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and

THE WORLD'S POULTRY SCIENCE ASSOCIATION
(Australian Branch)

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LAYING HEN WELFARE IN AUSTRALIA

P.H. HEMSWORTH[1]

Summary

A series of reviews of the scientific literature on layer hen welfare will be published in a special issue of Animal Production Science in early 2021. The aim of this conference presentation is to summarise some of findings and recommendations from this series of reviews on three contentious welfare topics in the egg industry: animal welfare assessment; housing systems; and the so-called 'natural behaviours' and injurious behaviours.

A variety of measures, particularly behavioural, physiological and fitness measures, are used to assess animal welfare. Multiple indicators are often used to assess animal welfare; however the relative importance of these individual indicators has yet to be clarified.

Research comparing housing systems generally indicates that hens in conventional and furnished cages have lower (or similar), but not higher, levels of stress based on glucocorticoid concentrations than hens in non-cage systems. Caged hens generally have lower mortality rates with less variability than hens in non-cage systems. However, the behavioural repertoire of laying hens in conventional cages is more compromised than those in non-cage systems. In contrast to conventional cages, furnished cages provide opportunities for perching, dust-bathing, foraging and nesting in a nest box, activities that may elicit positive affective states.

Non-cage systems, such as barns and free-range, offer freedom of movement and an opportunity to display a repertoire of behaviours. Most hens in free-range systems access the range, which appears to be associated with some health benefits (e.g., improved plumage condition and reduced footpad dermatitis), but there are also health risks, such as greater susceptibility to disease, predation and potentially parasites in comparison to barn or cage housing systems. Loose housed hens in general are more susceptible to feather pecking and cannibalism outbreaks.

Research examining hen welfare in common housing systems highlights the importance of the design and management of the housing system, as well as the husbandry skills, knowledge and willingness of stockpeople to effectively care for and manage their animals.

I. INTRODUCTION

In accepting the invitation to present a paper on laying hen welfare at APSS 2021, I thought that I would provide an overview of a special issue of Animal Production Science on layer hen welfare that will be published in early 2021. This special issue consists of 19 review papers on the welfare implications of: the production system; development, growth and production; husbandry and behaviour; and health. I had planned to report on the main findings of the authors and their recommendations on industry adoption and future research. However, this is too ambitious for APSS 2021 since such a report on the main findings and recommendations arising from 19 papers is beyond the length of either an oral or written presentation. Therefore, my aim with this paper is to first, briefly introduce the special issue papers (titles and authors) and second, focus on highlights (findings and recommendations) in the special issue that address three contentious welfare topics in the egg industry: animal welfare assessment; housing systems; and 'natural behaviours' and injurious behaviours.

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II. 2021 SPECIAL ISSUE OF ANIMAL PRODUCTION SCIENCE ON LAYER HEN WELFARE

A group of Australian researchers with a wide discipline interest and expertise in poultry science were commissioned by Australian Eggs Limited to undertake a comprehensive independent review of the scientific literature on layer hen welfare. The objective of the series of reviews was to inform both current welfare discussions and future investment in welfare research and development in Australia. In considering their recommendations, the reviewers reflected on the context of egg production in Australia. This consideration of the characteristics of Australian egg production is very relevant since a considerable amount of the research on laying hen welfare has been and continues to be conducted by European researchers and thus is most relevant to European conditions and production systems. As Fraser (2008) stated "To date, animal welfare science has tended to be a somewhat Eurocentric field. Much of the work has been done on production systems typical of agriculture in Europe and countries with European-derived culture, and the direction of some research has been influenced by actual or potential European regulations."

Nineteen review papers will be published in this special issue of Animal Production Science on layer hen welfare. The abbreviated titles, authors and the general subject area are presented in Table 1.

Table 1 - Abbreviated titles, authors and the general subject area of the review papers that are to be published in 2021 in the special issue of Animal Production Science on layer hen welfare.

General subject area and abbreviated titles	**Authors**
A. Welfare implications of the production system	
1. Cage housing	Paul Hemsworth
2. Barn and aviary	Mini Singh and Peter Groves
3. Free-range	Dana Campbell, Saiful Bari and Jean-Loup Rault
B. Welfare implications of development, growth and production	
4. Genetic selection and breeder practice	Greg Underwood, Daniel Andrews and Tin Phung
5. Incubation and hatchery practice	Greg Underwood, Daniel Andrews and Tin Phung
6. Nutrition, feeding and laying hen welfare	Wayne Bryden, Xiahua Li, Isabelle Ruhnke, Dagong Zhang, and Shaniko Shini
7. Rearing conditions	Ellen Jongman
8. Skeletal health	Dana Campbell
C. Welfare implications of husbandry and hen behaviour	
9. Stress, health and the welfare	Alan Tilbrook and Andrew Fisher
10. Natural behaviours and their drivers	Paul Hemsworth and Lauren Edwards
11. Husbandry and management decisions	Lauren Edwards and Paul Hemsworth
12. Feather pecking	Greg Cronin and Phil Glatz
13. Beak trimming	Phil Glatz and Greg Underwood
14. Moulting	Phil Glatz and Alan Tilbrook
D. Welfare implications of health	
15. Disease	Peter Groves
16. Non-infectious diseases	Peter Groves
17. Bacterial and viral infectious diseases	Amir Noormohammadi
18. Antibiotic use	Peter Groves and Greg Underwood
19. Euthanasia	Ellen Jongman and Andrew Fisher

III. ANIMAL WELFARE ASSESSMENT

a) Review findings

Accurate assessment of animal welfare is crucial in understanding how laying hens should be housed and managed to achieve best practice animal welfare. According to our current conception,

welfare is a state within an animal, and most directly relates to what the animal itself experiences (Mellor et al., 2009).

Animal welfare assessment is a topic of some debate, particularly in the public but also to a lesser extent in the scientific community. There is no single measure or indicator of welfare and the assessment of animal welfare currently uses a variety of measures, particularly behavioural, physiological and fitness measures. Although animal welfare science has made major contributions to understanding animal welfare and its assessment, often by the use of multiple indicators from multiple disciplines, their relative importance has yet to be clarified (Fraser 2008; Nicol et al., 2011; Hemsworth et al., 2015; Sandoe et al., 2019). The use of only one or several of these indicators of animal welfare provides a less holistic view of the animal's welfare state.

Several review papers in the special issue of Animal Production Science on layer hen welfare consider in some detail animal welfare assessment (papers 1, 9 and 10, Table 1). A number of papers also consider the main hen health problems in the Australian egg industry, their welfare implications and their prevention (papers 8, 9, 15, 16, and 17, Table 1). The main findings and recommendations from these review papers are summarised here.

The majority of the welfare studies conducted on farm animals, including laying hens, have employed what is often called the biological functioning framework. The rationale for this framework is that difficult or inadequate adaptation generates welfare problems for animals and that suboptimal biological functioning accompanies negative affective states, such as fear, pain, sickness, hunger, thirst, helplessness and frustration. In assessing risks to animal welfare, extreme coping attempts are measured using behavioural and physiological stress responses, as well as health and other fitness variables. Many of the review papers in the special issue of Animal Production Science on layer hen welfare refer to these indicators when considering risks to hen welfare. The most common physiological measure in laying hens is circulating corticosterone concentration (or its metabolites in eggs, excreta, feathers), and measures of immune function, such as heterophil to lymphocyte ratio and packed cell volume, are often used. Abnormal behaviours, such as pacing, feather, object and cohort pecking, and head shaking, are also commonly used to study hen welfare.

Flock mortality is one of the most important animal welfare indicators for laying hens. As with disease, the implications of the noxious subjective experiences associated with mortality on animal welfare are not always recognised by the public or some non-government organisations. Health and injuries are an important part of welfare and whenever an animal is injured or diseased, welfare is poorer. Similarly, hypothermia, hunger, thirst, sickness and pain associated with mortality are considered potentially noxious affective (subjective) experiences.

In addition to health, stress can have significant biological costs, leading to growth and reproductive impairments, which may reflect and/or result in welfare problems for the animal. Fitness measures studied in laying hens include bodyweight, feed conversion, hen day production, condition of the skin and plumage, keel-bone damage, foot health score and mortality.

Since what the animal experiences is central to understanding animal welfare, the conceptual framework often called the affective state framework, emphasises that the welfare of an animal derives from its capacity for affective (subjective) experiences. Motivation tests involve 'asking' the animal whether it will work (perform some arbitrary task such as pecking a key or pushing a door) to obtain something it wants or wants to do (positive reinforcers leading to a positive affective state) or of avoiding something it does not want (negative reinforcer leading to a negative affective state). For example, motivation tests have been used to measure the value that laying hens place on accessing a nest box for oviposition, substrates for foraging and dust bathing, and perches for roosting.

Only a few studies have utilised both these frameworks in studying hen welfare, that is examining both the choice behaviour of laying hens and the biological functioning consequences of depriving laying hens of this opportunity (e.g., Nicol et al., 2009; Engel et al., 2019).

b) Review recommendations

Some of the key recommendations from authors of reviews in the special issue of Animal Production Science on layer hen welfare are as follows.

In appreciating the impact of housing, management and husbandry practices on hen welfare, it is important to understand (1) how motivated they are to choose an environmental option or perform a type of behaviour or avoid an environmental or husbandry option and (2) the consequences of depriving them of their choice on behavioural and physiological stress responses as well as fitness variables, including health.

However, there remain substantial gaps in our understanding of stress in laying hens. In particular, a major gap exists with respect to understanding the regulation of physiological stress responses. A sound understanding of this is required in order to assess physiological stress and its impact on normal physiological and behavioural functioning.

The public is a key driver of animal welfare change and thus there needs to be a clear articulation of the effects of housing, management and husbandry practices on hen welfare. Since the welfare consequences arising from illness, injury and mortality are often not well recognised, a more effective communication between government, industry and the public sectors would benefit from a better informed public on the full array of behavioural, physiological and fitness measures used by researchers in examining the effects of industry practices on hen welfare.

IV. HOUSING SYSTEMS AND LAYING HEN WELFARE

a) Review findings

The main concerns that the public has about animal production appear to focus on the conditions that guarantee food security, public health, environmental quality and animal welfare (Vanhonacker et al., 2012). In relation to animal welfare of farm animals, it is a controversial topic for many, particularly because of the perceived negative effects of intensification of animal production on the animal, such as a general lack of social contact, a general lack of space, an inability to exercise, ‘barrenness’ of the environment, abnormal behaviour and the reliance on technology (Barnett et al., 2001; Te Velde et al., 2002; Fraser 2005, 2008; Vanhonacker et al., 2009; Hemsworth, 2018). In contrast, farm animal welfare problems in extensive systems, such as problems with extreme cold and heat, parasites, and poor access to feed and water, have received less attention (Fraser 2008).

Several review papers in this special issue of Animal Production Science consider one or a number of the three main housing systems, cage, barn and free range and the design and management of these systems (papers 1, 2, 3, 10 and 11, Table 1). The main findings and recommendations from these review papers are considered here.

Comparisons of housing systems, particularly in commercial settings, are complex because of potentially confounding differences in physical, climatic and social environments, genetics, nutrition and management. Furthermore, some of the confounding factors are inherent to some specific housing systems.

Nevertheless, research in commercial and experimental settings comparing housing systems generally indicates that hens in conventional and furnished cages have lower (or similar), but not higher, levels of stress based on glucocorticoid concentrations than hens in non-cage systems. Furthermore, caged hens generally have lower mortality rates with less variability than hens in non-cage systems. However, the behavioural repertoire of laying hens housed in conventional cages is clearly more compromised than those hens in non-cage systems. Abnormal behaviours, such as pacing, feather pecking, spot pecking and head

shaking, are commonly seen in barren production environments and clearly the use of enrichment in indoor systems requires ongoing research.

In contrast to conventional cages, furnished cages provide opportunities for perching, dust-bathing, foraging and nesting in a nest box, activities that may elicit positive affective states in laying hens. While hens may prefer to distance themselves from other birds, their strength of motivation to do so has not been thoroughly investigated and appears to depend on the activities in which hens are engaged.

Barn systems in Australia are indoor with single-level (flat deck) or multilevel housing (aviary). These systems offer hens freedom of movement and an opportunity to display a repertoire of behaviours. Barn systems in comparison to free-range systems offer better biosecurity due to lack of direct access to wild birds and their faeces. In addition to protection from predators, barn systems also offer protection from diseases and possibly parasites that can be contracted from range areas. However, injurious behaviours, such as feather pecking and cannibalism, can occur, as well fractures and injuries arising from collision or falls from elevated structures. Barns generally have poorer air quality (dust and NH3 concentrations) and thus greater compromised hen health and welfare than cage systems.

Housing in free-range systems are either single-level (flat deck) or multilevel (aviary). The majority of hens in free-range systems access the range, spending most of this time foraging outside. Use of the range will depend on the range design but may also depend on the indoor shed design, and this is currently not well understood. Range access appears to be associated with some health benefits such as improved plumage condition and reduced footpad dermatitis, but there are also health risks associated with free-range systems such as greater susceptibility to disease (e.g. spotty liver disease), heat stress, predation, and potentially parasites in comparison to barn or cage housing systems. Design of the range area, indoor shed, management practices and rearing environments can all influence how hens utilise free-range housing systems.

