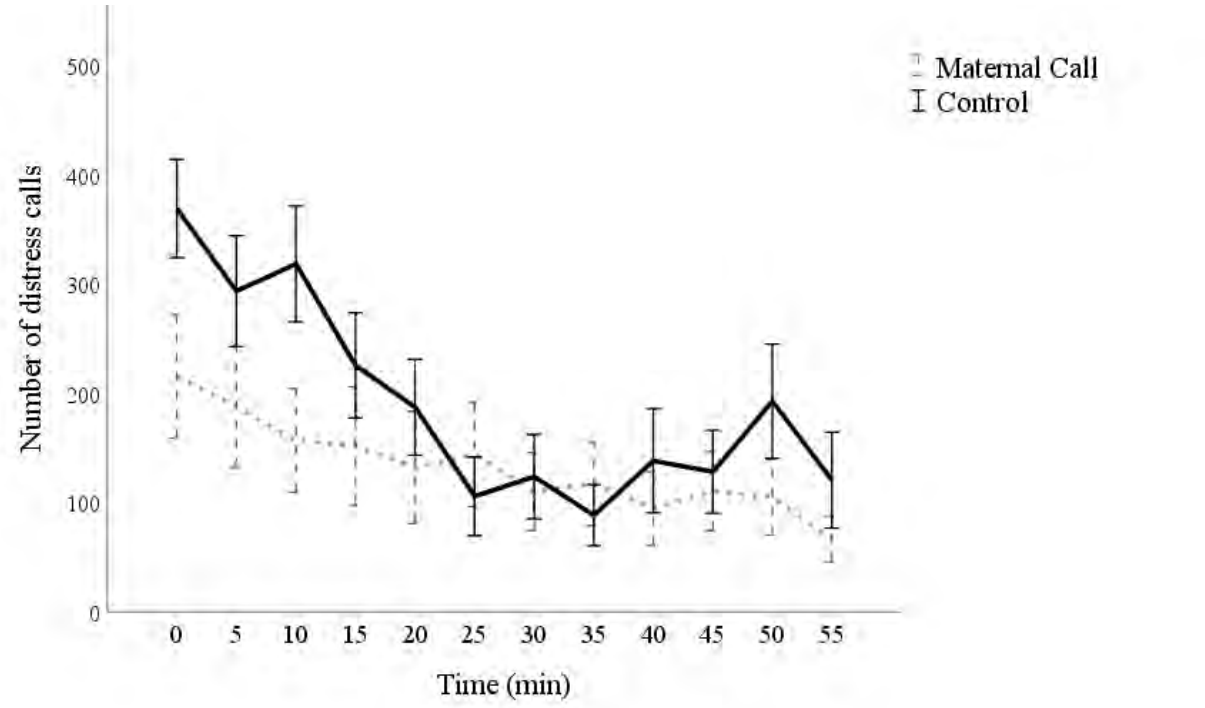

Figure 1. - The mean number of distress vocalizations (± SEM) from chicks during the anxiety-depression model test. Results are presented in 5-minute increments for chicks receiving maternal feed calls (grey dashed line) or white noise (black solid line) from day old.

IV. DISCUSSION

Previous pharmacological interventions to validate the chick anxiety-depression model shows that birds in an anxiety state will frequently emit distress calls and birds in a depressive-like state will rapidly reduce the number of distress calls over time (Salmento et al., 2011; Sufka et al., 2006). We provide evidence that playing maternal feeding calls at hourly intervals throughout the first 5 days of life reduced anxiety and depression-like states in meat chickens, suggesting that playing maternal calls may be an effective way to improve the welfare of meat chickens.

ACKNOWLEDGEMENTS: We would like to thank AgriFutures for funding this work and Haylee Herriot for her assistance with the daily animal care and experimental data collection.

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BIOLOGICAL CONTROL OF SALMONELLA IN THE POULTRY INDUSTRY – A EUROPEAN PERSPECTIVE

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Summary

Salmonella remains one of the most important foodborne bacterial pathogens worldwide, and is frequently linked with the consumption of contaminated poultry meat and eggs. Despite some noted successes in reducing particular serotypes and strains, other serotypes have become more problematic. Progress has also been frustrated by increasing resistance to antimicrobials and biocides. Biological control using bacteriophages or predatory bacteria is an alternative approach to *Salmonella* control in poultry which has been effective in laboratory, experimental and/or field trial settings. The European Union is currently considering authorizing the first phage-based commercial product for the treatment of *Salmonella* in poultry. Questions remain about the regulation and commercial status of such products, as well as their long-term efficacy; but their ability to specifically target multidrug resistant bacteria may provide a new way of reducing our reliance on antimicrobials and decrease the burden of disease in both animals and humans in the future.

I. INTRODUCTION

Salmonellosis remains one of the most frequent food-borne zoonoses, constituting a worldwide major public health concern. Although *Salmonella* can be acquired from a range of foods, poultry meat and eggs remain prominent (Antunes et al., 2016). Human salmonellosis cases in the US are estimated at 1.35 million per year (Centers for Disease Control, 2019a). Likewise, cases in the UK and EU have not changed significantly over the past decade.

Success in controlling some *Salmonella* serotypes (e.g. Enteritidis) in poultry through vaccination and biocontrol have been punctuated by failure to control other serotypes. A recent example of this was an outbreak of *Salmonella* Infantis in the United States in 2018, affecting 32 states (Centers for Disease Control, 2019b). In the EU, *Salmonella* Infantis is now the 4th most common serotype from human infections, and the most frequent serotype in broilers flocks (45.6%) and broiler meat (50.6%) in Europe (Alba et al., 2020). More worryingly, multidrug-resistant isolates are becoming increasingly common in Europe, particularly those which have acquired a pESI megaplasmid encoding resistance to multiple classes of antibiotics as well as virulence genes and enhanced tolerance of heavy metals and biocides (Tyson et al., 2021).

One alternative approach to *Salmonella* control is the use of biological control agents; either viruses which specifically infect *Salmonella* – bacteriophages – or use of predatory bacteria such as *Bdellovibrio bacteriovorus*. In a recent review of alternatives to antibiotics, funded by the Wellcome Trust and UK Department of Health, bacteriophage-based treatments comprised three of the ten Tier 1 (most promising) new technologies (Czaplewski et al., 2016). Although less developed, *Bdellovibrio* has been used successfully to control infection in multiple animal models of disease, including the treatment of *Salmonella* in poultry (Atterbury et al., 2011).

Biological control has unique advantages over antimicrobials. These agents are both self-replicating and self-limiting – reproducing only when susceptible bacteria are present. Unlike broad-spectrum antibiotics, they target a specific genus, species or strain of bacterium,

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avoiding potential harmful dysbiosis in the patient. Likewise, several studies have found that *Bdellovibrio* can prey upon multidrug resistant pathogens in biofilms (Atterbury and Tyson, 2021; Sun et al., 2017). In addition, *Bdellovibrio* can survive for up to 24 h inside eukaryotic cells without adverse effects, suggesting that it could be applied to control pathogens with an intracellular component to their lifecycle, including *Salmonella* (Atterbury and Tyson, 2021).

II. METHOD

Candidate therapeutic bacteriophage are usually isolated from an environment where their bacterial hosts are abundant. In the case of *Salmonella*, this is often poultry feces, wastewater or human sewage. Once purified, these phages are screened against a large panel of target serotypes and strains which should be chosen to reflect the diversity of pathogens affecting the population. Phages that infect a broad range of bacteria, either separately or in combination (cocktail) are usually selected for genome sequencing and analysis. This step allows the identification of undesirable phage e.g. temperate phage which do not underdo a direct and predictable lytic infection cycle. It also identifies phage carrying undesirable genes such as those associated with virulence. More detailed *in vitro* characterization of the phage may be performed in parallel, such as determining the burst size and replication kinetics of infection. For promising candidates, the specific phage receptor(s) on the host bacterium may be identified, along with an assessment of how frequently resistance to phage occurs.

Increasingly, bioinformatics is being used to facilitate the characterization and selection of phage. There are tools available to predict the taxonomy, DNA packaging mechanisms (Garneau et al., 2017), lifecycle (McNair et al., 2012), receptor specificity and host range (Zhang et al., 2017) of candidate phage. Once a phage is characterized, online tools can be used to compare it with others uploaded to the database which may be useful for cross-validation (Rangel-Pineros et al., 2021). Further confirmatory tests can then be performed in the laboratory, for example to establish the phage receptors predicted *in silico*. Thereafter, the phage candidates can be tested for efficacy in simple animal models such as *Galleria mellonella* and their stability in commercial preparations before taking forward to experimental and field trials in poultry.

Bdellovibrio, like phage, is most often isolated from environments where their prey are abundant. This is often in aquatic environments or sewage. Unlike most bacteriophage, *Bdellovibrio* targets multiple genera of Gram-negative pathogens, and resistance to predation is rare and transient (Marine et al., 2020). There is evidence that *Bdellovibrio* or like organisms are present in the intestinal tracts of a range of animal species, including poultry (Schwudke et al., 2001). However, these bacteria have been difficult to isolate and exploit directly. *Bdellovibrio* are metabolically active and chemotactic towards their prey. As such, unlike phage they are not reliant on passive diffusion to spread through a bacterial population. However, it also means the options available for therapeutic delivery are more limited than with phage and commercial preparations may be more challenging.

III. RESULTS

Salmonella is frequently a target of biological control trials owing both to its importance as a human pathogen and as a cause of significant disease and production losses in livestock. Most effort has been directed towards controlling important non-host-restricted serotypes of *Salmonella*, such as Enteritidis and Typhimurium which are more significant from a regulatory perspective in the EU and elsewhere.

Cocktails of lytic phage have been used to reduce intestinal carriage of *Salmonella* by between 1 and 3.5 $\log_{10}$ CFU/g (Atterbury et al., 2007; Sklar and Joerger, 2007). These reductions were often accompanied by marked improvement or elimination of clinical signs of disease

(Sklar and Joerger, 2007). Phage therapy has been combined with seeder models of infection to demonstrate how effective phage can be in preventing horizontal infection of chicks by *Salmonella*. Lim et al. (2012) showed that treatment with up to 10^9 PFU/g of phage in feed could reduce intestinal colonization by up to 1 $\log_{10}$ CFU/g, while 70% of the contact chickens had no detectable *Salmonella* Enteritidis colonization. Henriques et al. (2013) significantly reduced the spread of *Salmonella* Enteritidis in chicks by treating fertilized eggs with a cocktail of two phages. Clinical signs of disease at the end of the eight-day study were not significantly different from the control group.

More recently, two studies have examined the impact of applying therapeutic phage on the chicken microbiome. Kosznik-Kwaśnicka et al. (2022) found that both phage and antibiotics (enrofloxacin and colostin) were all able to reduce *Salmonella* to below detectable levels in infected chickens. However, antibiotic use was associated with significant and prolonged changes in the microbiome whereas the effects of phage were transient and normalized several weeks after treatment. Similarly, Clavijo et al. (2022) used a proprietary *Salmonella* phage product in a commercial broiler farm to assess the effect on the gut microbiome. No significant effects of phage on the maturation of the microbiome were detected.

Bdellovibrio has been isolated previously from the intestinal tracts of chickens as well as humans and other vertebrates (Schwudke et al., 2001). Atterbury et al. (2011) used *Bdellovibrio* to treat chickens experimentally infected with *Salmonella* Enteritidis. Significant reductions of over 1 $\log_{10}$ CFU/g were recorded in the ceca of treated birds compared with buffer-treated controls. *Bdellovibrio*-treated birds also had significantly fewer cecal abnormalities compared with both untreated controls and birds treated with a non-predatory *Bdellovibrio* mutant. Interestingly, *in vitro* experiments using both phage and *Bdellovibrio* showed that the combination of both virus and predator was able to eliminate pathogen populations much more effectively than either used independently (Hobley et al., 2020)

IV. DISCUSSION

Bacteriophages have been shown to significantly reduce the intestinal carriage of multiple serotypes of *Salmonella* in broiler and layer chickens. These reductions were frequently accompanied by improvements in clinical manifestations of disease (reduced perihepatitis, pericarditis, typhlitis) as well as feed conversion ratios. Although phage-resistant variants of *Salmonella* have been recorded, these phenotypes are often transient, and associated with reduced fitness in animal models of infection. Resistance to *Bdellovibrio* on the other hand is extremely rare, and only conferred by the presence of an intact S-layer which is metabolically expensive to maintain. Interestingly, when *Bdellovibrio* and bacteriophage are used in combination, they can act synergistically for a greater reduction in the target pathogen without the development of resistance.

Despite their promise, there remain some technical, commercial and regulatory hurdles which need to be overcome before bacteriophages and *Bdellovibrio* could be used more widely in the UK/EU. These agents do not fit easily within existing regulatory frameworks which were designed for defined chemotherapeutics. Likewise, the ability to protect intellectual property relating to naturally-occurring biological entities is an open question. Despite these obstacles, commercial phage products targeting food-borne pathogens are available in the United States and the EU is in advanced discussions to authorize the first bacteriophage treatment for livestock in the bloc. Given the pressing need to control multidrug-resistant infections, and the reducing efficacy and availability of antibiotics to treat them, biological control agents such as *Bdellovibrio* and bacteriophage offer a potential solution.

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SEEDER BIRD RESEARCH IN YOUNG LAYERS - EFFECTIVELY CHALLENGING WITH SALMONELLA TYPHIMURIUM

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Summary

Salmonella infection challenge models by oral, intracloacal, intratracheal, intraocular, navel, intravenous and aerosol administration have been developed for research (Gast and Porter 2020). This study was developed to assess the seeder challenge model in immature layers in line with current vaccination procedures followed in Australia using a live *Aro-A* deletion *Salmonella enterica* serovar Typhimurium vaccine. Commercial layers were followed to 35 days of age to assess the success of using a seeder model to replicate the transfer of *S.* Typhimurium between young commercial layers and the effectiveness of the vaccine protocol used. The use of seeder birds allowed the evaluation of using a horizontal transmission for the infection of layers with *S.* Typhimurium with the expectation it will more closely resemble a natural challenge situation. Isolation of *S.* Typhimurium from the cloacal swabs of the control and vaccinated birds showed that there was a consistent uptake in the Day 0 challenged birds. The week 3 challenge was not successful in infecting most of the birds, even though the colonisation of the seeder birds at 3 weeks was successful. There was no statistical significance in the transmission of S. Typhimurium between the vaccinated and unvaccinated groups.

I. INTRODUCTION

To successfully develop a challenge model to simulate actual infection as seen in production practices, a seeder challenge model was proposed and previously shown to be an effective method to infect poultry (Cox et al., 1996, 2020). However, applying it in this way to young layers is novel. This pilot study was developed with the objective of determining the success of this challenge method. A second objective was to determine the base line effectiveness of the vaccine against the seeder challenge method. Once established, the methodology could be used to assess any confounding effect of a live *Salmonella* vaccine protection against wild *Salmonella*. Identifying the best challenge period, day 0 or week 3, in young birds will set the basis for a successful challenge and control with the current vaccine programs available in Australia.

II. METHOD

The intention was to demonstrate if seeding birds at day old or 3 weeks will achieve colonisation of the control birds and if the vaccination protocol demonstrates protection at this point (a colonisation inhibition effect from the oral vaccine was anticipated following application). Pilot study design (looking at different challenge times under vaccination) was: live *Aro-A* deletion *S. enterica* serovar Typhimurium vaccine administered by coarse spray at day 0 and orally (gavage) at day 19 at single label dose rate per bird.

There were two floor pens allocated for each group of 8 birds (day old layer chicks). Pens for groups 1 and 3 were separated from groups 2 and 4 and biosecurity practices enacted between these to prevent cross contamination before the three-week challenge. Seeder birds were housed two per pen in suspended cages above the birds in each challenge pen for 5 days. The seeders were given an oral inoculation with 10^6 live *S.* Typhimurium DT 135 at either day

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0 or at week 3. After 5 days, the seeder birds were released into the pens. Cloacal swabs were collected at 10, 17, 24, 31 days of age with caeca collected at 35 days of age and cultured for the presence of *S*. Typhimurium.

All microbiological testing was performed at NATA accredited laboratory (Birling Avian Laboratories, Bringelly, NSW). Rapid finder Multiplex PCR was used to detect and implemented for testing. A selection of positive results was culture confirmed (XLD/Hektoen agar plates).

III. RESULTS

Horizontal transmission from the seeder birds resulting in infection of the challenge birds is shown in Table 1.

Table 1 - Floor pen birds; The number of cloacal or caecal samples positive for *Salmonella* Typhimurium. Control groups challenged at day 0 or 3 weeks of age (10^6 cfu). Vaccinated groups challenged at day 0 or 3 weeks of age (10^6 cfu).

Pilot Study Treatments			No. Positive Results (n=16)				
N= 16 per treatment	Description	Challenge	10 Days Cloacal Swab	17 Days Cloacal Swab	24 Days Cloacal Swab	31 Days Cloacal Swab	35 Days Caecal Samples
Control (Pen 1)	ST Challenge	Day 0	16	16	14	12	10
Control (Pen 2)	ST Challenge	3 Weeks	0	0	0	3	2
Vaccinated (Pen 3)	ST Challenge	Day 0	15	16	15	15	11
Vaccinated (Pen 4)	ST Challenge	3 Weeks	0	0	0	0	1

Seeder birds challenged at day 0 clearly showed better uptake than at 3 weeks for unvaccinated and vaccinated birds in this trial, Table 2.

Table 2 - Seeder Birds; The number of cloacal or caecal samples positive for *Salmonella* Typhimurium. Groups challenged at day 0 or 3 weeks of age (10^6 cfu).

Seeder Birds Unvaccinated			No. Positive Results (n=4)		
N= 4 per treatment	Description	Challenge	4 Days Cloacal Swab	31 Days Cloacal Swab	35 Days Caecal Samples
Pen 1	ST Challenge	Day 0	4	3	4
Pen 2	ST Challenge	3 Weeks	0	4	2
Pen 3	ST Challenge	Day 0	4	3	4
Pen 4	ST Challenge	3 Weeks	0	3	3

The day 0 coarse spray vaccination program used in this study was unsuccessful against the seeder challenge at day 0 with no significance difference in the proportion of vaccinated and control birds returning a positive cloacal swab at each sampling point. Oral vaccination at 19 days did not provide any further protection against challenge from day 0.

The week 3 challenge achieved colonisation in only three out of 16 control birds (19%) at 31 days and only two birds from 16 birds at day 35 from the caeca. This was not significantly different from the vaccinated group. The colonisation of the seeder birds at 3 weeks was successful (7/8 birds with positive cloacal swabs at 4 days post infection) but this did not transmit effectively to the treatment birds.

IV. DISCUSSION

Horizontal transmission results from seeder birds have been shown to facilitate gradual intestinal colonization (Muir et al., 1998). Isolation of *S.* Typhimurium from the cloacal swabs of the control and vaccinated birds showed that there was consistently successful uptake by day 0 challenged birds. Young chicks (under 3 weeks of age) are regarded as much more susceptible to *Salmonella* infection than older birds and an infection establishing at this early age can allow continuous presence of the organism for long periods (Gast and Porter, 2020). Overall, the study demonstrated that the model of using seeder birds from day 0 could successfully horizontally transmit *S.* Typhimurium mimicking an early natural infection. Although infection of the seeders in cages at 3 weeks of age was successful, this did not transmit successfully to the birds on litter at 3 weeks of age. The caged seeders remained susceptible to the infection while the birds reared on the floor achieved resistance to infection, perhaps due to differences in establishment of a gut flora due to having access to the floor. The day 0 administration of the live *Aro-A* deletion *S. enterica* serovar Typhimurium vaccine was overpowered by this challenge method.

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NEWCASTLE AND INFLUENZA A DISEASE BURDEN IN COMMERCIAL AND BACKYARD POULTRY FARMS IN NEPAL

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Newcastle Disease (ND) and Influenza A (IA) are major poultry diseases affecting both commercial and backyard poultry production worldwide[1]. We conducted a Nepal's first nationwide ND and IA prevalence study in 2018, collecting samples from both in commercial and backyard poultry farms. In commercial and backyard farms prevalence of ND was 70% and 17.5% respectively. We also determined the prevalence of IA in these farms (commercial= 27.5%, backyard= 7.5%). Genotype II was the most prevalent strain of ND virus found in the commercial farms, and Genotype I was detected in the backyard samples. We also identified and characterized the ND virus variant (Genotype VIIc) that caused a nationwide ND outbreak in 2021.

Poultry farming plays an important role as an income generating enterprise in a developing country like Nepal[2]. There were more than 90 reported cases of ND outbreaks in Nepal in 2018[1]. In 2019, Nepal reported three highly pathogenic Influenza A (H5N1) outbreaks in commercial layer farms (n=2) and backyard birds (n=1)[3]. Although there is an active nationwide IA surveillance, this is a first study that assessed a comprehensive ND and IA prevalence in both commercial and backyard poultry farms in Nepal.

We collected samples (oral, cloacal and blood) from commercial (n=40 farms, total birds= 600) and backyard (n=36 farms, total birds= 108) poultry farms from across the major poultry production hubs of Nepal in 2018. We used commercially available ELISA kits to detect antibodies (Nucleoprotein) against NDV and Influenza A; and conducted PCR and DNA sequencing based molecular assessments to characterize ND virus variants.

Of 40 commercial farms (each farm=15 birds) tested, both ND (n=28, 70%) and IA (n=11, 27.5%) antibodies were detected. In 36 backyard farms, sero-prevalence of ND was 17.5% (n=7) and IA was 7.5% (n=3). In the commercial farms, we were able to detect live ND virus (n=31, 78%) and IA virus (n=15, 38%) by using PCR. We found ND- Genotype II to be most common variant in the commercial farms; and ND-Genotype I was detected in some backyard poultry samples. The identified Genotype I is reported for the first time and could be an endemic ND virus variant found in Nepal. Additionally, ND- Genotype VIIc variant was found to cause 2021 ND outbreak in Nepal.

Prior to this study, there was a limited information on disease burden and epidemiological dynamics of ND and IA in Nepal. We detected higher prevalence rate of ND in commercial farms than in backyard farms. Usage of live virus vaccine for ND probably resulted in a high sero-prevalence of ND in commercial farms. The Government of Nepal has banned vaccination against IA, however, detection of IA virus (Genotype II) in the commercial farms could indicate practice of illegal use of live IA vaccines in the farms.

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DISPARITY IN THE GUT WALL PENETRATION BY *ESCHERICHIA COLI* DURING CO-INFECTION WITH *HISTOMONAS MELEAGRIDIS* IN CHICKENS AND TURKEYS

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Histomonas meleagridis and *Escherichia coli* exhibit an outstanding interplay *in vitro* and are well recognized pathogens in poultry causing histomonosis and colibacillosis, respectively. *H. meleagridis* and *E. coli* reside in the intestine of birds, thus, to understand their interaction *in vivo*, gut microbial changes and caecal wall penetration of *E. coli* were investigated separately in experimentally infected chickens and turkeys (Abdelhamid et al., 2020, 2021). In the first trial, commercial Lohmann Brown layers (23 weeks old) were orally infected with a bioluminescent labelled pathogenic strain of *E. coli* with or without virulent strain of *H. meleagridis* ($6x10^5$ cells). Infected birds along with mock-inoculated negative controls were sequentially killed at 7, 10, 14 and 28 days post infection (dpi) for sampling. In the second trial, turkeys were inoculated with attenuated and/or virulent strains of *H. meleagridis*, using the same strain and dose of the parasite as in layers. Negative control birds were left uninfected. Sampling was done in birds killed at 7, 14 and 21 dpi. High throughput amplicon sequencing of 16SrRNA in caecal samples, histopathology, quantification of tagged or total *E. coli* and immunohistochemistry for the detection of *E. coli* were performed. Results showed that, infection of layers with *E. coli* alone did not induce lesions in the gut and had no pronounced effect on the caecal microbial population. In contrast, inoculation of *H. meleagridis*, both in chickens and turkeys, led to substantial shift in caecal microbiota, coinciding with severity of lesions. In general, bacteria such as *Escherichia* or *Helicobacter* were higher and commensals (e.g. *Lactobacillus* spp.) were lower in their abundance. Typhlitis leading to caecal wall destruction due to *H. meleagridis* was obvious in chickens and turkeys. However, increased penetration of *E. coli* from the caecal lumen towards peritoneum was only observed in chickens but not in turkeys. The lack of tendency of intestinal *E. coli* to penetrate the caecal tissues of turkeys even in the presence of severe mucosal destruction was further confirmed in birds from field cases of histomonosis as well.

In conclusion, *H. meleagridis* influenced the gut integrity and relative *E. coli* population in the caeca but the bacterial cells showed different tendency to infiltrate into the caecal wall of chickens and turkeys.

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ALIGNING *IN VIVO* PATHOGENICITY OF *ESCHERICHIA COLI* IN EMBRYOS AND CHICKENS WITH GENOME CHARACTERISTICS

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Escherichia coli resides as a gut commensal in healthy chickens but also causes the extraintestinal disease called colibacillosis. Pathotype definition is primarily based on the clinical condition of the host, but it is still not definite due to the lack of genetic traits to differentiate between commensal and harmful isolates, which has led to divided views with regard to the pathogenicity of *E. coli* in poultry. In this study, embryos and chickens were experimentally infected with *E. coli* isolates from birds with well-defined clinical history and outcomes were aligned with antimicrobial sensitivity profiles and global genetic traits of isolates revealed by whole genome sequencing. Fifteen isolates (O78:K80) from the femur of broilers with or without femoral head necrosis (Gaußmann et al., 2018) and one from the ovary of a layer with colibacillosis (O1:K1, PA14/17480/5-ovary; Zloch et al., 2018) were selected for the embryo lethality assay. *E. coli* isolates greatly differed in terms of embryo mortality, which ranged from 33–100%. No correlation was observed among the severity of lesions in the femur, resistance against certain antibiotics and embryo lethality. Subsequently, the pathogenicity of PA14/17480/5 ovary and three broiler isolates (PA15/19103-3, PA15/25396-3-right, PA15/24960-2) that showed the highest embryo mortality rate was tested in two-weeks old chickens. Following infection, clinical signs, macroscopic and microscopic lesions as well as bacterial re-isolation from organs were investigated. Based on these parameters, PA15/19103-3 was the most pathogenic among broiler isolates, similar to PA14/17480/5-ovary. Genetically, all four isolates displayed similarity in the genome length and number of genes contained. PA14/17480/5 ovary and PA15/19103-3 belonged to the phylogroups B2 and C, respectively, whereas the remaining two strains were assigned as phylogroup G. In total, 259 virulence factors were present in at least one of the four *E. coli* isolates. Out of these, 128 were commonly present in all four strains, which included genes that were previously reported to be associated with avian pathogenic *E. coli* (APEC). Finally, the PathogenFinder tool predicted the high pathogenic potential of all four isolates in human.

In conclusion, discrepancies were observed in terms of pathogenicity in embryos and chickens among *E. coli* isolates that contained a similar set of genes. It highlights the importance of the natural host for the determination of the pathogenicity of avian *E. coli* isolates.

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DISPLACEMENT OF A20 AND SERVA INFECTIOUS LARYNGOTRACHEITIS VACCINES FOLLOWING CHALLENGE WITH VIRULENT VIRUS

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We investigated the kinetics of vaccine (A20 and Serva) and wild-type (Class 9) infectious laryngotracheitis virus (ILTV) strains in dust samples after challenge as part of experiments investigating the level of protection provided by variable initial vaccination coverage against the adverse effects of virulent challenge.

Two isolator studies using day-old Cobb broiler chickens were conducted to test the effects of partial vaccination with A20 (Study 1, 15 birds/per isolator) or Serva (Study 2, 10 birds/per isolator). Variable proportions of chickens (0%, 7 or 10%, 20%, or 100%) were eye-drop vaccinated at 7 days of age. Virulent Class 9 ILTV challenge occurred on days 7 and 21 or 25 post-vaccination by the introduction of two ILTV-infected chicks per isolator 4 days after infection. Dust samples were collected within isolators and tested for ILTV DNA using qPCR. ILTV qPCR positive dust samples containing 10^4 ILTV genome copies or higher per µl (n = 110) were submitted to Birling Avian Laboratories for typing to differentiate between vaccine and challenge virus as described by Assen *et al.* (2022a).

Overall, vaccination with the A20 or Serva vaccine provided a high level of protection against the increase in shedding of the Class 9 ILTV following the challenge (Assen *et al.*, 2022b). The virus types identified on different days post-challenge (DPC) are shown in Fig.1.

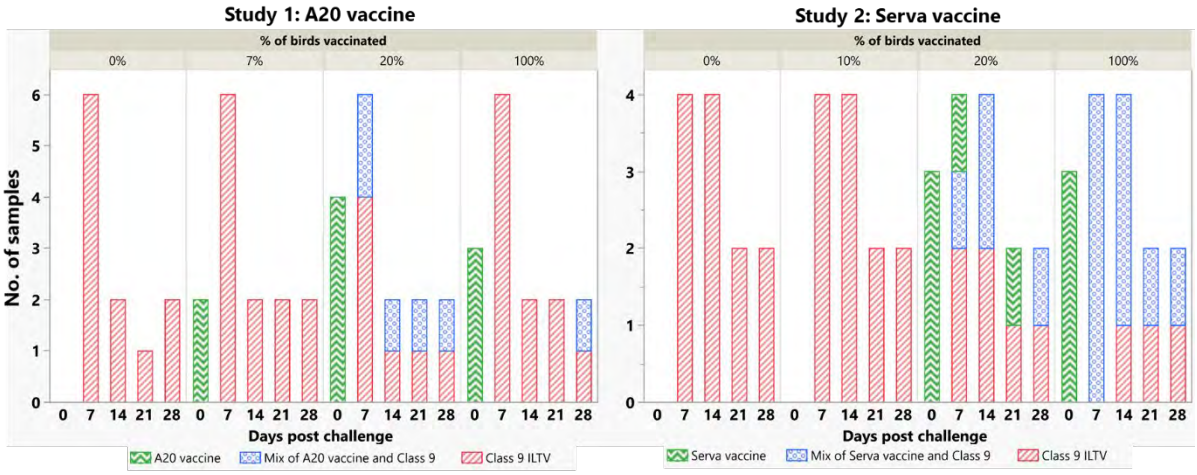

Figure 1 - ILTV strains identified by typing at different DPC in the two variable vaccination experiments.

While both vaccines markedly reduced ILTV shedding post-challenge, the Serva vaccine was detected for longer duration post-challenge than the A20 virus which was rapidly displaced by the virulent virus. This may provide additional opportunity for recombination between the Serva vaccine virus and other circulating strains. The ability to type ILTV in dust samples from chickens with potentially mixed infections (vaccine and wild-type virus) is a further useful epidemiological application of testing dust samples for ILTV DNA.

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INFLUENCE OF PHYTOGENIC SUPPLEMENT ON LAYER PRODUCTION DURING THE PEAK OF HEAT STRESS

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Summary

Phytogenic supplements are developing as an alternative to the use of antibiotics in livestock. Unlike other antibiotic alternatives, where the primary effect is mostly antimicrobial, plant phytogens as synthesised chemicals or natural essential oil products have a range of unexplored beneficial effects, making them popular in alternative medicine for thousands of years. Here we will present a mechanistic evaluation of a phytogen used to supplement layers during the peak of heat stress and Spotty Liver Disease (SLD) outbreak. Phytogenic product improved performance throughout the supplementation period, including during the SLD outbreak, increased the number of ileum goblet cells, reduced microbial functions related to pathogenicity, improved cardiovascular health and altered metabolism towards reduced fat storage.

I. INTRODUCTION

Phytogenic products are used as an antibiotic alternative, with other alternatives like prebiotics, probiotics, organic acids and other products with antimicrobial properties. The ability of phytogens to reduce the load of significant pathogens such as *Clostridium* (2020), *Escherichia* (Zou et al., 2016), and *Salmonella* (Abudabos et al., 2016), and improve the health and performance of chickens makes them increasingly popular. Phytogens also improve critical layer production parameters such as the quality and quantity of eggs and feed conversion ratio (Abou-Elkhair et al., 2018). Other effects reported include phytogen interaction with levels of cholesterol (Abou-Elkhair et al., 2018), gut-brain axis (Bajagai et al., 2021), levels of sex hormones and immune systems readiness (Wang et al., 2021). Despite the reported benefits of phytogenic products, running open-range production systems is still a major challenge for the poultry industry. The phytogenic blend comprised of a mixture of essential oil from the *Myrtaceae* and *Asteraceae* plant families and saponins (Phy) was used in this experiment. The effects of this product have been examined to investigate the health, performance, egg quality and intestinal microbiota composition, and changes in microbiota functional capability via shotgun metagenomics and gene expression via RNAseq under a free-range system at the time of the year corresponding to the highest heat stress and disease outbreak peak. The effects on performance, microbiota and metagenomics functional alterations have been published in the *Applied and Environmntal Microbiology* (Yu et al., 2022b) and the detailed RNAseq analyses on the effects of Phy on ileum gene expression in layers was published in *Antibiotics* (Yu et al., 2022a). Here we will present an overview of the most relevant findings from this study.

II. METHOD

The study was performed on a commercial layer farm in Queensland, Australia uisng Lohman-Brown breed and was approved by the Animal Ethics Committee of Central Queensland University under approval number 0000022879. When the pullets reached 16 weeks of age, they were moved to production sheds with ad libitum access to feed and water and with 20,000 birds each in the treatment and control group.

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The birds were fed a proprietary commercial layer diet as an unsupplemented (Ctr) or with phytogen-supplemented feed (Phy). The two groups were grown in special research sheds with groups housed on separate sides of the shed, divided by the utility room through the middle of the shed and by the fence in the range. There was no physical contact between the Ctr and Phy birds indoors or in the range. The supplementation lasted from week 16 to week 40 of bird age. The aim was to evaluate the efficacy of Phy against Spotty Liver Disease (SLD) reoccurring during the hottest summer period. The supplementation started just before the summer's height, and the birds' sampling was at 30 weeks of age. The birds were sampled at the end of the peak of the lay and precisely at the peak of summer heat, with the maximum daily temperature of 38 ˚C on the sampling day. Birds used in this study were randomly selected from different parts of the shed, rejecting the outlier birds.

At 30 weeks of age, 50 cloacal swabs were collected for microbiota analysis from both control and treatment sheds. Ten birds from each treatment were euthanised, and ileum tissue was collected for RNAseq analysis and histology. Ileum tissue for RNAseq was snap-frozen in liquid nitrogen and stored at −80 ˚C, and for histology, it was stored in 10% neutral buffered formalin. The V3-V4 region of 16S rRNA gene was amplified using forward primer 338F, and the reverse primer 806R. All samples were sequenced in Azenta Live Sciences (China). The alignment of the sequences with the chicken genome and transcriptome analysis was done using CLC Genomic Workbench 21.0.3 (Qiagen, Germany). The differential genes were selected using the DESeq2 R package, and further pathway investigation was done using Ingenuity Pathway Analysis (QIAGEN IPA, version 76765844). Genes included in IPA analysis were DESeq2 $P < 0.05$, with an absolute fold change of 1.2.

III. RESULTS

The SLD outbreak was confirmed at week 37 of bird age when the mortality climbed and birds were diagnosed with the disease. Figure 1 shows some of the most affected performance parameters, and details on performance are available in Yu et al., (2022b). The phytogen consistently reduced mortality and increased the rate of lay and average egg weight even during SLD outbreak.

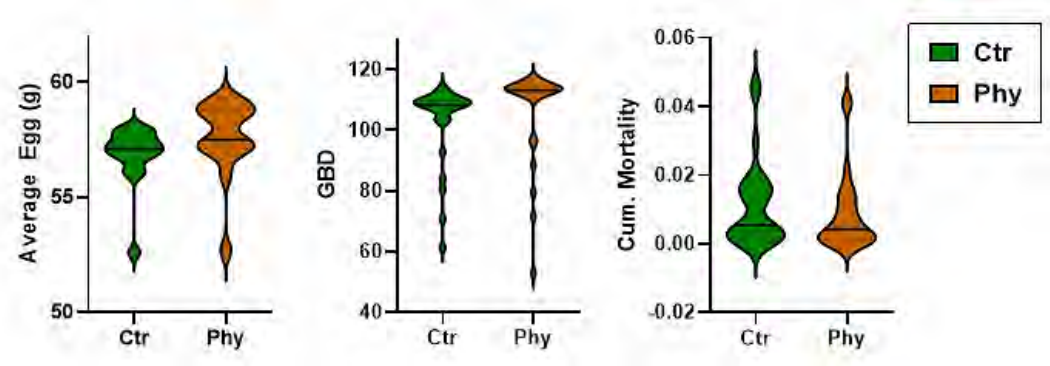

Figure 1 - Average egg mass, average feed intake (GBD = grams/bird/day), and cumulative mortality during the phytogen administration period. Paired weekly data are shown in violin plots.

Cumulative hen-housed eggs were higher throughout the phytogen application resulting in approximately 8,333 additional cartons of dozen eggs or expressed as an egg mass improvement, approximately surplus of 5.8 tons over the supplementation period (Yu et al., 2022b). The phytogen showed protective effects during the SLD outbreak via considerably lower mortality and larger eggs than control birds (Yu et al., 2022b).

Our data indicate that microbiota change targeted the specific pathogenic and growth-related functions across microbial community membership, affecting complete community

functional capabilities. Figure 2 shows the functional diversity of the microbial community (left) and RDA plot (right), representing the relationship between control and treatment functional profiles. Phy-supplemented birds show a more reproducible number of functions compared to variable functional diversity in Ctr.