Although feather-pecking is multi-factorial in its aetiology, in general loose-housed hens are more susceptible to feather pecking and cannibalism outbreaks as it spreads more easily between the large numbers of hens housed in contact with each other. Smothering, in which hens press tightly together and often on top of each other in such a way that results in death, is mainly reported in non-cage systems. Anecdotal reports suggest that smothering can account for a considerable proportion of mortality in Australian free-range systems, but its aetiology is poorly understood.

While not always fully appreciated by the public and some non-government organisations, management decisions markedly affect laying hen welfare, irrespective of the housing system. At the level of farm management, human resource-management practices, including employee selection and training, and animal management practices, such as best practice in housing and husbandry, and implementation of welfare protocols and audits, all affect hen welfare. At the stockperson level, together with the opportunity to perform their tasks well, stockpeople require a range of well-developed husbandry skills, knowledge and willingness (motivation and attitudes) to effectively care for and manage laying hens.

b) Review recommendations

Sustainability of the production system will depend on animal welfare legislation, public and consumer preference, cost of production, environmental footprint and suitable hen genetics.
Research on housing systems highlights the importance of the design of the housing system and management of the system: multiple management and system design factors will affect how hens adapt to the housing environment and the subsequent impacts on their welfare.

Space is a contentious issue for many and while floor space per laying hen is generally less in cage systems, the lack of a suitable site or resource for activities such as nesting, pecking and scratching a suitable litter substrate, dust-bathing and perching also restricts the behavioural repertoire of hens in conventional cages.

Furnished cages provides opportunity for nesting, foraging, roosting and dust-bathing. Some authors have suggested that the behavioural needs for these behavioural activities are not fully satisfied in furnished cages; however this requires testing. Our understanding of the strength of motivation of hens to distance themselves from other birds based on the specific behavioural activities that they are engaged in, is poor. A better understanding of the effects of floor space and group size on hen welfare in furnished cages is therefore recommended.

The multi-tier provision of an aviary structure in a barn system offers increased total useable surface and allows hens to disperse across several levels of living space. However, one of the major health and thus welfare concerns in barn systems (and aviaries in free-range systems) is the high incidence of fractures. Good design, placement and management of structures in the shed can help mitigate these to a large extent. Pullets should also be reared in housing systems that offer platforms, tiers and perches that train them to go up in a system to reach food and water. Genetics may also influence the use of space and distribution within an aviary. The other major concern in barn systems as with free-range systems is the prevalence of severe feather pecking. Although feather pecking is multi-factorial in its aetiology, in general loose-housed hens are more susceptible to feather pecking and cannibalism outbreaks.

The majority of hens in free-range systems access the range. The use of the range is associated with the range design, but may also depend on the indoor shed design, which is currently not well understood. Ranging may have some positive effects on welfare, but this needs to be further validated across commercial farms. Research is also required to better understand how pullet rearing strategies may affect adult range use. Mortality due to smothering is mainly reported in non-cage systems and research on its aetiology in Australian free-range systems is required.

V. ‘NATURAL’ BEHAVIOURS, INJURIOUS BEHAVIOURS AND LAYING HEN WELFARE

a) Review findings

In contrast to indoor housing, outdoor housing is typically extensive and, so, is considered by some to be inherently ‘good’ because it provides a more ‘natural’ environment and choice for the animal in performing several behaviours over a larger area, and the lower technological inputs provide for fewer equipment breakdowns that may adversely affect welfare (Hemsworth, 2018). Furthermore, some have suggested that the problems with modern animal production is not that the animals are unable to perform certain behavioural opportunities, but they are unable to fill the extra time available with limited behaviours when they have no need to find food, water, or shelter (Fraser 2005).

A common view amongst the public and some non-government organisations is that the provision of ‘natural’ aspects in the animal’s environment and the ability for the animal to perform its full ‘behavioural repertoire’ equates to safeguarding its welfare. However, as many authors have recognised, encouraging captive animals to perform all the behavioural patterns evident in the wild does not necessarily safeguard animal welfare because many of these patterns in the wild may be responses to adversity, such as shivering in harsh weather and fleeing from predators (e.g. Fraser 2003; Dawkins 2008; Mason and Burn 2018). Furthermore, while many of the ‘wild’ behaviours are perfectly natural, their absence in captivity should not necessarily raise welfare concerns because they are elicited by external stimuli or physiological

states that have already been fulfilled in animals whose safety, physical, social, health and nutritional needs are met (Dawkins 2008).

A number of papers in the special issue of Animal Production Science review hen behaviour in relation to hen welfare (papers 1, 2, 3, 10, 11 and 12, Table 1) and the main findings and recommendations from these papers are considered here.

Preference and motivation research indicates that laying hens value resources such as substrates for foraging and dust bathing, perches for roosting and particularly nest boxes for oviposition. Hens are generally motivated to dust bathe, but in contrast to nesting, the results of studies on the strength of motivation to access a dust-bathing substrate are conflicting. However, there is no convincing evidence that deprivation of any of these resources in inexperienced hens results in physiological stress. Furthermore, apart from adverse effects of the absence of perches on bone strength, there is no evidence that deprivation of nest boxes, perches, and foraging and dust-bathing substrates results in reduction in fitness such as reduced egg production or health. Nevertheless, preference research indicates that the opportunity to utilise these resources, particularly nest boxes, may elicit positive affective states in laying hens.

The behavioural repertoire of commercial laying hens, particularly when housed in conventional cages, is compromised and, together with barren environments, caged housing systems have been implicated in the development of so-called abnormal behaviours in laying hens, such as pacing, feather pecking, spot pecking and head shaking. While most of the enrichment studies have specifically examined feather pecking, further research is required to examine the implications of environment enrichment on other abnormal behaviours and welfare in laying hens, particularly in cage systems.

Feather-pecking behaviour has been identified as one of the most significant welfare concerns for laying hens, due to its high frequency of occurrence and damaging nature. Although feather pecking occurs unpredictably in all housing systems, severe feather pecking in non-cage systems is more problematic due to the greater difficulty of intervention. Feather-pecking is multi-factorial in its aetiology and challenging to eliminate, but there is evidence that alternative pecking opportunities, such as litter substrate and additional enrichment (such as straw and grain in litter, sand and peat straw in baskets, maize and pea-barley silage, deeper litter and strings) both during rearing and in adulthood, can reduce the development and prevalence of feather pecking.

There is also evidence that some complexity and, thus, stimulation during rearing and adulthood reduces fearfulness in laying hens. Furthermore, exploratory behaviour is regarded as a pleasurable activity in itself and, thus, the opportunity for exploration may be associated with a positive affective experience in laying hens.

b) Review recommendations

In contrast to indoor housing, outdoor housing is typically extensive and so is considered by some to be inherently ‘good’ because it provides a more ‘natural’ environment and choice for the animal to perform an extensive range of behaviours over a larger area. However, this concept of natural is usually too poorly defined to provide a sound basis for animal welfare assessment, and indeed when applied uncritically it may lead to poorer welfare instead of an improvement. Although the concept of natural living does not provide a rigorous basis for welfare assessment, it usefully draws attention to the potential welfare benefits of providing opportunities to engage in natural behaviours, such as nesting, perching and foraging.

Further research is required on the aetiology of severe feather pecking. Common recommendations in the literature to prevent or control severe feather pecking include stimulating feeding and foraging behaviour by providing high-fibre diets and suitable litter

from an early age onward, controlling fear and stress levels through genetic selection, reducing stress and improving the stockmanship skills of the stockperson, but clearly further research is required. Further research is also required to examine the implications of environment enrichment on other abnormal behaviours and welfare in laying hens, particularly in cage systems.

VI. CONCLUSION

Many of the public concerns and policy debates about farm animal welfare, including laying hen welfare, have generally focussed on intensive housing systems. This focus on housing systems has distracted research, development and education from some of the other important considerations in safeguarding and improving animal welfare. Indeed, the design and management of the housing system as well as the husbandry skills, knowledge and willingness of stockpeople to effectively care for and manage laying hens is generally more important for animal welfare than is generally recognised.

The public is a key driver of animal welfare change and thus there needs to be a clear articulation of the full array of behavioural, physiological and fitness measures that researchers utilise to examine the effects of industry practices on hen welfare and their findings.

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AUSTRALIAN COMMUNITY VALUES AND LAYER HEN WELFARE

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Summary

This paper summarizes key findings of a project performed with funding from Australian Eggs Ltd. in order to better understand how Australian community members apply values when considering key animal welfare issues in commercial egg production (Ankeny and Paxton, 2020). The main goal of the project was to gain a deeper understanding of the relative acceptability or unacceptability of various practices and developments in commercial egg production in Australia. A mixed qualitative and quantitative study was conducted to assess how community values are applied to difficult decisions involving outcomes for animal welfare, human health, and environmental sustainability. We focus in this paper on those findings likely to be of most interest to those engaging in avian health research. We found that members of the Australian community hold diverse views about animal welfare, reflected in varied prioritisation of animal welfare goals and expectations about how these are best achieved. Overall, meeting basic needs, permitting freedom to choose, and providing the hens with care and protection represented the highest welfare priorities for the majority of research participants. Animal welfare was nearly always prioritised ahead of environmental outcomes, in part because egg production was perceived as being relatively sustainable compared with other industries.

Our findings have broader implications for how research about hen welfare is framed and communicated to the general public, particularly because research participants tended not to revise their preferences in light of new knowledge or information. In addition, it is clear that care needs to be taken when designing research tools to explore animal welfare, given the tensions revealed between how members of the Australian community think and reason about animal welfare and their 'gut feelings' when making value judgements related to animal welfare. Finally, research participants tended to have different understandings of key terms related to animal welfare in comparison to how they are typically used in animal welfare science. Terms such as 'choice,' 'light,' and 'space,' which are likely to be understood by animal scientists and those in the egg industry as objective and measurable, tended to be value-based for study respondents. This finding suggests that such terms may have considerable impact when used in research or communications with members of the Australian community, and have considerable potential to result in miscommunication and misunderstandings.

I. INTRODUCTION

Most Australian households consume large numbers of eggs on an annual basis, but we know very little about what members of the broader community think about the practices associated with commercial egg production and the underlying values that they bring to these considerations. A previous study for Australian Eggs (Fisher et al., 2019) examined the values-based elements underlying the consensus in animal science across key areas related to laying hen welfare, and suggested how these values-based elements could be better exposed and examined to open the way for additional and new ways of researching key areas of hen welfare. This work revealed that there are numerous decisions associated with commercial egg production that require consideration of a balance of factors including both the best available

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scientific data and also community expectations and values. The study described in this paper also complemented ongoing studies done by CSIRO researchers for Australian Eggs focused on their Sustainability Framework and its implementation which provide data on community attitudes about commercial egg production (Moffat et al., 2018, 2019).

The current research project sought to explore community values underlying animal welfare in commercial egg production, and particularly where values might be in tension with current industry practices. This study explored how members of the Australian community weighed up different desirable (or undesirable) animal welfare outcomes and investigated the values that underlie public expectations regarding such outcomes. The current research explores community values underlying animal welfare in commercial egg production, with a particular focus on where values might be in tension with current practices in the industry, via focus on the following questions:

- What values issues related to animal welfare, environment, and human health in commercial egg production are most relevant to various types of people within the Australian community?
- How do various types of people within the Australian community apply values and express, explain and justify such application of values in situations where trade-offs between animal welfare, environmental, and human health outcomes exist?
- What are the impacts of these value applications on preferences for, or acceptability of, developments in commercial egg production in Australia?

It is critical to understand how values associated with layer hen welfare compare to and are weighed up against other valued outcomes, for instance the potential effects of egg production on human health and environmental sustainability, particularly where values may be in conflict and where there is no objectively or scientifically correct answer. Such potentially conflicting values are a key challenge for the Australian egg industry as it continues to strive to meet community expectations regarding what is acceptable animal welfare.

II. METHOD

Most people are familiar with making decisions based on trade-offs in their day-to-day lives but may find it difficult to articulate the processes that they use when doing so. Trade-offs that involve moral choices are particularly difficult to make, and are even more tricky to consider in contexts where most people are not likely to have deep knowledge about the underlying practices, or may have never thought about the issues at stake in any detail, as in the case of weighing up and trading off different animal welfare outcomes in relation to their underlying values.

Hence this project used two primary approaches to explore the key research questions, qualitative and quantitative methods. Qualitative methods are used to gain rich or detailed knowledge about people's thoughts, attitudes, or opinions, and allow a deeper dive into the problems or questions of interest. They can be used as exploratory research, and hence provide hypotheses that can be used as the basis of future quantitative research, but also can be used to better understand quantitative results. Qualitative methods are particularly useful when seeking to understand not just what people think, but what the reasons are underlying opinions or views, and the relations between various parts of people's worldviews and values. Although statistical methods cannot be utilised to determine the validity or reliability of qualitative research, there are well-established methods for making certain that qualitative results are high-quality and rigorous (e.g., see Lincoln and Guba, 1999), which include but are not limited to making certain to control for researcher or other biases, engaging in ongoing critical reflection on methods as data is collected and analysed, coding of data including comparison by multiple researchers to ensure consistency, clear and consistent decision-making throughout the processes of

collection and analysis, and repetition of data collection in order to ensure that different perspectives are represented until saturation occurs (namely no new themes emerge in subsequent research).

In this study, qualitative methods were used at two stages in the process. First, community-level focus groups were carried out at the start of the project to establish an overview of community priorities for layer hen welfare, including the diverse range of views held by members of the public. Focus groups were also used as a final stage in the overall research in order to deepen understandings of quantitative survey results, with a particular focus on how different sectors within the community make value trade-offs and how they respond to making difficult decisions between valued animal welfare outcomes.