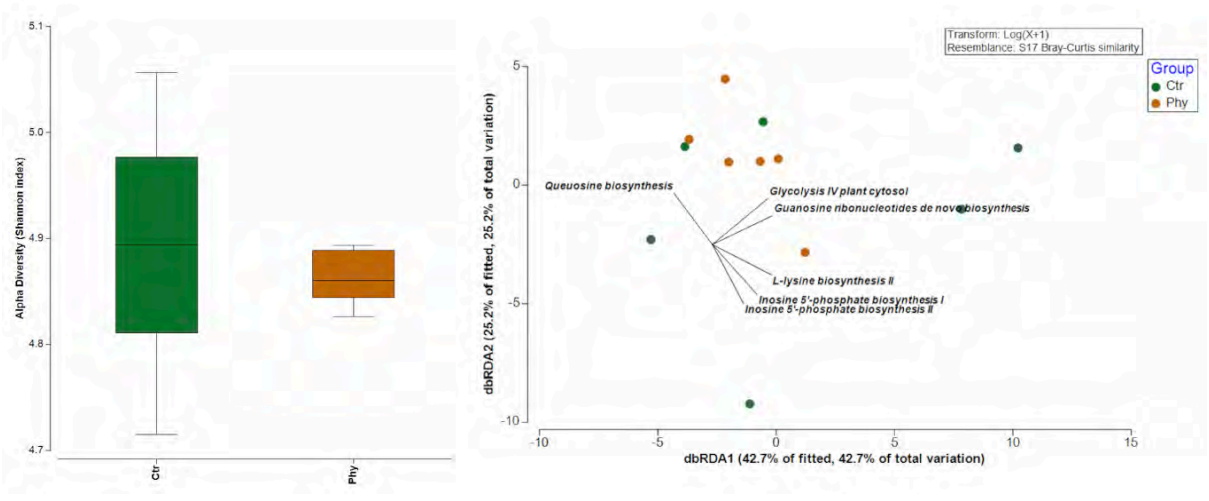

Figure 2 - Functional diversity assessed by Shannon index (left) and redundancy analysis (RDA) plot of gut microbiota functional profiles in control (Ctr) and phytogen (Phy) groups.

Phytogen supplementation had a significant effect on the ileum gene expression. Phy significantly inhibited major cholesterol pathways and altered disease prediction analysis towards improved cardiovascular health, reduced disease susceptibility for 26 cancer categories and improved intestinal health (Yu et al., 2022a). Major metabolic shifts were identified in lipid and carbohydrate metabolism, resulting in a decrease in the Obesity category, higher bird weight, and altering the ratio of fat and carbohydrate metabolism toward lower fat storage (Figure 3). RNAseq functional analysis identified genes involved in increased goblet cell function, which was confirmed using ileum histology (Yu et al., 2022a).

IV. DISCUSSION

Performance data established vital welfare and production-related improvements in a large-scale commercial layer system. Phytogen could not prevent the outbreak of SLD, but cumulative mortalities were constantly lower, and egg production was improved during the disease outbreak. Similarly, performance parameters, including the cumulative hen-housed eggs, and weight and number of individual eggs produced, were better in the phytogen-supplemented flocks, representing enhancements in general healthiness and performance. This is of high implication for the layer industry, where re-emerging of SLD can have serious consequences for bird welfare. *Campylobacter hepaticus* is frequently found in range soil, insects, rodents and wild birds.

Shotgun sequencing of cecal metagenomes shows that a range of essential vitamin, energy, and amino acid production functions was devastated in Phy-supplemented birds' bacterial community. Reducing the diversity of functional abilities have promoted hosts' productivity in heat and disease outbreak. This is well reflected in RNAseq data, especially in disease and function analysis, where the evidence pointed out better cardiovascular and intestinal health (significantly inhibited diseases categories) and, less relevant to poultry, reduced cancer predisposition due to supplementation. Histology confirmed an increase in the number of goblet cells identified via RNAseq disease and function algorithm, which is also highly relevant to overall intestinal health. Interesting rearrangements occurred in carbohydrate

and lipid metabolism leading to slightly heavier yet less fat birds. This outcome is relevant for use in poultry and other livestock where the fat percentage has a role in meat quality scoring.

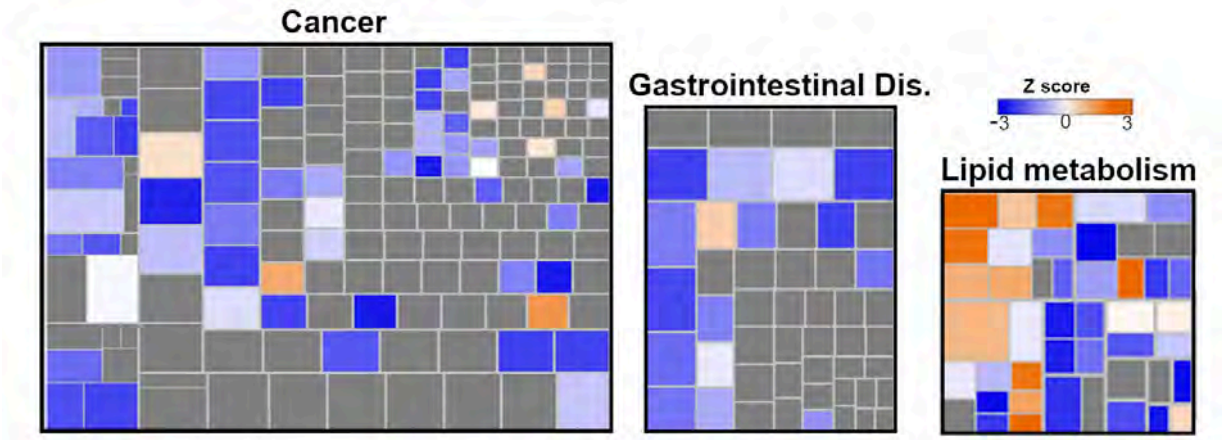

Figure 3 - Disease predisposition heatmap (IPA) coloured by the activation z-score. Orange colour indicates activated and blue inhibited disease of function categories.

Our data displays a wide range of the benefits of the plant phytogen antimicrobials supplementation in poultry and suggests that identifying the precise functional alterations in the microbiota community (loss of pathogenic functions, toxin production or motility in bacteria, for example) via shotgun metagenomics functional analysis, in combination with disease and vital metabolic functions analysis via RNAseq, can be the first step in personalised nutritional supplementation. This can be custom tailored for each farm, or even more precise, specific shed, by choosing a particular phytogenic supplement capable of best addressing particular issues, including shed disease outbreak history or performance issues.

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GUT HEALTH OF BROILERS IN RESPONSE TO DIFFERENT SOURCES AND LEVELS OF COPPER

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Copper (Cu) is a vital element involved in cellular metabolism and enzyme systems. At levels greater than nutritional requirements, dietary Cu addition enhances growth performance as a growth promoter (Pesti and Bakalii, 1996). Copper dosing at levels up to 250 mg/kg feed has been shown to improve intestinal structure and function, and alter the intestinal microbiota profile (Di Giancamillo et al., 2018). However, a high level of copper in the sulphate form ($CuSO_4$) which is chemically reactive, may irritate the gut lining and oxidize fat and other nutrients. Copper in the hydroxychloride form (CH) is comparatively less soluble and reactive than $CuSO_4$ at neutral pH, and presents higher bioavailability.

This study aimed to compare the effects of nutritional and growth-promoting dose of CH with $CuSO_4$, by examining intestinal morphology, caecal microbiota population, and jejunal gene expression. Ross 308 male day-old chicks (n = 864) were fed wheat-soy-based starter (d 0-14) and grower diet (d 14-35). There were eight dietary treatments replicated six times in 48 floor pens: negative control (NC) treatment with no supplemental Cu, 15 and 200 mg/kg Cu from $CuSO_4$ or 15, 50, 100, 150 and 200 mg/kg Cu from CH. On d 14, the pooled caecal contents from three sacrificed birds per replicate were collected for microbial group analysis by real-time quantitative PCR, a 1-cm section of jejunum from each bird was collected for gene expression analysis. Mid jejunum sections were collected for morphology measurement on d 35.

Birds fed diets supplemented with Cu from CH had longer villi compared to those fed $CuSO_4$ supplemented at either level (15 or 200 mg/kg) ($P < 0.01$), reflecting better absorption of nutrients in the intestine. Increasing dietary Cu linearly decreased the population of both beneficial bacteria - *Lactobacillus* ($P = 0.032$), and pathogenic groups - *Bacteroides* ($P = 0.033$) and *Enterobacteriaceae* ($P = 0.028$). Copper supplementation at 200 mg/kg from CH inhibited the growth of *Bacteroides* and *Enterobacteriaceae* compared with the NC diet and 200 mg/kg $CuSO_4$ supplemented diet. CH may have a higher concentration of free Cu-Cu ions at lower parts of the intestine which is soluble and can penetrate into the bacterial cells then change the enzyme activity of the bacteria, leading to bacterial death. Neither copper level nor source had an impact on the mRNA expression of jejunal genes involved in gut integrity (claudin-1, claudin-5, junctional adhesion molecule B, occludin, tight junction protein-1) ($P > 0.05$). This indicates that that the NC diet was not severely deficient in Cu, and a high level of Cu supplementation, up to 200 mg/kg, does not negatively affect gut permeability in broilers.

In conclusion, supplementation of broiler diets with copper at growth-promoting levels (up to 200 mg/kg) in the CH form alters gut microbiota composition but does not negatively affect gut integrity. Supplementation with copper from CH could be beneficial over $CuSO_4$ at improving intestinal morphology.

ACKNOWLEDGEMENTS: This study was funded by Trouw Nutrition, a Nutreco company.

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BACILLUS AMYLOLIQUEFACIENS CECT 5940 ALLOWED MAINTAINING OF PERFORMANCE AND ACCELERATED MICROBIOTA MATURATION OF BROILERS FED ALTERNATIVE INGREDIENTS

N. YACOUBI[1], M. KLUENEMANN[1], C. ADAMS[2] and C. STEFANELLO[2]

Increasing prices and low availability of common feed raw materials resulted in the use of alternative ingredients in animal production. These ingredients are often characterised by a high amount of soluble fibre resulting in lower performance and intestinal health issues that limits their inclusion rate in the diet. *Bacillus amyloliquefaciens* CECT 5940 (Ba) is a probiotic that can produce various metabolites and enzymes relieving the negative effects of soluble fiber and therefore it allows a higher inclusion rate of these ingredients resulting in superior economic success. An experiment was conducted to evaluate the effects of 2 alternative ingredients (rye and rice bran) in interaction with the Ba in diets for broilers until day 35 of age. The effect on growth performance and intestinal microbiota was investigated. A total of 672 one-day-old male Cobb 500 slow feathering broilers were used.

Dietary treatments were distributed in a 2 x 2 factorial arrangement, 6 pens of 28 broilers. A corn-soy basal diet was formulated with an inclusion rate of 10, 15, and 20% in starter, grower, and finisher diets of rye or defatted rice bran with and without supplementation of the Ba (1,000 g/ton). Growth performance was evaluated per week and from days 1 to 14, 1 to 21, 21 to 35, and 1 to 35. No interaction was observed between the ingredient and the probiotic. Birds fed the diet with rice bran had a higher BW (+4%) and FCR (-5.5%) compared to rye and probiotic supplementation improved BWG (+ 3.4%) and FCR (-5.2%) compared to non-supplemented groups ($P < 0.05$). Caecal microbiota composition was determined at days 7, 14, 21 and 35 using 16S amplicon sequencing. The 16S data were processed using QIIME and analyzed using R3.6.3 with base and vegan libraries. Species richness was positively correlated with age and with probiotic supplementation. The differential abundance analysis showed that the relative abundance of the *Clostridiales* order mainly the *Lachnospiraceae* family decreased, and the *Bacteroidales* order mainly the *Rikenellaceae* family increased with age. *Faecalibacterium* increased significantly until day 21, then it decreased again on day 35. The *Alistepes* genus had a positive correlation with time having an abundance between 0.65 and 1.4% when broilers were fed diets not supplemented or supplemented with the probiotic, respectively, and reaching 20.05 and 27.75% on day 35, respectively. On day 7, it was observed that groups supplemented with probiotic had a comparable microbiota composition to the group without probiotic in day 14, indicating that the probiotic may have accelerated the maturation of the microbiota during the early stages. The birds fed rye showed a significantly lower abundance of the *Lachnospiraceae* compared to the birds fed the rice bran diet on days 7 and 14.

In conclusion, these results confirmed that the probiotic *Bacillus amyloliquefaciens* CECT 5940 resulted in improved growth performance of broilers fed alternative ingredients such as rye and rice bran. Furthermore, maturation of the cecal microbiota in broilers is mainly driven by time and then affected by the feed ingredients and the probiotic.

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POTENTIAL OF ENZYMES TO IMPROVE PERFORMANCE AND HEALTH OF BROILERS UNDER A MILD COCCIDIAL VACCINE CHALLENGE

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Enzymes have shown promising effects on performance and intestinal health in broilers (Toghyani *et al*., 2022). However, the dosage effect of enzymes and combination of different enzymes have not been extensively evaluated under the coccidial vaccine challenge condition. Therefore, a feeding study was conducted to examine the effect of dosage of xylanase+glucanase and the effect of its combination with a high level of phytase on growth performance, health, and welfare of broilers under a mild coccidial vaccine challenge. A total of 640 d-old Cobb 500 broiler chicks of mixed sex were assigned to 40-floor pens with eight replicates of 16 birds per dietary treatment. The five treatments were: unchallenged birds fed a basal diet containing 500 FTU/kg phytase (UC); challenged birds fed a basal diet containing 500 FTU/kg phytase (CC); CC + xylanase and glucanase at 100 g/t feed (XG100); CC + xylanase and glucanase at 200 g/t (XG200); CC + xylanase and glucanase at 100g/t and an additional 500 FTU/kg phytase, resulting in total phytase of 1000 FTU/kg (XG100+PHY) in starter, grower and finisher phases. Birds were fed pelleted feed and diets were based on wheat, soybean meal, barley and rye and formulated to meet breed standards considering the matrix values of phytase at 500 FTU/kg. The additional enzymes were added on top during feed mixing. Birds were given 1 mL/bird *per os Eimeria* spp. vaccine consisting of *E. acervulina* 5000, *E. maxima* 5000 and *E. brunetti* 2500 oocysts on d 9. The sex of the sampled birds was determined following the method described by England *et al*. (2021). The measured and analysed parameters were bird performance (d 0-8, 9-19, 20-35 and 0-35), where the female percentage was used as a covariate, excreta *Eimeria* spp. oocyst counts on d 14, intestinal lesions on d 16, litter moisture on d 17 and 35, and footpad dermatitis score (FPD) on d 35.

Reduced average weight gain (AWG), feed intake, increased FCR, *Eimeria* spp. oocyst counts, duodenal lesion scores and litter moisture observed in the CC group (d9-19; $P < 0.05$) indicates a successful coccidial vaccine challenge of the birds. XG100 enhanced ($P < 0.05$) AWG on d 20-35 and d 0-35 compared to the CC group. XG100 lowered ($P < 0.05$) FCR on d 0-8, d 9-19, d 20-35 and d 0-35 compared to the CC group. XG100 reduced ($P < 0.05$) litter moisture content compared to the UC group and decreased ($P < 0.05$) FPD scores compared to the CC group on d 35. Birds fed XG100 had similar ($P > 0.05$) performance, FPD and litter moisture compared to XG200 and XG100+PHY, except for FCR on d 9-19 and FPD on d 35 whereby birds fed XG100+PHY had a higher ($P < 0.05$) FCR and FPD compared to the XG100 group. In addition, birds fed XG200 had a higher ($P < 0.05$) FPD on d 35 compared to the XG100 group. These findings suggest that dietary supplementation of XG100 on a basal diet based on wheat-barley-rye-soybean meal and containing phytase (500 FTU/kg) may enhance performance by increasing AWG and reducing FCR, and improve welfare by decreasing litter moisture content and FPD of birds under a mild coccidial vaccine challenge. However, increasing doses of the enzymes to XG200 and 1000 FTU/kg PHY may not additionally improve bird performance and health compared to XG100.

ACKNOWLEDGMENTS: The authors would like to thank BASF SE Germany, Poultry Hub Australia, and Eimeria Pty. Ltd., Australia, for supporting this project.

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EFFICACY OF A PHYTOGENIC FEED ADDITIVE IN BROILERS CHALLENGED WITH SUBCLINICAL NECROTIC ENTERTIS

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Summary

The objective of this study was to investigate the effect of a phytogenic feed additive (PFA) product on performance and intestinal gene expression in chickens under subclinical necrotic enteritis (NE) challenge. The three treatments of this study were; 1) non-challenged birds - no additive, 2) NE challenged birds - no additive, 3) NE challenged- PFA (100g/T). Results indicated that the NE challenged birds had reduced WG and increased FCR ($P < 0.001$) compared to the non-challenged birds. The PFA supplementation reduced FCR ($P < 0.001$) and downregulated the expression of CASP3 ($P < 0.001$), compared to the challenged birds fed with diets containing no additives. These findings suggest that supplementation of this PFA product can help to improve performance and gut health in broilers under a subclinical NE challenge.

I. INTRODUCTION

Necrotic enteritis (NE) in broiler chickens is an economically important disease which can be controlled by antibiotics (Timbermont, et al., 2011). The occurrence of necrotic lesions in the small intestine is associated with proliferation of *Clostridium perfringens*, which leads to lower growth rate in subclinically infected chickens. Increasing concerns regarding antibiotic resistance and the presence of drug residues in animal products have led many countries to ban the use of antibiotic growth promoters which has therefore triggered a search for viable alternatives to antibiotics in the animal. Phytogenic feed additives (PFAs) are plant derived extracts that have been investigated extensively as alternatives to antibiotic growth promoters due to their strong antibacterial activity, antioxidant capacity and their beneficial impact on health and performance in broiler production systems (Delaquis, et al., 2002; Paraskeuas, et al., 2017). PFAs are categorized as sensory and flavoring compounds, which consist mainly of plant extracts (essential oils, oleoresins, and flavonoids) and their active principles. Essential oils present in PFA, which contain most of the active substances of the plant, have been shown to improve gut health by modulating the intestinal microbiota and effecting intestinal gene expression and animal performance (Paraskeuas, et al., 2017, Paraskeuas and Mountzouris, 2019). Supplementation of essential oils to poultry has been shown to stimulate the production of endogenous enzymes such as lipase and amylase (Platel and Srinivasan, 2000). The PFA used in this study is a blend of essential oils including carvacrol. Carvacrol has strong anti-inflammatory and antibacterial activity and has previously shown to reduce *C. perfringens* load in NE infected broilers (Liu, et al., 2016). We aimed to evaluate and compare the effects of a PFA product, a blend of essential oil (EOs) on growth performance and intestinal gene expression in broiler chickens subjected to a subclinical necrotic enteritis challenge.

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II. METHOD

A total of 384 d-old Cobb 500 chicks (as hatched) were vent sexed, and 16 birds (8 males and 8 females) were randomly allocated to floor pens covered in wood shavings. The experiment consisted of three treatments, with eight replicates per treatment. The three treatments were: 1) non-challenged birds - no additive, 2) NE challenged birds - no additive, 3) NE challenged - PFA. The dietary dose of PFA (Activo®, supplied by EW Nutrition) was 100 g/T. All diets were formulated based on Cobb 500 nutrient specifications (Cobb-Vantress, 2018). Diets were based on wheat and soybean meal and were fed *ad libitum* for the duration of the trial period. The PFA product used in this study is a mixture of different phytomolecules, including carvacrol. The experiment was performed with three periods: starter (d 0-10), grower (d 11-24) and finisher (d 25-35). On d 9, birds in the challenged groups were subjected to an oral gavage of 1 ml *Eimeria* species (*E. acervulina* at 5,000 oocytes/mL, *E. maxima* at 5,000 oocytes/mL, *E. brunetti* at 2,500 oocytes/mL). The birds in the non-challenged group were inoculated with 1 ml of PBS. On days 14 and 15, birds in the challenged treatments were inoculated with approximately 10^8 CFU of *Clostridium perfringens* NE18 strain, and control birds were inoculated with 1mL of sterile thioglycollate broth. Average body weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) were calculated considering the mortality. Performance data were analysed for the treatment effect with male percentage (corrected to dead birds) as a covariate. On d 16, two birds from each pen were humanely euthanised and jejunum tissue was collected for gene expression analysis. For each sample, total RNA was extracted after homogenization in TRIsureTM (Bioline, Sydney, Australia) following the manufacturer's instructions. The quantity and purity of the samples were measured with NanoDrop ND-8000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA), and the RNA Nano 6000 kit was used to measure RNA integrity on the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Waldron, Germany). The extracted RNA of each sample was reverse transcribed with the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Amplification and detection were carried out in duplicates using an SYBR Green kit SensiFAST SYBR No-ROX (Bioline, Sydney, Australia) with Rotorgene 6000 real-time PCR machine (Corbett Research, Sydney, Australia). The data were analysed with SPSS software Version 28 using oneway-ANOVA and the significant differences between treatments were examined with Tukey's HSD post hoc test.

III. RESULTS

As illustrated in Table 1, during day 0-35 non-challenged birds presented significantly higher WG and lower FCR compared to challenged groups. However PFA supplementation improved FCR and WG significantly ($P < 0.001$) compared to the challenged birds fed with non-supplemented diets.

Table 1 - Effect of phytogenic additive on the performance (day 0-35) and day 16 jejunal lesion score of broilers subject to subclinical necrotic enteritis challenge.

NE	additive	Day 0-35		
		WG	FI	FCR
Non-challenged	No additive	2616 [a]	3700	1.414 [c]
Challenged[1]	No additive	2303 [c]	3594	1.561 [a]
Challenged	PFA	2414 [b]	3636	1.506 [b]
P-value		< 0.001	0.278	< 0.001

[1]*Clostridium perfringens* strains (10^8 CFU/mL), WG: Weight gain, FI: Feed intake, FCR: Feed conversion ratio, PFA: phytogenic feed additive, 100g/T. [a–c] Means sharing the same superscripts are not significantly different from each other at $P < 0.05$.

Jejunal gene expression is presented in Table 2. Challenged birds fed with non-supplemented diets showed a significantly higher expression (P= 0.002) of CASP3 compared to other groups. Addition of PFAs in the diet reduced the expression of CASP3 in the challenged birds indicating alleviated damage to the intestine. Furthermore, the non-supplemented challenged birds presented a significantly lower expression of OCLN (P < 0.001), SOD1 (P = 0.018) and APN (P= 002) compared to the non-challenged birds.

Table 2 - Effect of phytogenic additive on the jejunal expression level of broilers subject to subclinical necrotic enteritis challenge on day 16

NE	additive	Gene expression level day 16			
		CASP3	OCLN	SOD1	APN
Non-challenged	No additive	0.823 [b]	1.786 [a]	1.275 [a]	1.311 [a]
Challenged[3]	No additive	1.844 [a]	0.645 [b]	0.884 [b]	0.843 [b]
Challenged	PFA	1.127 [b]	0.980 [b]	1.002 [ab]	1.019 [b]
P-value		0.002	< 0.001	0.017	0.002

[1] *Clostridium perfringens* strains (10^8 CFU/mL). CASP3: Caspase 3, OCLN: Occludin, SOD1: Superoxide dismutase 1, APN: Aminopeptidase N, PFA: Phytogenic feed additive, 100g/T

[a–c] Means sharing the same superscripts are not significantly different from each other at $P < 0.05$.

IV. DISCUSSION

This study investigated the responses of NE challenged broilers supplemented with a PFA product, and compared their responses to non-challenged and NE challenged birds which were fed with basal diet or diets supplemented with PFAs. In the present study, a successful subclinical NE infection was introduced in challenged groups, as typical signs such as impaired FCR and BW were observed in the challenged birds (M'Sadeq, et al., 2015). The effect of the challenge can also be observed with the downregulated expression of OCLN, an important protein in the tight junction complex, implying compromised gut health by the challenge. The PFA supplemented group showed an improved FCR compared to the challenged birds fed with control diets. Furthermore, weight gain and expression of genes related to cell death (CASP3) and antioxidant capacity (SOD1) in the PFA supplemented birds did not differ from the non-challenged treatments suggesting a positive effect of PFA in maintaining the health of the intestinal epithelial cells. Phytogenic feed additives are plant compounds that can stimulate appetite and endogenous secretions, and have antimicrobial and coccidiostatic activities in monogastric animals (Wenk, 2003). Previous studies have shown the positive effect of carvacrol on the upregulation of tight junction proteins in broilers under lipopolysaccharide challenged (Liu, et al., 2020). Although we have not observed a significantly higher expression of these genes in the PFA supplementation groups, the numerically higher expression of the tight junction gene (occludin, OCLN) could be a sign of a moderately improved gut environment and lower damage to the epithelium cells creating a stronger tight junction bond between cells. The downregulation of genes related to cell death (caspase 3, CASP3) observed in the PFA treated birds, could be an indication of a healthier epithelial lining. Similar results have been reported by Mousavi, et al. (2020), where carvacrol, reduced CASP3 expression in the intestine of *Campylobacter* infected mouse. The beneficial effects of the PFA on broiler performance observed in the study could be attributable to improved gut health conditions. It is not yet clear if this PFA is affecting *C. perfringens*, Eimeria or both of these disease agents. However, it can be concluded that supplementation of this PFA in diets can help improve performance through modulation of gut health in broilers under subclinical NE challenges.

ACKNOWLEDGMENTS: We acknowledge EW Nutrition GmbH© for funding this project, Bioproperties for providing Eimeria, and Prof. Robert Moore for providing *Clostridium perfringens* EHE-18 strain. This project was co-funded by AgriFutures and managed through Poultry Hub Australia.

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FROM *IN VITRO* TO *IN VIVO*: DEVELOPING A NEW PROBIOTIC WITH MULTI-PATHOGEN ACTIVITY

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Summary

In this paper, a concurrent *in vitro* and *in vivo* screening process to develop a new probiotic product is described. In the *in vitro* screening, pathogenic *E. coli* strains and several *Salmonella* strains from various locations were included. The inhibitory effect of the probiotic strains was measured using growth inhibition kinetics. Next to the inhibitory effect, other qualitative parameters of the potential probiotic organisms were tested, such as pH and heat tolerance, cytotoxicity, absence of antimicrobial resistance (AMR) genes and production yield. At the same time, the best performing strains were also subjected to a range of *in vivo* screening trials in broilers. In these trials, the broilers were infected with an oral gavage of either avian pathogenic *E. coli* or *Salmonella* Heidelberg. In addition, the efficacy against *Clostridium perfringens* by the new probiotic was also assessed. From the combined results from the *in vitro*, *in vivo* and qualitative screening, a final three-strain probiotic product was developed, consisting of *Bacillus* sp. PB6 and two other *Bacillus* spp. (FXA and G3). The new strains were able to reduce the growth of both *E. coli* and *Salmonella* in the kinetic studies, reduce mortality and performance losses due to avian pathogenic *E. coli* and reduce *Salmonella* load in the ceca of broiler chickens. In addition, the inclusion of *Bacillus* sp. PB6 in the new product also resulted in the prevention of mortality and intestinal lesions due to necrotic enteritis. Furthermore, the strains were shown to be tolerant to intestinal pH conditions and to high temperature conditions, they were non-cytotoxic, showed no presence of AMR genes and had a good production yield, showing their potential as probiotics.

I. INTRODUCTION

Continuous interest in new probiotics to alleviate intestinal health problems is emerging, especially since antibiotics become more restricted in use. Various *Bacillus* spp. are known to improve broiler performance and health due to their ability to produce antimicrobial substances, modulate the immune system, change the intestinal microbiota or increase nutrient digestion and utilization (Lee *et al.*, 2010; Caulier *et al*., 2019; Giurescu *et al.*, 2020; Jha *et al*., 2020; Zaghari *et al*., 2020). Several years ago, the probiotic organism *Bacillus* sp. PB6 was isolated from the intestinal tract of healthy chickens, and it was shown that this strain has an effectivity against *Clostridium perfringens* (Teo and Tan, 2005; Abudabos et al, 2013). During the past years, an extensive *in vitro* and *in vivo* screening process took place to find new strains to add together with this *Bacillus* sp. PB6, in order to extend its target range from only *Clostridium* to Enterobacteriaceae like *E. coli* and *Salmonella*. The current paper will describe a selection of these trials.

II. METHOD

In vitro trials. In the *in vitro* screening, 23 pathogenic *E. coli* strains from several locations (US and Asia) and 10 *Salmonella* strains (US and Asia, serovars Enteritidis, Typhimurium,

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Worthington, Pullorum, Gallinarum, Choleraesuis and London) were included. The inhibitory effect of the probiotic strains was measured using growth inhibition kinetics, where the pathogen culture was incubated with the probiotic cell free supernatant for 20 hours at 37°C and the optical density was measured at 600 nm every 20 minutes. Next to the inhibitory effect, also other qualitative parameters of the potential probiotic organisms were tested, such as pH (pH 3 and 6) and heat tolerance (pelleting at 80°c and 90°C), cytotoxicity to Vero cells, absence of antimicrobial resistance (AMR) genes (checked by full genome sequencing) and production yield (small scale fermentation).

In vivo Trial 1. *E. coli* challenge trial. In total, 100 one-day-old commercial broiler chickens (Ross-308) were divided over five isolators under negative pressure (20 birds/isolator). Each group of birds in an isolator received a different diet: (1) multi-strain probiotic dose 1, $3x10^8$ CFU/kg feed, (2) multi-strain probiotic dose 2, $3x10^9$ CFU/kg feed, (3) single-strain probiotic Bacillus sp. PB6, $3x10^8$ CFU/kg feed, (4) not supplemented, challenged control, (5) not supplemented, not challenged control. At 14, 16 and 18 days of age, birds in group 1-4 were infected with $4.5\text{-}5.7x10^8$ CFU avian pathogenic *E. coli*. At 21, 28 and 35 days of life, 5 birds from each group were killed for necropsy and bacteriological investigation. *E. coli* was counted using MacConkey agar plates. Feed consumed by birds in each group and body weight of all the birds were measured at weekly intervals and the body weight gain and FCR were calculated.

In vivo trial 2. *Salmonella* challenge trial. 240 one-day-old male Ross 708 broilers were divided over 2 groups, housed in cages (12 birds/cage; 10 cages/treatment): (1) challenged control, (2) multi-strain probiotic $3x10^8$ CFU/kg feed. At day 7, the birds were challenged with 3.7×10^6 nalidixic acid-resistant *Salmonella* Heidelberg. At the end of the trial, at 42 days of age, 10 birds per treatment were taken from each individual cage (in total 100 samples per treatment), euthanized and the ceca were aseptically removed and the presence of *Salmonella* was checked.

In vivo Trial 3. *Clostridium perfringens* challenge trial. 336 one-day-old male Cobb 500 broilers were divided over 3 groups, housed in cages (8 birds/cage; 14 cages/treatment) : (1) not supplemented, not challenged control, (2) not supplemented, challenged control, (3) multi-strain probiotic $3x10^8$ CFU/kg feed. At day 14, the birds were challenged with coccidia (5000 oocysts of *Eimeria maxima*) and at 19, 20 and 21 days of age with 10^8 CFU *Clostridium perfringens*. At the end of the trial, at 28 days, the lesion scoring due to necrotic enteritis was assessed, as well as general performance and mortality.

III. RESULTS

In vitro trials. The new strains were able to reduce the growth of both *E. coli* and *Salmonella* in the respective kinetic studies. One of the obtained results can be found in Figure 1. Furthermore, the strains were shown to be tolerant to intestinal pH conditions and to high temperature conditions, they were non-cytotoxic, showed no presence of AMR genes and had a good production yield, showing their potential as probiotics.

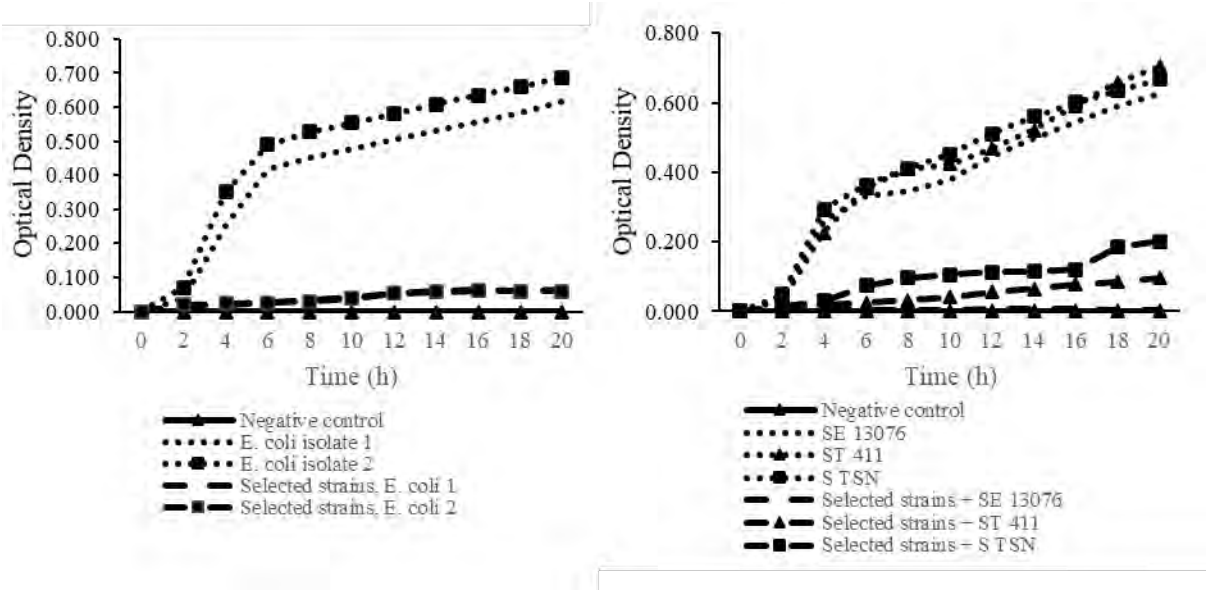

Figure 1 - Effect of probiotic supernatant (mixture of the two new *Bacillus* strains FXA and G3) on *E.coli* (left, avian pathogenic field isolates) and *Salmonella* (right, SE: *Salmonella* enteritidis, ST: *Salmonella* thyphimurium, TSN: unknown field isolate) growth in an inhibition kinetic experiment.

In vivo trials. In the *E. coli* challenge trial, a significant decrease in cecal *E. coli* counts, compared to the infected control could be observed in the groups treated with both dosages of the multi-strain probiotic at 21 and 35 days of age, and for the highest dosage at day 28 (Figure 2). In contrast, this significant reduction in cecal *E. coli* counts was not observed with the single strain probiotic product. In addition, the highest body weight gain and lowest FCR during the last week of the trial was noted for group 2 (multi-strain probiotic dose 2).

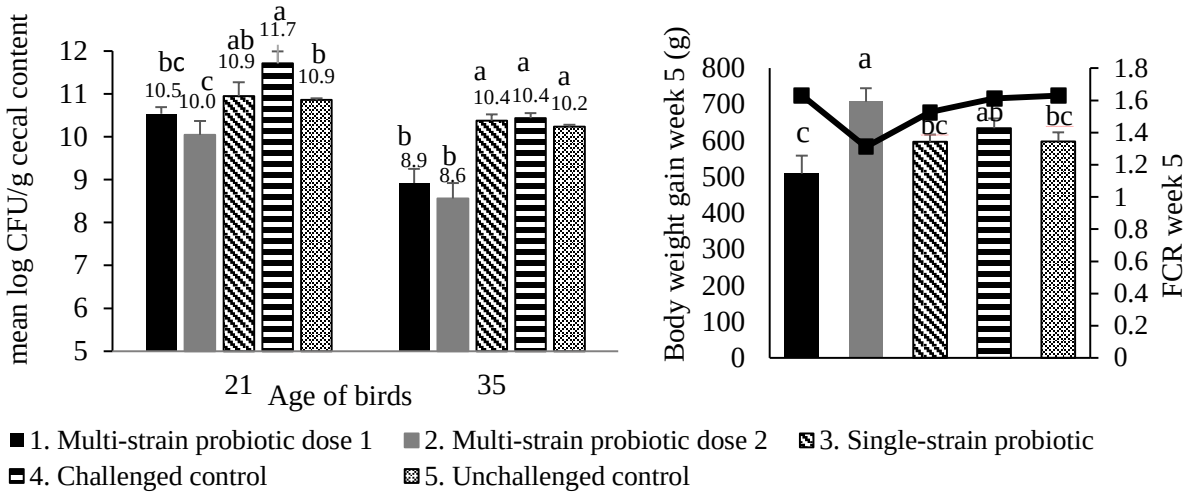

Figure 2 - Effect of different treatments on the counts of *E. coli* in the cecum of broiler chickens (CFU/g) (left) and on broiler performance (right). Error bars show the standard error of the mean. Bars with different superscripts indicate significant differences ($P < 0.05$).

In the *Salmonella* challenge trial, the probiotic treatment numerically reduced the number of Salmonella positive caeca at the end of the trial whereas in the *Clostridium* challenge trial, the probiotic treatment resulted in significantly lower lesion scores and mortality (Table 1).

Table 1 - Selection of results from the *Salmonella* and *Clostridium perfringens* challenge trials. Numbers with different superscripts within one column indicate significant differences (P < 0.05).