Quantitative research is typically used to generate data that can be transformed into usable statistics, particularly to be able to generalise results from a sample to a larger group or even the general population. Questions tend to be close ended, and data generated can include attitudes, opinions, behaviours, or other defined variables, from which patterns or correlations are sought. The quality of quantitative research results can be measured using a range of statistical techniques, but the main goal is to assess how well the methods used measured whatever was of interest. Most quantitative research is assessed in terms of reliability (the consistency of a measure, in other words the extent to which the results can be reproduced if the research were to be repeated under the same conditions) and validity (the extent to which the results really measure what they are supposed to measure).

In this project, we performed a large-scale representative survey of Australian community values and value trade-offs between different potential animal welfare outcomes, and analysed the data to determine patterns in animal welfare priorities within members of the Australian community as well as whether consistent underlying values can be identified that are drivers of these priorities.

When performed correctly, combining qualitative and quantitative techniques permits better understanding of the research questions and the data generated than any one method on its own, and this approach is known as ‘mixed methods research.’ Such an approach allows researchers to gain more depth and breadth in their understanding of the data, as well as corroboration of findings across various methods used. Most importantly, mixed methods allow the weaknesses inherent in each approach performed on its own to be offset, for instance via triangulation (use of several means or methods to explore the same phenomenon or question of interest) together with careful and reflective analysis about the types of data produced using each method, and the strengths and limitations of each method. This project relied on this type of mixed methods approach in order to produce both a much richer context for understanding what might sometimes appear to be contradictory community responses to various types of egg production practices, as well as actionable findings pointing to areas that require more research, improved communication and transparency, and more public and industry consideration and debate.

III. RESULTS

According to results of our representative survey, members of the Australian community overwhelmingly considered protecting the welfare of chickens in commercial egg production in Australia to be an important issue (mean score = 5.84 on a seven-point scale where 1= not important and 7 = very important). Over half (58%) of survey respondents also agreed that animal welfare is a main influence on their choice of whether or not to buy eggs, or of which types of eggs to buy. A similar number (56%) of respondents believed that most people make efforts to buy eggs produced under high animal welfare conditions. Overall, the majority of survey respondents signalled that layer hen welfare is a moral issue, although they were mixed

in their perceptions of whether it is morally right or wrong to negatively affect the quality of life for layer hens in order to produce reasonably priced eggs. The belief that negatively affecting animal welfare in order to produce reasonably priced eggs is absolutely morally wrong was also significantly correlated with lower levels of measured knowledge about commercial egg production in Australia.

Our findings from both the community survey and focus groups revealed that members of the Australian community hold diverse views about animal welfare, which are reflected in varied prioritisation of animal welfare goals and expectations about how these are best achieved. Community members expected layer hens' basic needs to be fulfilled as a minimal welfare requirement. Some research participants considered access to an outdoor range to be a basic need and right for layer hens and resisted the idea that good welfare could be achieved without such access. However, among community members who held more moderate views, it was important that hens have the freedom to choose how and where they spend their time. Overall, meeting basic needs, permitting freedom to choose, and providing the hens with care and protection represented the highest welfare priorities for the majority of research participants. Notably in both the survey and focus groups, research participants nearly always prioritised animal welfare ahead of environmental outcomes, in part because egg production was perceived as being relatively sustainable compared with other industries.

In order to examine why different animal welfare, human health, and environmental sustainability preferences may exist within the Australian community, we assessed the tendencies among survey respondents to anthropomorphise layer hens, that is, their tendencies to believe that layer hens possess qualities traditionally associated with human beings such as a humanlike mind, free will, intentions, consciousness, and emotions (this description of anthropomorphism, its constituent categories, and the method for assessing individual tendencies to anthropomorphise, were derived from work by Waytz et al., 2010).

Overall, survey respondents tended to agree that hens possessed anthropomorphic qualities, although they disagreed about the extent to which they possessed these qualities. Across all anthropomorphism questions, survey respondents scored on average 30.38 out of a possible 55 on the anthropomorphism scale, with only a small percentage of respondents located at either extreme of the scale. Respondents were somewhat more likely to believe that hens have consciousness and experience emotions than they were to attribute free will to hens.

Respondents who tended to anthropomorphise layer hens were significantly more likely to prefer outcomes that they viewed as providing hens with more natural living conditions and improved emotional well-being. While the tendency to anthropomorphise layer hens was also associated with greater knowledge of commercial egg production, increased knowledge was not strongly associated with a preference for outcomes affecting particular categories of animal welfare. Research participants also tended not to revise their preferences in light of new knowledge or information, although this did more infrequently occur in the deliberative focus group setting. Somewhat surprisingly, there were also no consistent differences in the trade-off preferences of survey respondents living in urban and rural areas, or according to most demographic indicators, with the exception of age and gender.

A core purpose of this research was to investigate how members of the Australian community decide between conflicting animal welfare outcomes in trade-off situations. Trade-offs occur in situations where the benefits gained by choosing one outcome necessarily means that the benefits of another outcome are reduced. In the online survey, we asked respondents to indicate their degree of preference in a range of trade-off scenarios (using a scale from 1 = strong preference for option A to 7 = strong preference for option B). Each scenario described a trade-off situation between two outcomes related to animal welfare, human health, or environmental sustainability. Overall, survey respondents' preferences for the major categories of layer hen welfare (i.e., biological function, affective state, and natural living) were relatively

mixed. Nevertheless, members of the Australian community tended to have moderate or strong preferences for welfare outcomes that provided layer hens with what they viewed as more natural living conditions or improved their emotional experiences over physical health outcomes.

While members of the Australian community also expressed mixed preferences in trade-offs between animal welfare, human health, and environmental sustainability, a critical finding is that animal welfare was always prioritised ahead of environmental outcomes in our survey data. The most notable example of this preference involves a trade-off between improving hens' affective states by providing enough space for them to relax without interruption and limiting the use of land and energy resources. In this trade-off scenario, nearly three-quarters of respondents preferred to improve animal welfare, while relatively few had no preference or prioritised potential environmental outcomes. Considering the mix of responses as to whether Australian egg producers are perceived as environmentally responsible, it is unclear whether the low priority accorded to environmental outcomes is due to a belief that egg production does not (overly) impact environmental sustainability or to some other set of beliefs. For trade-offs between layer hen welfare and human health, survey respondents tended to accept risks to hens' biological function and forgo opportunities to improve their affective states in order to protect human health. However, providing the hens with a natural living environment in which they have space to move around was a clear priority for respondents as compared with reducing risks to stockperson health.

Several notable issues emerged during discussions in the focus groups which affected how we interpret these results and highlight the need for further in-depth research to understand how members of the Australian community draw on both knowledge and values to interpret information and make decisions about animal welfare. First, research participants tended to express their values differently when stating abstract preferences as compared with how they described their values when presented with concrete trade-off situations. In particular, research participants tended to prefer improving hens' affective states as an outcome in trade-off situations, while this was not prioritised in exercises that ranked preferences or when respondents were asked directly about affective states. Focus group discussions indicated that participants empathised with the hens' situations in more concrete situations but found it difficult to imagine a hen having abstract mental and emotional needs and experiences. This finding indicates that there may be a tension between how members of the Australian community think and reason about animal welfare and their 'gut feeling' when making value judgements related to animal welfare. It also suggests that care needs to be taken when designing research tools in order to be able to explore both of these aspects of participants' reasoning.

Second, research participants tended to have different understandings of key terms related to animal welfare in comparison to how they are typically used in animal welfare science. Terms such as 'choice,' 'light,' and 'space,' which may be understood by animal scientists and those in the egg industry as objective and measurable, tended to be value-based for study respondents. Focus group discussions revealed that participants had different understandings of key terms in comparison to how they are typically used in animal welfare science which in turn had important implications for our interpretations of values and preferences in the community survey. These findings suggest that such terms have considerable impact when used both in research or other communications with members of the Australian community and have considerable potential to result in miscommunication and misunderstandings as a result.

Focus group participants differed in their willingness to accept any compromises to what they viewed as best animal welfare practices in order to make the Australian egg industry sustainable. Most focus group respondents did accept that some compromises were likely to be

necessary. For example, if farmers did not have adequate outdoor space or were unable to prevent the risk of hens contracting diseases from local wildlife, 'proper treatment' in indoor systems was often viewed as 'the next best thing' by focus group participants. However, a minority of participants questioned why egg production should take place at all if farmers were unable to guarantee safe outdoor access and, by extension, to provide what the participants believed to be the only conditions under which the farmers could ensure adequate welfare for their hens. Overall, participants' willingness to accept compromises to animal care that would still allow a good standard of welfare and make the egg industry more sustainable depended on the perceived intent of egg farmers. Focus group participants showed little acceptance of compromises to animal welfare which they perceived to be income maximising, such as having more hens in a particular sized space which was legal but more than usual. They were sceptical that farmers would improve animal welfare without community pressure (despite their unwillingness to participate in such activities beyond making purchasing decisions) and tended to believe that farmers were primarily profit motivated. As a result, they emphasised the need to make and enforce layer hen welfare standards and to strictly monitor labelling schemes to ensure accountability. Focus group participants accepted that the demand for high-welfare eggs (and implicitly their sale at a higher price point) would compensate for stricter welfare standards and increased oversight, and therefore expressed limited sympathy for farmers who were unable to meet those higher welfare criteria.

The meanings of terms such as 'choice,' 'light,' and 'space,' which may be understood by animal scientists and those in the egg industry as objective and measurable, tended to be value-based among respondents. For example, participants felt that hens ought to have access to what they described as 'proper' sunlight, which was often associated with the experience of freedom and contrasted with being 'cooped up' or 'stuck indoors' even if natural light was provided. Participants characterised spending time in sunlight as a basic right for living beings and its absence as a deprivation. Similarly, the term 'space' was used to refer to an abstract need which was also associated with freedom and openness, rather than a defined and measurable area. Spaces in cages or barns were perceived as inherently 'worse' or 'less than' outdoor spaces, and farmers were believed not to care about animal welfare if they 'maximise the number of animals in the space' even if the space was strictly speaking within regulatory or agreed best practice standards.

Additionally, participants often conflated the terms 'cage' and 'barn' to mean any type of enclosure. This confusion was in part due to a lack of knowledge about current Australian egg production standards, but also served to escalate or diffuse the moral weight of certain trade-off options. For example, one respondent described barns as "still a cage," while another considered cages that house multiple birds as being "pretty close to free-range, because even though they're free-range they're still within an enclosed environment." These types of comments suggest that the meanings or understandings associated with these terms for members of the Australian community are not based in literal or formal definitions, but rather on their underlying values relating to animal welfare, and in particular their negative responses to 'cages' and positive responses to outdoor or 'free-range' living.

While we were conscious in designing the survey to avoid making explicit references to different types of production systems, the terms 'light,' 'sun-bathing,' and 'space' were used in questions that aimed to identify community values and preferences. For example, one option in a trade-off scenario involved providing "space for hens to relax without interruption." Our focus group discussions suggest that the term 'space' may have carried greater weight in the scenario than we had intended based on its literal interpretation. In other words, our focus groups revealed that what hens have space to do may be less important to members of the Australian community than the fact that they have the space to do it, namely whatever it is that they wish to do. This finding suggests that such terms may have considerable impact when

used in social science research or other communications with members of the Australian community and have considerable potential to result in miscommunication and misunderstandings as a result.

IV. DISCUSSION

Both our quantitative and qualitative findings suggest that a multi-pronged approach is necessary in order to meet diverse community expectations for layer hen welfare in commercial egg production in Australia. In addition to prioritising different layer hen welfare goals, findings from the qualitative focus group discussions indicate that even shared priorities draw on a range of values related to layer hens' needs and farmers' intentions, as well as different understandings of key terms.

Several findings from this study suggest a need to put in place processes that will allow building of the community's capabilities to take on board new information, so that they can use such information to more clearly articulate and advocate for their values related to layer hen welfare, human health, and environmental outcomes of commercial egg production. Simply providing more information is not a solution, as we have shown that information provision alone does not result in engagement with the new information, in parallel to the extensive literature available from the field of public understanding of science on the 'deficit model' (Sturgis and Allum, 2004; Simis et al., 2016).

In addition, value-based understandings of key terms impact how this information is interpreted and may lead to miscommunication and misunderstandings. These findings highlight the need for further research on how scientific or industry concepts are interpreted by members of the community and how these interpretations may be shaped by different forms of media or a 'vocal few' who are active in public discourse, just to note a few examples. Community members' tendencies to maintain their initial value-judgements in light of new knowledge and avoid engaging with complex issues suggest that there is a need to identify learning systems or processes that are sensitive to different value frameworks and stakeholder interests. The focus group discussions that were conducted as part of this research provide some hints as to how deliberative or dialogical approaches can help participants to begin to embed new information within their current knowledge and value frameworks and introduce them to novel perspectives that may challenge those frameworks.

Further exploration is required about how general values associated with particular moral outlooks play out in the domain of animal welfare particularly in relation to understandings of the role of the farmer and his or her moral duties within a commercial production system. The need for such research is underscored by the generally negative perceptions among research participants that commercial egg producers do not care about the welfare of their layer hens, as well as the finding that farmer or stockperson health is considered to be less of a priority than is production animal welfare. These findings raise the question as to how such negative perceptions impact opportunities to improve layer hen welfare.