Challenge	Treatment	Endpoints	
Salmonella		No. Positive caeca (%)	
	Challenged control	48.5	
	Multi-strain probiotic	36.1	
Clostridium		Lesion Score	Mortality
	Unchallenged control	0.0 c	0.0 c
	Challenged control	2.0 a	19.6 a
	Multi-strain probiotic	1.1 b	6.3 b

IV. DISCUSSION

The results showed that the new strains were able to reduce the growth of both *E. coli* and *Salmonella* in the kinetic studies. This observed effect can be explained by antimicrobial substances produced by the probiotic strains, such as lipopeptides and polyketides (Caulier *et al.*, 2019). In addition, the strains were tolerant to intestinal pH conditions and to high temperature conditions, were non-cytotoxic, showed no presence of AMR genes and had a good production yield, all important characteristics of in-feed probiotics. The newly developed multi-strain probiotic was also able to reduce mortality and performance losses due to avian pathogenic *E. coli*, reduce *Salmonella* load in the ceca of broiler chickens and prevent mortality and intestinal lesions due to necrotic enteritis. The effect against *Clostridium perfringens* has been shown before for the *Bacillus* sp. PB6 (Teo and Tan, 2005; Abudabos et al., 2013), and this effectivity was maintained with this multi-strain probiotic. The ability of the *Bacillus* strains to affect Enterobacteriaceae, such as *E. coli* and *Salmonella* is in accordance with previous literature (Amerah *et al.*, 2013; Thirabunyanon and Thongwittaya, 2011; Shanmugasundaram *et al*., 2020;) and can be explained by antimicrobial substances formed, as shown in the *in vitro* study, and in addition, the probiotic strains might also favor a more beneficial development of the intestinal microbiota, modulate the immune system and improve nutrient digestibility, as explained in the introduction.

In conclusion, this research describes the development process of a new multi-strain probiotic targeting multiple potential pathogens at once.

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OPTIMAL ENERGY DENSITY AND A BALANCE BETWEEN ESSENTIAL AND NON-ESSENTIAL AMINO ACIDS IN REDUCED PROTEIN DIETS CAN RESTORE BIRD PERFORMANCE

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Feeding meat-chicken with reduced crude protein (CP) diets commonly reduces N release in the environment. However, this is often associated with poor performance and a heightened body fat content due to a high energy-to-protein ratio. Reducing feed CP content affects all amino acids (AA), but the most limiting essential AA (EAA) are commonly considered during feed formulation. It was realised that a chicken body has a constant composition in EAA and non-EAA (NEAA), or EAA-to-true protein (EAA:TP) ratio, whereby TP = EAA + NEAA (Heger, 2003). Therefore, feeding chickens with reduced CP-diets added with some EAA without considering all AA content may cause imbalanced EAA:TP, leading to the N utilisation and growth rate impairment (Pesti, 2009).

The current study examined the impact of AA balance and energy density in reduced-CP diets on nutrient utilisation and performance of Cobb 500 broilers. A 2 × 2 × 2 factorial arrangement of treatments was used, with factors including CP (16% and 18%), net energy (NE, 9.9 and 10.4 MJ/kg) and EAA:TP ratio (0.56 or 0.60). Thus, 8 finisher diet treatments were formulated and tested into 8 replicates, with 16 mixed sex birds per replicate. All diets were formulated to meet Cobb 500 (2018) specifications. Crystalline NEAA supplement was used to lower EAA:TP ratio. The TP contribution of each feed ingredient (other than purified AA) was estimated using a specific N-to-protein conversion factor, also known as K_A. The K_A value of 6.25 was used for all purified AA used in feed formulation (Pesti, 2009). Birds were fed diets in three phases; starter (d0-8), grower (d9-18) and finisher diets as the treatments (d19-35). Birds and feeds were weighed on d19, 28 and 35. On d35, 4 birds (2 males and 2 females) per pen were sampled to evaluate carcass quality. All data were analysed using JMP software, with gender as a covariate for the performance data.

Energy content or EAA:TP did not significantly ($P > 0.05$) affect animal performance when CP was 18%. Moreover, applying 10.4 MJ/kg NE and 0.60 EAA/TP ratio in a 16% CP diet removed the difference ($P > 0.05$) in bird performance (weight gain, feed intake and feed conversion ratio) compared to the 18% CP diets. However, reducing CP content from 18% to 16% increased ($P < 0.001$) body fat pad for all treatments, regardless of energy and EAA/TP levels. These results show that balancing all amino acids in 16% CP diets, with an energy content of about 10.4 MJ/kg can restore bird performance, but further studies are needed to validate these findings.

ACKNOWLEDGEMENTS: This study was funded by Adisseo France in partnership with AgriFutures Australia and Poultry Hub Australia.

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VERSATILITY OF DIETARY ZINC LEVELS FROM ZINC HYDROXYCHLORIDE IS BENEFICIAL IN BROILER CHICKEN PRODUCTION

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Zinc is an essential trace mineral in broiler nutrition. However, in commercial practice it is often fed above requirements to prevent any deficiencies. To prevent deficiencies, Zinc hydroxychloride (HTM) (IntelliBond®, Trouw Nutrition, Netherlands) provides versatility to optimise the producer's trace mineral program and allow them to feed levels lower than those commercially used today in the form of sulfate trace minerals (STM).

Raw data of nine studies was combined into one dataset to be used in the meta-analysis. All nine studies included a comparison between HTM and STM fed at 80 mg/kg and/or 20 mg/kg Zn. All studies were performed in Europe in experimental conditions mimicking commercial practice by using a wheat-based diet, high stocking density, and/or environmental challenges. The meta-analysis was conducted in SAS using PROC MIXED for growth performance and PROC GLIMMIX for carcass yields. Three comparisons were made between 80 mg/kg Zn from HTM (HTM80) and STM (STM80), between 20 mg/kg Zn from HTM (HTM20) and STM (STM20), and between HTM20 and STM80.

The results show that, at current commercial levels, HTM80 outperformed the STM80 group, resulting in a higher BW (2689.7 g for HTM80 vs 2664.8 g for STM80, P=0.0374), and ADG (70.4 g/bird/day for HTM80 vs 69.6 g/bird/day for STM80, P=0.0294). Similar results were obtained when Zn levels were reduced to 20 mg/kg. At lower levels, HTM outperformed STM resulting in a higher BW (2695.1 g for HTM20 vs 2654.5 g for STM20, P=0.0245), ADG (70.5 g/bird/day for HTM20 vs 69.4 g/bird/day for STM20, P=0.0249), ADF (97.9 g/bird/day for HTM20 vs 96.3 g/bird/day for STM20, P=0.0413) and breast meat yield (27.1% for HTM20 vs 26.6% for STM20, P=0.0202). Comparing 20 mg/kg Zn from HTM to the commercially used dosage of 80 mg/kg STM, resulting in a tendency towards a higher BW (2695.1 g for HTM20 vs 2664.8 g for STM80, P0.0694), ADG (70.5 g/bird/day for HTM20 vs 69.6 g/bird/day for STM80, P=0.0674) and ADFI (97.9g/bird/day for HTM20 vs 96.5 g/bird/day for STM80, P=0.0531), while breast meat yield was significantly improved compared to 80 mg/kg Zn fed as STM (27.1% for HTM20 vs 26.7% for STM80, P=0.0169).

Although no significant difference in mineral depositions could be found in the animals (Olukosi et al., 2018), the above obtained results indicate a difference in mode of action between HTM and STM. Another study looked at the effects of HTM and STM on the microbiota in the ileum and caecum (van Kuijk et al., 2021). These authors showed a modulation of the microbiota by HTM which was most visible in the caecum between HTM20 and HTM80. This corresponded with significantly higher levels of Zn in the caecum in the HTM80 group compared to the HTM20 group. Although faecal excretion was not measured, the latter results indicated that feeding lower levels in the form HTM not only can be beneficial for the growth performance, but also for the environment.

From these results it can be concluded that feeding HTM resulted in a better growth performance, at commercial Zn levels but also at lower Zn levels.

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NUTRITIONAL EMULSIFIERS AND THEIR EFFECT ON BROILER PERFORMANCE, A BENCHMARK STUDY

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Usage of nutritional emulsifiers in animal feed offers a valid strategy to improve energy, fat and protein digestibility. There are numerous nutritional emulsifiers currently on the market containing a range of active ingredients. Most emulsifiers contain phospholipids or lysophospholipids (LPL), present in lecithin or lysolecithin. An alternative active ingredient is glyceryl polyethylene glycol ricinolate (GPGR). It is important to know the hydrophilic-lipophilic balance (HLB) value of these active ingredients, as this provides crucial information on the activity of the nutritional emulsifier in a specific environment. A nutritional emulsifier with a high HLB value will be more potent to making fat in water emulsions (e.g. fat emulsification in the gastro-intestinal tract), and this will be reflected in the performance of the animal. The objective of this study was to evaluate the effects of three different nutritional emulsifiers on performance in broilers.

This trial was conducted at the Flemish research institute ILVO in Belgium. A total of 1080 one-day old male Ross 308 chicks were divided into four treatments, with 9 replicates of 30 birds per pen per treatment. The treatments were T1-control, T2-GPGR^{A} (Excential Energy Plus, supplied by ORFFA Additives BV; 350 ppm supplemented 'on-top'), T3-LPL (500 ppm supplemented 'on-top') and T4-GPGR^{B} (500 ppm supplemented 'on-top'). All tested nutritional emulsifiers had a different HLB value, with the LPL nutritional emulsifier having the lowest. Diets were fed in pelleted form and were wheat-based. A challenge was provided to all the groups, including the control, by adding rye, rapeseed meal and an increased crude protein level without NSP enzyme. Feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) in accordance with the different phases (0-9, 9-23 and 23 to 35 days) were recorded. The FCR data were corrected for mortality using the number of 'broiler days' (number of broilers x days alive). All zootechnical parameters were analyzed by a General Linear Model (GLM) with treatment as a fixed factor and block as a random factor. A Tukey HSD test was used to compare the treatments if the GLM determined a significant ($P < 0.05$) treatment effect or a trend ($0.05 < P < 0.1$).

Considering the entire evaluated period (0-35 days), the results (see table 1) showed a numerically higher weight/bird in birds fed GPGR^{A} and LPL compared to those fed the control diet. Birds fed GPGR^{B} showed significantly lower weight/bird when compared to those fed all other treatments. Lowest feed conversion ratio (FCR) was seen in birds fed GPGR^{A} whilst the highest feed conversion ratio was seen in birds fed LPL.

In conclusion, supplementing broiler diet with the GPGR^{A} nutritional emulsifier resulted in the best bird performance overall. The low performance in birds fed GPGR^{B} could not be attributed to chicks, pellets or pellet quality as these parameters were similar amongst the treatments.

Table 1 - Broiler performance when fed different nutritional emulsifiers. Standard deviation (SD) between brackets.

	T1-control	T2-GPGR^{A}	T3-LPL	T4-GPGR^{B}	P
Weight/bird (35 days)	2326.3^{a} (82.7)	2387.7^{a} (94.6)	2372.0^{a} (58.8)	2222.3^{b} (96.7)	< 0.001
Feed intake (g/d, 0-35 days)	93.1^{ab} (4.9)	93.4^{ab} (5.6)	96.4^{a} (2.5)	89.6^{b} (3.6)	0.004
FCR	1.428^{ab} (0.042)	1.396^{a} (0.043)	1.451^{b} (0.034)	1.441^{ab} (0.025)	0.009

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PHYTASE SUPPLEMENTATION, IRRESPECTIVE OF DIETARY PHYTATE LEVELS, IMPROVES DIGESTIBILITY OF AMINO ACIDS IN FINISHER BROILER CHICKENS

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All grains, vegetable-based protein meals and their by-products used in poultry diets contain some levels of phytate phosphorous (PP). Depending on the ingredient type and source, a typical broiler chicken diet would contain between 2.2 to over 3.5 g/kg dietary PP. Research has shown that phytate anti-nutritional effects can reduce the digestibility of amino acids (AA) and minerals. Previously the effect of phytase on AA digestibility was reported in broilers at 21 days of age (Toghyani et al., 2021). This study reports the AA digestibility in the finisher phase.

The current study evaluated the effect of incremental doses (0, 500, 1000, 2000 and 4000 FTU/kg) of a novel consensus bacterial 6-phytase variant (PhyG) on AA digestibility in broilers at 35 days of age. The basal diets were formulated to contain either 2.45 g/kg, 2.95 g/kg or 3.45 g/kg phytate phosphorus (PP). However, the analyzed PP levels were greater than formulated, being 2.85, 3.43 and 3.87g/kg, in low, medium and high PP diets, respectively. A total of 1800 (Ross 308) day-old male chicks were randomly allocated to 90 battery cages with 6 replicate cages of 20 birds per treatment, creating a 3 × 5 factorial arrangement. Birds were offered diets deficient in available P (AvP) and Ca based on the expected contribution of 1000 FTU/kg PhyG, 2.77 g/kg AvP and 7.6 g/kg Ca in starter (0-10d), 2.25 g/kg AvP and 6.4 g/kg Ca in grower (10-21d), and 1.73 g/kg AvP and 5.5 g/kg Ca in finisher (21-25d). All the diets were formulated to be iso-nitrogenous and iso-caloric and balanced for all the essential AA based on Ross 308 broiler recommendations. On day 35, four birds per cage were euthanized to collect distal ileum content. Samples were pooled per replicate cage and analyzed for AA to calculate ileal digestibility. Increasing dietary PP decreased ($P < 0.001$) the average ileal digestibility of all the AA tested, by 1.5 and 2.2 % in medium and high PP groups, respectively, compared to low PP. There was no interaction of phytase and PP levels for the digestibility of any of the AA. Phytase inclusion, as the main effect, improved ($P < 0.001$) the ileal digestibility of all essential and non-essential AA tested, regardless of PP levels. Fitting exponential models predicted an average digestibility improvement, above negative control diets with no phytase, of 0.5 to 3.1 % at 500 FTU/kg, up to an average of 2.7 to 8.4 % at 4000 FTU/kg. Amongst the essential AA and at each level of phytase inclusion, methionine had the lowest (0.5 to 2.8 %) and threonine had the highest (2.7 to 8.4 %) predicted digestibility improvement. For the non-essential AA, the lowest and the highest digestibility improvement were predicted for glutamine (0.98 to 3.0 %) and tyrosine (3.1 to 8.1 %), respectively. The data obtained in this study indicate that dietary phytate, even in finisher broiler chicks, decreases AA digestibility, and exogenous phytase, at each level of inclusion (from 500 to 4000 FTU/kg) improves AA digestibility, irrespective of dietary PP levels.

ACKOWLEDGEMENTS: The authors would like to thank Danisco Animal Nutrition & Health (IFF) for financially supporting this research trial.

Toghyani M, Macelline SP, Chrystal PV, Selle PH, Dersjant-Li Y, Bello A & Liu SY (2021) *Proceeding of the Australian Poultry Science Symposium* **32:** 130-133.

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THE EFFECT OF CHELATED TRACE MINERALS AND PROTEIN SOURCES ON GROWTH PERFORMANCE, BREAST MUSCLE VASCULARISATION AND MINERAL EXCRETION IN BROILER CHICKENS

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Trace minerals manganese (Mn), copper (Cu), and zinc (Zn) are essential for multiple cellular functions and optimal growth and health in production animals. Inorganic sources of these minerals often provide poor relative bioavailability for the animal, primarily due to numerous antagonisms and interactions between them and other components of the digesta, such as phytic acid and fibre, resulting in a high excretion into the environment through the faeces. Chelated trace minerals can be a tool to counteract these effects, increasing mineral utilization by the animals while decreasing their excretion. The objective of this study was to evaluate the effect of trace mineral forms in diets with different protein sources on performance, breast vascularization, and mineral retention.

Six hundred, one-day-old male Ross 308 chicks were randomly allocated into 6 dietary treatments with 10 replications each (10 birds per pen). The study consisted of a 3x2 factorial design, including three different protein ingredients (soybean meal, rapeseed meal or yellow lupin meal) as the sole protein source, and two Zn, Cu and Mn sources (inorganic or chelate). Metal methionine hydroxy analogue chelate (MMHAC) was used as the source of chelate. The level of inorganic trace minerals in feed met the recommendations for broiler chickens following AVIAGEN (2014). MMHAC minerals were provided at reduced rates based on bioefficacy. Feed was distributed ad libitum following a 3-phase feeding program (starter 1-10d; grower 11-24d; and finisher 25-42d). Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured for each period. Breast vascularization, ileum villus height as well as the content of Zn, Cu and Mn in excreta were analysed at the end of the experiment.

No interactions between protein sources and mineral forms were observed for broiler performance and breast vascularization. The use of MMHAC chelated trace minerals made no difference to bodyweight gain, however reduced feed intake resulting in significant FCR reduction from 0-42 days 1.48 to 1.45 ($P < 0.05$), and significantly increased breast vascularization and ileum villus ($P < 0.05$). There was an interaction between protein sources and mineral form on Cu content in the excreta. MMHAC mineral form significantly decreased the content of Cu in excreta only in treatments containing rapeseed meal or yellow lupin meal. In the case of Zn and Mn, their concentration in the excreta was significantly reduced in all the experimental diets when supplemented as chelates. In this study, chelated trace minerals had a positive effect on feed conversion efficiency, breast vascularization, and ileum villus development, regardless of the protein source used in broiler diets. The use of Cu, Zn, and Mn as chelates increases their utilization in broiler chickens, reducing the excretion into the environment.

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COMMERCIAL PHYTASES DIFFER IN THEIR ABILITY TO WITHSTAND EXTREMELY LOW-, YET PHYSIOLOGICALLY-RELEVANT PH

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Summary

An *in*-vitro assay was conducted to test the stability of three commercial phytases under low pH conditions. Three commercial phytases (A, B, and C) were incubated, with or without pepsin, at pH 1.25 or 1.5 for varying time intervals; 0 min (T0, Control), 10 min (T10), 30 min (T30), 60 min (T60) and 240 min (T240). Phytase A displayed high resilience to pH 1.25 and 1.5, both in the absence or presence of pepsin, as shown by no loss in activity to extended incubation up to T240. Phytase B showed fair resistance when pepsin was absent, while in the presence of pepsin, phytase B lost 80 and 50% of its activity, respectively, at pH 1.25 and 1.5 as early as T10. In the presence of pepsin, phytase C ran below the limit of detection at T10, independent of the assay pH. Phytase C displayed weak resilience to pH in the absence of pepsin and retained ~15 and 27% of its initial activity at T240, respectively at pH 1.25 and 1.5. These results suggest remarkable differences in stability of commercial phytases under the conditions of the assay and warrants further studies to explore implications of these differences on in-vivo efficacy of phytases.

I. INTRODUCTION

Exogenous enzymes are susceptible to denature when exposed to low-pH conditions, normally prevailing in the gastric phase. Hence, resistance to low-pH is considered one of the key criterion for the suitability of commercial phytase enzymes for animal feed application. The pH-stability is assessed *in*-vitro by measuring the recovery of enzyme activity after exposing enzyme protein to low-pH buffers. To mimic gizzard conditions, pH 2.5-3.5 with added pepsin is often employed as the standard for these assays (Menezes-Blackburn et al. 2015). Using real-time measurement of pH over time in live individual broiler chickens, Lee et al. (2017) noted that gizzard pH was not a constant and, invariably, fell below 1.5 or even 1.0. These observations were considerably lower than those representing published estimates of average gizzard pH in poultry (Svihus, 2011) and posed a question if the conventional method of measuring pH in killed birds was confounded by the post-mortem changes. It also warrants a reevaluation of pH-stability of commercial phytases at more realistic low-pH conditions. This communication presents results of an in-vitro assay designed to study the behavior of three commercial phytases under two assay pH conditions i.e., 1.25 and 1.5, each with or without pepsin.

II. METHOD

Reactions were carried out in 100mM glycine-HCl buffer pH 1.25 or pH 1.5 containing 2g/l BSA. 1g of pepsin (Sigma P7000) was dissolved in 60ml of glycine buffer (pH 2.5) and frozen in 1.5ml aliquots. 1ml of the frozen aliquot was diluted in 25ml glycine buffer pH 1.25 or pH 1.50 and 5ml of the dilution was added to the reaction.

The final pepsin concentration was 3.33mg in the 50ml reaction. The reactions were carried out on a multi-stirrer plate in a water bath at 40ºC.At T0, 1ml of enzyme dilution was added to each reaction (50ml total). The sample was left to stir for ~20 seconds to allow the

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enzyme to be distributed evenly throughout the reaction before 1ml was taken and stopped in 4ml pH 5.5 acetate buffer on ice. Samples (1ml) were then taken (and stopped in 4ml pH 5.5 acetate buffer on ice) at 10, 30, 60 and 240 minutes. Phytase activity was determined by incubating the enzyme solution sodium phytate at pH 5.5 and 37°C, liberating inorganic phosphate from the substrate. The reaction is terminated by the addition of an acid molybdate / vanadate reagent which also produces a coloured complex with the phosphate produced. The colour of the yellow vanado-molybdo-phospho-complex, which is a measure of the amount of phosphate released, is measured at a wavelength of 415 nm and related to a phosphate standard curve.

III. RESULTS & DISCUSSION

Phytases differ significantly in their ability to withstand low pH conditions. Phytase A retained its activity at both pH's, with or without pepsin, and at all time intervals. In the presence of pepsin, phytase B lost 80 and 50% of its activity, respectively at pH 1.25 and 1.5 at as early as T10. The activity fell further and went below the limit of detection at T30 and T60, respectively at pH 1.25 and 1.5. Phytase B showed a fair degree of stability in the absence of pepsin as reflected by residual activity of ~50 and ~65% at T240, respectively at pH 1.25 and 1.5. In the presence of pepsin, phytase C was below the limit of detection at T10, independent of the assay pH. Phytase C displayed weak resilience to pH in the absence of pepsin and retained ~15 and 27% of its initial activity at T240, respectively at pH 1.25 and 1.5.

These data suggest that conventional pepsin stability tests may erroneously suggest a phytase is stable under the conditions prevailing in the gizzard when it may not be and thus this warrants further investigation. Furthermore, feeding practices and/or dietary ingredients which alter the pH minimum encountered in the gizzard likely degrade the stability of some phytases far more than others.

Table 1 - Activities of phytases at different time points (0, 10, 30, 60 and 240 minutes), pH (1.25 or 1.50), with/without pepsin.

			Activity at time points (FTU/kg)				
Phytase	pH	Pepsin	T0	T10	T30	T60	T240
A	1.25	-	6370	6490	6240	7000	6680
		+	6240	6460	6240	7060	6680
	1.50	-	6690	6480	6590	7250	7280
		+	6310	6220	6190	6870	6710
B	1.25	-	16600	14600	13400	12700	8930
		+	12600	2120	low**	Low	Low
	1.50	-	15500	14200	13300	12900	10300
		+	13700	6170	2050	Low	Low
C	1.25	-	8390	6320	6610	3610	1260
		+	5130	Low	Low	Low	Low
	1.50	-	10400	7520	4680	5430	2800
		+	6940	Low	Low	Low	Low

*****T0 = 0 minutes – actually ~20 seconds to allow phytase to mix fully in the reaction.
** low = absorbance reading below lower limit of detection

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EFFECT OF A MULTI-PROTEASE SUPPLEMENTATION IN SOYBEAN MEAL ON APPARENT METABOLIZABLE ENERGY AND AMINO ACID DIGESTIBILITY IN BROILERS

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Summary

Inclusion of exogenous enzymes such as proteases to poultry feed is one approach of optimizing protein and amino acid (AA) digestibility. Male broiler (Ross 308) chicks were used in two independent experiments to investigate the effect of a multi-protease supplementation on the apparent metabolizable energy (AME; Experiment 1) and AA digestibility (Experiment 2) of a soybean meal (SBM) in broilers. For the AME assay, a corn-soybean meal basal diet was formulated, and the test diet was developed by replacing 300 g/kg (w/w) of the basal diet with SBM sample. From the basal and test diets, four experimental diets were developed using different doses (0 and 300g/t) of protease. In addition, to determine the AA digestibility of SBM sample, an assay diet based on corn starch and test soybean meal as the only source of protein was formulated to supply 18% crude protein in the diet. The assay diets with different doses (0 and 300g/t) of protease also contained titanium dioxide at 5 g/kg as an indigestible marker. In conclusion, there was a positive numerical improvement in AME (0.29 MJ/kg) and AMEn (0.27 MJ/kg) with protease supplementation. Standardized ileal digestibility of several AA was significantly improved by supplementation of protease. Overall, the addition of exogenous protease can increase the nutritional value of feed ingredients through improved nutrient digestibility and energy utilization.

I. INTRODUCTION

Soybean meal (SBM) is the most important plant protein source in broiler diets. Although most of the proteins in SBM are highly digestible, some proteins including glycinin, protease inhibitors, and antigenic proteins are indigestible and can cause intestinal damage and impair immune functions resulting in sub-optimal growth performance (Pan et al., 2016). The supplementation of SBM with appropriate commercially available proteases provides a potential strategy to enhance the utilization of SBM proteins. The benefits and efficacy of exogenous microbial protease have also been well-reported to improve performance of animals through the control of protein and amino acid (AA) digestibility (Angel et al., 2011; Cowieson et al., 2017; Cowieson et al., 2019).

Research on exogenous proteases in poultry diets has focused on protein and AA digestibility but the effect on apparent metabolizable energy (AME) is not well-studied. Although an increase in AME is expected with the increase in protein and AA digestibility, the improvements in AME are typically greater than the sum of energy contributed by AA digestibility, indicating an improvement in energy partitioning (Cowieson et al., 2019). Therefore, this study was undertaken to determine whether the supplementation of a multi-protease could deliver better energy utilization and AA digestibility in soybean meal.

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II. METHOD

Two experiments were carried out at Monogastric Research Centre, Massey University, New Zealand. In the first experiment to determine the AME, a total of 256 one-day-old male Ross 308 broiler chicks were allocated into four treatments with eight replicates per treatment using a completely randomized design. A basal diet based on corn-soybean meal was formulated and the test diet was developed by replacing 300 g/kg (w/w) of the basal diet with SBM sample using the substitution method (Table 1). From the basal and test diets, four experimental diets were developed using different doses (0 and 300 g/t) of Kemzyme^{TM} Protease and no phytase was used in both experiments. The AME values were determined using the classical total collection method. Each diet was fed to eight replicate cages (eight 14-day old broilers/cage) for 7 days (from day 14 to 21) with the first 3 days serving as an adaptation period. During the last 4 days (day 17 to 21), feed intake was monitored, and the excreta was collected daily, weighed, and pooled within a cage. Pooled excreta were mixed well (using a blender) and representative samples were obtained and freeze-dried for dry matter (DM) determination. Dried excreta samples were ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at 4°C for chemical analyses. The DM, gross energy (GE) and nitrogen (N) of the diets, excreta samples, and soybean meal sample were determined.

Table 1 - Percentage composition of the basal diet used in Experiment 1.

Ingredient	Inclusion (%)
Corn	60.44
Soybean meal	33.81
Soybean oil	1.42
Dicalcium phosphate	1.58
Limestone	1.04
Sodium chloride	0.10
Sodium bicarbonate	0.39
DL Methionine	0.31
Lysine HCl	0.37
L Threonine	0.20
L Valine	0.07
Vitamin premix	0.10
Mineral premix	0.07
Choline chloride 60%	0.07

In the second experiment to determine apparent ileal amino acid digestibility of soybean meal with or without protease supplementation, a total of 128 one-day-old male Ross 308 broiler chicks were allocated into two treatments with eight replicates per treatment using a completely randomized design. The AA digestibility coefficients were determined by the direct method. An assay diet, based on corn starch and test soybean meal as the only source of protein was formulated to supply 18% crude protein in the diet (Table 2). The assay diets contained titanium dioxide at 5 g/kg as an indigestible marker. Each diet was offered ad libitum to eight cages (eight birds/cage) of male broilers from 17 to 21 days of age. On day 21, all birds were euthanized by an intravenous injection of sodium pentobarbitone solution, and the contents of the lower half of the ileum were collected by gently flushing with distilled water into plastic containers. The ileum was defined as that portion of the small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileocaecal junction. Samples from all birds within a cage were pooled, frozen immediately after collection and subsequently freeze-dried. Soybean meal sample, diets and ileal digesta samples were ground to pass through a 0.5

mm sieve and stored in airtight containers at 4°C for chemical analyses (DM, titanium, N and amino acids, including sulphur-containing amino acids, but not tryptophan).

Table 2 - Percentage composition of the basal diet used in Experiment 2.

Ingredient	Inclusion (%)
Test soybean meal	41.25
Corn starch	52.50
Soybean oil	2.00
Dicalcium phosphate	1.90
Limestone	1.00
Sodium bicarbonate	0.25
Sodium chloride	0.20
Trace mineral premix	0.20
Vitamin premix	0.20
Titanium dioxide	0.50

All analyses were conducted in an ISO17025 accredited laboratory (Nutrition Laboratory, Massey University). Dry matter content was determined in a convection oven at 105°C (AOAC 930.15; AOAC 925.10). Nitrogen content was determined by the combustion method using a CNS-2000 carbon, N and sulphur analyser (LECO® Corporation, St. Joseph, Michigan, USA). Gross energy was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, UK) standardised with benzoic acid. Crude fat was measured using a Soxhlet extraction procedure (Method 2003.06; AOAC, 2005). Titanium content was measured on a UV spectrophotometer following the method of Short et al. (1996).

Amino acids were determined by hydrolysing the samples with 6 N HCl (containing phenol) for 24 h at 110 ± 2°C in glass tubes sealed under vacuum. Amino acids were detected on a Waters ion-exchange HPLC system, and the chromatograms were integrated by using dedicated software (Millenium, version 3.05.01, Waters, Millipore, Milford, MA) with the amino acids identified and quantified by using a standard amino acid mixture (product no. A2908, Sigma, St. Louis, MO). Amino acids were eluted by a gradient of pH 3.3 sodium citrate eluent to pH 9.8 sodium borate eluent at a flow rate of 0.4 mL/min and a column temperature of 60°C. Cysteine and methionine were analysed as cysteic acid and methionine sulfone, respectively, by oxidation with performic acid for 16 h at 0°C and neutralisation with hydrobromic acid before hydrolysis (Ravindran et al., 2009).

III. RESULTS

Protease supplementation (300 g/t) did not significantly increase the AME as compared to the diet without protease ($P > 0.05$), but numerically increased AME by 0.29 MJ/kg (~69 kcal/kg) and AMEn by 0.27 MJ/kg (~64.5 kcal/kg).

The effect of supplementation of protease on AA digestibility is presented in Figure 1. Significant improvements were observed in the apparent ileal digestibility of nitrogen and twelve amino acids (particularly Aspartic Acid (Asp), Threonine (Thr), Serine (Ser), Proline (Pro), Glycine (Gly), Valine (Val), Leucine (Leu), Histidine (His), Lysine (Lys), Arginine (Arg) and Cysteine (Cys)) with supplementation of protease ($P < 0.05$).

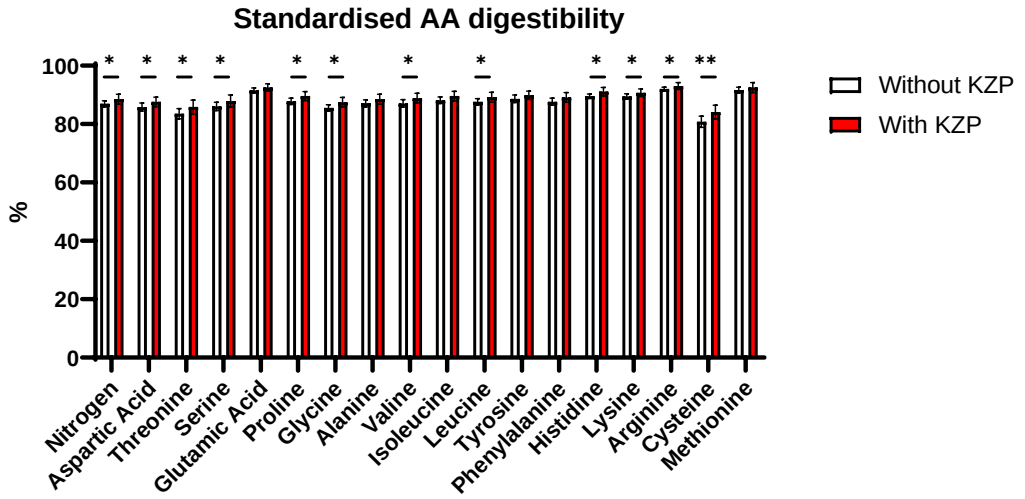

Figure 1 - Effect of Kemzyme™ Protease on standardized ileal digestibility of nitrogen and amino acids of soybean meal in broilers. Results were expressed as amino acid digestibility coefficients measured on day 21 post-hatch. Data represents mean ± SEM from 8 replicates (8 birds per replicate). Statistical significance was measured using multiple unpaired t test. * $p < 0.05$; ** $p < 0.01$.

IV. DISCUSSION

In both experiments, it was evident that the addition of protease had significant benefits to the digestibility of nitrogen and amino acids. While there was no significant increase in AME, a positive numerical improvement of AME was observed. Taken together, the addition of exogenous protease in the diet formulation complemented that of the endogenous protease to increase AME and to improve protein digestibility in diets.

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β-MANNANASE SUPPLEMENTATION ENHANCES FIBER DIGESTION AND APPARENT METABOLIZABLE ENERGY OF BROILERS FED HIGH CASSAVA DIETS

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Summary

Cassava is an alternative feed ingredient that is widely used in broiler industry as a good source of energy, but it contains relatively lower content of protein and methionine than some other feed ingredients. As a result, a cassava-based diet would need more protein sources such as SBM. However, SBM contains β-mannans, an indigestible non-starch polysaccharide that possesses anti-nutritional properties. The present study was conducted to investigate the effects of β-mannanase supplementation on nutrient digestibility of broilers fed cassava-corn-based diets with hulled and dehulled SBM. Results showed no significant interaction between SBM source and β-mannanase supplementation on ileal protein digestibility of broilers ($P > 0.05$). However, ileal crude fiber digestibility ($P < 0.05$) of broilers was improved when β-mannanase was added to the diet containing dehulled-SBM. The highest AME was observed in birds fed dehulled SBM with β-mannanase supplementation, which was significantly higher than in birds fed hulled SBM with no enzyme supplementation ($P < 0.05$) but not the rest of the groups. Regardless of SBM source, β-mannanase supplementation improved the AME of the broilers by approximately 0.57 MJ/kg. This could possibly be due to β-mannanase increased fiber digestion which led to a reduction in anti-nutritional effects in the broiler diet containing dehulled SBM as a sole source of protein.

I. INTRODUCTION

A shortage and rising cost of major feed ingredients triggers more pressure on local poultry industries to maximize the use of alternative feed ingredients. Cassava is a potential alternative to corn. It is a good source of energy, with 60-70% of starch & 2-4 % of crude fiber (Staack et al, 2019). However, it has low protein (2%) with low methionine content (0.03%) (Morgan and Choct, 2016). When cassava meal is used at a high level, more SBM is needed to meet protein requirements. Soybean products and co-products are the major sources of β-mannans in the poultry diet. β-mannans in SBM are mainly associated with the hull (~5% β-mannans) and heat-resistant compounds that remain after the drying-toasting phase of processing soybeans (Hsiao et al., 2006). β-mannan content in SBM ranges from 0.7% DM in dehulled SBM (~48% CP) to 2.1% DM in 44% CP hulled SBM (Knudsen 1997; Knudsen, 2011). Exogenous β-mannanase enzyme can hydrolyze β-mannans and potentially eliminate the negative effect of these anti-nutritional factors (Vangroenweghe et al., 2021; Yaqoob et al., 2022). The present study was conducted to investigate the effects of β-mannanase supplementation on the nutrient digestibility of broilers fed cassava-corn-based diets with hulled and dehulled SBM.

II. METHODS

A total of 256 Ross 308 broiler chicks were allocated to 4 treatments with 64 birds per treatment. All birds of each treatment were be raised together in one floor pen and fed the experimental diet until 16 day of age (DOA) then divided into 8 replications (8 birds/replicate) and raised in metabolic

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cages until 24 DOA. The experimental design was a 2 x 2 factorial in a completely randomized design with 2 SBM sources (hulled, 44% CP and dehulled, 48% CP) and 2 levels of β-mannanase supplementation (0 and 250 g/ton). Cassava meal was incorporated at the level of 30% in all starter and grower diets. The trial was run from 1 to 24 day of age (DOA) using 2-phase feeding. Starter diet was offered during 1-16 DOA, then changed to grower diets during 17-24 DOA. Titanium dioxide (0.3%) was added to all grower diets as an indigestible marker for nutrient digestibility study. All birds were kept in an environmentally controlled house. Feed in mash form and water were provided *ad-libitum* throughout the experimental period. Diet compositions and proximate analysis are shown in Table 1. The excreta were collected daily during the last three days of the balanced period (from day 21 to day 23). At 24 DOA, 2 birds per cage were selected for ileal digesta collection. Statistical analysis of all data was conducted using Analysis of Variance. Duncan's new multiple range test of SAS University Edition (2018) was applied to compare treatment mean comparisons. Differences where $P < 0.05$ were considered significant, while differences where $P < 0.01$ were considered highly significant.

Table 1 - Ingredient composition and chemical analysis of experimental diets[1].

Ingredient	Grower (17-24 DOA)			
	Hulled SBM 44%CP		Dehulled SBM 48%CP	
	None	β-mannanase	None	β-mannanase
Corn	24.86	24.86	27.13	27.13
Cassava meal	30.00	30.00	30.00	30.00
SBM 44%CP	31.37	31.37	-	-
SBM 48%CP	-	-	29.65	29.65
Full fat soybean	8.00	8.00	8.00	8.00
Crude rice bran oil	2.11	2.11	1.17	1.17
Monodicalcium phosphate	1.65	1.65	1.68	1.68
Calcium carbonate	0.83	0.83	1.19	1.19
Salt	0.31	0.31	0.31	0.31
DL-Methionine	0.39	0.39	0.38	0.38
L- Lysine	0.22	0.22	0.23	0.23
Choline Chloride 60%	0.01	0.01	0.01	0.01
Premix	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated analysis				
ME, MJ/kg	12.66	12.66	12.66	12.66
Protein, %	20.00	20.00	20.00	20.00
Chemical analysis (proximate)				
Dry matter, %	90.74	90.75	90.33	90.54
Protein, %	19.61	19.63	19.52	19.45
Fiber, %	3.13	3.12	3.83	3.84
Ash, %	2.91	2.61	2.20	2.33
Calcium, %	0.95	0.96	0.97	0.95
Phosphorus, %	0.64	0.62	0.63	0.65
GE, MJ/kg	17.44	17.43	17.16	17.29

[1] All diets were formulated according to Ross 308 recommendation.

III. RESULTS

The effect of SBM source and β-mannanase supplementation in broilers fed a high level of cassava diets on growth performance is presented in Table 2. During 17-24 DOA, there was no significant interaction between SBM source and β-mannanase supplementation on final weight,

body weight gain, feed intake, feed conversion ratio, mortality and culling of broilers (P > 0.05). There was no significance effect of either main effect (SBM source and β-mannanase supplementation) on overall growth performance of broilers (P > 0.05). This may be due to the period of enzyme supplementation not being long enough to elucidate the positive effect on the growth performance of broilers. Although no significant difference was observed, the lowest FCR was found in broilers fed the diet containing dehulled SBM supplemented with β-mannanase which was 4 points lower than birds fed dehulled SBM with no β-mannanase and hulled SBM with β-mannanase, and 8 points lower than that of hulled SBM with no β-mannanase.

Table 2 - Effect of SBM source and β-mannanase supplementation on growth performance of broilers (17-24 DOA).

	Initial weight (g)	Final weight (g)	Body weight gain (g)	Feed intake (g)	FCR	Mortality (%)
SBM x Enzyme						
Hulled SBM 44% CP - Enzyme	621.18	1191.84	570.67	847.54	1.485	2.08
Hulled SBM 44% CP + Enzyme	621.29	1205.18	583.90	838.75	1.436	4.16
Dehulled SBM 48% CP - Enzyme	621.75	1194.84	573.09	825.20	1.440	0.00
Dehulled SBM 48% CP + Enzyme	621.57	1207.15	585.58	818.59	1.398	4.16
Source of variation				*P*-value		
SBM x Enzyme	0.975	0.747	0.760	0.732	0.490	0.489
Pooled SE	0.46	5.72	5.83	9.66	0.02	1.08

There was no significant interaction of SBM source and β-mannanase supplementation on ileal protein digestibility (P > 0.05). However, there was a significant interaction of SBM source and β-mannanase supplementation on ileal crude fiber digestibility and AME (P < 0.05). The supplementation of β-mannanase improved ileal crude fiber digestibility of birds fed the diet containing dehulled SBM but not of birds fed hulled SBM (P < 0.01). The supplementation of β-mannanase significantly improved AME of broilers fed dehulled SBM when compared to that of hulled SBM with no β-mannanase, but not the other treatments (P > 0.05).

Table 3 - Effect of SBM source and β-mannanase supplementation on AME and nutrient digestibility of broilers.

	Ileal protein dig.(%)	Ileal crude fiber dig. (%)	AME (MJ/kg)
SBM x Enzyme			
Hulled SBM 44% CP – Enzyme	75.59	10.47^{b}	11.76^{b}
Hulled SBM 44% CP + Enzyme	76.70	15.25^{b}	12.33^{ab}
Dehulled SBM 48% CP – Enzyme	76.21	26.77^{ab}	12.27^{ab}
Dehulled SBM 48% CP + Enzyme	76.91	36.66^{a}	12.84^{a}
SBM			
Hulled SBM 44% CP	76.14	12.86^{b}	12.00
Dehulled SBM 48% CP	76.70	32.70^{a}	12.53
Enzyme			
None	76.14	16.99^{b}	12.01^{b}
β-mannanase	76.71	25.96^{a}	12.60^{a}
Source of variation		*P*-Value	
SBM	0.733	<0.001	0.051
Enzyme	0.465	0.025	0.030
SBM x Enzyme	0.874	<0.001	0.036
Pooled SE	0.58	3.87	31.20

^{a,b} Means within the same column with different superscripts differ significantly (P < 0.05)

IV. DISCUSSION

The supplementation of β-mannanase improved ileal crude fiber digestibility of birds fed the diet containing dehulled SBM which led to an increase in AME thus improving FCR. It is likely that β-mannanase increased the nutrient digestibility of broilers due to enzymes breaking down mannan, thereby sparing energy (Kiarie et al., 2022) and releasing the nutrient contents (Bedford, 2000; Chang et al 2020). β-mannanase also can reduce intestinal viscosity, increasing digesta flow, ameliorating the mixing of the substrate with the digestive enzymes, enhancing nutrient contact with the intestinal wall, and thereby improving nutrient utilization and contributing to high energy values (Shastak et al., 2014; Jiang et al., 2022). Dehulled SBM has lower β-mannans than hulled SBM which creates less of an antinutritional effect for broilers. The supplementation of β-mannanase, therefore, improved nutrient utilisation and feed conversion ratio of broilers.

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COMPARISON OF THREE DIFFERENT PHYTASE PRODUCTS ON BROILER PERFORMANCE AND TOE ASH

Y.G. LIU[1], F.X. XU[1], B. GUO[1] and B. YAVUZ[2]

Summary

This study used 4,356 day old male broiler birds to compare the efficacy of three commercial phytase products (A, B and C) applied at graded doses of 0, 250, 500 and 1000 FTU/kg feed, in compensating for a pre-determined down-spec of essential nutrients, i.e. calcium and available phosphorus 0.2 percental units each, digestible amino acids 2% plus ME 0.084 MJ/kg, in a factorial design of 11 dietary treatments. The results showed that the positive control (PC) diet supported expected growth performance whilst the reduction of the three nutrients in the negative control (NC) resulted in decreases in feed intake (FI), weight gain (WG), performance index (PI) ($P < 0.05$), and toe ash ($P < 0.05$). Supplementation with any one of the phytase products improved performance (net gain and feed conversion ratio (FCR)) in a dose-dependent pattern. Among the three products, Phytase A showed advantages in restoring performance especially during the starter phase, and the birds fed with Phytase A produced more breast muscle and less abdominal fat.

I. INTRODUCTION

Phosphorus (P) is an essential nutrient in metabolic processes and the third most costly nutrient in monogastric feed after energy and protein. All plant ingredients contain phytate (salt of phytic acid, *myo*-inositol hexakisphosphate, IP6) which is a source of phosphorus for farm animals. However, phytate has poor bioavailability for monogastric animals due to inadequacy of endogenous phytase activity and lower microbial degradation capacity in the digestive tract (Li *et al.*, 2022). This leads to routine supplementation with inorganic P sources to animal diets which not only increases feed cost but also P excretion resulting in environmental pollution. Moreover, phytate possesses substantial antinutritional impact, due to its interactions with other components in the digestive tract, forming insoluble complexes with positively charged mineral ions, such as calcium, and other trace minerals as well as amino acids (Selle *et al.* 2012) causing a negative impact on overall feed digestibility.

The recent significant inflation in the cost of feed ingredients, along with the global demand on sustainability, is driving the animal industries to review P levels in diets and to search for more effective phytases with higher efficacy to enhance P utilisation. This study aimed to compare the efficacy of two new-generation commercial phytase products versus a currently available commercial phytase.

II. MATERIALS AND METHODS

The study was conducted following a complete randomised design, plus a positive control (PC) and a negative control (PC), which contained no added phytase, to serve as a baseline for the comparison. In total of 4,356 day-old male broiler chicks (Lohmann Indian River) were allocated into 11 dietary treatments, consisting of PC and NC, plus 3 different phytases (A, B and C) at 3 application dosages (250, 500 and 1000 FTU/kg diet). The PC diet was formulated to meet the requirements of nutrients to support normal growth as specified by the breeding

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firm (Lohman Indian River), and the NC was formulated to create marginal deficiency of nutrients in order to observe performance differences, whilst avoiding significant losses of growth and health of the birds. The levels of down-spec were ME 0.084 MJ/kg, 2% in digestible amino acids, and 0.2 %-unit in calcium and available and finally 0.03 %-unit in sodium. Each treatment had 11 replicates with 36 birds per replicate, in total 396 birds per treatment. The birds were raised in floor pens of 1.70 x 1.44 m^2, in an enclosed pen house. As the purpose of the study was to evaluate P and phytase efficacy, a 2-phase feeding program was chosen: 1-21 days of age as starter and 22-35 days of age as finisher. Fine crumble was provided during the starter and pellet was given during the finisher phase. The birds had free access to feed and water.

Diet formulation is shown in Table 1. Three commercial phytase samples (A, B and C) were collected from the market in Southeast Asia. Phytase A and B both are newly launched bacterial consensus 6-phytase variant, expressed in *Trichoderma reesei*, and C was an enhanced *E.* coli phytase. All the three phytase samples were analyzed according to standard procedure and all samples met their declared specification.

Table 1 - Diet formulation and nutrients (phytase was added on top).

	Starter, 1-21 d		Finisher, 22-35 d	
INGREDIENTS, g/kg	PC	NC	PC	NC
Corn	531.4	557.4	614.7	648.2
DDGS	50.0	64.8	27.6	31.8
Soybean meal	341.7	326.1	273.4	261.5
Palm olein	32.8	20.0	50.0	36.4
Limestone (coarse)	12.9	11.25	9.50	7.80
MCP	12.87	3.49	9.21	-
Salt	2.97	3.01	2.90	2.83
Lysine sulphate 70%	4.20	4.24	3.69	3.71
DL - Methionine (99%)	3.75	3.53	3.01	2.85
L - Threonine (98.5%)	1.39	1.29	1.09	1.02
L - Valine (96.5%)	0.33	0.20	0.35	0.27
Potassium carbonate		0.26		0.21
Sodium bicarbonate	2.30	1.00	1.20	0.07
Trace minerals	0.65	0.65	0.60	0.60
Choline-Cl 60%	0.26	0.24	0.26	0.27
Vitamin mix	2.50	2.50	2.50	2.50
TOTAL	1000.0	1000.0	1000.0	1000.0
Nutrients, g/kg				
ME, MJ/kg	12.34	12.26	13.18	13.10
Fats	61.01	50.04	78.68	66.53
Fibre	27.44	28.50	24.80	25.33
Ash	60.65	50.59	48.78	38.67
C. Protein	221.11	219.64	188.42	186.38
Dig. Lys.	12.50	12.25	10.50	10.29
Dig. Met.	6.62	6.42	5.54	5.38
Dig. M+C.	9.38	9.19	7.98	7.82
Dig. Trp.	2.29	2.25	1.91	1.87
Dig. Thr.	8.38	8.21	7.04	6.89
Calcium.	8.00	6.00	6.00	4.00
P total	6.41	4.33	5.15	3.07
P available	4.00	2.00	3.00	1.00

The birds were weighed on a pen basis on days 0, 21 and 35. Feed intake was measured at the end of each phase, and feed conversion ratio (FCR) was calculated (including live weight and mortality correction) accordingly. The performance index was calculated at day 35. Mortality, culling number, and dead weights were recorded daily. Toe ash was measured at day 21 over one sample pooled by 3 birds/pen (total of 121 pooled toe ash samples). Carcass % and abdominal fat pad were measured at day 35 over a total of 363 samples, 3 birds/pen.

III. RESULTS AND DISCUSSION

a) Performance and toe ash at Day 1-21

Table 2 shows the results of performance and toe ash contents at the end of the starter phase. As expected, all performance parameters (FI, BW and FCR) of the PC group reached their breeder standard, confirming adequate diet formulation and trial management. Significant decrease in performance was observed in the NC. The reduction of the three essential nutrients led to a decrease of feed intake ($P < 0.05$), and consequently lower net gain ($P < 0.05$), and performance index ($P < 0.05$) and increase in FCR ($P < 0.05$). Significantly lower ($P < 0.05$) toe ash content in the NC groups with a very low mortality rate indicates that the final avP level in NC was appropriate. All performance parameters were improved with phytase supplementation and there were no interactions between phytase source and dose ($P > 0.05$). Among the phytases tested, Phytase A displayed a tendency of more effectiveness over the other two products. Moreover, the results showed a steady and significant ($P < 0.05$) dose response in terms of weight gain, FCR and PI.

Table 2 - The effect of phytase on broiler performance and toe ash during 1-21 days of age[1].

	Net gain, g	Feed Intake, g	mFCR[2]	Mortality, %	Perf. Index	Toe ash, %
Positive control	1033.0 [a]	1288.8 [a]	1.246 [b]	1.01	391 [a]	12.32 [a]
Negative control	986.0 [b]	1252.3 [b]	1.265 [a]	0.76	366 [b]	10.86 [b]
Phytase products						
A	1036.0 [a]	1303.9	1.256	0.76	389	11.44
B	1015.0 [b]	1248.3	1.262	0.67	379	11.50
C	1022.8 [ab]	1295.2	1.263	0.59	382	11.50
Phytase dose, FTU/kg						
250	1012.6 [b]	1289.1	1.270 [b]	0.67	376 [b]	11.41
500	1025.7 [ab]	1294.5	1.259 [a]	0.76	384 [ab]	11.41
1,000	1035.5 [a]	1299.8	1.252 [a]	0.59	390 [a]	11.62
Effect						
Phytase source	0.022	0.074	0.099	0.907	0.054	0.792
Phytase dose	0.012	0.454	0.000	0.907	0.004	0.067
Source x Dose	0.825	0.964	0.085	0.940	0.729	0.149

The live weight of Day 1 chicks was 46 g/b without statistical difference among treatments.
Feed conversion ratio (FCR) was corrected for mortality.
Means in the same category without bearing the same superscript letter differ ($P<0.05$).

b) Performance and carcass traits at Day 35

Table 3 presents the performance results and carcass measurements at the end of the study. While the performance of the PC group met the breeder standard, the reduction in the essential nutrients resulted in numerical decreases in feed intake, and net gain, and a significant decrease in performance index ($P < 0.05$). FCR was maintained, most likely due to a well-balanced

reduction of all major nutrients, leading to "eat less and grow less" situation and therefore similar feed efficiency to the PC group. The sub-adequate performance due to the down-spec nutrients created room for the birds to reflect the effect of additional phosphorus and nutrients released by phytases, which improved FCR in a quadratic manner as reported by Jlali et al. (2022). Weight gain, and PI were also improved ($P < 0.05$) over the NC group.

No statistical differences were observed among the phytase products in terms of performance. Phytase A yielded significantly more breast muscle ($P < 0.05$) than the other two products, with less abdominal fats ($P < 0.05$). This may be explained by a more efficient digestion and utilisation of dietary energy for protein synthesis rather than fats. On the other hand, a phytase dose response was observed as the groups receiving higher doses grew more weight, at significantly lower FCR ($P < 0.05$) and higher PI ($P < 0.05$), these results are well in line with the findings of Li *et al.* (2022) and Jlali *et al.* (2022).

Table 3 - The effect of phytase products on broiler performance of 1-35 days of age.

	Net gain, g	Feed Intake, g	mFCR[1]	Perf. Index	Breast, %	Abd. Fats, %
Positive control	2400	3446	1.432	469 [a]	26.40	1.67
Negative control	2317	3386	1.438	437 [b]	26.94	1.65
Phytase type						
A	2405	3493	1.433	460	27.22 [a]	1.61 [b]
B	2392	3459	1.433	462	26.62 [b]	1.74 [ab]
C	2377	3467	1.444	456	26.61 [b]	1.83 [a]
Phytase dose, FTU/kg						
250	2365	3473	1.445 [b]	445 [b]	26.71	1.76
500	2394	3469	1.434 [ab]	461 [a]	26.85	1.70
1,000	2415	3476	1.430 [a]	471 [a]	26.89	1.72
Effect						
Phytase source	0.530	0.463	0.074	0.661	0.009	0.000
Phytase dose	0.136	0.970	0.039	0.002	0.702	0.554
Source x Dose	0.553	0.846	0.026	0.504	0.026	0.940

Feed conversion ratio (FCR) was corrected with mortality.
Means in the same category without bearing the same superscript letter differ ($P<0.05$).

In conclusion, the results demonstrated that the supplementation of all three phytase products was effective in restoring performance and toe ash, and there are clear dose responses from 250, 500 to 1000 FTU/kg. Among the three products, Phytase A appears to have displayed more effectiveness during the starter phase, but such advantage did not last until the end of the growth cycle. Moreover, the birds fed on Phytase A produced more breast muscle and less abdominal fat.

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HYDROXY SELENOMETHIONINE CAN RESCUE BROILER GROWTH PERFORMANCE AND MEAT QUALITY UNDER HEAT STRESS THROUGH ENHANCED ANTIOXIDATIVE CAPACITY

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Summary

Heat stress (HS) brings large economic losses for the poultry industry, as HS leads to significantly impaired growth performance and meat quality in broilers. The overloaded oxidative stress triggered by HS is the main reason for such loss. In this study, 600,000 birds were used to evaluate the effect of hydroxy selenomethionine on overall growth performance and meat quality in commercial broiler farms in Central Thailand under HS conditions during summertime. The results indicated that HS significantly reduced both the growth parameters and most of the meat quality traits. However, OH-SeMet treatment at 0.03 ppm level can effectively eliminate the negative impact of HS hence and improve the profitability of commercial broiler producers.

I. INTRODUCTION

Poultry meat is the second-largest meat source with excellent quality of proteins and essential micronutrients for human nutrition globally (Pawar et al., 2016). However, the pursuit of increased meat yield, lean mass ratio and large breast muscle has had significant impacts on meat quality in broilers. Moreover, in modern commercial broiler rearing systems, high stocking density and various stress factors also inevitably lead to impaired growth performance and meat quality (Liu et al., 2020). As well as being a serious animal welfare issue, HS in broiler production can cause significant economic losses due to its negative impact on growth performance and health conditions (Liu et al., 2020). During HS, the high environmental temperature exposure has been shown to induce severe oxidative stress in key metabolic organs including muscle, liver, and intestine. It is well documented that post-mortem (PM) meat quality traits, such as pH, meat colour, water-holding capacity, and tenderness are closely related to the oxidation status in the muscles (Zhang et al., 2020). Enhancing the muscular anti-oxidative capacity can therefore maintain the PM meat quality by reducing oxidation of phospholipid of cell membranes and myoglobin, resulting in less drip loss and more favourable meat colour (Zhang et al., 2020). In past decades, many molecules were tested to protect poultry against HS, including minerals, plant polyphenols, and probiotics. Among these solutions, selenium (Se) has been shown to increase Se deposition and provide defence against oxidative stress in animals, especially broilers (Vieira et al., 2022). Basically, Se exerts its biological function by interacting with a large set (25) of selenoproteins, and consequently increase the activity of some anti-oxidative enzymes, including glutathione peroxidases and melanoproteins. In the present study, OH-SeMet, an organic Se resource, was used to validate its impact on meat quality and growth performance in commercial broilers under chronic HS.

II. MATERIALS AND METHOD

a) Animals, diet, and experimental design

A total of 600,000 broilers (ROSS 308) were allocated into two groups. All animals were reared for 42 days in a commercial broiler farm. The diets for the two group were a. Control group (CTR) fed with broiler basal feed; b. Treated group (TRT) fed with basal diet supplemented with Hydroxy-

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selenomethionine (Selisseo 0.2%, Adisseo France SAS) 150 gram/ton of feed (0.3 ppm). The selenium supplements were added to the diet via premix, and both the CTR and TRT feeds were measured for the actual Se content (Table 1). The basal diet was supplemented with a mineral-vitamin premix without selenium. The feeding trial was carried out as planned for 6 weeks from March to May 2022 in Lopburi area (central area of Thailand) to take advantage of the high temperature and high humidity environment, which is normally recorded in this region during summertime over the past few decades. Therefore, the birds used in this trial were exposed to natural HS. The barn temperature and humidity were measured by automatic temperature and humidity detector on a daily basis. Throughout the trial, the birds were exposed to at least 28.5 °C and 81.5% humidity for minimal 6 hours per day.

Table 1 - Diet formulation and nutrients.

Ingredients	Content, g/kg
Corn	638.0
Roasted soybean	300.0
$CaCO_3$	10.0
$CaHPO_4$	21.0
Salt	3.0
Choline	2.0
Mineral premix[1]	5.0
Vitamin premix[2]	0.5
Amino acid premix[3]	19.6
Nutrient composition (calculated)	
Metabolic energy, MJ/kg	13.4
Crude protein, %	18.0
Lysine, %	1.08
Methionine, %	0.69
Methionine + cysteine, %	0.92
Calcium, %	1.02
Available phosphorus, %	0.47

[1]Trace mineral premix nutrients for per kg diet: $FeSO_4 \cdot 7H_2O$, 379 mg; $CuSO_4 \cdot 5H_2O$, 31.3 mg; $ZnSO_4 \cdot 7H_2O$, 177 mg; $MnSO_4 \cdot 5H_2O$, 154 mg; and KI, 0.5 mg.

[2]Vitamin premix nutrients for per kg diet: retinyl acetate, 1500 IU; cholecalciferol, 200 IU; rac-α-tocopheryl acetate, 50 mg; menadione,5 mg; thiamine, 1.8 mg; riboflavin, 3.6 mg; calcium pantothenate, 10 mg; niacin, 35 mg; pyridoxol, 3.5 mg; d-biotin, 0.15 mg; and folacin, 0.55 mg.

[3]Amino acid premix nutrients for per kg diet: L-lysine, 4,630 mg; DL-methionine, 4,160 mg; L-threonine, 2,600 mg; L-tryptophan, 355 mg; L-isoleucine, 2,010 mg; L-valine, 2,435 mg; L-phenylalanine, 1,365 mg; and L-arginine, 2,570 mg

b) Growth performance

The final body weight, feed conversion ratio (FCR), average daily gain (ADG), performance index (PI) and mortality of broilers were measured and calculated at D42 as per the procedure in the previous report by Zhao et al. (2017).

c) Total Se concentrations

One side of the breast muscle was collected from 10 randomly selected birds in each group. Concentration of Se in the breast was measured as previously described (Zhao et al., 2017). These samples were also used to measure meat quality parameters.

d) Meat quality traits

In this trial, pH, meat colour, drip loss, thawing loss and cooking loss of breast samples as key meat quality traits were measured at different time points after slaughter. The pH was measured at 1h and 24 h post-mortem (PM) using a portable pH meter (HI9025, HANNA, Co. Italy). The meat

colour, drip loss, thawing loss and cooking loss were measured in triplicate for each breast samples and the results were obtained to determine an average value at different time points (Table 4).

e) Thiobarbituric acid reactive substance (TBARS)

2-thiobarbituric acid (TBA) levels were measured by the method adapted from previous report (Rahman et al., 2015) and a UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan) at 532nm wavelength. The TBA values were calculated from a standard curve and expressed as mg malonaldehyde per kilogram (MA/kg) for both fresh and dried samples on D1 and D7 PM.

III. RESULTS AND DISCUSSION

a) Growth performance

As shown in Table 2, humid and hot whether led to a significant drop in overall growth performance, especially for FCR in both CTR and TRT groups, demonstrating the reliability of the HS model that was set up in Thailand. However, OH-SeMet brought significant improvements for FCR ($P = 0.04$), ADG ($P < 0.01$) and PI ($P < 0.01$) in TRT compared with CTR, although we only observed an improved trend on BW ($P=0.08$) and no difference for mortality between the two groups. This is consistent with the previous report that organic selenium can rescue broiler performance under HS in both experimental and commercial rearing systems (Surai et al., 2018).

Table 2 - Growth performance in D42.

Index	CTR	TRT	SEM	P- value
BW, kg	2.64	2.90	0.09	0.08
FCR	1.612[a]	1.566[b]	0.01	0.04
ADG, g	61.34[a]	69.33[b]	1.57	<0.01
PI	372.58[a]	437.08[b]	14.11	<0.01
Mortality, %	1.932	2.048	0.37	0.87

b) Selenium content in breast muscle and meat quality

As shown in Figure 1, there was a significant increase ($P<0.01$) in selenium deposition in breast muscle from TRT compared with CTR , which is in line with the previous findings (Surai et al., 2018).

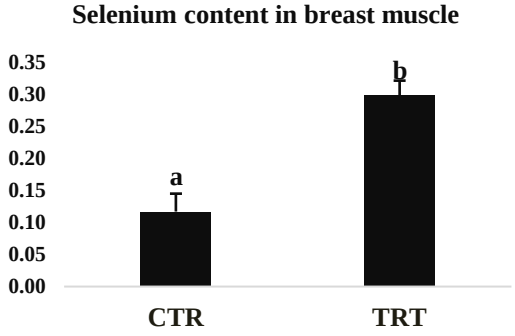

Figure 1 - Selenium content in breast muscle

As the key molecule of 25 selenoproteins, selenium with enhanced content in breast muscle can establish a stronger antioxidative capacity, and hence improve PM meat quality traits. Similarly, the loss of muscle protein caused by oxidation and over-hydrolysis during HS can also be considerably prevented by OH-SeMet, leading to better growth performance. The elevated antioxidative capacity brought by higher Se content in breast muscle was partially demonstrated by

the significant lower TBARS values (P value ranging from <0.01 to 0.04) in both fresh and dry meat samples in PM D1 and D7 (Table 3).

Table 3 - Post-mortem TBARS in both fresh and dry meat samples, MA/kg.

PM time	Meat type	CTR	TRT	SEM	P- value
D1	fresh	0.40[a]	0.32[b]	0.02	0.04
	dry	1.85[a]	1.33[b]	0.10	<0.01
D7	fresh	0.69[a]	0.58[b]	0.03	0.03
	dry	2.72[a]	2.26[b]	0.12	0.04

Impaired meat quality traits were also caused by HS. Observing the dramatically affected L value (meat lightness), 24h PM pH and drip loss (Table 4), it is clear that breast muscle in both groups had the properties to be defined as pale, soft, exudative (PSE) like meat. Together with the decreased growth performance, HS in the commercial broiler farm, even if it was relatively moderate, can lead to significant economic loss due to the reduced proportion of marketable meat, or overall value of the carcass (Zhang et al., 2020). In the current trial, OH-SeMet treatment can improve the meat quality traits as shown in Table 4. The significantly improved L value ($P < 0.01$), drip loss ($P < 0.01$), thawing loss ($P < 0.01$) and cooking loss ($P < 0.01$) can help maintain a reasonable retail price for the meat cut or broiler carcass.

Table 4 - Post-mortem meat quality traits in breast muscle samples.

Index	PM time, h	CTR	TRT	SEM	P- value
pH	1	6.56	6.63	0.04	0.37
	24	5.90[a]	6.17[b]	0.05	<0.01
L*	1	60.42[a]	57.23[b]	1.68	0.02
drip loss, %	24	4.70[a]	2.81[b]	0.29	<0.01
thawing loss, %	24	14.14[a]	12.63[b]	0.32	<0.01
cooking loss, %	24	35.56[a]	30.80[b]	0.76	<0.01

In conclusion, growth performance and meat quality of broilers reared in an extensive commercial feeding system under HS were inevitably affected. Using OH-SeMet can reduce the oxidative stress triggered by HS, thereby improving the profitability of commercial broiler producers significantly.

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EFFECT OF MULTI-ENZYME PREPARATION ON GROWTH PERFORMANCE AND ITS MATRIX VALUES VALIDATION IN BROILER

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Summary

This study used 3,900 day-old broiler birds to investigate the efficacy of a commercial multi-enzyme preparation (MEP) containing both carbohydrase and phytase, and combination of separate commercial carbohydrase and phytase at recommended doses. Both the moderate and aggressive reduced specifications (down-specs) of essential nutrients were used to evaluate these enzymes in a randomised complete block design of six dietary treatments. The results showed that the on-top application of the MEP brought significant improvements in feed intake, weight gain, feed conversion ratio and performance index ($P < 0.05$) in the corn-DDGS-SBM based diet. Also, this MEP can adequately compensate the different levels of nutrient down-specs to restore the performance of the birds to the negative control level. A reliable and practical matrix value of this MEP was therefore validated. This MEP also showed the same efficacy as the combined premium carbohydrase and phytase to eliminate the negative impact on broiler growth performance from a very aggressive down-spec scenario.

I. INTRODUCTION

Non-starch polysaccharides (NSPs) are major components of dietary fibre found in feed ingredients that consist of both soluble and non-soluble polysaccharides. NSPs possess anti-nutritive properties that can hinder bird performance and alter gut physiology. These anti-nutritive properties include high gut viscosity, encapsulation of starch and amino acids, sticky droppings, modification of gut physiology, and an overall reduction in bird performance. Feed enzymes containing multiple components targeting different NSPs can effectively eliminate these negative impacts (Woyengo and Nyachoti, 2011). In addition, another major anti-nutritional factor in the form of phytic acid (phytate) is also widely presented in cereal ingredients, representing the predominant portion (60 – 80%) of the total phosphorus. Phytate is a complex of Ca or Mg with myo-inositol and binds with phosphorus and other important nutrients and thereby decreases their availability. As an efficient enzyme to hydrolyse phytate, phytase has become an essential additive in poultry feed since the early 2000s (Dersjant-Li et al., 2015). As all plant-based feed ingredients contained protein, polysaccharides and other nutrients encapsulated in cell wall, it is meaningful to examine the interaction between carbohydrase and phytase in poultry diets. Early research in poultry suggested such interaction did exist and the degree of interaction seemed to be related to the main ingredients used in the feed formulation (Schramm et al., 2017). For instance, a study using fast growing broilers reported additional daily weight gain and improved feed conversion ratio following the supplementation with multi-NSPase (enzymes targeting on NSPs) to the corn-soya diets that already contained high dose phytase (1000 FTU/kg) (Schramm et al., 2017).

This study was designed to examine the effect of multi-enzyme preparation (MEP) containing both carbohydrase and phytase, and compared the efficacy of different phytases in the feed which already contained a premium carbohydrase. The precise estimation of matrix values of this MEP in corn-DDGS-SBM based broiler diet was also explored.

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II. MATERIALS AND METHODS

The trial was carried out at the experimental farm of PT Malindo Feedmill Tbk in Indonesia. 3900 birds (Ross 308) were divided into 6 treatments with 10 replication per treatment and 65 birds per floor pen, with randomised complete block design (RCBD). Each experimental unit contained 65 birds and the male:female ratio was close to 50:50. The following enzyme preparations were used in this trial: Rovabio® Advance Phy T (Enzyme product A) contains mainly xylanases, beta-glucanases and arabinofuranosidases combined with 6-phytase (Adisseo France S.A.S). Rovabio® T-Flex (Enzyme product B) contains mainly xylanases, beta-glucanases and arabinofuranosidases (Adisseo France S.A.S), and phytase A is a modified *Escherichia coli* 6-phytase expressed in *Trichoderma reesei* (United Kingdom). The trial design was shown in Table 1 and the standard diet specifications were shown in Table 2.

Table 1 - Diets and enzyme applications for each group.

Treatment	Diet down-specs	Enzymes
T1 as negative control	Standard diet	N/A
T2	Standard diet	Enzyme product A (100 g/mt)
T3	T1 - AME 40, dAA 2%, Ca/avP 0.16%	Enzyme product A (100 g/mt)
T4	T1 - AME 60, dAA 3%, Ca/avP 0.18%	Enzyme product A (100 g/mt)
T5	T1 - AME 80, dAA 4%, Ca/avP 0.20%	Enzyme product A (100 g/mt)
T6	T1 - AME 80, dAA 4%, Ca/avP 0.20%	Enzyme product B (50 g/mt) +Phytase A 1000 FTU/mt

A three-phase feeding program was applied in the current trial, including starter feed from 0-10 days, grower feed 11-21 days, and finisher feed 22-35 days, which was mainly based on ROSS 308 nutrition specifications. The starter feed was produced as fine crumble (1-1.5 mm), grower feed as crumble (1.8-2 mm), and finisher as pellet (3 mm). The pelleting temperature was ranging from 80 ^{o}C to 85^{o}C with conditioning time at 40 seconds. The feeds for all treatments were manufactured and packed in 50 kg bags. The main ingredients in all feeding phases contained corn, corn DDGS, and soybean meal (SBM).

Table 2 - Standard diet specification.

Parameter	Starter	Grower	Finisher
AME (kcal)	2950	3050	3150
dLysine, %	1.25	1.15	0.95
Av.	0.38	0.32	0.31
Ca, %	0.80	0.70	0.60
Na, %	0.20	0.18	0.16

Proximate analysis of feed samples from all the six groups for starter, grower and finisher diets were done at Adisseo laboratory in Singapore (Adi-lab) using NIR machine (Bruker MPA - 2I050105, Germany).

Live weight and feed intake were recorded on D10, D21 and D35 on per pen bases, and depletion was recorded daily. FCR and average daily gain (ADG) were analysed for all the three phases and throughout the whole phase from D0 to D35. Performance index (PI) was also calculated on D35.

Table 3 - Proximate values for each group.

Treatment	Crude Protein (%)			Calcium (%)			Phosphorous (%)		
	Starter	Grower	Finisher	Starter	Grower	Finisher	Starter	Grower	Finisher
T1	22.8	21.2	18.2	1.12	1.10	0.87	0.76	0.49	0.40
T2	22.3	21.1	18.1	1.12	1.08	0.87	0.78	0.50	0.37
T3	23.1	21.5	18.3	1.17	1.11	0.86	0.60	0.45	0.37
T4	22.8	21.1	19.7	1.17	1.05	0.90	0.53	0.43	0.39
T5	23.6	21.6	19.3	1.20	1.11	0.92	0.53	0.45	0.41
T6	23.8	21.5	18.6	1.22	1.08	0.89	0.53	0.44	0.39
Treatment	Ash (%)			Fat (%)			Fibre (%)		
	Starter	Grower	Finisher	Starter	Grower	Finisher	Starter	Grower	Finisher
T1	5.94	5.26	4.90	3.51	5.37	6.99	3.02	2.40	3.04
T2	5.96	5.28	4.84	2.98	5.50	6.58	3.03	2.49	2.88
T3	5.75	5.20	4.83	3.72	5.01	6.38	2.90	2.88	3.44
T4	5.61	5.13	5.04	3.98	4.77	5.19	2.83	3.15	3.30
T5	5.77	5.18	5.04	3.82	4.49	5.51	2.74	3.10	3.62
T6	5.63	5.20	4.94	3.65	4.49	6.42	2.62	3.17	3.53

III. RESULTS AND DISCUSSION

a) Growth performance in each three phases

Table 4 - Effects supplementation enzymes in broiler performance parameter in each phase[1].

Treatment	Starter 0-10 day				Grower 11-21 day				Finisher 22-35 day			
	BW, g	ADG, g/d	FI, g/d	FCR	BW, g	ADG, g/d	FI, g/d	FCR	BW, g	ADG, g/d	FI, g/d	FCR
T1	343^{bc}	29.46^{bc}	310^{ab}	0.904^{a}	769^{ab}	69.89^{ab}	1032	1.347^{bc}	1434	102.42	2518^{ab}	1.766^{ab}
T2	353^{a}	30.54^{a}	314^{a}	0.888^{b}	796^{a}	72.36^{a}	1049	1.325^{c}	1484	105.96	2578^{a}	1.752^{ab}
T3	348^{ab}	30.03^{ab}	314^{a}	0.901^{a}	784^{ab}	71.30^{ab}	1049	1.344^{bc}	1478	105.55	2539^{ab}	1.729^{ab}
T4	350^{ab}	30.16^{ab}	313^{a}	0.896^{ab}	768^{ab}	69.77^{ab}	1041	1.364^{ab}	1458	104.11	2487^{b}	1.716^{b}
T5	340^{c}	29.19^{c}	308^{b}	0.906^{a}	757^{b}	68.85^{b}	1036	1.375^{a}	1449	103.51	2550^{ab}	1.771^{a}
T6	349^{ab}	30.08^{ab}	314^{a}	0.900^{ab}	761^{b}	69.19^{b}	1050	1.387^{a}	1441	102.93	2539^{ab}	1.772^{a}
SEM	0.939	0.094	0.699	0.002	3.231	0.294	2.960	0.004	5.923	0.423	7.707	0.006
P-value	<0.01	<0.01	0.039	0.002	0.002	0.002	0.350	<0.01	0.074	0.074	0.010	0.007

The live weight of Day 1 chicks was 47.9 g/b without statistical difference among treatments.