Finally, in light of industry efforts to improve the sustainability of commercial egg production, a key finding of this research is that members of the Australian public tend to prioritise animal welfare ahead of environmental outcomes. This preference seems to be rooted in the perception that egg production already has a relatively low impact on the environment as well as tendencies among research participants to conflate environmental sustainability with hens' 'natural' living environments. A priority for future research may be to investigate how community members' perceptions of environmental sustainability and animal welfare are linked, and in particular how they would view more technological approaches to sustainability compared to visions of the 'old fashioned' farmer doing 'the right thing.' Thus this study provides important data for future policy and practice decisions both by producers and at an

industry-wide level, and has implications not only for the egg industry, but for animal-based production and research about it more generally.

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TRANSMISSION OF INFECTIOUS LARYNGOTRACHEITIS VIRUS

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Infectious laryngotracheitis (ILT), caused by *Gallid herpesvirus type 1*, is a highly contagious upper respiratory and conjunctival disease of poultry. The virus (ILTV) is thought to exit the host in respiratory aerosols and enter by inhalation of these. High levels of ILTV DNA have been detected in excreta, dust, blood or plasma and in various organs outside the respiratory tract; raising the possibility of alternative routes of shedding from the host. However, it is not known whether the ILTV DNA in excreta, dust and blood or plasma represents infective virus. Wind borne transmission is also implicated in the epidemiology of ILT. However, despite the widespread acceptance of airborne transmission of ILTV there appear to have been no controlled experiments to investigate the efficiency of airborne transmission.

We investigated transmission of wild type and vaccinal ILTV from infected to susceptible commercial meat chickens in multiple experiments in a PC2 isolator facility. Airborne transmission (Class 9, Class 10, SA2, A20 and Serva) and transmission by direct contact (Class 9, A20 and Serva), infection by inoculation of blood/plasma (Class 9, A20 and Serva) or dust and extracts of dust (Class 9) or excreta (Class 9, Class 10, SA2, A20 and Serva) were tested. There was no transmission of ILTV in extracts of excreta or dust from infected birds administered via eye drop, by fresh dust (~10mg) collected from infected isolators and insufflated into the nares, choanal cleft and trachea or blood (1ml) from infected birds inoculated intra coelomically with plasma (60 µl) administered by eye drop. We also carried out a preliminary investigation of coarse spray vaccination with Serva and A20 vaccines.

All strains of ILTV transmitted by the airborne route, whether through a 2 m air hose between isolators, or by sharing a common airspace without physical contact. Wild type virus transmitted very effectively by this route, but transmission of the vaccine viruses was significantly less efficient, particularly when via the 2 m air hose. The field viruses induced clinical signs, pathology, and greatly elevated ILTV genome copies in airborne exposed birds. However, clinical signs were less severe and delayed compared to birds infected by eye drop, or in direct contact with infected birds. This was also reflected in reduced and delayed ILTV GC in choanal cleft swabs. When birds shared a common airspace, transmission of Serva vaccine virus was significantly greater than that of the A20 vaccine virus as assessed by ILTV GC. Birds in direct contact with each other had significantly higher ILTV GC than birds sharing a common airspace ($P < 0.0001$). Coarse spray vaccinated chickens (one dose in 1ml/bird) showed no adverse reaction and greater vaccine take with A20 than Serva.

These findings confirm the suspected airborne transmission of ILTV, demonstrate differential transmission potential between wild type and vaccine strains by this route, and the importance of degree of contact between chickens on the transmission of ILTV. ILTV GC detected in excreta appears to reflect non-infective virus inactivated by passage through the gut with no role in ILTV transmission. As excreta is the main component in poultry dust (Ahaduzzaman et al., 2021, these proceedings), this inactivated ILTV may explain the failure to transmit ILTV in dust or extracts of dust from infected birds. Similarly, the detection of ILTV GC in blood seems to be due to circulation of a non-infective form of ILTV and does not appear to be infectious.

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IDENTIFICATION OF BROILER POULTRY HOUSE DUST COMPONENTS USING CHEMICAL AND PHYSICAL ANALYSIS

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Dust found in poultry housing is a complex substance most likely comprised of a mixture of excreta, feather, feed and bedding material. Exposure to poultry dust can impair the health of both poultry and poultry farmworkers as it serves as a medium for survival and spread of pathogenic microorganisms and inhalation of respirable particles and toxins (Just et al. 2009; Viegas et al. 2013). It has also proven useful as a population level sample material for tracking pathogen incidence and assessing vaccination efficacy (Walkden-Brown et al., 2013; Ahaduzzaman et al., 2019). Given these aspects, it is important to better understand the composition of poultry dust and the potential impacts on bird and human health. The composition of dust in each shed, and each batch, can vary and be expected to change over time. This study was therefore designed to determine whether chemometrics (the application of multivariate statistical techniques to chemical analysis data) and scanning electron microscopy (SEM) could be used to determine the ratio of different originating materials in dust samples.

To enable us to predict the source components of dust, individual materials comprising poultry dust (feed, excreta, feathers and bedding) as well as defined mixtures of the materials, were analysed to establish their elemental character. Settled dust collected late in the batch (35-49d) from 28 broiler flocks were also tested for comparison. Total chemical elemental concentrations (C, N, Al, B, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Se and Zn) were determined using combustion and inductively coupled plasma optical emission spectrometry (ICP-OES) analyses and a chemometrics approach was applied to predict the contribution of source material in defined mixtures and dust samples. SEM was also used to characterise the particulates in dust samples and to validate the chemometrics results. Excreta was found to be the main component (>50%) of late batch (>35d) broiler dust samples, both by SEM imagery and chemometric analysis. SEM imagery of weekly dust samples collected from an experimental flock between 7 and 35 days of age revealed that the contribution of excreta to dust increased with age from 60% at 7d to 95% at 28d ($P<0.001$). The proportion of bedding and feed in dust declined from 22% and 12% respectively at 7d, to low levels (2% and 1%, respectively) after 21d while the contribution of feather material remained rather constant throughout (5-9%). This study demonstrates that excreta provides the bulk of the material in poultry dust samples with bedding, feed and feather material providing lower proportions. The relative contributions of these materials varies with age of birds at dust collection. Additional research is required to determine the health and diagnostic implications of this variation.

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ONSET OF VIRAL SHEDDING AND CLINICOPATHOLOGICAL FINDINGS IN CHICKENS INFECTED WITH DIFFERENT STRAINS OF INFECTIOUS LARYNGOTRACHEITIS VIRUSES

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Infectious laryngotracheitis (ILT) caused by infectious laryngotracheitis virus (ILTV), is a highly contagious upper respiratory disease of poultry causing great economic losses to the chicken industry. New strains have been reported to emerge due to recombination of vaccine and field strains, some of them replacing less virulent strains because of their high virulence. The present study aimed to evaluate the viral shedding and clinic pathological findings in chickens infected with different strains of ILTV. For that, meat and layer chickens were divided into 8 groups (each group with 15-16 chickens) and inoculated with 10^4 $TCID_{50}$ of ILTV class 9, 10 or a putative class 14 isolate (Nazir *et al*., 2020) or sham-inoculated by eye drop at 15 or 22-day age. Four chickens from each treatment were euthanized at 5 days post-infection (dpi) and the remaining birds at 9 dpi. Chickens were observed for clinical signs from 2 to 9 dpi. Viral DNA shedding was measured in oropharyngeal (OL) and cloacal swabs (CL) and in dust samples at 2, 4, 6 and 9 dpi using quantitative PCR. Microscopic lesions in trachea and conjunctiva were scored at 5 and 9 dpi. In addition, feather tissues from inoculated chicken were collected at 5 and 9 dpi and used for examination of ILTV antigen using immunohistochemistry. Class 9 and class 10 induced severe clinical signs with marked bilateral severe conjunctivitis and caused characteristic cytolytic lesions in the conjunctiva. The mortality/euthanasia induced by class 9 (41.9%) and 10 (26.7%) was significantly higher ($P < 0.0001$) than class 14 (0%). ILTV was detected in OL and CL and in dust samples of chickens inoculated with ILTV classes 9, 10, and 14 from 2 to 9 dpi. The overall levels of ILTV DNA in OL and CL and the proportion of positive samples in chickens infected with class 9 (detection rate of 26/26 in OL and 23/26 in CL) and 10 (OL 31/32; CL 28/32) were significantly higher ($P < 0.0001$) than in chickens infected with class 14 (OL 11/32; CL 10/32). Likewise, dust samples collected from isolators from chickens inoculated with class 9 or class 10 showed a trend towards higher viral load than class 14 isolator samples. The higher virulence and shedding of class 9 and 10 strain leading to better dissemination and transmission of these strains compared to class 14 could explain the disappearance of the latter from the field after its detection for a brief period in the central coast of NSW. A significant interaction of bird type and ILTV strain was observed with class 9 and 10-inoculated chickens, with class 10 causing more severe disease in broilers and class 9 in layers. This may reflect immunological differences between meat and layer chickens. None of the sections of feathers of ILTV-infected birds were positive for ILTV antigen by immunostaining. Although the molecular testing of feather tips has been used to measure ILTV vaccine uptake (Davidson *et al*., 2016), the absence of microscopic changes and ILTV antigen staining in feather follicles of infected chickens suggest that there is no active replication of the virus despite the presence of ILTV DNA. Further investigations are needed to determine the differences in the three strains at the genomic level and to determine the source of ILTV DNA detected in feather follicles.

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A STANDARDISED BLEND OF PLANT-DERIVED ISOQUINOLINE ALKALOIDS (IQA) IN LAYING HENS MITIGATES THE IMPACT OF *CAMPYLOBACTER HEPATICUS* (SPOTTY LIVER DISEASE) CHALLENGE

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Summary

Spotty Liver Disease (SLD) is a serious condition affecting extensively housed laying hens caused by *Campylobacter hepaticus.* In this project, the efficacy of a standardised blend of plant-derived isoquinoline alkaloids (IQA, containing 0.5% sanguinarine, Phytobiotics) in amelioration of the impact of SLD was assessed. A reduction in the number of miliary lesions on the liver surface and reduced lesion scores in the treated groups compared with untreated hens after oral challenge with *C. hepaticus* was detected. While a significant reduction of egg weights was detected after infection in the positive control group, there was an increase in the egg weights in the high dose phytobiotic group during the same period. Egg production in the group with high-dose of IQA was improved. Other minor changes in production indicators included an increase in feed consumption and an increase in body weight of the treated hens. The present study demonstrated a reduction in disease indicators in hens exposed to a SLD challenge and supplemented with a feed additive containing isoquinoline alkaloids.

I. INTRODUCTION

Spotty Liver Disease (SLD) is characterised by increased mortality, particularly around the time of peak egg production, the occurrence of multiple grey/white lesions in the liver, and reduction in egg output. It is caused by *Campylobacter hepaticus* (Van et al., 2016), which responds to therapeutic antibiotics, although resistance has been reported (Grimes and Reece, 2011). In this study, the ability of a feed-additive containing a standardised blend of isoquinoline alkaloids to modify the progression of SLD in hens exposed to *C. hepaticus* was assessed. Before and after exposure of the treated birds to *C. hepaticus*, production parameters were measured. A necropsy examination of the hens was performed to assess the degree of liver damage as an indicator of disease caused by *C. hepaticus*.

II. MATERIALS AND METHODS

Ethics approval: WSIAEC – 19.17. One hundred and thirty-two 21-week-old Hy-Line laying hens were distributed into: Negative control group (NC, 28), not treated and not exposed; positive control group (PC, 36), not treated and exposed; low dose IQA (LD-IQA, 100 mg of product/kg of feed or 1.4 ppm of IQA, 32) and high dose IQA (HD-IQA, 200 mg of product/kg of feed, equivalent to 2.8 ppm of IQA, 36). Hens were free of *C. hepaticus*. Each group was subdivided into “Short” and “Long”, determining the time of autopsy post-exposure; 6 days for the Short and 29 days for the Long groups. Short groups were used to assess the liver lesions of the hens, while Long groups were used to compare production parameters between groups ($\chi 2$ and two-sided Fisher’s exact test). Hens were fed with the phytobiotic supplemented diets

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for a period of 28 days. After that period, hens were orally exposed to a broth containing 1×10^9 cfu of the *C. hepaticus* strain $HV10^T$, as described by Van et al. (2017), or sterile broth in the case of the NC group.

III. RESULTS AND DISCUSSION

a) Egg weights

Egg weights are presented in Table 1. The egg-weights increased over time in the NC group. The egg-weights in the PC group decreased significantly between 3 and 6 days after exposure (DAE; P = 0.02). There was a recovery in the egg-weight at 14 DAE (4.12%). However, egg-weights were not significantly higher compared with 3 and 6 DAE (P = 0.86 and 0.07, respectively). In the LD-IQA group, the egg-weight had no significant change between 3, 6 and 14 DAE. HD-IQA group egg weights increased numerically between 3 and 6 DAE and increased significantly by 14 DAE compared with 3 DAE (P = 0.01) and 6 DAE (P = 0.03), respectively. Diets with 2.8 ppm IQA prevented the egg-weight loss in SLD challenged birds.

Table 1 - Average egg weights per group and their percentage of change between different sampling days.