Table 4 shows the results of key growth performance indicators for starter (0-10 day), grower (11-22 day) and finisher (22-35 day) periods. As expected, results in T1 showed that birds can reach their genetic potential in each stage compared with the breeder standard, indicating sufficient nutrients and appropriate trial management level. Using Enzyme product A as on-top application (T2) brought significant improvements for BW, ADG and FCR ($P < 0.01$) when compared with T1 in the starter period. While in the grower phase this effect tended to attenuate, until the finisher period there were almost no significant differences for all the key growth indicators observed (Table 4). The results are supported by the previous findings that 1-2 weeks after hatching the digestive systems are not well developed, and bone development requires critical nutrients supply, especially calcium and phosphorus (Sklan, 2001; Sanchez-Rodriguez et al., 2019) in poultry. As shown in Table 3, the reformulation successfully reduced the total phosphorus and crude fat level in T3 to T6, leading to the elevated risk of delaying the body development of birds. This creates the opportunity for both carbohydrase and phytase to help eliminate the anti-nutritional factors in the feed, and release more essential nutrients for birds in the early developmental stages. Also, enzyme treatments in T3 to T6 all restored the growth parameters to the T1 level, regardless of the reformulated nutrients levels and enzyme applications in all three phases. However, there was a tendency for the major reduction of all

three key nutrients in T5 and T6 to induce nutritional deficiency stress in both the starter and finisher phases, which is correlated with the highest FCR among all the treated groups. In particular, FCR in both T5 and T6 was significantly affected compared with T1 ($P < 0.01$) in the grower phase. This may be due to the imbalanced nutrients caused by the aggressive down-spec when the birds need to deposit proteins in organs, therefore reaching the limit of enzyme application to rebalance the diets.

b) Growth performance for the whole phase

As shown in Table 5, throughout the whole phase (0-35 day) on top application of Enzyme product A (T2) can also bring significant improvement for final BW ($P = 0.002$), ADG ($P = 0.002$) and FCR ($P < 0.001$) compared with T1, with no significant difference ($P = 0.35$) in feed intake. Feed utilisation was optimised by this effective MEP Enzyme product A throughout the whole growing phase, with feed intake being the same or less but with birds gaining more weight. In addition, we observed no significant difference for the growth parameters between T3-T6 and T1. However, T4 with 60 kcal/kg AME, 3% dAA and 0.18% Ca/avP nutrients reduction and Enzyme product A treatment delivered the most economical solution, showing the lowest FCR among all the treated groups. In this case, it is estimated that the optimal matrix value of Enzyme product A in corn-DDGS-SBM based broiler diet in practice can be starting from T4 down-spec and fine-tuned accordingly. Finally, T5 and T6 delivered very close results, meaning Enzyme product A can work as well as the top tier carbohydrase and phytase combination.

Table 5 - Effects supplementation enzymes in broiler performance parameters from 0-35 day[1].

Treatments	0-35 day				
	BW, g	ADG,	FCR	FI, g/d	IP
T1	2545^{b}	71.35^{b}	1.517^{ab}	1032	444
T2	2633^{a}	73.85^{a}	1.497^{b}	1049	455
T3	2610^{ab}	73.21^{ab}	1.495^{b}	1049	452
T4	2575^{ab}	72.19^{ab}	1.492^{b}	1041	447
T5	2552^{b}	71.56^{b}	1.528^{a}	1036	436
T6	2551^{b}	71.52^{b}	1.528^{a}	1050	438
P-value	0.002	0.002	<0.001	0.350	0.470
SEM	8.104	0.232	0.003	2.960	3.080

The live weight of Day 1 chicks was 48 g/b without statistical difference among treatments.

In conclusion, the results demonstrated Enzyme product A was able to reverse the negative effect of both moderate and aggressive reformulations in broiler diets. Also, the carbohydrase and phytase in Enzyme product A had a clear synergetic effect to release more nutrients in broiler feed thus reducing feed cost. A reliable matrix value of Enzyme product A was also validated and recommended as a solid basis for its practical application in broiler feed.

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SUPPLEMENTATION OF CARBOHYDRASES AND BUFFERED FORMIC ACID TO BROILER DIETS BASED ON WHEAT OR MAIZE

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The formation of a viscous gut content in the small intestine due to the presence of non-starch polysaccharides in feed can markedly restrict efficient nutrient digestion in poultry. This adverse effect may be mediated through the supplementation of exogenous feed additives. The present study investigated the effects of carbohydrases and a buffered formic acid on growth performance and ileal digesta viscosity in broiler chickens fed a wheat- or maize diet. A 2 × 4 factorial arrangement of treatments with eight replicates per treatment was conducted using 640 Cobb 500 mixed-sex broiler chicks. Factors were diet base: wheat or maize; supplement: xylanase (5,600 TXU/g)/β-glucanase (16,000 TGU/g) mixture (XG), mannanase (M;8,800 TMU/g) or buffered formic acid (FA; 0.42%). On day 21, ileal digesta was collected from 24 birds per treatment (4 birds per pen; 6 replicates), and the digesta viscosity was measured. Overall (d 0 – 21), birds fed the wheat diet were heavier (P = 0.010) than those fed the maize diet, and the maize-based diet resulted in a lower FCR than the wheat-based diet (P < 0.001). All feed additives improved (P < 0.001) weight gain, with no significant difference between the additives. Supplements M and FA improved (P < 0.001) overall FCR compared to the control. A two-way diet × supplement interaction occurred for ileal digesta viscosity at d 21 (P < 0.001). A marked reduction of ileal digesta viscosity due to supplement XG was observed in birds fed the wheat-based diet whereas none of the feed additives altered the viscosity in birds fed the maize-based diet. Collectively, although supplement XG was only effective in reducing digesta viscosity when supplemented in the wheat-based diet, all supplements improved growth performance independent of diet type. This suggests the presence of a mechanism besides viscosity reduction. Understanding the relative contributions of different feed additives in birds fed diets based on viscous and non-viscous grains would further help to develop tailored feeding strategies when multiple additives are used in a single feed formulation.

ACKNOWLEDGEMENT: This study was funded by BASF SE.

Table 1 - Effects of feed additives on overall growth performance (d 0 – 21) and ileal digesta viscosity at d 21 in broiler chickens fed wheat- or maize-based diets.

		d 0 - 21		Ileal digesta
Diet type	Supplement	Weight gain, g	FCR, g/g	viscosity, mPa·s
Wheat	Control	967	1.47	3.32^{ab}
	Xylanase/β-glucanase (XG)	1,013	1.42	2.39^{d}
	Mannanase (M)	1,005	1.40	3.15^{abc}
	Buffered formic acid (FA)	1,016	1.38	3.50^{a}
Maize	Control	931	1.37	2.59^{d}
	XG	989	1.29	3.13^{abcd}
	M	973	1.27	2.93^{bcd}
	FA	963	1.27	2.72^{cd}
Main effect				
Diet type	Wheat	1,000^{a}	1.42^{b}	3.09
	Maize	964^{b}	1.30^{a}	2.84
Supplement	Control	949^{b}	1.42^{b}	2.96
	XG	1001^{a}	1.36^{ab}	2.76
	M	989^{a}	1.34^{a}	3.04
	FA	989^{a}	1.33^{a}	3.11
SEM		4.9	0.006	0.082
Probability				
Diet type		0.010	<0.001	0.230
Supplement		<0.001	<0.001	0.193
Diet type × supplement		0.648	0.837	<0.001

$^{a-d}$Different superscript letters in a column indicate statistical differences (P < 0.05)
SEM; standard error of the mean

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DIETARY SUPPLEMENTATION OF VALERIN AND BUTYRIN IMPROVES THE GROWTH PERFORMANCE OF BROILERS

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Tri-butyrin is regarded as an ideal molecule to supply both butyrate and mono-butyrin to the small intestine (SI) to maintain intestinal function, modulate the immune response and improve broiler growth performance (Moquet et al., 2016; Moquet et al., 2018). Valerins have been shown to positively affect the morphology of the SI mucosa and reduce the incidence of necrotic enteritis (Onrust et al., 2018). To date, little information is available as to the extent to which valerin and butyrin in combination affect the morphology of the SI and improve broiler performance with or without antibiotic supplementation. This study was conducted to evaluate the effects of a synergic combination of valerin and butyrin (Gastrivix™ Avi) with or without antibiotics using a total of 3520 as-hatched d-old chicks (Vencobb 430Y). These additives were added to the corn/soybean meal-based diet individually or in combination at various doses. Four dietary treatments (eight replicates/treatment with 110 birds/replicate) were: T1: Basal diet control; T2: T1 + Bacitracin Methylene Disalicylate (BMD; 500 g/MT throughout all the phases); T3: T1 + valerin/butyrin combination (500, 500 and 250 g/MT in starter, grower and finisher phases, respectively) and T4: T2 + valerin/butyrin combination (500, 500 and 250 g/MT in starter, grower and finisher phases, respectively). Feed refusal and body weight of birds were measured every week until d42. On d14, 28 and 42, eight birds (one bird/replicate) were randomly selected from each pen and euthanised for jejunum and ileum morphology analysis.

Table 1 - d1 to d42 performance of broilers fed different diets and d42 Jejunum and Ileum Villi length and crypt depth (data expressed as mean ± standard deviation).

Treatment	FI (kg/bird)	BWG (kg/bird)	FCR	Mortality (%)	Jejunum villi length (µm)	Ileum villi length (µm)
T1	3.63^{a}±0.120	2.14^{a}±0.079	1.70^{a}± 0.054	5.9^{a}±3.15	1504.5^{a}±2.04	998.1^{a}±1.35
T2	3.68^{a}±0.103	2.24^{b}±0.055	1.64^{b}±0.024	2.5^{b}±1.44	1549.2^{b}±2.23	1008.9^{b}±1.66
T3	3.80^{b}±0.051	2.32^{c}±0.042	1.64^{b}±0.040	1.6^{b}±1.64	1631.5^{c}±2.78	1078.2^{c}±1.89
T4	3.84^{b}±0.031	2.51^{d}±0.045	1.53^{c}±0.036	1.6^{b}±1.59	1699.1^{d}±2.55	1100.8^{d}±3.98

Means within a column not bearing a common superscript are different ($P<0.05$)

Results showed that, during the entire trial period (d1-42), compared to birds offered T1 diet, BMD supplementation alone (T2) reduced FCR by 6 points and improved BWG by 0.1 kg. Birds fed diet containing valerin/butyrin combination alone (T3) had 6 points lower FCR and improved BWG by 0.18 kg. Compared to the BMD supplemented group (T2), valerin/butyrin combination in combination with BMD (T4) lowered FCR by 11 points and increased BWG by 0.27 kg. In conclusion, under the current trial management condition, valerin/butyrin combination at the suggested dosage performed as well as an in-feed BMD in sustaining bird feed utilisation efficiency and growth performance. Combining valerin/butyrin combination with BMD further improved growth potential and feed efficiency of birds. Such an effect was achieved by the improved morphology of SI, likely with more efficient nutrient absorption.

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XYLO-OLIGOSACCHARIDES IMPROVE FEED EFFICIENCY IN BROILER CHICKENS BY SLOWING DOWN JEJUNUM CELL ACTIVITY/TURNOVER

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Xylo-oligosaccharides (XOS) are xylose-based oligomers, which have been described to affect the gut microflora, and gut function in chickens (Zhou et al., 2021). This, in turn, may have an impact on intestinal epithelial cell turnover, which accounts for a significant part of the energy of maintenance required by the gastrointestinal tract. The aim of this study was to evaluate the effect of dietary XOS on gut function and epithelial cell turnover in broiler chickens. It was hypothesized that XOS would improve gut health and reduce maintenance requirements associated with cell renewal in the small intestine.

A total of 128 one-day-old broiler chickens were assigned to a corn/soy-based mash diet with or without supplementation of XOS (0.5%). Each treatment was randomly distributed across eight pens (n=8). Performance parameters were recorded weekly. On day 42, one chicken per pen was euthanizsed and jejunum samples collected for proteomic analysis. Samples were processed with FASP and SWATH methods. Performance data was analyzed using a t-test in R (RStudio, Inc., USA). Proteomic data was analyzed using MSstats in R. Functional enrichment analysis was performed in DAVID Bioinformatics Resources. The results showed that XOS supplementation improved feed efficiency ($P < 0.05$) from day 1 to 42 compared to the control group (1.73 ± 0.01 vs 1.82 ± 0.04). During that period, there were no significant differences ($P > 0.05$) between XOS supplemented and control group for ADG (58.1 ± 0.87 vs 57.4 ± 0.69) and ADFI (100.6 ± 1.6 vs 104.5 ± 2.2). The proteome analysis uncovered 346 differentially abundant proteins (DAP) associated with XOS supplementation, of which 310 showed a significant ($P < 0.05$) lower abundance. These DAP translated into decreased activities in several biological pathways involved in cell migration and energy metabolism in the jejunum, including a lower actin filament-based movement, glycolysis, and gluconeogenesis (Table 1). Overall, decreased cell metabolism together with improved feed efficiency indicated a lower maintenance requirement reflecting slower epithelial cell turnover.

Table 1 - Proteins showing significantly (P<0.05) decreased abundance (↓) and downregulated associated biological pathways in the jejunum of chickens fed a dietary supplement of 0.5% xylo-oligosaccharides.

Database	Pathway	*P* value	Proteins
Gene Ontology	Gluconeogenesis	0.0020	↓ TPI1, GOT2, FBP1, GPD2, PGK1
	Actin filament polymerization	0.0000	↓ RAC1, ARPC4, COTL1, VIL1, GSN, CTTN
	Actin filament-based movement	0.0191	↓ MYH9, MYO6, MYO1A
Kyoto Encyclopedia of Genes and Genomes	Citrate cycle (Tricarboxylic acid cycle)	0.0000	↓ PDHB, OGDH, IDH1, ACO1, MDH2, IDH3B, IDH2, ACO2
	Glycolysis / Gluconeogenesis	0.0016	↓ PDHB, LDHA, TPI1, PKM, FBP1, AKR1A1, PGK1, ADH1C

In conclusion, dietary XOS supplementation improves feed efficiency by reducing cell activity (interpreted as lower cell turnover) in the gut epithelia in broiler chickens.

ACKNOWLEDGEMENTS: This study has been partially funded by AB Vista.

Zhou J, Wu S, Qi G, Fu Y, Wang W, Zhang H & Wang J (2021) *Anim Nutr*. **7**: 152-162.

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ORDER OF LIMITING AMINO ACIDS IN WHEAT-SORGHUM BASED REDUCED PROTEIN DIETS FOR LAYING HENS

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Reduced protein (RP) diets have received increasing interest in poultry nutrition due to the potential benefits in feed cost and environmental footprint (Liu et al., 2021). Understanding the order of limiting amino acids (AA) in RP diets is critical to ensure requirements are met cost effectively. To date, only the first three limiting AA (including lysine [Lys], methionine [Met], and threonine [Thr]) have been demonstrated in RP layer diets, while the order of the next most limiting AA is still controversial. Thus, it is unclear which AA should be given priority when formulating RP diets. Therefore, the limiting order of eight crystalline AA in wheat-sorghum-based RP laying hen diets were determined.

A total of 330 Hy-Line Brown laying hens were randomly assigned to 11 dietary treatments (30 replicates of a single bird per treatment) from 20 to 39 weeks of age. The first treatment (control diet) was a standard protein (SP, 17% crude protein) diet with a sufficient level of all AA and other nutrients according to the nutritional recommendations of Hy-Line Brown laying hens. The second treatment was an RP diet (15% crude protein) containing sufficient levels of Lys, Met, and Thr and deficient levels of eight essential AA (EAA) including tryptophan (Trp), valine (Val), isoleucine (Ile), arginine (Arg), leucine (Leu), histidine (His), phenylalanine (Phe), and glycine$_{equivalent}$ (Gly). The third treatment contained sufficient levels of Lys, Met, and Thr and sufficient levels of eight essential AA (EAA). Then, each of these eight EAA was individually deleted from the RP-EAA diet by removing its supplemental level to generate treatments 4 to 11, following the deletion method described by Fernandez et al. (1994). Mash feed and water were provided *ad libitum* throughout the study. Eggs were collected and weighed daily, feed consumption recorded weekly and feed conversion ratio (FCR) calculated. Egg quality traits including egg shape index, eggshell reflectivity, eggshell breaking strength, eggshell thickness, albumen height, yolk index, yolk colour, and Haugh unit were measured at 29 and 39 weeks. Data were analysed using IBM SPSS statistical software with significance at 0.05.

The limiting order of AA were ranked based on egg mass and FCR, due to the commercial importance of these parameters. Hens fed the RP diet and RP diet deficient in Val or Ile had significantly lower egg mass (mean ± SEM: 53.7 ± 0.28 g, 54.0 ± 0.28 g, and 53.9 ± 0.28 g, respectively) compared to hens fed the SP diet (mean ± SEM: 57.5 ± 0.28 g) and RP diets deficient in His (mean ± SEM: 57.7 ± 0.28 g) and Gly (mean ± SEM: 58.4 ± 0.28 g) ($P < 0.001$). Moreover, hens fed the RP diet and RP diet deficient in Val had a higher FCR (mean ± SEM: 2.38 ± 0.01 and 2.33 ± 0.01, respectively) compared to those offered the RP-EAA diet (mean ± SEM: 2.20 ± 0.01) and RP diets deficient in Leu (mean ± SEM: 2.21 ± 0.01), Phe (mean ± SEM: 2.21 ± 0.01) and Gly (mean ± SEM: 2.19 ± 0.01) ($P < 0.05$). FCR was not affected by deficiency of Trp, Ile, Arg and His in RP diet (mean ± SEM: 2.26 ± 0.01, 2.31 ± 0.01, 2.26 ± 0.01, and 2.27 ± 0.01, respectively) compared to SP diet (mean ± SEM: 2.24 ± 0.01) ($P > 0.05$). Egg quality traits were not different between the dietary treatments at both 29 and 39 weeks (all $P > 0.05$).

Thus, for egg mass, Ile was the fourth and Val the fifth limiting AA after Lys, Met and Thr in laying hens fed on wheat-sorghum based RP diets. For FCR, Val was the fourth limiting AA and Trp, Ile, Arg and His may be considered as co-fifth limiting AA in wheat-sorghum based RP diets for laying hens. Overall, it may be concluded that Val and Ile are the most important AA, after Lys, Met and Thr; while Leu and Gly are the least important AA, in wheat-sorghum based RP diets for laying hens.

ACKNOWLEDGMENTS: The authors would like to acknowledge and thank Poultry Hub Australia for funding this project and their guidance, encouragement and support.

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EVALUATION OF MICROBIAL MURAMIDASE SUPPLEMENTATION ON THE PERFORMANCE OF LAYING HENS

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Summary

The cell walls of bacterial residues in the GIT, contain peptidoglycans (mainly Muramic acid). These residues can interfere with normal absorption in the gut. Muramidase cleaves the β-1,4-glycosidic linkages between N-acetylmuramic acid and N-acetyl glucosamine, the basic elements in the carbo-hydrate backbone of PGN from bacterial cell walls (Sais, et al., 2020). The current study evaluated a microbial muramidase on the performance of hens (Muramidase 007, DSM Nutritional Products). It was carried out at the Center of Teaching, Research and Extension in Poultry Production of the Veterinary College of the National University of Mexico in Mexico City, Mexico (16°C annual temperature average and 60% relative humidity). Five hundred and twenty-eight one-day-old Bovans pullets were randomly assigned to 5 experimental treatments. During production, 8 groups of 12 birds per treatment were evaluated. Four corn-soybean meal experimental diets were produced without (control diet -CD) and with different dosages of muramidase: low (LD 25,000 LSU (F)/Kg), medium (MD 35,000 LSU (F)/Kg), High (HD 45,000 LSU (F)/Kg) and low (L95 25,000 LSU (F)/Kg with 95% of the requirements for energy and protein. Diets were fed from the one-day-old until 32 w. The pullets were raised in cage and transferred to conventional cages at 16 weeks of age. All the birds received a simultaneous Newcastle vaccination at 10 days and 8 weeks old. The light program was established to 16 hrs. of photoperiod. Data were subjected to ANOVA analysis using the GLM procedure, and the means were separated by Tukey´s test. During the rearing period (wk 1-18) no differences were observed among treatments. The main results are shown in Table 1 for the whole production period. period with no significant differences (P>0.05) among treatments. In production, the L95 treatment showed a higher egg production in nine of the 13 weeks (19-32 wk) evaluated (P≤0.05), but not for the overall period. In the whole experiment the CD treatment showed a higher feed consumption consistently, being statistically higher in 5 experimental weeks, but not overall. In conclusion, the use of a muramidase at low level, allowed the dilution of nutrient density in the diet, without deleterious effects on production parameters in Bovans laying hens.

Table 1 - Effect of a microbial muramidase on the performance of laying hens (19-32 weeks).

Treatment	Control	LD	MD	HD	LD95	MSE
Dose (MU/Kg)	0	25000	35000	45000	25000	
% Nutritional requirement	100	100	100	100	95	
ADFI, g	105.3	100.1	101.2	102.2	105	1.73
FCR	2.41	2.37	2.33	2.30	2.33	0.047
Egg mass (g/d)	40.3	38.8	39.8	40.7	41.62	1.15

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EFFECTS OF BUTYRATE, BUTYRIC GLYCERIDES IN COMBINATION WITH DIFFERENT SELENIUM SOURCES AS STRATEGIES TO IMPROVE PERFORMANCE OF THE AGED LAYING HENS

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In recent years, the egg industry is aiming to prolong the life cycle of laying hens, both for reasons of profitability and for sustainability. However, the goal to produce 500 eggs in a 100-week cycle is hindered by the declining performance of older hens. It is hypothesised that nutritional strategies can be implemented to support the health of these animals, thereby improving laying persistency. The objective of this trial was to investigate the effects of two sources of butyrate (precision delivery-coated sodium butyrate and butyrate glycerides, Adisseo France) either with or without the addition of an organic selenium (OH-SeMet, Adisseo, France) on the performance of aged laying birds. 900 forty-five-week-old Hy-Line brown hens with similar performance were randomly allocated to five treatment groups with 10 replicates of 18 animals each. The experiment lasted 20 weeks. The five experimental diets were based on commercial feeds supplemented with 0.3 mg/kg of sodium selenite (Control), and with 240 mg/kg butyric acid, either as coated butyrate (a 24% butyric acid product) (T2), or as butyric glycerides (a 40% butyric acid product) (T3). In treatments 4 and 5, 0.3 mg/kg of Se from OH-SeMet was used, combined with the coated butyrate (T4) or with the butyric glycerides (T5), both at 240 mg/kg butyric acid. Data was evaluated using a one-way analysis of variance (ANOVA). Significant differences were considered at $p \leq 0.05$, and a Tukey test was used to compare separated means. Results showed that the supplementation with the different nutritional strategies supported laying performance in a T5 > T4 > T3 > T2 > Control trend (Figure 1a), with T4 and T5 performing significantly better than the Control. Compared to the Control group, the feed/egg weight ratio was improved by supplementation of butyrate-based products: we observed a numerical improvement for T2 and a significant difference in T3. This effect was further increased with the additional inclusion of OH-SeMet in the diet, as shown on Figure 1b. No significant difference in performance was detected between the dietary butyrate treatments. However, as these two additives will release butyrate in different parts of the digestive tract, we speculate that distinct modes of action underly their beneficial effect on performance. In summary, this trial adds evidence to the potential of butyrate additives, alone or in combination with OH-SeMet, as effective nutritional strategies to support a longer laying cycle.

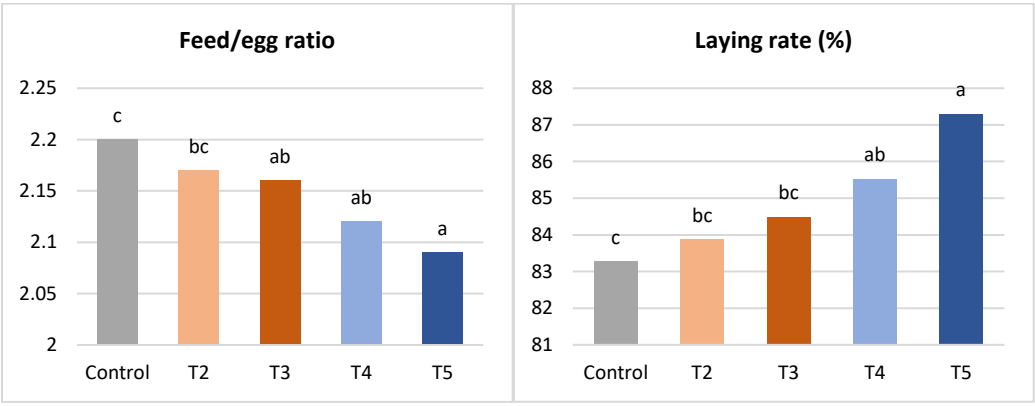

Figure 1 - (a) Laying rate of birds fed one of the 5 treatments ($p < 0.05$). (b) The effect of the different treatments on FCR of layers.

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EFFECT OF HIGH *BUTTIAUXELLA* PHYTASE DOSE SUSTAINS PERFORMANCE AND BONE QUALITY OF LAYING HENS FED NUTRIENT COMPROMISED DIETS

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Summary

The present experiment was conducted in order to evaluate the effect of increasing dose rate of a *Buttiauxella* phytase to 600 FTU/kg feed in diets of laying hens from 24-80 weeks of age. The inclusion of higher levels of phytase in diets reduced in energy, minerals and amino acid requirements, maintained performance, eggshell quality and bone quality of laying hens compared to a typical commercial laying hen diet containing a 300 FTU/kg dose of the same phytase.

I. INTRODUCTION

The inclusion of alternative ingredients such as rice bran, DDGs and sunflower meal in layer diets, to reduce feed cost, can result in higher phytate levels, potentially limiting availability of minerals, protein and energy, all of which are important to maintain egg production, eggshell strength and bone strength (Bello and Korver, 2019). Phytase enzymes are commonly used in the animal feed industry primarily to increase phosphorus (P) availability from plant ingredients. Additionally, phytase enzyme improves availability of non-P nutrients like Ca, amino acids (AA), and energy (AME) by facilitating the breakdown of phytate P and thus negating its antinutritional effects (Dersjant-Li *et al.*, 2015). As phytase enzyme can increase the availability of phosphorus, calcium, sodium, amino acids, and energy content of diets, these nutrients may be decreased in the feed formulations as 'matrix values' without adverse effects on the bird's performance. This will reduce the feed costs while maintaining the same level of performance. Currently, a dose level of 300 FTU/kg feed of phytase enzyme is often used in commercial laying hen diets. Increasing the dose of phytase would reduce feed costs further due to higher matrix values but there have been concerns over whether this can be done without compromising laying hen performance. A trial was conducted to determine the efficacy in laying hens of higher inclusion levels of phytase with corresponding matrix application.

II. METHODS

A total of 180 Lohmann LSL Lite hens (20 replicates/treatment, three hens/cage) were distributed into four treatment groups at 24 weeks of age. Distribution was a completely randomized design to achieve uniform bodyweight across the treatments at the beginning of the feeding trial. Diets were corn/soybean meal based and fed as mash, *ad libitum*. A pullet/grower adaptation diet supplemented with a *Buttiauxella* phytase (Axtra® PHY, Danisco Animal Nutrition) at 300 FTU/kg was fed from 12-23 weeks of age. During the laying stage, the hens were maintained on two diets formulated based on common commercial practices: phase 1 (24-50 weeks of age) and phase 2 (51-60 weeks of age). The positive control (PC) diet contained 3.8% Ca and 0.42% available P (AvP) during phase 1 and 4.0% Ca and 0.41% AvP during phase 2 (the values included the matrix contribution from the phytase at the given dose which was equivalent to 0.17% Ca, 0.16% AvP, 58 kcal/kg AME and 0.35% crude protein)

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and was supplemented with a *Buttiauxella* phytase at 300 FTU/kg. The PC diet was reformulated to generate the negative control (NC) diet in which the AvP and Ca contents were decreased by 0.19%, and 0.2% respectively along with a 63.5 kcal/kg reduction in AME and a 0.65% reduction in CP content, compared to the breeder recommended levels. The NC diet was fed either as such or was supplemented with the phytase at 600 FTU/kg. Egg production and feed intake were measured daily and weekly respectively, and all eggs were collected from each replicate cage to determine egg weight at weekly intervals. Every 4 weeks, 4 eggs were randomly selected from each cage to determine eggshell breaking strength. At 80 weeks of age, 1 bird per cage was euthanized and the tibia were collected for morphometric analysis (diaphysis length and mid shaft diameter) and determination of tibia ash. Data was analysed across age and treatment by analysis of variance. Means were compared using ANOVA and Tukey's test (JMP, SAS software). Significance was taken as $P<0.05$.

Table 1 - Nutrient down spec in the experimental diets with varying phytase dose.

Treatment	Downspec			
	AvP (%)	Ca (%)	AME (kcal/kg)	CP (%)
PC* + *Buttiauxella* phytase at 300 FTU/kg	0.16	0.17	58	0.35
NC**	0.19	0.20	63.5	0.65
NC** + *Buttiauxella* phytase at 600 FTU/kg	0.19	0.20	63.5	0.65

* PC diet formulated to be reduced according to 300 FTU/kg matrix vs breeder recommendations and supplemented with *Buttiauxella* phytase at 300 FTU/kg.
** NC diet formulated to be reduced according to 600 FTU/kg matrix vs PC diet.

III. RESULTS

Feeding the NC diet with reduced nutrient specifications significantly decreased ($P < 0.05$) hen-day production (HDP) by 3.4 % points, egg weight by 1.6% and egg mass by 5.2% versus the positive control fed birds. Feeding the NC diet also significantly increased ($P < 0.05$) feed intake by 1.8% and FCR by 13 points versus the positive control across the trial period of 24-80 weeks (Table 2). Supplementing the NC diet with the phytase at 600 FTU/kg significantly improved ($P<0.05$) all the above-mentioned performance traits to the levels obtained with the PC diet. With the PC diet the feed cost/dozen eggs was $0.43 which decreased to $0.42 when the phytase supplemented NC diet was fed (Table 2). Egg breaking force was significantly ($P<0.05$) reduced by 10% when birds were fed the un-supplemented NC diet compared to the PC (Table 3). The addition of *Buttiauxella* phytase at 600 FTU/kg to NC significantly ($P<0.05$) improved egg breaking force by 9.4% back to the level of the PC. Feeding the NC significantly ($P<0.05$) decreased tibia length, diameter and ash by 8mm (7%), 0.45 mm (8.3%) and 5.5 % points respectively versus the PC. Supplementing the NC diet with 600 FTU phytase significantly improved tibia length by 7mm (6.6%) compared to the NC, and numerically improved tibia diameter and ash by 0.25 mm (5.1%) and 4.2 % points respectively versus the NC. All tibia parameters were restored to the level of the PC ($P>0.05$) with supplementation of the NC with 600 FTU phytase.

Table 2 - Performance of laying hens fed diets containing varying phytase dose.

	PC*	NC	NC + *Buttiauxella* phytase at 600 FTU/kg
Hen-day egg production (%)	95.3[ab]	91.9[c]	96.0[a]
Egg weight (g)	60.8[a]	59.8[b]	61.0[a]
Egg mass (g/bird/day)	58.0[a]	55.0[b]	58.5[a]
Feed intake (g/bird/day)	97.3[b]	99.1[a]	96.6[b]
FCR (g feed: g egg)	1.68[b]	1.81[a]	1.65[b]
Feed cost /dozen eggs ($)**	0.43	0.45	0.42

[abc] Values without a common superscript are significantly different (P < 0.05).
*PC diet formulated to be reduced according to 300 FTU/kg matrix vs breeder recommendations and supplemented with *Buttiauxella* phytase at 300 FTU/kg.
**Feed cost/dozen eggs ($) based on representative 2021 US feed cost.

Table 3 - Egg breaking force and tibia quality.

	PC*	NC	NC + *Buttiauxella* phytase at 600 FTU/kg
Egg breaking force (kgF)	4.96[a]	4.46[b]	4.88[a]
Tibia length (mm)	114[a]	106[b]	113[a]
Tibia diameter (mm)	5.40[a]	4.95[b]	5.20[ab]
Tibia ash (%)	57.5[a]	52.0[b]	56.2[ab]

[ab] Values without a common superscript are significantly different (P < 0.05).
*PC diet formulated to be reduced according to 300 FTU/kg matrix vs breeder recommendations and supplemented with *Buttiauxella* phytase at 300 FTU/kg.

IV. DISCUSSION

As expected, diluting the NC diet by 0.19% AvP, 0.20% Ca, 63.5 ME kcal/kg and 0.65% CP, and only supplementing 300 FTU of phytase, decreased performance of laying hens for parameters including HDP, eggshell quality and tibia ash. Supplementing the NC diet with 600 FTU/kg phytase restored all the above-mentioned performance traits to the level of that obtained with the PC group. This is in agreement with earlier findings (Martinez Rojas et al., 2018) that showed performance of birds fed diets reduced in available P could be maintained with phytase supplementation. Dersjant -Li et al., 2022 also demonstrated using a meta-analysis of 13 digestibility studies that phytase gives consistent improvements in amino acid digestibility. One of the important factors that governs the productive performances of laying hens is the Ca to AvP ratio. Previous studies (Augspurger et al., 2007; Meyer and Parsons, 2011; Kim et al., 2017) have shown that increasing the phytase activity in the diet did not show any beneficial effect on egg production traits in laying hens and the most plausible reason for the productive traits being refractory was the altered Ca to AvP ratio. It is postulated that owing to the antagonistic effects that Ca exerts on availability of dietary P when the Ca to AvP ratio widens it mutes the effects of phytase and this was the case in the earlier reported experiments. In the present study, both AvP levels and dietary Ca levels were decreased according to the phytase matrix in both the PC and the NC groups, helping to maintain the Ca to P ratio. This approach facilitated a more efficient utilization of dietary P. Jing et al., 2021 found that performance of laying hens could be maintained with dietary AvP as low as 0.1% if the diet was fortified with 1000 FTU/kg of a phytase. Increasing the dose of phytase should facilitate more complete breakdown of phytate P thus releasing greater quantities of entrapped nutrients (amino acids, trace minerals and starch), reducing the anti-nutritional effects of phytate P and improving the productivity of the hens fed with the phytase supplemented NC diet. In the current study tibia ash content and the eggshell breaking strength of the birds fed the phytase

supplemented NC diet were comparable to those of layers fed the PC diet. Supplementation of 600 FTU/kg phytase to the NC diet significantly improved the mineral matrix of eggshell and tibia which could not be possible unless the utilization of efficiency of Ca and P was increased.

V. CONCLUSIONS

The study demonstrated that it was possible to sustain egg production, FCR and eggshell quality of laying hens with diet containing as low as 0.22% AvP when supplemented with 600 FTU/kg *Buttiauxella* phytase from 24-80 weeks of age. This approach not only helped in bringing down feed cost significantly but should also be viewed as a potential tool to decrease the usage of inorganic P in poultry diet to bring down P excretion into the environment. Dietary inclusion of *Buttiauxella* phytase was able to replace up to 0.19% AvP, 0.20% Ca, 63.5 ME kcal/kg and 0.65% CP in laying hen diets, reducing feed cost per dozen eggs, whilst maintaining performance.

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IMPACT OF THE LIGHTING AND FEEDING REGIMEN DURING REARING ON HY-LINE BROWN PULLET GROWTH AND START OF LAY.

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Summary

The impact of two lighting and three feeding programs during rearing on pullet body weight (BW), feed intake (FI), organ characteristics and start of lay was evaluated. Nine hundred Hy-line Brown chicks were housed in floorpens from day old to 16 weeks of age (WOA) under two lighting programs i.e. standard lighting (SL) of 10h Light (L)/day from 7 WOA or, rapid light reduction (RLR) of 9h L/day from 4 WOA. Feed was provided *ad libitum* until 4 WOA, then three feeding regimens i.e. *ad libitum*, feeding to Hy-Line Brown breed standard weight (BSW), and feeding to 88% BSW(Managed) for age were introduced into each lighting program and continued to 16 WOA. Hen BW and FI were measured from 4-16 WOA. At 16 WOA a subset of pullets were sampled for carcass and reproductive tract assessment. Remaining pullets were then housed individually in a caged layer facility, with *ad lib* feeding under increasing photoperiod. Hen FI, BW, age of first egg and average weight of the first 3 eggs was recorded. From 4-16 WOA BW differed due to an interaction of feeding and lighting with *ad lib* fed birds being the heaviest and Managed feeding birds lightest. *Ad lib* FI was higher in SL compared to RLR. In the layer shed birds from Managed feeding during rearing had higher FI during 16-17 WOA, but *ad lib* fed hens sustained highest BW and, Managed feeding lowest BW through to 19 WOA. Feeding *ad lib* and to BSW during rearing generated higher breast muscle scores and keel curvature. Ovary and oviduct development were most advanced at 16 WOA with *ad lib* feeding during rearing. Pullets fed *ad lib* under RLR started to lay eggs first and, Managed feeding under SL were the last to start laying. The average weight of first three eggs was highest in both SL and, with feeding to BSW. Hence lighting and feeding programs during rearing altered FI, BW and age at point of lay in current day brown pullets. The hens will be followed through to 100 WOA to determine the effects of lighting and feeding during rearing on egg production, egg quality and hen health.

I. INTRODUCTION

A hen's physiological patterns including FI and BW trajectory are established by early lay (Muir et al. 2022). Therefore, management tools such as lighting and feeding programs during rearing may offer opportunities to regulate bird size and establish feeding habits by the end of rearing. This may also influence persistency of lay, egg quality and hen health during an extended laying period. The lighting program during rearing can modify bird age at sexual maturity, the number of eggs produced and egg size (Santiago-Anadón and Latorre-Acevedo, 2004). For example, compared to a standard lighting (SL) program, more rapid light reduction (RLR) provides fewer hours of light/day, which slows chick growth and, on light stimulation, initiates earlier sexual maturity (Arango et al., 2007). Hens reared under RLR also produced more eggs of larger size at 66 WOA. While bone mineralization did not differ (Hester et al. 2011), other features of egg quality and bird health were not reported but are worthy of evaluation.