Days after challenge	Negative control	Change (%)	Positive control	Change (%)	LD-IQA	Change (%)	HD-IQA	Change (%)
3	55.64^{a}		57.14^{a}		57.65		56.74^{a}	
6	57.97^{ab}	4.19%	54.38^{b}	-4.83%	56.70	-1.65%	57.11^{a}	0.64%
14	59.70^{b}	2.98%	56.62^{ab}	4.12%	58.55	3.27%	59.72^{b}	4.58%

Numbers with different superscript letters in the same column differ significantly ($p < 0.05$).

b) Egg production

Egg production was significantly affected by SLD (PC versus NC groups, Table 2). Production was significantly higher in the HD-IQA group but not different in the LD-IQA group compared with PC group. The high dose of IQA was able improve egg production after a SLD challenge.

Table 2 – Number of eggs expected for each group to be produced after exposure calculated using the egg production data before exposure.

	NC	**PC**	**LD-IQA**	**HD-IQA**
Expected	379	383	310	367
Difference produced with expected	20	4	-9	15
Significance*	A	B	B	A

* Different letters between columns represent significant differences ($P < 0.05$), calculated by $\chi 2$ and two-sided Fisher's exact test.

c) Postmortem findings

During postmortem examination, an estimation of the number of liver lesions and lesions score was made. Results are summarised in Figure 1. There were no lesions present in the NC group. The average numbers of lesions ± SD in the PC and LD-IQA groups were 201.1 ± 327.6 and 183.6 ± 318.4, respectively, being significantly higher compared to the NC group. The average number of lesions in the HD-IQA group was 41.7 ± 67.3, which was not significantly higher than the NC (P = 0.22), but not significantly lower than the average number in the PC group. Similarly, for lesion scores the median liver lesion score of the hens from the HD-IQA group was not significantly higher than the NC group, although not significantly lower compared with the PC group. The median scores of both the PC and LD-IQA groups were statistically higher compared to the NC. However, to include the negative control in the comparison is

misleading, as the number of liver lesions in that group was zero. When the NC and LD-IQA groups are excluded from the calculations, and only the HD-IQA and PC groups are compared, the difference between the number of lesions was statistically significant (P = 0.04), but not the SLD liver scores (P = 0.11, Figure 1).

These results demonstrate that the phytobiotic produced a reduction of the pathological changes in the liver induced by SLD. Previous field reports describe the use of two phytobiotics (oregano and isoquinoline alkaloids) on multiple free-range operations which led to a trend of reduction in the incidence, the age of onset and severity of SLD clinical signs (Scott et al., 2020). The positive effect of diets containing isoquinoline alkaloid extracts in reducing the negative impact of gut bacteria has been previously described (Xue et al., 2017).

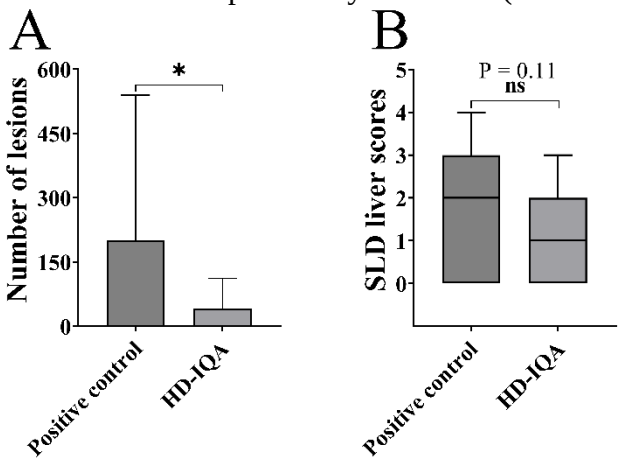

Figure 1 - Summary of the analysis of the livers from the *post mortem* analysis of the hens of the Short group, euthanised 7 DAE *to C. hepaticus* (PC and HD-IQA groups only). A, Columns, average number of lesions with SD (error bars). B, Central line on each rectangle, median score; margins of the box, interquartile range; external lines, minimum and maximum values. *, P < 0.05; ns, not significant

d) Feed consumption

All hens had a modest increase in the average feed consumption during the first week after the exposure (Figure 2A). However, the increase was more prominent in the NC compared with the other three groups. In all Long groups, there was an increase in the average feed consumption post-exposure. HD-IQA was the only group where the increase in feed consumption at 29 DAE was significantly higher (Figure 2B). This could indicate an increase in the palatability of diets containing the phytobiotic.

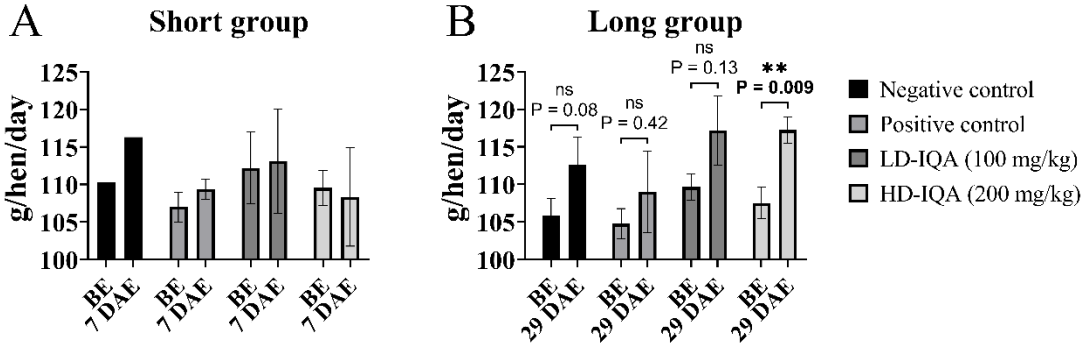

Figure 2 - Average feed consumption by group and sampling period. Each column represents the average feed consumption from each group, and the black lines represent the SD. BE, Before exposure; DAE, days after exposure. Ns, not significant. **, P < 0.01.

e) Weight gain

Hens were blocked by weight at the start of the study so there were no differences between treatment groups. Hens fed with the higher dose of IQA exhibited a significant increase in feed consumption between pre-exposure and 29 days post exposure (not seen in the NC and PC groups), and a significantly higher final body weight compared with the NC and PC groups (Figure 3). It can be concluded that IQA produced an increase in the appetite of the hens. A previous study related some beneficial effects obtained by phytobiotics with an enhancement of feed intake, improved nutrient digestion, increases in digestive enzymes and a greater absorption of nutrients in the intestines (Abudabos et al., 2018).

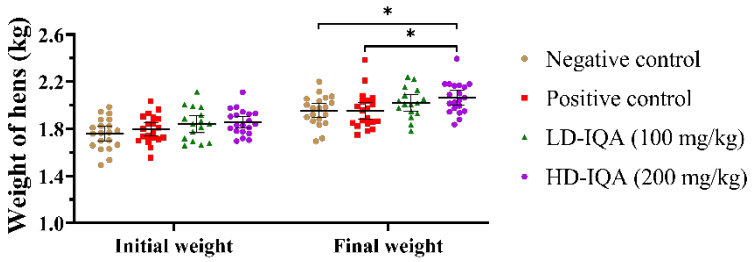

Figure 3 - Weights of the hens from the Long groups at the beginning and the end of the study. *, $P < 0.05$.

Some dose-response to IQA concentration was observed in the present study with egg-weights immediately post-challenge, lesion scores and lesion numbers improved in the HD-IQA group compared to the LD-IQA group, indicating that inclusion rates may be critical in obtaining the benefits seen in this study when this phytobiotic is included in commercial layer-feed.

IV. CONCLUSIONS

Feed rations containing the IQA at a dose rate of 200 mg/kg of feed or 2.8 ppm (1 ppm of sanguinarine and 1.8 ppm of other alkaloids) was capable of reducing the negative impact of SLD in layer hens, including higher egg weights and production, a reduction in the number of liver lesions, and an improvement in the weight of the hens and feed consumption. Further field and laboratory exposure studies should be undertaken to better define the benefits of this feed additive in ameliorating SLD and the mechanisms of action.

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THE MITIGATION OF LPS-STIMULATED IMMUNE RESPONSE IN IPEC-J2 CELLS BY AN ANTI-MYOCOTOXIN PRODUCT

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Summary

As mycotoxin-contaminated feeds are frequently reported to be more toxic compared to the pure mycotoxins in animals, it can be inferred that those effects come from possible interactions between mycotoxins or with other factors existing possibly in the gastrointestinal tract, such as bacteria. The effects of mycotoxins in increasing Gram-negative bacteria (GNB) numbers in the intestine are known. With this, an increase of luminal lipopolysaccharides (LPS) is a risk. LPS may induce inflammation and fever, lowering animal performance; in some cases this is followed by shock and, eventually, death. Intestinal porcine epithelial-jejunum (IPEC-J2) cells were exposed to a challenge of LPS from *E. coli* and the LPS binding capability of a specific anti-mycotoxin product (MS) was evaluated. The challenge increased significantly ($P < 0.05$) the secretion of interleukin (IL)-8, a pro-inflammatory cytokine in IPEC-J2 cells 24 hours after the challenge and showed that MS alone did not induce inflammation, indicating that is an effective intervention in mitigating the risk of increased LPS.

I. INTRODUCTION

Mycotoxin-contaminated feeds are frequently reported to be more toxic for animals than their purified forms, indicating possible interactions with each other or with the micro-organisms in the animal's gastrointestinal tract (JECFA, 2002) as bacteria (Galarza-Seeber et al, 2016). The gastrointestinal tract is the largest immune organ, where several immuno-regulatory mechanisms simultaneously defend the body (Belkaid & Hand, 2014). Yet, it is also home to a diverse community of bacteria, fungi, protozoa, and viruses. GNB are part of the microbiota, thus LPS 'the major constituent of the outer membrane of all GNB' are present in the intestine as GNB continuously multiply and die (Van Amersfoort, 2003). Under eubiosis, LPS does not affect animals because intestinal epithelial cells are poorly responsive to LPS when stimulated from the luminal side. However, when LPS crosses the intestinal barrier, reaches the bloodstream or emerges in the basolateral (BS) membrane, inflammatory cytokines are upregulated and stimulate the expression of toll-like receptor (TLR)-4 in the apical (AP) side of the intestinal cells. The immune response starts and changes in the epithelial structure and functionality occur (Ghareeb et al., 2016; Vamadevan et al., 2010; Zhang et al., 2017).

LPS in the gut can enter the circulation mostly by paracellular transport in the intestinal epithelium 'enhanced by a dysfunctional barrier' but also by transcellular transport without a barrier disruption (Mani, 2012). Once LPS is in the bloodstream, it induces inflammation and fever, sometimes followed by shock and, eventually, septicemia and death. These inflammatory responses require increased cytokine levels, whose activation is mediated by TLR-4, the immune receptors that recognize LPS (Sampath, 2018) and culminates with phosporylation and activation of the transcription factor NF-kB. A high portion of energy is utilized to sustain the elevated immune response (Li et al., 2015); thus, LPS depress growth performance and feed efficiency. LPS also triggers or exacerbates diverse diseases or disorders that hinder animal health, such as heat stress and oxidative stress (Huang, 2017) and they mediate immune-pathological alterations in the liver, the bursa, and the intestine, as well as in the reproductive tract.

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Compounds known as mycotoxin binders can adsorb small molecules during passage through the digestive tract, resulting in the excretion of a toxin-adsorbent complex in the faeces (Kolosova and Stroka, 2011). A mixture of clay, yeast cell walls and phytomolecules has recently been shown to have efficacy in reducing the effects of feed-borne mycotoxins in poultry (Janječić et al., 2020). Likewise, some compounds have been tested for their ability to mitigate the adverse effects of LPS (Basauri et al, 2019; Farkas et al., 2014), such as sodium bentonite, palygorskite and organoclays, which have also been tested for their ability to bind mycotoxins (Chen et al, 2020; Schaumberger et al, 2014; Zheng et al, 2020). These results indicate the opportunity to use specific toxin binder products as a practical and economic measure to reduce LPS challenges under conditions of stress and/or mycotoxin exposure in animals including poultry.

The objectives of the current study were to evaluate the effect of LPS in an *in vitro* model of IPEC-J2 cells and the possible mitigating and anti-inflammatory activity of an anti-mycotoxin product in the same model.

II. METHOD

Intestinal porcine epithelial-jejunum (IPEC-J2) cells were seeded and differentiated on semipermeable Transwell filters of 12 mm diameter, 0.45 μm pore diameter, fitting in 12-well plates. This filter system allows the separation of the AP from BL compartments. Four different solutions were prepared using serum-free cell culture medium, the IPEC-J2 growth factor solution (GFS): Control (C): only growth factor solution (GFS); GFS + an anti-mycotoxin product (Mastersorb Gold, from EW Nutrition GMBH (MS)); LPS: GFS, 20μg/ml lipopolysaccharide (LPS; serotype O111:B4 from *E. coli*), and LPS+MS: GFS, 20μg/ml lipopolysaccharide (LPS; serotype O111:B4 from *E. coli*), 1mg/ml MS (≙0.1%).

All solutions were incubated for 2.5 h at 37°C with shaking. After centrifugation, the supernatants were added to the AP side of the filters containing the IPEC-J2, and incubated for 4 and 24 hours at 37°C. The solutions of all samples were collected, NF-kB activation after 4 hours was evaluated by immunofluorescence and Interleukin-8 (IL-8) after 24 hours was quantified by ELISA.

Cell monolayers were stained with fluorescent specific antibodies targeting occludin (as marker of tight junction integrity) and P-p65 (indicating the activation -phosphorylation- of NF-kB). To localize the proteins, rabbit polyclonal anti-P-p65 antibody and mouse monoclonal anti-occludin antibody were used. For secondary detection, Tetramethylrhodamine (TRITC) was used for P-p65, and Fluorescein-5-isohiocyanate (FITC) for occludin. Nuclei were stained with 4', 6-Diamidino-2- Phenylindole (DAPI). Stained monolayers were mounted on glass slides and analyzed under a confocal microscope.

All experiments were performed in triplicate. Prior to analysis, normal distribution and homogeneity of variance of all variables were assumed with Shapiro-Wilk's and Levene's tests. Statistical significance followed by post hoc Tukey honestly significant difference (HSD) test.

III. RESULTS

The immunofluorescence experiments showed nuclear localization of phospho-NF-κB p65 (P-p65) upon LPS stimulation, that was not present in cells simultaneously treated with LPS and clay (Figure 1). The P-p65 translocation did not occur after the addition of the clay alone to the cells.

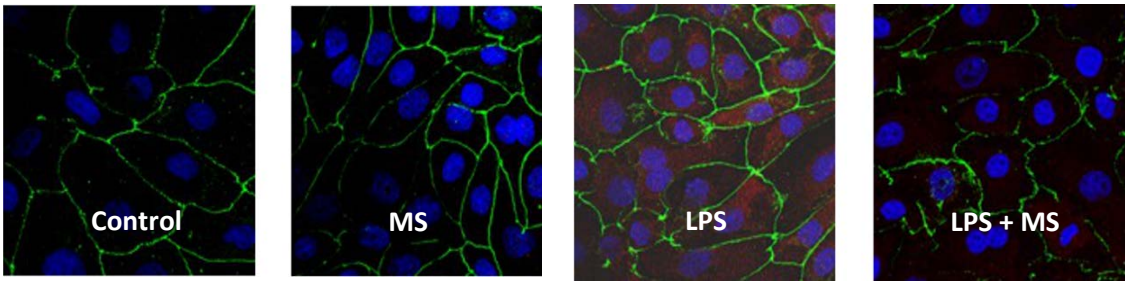

Figure 1 - Immunofluorescence of NF-kB (P-p65) in IPEC-J2 cells treated for 4 h either with and without an LPS challenge and with and without an anti-mycotoxin product. Red: p-p65-TRITC (Tetramethylrhodamine). Green: occludin-FITC (Fluorescein-5-isothiocyanate). Blue: nuclei-DAPI (4', 6-Diamidino-2- Phenylindole).

The challenge of 20µg/ml *E. coli* LPS (serotype O111:B4) increased significantly ($P < 0.05$) the production of IL-8 24 hours after the challenge (Figure 2). MS alone did not increase inflammation and lower amounts of IL-8 were detected in the solution collected from the IPEC-J2 cells exposed to the LPS together with MS.

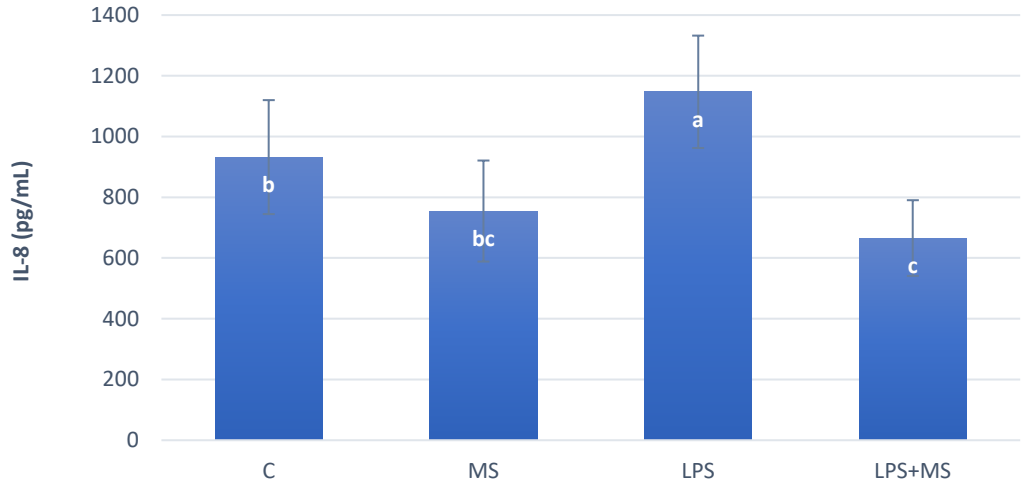

Figure 2 - IL-8 secretion of IPEC-8 cells 24 after an exposure to different solutions with and without an LPS challenge and with and without an anti-mycotoxin product. Different letters indicate significant differences ($p \leq 0.05$).

IV. DISCUSSION

IPEC-J2 cells have been used as an intestinal *in vitro* cell model showing inflammatory reactions (Xu et al., 2020). The increased expression and circulation of pro-inflammatory cytokines triggered by LPS is detrimental for the animal (Rauber et al., 2014). The results of the experiment, in regards with induction of inflammatory response, confirmed those of Palocz et al. (2016) who found significant increase of IL-8 in IPEC-J2 cells 24 hours after LPS treatment (10 µg/ml). In the present experiment, an increase of 20% in IL-8 release was found with a higher dose of LPS, but lower doses also increased IL-8 as shown by Geens and Niewold (2010). Moreover, using a chicken ileal explant culture model, Zhang and his team (2017) observed that a challenge of 20µg/ml induces an acute inflammatory response after 2 hours of incubation, including an increase of 80% in IL-8.

Binding LPS in the gut lumen may reduce the load of endotoxins and the risk of them entering the bloodstream (Ditter et al, 1983). An evaluation of binding substances in *in vitro*

conditions provides a screening point and higher insights into the mode of action (Schaumberger et al, 2014). The binding of LPS by commercial products is so far, not widely explored; a few traditional binding experiments were performed in artificial intestinal fluid quantifying endotoxins with the Limulus Amebocyte lysate (LAL) test (Schaumberger et al, 2014).

The use of the IPEC-J2 cell model, in this experiment, confirmed that MS is innocuous to the cell-line and demonstrated the beneficial effect that MS potentially has at intestinal level in binding the LPS and reducing inflammation, evidenced by the lower immunofluorescence of NF-kB and IL-8 levels in the MS and in the MS+LPS groups, respectively.

Reducing inflammation factors, such as LPS, in production animals, increases health and welfare and helps to ensure that nutrients are being used for growth and muscle development instead of fighting the challenge.

Further experiments with avian intestinal cell lines should be performed to confirm the application of the results of the study in poultry. However, the use of healthy intestinal cell line-models such as IPEC-J2, to study the mechanisms of existing harmful substances and their mitigation strategies, is an advantageous approach. The findings can be extrapolated to other species (Palocz et al., 2016), experimental costs are reduced and ethical considerations of animal welfare are moot, as no animals are required.

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PROBIOTIC BACILLUS LICHENIFORMIS SIGNIFICANTLY IMPROVES LAYER PERFORMANCE

W. VAN DER VEKEN[1], V. HAUTEKIET[2] and R. SERWATA[3]

Summary

Adding probiotic *Bacillus licheniformis* DSM 28710 to layer diets was shown to improve layer performance, both in commercial as well as in research conditions. This was confirmed in a recently conducted experiment in laying hens. Addition of the probiotic significantly improved egg production, without increasing average daily feed intake. This is in line with the available peer-reviewed literature regarding probiotics and their beneficial effect on animal performance, indicating their relevance in modern animal production. Probiotics can be applied as an important management tool to secure healthy animals, and thus improve production performance.

I. INTRODUCTION

The importance of the microbiota and general gut health, in relation to animal health and thus performance, cannot be underestimated. This has led to the development of management tools to influence and support the gut microbiota, as well as gut health in general. Probiotics, defined by the FAO/WHO (2002) as "live microorganisms which when administered in adequate amounts confer a health benefit on the host", are a good example thereof. Specifically of interest is the probiotic *Bacillus licheniformis* strain DSM 28710. The specific strain has previously shown efficacy in poultry, including broilers and turkeys. In these species, the probiotic strain was able to improve the general technical performance. This was achieved by mitigating challenges such as pathogens and stress situations, whilst supporting general gut health. These observations were highlighted by a variety of investigated beneficial effects, ranging from improvements of microbiota compositions and gut morphology to digestion efficiency and mortality percentages. To evaluate the efficacy of the probiotic strain in layers, an independent research trial was conducted, supplementing dietary *Bacillus licheniformis* DSM 28710 to birds in lay.

II. METHOD

The trial was carried out at the experimental centre of Roslin Nutrition (Scotland), using 768 laying hens (Hy-line Brown) randomly allocated to the treatments. In the set-up, 192 cages were used in total, resulting in 96 cages per treatment with four birds per cage. The trial ran for a duration of 168 days, equal to 24 weeks (from week 21 to 45 posthatch). Treatments consisted of a control group fed a commercial basal diet and a *B. licheniformis* DSM 28710 (B-Act®, Huvepharma®) group, fed the same commercial basal diet but supplemented with $1.6x10^{12}$ CFU *B. licheniformis* DSM 28710 per metric ton of feed. To evaluate the impact of the strain on technical performance, the following parameters were assessed during the trial period (week of age 21 to 45): average daily feed intake (ADFI, g/hen/day), egg production (expressed as percentage, amount of eggs per day/hens on that day) and egg numbers (total eggs/cage) were measured and reported on a four-weekly basis, with the first measurements taken in week of age 21.

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III. RESULTS

Egg production for the probiotic group was significantly better, with a percentage of 93.9% versus the control's 92.5% (evaluated over the whole trial period; P = 0.007). This was reflected in egg numbers, being significantly greater in birds supplemented with *B. licheniformis* DSM 28710: 630.9 eggs versus the control's 621.8 (total number of eggs/cage over the 168 days; P = 0.01). These results were achieved without an increase in ADFI: control animals consumed 123.2 g of feed per hen per day, with the animals supplemented with the probiotic consuming a comparable 123.4 g of feed per hen per day (P = 0.48).

IV. DISCUSSION

The above results are in line with previously conducted animal studies, where the same probiotic DSM 28710 strain was supplemented to layers as well as other poultry. In addition to the beneficial effects on performance, it was recorded that manure nitrogen significantly decreased in these trials whilst litter quality improved (P<0.05; internal research). The reduced manure nitrogen is indicative of enhanced protein digestibility, with improved litter quality linking back to general gut health status. Although the precise probiotic mode of action was not evaluated in the trial at hand, the observations mentioned above might offer a potential explanation for the noted improvements in performance. The peer-reviewed literature has also described multiple potential mode of actions for probiotics, one of which being the improvement of general gut health (Simon *et al*., 2001; Deng *et al*., 2012). This includes, but is not limited to, an improved utilisation of ingested feed, which in turn has a positive impact on performance.

In the trial at hand, egg production increased in the probiotic treatment group, but average daily feed intake remained the same when compared to the control. It thus makes sense to follow the same logic here regarding improvement of performance by improving feed utilisation, as already explained in the general peer-reviewed literature. The improved performance recorded in this trial can be considered an indirect effect of a more efficient feed utilisation, thanks to the probiotic supplementation. Although the exact mode of action was not investigated into detail here, the potential of *Bacillus licheniformis* DSM 28710 to support layer performance is clear. The probiotic should thus be considered in the overall feed management plan, supporting the animals and final productivity.

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A NOVEL MICROBIOME METABOLIC MODULATOR IMPROVED GROWTH PERFORMANCE AND INTESTINAL MORPHOLOGY DURING A COCCIDIOSIS OUTBREAK

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Summary

The present study was designed to investigate the effects of a precision glycan Microbiome Metabolic Modulator (MMM) on growth performance and gut health parameters in broiler chickens. The MMM nutritional product was compared against conventional essential oil products and an antibiotic positive control. During the study, a natural coccidiosis outbreak appeared, obliging us to conclude the study at 28 days for welfare purposes, but providing us an opportunity to evaluate treatment effects on the emergent coccidiosis. Day-old Ross 308 broiler chicks were randomly allocated to one of six treatments with 8 replicate pens per treatment and 15 birds per pen. Dietary treatments included: 1) negative control (NC); 2) NC with Avilamycin (10 ppm); 3) NC with essential oil product #1 (commercial dose, 40 ppm); 4) NC + essential oil product #2 (commercial dose, 150 ppm); 5) NC + essential oil product #3 (commercial dose, 300 ppm); and 6) NC with MMM (400 ppm in final feed). Body weight (BW), feed intake (FI), and mortality rate were recorded throughout the trial. Feed conversion ratio was corrected for mortality and adjusted to a common body weight (cFCR). Intestinal lesions were scored at the end of the study. Histology samples were also fixed for intestinal morphological determination. In this study, the MMM treatment improved cFCR versus the NC (P<0.05). At the level of the entire intestine, the MMM treatment resulted in reduced intestinal lesions compared to the essential oil treatments and similar to the antibiotic treatment (P<0.05). At the same time, in the duodenum, both the MMM and essential oil treatments reduced the severity of intestinal lesions compared to the NC. Both the MMM and Avilamycin treatments resulted in greater mucosa thickness in the ileum (P=0.02) and villus length (P=0.04) compared to the essential oil treatments. In conclusion, the supplementation of diets with MMM resulted in similar and in some cases superior performance to the Avilamycin treatment and in all cases superior performance to the essential oils treatments. Harnessing the functionality of the microbiome through modulation of microbial metabolite output is a promising new approach to supporting the nutritional health, performance and sustainability of broiler production.