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Comparison of feeding programs during rearing undertaken during the early stages of intensification of egg production show that managing feeding can impact number of smaller eggs produced during early lay, the rate of decline in egg production from peak lay and bird mortality (Lee et al. 1971; Balnave, 1973). Such features have not been evaluated in current day Brown egg laying hens. As the industry explores extending layer hen production cycles these management options require re-evaluation in today's layer hens. This experiment compared Hy-Line Brown pullets that had been reared under two lighting and three feeding programs at point of lay.

II. MATERIALS AND METHODS

Nine hundred, Hy-line Brown day-old chicks, were placed in groups of 30 in floorpens (7 m^2) at the Zootechny research facility, Austral, NSW, Australia. All chicks were beak trimmed and vaccinated for Newcastle Disease, Infectious Bronchitis and Marek's Disease Virus. Each pen had a perch, automatic nipple drinkers and manually filled feed hoppers. The shed was brooded with space heaters, and had side curtains, foggers, and dimmable lights with photoperiod control for each end of the shed. A light proof curtain traversed the centre of the shed separating the two lighting treatments, with 15 pens per side. All birds were held under intermittent lighting during the first week (4hLight(L):2hDark(D)) then 20hL:4hD in the second week. For RLR the photoperiod was reduced as 16hL:8hD, then 12hL:12hD and finally held at 9hL/day from 4-16 WOA. Under SL program 20hL:4hD was maintained through to 3 WOA then reduced gradually to reach 10hL:14hD by 7 WOA, which was maintained through to 16 WOA. The study was a 2 × 3 factorial arrangement of 2 lighting and 3 feeding programs. All birds were fed *ad libitum* until 4 WOA when three feeding programs of five pens per lighting treatment were introduced. The three feeding regimes were: *ad libitum* feeding; feeding to achieve breed standard weight (BSW); and feeding to achieve 88% breed standard weight (Managed) for age (Lee et al. 1971). . All birds receive the same commercial crumble pullet starter (0-5 WOA) and grower (5-12 WOA) (Barastoc, Australia), then a Developer mash (12-16 WOA). From 16-17.4 WOA all pullets received a Pre-lay mash and then an Early lay mash both being fed *ad libitum*. All birds were weighed individually from 4-16 WOA. Based on their BW the amount of feed/pen/day for each BSW and Managed feeding pen was weighed out for the following week. At 16 WOA, 15 pullets from each treatment were euthanased to evaluate breast muscle, keel curvature, keel length, abdominal fat, liver weight and ovary development, ovum width, oviduct appearance and length.

Seventy hens/treatment group were then moved to individual pens in the high-rise layer shed and held under a common lighting program with gradual increase in photoperiod as per Hy-Line Brown program. The age of first egg and weight of the first three eggs was recorded. Experimental data were analysed using a factorial ANOVA with lighting and feeding programs as the main effects.

III. RESULTS AND DISCUSSION

At 16 WOA BW was highest in *ad lib* feeding under SL and lowest in Managed feeding under both SL and RLR (Table 1). Of the *ad lib* fed birds, SL allowed for the highest FI and RLR the lowest. Not surprisingly when all birds were on *ad lib* feeding in the layer shed, those that had been on Managed feeding during rearing had the highest FI between 16-17 WOA. Weekly FI varied between rearing treatment groups through to 19 WOA (Table 1). Despite this, at 19 WOA hen BW remained heaviest in *ad lib* feeding and lightest in birds of Managed feeding during rearing. Age of first egg was altered by both lighting and feeding (Table 1) with pullets fed *ad lib* under RLR being the youngest at age of first egg (19.16 WOA) and Managed feeding under SL the oldest (20.33 WOA). The lighter BW together with the later age of first egg in

Managed birds concurs with the minimum BW for sexual maturity identified by Brody et al., (1984). Both SL and feeding to BSW during rearing independently generated the heaviest first three eggs.

Table 1 - Body weight, feed intake, age and weight of first eggs in Hy-Line Brown pullets reared under different lighting and feeding regimens.

Treatments during rearing	BW (kg) wk 16	FI (kg) wk 4-16	FI (g/d) wk 16- 17	BW (kg) wk 17	FI (g/d) wk 17-18	BW (kg) wk 18	FI (g/d) wk 18- 19	BW (kg) wk 19	Age (wk) first egg	Weight (g) first 3 eggs
Lighting										
SL	1.37	4.63	56.1	1.49	53.1	1.54	92.9	1.66	19.9	49.5
RLR	1.36	4.50	55.2	1.49	54.3	1.53	95.0	1.66	19.6	48.3
Feeding										
AD	1.50^{a}	5.40	48.5^{a}	1.55^{a}	52.6	1.66^{a}	91.3^{b}	1.78^{a}	19.3^{c}	48.0^{b}
BSW	1.37^{b}	4.47	55.7^{b}	1.48^{b}	53.7	1.53^{b}	97.4^{a}	1.66^{b}	19.7^{b}	49.5^{a}
M	1.22^{c}	3.87	62.8^{c}	1.45^{c}	54.7	1.42^{c}	93.3^{b}	1.55^{c}	20.0^{a}	49.2^{ab}
Interaction										
SL*AD	1.52^{a}	5.53^{a}	48.8	1.56	51.4	1.67	91.0	1.79	19.53^{c}	48.5
SL*BSW	1.37^{c}	4.43	57.2	1.48	53.7	1.54	97.1	1.67	19.87^{b}	50.0
SL*M	1.21^{d}	3.97	62.4	1.44	54.1	1.43	90.8	1.54	20.33^{a}	49.9
RLR*AD	1.48^{b}	5.23^{b}	48.2	1.55	53.9	1.65	91.5	1.76	19.16^{d}	47.6
RLR*BSW	1.37^{c}	4.50	54.2	1.49	53.7	1.51	97.7	1.66	19.67^{b}	48.9
RLR*M	1.23^{d}	3.80	63.3	1.46	55.3	1.42	95.8	1.56	19.73^{ab}	48.4
P- Values										
Lighting	0.078	-	0.387	0.554	0.188	0.115	0.078	1.000	< 0.001	0.007
Feeding	< 0.001	-	< 0.001	< 0.001	0.166	< 0.001	0.001	< 0.001	< 0.001	0.021
Interaction	0.001	0.007	0.350	0.313	0.566	0.618	0.201	0.092	0.038	0.793

Lighting: Lighting program*;* SL*:* Standard lighting program; RLR: Rapid light reduction program.
Feeding: Feeding program; AD: *Ad libitum* feeding; BSW: Fed to achieve Breed standard weight for age.
M: Managed feeding to achieve 88% BSW for age; BW: Body weight; wk: Week; FI: Feed intake.
As BSW and M feeding quantities were controlled by the research team, statistical analysis is not valid.

At 16 WOA, breast muscle score, fat pad weight and keel curvature were highest in birds fed *ad lib* and lowest in birds receiving Managed feeding (Table 2). Heavier birds may be less able to control their flying and landing, colliding more often with the perch, feeder or pen mates (Wilkins et al 2011), resulting in damage to the keel, but this remains to be confirmed. Proportional to bird weight the Managed feeding birds had a higher percent liver weight. Features of the reproductive tract including ovary score, ovum width, oviduct appearance and oviduct length were highest in *ad lib* fed birds (Table 2), which concurs with their younger age at first egg and lighter weight of first three eggs (Table 1). Hence lower weight gain through controlled feeding delayed the development of sexual organs, postponing the onset of lay, also found by Bruggeman et al. (1999), with the production of heavier eggs at the start of lay. These observations are not dissimilar to those of Lee at al. (1971) and Balnave (1973) in earlier strains of egg laying hens.

These findings indicate that the management of FI and lighting during rearing can alter pullet BW, the onset of lay and the weight of the first eggs in current day egg laying hens. The effects of these rearing conditions on persistency of lay, egg quality and hen health through an extended laying cycle will be determined with the flock being followed through until hens are 100 WOA.

Table 2 - Organ characteristic of 16 week old Hy-line Brown pullets reared under different lighting and feeding regimens.

Treatments during rearing	Breast muscle (score 0-3)	Keel curvature (score 1-4)	Keel length (cm)	Ovary (score 0-3)	Ovum width (cm)	Oviduct appearance (score 1-3)	Oviduct length (cm)	Fat pad weight (%)*	Liver weight (%)*
Lighting									
SL	1.80	1.47	10.7	1.07	2.89	1.18	8.67	0.77	1.21
RLR	1.84	1.49	10.8	1.09	2.98	1.40	9.60	0.81	1.17
Feeding									
AD	1.97^{a}	1.60^{a}	11.2^{a}	1.50^{a}	3.83^{a}	1.73^{a}	11.7^{a}	1.76^{a}	1.05^{b}
BSW	1.93^{a}	1.57^{a}	10.8^{b}	1.07^{ab}	2.77^{b}	1.20^{b}	8.08^{b}	0.42^{b}	1.24^{a}
M	1.57^{b}	1.27^{b}	10.4^{c}	0.67^{b}	2.20^{b}	0.93^{b}	7.58^{b}	0.21^{b}	1.29^{a}
Interaction									
SL*AD	1.93	1.67	11.1	1.60	4.00	1.53	11.0	1.71	1.04
SL*BSW	1.93	1.47	10.8	0.87	2.67	1.00	7.54	0.40	1.27
SL*M	1.53	1.27	10.2	0.73	2.40	1.00	7.45	0.21	1.33
RLR*AD	2.00	1.53	11.2	1.40	3.67	1.93	12.5	1.81	1.06
RLR*BSW	1.93	1.66	10.8	1.27	3.27	1.40	8.63	0.43	1.21
RLR*M	1.60	1.27	10.5	0.60	2.00	0.87	7.70	0.20	1.25
P- Values									
Lighting	0.651	0.837	0.276	0.885	0.763	0.052	0.187	0.667	0.136
Feeding	0.001	0.025	<0.001	0.001	0.001	<0.001	<0.001	<0.001	<0.001
Interaction	0.949	0.450	0.662	0.222	0.096	0.089	0.771	0.871	0.271

Lighting: Lighting program*;* SL*:* Standard lighting program; RLR: Rapid light reduction program.
Feeding: Feeding program; AD: *Ad libitum* feeding; BSW: Fed to achieve Breed standard weight for age.
M: Managed feeding to achieve 88% BSW for age; * weight as a percent of live body weight.

ACKNOWLEDGMENT: We thank Australian Eggs for funding this project.

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THE RELATIONSHIP BETWEEN WINTERGARDENS AND SMOTHERING

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Smothering is a form of mortality which results from groups of hens pressing together in such a way that some hens are killed presumably due to suffocation. A recent Australian epidemiological study reported that smothering accounted for 11% of mortality across 3 separate organisations and in two separate climate zones in Australia (Hemsworth et al., 2022). Previous surveys of producers in the UK reported a positive correlation between range use on a sunny day and smothering and suggested wintergardens (or verandas), by providing a graduation of light intensity from inside to outside and thus encouraging ranging behaviour, may minimise smothers (Rayner et al., 2016). This study utilizes some of the data from the Australian epidemiological study (Hemsworth et al., 2022) to explore the relationships between smothering and the presence of wintergardens in commercial free-range laying hen flocks and to generate hypotheses for future controlled studies.

Data from 24 Hyline brown free-range flocks managed by one large commercial free-range egg organisation were included in this study. Flock sizes ranged from 16,900 to 19,651 hens. All flocks were housed in flat-deck systems (with all feed and water provided on a single level) however 15 flocks were housed in sheds with wintergardens (an enclosed outdoor area with a solid roof) and 9 flocks were housed in sheds with no wintergardens. Hens were housed in these sheds from approximately 17 weeks of age through to approximately 78 weeks of age with access to the range from 22 weeks of age. The number of hens smothered and the location of the smothering event (inside the shed or on the range) were recorded daily by stockpeople for each flock. Wintergardens were considered to be part of the range in the data collection. Data were analysed using a two-sample t-test conducted in R 4.2.1. All data were square root transformed prior to analysis, untransformed means are presented.

Overall, the mean number of hens smothered (per 100 hens) was greater for flocks that did not have wintergardens compared to those that did ($\bar{X}_{\text{no wintergarden}}$=1.86, $\bar{X}_{\text{wintergarden}}$= 0.94, t=2.89, p=0.01). There was no difference in the mean number of hens smothered indoors between the two groups ($\bar{X}_{\text{no wintergarden}}$=0.38, $\bar{X}_{\text{wintergarden}}$= 0.41, t=1.10, p=0.28). However, the mean number of hens smothered outdoors was higher for the flocks that did not have wintergardens compared to those that did ($\bar{X}_{\text{no wintergarden}}$=0.90, $\bar{X}_{\text{wintergarden}}$= 0.31, t=2.11, p=0.05).

A recent study investigating the relationship between sunlight and range use of commercial free-range laying hens found that hens avoid high light intensities (Rana et al., 2022). While the presence of a wintergardens would have a clear impact on the light intensity around the pop holes, it is important to note that this was an opportunistic exploration of data collected within a broader research project and the relationship observed between smothering (particularly in the range) and the presence of a wintergarden could be a result of a number of other factors including other environmental factors (such as wind, temperature, predator avoidance) and differences in management practices, which could not be controlled within the design of this study. Further research, using controlled experiments, is required to test this hypothesis on the effects of light intensity around the pop holes on smothering.

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BARRIERS TO ADOPTION OF BEST PRACTICE IN EGG PRODUCTION ENTERPRISES

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The Australian Egg Industry has identified opportunities to optimise production performance, health, flock consistency and egg quality through best practice. Using a cross-sectional survey design for 3 stakeholder groups (farm staff, managers, and consultants), we assessed current knowledge, attitudes, practices, barriers and enablers of adoption of best practices in poultry welfare, health, biosecurity and production performance.

Of the 61 manager surveys posted, 27 were returned; of 169 staff surveys, 64 were returned, and of the 6 third party surveys posted, all 6 were returned. Of these, seven free range sites, 3 cage sites, 1 barn site and 2 office sites responded across a total of 5 producers. A further 8 sites across 4 producers expressed interest in participating but were unable to respond due to lack of time and COVID-19 constraints.

Items were assessed on a 5-point Likert scale ranging from 1 = untrue to 5 = very true, and were averaged to compute a composite score for each of the subscales. Results showed that more than 60% of the farm staff surveyed believed that they knew their work role, task and what they were expected to do, while 77.8% of farm managers believed that they were familiar with their work task and role. Only 21.6% of farm staff believed that they regularly received constructive feedback from their supervisor compared to 44.4% of managers. Only 16.5% of the farm staff believed that their supervisor allowed them to determine how their work should be done. While 33.3% of the managers believed that they were leading by an example, only 27% of staff felt that their supervisor lead by example.

The mean score for all psychological variables were calculated using a 5-point scale, where 1 = strong negative perception and 5 = strong positive perception. On average, participants across staff, managers and consultants reported positive attitudes towards their work (4.4, 4.4, and 4.7 respectively), felt supported from others to do their work (4.1, 4.2, and 4.4, respectively), and felt they were having control over their work (4.2, 4.3, and 4.4%, respectively).

Both managers and consultants reported high levels of personal financial efficacy (4.0 and 4.0, respectively), while staff reported lower levels of financial efficacy (3.8). Time orientation (reflecting an attention bias towards past, present or future outcomes through which an individual makes meaning from experiences and external events) showed that, on average, consultants reported high levels of future thinking, followed by farm managers and staff (4.2, 3.7, and 3.5, respectively).

A higher proportion of farm managers indicated that they were very concerned about animal diseases (27.8%) while only 10.8% of farm staff were highly concerned about this. More than 30% of farm managers were concerned about the need of work training, high workload at peak times and other family commitments affecting their work performance. While comparing the concerns, the most concerning factors affecting the work performance of farm staff were financial problems (27%) while for the managers it was family commitments (50%).

Overall, our findings suggest that the areas of greatest opportunity for improvement can be found in increased training opportunities for management, addressing labour shortages, and clarifying expectations.

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DIFFERENT DOSES OF COMMERCIAL EIMERIA VACCINES WERE NOT EFFECTIVE TO INDUCE A SUCCESSFUL NECROTIC ENTERITIS CHALLENGE MODEL IN BROILERS

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Necrotic enteritis (NE) is often induced in broilers after getting infected with coccidiosis. However, not all sources of Eimeria, as a causative agent of coccidiosis, can successfully induce NE.

This study was conducted to assess whether different doses of Eimeria combinations predispose broiler chickens to NE, compared with the NE challenge model used at UNE. A total of 768 d-old Cobb 500 broiler chicks were assigned to 48 floor pens each stocked with 16 birds replicated 6 times per treatment. The treatments were: unchallenged birds (NC); Eimeria challenged birds with vaccine strains used at UNE model (*E. acervulina* 5000, *E. maxima* 5000 and *E. brunetti* 2500 oocysts, Eimeria Pty Ltd.) (EUNE); NE challenged birds with Eimeria used at UNE (*E. acervulina* 5000, *E. maxima* 5000 and *E. brunetti* 2500 oocysts) plus *C. perfringens* (UNEM); challenged birds with Emeria vaccine (*E. acervulina* 5000, *E. maxima* 5000 and *E. brunetti* 2500 oocysts) and *C. perfringens* plus supplemented with in-feed antibiotic and anticoccidial drugs (PC); challenged birds with higher dose of vaccine A (*E. acervulina* 5000, *E. maxima* 10000 and *E. tenella* 15000 oocysts) plus *C. perfringens* (HVACP); challenged birds with lower dose of vaccine A (*E. acervulina* 2500, *E. maxima* 5000 and *E. tenella* 7500 oocysts) plus *C. perfringens* (LVACP); challenged birds with higher dose of vaccine B (*E. acervulina* 5000, *E. maxima* 10000, *E. mitis* 10000 and *E. tenella* 15000 oocysts) plus *C. perfringens* (HVBCP); challenged birds with lower dose of the vaccine B (*E. acervulina* 2500, *E. maxima* 5000, *E. mitis* 5000 and *E. tenella* 7500 oocysts) plus *C. perfringens* (LVBCP). The challenged birds were orally inoculated with *Eimeria* on d 9 and *C. perfringens* on d14. Bird performance was measured on d 8-19 and 0-35 and oocyst counts were determined in pooled excreta collected during d15 to17, and in ileal and caecal contents at d 16. The JMP 16.0 statistical package was used for analyzing the data and the female percentage was used as a covariate for performance data after the feather DNA sexing was employed to determine the sex of the birds.From d 0- 35, weight gain (WG) reduced ($P < 0.001$) in EUNE (2351g) and UNEM (2360) birds compared to NC (2573), PC (2539) and HVBCP (2527) birds, with other challenged groups being intermediate. FCR increased ($P < 0.01$) in UNEM (1.481) birds compared to NC (1.398), PC (1.421), LVACP (1.421), HVBCP (1.419) and LVBCP (1.423) birds, with the other groups being intermediate. No significant differences for FI were observed between any groups on d 35. From d 8-19, FCR increased ($P < 0.001$) in EUNE (1.343), UNEM (1.343) and PC (1.365) groups compared to NC (1.268) and LVACP (1.276) groups, with other groups being intermediate. FI increased in PC (885g) birds compared to EUNE (810), LVBCP (807) and LVACP (797) birds, with other groups being intermediate. In terms of oocyst counts, the EUNE and UNEM groups had higher oocyst counts ($P < 0.001$) in caeca (455222, 455222), ileum (38767, 60833) and excreta (117458, 86958) compared to NC (133, 33, 39), PC (967, 442, 74), HVBCP (7558, 8442, 13060) and LVBCP (1767, 1317, 12244) birds. No significant differences were observed in caecal and ileal oocyst counts between EUNE, UNEM, HVACP and LVACP birds.

The data obtained in this experiment suggest that birds received Eimeria used at UNE, successfully induced NE impairing bird performance and increasing the number of occysts in caecal, ileal and excreta contents but not vaccines.

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IN-OVO INJECTION OF OREGANO ESSENTIAL OIL UP TO 10µl SHOWED NO IMPACT ON EMBRYO SURVIVABILITY IN BROILER CHICKENS

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Oregano essential oil (OEO) and carvacrol, the main compound of OEO, are considered as a reference for biological activities of essential oils (EO). OEO has positive effects on gut and immune system developments potentially related to antimicrobial and antioxidant activities in chickens (Brenes and Roura, 2010). Embryonic development accounts for one-third of the lifespan of modern broiler chickens, and has the potential to determine performance in later life. In-ovo injection is a powerful tool for delivering biologically active compounds such as EOs. However, little is known about the safety margins of injection of EOs on hatchability regarding the injected concentration and volume. The objective of this study was to identify the maximum safe concentration of OEO that can be administered to broiler chickens during embryonic development.

A dose response of OEO including 0 (injected control), 5, 10, 20, 30, 40, or 50µl of oil emusified using 1:1 polysorbate 80 into 1000µl physiological saline solution were injected into the amnion of 48 eggs (each treatment) at day 17.5 of incubation. A non-injected control group was also included. Hatchability and post-hatching performance until day 7 were measured.

Results showed that in-ovo injection of OEO at levels above 10µl dramatically reduced hatchability ($P < 0.01$; Table 1). The OEO injection up to 10µl showed no significant ($P > 0.05$) impacts on hatchability, growth, and feed intake until day 7. These results are in contrast with toxicity thresholds published in rats showing a safety margin of oral administration of OEO up to 200mg/kg body weight (Llana-Ruiz-Cabello et al, 2017). Thus, this study showed that chicken embryos are remarkably more sensitive to toxicity of OEO compared to rats.

In conclusion, in-ovo injection of OEO up to 10µl did not have any significant effect on hatchability and post-hatching performance. Impact of non-toxic levels of OEO on embryonic development and particularly gut functionality and the immune system warrant further invistigation.

Table 1 - Effects of in-ovo injection of different amounts of oregano essential oil (OEO) on hatchability and performance parameters up to 7 days post-hatching. OEO was injected into the amnion of fertile eggs at day 17.5 of incubation (n = 48 per treatment).

Injected OEO	Hatchability (%)	BW0 (g)	BW7 (g)	Feed intake (g)	FCR (g/g)
Not injected	95.7 ^{a}	43.4 ^{ab}	195.4	169.9	0.869
0 µl	89.6 ^{a}	44.8 ^{a}	191.4	179.5	0.937
5 µl	89.6 ^{a}	44.0 ^{a}	189.7	170.1	0.896
10 µl	83.3 ^{a}	43.5 ^{a}	192.5	173.0	0.901
20 µl *	22.9 ^{b}	43.5 ^{ab}	-	-	-
30 µl *	16.7 ^{b}	40.0 ^{b}	-	-	-
40 µl *	2.1 ^{c}	41.3 ^{ab}	-	-	-
50 µl *	4.2 ^{bc}	41.6 ^{ab}	-	-	-
SEM	-	1.01	4.40	7.74	0.027
P value	<.0001	0.0007	0.8282	0.8019	0.4362

BW0: Body weight at hatch, BW7: Body weight at day 7, FCR: Feed conversion ratio
*post-hatching performance could not be recorded due to low number of chicks hatched in these treatments

ACKNOWLEDGEMENTS: This work was partially funded by AgriFutures Australia and Delacon Biotechnik GmbH.

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A BLEND OF PROTECTED ORGANIC ACIDS + ESSENTIAL OILS PROMOTES INTESTINAL HEALTH, NUTRIENT DIGESTIBILITY, IMMUNE RESPONSE AND GROWTH PERFORMANCE OF BROILER CHICKENS UNDERGOING AN INTESTINAL CHALLENGE

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Summary

The growing concern over the use of antibiotic growth promoters (AGP) indicates a downward trend in the use of these substances. Feed additives as organic acids (OA) and essential oils (EO) are effective tools to maintain intestinal homeostasis and to avoid performance losses. We investigated the dietary supplementation of protected organic acids + essential oils [P(OA+EO)] in broiler chickens undergoing an intestinal challenge while comparing it to an AGP. A total of 1,080 Cobb 500 male broilers were randomly distributed in four treatment groups with 10 replicates (27 birds/each) for 42 days. Treatments were as follow: unchallenged control (UC), challenged control (CC), AGP (Enramycin at 10 g/t), and P(OA+EO) at 300 g/t. Except the UC group, all birds were challenged with *Eimeria* spp. at 1 day and *Clostridium perfringens* at 11, 12 and 13 days. Feed intake, body weight (BW), body weight gain (BWG) and feed conversion ratio (FCR) were evaluated weekly. At 17 days, one bird per pen was orally gavaged with fluorescein isothiocyanate-dextran (FITC-d) to evaluate the intestinal permeability. At 21 days, nutrient and ileal digestible energy (IDE), macroscopic and histologic alterations in the intestine using the I See Inside methodology (ISI), and jejunal gene expression of mucin-2 (MUC2), claudin-1, occludin, and interleukin-12 (IL-12) were evaluated. P(OA+EO) improved intestinal integrity by reducing intestinal permeability ($P < 0.001$) and increasing MUC2, claudin-1 and occludin gene expressions ($P < 0.05$), compared with CC group. Broilers on P(OA+EO) had lower total ISI histologic scores ($P < 0.05$) compared with the other treatments, which can be translated in better intestinal health. In accordance with the lower ISI scores, broilers on P(OA+EO) presented a lower expression of IL-12, which resulted in the superior BWG and FCR, compared to the CC group at 21 days. Broilers on P(OA+EO) had higher dry matter (3.3%) and IDE (0.44 MJ/kg) compared to CC group ($P < 0.05$). At 42 days, broilers on P(OA+EO) had BW and FCR (1-42 days) similar to the UC group and even higher BW (2.9%) than broilers on AGP group ($P < 0.05$), which was supported by the better intestinal health and digestibility. In conclusion, the blend of P(OA+EO) showed better or similar effects to an AGP on improving intestinal health, nutrient and energy digestibility, and modulating the immune response to benefit the growth performance of broiler chickens undergoing an intestinal challenge.

I. INTRODUCTION

Maintenance of intestinal integrity is of fundamental importance to the overall health and growth performance of the bird. Disturbances in the homeostasis of the gastrointestinal tract can result in inflammatory process, loss of intestinal integrity, low nutrient digestibility and poor growth performance. In a situation of intestinal inflammation, pro-inflammatory interleukins such as interleukin-12 are produced with the objective to defending the host from any aggression. However, pro-inflammatory interleukins have a high potential to reduces host

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homeostasis, causing fever, muscle catabolism and disruption of the intestinal tight junction proteins (Al-Sadi et al., 2013). Claudin-1 and occludin are tight junction proteins that help seal the paracellular space between enterocytes, preventing the translocation of microorganisms from the intestinal lumen into the bloodstream. In addition, there is Mucin-2 (MUC2), the main mucin produced by the Goblet cells, that prevents microbial adhesion to the mucosa. Thus, the higher the intestinal expression of MUC2, claudin-1 and occludin, the better the intestinal integrity. For these reasons, unnecessary overstimulation of the inflammatory leading to a low intestinal health response may negatively affect growth performance and should be alleviated.

A common practice to improve birds' intestinal health has been the use of feed additives. In AGP-free poultry production, protected OA and EO are strong tools to maintain intestinal homeostasis (Wang et al., 2019). Organic acids and EO act through different modes of action, however when combined, may have a synergistic effect on the intestinal health and growth performance by modulating the microbiota (Adewole et al., 2021) or signaling molecules that influence diverse regulatory functions on host's physiology, metabolism regulation, inflammation, and immunity (D´Aquila et al., 2020). In addition, the use of protective technology allows these compounds to be slowly released along the digestive tract and reach the posterior portion, where most of the pathogenic bacteria is present. Therefore, the objective of the study was to evaluate the effects of a blend of a protected organic acid + essential oils on growth performance, nutrient digestibility, intestinal health, and immune response of broiler chickens undergoing an intestinal challenge while comparing it to an AGP.

II. METHOD

A total of 1,080 1-day-old Cobb 500 male broiler chicks (body weight: 43 ± 1 g) were randomly distributed in four experimental treatments (10 replicates with 27 birds/each) for 42 days, as follows: T1 – unchallenged control (UC), T2 – challenged control (CC), T3 – challenged supplemented with antibiotic growth promoter (AGP, Enramycin at 10 g/t) and, T4 – challenged and supplemented with a blend of protected organic acids + essential oils [P(OA+EO) at 300 g/t, Jefo Nutrition Inc.]. The P(OA+EO) consists of fumaric, sorbic, malic, and citric acids and thymol, vanillin, and eugenol. Except the NC group, all birds were challenged at 1 day, via individual oral gavage with a commercial coccidiosis vaccine at 10× the regular dose (Bio-Coccivet live vaccine, containing *E. acervuline*, *E. brunetti*, *E. maxima*, *E. necatrix*, *E. praecox*, *E. tenella*, and *E. mitis*; Biovet Vaxxinova). At 11, 12, and 13 days of age, birds were individually orally gavaged with 1 mL/bird of *Clostridium perfringens* (10^8 CFU/bird). All procedures were approved by the Ethics and Research Committee of the Federal University of Santa Maria, Santa Maria, Brazil (number 5404280717).

Body weight (BW), feed intake, body weight gain (BWG), and feed conversion ratio (FCR) were determined by week. Mortality was recorded daily. On day 17 of age, one bird/pen (n = 20 birds/treatment) was orally gavaged with 2.2 mg/bird of systemic fluorescein isothiocyanate-dextran (FITC-d). Blood samples were collected 1 h after gavage to detect FITC-d level in serum. At 21 days, mucosa samples of one bird/pen from the middle of jejunum were collected to evaluate the following markers: mucin-2 (MUC2), claudin-1, occludin and, pro-inflammatory interleukin-12 (IL-12) mRNA expression. Macroscopic (3 birds/replicate, n = 30 birds/treatment) and histological (2 birds/replicate, n = 20 birds/treatment) intestinal alterations were evaluated by a blinded assessor using the *I See Inside* methodology (ISI®). In this methodology, an impact factor (IF) ranging from 1 to 3 is defined for each alteration in macro/histologic analysis according to the reduction of the organ's functional capacity, where 3 is the most significant alteration for the organ function. In addition, the intensity and extension of each alteration are evaluated and a score from 0 to 3 is assigned, 0 being the absence of lesion. To reach the final value of the ISI® index, the IF of

each alteration is multiplied by its respective score number and the results of each individual alteration are summed (Kraieski et al., 2016). A low ISI score represents better intestinal health (Belote et al., 2019). To determine ileal digestibility, digesta (4 birds/replicate = 40 birds/treatment) was collected from the distal two-thirds of the ileum. Celite at 1% was used as an indigestible marker in the starter diets and this diet was provided 48h prior to ileal digesta collection. The parametric data were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS Institute. Means were analyzed by Fisher LSD. The non-parametric data were submitted to the Kruskal-Wallis. Significance was accepted at $P < 0.05$ and tendency at $P < 0.10$.

III. RESULTS

Broilers on P(OA+EO) had better intestinal barrier integrity due to the higher MUC2, claudin-1 and occludin gene expression ($P < 0.05$) and lower levels of serum FITC-d ($P < 0.05$), compared to CC group (Table 1). Still compared to CC group, broilers on P(OA+EO) had better intestinal health due to the lower total histologic ileal ISI index ($P < 0.05$; Table 1) and tendency towards a lower macroscopic ISI index ($P < 0.10$). Broilers on P(OA+EO) had higher dry matter (3.3%) and IDE (0.44 MJ/kg) compared to CC group, and no difference compared to AGP group ($P < 0.05$; Table 1).

Table 1 - Jejunal gene expression of intestinal biomarkers, serum FITC-d, ISI® index, apparent ileal digestibility and growth performance of broilers undergoing an intestinal challenge.

	Unchallenged Control	Challenged Control	Challenged + AGP	Challenged + P(OA+EO)	P-value
Jejunal gene expression					
MUC2	1.21^{a}	0.81^{b}	1.10^{ab}	1.34^{a}	$P < 0.05$
Claudin-1	1.35^{b}	0.90^{b}	2.35^{a}	2.45^{a}	$P < 0.05$
Occludin	1.18^{b}	0.78^{b}	1.24^{b}	2.22^{a}	$P < 0.05$
Intestinal integrity					
FITC-d, µg/mL	0.169^{bc}	0.191^{c}	0.148^{ab}	0.142^{a}	$P < 0.05$
Total ISI index					
Macroscopic	12.67^{y}	11.63^{y}	10.63^{xy}	9.34^{x}	$P < 0.10$
Histological	7.07^{b}	6.88^{b}	6.81^{b}	6.18^{a}	$P < 0.05$
Apparent ileal digestibility					
Dry matter, %	64.2^{a}	61.0^{b}	63.7^{a}	64.3^{a}	$P < 0.05$
Crude protein, %	81.6	79.8	81.6	82.0	$P > 0.10$
Energy, MJ/kg	13.68^{a}	13.16^{b}	13.55^{a}	13.60^{a}	$P < 0.05$
Growth performance					
BW at 42d, g	3342^{a}	3096^{c}	3213^{b}	3309^{a}	$P < 0.05$
FCR 1-42, d	1.409^{a}	1.455^{b}	1.395^{a}	1.387^{a}	$P < 0.05$

At 21 days old, broilers on CC group had an overstimulation of the inflammatory response (higher IL-12) that resulted in lower BWG and higher FCR ($P < 0.05$; Figure 1). Pro-inflammatory IL-12 increases during dysbiosis and has a high potential to disrupt host homeostasis and negatively affect growth performance. Meanwhile, compared to the CC groups, P(OA+EO) was able to alleviate the inflammatory response (lower IL-12) and resulted in a superior growth performance. Furthermore, P(OA+EO) provided a similar response to the AGP group for the pro-inflammatory response while having a lower FCR. At 42 days, the reduced growth performance of the CC compared to UC group ($P < 0.05$; Table 1) show that the applied challenge model was efficient. Broilers on P(OA+EO) had BW and FCR (1-42 d)

similar to the UC group and even had 2.9% higher BW than birds on AGP group ($P < 0.05$; Table 1).

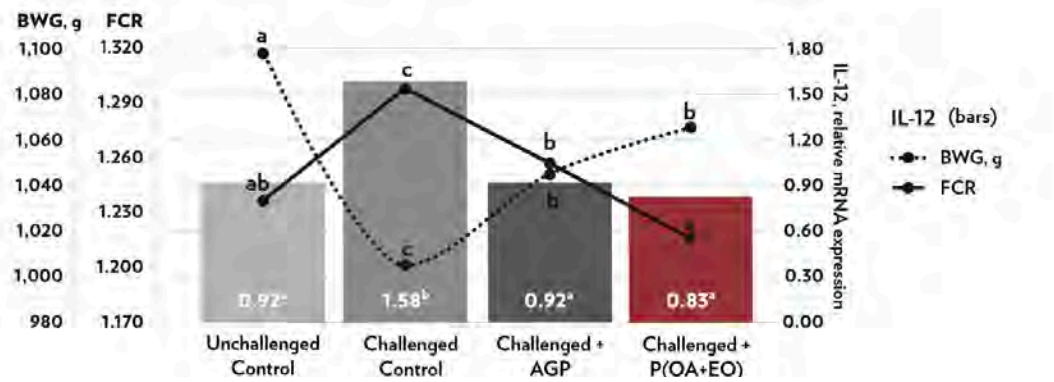

Figure 1 - Jejunal gene mRNA expression of IL-12, BWG (dotted black line) and FCR (straight black line) on 21-day-old broiler chickens undergoing an intestinal challenge ($^{abc}P < 0.05$).

IV. DISCUSSION

The intestinal challenge model was efficient to cause a disturbance in the intestinal homeostasis negatively affecting intestinal health, nutrient digestibility and, consequently, the growth performance of broiler chickens. The higher ISI scores, FITC-d serum level and pro-inflammatory IL-12 expression combined with the lower expression of the tight junction proteins and lower nutrient digestibility observed in CC group justify the poor growth performance of these birds. However, challenged broilers fed diets supplemented with P(OA+EO) had better growth performance, which is in line with the results of better intestinal barrier integrity, nutrient digestibility and health status, the latter being observed through lower ISI scores. Belote et al. (2019) demonstrated that the ISI histological analysis has a strong correlation with growth performance, and that the higher the ISI score is, the poorer the performance of the broiler chickens.

The reduced ISI scores observed in broilers fed P(OA+EO) can also be an indication of a lower demand for mucosal renovation, which may explain the higher expression of MUC2 in this group. Additionally, FITC-d blood levels were lower for broilers on P(OA+EO) group, indicating that even under a dysbiosis model, their intestinal barrier seemed to be less compromised, suggesting a low intestinal leakage and less chance of bacterial and toxins translocation from intestinal lumen to bloodstream. In conclusion, the blend of protected organic acids + essential oils evaluated showed better or similar effects to an AGP in improving intestinal health, nutrient and energy digestibility and modulated the immune response to benefit the growth performance of broiler chickens undergoing an intestinal challenge.

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NUTRITIONAL STRATEGIES TO MITIGATE COCCIDIOSIS

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Coccidiosis is a disease with substantial economic impact (Muthamilselvan et al., 2016), particularly due to the push to ban anticoccidials. Vaccines are available but can be expensive, and are often implemented for free range and breeder flocks only. Thus, it is imperative to find effective nutritional alternatives to reduce the impact of coccidiosis on broiler chickens. The aim of this experiment was to determine if the nutritional strategies of post-pellet whole wheat (WW), xylooligosaccharide (XOS), high fat (HF, vegetable oil), high carbohydrate (HC), supplementation of threonine and branched-chain amino acids (Thr + BCAA) and short-chain fatty acid (SCFA) inclusions may assist broilers to combat the severity of coccidiosis challenge, in comparison to a ground grain, negative control (unchallenged, NC) and positive control (challenged, PC) diets, containing breed recommended nutrient levels. A total of 648 day-old males from the female Ross 308 parent line (six replicates, 12 birds/pen) were allocated to one of the nine dietary treatments on the basis of initial body weight. Birds were offered starter (d 1 – 10), grower (d 10 – 21) and finisher (d 21 – 35) diets. Birds in the challenge treatments were dosed with *E. maxima* and *E. acervulina* (Eimeria Pty.) in 1 ml sterile phosphate-buffered saline (PBS), while un-challenged birds were dosed with 1 ml PBS on d 14. Birds had unlimited access to feed and water in an environmentally controlled facility. Lighting and temperature followed Ross 308 guidelines. Fecal collection was performed daily from d 17 to 28 for coccidial oocyst counts. Feed intake, weight gain and FCR were calculated for each dietary phase. Four birds/pen were sampled on d 21 to assess intestinal lesion scores. The challenge had the greatest impact on performance during the finisher phase, where weight gain of the NC treatment was numerically higher than the PC treatment (1,502 vs 1,421 g; P = 0.129). During this period, the NC treatment tended to have a better FCR than the PC treatment (1.572 vs 1.736; P=0.06). Through this phase, the XOS treatment maintained weight gain (1,422 vs 1,502 g; P > 0.05) and FCR (1.740 vs 1.572; P > 0.05) in comparison to the NC treatment. Over the entire study, the WW and HF treatments had the lowest weight gain (2,236 and 2,252 vs 2,431 g; P < 0.001) and the poorest FCR (1.883 and 1.840 vs 1.510; P < 0.001) compared to the NC treatment. There was no significant effect of the treatments on intestinal lesion score on d 21. Fecal samples on d 21 contained no oocysts in the NC treatment whereas the PC treatment had the highest oocyst counts of *E. maxima* and *E. acervulina* (650 and 11,467 vs 0 OPG; P < 0.001). XOS (280 vs 650 OPG; P < 0.001) diets had lower counts of *E. maxima* oocysts in feces compared to the PC. WW diet significantly reduced *E. acervulina* oocysts (3,600 vs 11,467 OPG; P < 0.001) in feces compared to the PC diet. No significant difference on mortality was found in between treatments. Overall, XOS and WW diets had potential to reduce the number of *E. maxima* and *E. acervulina* oocysts in feces.

ACKNOWLEDGEMENTS: The authors would like to thank Poultry Hub Australia, AB Vista andEimeria Pty. Ltd., Australia, for funding this project. They also would like to acknowledge Greg Underwood, Petrina Young and Michelle Benham of Eimeria Pty. Ltd for their laboratory support.

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SUPPLEMENTATION OF ENZYME AND PROBIOTIC IMPROVED GROWTH PERFORMANCE IN BROILERS UNDER NECROTIC ENTERITIS CHALLENGE

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Feed additives have a promising impact on broiler production in improving performance and reducing the incidence of diseases in the post-antibiotic era (Ayalew et al. 2022). However, there are limited data that illustrate the effect of feed additives on broiler performance under necrotic enteritis (NE) challenge. Therefore, this study was conducted to determine the impact of an enzyme (xylanase, 3000 U/kg) and a probiotic (*Bacillus subtilis*, 2.2×10^8 CFU/g) on growth performance and intestinal lesions of broilers under the NE challenge. A total of 630 d-old Cobb 500 mixed-sex broiler chicks were randomly distributed into five treatment groups, with nine replicates of 14 birds per pen in a completely randomised design. The treatments were: Non-challenged (NC); NE challenged control (CC); CC+Xylanase 300 g/t (CC+Xy); CC+Probiotic 500 g/t (CC+Pb); and CC+ Xylanase+ Probiotic (CC+Xy+Pb) in the starter, grower, and finisher phases. Birds were fed a corn-soy-based diet supplemented with phytase (100 g/t and 500 FTU/kg). Challenged birds were gavaged with *Eimeria spp*. on d 9 and *Clostridium perfringens* EHE-NE18 on d 14 and 15 according to Rodgers et al. (2015). Birds were sexed according to the method described by England et al. (2021) and the female percentage was used as a covariate for the performance analysis. Intestinal NE lesions were scored and recorded at d 16 and growth performance was measured from d 0 to 35. Data were subjected to a one-way analysis of variance using JMP 14.0 and significance was determined at $P < 0.05$ by the Tukey HSD test.

Before the challenge (d 0-8), average body weight gain (AWG), average feed intake (AFI) and feed conversion ratio (FCR) were not different ($P > 0.05$) between control groups, NC and CC. Birds in the groups CC+Xy, CC+Pb, and CC+Xy+Pb had a lower ($P < 0.05$) FCR compared to the control groups, whereas, only CC+Xy had a higher ($P > 0.05$) AWG than the control groups. Effective induction of the sub-clinical NE challenge was confirmed by decreased AWG, increased FCR, and duodenal lesions in the CC group (P 0.05) after the challenge. In the finisher phase (d 19-35), birds in the CC+Xy+Pb treatment had a lower ($P < 0.05$) FCR compared to the CC group. For the overall period (d 0-35), the CC group had a lower ($P < 0.05$) AWG and a higher ($P < 0.05$) FCR compared to the NC group. Birds in the treatment CC+Xy+Pb had a lower ($P < 0.05$) FCR compared to the CC group and maintained a similar ($P > 0.05$) AWG compared to the NC group and other additive groups. On d16, the birds in the group CC+Xy+Pb had a lower ($P < 0.05$) duodenal lesion score in female birds compared to the CC group and had similar ($P > 0.05$) duodenal, jejunal, and ileal lesions in both male and female birds compared to the NC group. These results suggest that the supplementation of xylanase in the starter phase may improve broiler performance. Overall, the combination of enzyme and probiotic in NE-challenged birds may enhance performance by improving AWG and FCR. This could be a result of the lower intestinal lesions and enhanced gut environment, which may have facilitated the maintenance of growth response in the CC+Xy+Pb group compared to the non-challenged treatment.

ACKNOWLEDGMENTS: The authors would like to thank Kemin Animal Nutrition and Health, Asia Pacific, Poultry Hub Australia, and Eimeria Pty. Ltd., Australia for supporting this project.

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A COMPARISON OF MULTI-SPECIES *BACILLUS* PROBIOTICS ON EARLY BROILER PRODUCTION PERFORMANCE, GUT HEALTH, AND NUTRIENT DIGESTIBILITY

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Summary

The use of antimicrobials as antibiotic growth promoters (AGPs) has been banned in most countries to prevent further development and spreading of antimicrobial resistance. Probiotics have increased in popularity due to their potential to improve animal performance and welfare particularly in the absence of AGPs. This trial investigated several *Bacillus* probiotic formulations on broiler production performance, gut health, and nutrient digestibility compared to a diet with AGPs (Tylosin). Results revealed that formulation 1 (T3) significantly improved production performance compared to the other treatment groups ($P<0.05$) and tended to decrease mortality ($P<0.09$). Microbial profiling revealed a significant reduction ($P<0.05$) of the phylum Bacteroidetes in T1, whereas the probiotic treatments were able to recover these bacteria. Nitrogen and crude protein digestibility were also significantly increased ($P<0.05$) when broilers were fed T3. In conclusion, supplementing feeds with *Bacillus*-based probiotics can improve performance and health in broiler chickens.

I. INTRODUCTION

The ban on the use of AGPs together with good antibiotic stewardship practices by intensive livestock industries resulting in a decline of antimicrobial resistance globally (Salim et al., 2018). Probiotics have been widely studied partially as a replacement of AGPs. Some probiotics are able to improve production performance whilst improving gut health and decreasing the likelihood of mortality due to infection. This study investigated the effects of various novel *Bacillus* probiotic formulations selected through various *in vitro* metrics including enzyme production, on broiler chicken growth performance, microbial profile, gut morphology, and nutrient digestibility to determine the most viable AGP alternative.

Table 1 - *Bacillus* species formulation combinations ($6x10^8$ CFU/g).

Formulation 1	Formulation 2	Formulation 3	Formulation 4
BAM1	BAM1	BAM1	BAM1
BAM3	BAM4	BAM4	BAM2
BAM4	BCON	BLIC2	BLIC1
		BCON	BSUB

BAM1: *Bacillus amyloliquefaciens* strain 1, BAM2: *Bacillus amyloliquefaciens* strain 2, BAM3: *Bacillus amyloliquefaciens* strain 3, BAM 4: *Bacillus amyloliquefaciens* strain 4, BLIC1: *Bacillus licheniformis* strain 1, BLIC2: *Bacillus licheniformis* strain 2, BSUB: *Bacillus subtilis*, BCON: *Bacillus coagulans*

II. METHOD

The animal experiments developed in this project were approved by The University of Queensland (UQ) Production and Companion Animal Ethics Committee with the certificate number 2020/AE000235. A total of 576-day-old ROSS308 broiler chicks (mixed sex) were

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transferred to the Queensland Animal Science Precinct (QASP) facility at UQ, Gatton for a 21-day preliminary trial. All chicks were weighed, and randomly distributed to 72 floor pens, 8 chicks per pen. Pens were randomly allocated to six corn-soybean-based treatments; T1: Negative control (NC), T2: Positive control (NC + Tylosin), T3: T1 + formulation 1, 0.15%, T4: T1 + formulation 2, 0.15%, T5: T1 + formulation 3, 0.15%, T6: T1 + formulation 4, 0.15% (12 pens per treatment, n=96) (Table 1). Feed intake and body weight were measured weekly, and mortality was recorded daily. Ileum, duodenum, jejunum, and ileal contents were collected for gut morphology, microbial profiling, and nutrient digestibility, respectively. In the assay for protease, clearance zones were observed on the skim milk agar; whereas the assay for α-amylase was conducted on starch agar.

III. RESULTS

The performance results have been summarised in Table 2. Formulation 1 (T3) compared to formulation 3 (T5) showed significantly ($P < 0.05$) higher body weight at day 21 and higher average daily gain d1-21. The European feed efficiency index demonstrated that T3 had a significantly higher performance than T5 ($P < 0.05$). In addition, F1 strain (T3) tended ($P<0.10$) to show the lowest mortality among other treatments. The gut morphology, however, did not reveal significant differences ($P > 0.05$). Microbial profiling revealed that at the genus level, *Lactobacillus*, *Alistipes*, *Akkermansia*, *Turicibacter* and *Bacteroides* have low abundance in T1 compared to the remaining treatment groups ($P < 0.05$), suggesting the administration of Tylosin had a significant impact on the growth of these genera. However, the addition of probiotic strains *Bacillus* seemed to restore the genera abundance (T3, T4, T5, T6.) T3 revealed the highest N digestibility, which was significantly higher than the negative control, T1. T2, T5, and T6 also had significantly higher N than T1 ($P < 0.05$). The same result was also reflected in the crude protein digestibility ($P < 0.05$).

Table 2 - Effect of different Bacillus strains on performance parameters of broiler chickens from day 1 to 21 (n=96).

	Treatment group							
	T1	T2	T3	T4	T5	T6	SEM	P value
BW0 (g)	41.8	41.3	42.1	42.4	42.1	42.8	0.39	0.1427
BW21 (g)	808.1^{ab}	834.8^{ab}	847.0^{a}	814.4^{ab}	787.4^{b}	843.4^{ab}	14.0	0.0271
ADG0-21 (g)	36.4^{ab}	37.7^{ab}	38.3^{a}	36.7^{ab}	35.4^{b}	38.1^{ab}	0.66	0.0419
ADFI0-21 (g)	60.3	58.0	55.9	62.4	57.8	57.0	1.77	0.1225
FCR0-21 (g/g)	1.66	1.54	1.46	1.70	1.64	1.50	0.06	0.0683
CV21 (%)	12.6	11.7	12.4	13.1	13.7	9.0	1.17	0.0912
Mortality (%)	2.1	4.2	0.0	2.1	7.3	4.2	0.85	0.0858
EPEF	219^{ab}	234^{ab}	264^{a}	215^{ab}	207^{b}	243^{ab}	12.6	0.0239

T1: Negative Control (NC: standard commercial feed without antibiotics); T2: Positive Control (NC + antibiotics Tylosin, 20 g/ton); T3: NC + F1 strain (0.15%); T4: NC + F2 strain (0.15%); T5: NC + F3 strain (0.15%); T6: NC + Natupro (0.15%). BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio; CV: Coefficient of variation (indication of growth uniformity calculated as a ratio of the standard deviation to the mean); EPEF: European Production Efficiency Factor (ADG × survival rate)/FCR × 10).

^{a,b} means with different letters in each row are significantly different at $P<0.05$ level.

IV. DISCUSSION

This trial revealed that the inclusion of *Bacillus* based probiotics into broiler diets can improve growth performance, gut health, and nutrient digestibility compared to a positive control and AGP diet. Formulation 1, a combination of 3 *Bacillus amyloliquefaciens* strains, was the most effective in broiler diets, which was also demonstrated by Ahmat et al. (2021) who found that broiler production performance and immune response was improved in broilers fed this species

of *Bacillus*. As *Bacillus amyloliquefaciens* produce protease and amylase as by-products, the digestibility of nutrients such as crude protein are improved, as well as increasing resistance to gut infection (Stefanello et al., 2017). The administration of probiotic strains *Bacillus* can be used to recover the abundance of helpful genera, which play an important role in fibre fermentation and promote health such as *Lactobacillus* (Alloui et al., 2013). The lack of differences between treatments in gut morphology contradicts the findings by Rodjan et al (2018). The discrepancy might be due to short-term termination at 21 days of our study. This study aimed at screening several probiotic formulations hence the shortened reading period. Thus, this study has identified formulation 1 to be a candidate for further testing to improve broiler production performance, gut health, and nutrient digestibility in the absence of in-feed antimicrobials.

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NEW GENERATION OF WATER-SOLUBLE PROBIOTICS TO ALLEVIATE GUT HEALTH CHALLENGE DURING FEED TRANSITION PERIOD IN BROILER PRODUCTION

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Probiotics are included in the feed and administered continuously during the entire production cycle to maintain intestinal integrity and strengthen the immunity of the bird, thus reducing the need for repeated medication. Alternatively, water-soluble probiotics are often applied to quickly react to upcoming stress situations in the flock – but they can negatively impact the formation of biofilm in the water lines. For this reason, formulations that reduce the formation of biofilms while remaining efficacious are required.

The new formulation is an effervescent tablet loaded with a natural, fast-growing *Bacillus amyloliquefaciens* CECT 5940 ($5E^{+8}$ CFU/L), ensuring a fast dissolution rate (10 min in average) as well a homogenous distribution of the spores in water without additional stirring. In this study, we tested the efficacy of the new formulation under field conditions in a commercial farm. The farm, located in the UK, was composed of six houses of 36,500 Ross 308 birds each, with same stocking density, lighting, drinking and ventilation system. The different houses were randomly allocated to the different treatments: three control houses (C) and three houses received the tablets in water (P). One tablet was dissolved in a 20 L stock solution and then supplemented to the drinking line at a concentration of 2%. The target is to have one tablet/1,000L and a final concentration of 5 E^{+8} CFU/L of water. The tablets were administered on an intermittent model during a stress period. The birds received the probiotic in drinking water for three days after hatch and during the feed transition for approximately 12 hours/day for three days to follow. Animal performance: feed and water consumption, average body weight (Av BWG), feed conversion ratio (FCR) and mortality were recorded daily during the entire rearing period. Data were submitted to one-way analysis of variance (α=0.05). Results showed that applying the new formulation of the probiotic resulted in an increased av BWG by 34g compared to the control houses ($P < 0.01$). Hock-burn and pododermatitis scores were improved by approximately 5% in all three treatment houses as well as less factory rejects (-0.1% ~1095 Birds) compared to the control. The lower incidence of hock and pododermatitis in the treatment houses may be attributed to the lower moisture content of the litter as indicated by the decrease in feed: water ratio (1.85 and 1.78 for C and P, respectively). During the study, each two houses (C and P) suffered badly from chronic yolk sac infection. On day 3, high mortality of 3.39% was reported in house C and the veterinarian made the decision to medicate with antibiotics. On the other hand, the P house had lower mortality (0.82%) and no antibiotic treatment was required. The veterinarian responsible for the farm stated that the probiotic had a protective effect on the birds and mitigated the losses on house P, avoiding the necessity for antibiotic treatment. The 2 houses were from the same flock and the same parent flock, and assuming that they would have been equally affected by the hatchery related issues leading to the subsequent development of retained yolk sac.

The economic evaluation showed a positive return on investment (ROI) of 5,98. In conclusion, the new probiotic effervescent tablet, allowed an easy and quick application via water supply during stress period. It resulted in a better animal performance, less medication and improved animal welfare.

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IS THE PROBIOTIC EFFICACY OF *BACILLUS AMYLOLIQUEFACIENS* STRAIN H57 DOSE DEPENDENT?

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The effect of probiotics on animal performance is not consistent and may be impacted by multiple factors, such as dose, route of administration, disease stress, and management. We have previously examined the efficacy of the probiotic *Bacillus amyloliquefaciens* strain H57 (H57) in a disease stress model with subclinical necrotic enteritis (NE) (Shini *et al.,* 2020); in the study only one dose of H57 (10^8 CFU/g feed) was used. The purpose of present study was to evaluate if the effect of H57 on performance and gut health of broiler chicks challenged with subclinical NE is dose dependent.

Day-old, male, Ross 308 broiler chicks were assigned to 48 cages by stratified randomization with 10 chicks per cage in an environmentally controlled room. Day-old chicks were fed a wheat/soybean basal diet that was also supplemented with graded doses of H57 at 0, 10^6, 10^7, and 10^8 CFU/g feed, respectively; Treatments 1, 2, 3 and 4. Treatments 5-8 replicated Treatments 1-4, but were challenged by NE. Each treatment diet was fed to 6 cages and the procedure to induce NE, as detailed by Shini *et al.* (2020) was followed. Briefly, at 9-days of age, the birds were challenged with *Eimeria* spp. vaccine with 20 times the manufacturer's recommended dose by drinking water. At 14-days of age, *Clostridium perfringens* inoculated broth was mixed into the diets. Body weight and feed intake were recorded weekly. The experiment ended on day 21 when samples were collected from two birds per replicate, including liver, spleen, bursa of Fabricius, pooled ileal digesta, and ileal tissue for histomorphology. The birds were also visually scored for NE gut lesions and the following day, the NE gut lesions of the remaining birds were scored.

Body weight was not significantly different ($P>0.05$) between birds fed graded levels of H57 (Treatments1-4). However, the body weight of NE-birds treated with H57 at 10^7 CFU/g was the highest ($P < 0.05$) compared with other challenge groups. H57 improved the FCR of both non-challenged and challenged birds. Challenged birds fed H57 at 10^8 CFU/g had a similar FCR to the control group. The ratio of villi height and crypt depth of NE-birds supplied with 10^7 CFU/g H57 was significantly ($P < 0.05$) lower than NE-only birds. NE-birds supplied with H57 at 10^8 CFU/g had the lowest gut lesion score amongst challenged groups and were not significantly different to the non-challenged groups. Digesta pH of challenged groups was significantly ($P < 0.05$) lower than the non-challenged groups. NE challenged birds did not have a significantly different ($P > 0.05$) digesta pH compared with non-NE birds when fed H57 at 10^7 CFU/g and 10^8 CFU/g.

The data showed that there was a dose effect with dietary addition of H57 with respect to the parameters measured. However, birds receiving the highest dose (10^8 CFU/g) did not always outperform the birds receiving 10^7 CFU/g feed. It was demonstrated that H57 reduced enteric disease stress, maintained gut health and integrity, and growth performance.

ACKNOWLEDGEMENT: This study was supported by an Advanced Queensland Industry Partnership Grant.

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IN-OVO INJECTION OF OREGANO ESSENTIAL OIL DID NOT AFFECT HATCHABILITY OR POST-HATCHING PERFORMANCE IN BROILER CHICKENS

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Plant-derived essential oils (EOs) contain functional phytochemical components that are used as feed additives to improve gut health and reduce the need for antibiotics. The injection of EOs into fertile eggs has the potential to influence the developing embryo including the gastrointestinal tract. This in turn may mitigate intestinal susceptibility and regulate gut microbial composition, resulting in long-term benefits in the chicken's life. However, some gaps in the in-ovo injection methodology such as the viable volume range without affecting embryonic development warrant further investigation. This study aimed to establish a range of volume injected of a fixed amount of EO without a negative impact on hatchability, embryonic development, and post-hatch growth. We hypothesized that high injection volumes would be detrimental to embryonic development due to the tight space in the egg and/or the excessive hydration of the egg compartments such as the yolk impairing fat mobilization.

In this study, 300, 600, 900, 1200, or 1500 µL of saline solutions all containing 0.5µL of oregano essential oil (OEO) were injected into the amnion of fertile eggs at day 17.5 of incubation (n=96 eggs per treatment). Dilutions were made with sterile saline using polysorbate 80 as an emulsifier. Hatchability and performance metrics up to 7 days post-hatching were compared between treatments using analysis of variance in SAS 9.4's generalized linear model procedure. Table 1 shows the main results. In-ovo injection of a fixed amount of OEO at varying volumes had no significant influence on hatchability ($P > 0.05$). The largest volume injected, 1500 µL, significantly ($P < 0.001$) improved BW_0 compared to the control. However, by the end of the first-week post-hatch, there was no significant impact of the treatments on performance ($P > 0.05$). It is tempting to speculate that the excess water available in-ovo following the injection of the largest volume improved the hydration of the hatching embryo. After-hatch access to water seems to allow the other treatments to swiftly catch up. In conclusion, in-ovo injection of OEO at different volumes did not affect hatchability or early post-hatching performance in broiler chickens.

Table 1 - Effects of in-ovo injection of oregano essential oil (OEO) on hatchability and post-hatching performance (n=96). A fixed amount of OEO (0.5µL) was injected into the amnion at different saline solution volumes (from 300 to 1500 µL) at day 17.5 of incubation.

Volume of injection of OEO	Hatchability (%)	BW_0 (g)	BW_7 (g)	Feed intake (g)	FCR (g/g)
Non-injected	87.1	46.4^{bc}	207	159	0.984
Saline $_{100µL}$	94.7	46.4^{bc}	211	163	0.980
0µL	92.4	46.1^{c}	203	156	0.996
300µL	90.4	46.2^{c}	201	156	1.000
600µL	88.3	46.9^{ab}	213	159	0.948
900µL	86.2	46.7^{bc}	203	160	1.012
1200µL	90.6	47.5^{ab}	210	159	0.972
1500µL	87.2	48.0^{a}	208	160	0.997
SEM	-	0.28	3.8	3.6	0.021
P value	0.6707	< 0.0001	0.2690	0.9225	0.5353

OEO: oregano essential oil; BW_0; body weight at hatch; BW_7; body weight at day 7 post-hatch; FCR; feed conversion ratio.

ACKNOWLEDGEMENTS: This study has been partially funded by AgriFutures and Delacon Biotechnik GmbH.

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BENTONITE PLUS YEAST CELL WALL FRACTION IMPROVES THE PERFORMANCE AND HEALTH OF BROILERS UNDER MYCOTOXIN-CHALLENGED CONDITIONS

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Mycotoxins are a major problem in the poultry industry causing significant negative effects on animal health, productivity, and the economics of production. Binders such as bentonites are typically added to poultry diets to adsorb mycotoxins in the gastrointestinal tract and protect the birds against the harmful effects of mycotoxins. Bentonites are effective in preventing aflatoxicosis, however, their activity against zearalenone, ochratoxin, and trichothecenes is limited (Bhatti et al., 2018). Under multiple mycotoxin exposure, the use of binders with multi-mycotoxin adsorption seems to be a more effective solution. Bentonite plus yeast cell wall fraction (TOXO-XL, Trouw Nutrition) is a binder comprising a synergistic blend of smectite clay, glucose biopolymers, and inactive yeast cell wall fractions with a multi-binding capacity towards different mycotoxins.

This study aimed at investigating the effect of Bentonite plus yeast cell wall fraction on broilers' growth performance, serum biochemical parameters, and antioxidant status under multiple mycotoxin-challenged conditions. One-day-old male broiler chicks (n=450) were allocated to three treatments with 10 pens of 15 birds each. The treatments tested included 1) a negative control (NC, basal diet), 2) a positive control, (PC, as NC + 125 ppb Aflatoxin, 100 ppb Ochratoxin and 100 ppb T-2 toxin), and 3) PC + bentonite plus yeast cell wall fraction (TXL). Broilers were reared on floor pens in an open-sided housing and were given *ad libitum* access to feed and water throughout the 42-d experimental period. The broilers' body weight and feed consumption were measured weekly, and the body weight gain (ADG) and FCR were calculated. The mortality was recorded throughout the trial. The concentrations of aspartate aminotransferase (AST) and alanine transaminase (ALT) were measured in blood samples collected at d21, while the levels of serum superoxide dismutase (SOD) and malondialdehyde (MDA) were measured at d42. Data were analyzed using the MIXED procedure in SAS.

Results indicated that the mycotoxin challenge, compared with the NC, caused a significant decrease in weight gain (-25%) and an increase in FCR (+32%), but did not significantly affect the feed intake. Feeding diets with TXL significantly improved ADG (+16%, 47.58 vs 40.90 g) and FCR (-14.6%, 2.038 vs 2.386) compared to PC. In addition, TXL significantly decreased the concentrations of ALP (24.43 vs 32.26 U/L) and AST (229.5 vs 270.6 U/L) indicating an improvement in liver and kidney functions. Furthermore, TXL significantly enhanced serum SOD (150 vs 126 U/mL) and reduced serum MDA (3.3 vs 3.9 nmol/mL) suggesting an improvement in the antioxidant status of broilers. However, its effect on mortality was not significant. In conclusion, bentonite plus yeast cell wall fraction supplementation in contaminated feed alleviated the adverse effects of multiple mycotoxins on the performance and health of broilers and could be used as an effective solution against mycotoxicosis.

ACKNOWLEDGEMENTS: This study was funded by Trouw Nutrition, a Nutreco company.

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MICROBIOME MODULATION BY A PRECISION BIOTIC IN BROILER CHICKENS: A FIELD STUDY VALIDATION

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Summary

The objective of the present study was to evaluate the effect of the supplementation of precision biotic (PB) on the growth performance, and cecal microbiome modulation of broiler chickens raised under field conditions. A total of 190,000-day-old Ross 308 straight-run broilers were randomly assigned to two dietary treatments. There were 5 houses per treatment with 19,000 birds per house. At 42 d of age, growth performance and cecal microbiome were evaluated. The PB significantly improved (P = 0.04) the cFCR by 2.2 points and the EPI (P = 0.04) by 13 points. The abundance of pathways modulated by PB involve those associated with amino acid fermentation and putrefaction, particularly from lysine, arginine, proline, histidine and tryptophane. In conclusion, the results presented herein show that the PB can efficiently modulate microbiome pathways related to protein fermentation and putrefaction, leading to beneficial effects on the growth performance.

I. INTRODUCTION

The increased recognition of antimicrobial resistance as a public health risk and, therefore, the imposed restrictions on the use of antimicrobial growth promoters (AGP), has driven the search for novel nutritional strategies for broiler chickens. The advances in molecular biology, analytics, and data science in the past years have enhanced our understating of the gastrointestinal tract (GIT) microbiome of chickens (Oakley et al., 2014; Sun et al., 2021). These novel approaches have led to the development of precision biotics (PB) that are able to specifically modulate microbiome pathways of the GIT of chickens (Walsh et al., 2021). It has been found that the targeted modulation of microbiome pathways, mainly related to protein metabolism and utilization, and short chain fatty acid (SCFA) production, improves the growth performance of chickens (Walsh et al., 2021; Jacquier et al., 2022), and increase the resistance against enteric stress (Blokker et al., 2022).

Precision biotics (PB) are glycans with specific glycosidic linkages (Jacquier et al., 2022) that can redirect the functions of the microbiome towards increased beneficial outputs, such as higher propionate production and nitrogen utilization (Walsh et al., 2021), regardless of the taxonomic composition of the microbial community. The objective of the present study was to evaluate the effect of the supplementation of PB on the growth performance, and cecal microbiome modulation of broiler chickens raised under field conditions.

II. METHOD

A field trial was carried out at a commercial farm in Weifang City, Shandong Province, China. A total of 190,000-day-old Ross 308 straight-run broilers were randomly assigned to two dietary treatments. There were 5 houses per treatment with 19,000 birds per house. The two

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dietary treatments included a control diet (a commercial broiler diet) and a PB supplemented diet at 0.9 kg/MT (Symphiome^{TM}, DSM Nutritional Products, Switzerland).

At 42 d of age, the bird weight (BW) and feed intake (FI) of each house were recorded, the feed conversion ratio (FCR) was calculated and corrected with the final body weight (cFCR). Additionally, 40 birds/experimental group (80 birds in total) were randomly selected and the cecal content was aseptically collected. The samples were then sent to the lab and frozen at -80^{o}C until further processing (DNA isolation and sequencing).

The microbial DNA from the cecal content sample was extracted using MagPure Stool DNA KF Kit B (Magen, Wuhan, China) following the manufacturer's instructions. After DNA extraction, DNA was sequenced on the Illumina Hiseq platform (BGI-Shenzhen, China). The Functional Metagenomic Profiling and Microbiome Protein Metabolism Index (MPMI) was done as following: top microbial metabolic reactions (EC Numbers) and KEGG pathways responsible for distinguishing PB treated birds from Control were identified by sorting EC Numbers by "Mean Decrease in Accuracy" using the truncated random forest classifier. Each EC Number was then annotated and regrouped by KEGG Pathway.

Growth performance data were subjected to a student's t-test using JMP Pro v. 16.0 (SAS Institute, Cary NC). House served as the experimental unit. Statistical significance was considered at $P \leq 0.05$.

III. RESULTS

The supplementation of PB significantly improved the cFCR by 2.2 points (P = 0.04) and the EPI by 13 points (P = 0.04; Table 1).

Table 1 - Efficacy of a precision biotic on the growth performance of broilers from 1 to 42 days of age[1].

Treatment	BW, g/bird	FI, g/bird	FCR	cFCR	Mortality, %	EPI
Control	2,735	3,986	1.457	1.471[a]	1.62	450[b]
Precision Biotic	2,787	4,029	1.446	1.449[b]	1.56	463[a]
SEM	28.1	53.7	0.01	0.01	0.09	4.3
P-value	0.23	0.62	0.36	0.04	0.70	0.04

[1]Data were collected from all the birds of each house, with 5 replicate houses per treatment.
BW: body weight, FI: feed intake, FCR: feed conversion ratio, cFCR: FCR corrected with body weight, EPI: European production index.

The Local Fisher Discriminant Analysis (LFDA) of functional profiles (Figure 1A), showed a clear and significant qualitative separation in the cecal microbiome metabolism between control and PB supplemented birds. The abundance of pathways modulated by PB involved those associated with amino acid fermentation and putrefaction, particularly from lysine, arginine, proline, histidine and tryptophane (Figure 1B). Other pathways of importance related to purine, vitamins, carbohydrates, and ABC transporters were also modulated by the supplementation of PB. It was observed that the supplementation of PB significantly reduced the abundance of pathogen groups (*Escherichia coli* and *Salmonella enterica*) in the cecal microbiome (Figure 1C).

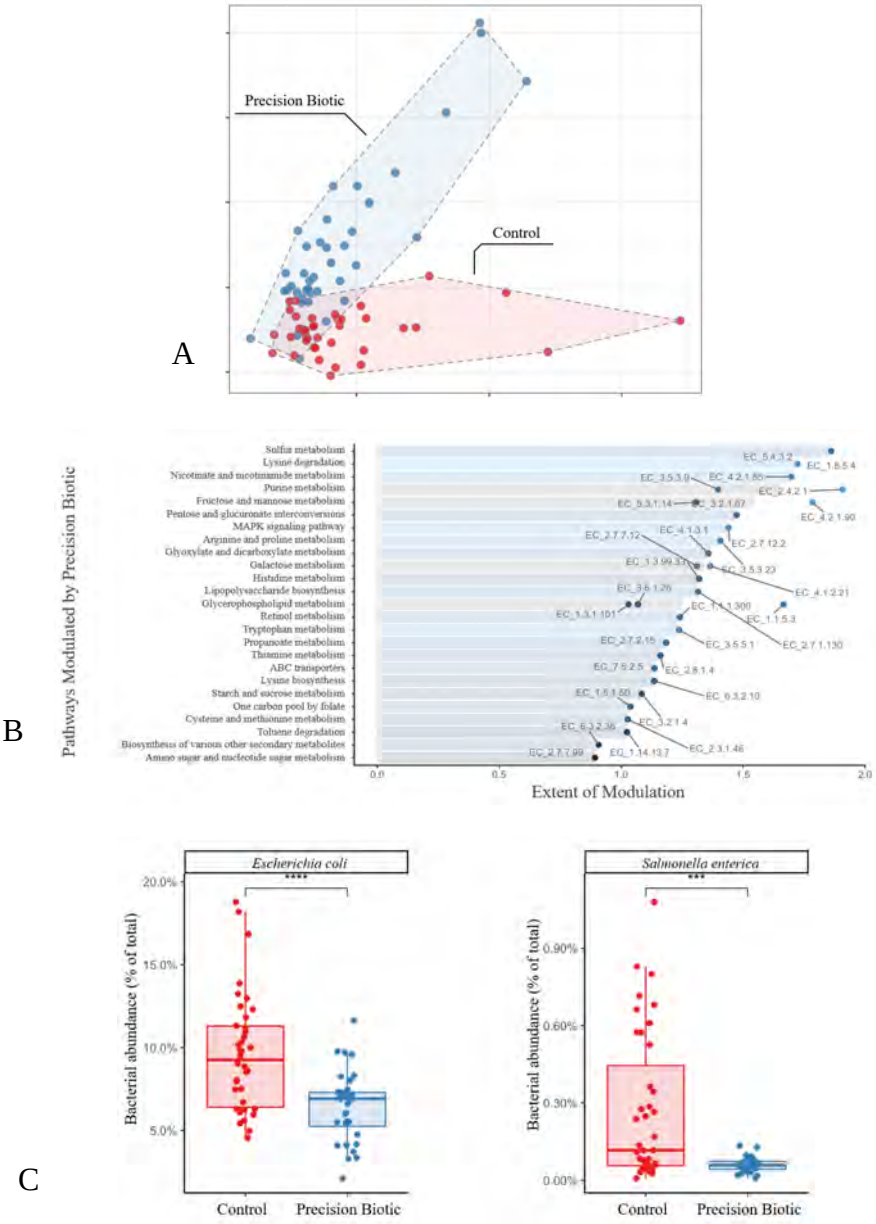

Figure 1 - Local Fisher Discriminant Analysis (LFDA) of functional profiles demonstrating distinct clusters between groups (A); 25 most modulated KEGG Pathways in the microbiome of broiler chickens supplemented with Precision Biotic (PB) relative to the microbiome of chickens fed the control diet (Set as 0). Enzymes (labeled points) were mapped back to KEGG pathways (B); Relative abundance (% of total) of Escherichia coli and Salmonella enterica in the cecal microbiome of broiler chickens supplemented or not with PB ($^{***}P < 0.01$; n = 40 samples/treatment group).

IV. DISCUSSION

The PB used in the present study has been previously shown, in experimental research settings, to modulate the pathways of the cecal microbiome (Walsh et al., 2021), led to improved growth performance and welfare (Jacquier et al., 2022), and improved resilience of chickens against enteric stress (Blokker et al., 2022). This is the first study, however, conducted in field conditions where we demonstrated that the output obtained in research trials with the supplementation of chickens with PB are translated into field conditions.

In a previous meta-analysis by Walsh et al. (2021) it has been reported how the PB used herein consistently improved the growth performance of broiler chickens. Also, Jacquier et al.

(2022) demonstrated that this PB not only improved the growth performance of chickens, but also had positive effects on the litter quality, which translated into enhanced gait score. In the present study, it was also observed that the supplementation of PB improved the cFCR by 2.2 points. The overall growth performance of the control group in the present study was satisfactory (final FCR of 1.457 vs 1.611 as the breed target) suggesting a good overall health of the birds. However, the cFCR was improved by 2.2 extra points with PB, which shows that by harnessing the full potential of the intestinal microbiome, precision nutritional ingredients may improve the performance of chickens to reach or to go beyond their genetic potential.

In conclusion, the results presented herein prove that the PB can efficiently modulate the intestinal microbiome of broiler chickens towards a beneficial metabolism related to protein metabolism and utilization with positive effects on the growth performance of the birds.