I. INTRODUCTION

Coccidiosis is caused by Apicomplexa protozoa of the family Eimeriidae. In poultry, most species responsible for coccidiosis belong to the genus *Eimeria,* which infect various sites in the intestine. Coccidiosis is recognized as one of the most common and economically important diseases in broilers, with an estimated $16,7 billion in losses globally per year. The average cost of coccidiosis per chicken produced is estimated to be $0.21 (Blake et al., 2020). Traditionally, coccidiosis has been controlled through the use of chemical coccidiostats, ionophores, and vaccines. But there is an ongoing effort to decrease the use of chemical and ionophore anti-coccidials due to consumer and regulatory pressure to reduce the overall use of medicated feed additives in broiler production. The industry therefore seeks alternative

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approaches to managing coccidiosis. This study evaluated the effect of a novel precision glycan microbiome metabolic modulator (MMM) on birds' resilience that encountered a natural (unintended) coccidiosis outbreak during the grow-out, compared to various common essential oil products.

II. METHOD

Seven hundred and twenty 1-day-old male broiler chicks (Ross 308) were randomly assigned to one of six dietary treatments with eight replicates of 15 chicks per treatment. The birds were fed either a basal diet without any additives as a negative control (NC), the NC diet supplemented with Avilamycin (10 ppm, Surmax 200) serving as a positive control (PC) or the NC diet supplemented with either 40 ppm essential oil product 1, or 150 ppm essential oil product 2, 300 ppm essential oil product 3 and 400 ppm MMM product. Diets were formulated to meet Ross 308 nutritional recommendations (Table 1) and fed over 3 phases including starter (1-10 d), grower (11-24 d) and finisher (25-28 d). The additives were added and mixed homogenously to the basal diets. All diets contained Ronozyme ProAct (200 ppm), Ronozyme HiPhos (2000FYT with only Ca (0.20%) and P (0.18%) and Na (0.02%) matrices applied) and Ronozyme Multigrain (100ppm), and were steam pelleted and starter diets were further crumbled. Feed and water were provided *ad libitum*. Birds were reared on reused litter materials from farms previously known to have had coccidiosis challenge. The used litter was topped up with fresh wood shavings to a depth of 3 cm prior to arrival. Chicks were individually weighed on arrival (37±0.5 g) and subsequently pen body weight and feed consumption were determined weekly for 4 weeks. Feed conversion ratio was corrected for mortality and adjusted to the study average body weight. Birds were individually weighed on days 7, 14, 21 and 28 to calculated coefficient of variation for body weight (CV%). Growth performance and histology data were subjected to a generalized linear mixed-effects models, with blocking as random effect, implemented in R. Lesions scores, noted from zero to four (zero for a normal appearance of the intestine and four being severe intestinal damage) were analyzed with a non-parametric Kruskal-Wallis test, using the kruskalmc function from the pgirmess package of R.

Table 1 – Composition of basal experimental diet and calculated nutrient composition

Ingredient (g/kg)	Starter 1-10 d	Grower 11-24 d	Finisher 25-28 d
Wheat	558.8	556.3	582.2
Soybean meal	281.2	225.6	172.4
Canola meal	42.5	60.0	70.0
Meat meal	40.0	32.0	25.2
Canola oil	26.0	35.5	40.8
Barely	20.0	40.0	50.0
Canola seed	10.0	30.0	40.0
Limestone fine	7.33	7.53	7.70
Salt	3.16	2.83	2.03
DL-Met	2.93	2.47	2.13
Lys-HCL	2.61	2.39	2.36
Vitamin-mineral premix	2.00	2.00	2.00
L-Thr	1.43	1.11	0.88
Na bicarbonate	1.03	1.20	1.35
Choline chloride (70%)	0.50	0.50	0.50
Protease	0.20	0.20	0.20
Phytase	0.20	0.20	0.20
Carbohydrase	0.10	0.10	0.10
Specifications			
Calculated nutrient AME (Kcal/Kg)			
NE (Kcal/Kg)	Starter	Grower	Finisher

Crude protein	2990	3100	3180
Dry matter	2362	2472	2551
Dig. Lys	231	213	194
Dig. Met	908.0	908.4	908.3
Dig. M+C	12.74	11.53	10.27
Dig. Thr	6.03	5.43	4.92
Dig. Iso	9.45	8.73	8.05
Dig. Leu	8.56	7.73	6.85
Dig. Trp	8.56	7.82	7.04
Dig. Arg	14.88	13.70	12.62
Dig. Val	2.56	2.36	2.11
Crude fat	14.06	12.74	11.30
Crude fibre	9.59	8.90	8.01
Calcium	51.85	70.00	79.69
Available phosphorous	34.67	37.63	38.54
Total phosphorous	9.00	8.50	8.00
Sodium	4.50	4.25	4.00
Chloride	4.99	4.69	4.34
Potassium	2.20	2.10	1.80
DEB meq/kg	3.30	3.02	2.49
	8.33	7.75	7.11
	215	204	190

III. RESULTS

The study was halted at 28 d due to a natural coccidiosis outbreak. Oocysts counts were performed and indicated that oocyst counts were above 4000 opg for all treatment groups. The growth performance results are summarized in Table 2. As there were no significant differences between the three essential oil products, the data were combined into a single treatment, presented as: NC + Essential oils. Under the conditions of the study, the feed intake and the final body weight at 28 d were not different between dietary treatments. Despite the high variability observed in cFCR, MMM significantly improved the cFCR compared to the negative control. There were no differences in mortality rate among treatments.

Table 2 – Effects of dietary treatments on feed intake (0-28 d), final body weight (28 d) and corrected FCR (0-28 d)

Treatments	Feed intake 0-28 d (g/b)	Final body weight 28 d (g/b)	cFCR 0-28 d	% Mortality 0-28d
Negative Control (NC)	2865	1409	2.236 b	10.0
NC + Avilamycin	2702	1489	1.917 ab	9.2
NC + Essential oils	2796	1432	2.038 ab	9.2
NC + MMM	2602	1469	1.859 a	10.0
SEM	207.1	29.4	0.115	2.74
P value	0.56	0.13	0.046	0.98

a,b, Within a column values with different superscripts are significantly different ($P < 0.05$)

Intestinal lesions scores on day 28 are reported in Table 3. Severity of lesions was greater in the duodenum than that in the ileum, suggesting that *E. acervulina* and *E. maxima* were the cause of this coccidiosis outbreak. Both MMM and Avilamycin reduced the degree of damage due to coccidia throughout the intestine, while the essential oil treatment group provided only a limited reduction. Specifically, in the duodenum, all treatments improved lesion scores compared to the NC treatment. However, in the ileum only the Avilamycin and MMM treatment reduced the lesion score compared to the NC treatment.

Table 3 – Effects of dietary treatments on and lesions score in duodenum, ileum, cecum, and full intestine (at 28 d)

Treatments	Duodenum lesion score	Ileum lesion score	Cecum lesion score	Full intestine lesion score
Negative Control (NC)	2.31 a	1.44 a	0.13	3.44 a
NC + Avilamycin	0.69 b	0.31 b	0.44	1.44 b
NC + Essential oils	1.29 b	1.08 a	0.71	3.06 a
NC + MMM	0.56 b	0.31 b	0.38	1.38 b
P value	< 0.001	< 0.001	0.07	< 0.001

a,b, Within a column values with different superscripts are significantly different (P < 0.05)

Intestinal morphological parameters are reported in Table 4. MMM significantly increased villus height and mucosa thickness compared to essentials oils and NC treatments, but was not different to the Avilamycin treatment. Villus height:crypt depth ratio was significantly higher in the Avilamycin treatment compared to all other treatments (*P*=0.03).

Table 4 – Effects of dietary treatments on ileum morphology at 28 d

Treatments	Mucosa thickness (μm)	Villus length (μm)	Crypt depth (μm)	Villus/ crypt
Negative Control (NC)	584.7 a	323.5 a	246.5	1.380 a
NC + Avilamycin	774.4 c	517.7 c	242.2	2.349 b
NC + Essential oils	676.6 b	401.3 b	261.4	1.636 a
NC + MMM	782.4 c	461.1 c	306.4	1.633 a
SEM	48.4	45.8	17.6	0.22
P value	0.019	0.039	0.058	0.033

a,b,c, Within a column values with different superscripts are significantly different (P < 0.05)

IV. DISCUSSION

In this study, MMM was more effective than essential oils at reducing intestinal damage and maintaining growth performance due to a coccidiosis outbreak. MMM resulted in similar increases in ileal mucosa thickness and villus length compared to the Avilamycin treatment. Interestingly, Blokker et al. (in Press) reported similar effects of MMM on gut morphology following a nutritional and vaccine overdose challenge. Authors suggested that MMM may contribute to reducing dysbacteriosis and promoting resilience to coccidiosis infection. This is consistent with the findings of this present study where intestinal lesions were reduced with MMM, reducing the severity of coccidia infection and loss in feed efficiency similar to the antibiotic treatment. In conclusion, modifying the functional pathways of the microbiome through the use of a precision glycan microbiome metabolic modulator was an effective tool at creating resilience to an enteric insult as seen through reduced performance losses and intestinal damage.

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BUGS HAVE NO BOUNDARIES: ANTIMICROBIAL RESISTANCE CHALLENGES OF AUSTRALIAN POULTRY

S. ABRAHAM[1]

Summary

Antimicrobial resistance (AMR) is one of the most prominent biosecurity issues affecting humans and animals. In recent decades, we have seen the emergence of bacteria resistant to critically important antimicrobials (last line drugs) in livestock production systems in Asia, Europe and North America. This predominantly includes resistance to critically important antimicrobials such as fluoroquinolones and extended spectrum cephalosporins among *E. coli* and *Salmonella* from pigs, poultry and cattle. The emergence of resistance to critically important antimicrobials in these regions is largely attributed to the direct use in livestock systems. Evidence accumulated over the last decade suggests that the ecology of AMR amongst *E. coli* and *Salmonella* isolated from Australian food-producing animals differs considerably to that observed in many other countries. This is attributable to Australia's unique geography, quarantine restrictions and unique constraints governing the use of critically important antimicrobials (CIAs) in food-producing animals. Recent surveys in *E. coli* and *Salmonella* in Australian livestock suggest absence or low levels of resistance to critically important antimicrobials. Genomic characterisation of some of these critically important resistant *E. coli* isolates demonstrated that the majority of the isolates have previously been reported in humans and wild birds overseas. These *E. coli* strains have not been identified previously in Australia either from humans or livestock. Their low frequency among clinical *E. coli* isolates from Australian livestock suggests that they have potentially been introduced via human carriers or migratory birds. This presentation identifies challenges of emerging critically important antimicrobial resistance particularly from “reverse zoonosis” and “migratory birds”.

I. INTRODUCTION

Antimicrobial resistance (AMR) is one of the most prominent health and biosecurity issues affecting animals and humans in modern society (WHO, 2017). Owing to the complex biology whereby AMR can develop and be harboured in a multitude of host animal species and the environment, it is arguably the biosecurity issue that best epitomises the need for a One Health approach to management. In recent decades, we have seen the emergence of critically important antimicrobial (CIA)- resistant *E. coli* and *Salmonella* in livestock production systems in Asia, Europe and North America (Mukerji et al., 2017). This predominantly includes resistance to drugs such as fluoroquinolones (FQs) and extended spectrum cephalosporins (ESCs) amongst isolates from pigs, poultry and cattle (Abraham et al., 2015; Kidsley et al., 2018; Lambrecht et al, 2018). Resistance to critically important antimicrobials (CIAs) amongst Enterobacteriaceae is shaping up as a key risk for livestock producers, particularly when it involves organisms with the potential to colonise humans, cause disease in humans or both. Internationally, there are growing calls for closer scrutiny of the pathways by which humans might be exposed to sources of CIA from animals. Outside of Australia, the emergence in livestock of bacteria resistant to CIAs, particularly those which are heavily relied on in human medicine (ESCs and FQs) has often been attributed to the routine use of such antimicrobials in livestock production systems (Lutz et al., 2011; Marshall and Levy, 2011).

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Evidence accumulated over the last decade suggests that the ecology of AMR amongst *E. coli* and *Salmonella* isolated from Australian food-producing animals differs considerably to that observed in many other countries (Abraham et al., 2014a, 2015, 2017; Barlow et al., 2015). This is attributable to Australia's unique geography, quarantine restrictions and unique constraints governing the use of CIAs in food-producing animals. For example, in Australia FQs have never been registered for use in food-producing animals and label directives limit the administration of ESCs to only individual animals (Cheng et al., 2012; Mukerji et al., 2017). Polymixins are only found in a single registered preparation that has low-level of use as a topical agent for ocular conditions. This multifaceted approach has been successful in minimizing the occurrence of CIA resistance among Gram-negative bacteria in food-producing animals.