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EFFECT OF A PROBIOTIC SOLUTION ON INTESTINAL HEALTH OF BROILER CHICKENS CHALLENGED WITH *SALMONELLA* AND MYCOTOXINS

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Summary

The objective of this study was to assess the effect of *Bacillus subtilis DSM 29784* on intestinal health in broilers challenged with *Salmonella* and fusarium mycotoxins. 322 day-old chicks were divided into 7 different groups and placed in separate isolators. They received either mycotoxins (mainly DON, FUM and T2) in the diet and/or *Salmonella Heidelberg* oral inoculation at d4. *Bacillus subtilis DSM 29784* was also tested in some treatments. At d4, d7, d14, d21, d28, 8 birds per treatment were sacrificed: blood, feces, liver and intestinal tissues and content were collected. Probiotic treated birds showed a decrease of plasma FITC-d and a reinforcement of the gut barrier. *Salmonella* spp prevalence in caeca decreased with the supplementation of the probiotic which also promoted the anti-inflammatory response with a greater IL-10 gene expression. LPO as a sensitive indicator of the redox balance, was also positively shifted by *Bacillus subtilis*. Villus height increased with the supplementation of the probiotic. Finally, beneficial bacteria such as butyrate producers firmicutes were also promoted in the probiotic groups. We can conclude from this study that mycotoxin can exert negative impacts on intestinal health and can increase salmonella infection. The addition of *Bacillus subtilis DSM 29784* can protect the animal from dietary and pathogen challenges.

I. INTRODUCTION

Mycotoxins are secondary metabolites produced by fungi (fusarium, aspergillus, penicillium mainly) that can cause serious health problems in poultry and may result in severe economic losses. They can exert negative impact on both performance (Kolawole et al, 2020) and intestinal health (Antonissen et al, 2014) depending on the type of mycotoxin, extent of exposure, its concentration, the age of the animals and their health status. Mycotoxins can also be a predisposing factor for other issues and increase susceptibility to infectious diseases like salmonellosis. Co-infection models have not been well studied over the past decades and only Ziprin and Elissalde (1990) explored the effect on broilers of T2-toxin with *S.* Typhimurium. Out of all mitigation strategies used to control salmonella prevalence in poultry, probiotics are seen as a promising technology with complementary modes of action (Keerquin et al, 2021). They can also be considered as potential solutions to control some inflammatory responses induced by mycotoxins (Rhayat et al, 2019). Therefore, the current study evaluated immune parameter modulation by a commercial probiotic solution, *Bacillus subtilis DSM 29784* (Bs29784) in a mycotoxin-Salmonellosis experimental model (Liew and Mohd-Redzwan, 2018).

II. METHODS

322 one-day-old Ross broilers were acquired from a commercial hatchery. The animals were placed in isolators (experimental unit) and randomly assigned to 7 different groups of 46 broilers each. The treatments consisted of a control group (ctrl), a mycotoxin (CM) and

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salmonella group (CS), a control group supplemented with the probiotic Bs29784 ($1x10^8$ CFU/kg feed; PRO), a co-infection mycotoxin-Salmonella group (CMS), a Salmonella group with Bs29784 (CS-PRO) and a mycotoxin-Salmonella group receiving Bs29784 (CMS-PRO). From 1 day-old until the end of the study at d28, the animals in CM, CMS and CMS-PRO groups received the mycotoxins in their diet (mainly fumonisins (12744 ppb), deoxynivalenol (1452 ppb) and T-2 toxin (358 ppb)). On the fourth experimental day, each animal of CS, CMS, CS-PRO and CMS-PRO groups received $1x10^8$ CFU Salmonella Heidelberg by oral gavage. All birds (feed and drinking water also) were initially confirmed to be *Salmonella* Heidelberg negative prior to the commencement of the study. Then, at d4, d7, d14, d21, d28, 8 birds per treatment were sacrificed and blood, liver and intestinal tissues, cecal content, and feces were collected. Intestinal permeability was evaluated by serum FITC-dextran, caeca content was collected to assess *Salmonella* spp. counts by Most Probable Number (MPN) and microbiota composition was determined using S16 sequencing. In caecal tonsil, the cytokine gene expression was measured, histomorphology was assessed from jejunum and caecum tissues and liver samples were also collected for lipoperoxidation (LPO) analysis and histopathology evaluation. Finally, anti-salmonella IgA was quantified in feces. Statistical analysis was performed by two-way ANOVA with Tukey's post-hoc test ($P < 0.05$).

III. RESULTS AND DISCUSSION

Mycotoxin presence in the feed significantly increased intestinal permeability ($P=0.0157$) compared to the control group and the *Bacillus subtilis* probiotic decreased the FITC-d concentration in blood by 13 and 11% ($P<0.05$) in the salmonella and salmonella/mycotoxin challenges, respectively. The mycotoxins are considered as "gate openers" which can increase the translocation of some toxins or pathogens through the intestinal barrier.

LPO as a biomarker of cell damage caused by free radicals' exposure, is sensitive to the presence of the *Bacillus subtilis* probiotic. The probiotic decreased the LPO concentration in liver tissue by 52% ($P<0.05$). It has been recently demonstrated (Kruse et al., 2021) that the *Bacillus subtilis* probiotic can produce high concentrations of antioxidants under challenge conditions which could explain part of its effect in controlling the lipoperoxidation process.

Both fusarium mycotoxins and Salmonella affect the innate immune system resulting in increased expression of several cytokines and activation of macrophage as shown by the increase of IL-6 pro-inflammatory cytokine relative to the control (+7% and +4% ($P<0.05$) with mycotoxins and salmonella, respectively). The presence of the *Bacillus subtilis* probiotic in the diet can limit the production of IL-6 by 11% ($P<0.05$) and then maintain the initial state before the challenge. Choi et al (2021) demonstrated that the *Bacillus subtilis* probiotic can facilitate the production of metabolites like hypoxanthine which is involved in cellular pathways leading to control the secretion of pro-inflammatory compound like IL-6.

Finally, the prevalence of *Salmonella* was also investigated by counting its numbers in the caecal content. The probiotic solution significantly decreased by 35% and 10% the *Salmonella* spp count in the intestine under the Salmonella and Salmonella-mycotoxin challenges, respectively.

IV. CONCLUSION

Fusarium mycotoxins have negative impacts on gut health and can increase susceptibility of broilers to *Salmonella* infection. Bs29784 has shown beneficial effect on gut permeability, salmonella shedding, redox status and on the control of inflammation, and can support the health of the animals in a preventive strategy against mycotoxin and *Salmonella* challenges.

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TOWARDS ANTIBIOTIC-FREE POULTRY PRODUCTION USING MULTI-STRAIN PROBIOTICS FOR AMELIORATION OF AVIAN PATHOGENIC E.COLI

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Summary

Currently, the use of probiotics for health benefits is attractive due to the search for safer products with protective effects against diseases. Based on in-house *in vitro* screening, three potential *Bacillus* isolates (*Bacillus subtilis* PB6, *Bacillus licheniformis* G3, and *Bacillus subtilis* FXA) were selected to be part of *in vivo* testing. An experimentally induced avian pathogenic *Escherichia coli* (APEC) strain challenge trial was conducted on broiler birds to evaluate the effect of single and multi-strain probiotics (combinations of three isolates) in controlling colibacillosis. The trial was conducted on Cobb day-old Cobb 430 male birds for a period of 35 days. A total of 432 birds were randomly divided into 6 groups with 6 replicates each and 12 birds per replicate. The groups include 2 controls and 4 treatments, 1) Uninfected control, 2) Infected control, 3) Infected bird with *Bacillus subtilis* PB6 at 500 g/ton, 4) Infected birds with *Bacillus licheniformis* G3 at 500 g/ton, 5) Infected birds with *Bacillus subtilis* FXA at 500 g/ton, 6) Infected birds with a multi-strain of FXA, G3, and PB6 at 500 g/ton. On day 7, birds were orally challenged with 1.0 mL (2×10^6 CFU/mL) of freshly grown APEC field strain, whereas the unchallenged group was administrated the same volume of saline solution. The highest reduction in overall mortality was observed in birds given the combination of FXA, G3, and PB6. This group also presented the highest final body weight and lowest FCR compared to the infected control.

I. INTRODUCTION

Growing concern over the use of antibiotics due to the rise in antimicrobial resistance and rising consumer demand for antibiotic-free products is increasingly becoming a challenge for poultry production. There is a need to identify natural and economical alternatives such as probiotics. The supplementation of probiotics as an antibiotic growth promoter (AGP) alternative in poultry production has the potential to improve poultry health, performance, growth, and feed efficiency (Reuben et al., 2021).

Avian pathogenic *Escherichia coli* (APEC) infections in poultry are associated with major economic losses to the poultry industry worldwide. Colibacillosis, caused by APEC, is typically a localized or systemic disease that occurs in poultry when host defenses have been compromised by virulent APEC strains (Lutful Kabir et al., 2010). The treatment or prevention of colibacillosis is accomplished by using antibiotics, such as colistin sulfate. However, the emergence of antibiotic-resistant bacteria has reduced the efficacy of antibiotics and may pose considerable risks to human health (Wang et al, 2017). Thus, there is a need for alternatives to antibiotics. Studies have shown that the translocation of these pathogenic bacteria may be reduced by the presence of probiotics in the intestine (Isroli et al., 2018). The present study intended to determine whether the feeding of *Bacillus* probiotic as a single strain and in combination would reduce the translocation of APEC and thereby reduce the severity of infection in the poultry model.

II. METHOD

A five-week broiler avian pathogenic *E. coli* challenge trial was conducted as part of screening studies for potential probiotics at the in-house Kemin poultry research farm, Gummidipoondi, Chennai, India. The trial was conducted using 432 one-day-old Cobb-430 male chicks. The chicks were procured from Komarla Hatcheries, Pollachi, India. The birds were divided into 6 groups with

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6 replicates and 12 birds per replicate in a total of 36 pens of 4 ft x 4 ft (1.18 sq. ft/bird). The details of the treatment groups are shown in Table 1. A completely randomized design was adopted to minimize the effect of environment and management on different groups. All the birds were reared under a deep litter system with paddy husk bedding throughout the experimental period. Paddy husk was processed by spreading the material in sun for about 4 days, after which bleaching powder, lime powder, and omnicide (disinfectant) were sprinkled and used for bedding. The *E. coli* strain was isolated from colibacillosis-infected birds on a commercial farm. On day 7, birds were orally challenged with 1.0 mL (2×10^6 CFU/mL) of freshly grown APEC (Internal ID - EC20UT) field strain isolated from the colibacillosis-infected birds using a 1 mL pipette or syringe. The unchallenged group was administrated with the same volume of saline solution.

Table 1 - Details of the probiotic treatment groups in APEC challenged and unchallenged broiler birds.

Group	Treatment	Dosage g/ton	Spore count (CFU/g of feed)
Uninfected Control	-	-	-
Infected Control	-	-	-
Treatment 1	*Bacillus subtilis* PB6	500 g/ton	2.00E+06
Treatment 2	*Bacillus licheniformis* G3	500 g/ton	2.00E+06
Treatment 3	*Bacillus subtilis* FXA	500 g/ton	2.00E+06
Treatment 4	Multi strains FXA, G3, and PB6	500 g/ton	2.00E+06

Table 2 - Ingredient of experimental diets.

Ingredients (g/kg)	Pre-starter	Starter	Finisher
Maize	530	598	625
Soya (45 % CP)	387	312	283
Rice Polish	20	30	30
Rice Bran Oil	25.4	25	32.4
Dicalcium phosphate	12.3	11.2	9.8
Calcite	10.8	9.8	8.1
DL-Methionine	3	2.8	1.9
L- Lysine	2.5	2.3	1.9
Salt	2.5	2.3	2.3
Soda	2.7	2.3	1.9
Choline Chloride (60 %)	0.5	0.5	0.5
Toxin Binder	1	1	1
L- Threonine	0.7	0.5	0.4
Vitamins	0.5	0.5	0.5
Organic TM	0.5	0.5	0.5
Antioxidant	0.15	0.15	0.15
Phytase (5000 FTU)	0.1	0.1	0.1
CMP -1 (Diclazuril) - Venkys	1	1	1
Total	1000.15	999.45	999.95

All birds were fed a starter diet from 1 to 14 days, a grower diet from 15 to 28 days, and a finisher diet from 29 to 35 days. The weekly parameters monitored were body weight, feed intake, feed conversion ratio (FCR), and mortality. Birds had access to *ad libitum* clean drinking water including sanitizer and acidifier. The birds were vaccinated for Newcastle Disease and Gumboro Disease on the 5^{th} and 12^{th} day, respectively.

Table 3 - Chemical composition of experimental diets.

Specification	Pre-starter	Starter	Finisher
Crude protein (g/kg)	219	195	183
Energy (MJ/kg)	12.14	12.45	12.79
Digestible Lysine (g/kg)	12.8	11	10
Digestible Methionine (g/kg)	6.1	5.5	4.6
Digestible Arginine (g/kg)	14	12	10.9
Digestible Methionine + Cysteine (g/kg)	9	8.2	7.1
Digestible Threonine (g/kg)	7.9	6.8	6.4
Digestible Tryptophan (g/kg)	2.2	1.9	1.7
Digestible Valine (g/kg)	9.4	8.4	7.9
Digestible Iso leucine (g/kg)	8.4	7.3	6.7
Crude fibre (g/kg)	37	35	33
Calcium (g/kg)	8.8	8	7
Available Phosphorous (g/kg)	4.6	4.3	4
Fat (g/kg)	52	56	64
Sodium (Na) (g/kg)	1.97	1.8	1.65
Chloride (Cl) (g/kg)	23	22	21

Statistical analysis of the data for cumulative body weight and FCR was performed using Statgraphics Centurion XVI.II software. Data were analyzed by one-way analysis of variance (ANOVA). A P value of < 0.05 is considered statistically significant.

III. RESULTS AND DISCUSSION

The performance on day 35 of birds receiving the probiotics treatments as well as the control are shown in *Figure 1*. Since this was a part of a screening trial with a low number of replicates, there was a high variance in performance due to mortality.

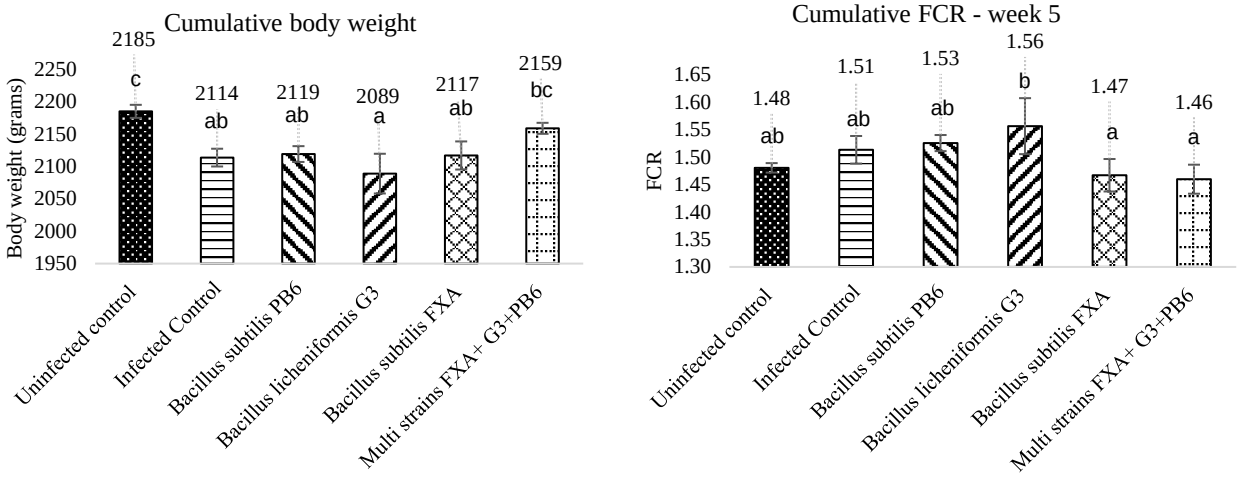

Figure 1 - Effect of multi-strain probiotics on body weight (left) and feed conversion ratio - FCR (right). Bars with different superscripts indicate significant differences ($P < 0.05$).

In terms of body weight gain, the multi-strain probiotics group showed improvement by 45 g in comparison to the infected control ($P > 0.05$). However, in terms of FCR, multi-strain supplemented groups showed 5 points difference while the uninfected control showed 3 points as compared to the infected control group ($P > 0.05$).

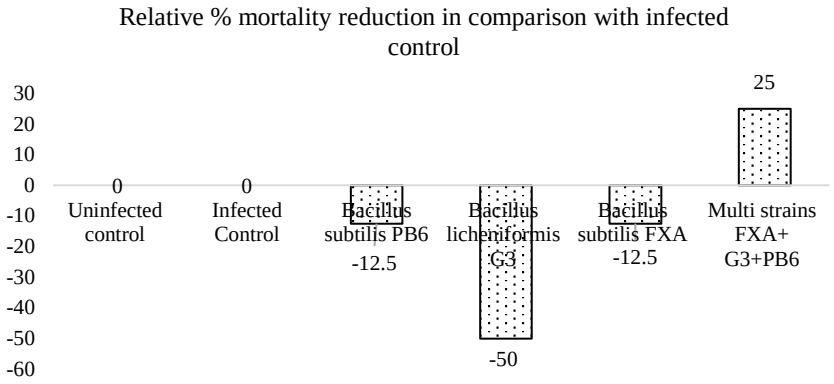

Figure 2 - Effect of multi-strain probiotics on mortality reduction.

Supplementation with the single strains group did not reduce mortality, whereas supplementation of the multi-strain probiotics resulted in a 25 % reduction in mortality compared to the infected control birds. The beneficial effect of *Bacillus* probiotics could be due to factors such as the production of organic acids and bacteriocins with a bactericidal action (Wang et al., 2017). Although single-strain probiotics are beneficial to the host, multi-strain probiotics might have additional benefits in terms of performance due to synergism and additive effects among the individual isolates (Kwoji et al., 2021).

IV. CONCLUSION

Overall, the present study results suggest that supplementation with multi-strain probiotics (FXA, G3, and PB6) can reduce mortality in colibacillosis-infected birds in comparison to single-strain supplementation. These findings indicate that multi-strain *Bacillus* probiotic supplementation has the potential to be used as an alternative to antibiotics, especially for the control of avian pathogenic *E. coli*. Further research on the effects of multi-strain *Bacillus* probiotics supplementation is needed to elucidate its effects on gut microbiota.

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AN EVALUATION OF A NATURAL OREGANO ESSENTIAL OIL-BASED FEED ADDITIVE ON THE WORM BURDEN AND PRODUCTIVITY OF BOVANS BROWN LAYING HENS IN A FREE-RANGE PRODUCTION SYSTEM

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Summary

Alternatives to anthelmintics for mitigating helminth infection and maintaining hen performance are much needed in free-range egg production systems. The objective of this study was to evaluate the effect of feeding a natural oregano essential oil-based feed additive (OS) to Bovans Brown laying hens in a free-range system, on intestinal helminth load, egg production and egg quality characteristics.

Pullets were fed either a Control (corn/SBM diet) or supplemented diet (Control + OS (300g/tonne)) through to 16 weeks of age. The laying phase, from 17-41 weeks of age, had three dietary treatment groups as follows: (1) Control in rearing phase (C) + Control in laying phase (C), CC; (2): Control in rearing phase (C) + OS in laying phase (O), CO; and (3) OS in rearing phase (O) + OS in laying phase (O), OO. Five hundred and forty hens were reared in a conventional slatted system until 12 weeks of age, then allowed to range with a mix of summer and winter forages providing three replicates per treatment in the production phase. Biweekly performance data was captured. Tapeworm, large roundworm, and caecal roundworm burden were measured at 16 weeks and termination of the trial at 41 weeks.

At the end of rearing, caecal roundworms were observed in lower numbers in pullets on O diet rather than C. At 41 weeks, a lower count of caecal roundworms and large roundworms were observed in hens in OO group compared to hens in CO and CC groups. Fewer hens in OO and CO groups were infected with tapeworms compared to CC hens. Feed conversion (kg feed consumption/dozen eggs) was six points lower for OO and CO than CC, and a larger proportion of USDA Grade A eggs were produced for OO than CO and CC.

The use of natural oregano oil in free range poultry systems offers a potential tool for mitigating helminth infections and maintaining hen performance.

I. INTRODUCTION

Consumer demand for organic and free-range poultry products is driving the commercial egg industry away from conventional housing systems towards free-range housing systems that allow birds access to the outdoors. Whilst these systems are considered higher welfare, they also carry more health risks that can impact the intestinal health of the bird, such as greater exposure to parasites as reported by Permin et al., (1999). Intestinal helminths such as nematodes and tapeworms may reduce hen performance and transmit disease. Challenges with preventing and treating such helminth infections include increasing resistance to anthelmintics and a limited number of anthelmintics available for laying hens. Therefore, finding alternatives for mitigating helminth infections and maintaining hen performance in free-range housing systems is important for production efficiency and hen welfare. Anti-parasitic properties of oregano essential oil have been reported previously (Force et al., 2000).

This study was conducted to provide an evaluation of feeding Orego-Stim®, OS to a brown egg breed, Bovans Brown (BB), in a free-range system, on intestinal helminth loads, egg production and egg quality characteristics.

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II. METHOD

Pullets were fed either a Control (corn/SBM diet) or supplemented diet (Control + OS (300g/tonne)) and were brooded and reared on slats until 12 weeks of age and then allowed on range through to 16 weeks, followed by the laying phase on range (929 cm^2/pullet) from 17-41 weeks of age. The laying phase had three dietary treatment groups as follows: (1) Control in rearing phase (C) + Control in laying phase (C), CC; (2): Control in rearing phase (C) + OS in laying phase (O), CO; and (3) OS in rearing phase (O) + OS in laying phase (O), OO. Five hundred and forty hens were reared in a conventional slatted system, then allowed on range with a mix of summer and winter forages that provided three replicates per treatment in the production phase. Biweekly performance data was taken during rearing and laying phases. Tapeworm, large roundworm, and caecal roundworm burden was measured at 16 weeks, just before the laying phase, and termination of the trial at 41 weeks by screening the intestinal contents of 10 hens from each treatment for adult helminths.

III. RESULTS

At the end of the rearing phase, no large roundworms or tapeworms were observed in any of the hens, but caecal roundworms were observed in lower numbers in pullets on O diet rather than C ($P > 0.05$) (Table 1).

There was no difference observed in pullet days (pullet number x days in rear), mortality, pullet end body weight, pullet body weight gain, feed consumption per pullet per day or feed conversion (g feed/g gain) in rearing phase between treatments ($P > 0.05$).

Table 1 - Rearing phase helminth summary.

Treatment	Caecal roundworm	
	(count/bird)	(SEM)
C	13.41	7.50
O	2.77	1.22

C, control in rearing phase; O, OS in rearing phase.
No significant differences were found between treatments ($P > 0.05$).

Large roundworm, caecal roundworm, and tapeworms were observed in all 3 treatment groups at 41 weeks. A lower count of caecal and large roundworms were observed in hens in OO group compared to CO and CC groups ($P > 0.05$). Fewer hens in OO and CO groups were infected with tapeworms compared to CC hens ($P > 0.05$) (Table 2).

In the laying phase, there was no difference in egg production percent (hen day or hen house), egg weight or age at 50 % production between treatments ($P > 0.05$). Feed consumption was lower for OO and CO than CC ($P < 0.05$) and the overall feed conversion (kg feed consumption/dozen eggs) was six points lower ($P < 0.05$) for OO and CO than CC (Table 3). A larger proportion of USDA Grade A eggs ($P < 0.05$) were produced for OO than CO and CC (Table 4).

Table 2 - Laying phase helminth summary.

Treatment	Caecal roundworm		Large roundworm		Hens infected with tapeworm	
	(count/bird)	(SEM)	(count/bird)	(SEM)	(%)	(SEM)
CC	79.30	24.05	14.90	3.45	56.67	9.20
CO	69.83	12.74	22.63	5.38	33.33	8.75
OO	68.07	12.24	11.07	2.67	43.33	9.20

CC, control in rearing and laying phase; CO, control in rearing and OS in laying phase; OO, OS in rearing and laying phase.
No significant differences were found between treatments ($P > 0.05$).

Table 3 - Laying phase production summary.

Treatment	Hen day production	Hen house production	Feed consumption	Feed conversion	Mortality	Egg weight	Age at 50% production
	(%)	(%)	(g/bird/day)	(kg feed /doz. eggs)	(%)	(g)	(days)
CC	87.03	86.86	115.71[a]	1.603[a]	0.093	59.19	126
CO	87.55	87.75	112.81[b]	1.547[b]	0.093	58.95	127
OO	87.97	86.51	112.53[b]	1.538[b]	0.556	58.66	126
SEM	0.890	0.772	0.964	0.016	0.223	0.243	0.816
*P-value	0.575	0.509	0.045	0.009	0.249	0.327	0.946

CC, control in rearing and laying phase; CO, control in rearing and OS in laying phase; OO, OS in rearing and laying phase.
* ab Different letters within column at each period represent significantly different means (P < 0.05) between treatments.

Table 4 - Laying phase egg quality summary.

Treatment	USDA grade A	USDA grade B
	(%)	(%)
CC	99.26[ab]	0.37
CO	98.33[b]	1.11
OO	99.82[a]	0.18
SEM	0.354	0.286
*P-value	0.016	0.064

USDA grade A, high quality eggs most often sold in stores;
USDA grade B, lower quality eggs usually used to make liquid, frozen and dried egg products.
* ab Different letters within column at each period represent significantly different means (P < 0.05) between treatments.

IV. DISCUSSION

The inclusion of a natural oregano oil-based feed additive numerically reduced helminth infections at the end of the rearing and laying phases. The anti-parasitic properties of plants are well characterized *in vivo* (Dhama et al., 2015), where natural oregano oil has been shown to significantly reduce gut lesion scores caused by intestinal parasite, *Eimeria* spp. in broilers (Giannenas et al., 2003). Natural oregano oil is documented to contain over 100 active components which together have a complex mode of action, exhibiting anti-microbial, anti-oxidant and anti-inflammatory properties (Gheisar and Kim, 2018). Rajkovic et al., (2019) showed carvacrol, a main active component of natural oregano oil, to be effective against large roundworms taken from the intestines of laying hens, with no evidence of resistance.

The most common genera of roundworms and tapeworms in poultry reside in the intestine and/or caeca. Infection can cause damage to the intestinal mucosa, reducing feed efficiency and subsequent egg production. In this study, feed conversion efficiency was improved by six points (P < 0.05) when hens were supplemented with natural oregano oil in either rearing or laying phase. In a recent experiment (Lund et al., 2020), intestinal morphology was improved in pullets fed diets supplemented with 300g/tonne OS. Supplemented pullets had increased villus height, deeper crypt depth and thicker intestinal lining, indicating better digestive function. Other studies in poultry have reported improved gut morphology and beneficial modulation of the gut microbiota following dietary supplementation with natural oregano oil at the same concentration as that used in this study (Mohiti-Asli and Ghanaatparast-Rashi, 2018; Soliman et al., 2016). Whilst intestinal health parameters were not assessed in this study, previous work would suggest natural oregano oil may have helped in improving feed efficiency and egg quality.

In conclusion, the inclusion of a natural oregano oil-based feed additive either during both rearing and laying phases, or the laying phase only, reduced helminth infections, whilst improving feed efficiency and egg quality. The use of natural oregano oil in free range poultry systems offers a potential tool for mitigating helminth infections and maintaining hen performance, which can be used to support conventional worm control programmes.

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BETA-GLUCAN EFFECTS ON VACCINE RESPONSES AND INNATE IMMUNITY IN LAYERS

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Summary

The aim of the study was to investigate the potentiation of vaccine responses achieved by adding Safglucan®, a beta-glucan product derived from yeast, to feed for layers. The objective of the study was to demonstrate that our yeast beta-glucan product can promote innate immune responses in layers and therefore strengthen vaccination protocols. The main read outs of study were antigen presenting cell counts, inflammatory cytokine production, T-cell response and antibody titres. Trial results confirm that supplementing the feed of layers with the beta-glucan product during the first half of the rearing period helps improve innate and adaptive immune system activity. This effect is called immune system training.

I. INTRODUCTION

The product tested is a purified yeast fraction which is highly concentrated in β-1.3/1.6 glucans (BG). β-1.3/1.6 glucans stimulate the immune system by triggering the Dectin-1 receptors located on the surface of phagocytes, stimulating a release of cytokines. Cytokines induce different immune pathways causing an immune response. When phagocytes that have previously been exposed to β-1.3/1.6 glucans, their immune responses toa pathogen is faster and stronger than innate cells that haven't encountered BG. This innate memory improvement is called trained immunity.

The aim of this study was to evaluate the effect of supplementing laying birds from D0 with beta-glucan product for a period of 8 weeks on inducing the training immunity from antigen presenting cells (APCs) in certain tissues such as the spleen and intestine as well as evaluating the enhancement of inflammatory cytokine production by the cells from these tissues. Having better priming APC should lead to an improvement of adaptive immune response such as T and B cells responses. To confirm this hypothesis antigen-specific immunoglobulins for Salmonella, Infectious Bronchitis (IB) as well as Newcastle Disease (ND) were evaluated in test and control birds using ELISA assay and a T cell was performed.

II. METHOD

This trial, which involved 47 Lohmann Brown layers, ran from Day 1 to 23 weeks of age. 27 birds were kept as a control (C) and 20 were given feed supplemented with 125 g/T of the beta-glucan product. Blood samples were taken at 3, 6, 9, and 23 weeks old, to assess antigen-specific immunoglobulins quantification by ELISA. Tissue samples (spleen and intestine) were collected at 3, 6, and 9 weeks old, to select different types of Antigen Presenting Cells (APC). This was done by flow cytometry. TNF-alpha cytokine relative expression was also evaluated. Birds from all groups were subjected to a commercially used vaccination regime (Table 2). All vaccinations were prescribed by a veterinarian and no on farm medicinal treatments such as anti-inflammatories or anti-microbials were rendered after arrival of chicks on the farm.

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Table 1 - Description of different investigational groups.

Group	Group name/product	Number of animals/group	Treatment
T01	Vaccinated control	27	-
T02	Vaccinated + Beta-glucan product phase 1	20	D0 – week 8

Table 2 - Vaccination schedule.

Age chickens	Vaccine	Application route	Live/inactivated
1 day	HVT+IBD+ND	IM	Live
	Marek		
	IB Ma5	Spray	
	IB 4/91		
+/-7 days	Salmonella	DW	Live
+/-14 days	ND-clon	Spray	Live
+/- 25 days	IB primer	Spray	Live
5 weeks	Rhino CV	Spray	Live
6 weeks	Salmonella + ND	DW + Spray	Live
8 weeks	IB QX	Spray	Live
9 weeks	ILT and AE+PD	Oculonasal + wingweb	Live
14 weeks	IB+ND	IM	Inactivated
16 weeks	Salmonella	DW	Live

At specified ages, blood was collected from assigned chickens in designated groups. Antibodies were determined by HI and ELISA methods. At sampling days, spleen and ileum were individually collected in small containers containing PBS at approximately 4 °C. Sera were also collected form each bird. Tissue samples were collected from all treatment groups on the same day. After cell isolation, APCs were quantified using a flow cytometer and gating on live $CD45^+$ (immune cells), $CD3^-$ (T cells), $Bu1^-$ (B cells), MHC II^+ (ID card specifically expressed on APC). Cells isolated were also stimulated with LPS for 24h to quantify the trained immunity ability of those cells.

In a second set of experiment, immune cells isolated from blood were stimulated using a specific antigen lysate from Newcastle disease (ND), or infectious bronchitis (ID) or Salmonella. Data analysis was performed using R (version 3.2.5) and data was collected on body weight (BW), average daily gain (ADG), daily feed intake (DFI) and feed conversion ratio (FCR). This data was analyzed using a linear regression model with treatment group as a fixed effect.

Investigational groups were compared to the negative control group as a standard. Statistical significance was assessed at $P \leq 0.05$. Immune results were analysed with graph pad prism software using a Mann-Whitney nonparametric test or a Kruskal-Wallis test (one-way ANOVA analysis) was used to compare significance between groups according to the data set. A $p<0.05$ was considered statistically significant.

III. RESULTS

Flow cytometry analysis showed a numerical increase of the phagocytes in the spleen and in the intestine after 6 weeks of beta-glucan feed supplementation compared to the control group

indicating that APCs in animal fed with the beta-glucan product are able to perform immunity have been significatively increased in the intestine.

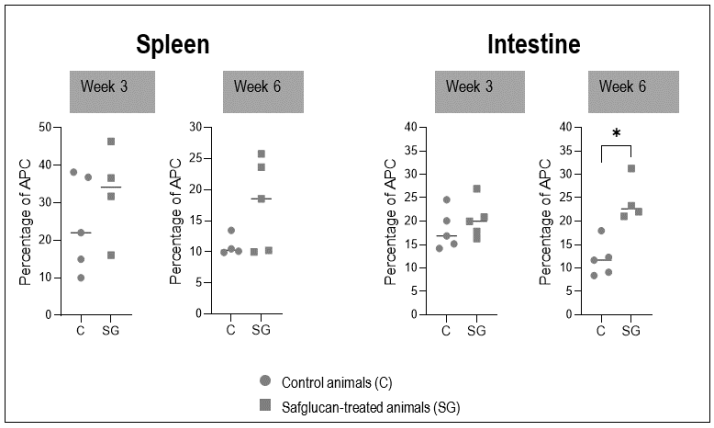

Figure 1 - Presence of Antigen Presenting Cells (APC) in the tissue.

The immune cells isolated from the intestine of animals fed with the beta-glucan product at 125g/t showed a higher production of TNFα only during the LPS challenge compared to immune cells isolated from the control group.

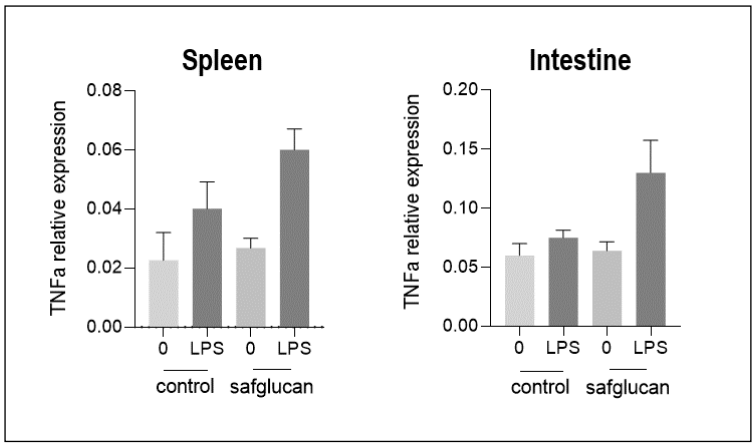

Figure 2 - Inflammatory cytokine production by immune cells (W6).

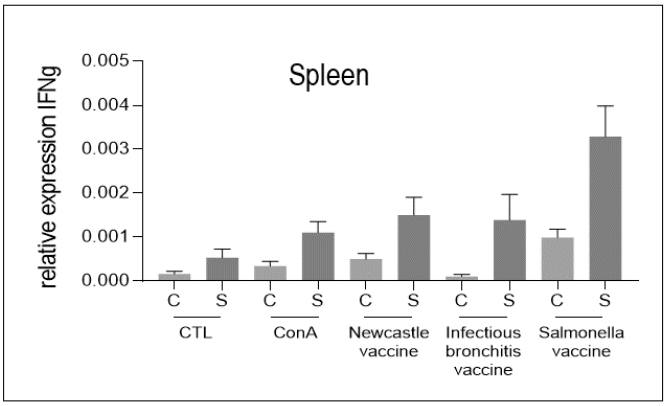

Figure 3 - Quantification of the immune cell response when stimulated by specific antigen.

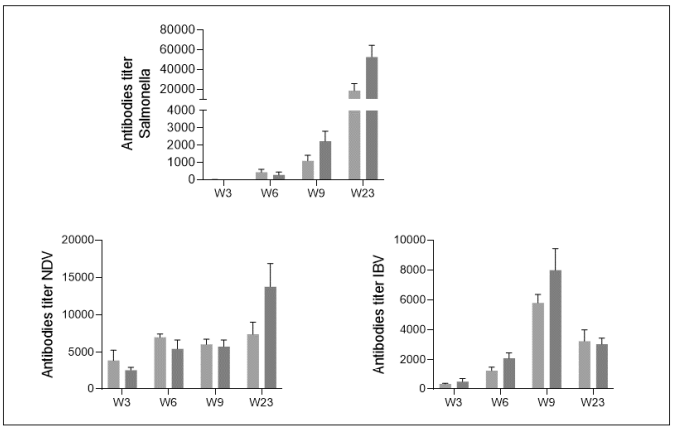

Figure 4 - Antigen specific immunoglobin quantification by ELISA.

IV. DISCUSSION

Flow cytometry analysis showed a numerical increase of APC in the spleen at 3 and 6 weeks old, and a significant increase of the same cells in the intestine of beta-glucan treated animals at 6 weeks (see graph 1). Analyzing the same tissues, the relative expression of TNF-alpha inflammatory cytokine, produced by immune cells, was evaluated and increased after a LPS challenge in the beta-glucan treated animals (see graph 2).

Serological analysis by ELISA showed an increase of antibodies in the beta-glucan group (see graph 3) specific to Salmonella at 9 and 23 weeks old; to Infectious Bronchitis virus (IBV) at 9 weeks, and to Newcastle Disease virus (NDV) at 23 weeks. Supplementation with the beta-glucan product from day 0 trained the immune system to react stronger to a pathogenic challenge, such as LPS, or to vaccinations with higher antibody titres. This effect is important in preparing the immune system to react better to vaccinations which are given before egg production.

Trial results show that by training the immune system, the beta-glucan product helps improve innate immunity with more Antigen Presenting Cells and more inflammatory cytokine, in response to a LPS challenge. It also helps improve adaptive immunity, creating more specific antibody titres at the beginning of egg production. Layers are then better protected against viral diseases and bacterial contamination during their egg production period.

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AUTHOR INDEX