Confirmation of the above status exists in the form of findings from cross-sectional studies that have demonstrated that Gram-negative bacteria obtained from Australian livestock have nil or low levels of resistance to a wide range of critically important antimicrobials (Abraham et al., 2014a,b, 2015; Barlow et al., 2015; Pande et al., 2015). In addition, recent AMR surveys in poultry also revealed low levels of resistance among key indicator bacteria such as *E. coli* and *Enterococci* spp. and food borne pathogen *Salmonella* spp. (Abraham et al., 2019; O'Dea et al, 2019; Sodagari et al., 2019). To date, there are no reports of resistance to carbapenems and colistin amongst *E. coli* and *Salmonella* from Australian livestock, and resistance to FQs and ESCs has been observed at only very low frequencies in such isolates. Genomic analysis has revealed that these FQ- and ESC-resistant strains are very dissimilar to those previously observed in clinically ill humans in Australia but have previously been observed amongst humans and wild birds overseas (Abraham et al., 2015, 2018). This provides grounds to suggest that the very few FQ- and ESC-resistant *E. coli* and *Salmonella* isolated from livestock in Australia are unlikely to have evolved locally but rather have been introduced into Australia from abroad, potentially via incursion mechanisms such as human carriers and/or wild birds.

II. CIA RESISTANT *E. coli* FROM AUSTRALIAN WILD BIRDS

More recently than the livestock studies above, we have reported the emergence of CIA-resistant *E. coli* among Australian seagulls (Dolejska et al., 2016; Mukerji et al., 2020), Following detection of the carriage of carbapenem resistance in *E. coli*, from a single, large, offshore seagull colony in New South Wales (Mukerji et al., 2020), a subsequent Australia-wide survey based on 562 faecal samples from six Australian states reported widespread occurrence of extended-spectrum cephalosporin (21.7%) and fluoroquinolone (23.8%) resistant *E. coli.* Comparative genomic characterisation revealed that gulls carry pandemic extraintestinal pathogenic *E. coli* -ST131 and globally emerging fluoroquinolone-resistant ST1193, both clones recognised as globally-distributed human pathogens. The rate of CIA-resistance among seagull *E. coli* was surprisingly high in a country where FQ and ESC resistant *E. coli* are typically rare or absent among food animals.

Another study further characterized the dynamics of drug-resistant *E. coli* in wildlife populations, where we investigated the carriage of critically important antimicrobial (CIA) drug-resistant *E. coli* in four bird species in a common environment. This study revealed that gulls (53%), little penguins (11%) and feral pigeons (11%) carried *E. coli* resistant to critically important antimicrobials. Genomic analysis also confirmed that these are human associated *E. coli* strains and genetic analysis of antimicrobial resistance genes indicated interspecies resistance transfer. Terns, representing a bird species that forages on natural food sources at sea and distant from humans, did not test positive for drug-resistant *E. coli*. This study

demonstrates carriage of CIA-resistant bacteria in multiple bird species living in areas commonly frequented by humans,

The carriage of diverse human associated CIA-resistant *E. coli* clones among seagulls and urban birds indicates that urban and peri-urban scavenging birds can indiscriminately accumulate and disseminate CIA-resistant bacteria of anthropogenic origins. These studies uniquely establish that Australian Silver Gulls and other urban birds are carriers of virulent CIA-resistant human-associated pathogenic *E. coli* clones. The carriage of diverse CIA-resistant *E. coli* clones, that strongly resemble pathogenic clones from humans, suggests that seagulls can act as ecological sponges indiscriminately accumulating and disseminating CIA-resistant bacteria over vast distances. The public health and animal health implications are yet to be determined; however, it is possible that both human and potentially livestock are likely to be exposed to pathogenic CIA-resistant *E. coli* from a wide range of other species including ducks and ibis that share ecological niches with scavenging birds.

The “reverse zoonosis” and “migratory bird” hypotheses represent important scientific challenges. The potential exists for these pathways to result in permanent colonisation of livestock systems with organisms carrying potent resistance determinants that undergo proliferation due to the use of first-line antimicrobials registered for use in livestock such as tetracyclines, through a phenomenon known as co-selection due to the carriage of multi-drug resistant genomic elements.

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ANTIBIOTIC STEWARDSHIP IN EAST AND SOUTH EAST ASIA: THE TRIALS AND TRIBULATIONS OF A FIELD VETERINARIAN.

D. MARKS[1]

Summary

The East and South East Asia region is recognised as a hotbed of antimicrobial resistance. There are many reasons for this situation, attributable to both human and agricultural use of antimicrobial products. In animal agriculture in the region, misuse of antimicrobial preparations is common and there is no doubt that this use can contribute to the antimicrobial resistance issues documented in the region. East and South East Asia is probably the most diverse region of the world, with 20 countries or dependencies ranging from very affluent and advanced societies to some countries at the other end of the socio-economic spectrum. There is little published data on why antibiotics are used and how they are used from a field perspective. This paper looks at the difficulties involved in implementing antibiotic stewardship programs in the region at the field level. My work address life is predominantly in East and South East Asia.

I.INTRODUCTION

To introduce myself, I am a veterinarian by profession, a poultry veterinarian by accident and I am a field veterinarian by choice. My role as a field veterinarian (along with other professionals and the management team) is to turn expensive feed into profitable meat as efficiently as possible. Thus, my focus on-farm is on bird health and flock management to achieve the best biological performance we can. But along with that brief is the 'social contract' that comes as a result of my profession, my training and my responsibility to the wider community, to the environment and to the animal.

I do not think there is any more doubt or argument that we in the animal food supply chain have contributed to issues around antibiotic resistance through our use and misuse of antibiotics; the question remains though as to how much of the resistance problem we are responsible for and the wider and long-term impact of that use. Whilst this of course is a legitimate question to ask, it is not the subject of this paper and the fact remains that antibiotic stewardship in animal agriculture is both prudent and necessary. Efficient flock health and biological performance can be achieved, side by side with the principles of Antibiotic Stewardship and even (ultimately) with antibiotic freedom. Antibiotic Stewardship in my definition is:

"Use as little as possible but as much as necessary"

"Reduce Refine Replace"

My paper today is probably somewhat different to what you have been used to here at APSS. I am not going to give you a paper on how we achieve antibiotic stewardship or freedom; there are many such papers available on the internet and elsewhere. I will be discussing the how and why of antibiotic use in East and South East Asia, relying in a greater part on what I see and what I have to deal with every day when I am out in the field. I will look at the difficulties a field veterinarian faces in advocating and implementing the principles of antibiotic stewardship. There will be a lot of personal anecdotes and opinions in my paper because this is what I face every day. Some will amuse you, some will shock you but they are

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all real and we have to understand what is behind them before we can change them. I will also give you a glimpse of what is being done in the region to address the situation of antimicrobial resistance in the poultry industry, both at regional and national levels, and also some of the incredible work being done locally. My observations and comments on the how and why apply to all countries within this region to some extent or another.

Some countries (and within countries some companies) are only starting the journey of antibiotic stewardship and some are well on the way. Some observations are more relevant, at this point in time, in some countries than others. But, in reality, what we see happening in this region with regards to antibiotic use and misuse is not that different from what is happening, and of course what has already happened, in all other poultry growing regions and countries around the world. In some countries the process of change happened much more quickly and quite some time ago due to a variety of reasons we cannot go into here, but we all went through it at some stage or are going through it. Whilst my comments are specifically about the poultry industry, there is no doubt at all that my colleagues in the other livestock areas are going through much the same process.

II.INDUSTRY STRUCTURE

The structure of the industry in the East and South East Asian region does in some way contribute to the lack of antibiotic stewardship. The market has been rapidly developing and large integrations are seen as the way ahead for the poultry industry, as the market and consumer base becomes more sophisticated and as the movement away from wet markets builds momentum.

In Vietnam, the most recent agricultural census (2018) showed only 26.1% of chickens were raised in what we could class as intensive farms. The rest were raised in both backyard and semi-intensive commercial farms. To some degree or other this figure is repeated around the region with estimated figures of 60-80% of production being in the semi-intensive and backyard systems. Integrators can generally afford the luxury of employing in-house technical people who are more likely to understand the principle of good management health and nutrition and they generally have the resources, the necessary ‘hardware and software’ required to implement and maintain the principles that lead to good antibiotic stewardship. As well as the true integrators, there are a number of larger independent farms who buy in day-old chicks and then sell finished broilers to a range of processors or intermediaries. These independents sometimes have good resources available to them, similar to the integrators, but in most cases do not or have limited resources.

In many countries, local or indigenous chicken breeds of both egg and meat varieties have some significant importance and can make up a considerable portion of the local chicken supply. The market supplied by these birds is primarily the wet bird market for broilers; however, their appeal to the local populations is such that up to 10% of the supermarket shelving in some regions is given over to these birds. They are viewed as having some health benefits that the ‘commercial white broiler’ does not have or that the ‘commercial layer breeds’ do not have. The meat and egg supplied by these breeds commands a premium and so there is significant interest in rural areas to supply the market with farm sizes being small (less than 2000 birds). One problem that results from this is that farms go in and out of production due to the prevailing market. On many farms, the income from this type of poultry production is a secondary or supplemental income. Management expertise on these farms therefore is not as good as it could be. Biosecurity is poorly understood and poorly implemented and vaccination and disease prevention can be haphazard. Also, these birds (broilers) are slow growing compared to the commercial white broilers and can be up to 2-5 months before processing.

These reasons can result in these birds having more disease problems than in the commercial white broiler market with a subsequent increased reliance on antibiotics.

III.ANTIBIOTIC USE IN THE REGION

a) Overview

There is little good published work on how much antibiotics are used to raise poultry around the world. Van Boeckel in 2015 estimated that 148 mg of antibiotics were used to raise 1 kg of chicken meat as a worldwide average (Van Boeckel et al., 2015). In the Mekong Delta of Vietnam, in semi commercial poultry production, an estimate of 260 mg/kg of poultry meat production was made (excluding in-feed antibiotics) (Carrique-Mas et al., 2014). Another study showed that, for 1000 chickens produced, 690.4 g of antimicrobial preparations were used (Carrique-Mas et al., 2014). Antibiotics are generally used prophylactically. Again, in a study in the Mekong delta of Vietnam, 84% of antimicrobial products were used for prophylactic reasons (Carrique-Mas et al., 2014). They are administered routinely at set times because there is a belief that "if we don't use them we will have a problem". I have often asked the question on farms 'Why do you routinely use antibiotics?' The answer reliably comes back as "If we don't use them we will see a problem with mortality." "Well when was the last time you didn't medicate with antibiotics, and what sort of mortality did you get?". "Oh we have never removed them, we have always used them".

Some farmers managers and even veterinarians believe that using the same medication at the same age without any presenting mortality, but used to prevent expected mortality, is actually a therapeutic use. The reason given is that if they do not use the medication they will get mortality. In some cases, though a minority in my opinion, the statement that if antibiotics are not used, is entirely justified and antibiotics are used to prevent mortality due to vaccine reactions and poor management, particularly poor ventilation practices.

Many of the countries I work in have hot humid climates but one of the major problems I see is a vaccine reaction due to chilling of the birds - a result of the lack of understanding of the principles of ventilation and of wind chill and its effect in young birds. In a number of the countries I work in, commercial white broilers receive antibiotics continuously in feed and water for the first 2 - 3 weeks, the water use being made up of five or so different antibiotics. They also get in-feed antibiotics. I have also seen the use of two different antibiotics used in the same day. On questioning as to why this occurs, I have been told that the morning antibiotic is for the clinical infections and the afternoon antibiotic is for subclinical infections.

'Native' chickens can also receive antibiotic at intervals throughout their life. As their life is generally much longer and can be up to five months, they are also more valuable on an individual bird basis. Breeder birds and layer commercial birds also can have continual antibiotic use for the first week or two and then at routine intervals or following vaccinations or periods of known or perceived stress or for mortality episodes. In two countries in the region, the supply hatchery takes responsibility for mortality up to four days of age. It is common therefore for the hatchery to supply antimicrobial medications with the chicks to avoid any potential compensation issues.

b) What Antibiotics are Used?

Twenty-eight types of anti-microbials belonging to 10 classes were reported being used in semi commercial flocks in the Mekong Delta of Vietnam. Sixty-three per cent of all commercial formulations contained at least two antimicrobials. Polypeptides, tetracyclines, penicillins and aminoglycosides were the antimicrobials used by most farms, whereas penicillins,

lincosamides, quinolones, and sulphonamides/ trimethoprim were quantitatively the most used compounds Carrique-Mas et al., 2014).

A study by Nguyen Van Trung et al looked at antimicrobial usage in chicken production in the Mekong Delta of Vietnam in 2012-2013 (Carrique-Mas et al., 2014). They found that 28 different types of anti microbial substances belonging to 10 classes were used. On one farm I was taking through the process of global food saftey accreditation, I was given a list of 59 different antimicrobial preparations that they wanted in their list of antimicrobial products that they could use. I have personally seen up to 12 different antimicrobial preparations in one farm's medication store belonging to 5 different antibiotic classes.

The most common types of antibiotics used as water medication include penicillins, nitrofurans, tetracyclines, macrolides, flouroquinalones, polypeptides, sulphonamides and polymyxins. In-feed medication is usually either bacitracin tetracyclines or enramycin (Van Guong et al., 2016). As you can see, antibiotics classified by the WHO as critical for human use are used in the poultry industry in this region.

IV. WHY IS THERE MISUSE OF ANTIBIOTICS?

There are many reasons why the situation with misuse of antibiotics exists in the Asian poultry industry. I have listed here what I consider to be the main reasons as I see them in my farm visits.

a) Easy availability of antibiotics and their cost.

Generally throughout the region, antibiotics for use in agriculture are readily available. Many are sold 'over the counter' and do not require veterinary intervention or authorisation. Many are sold by travelling salespeople who visit farms. Many of these salespeople are pseudo technical and pseudo veterinarians who make diagnoses and then recommend medications. In a lot of circumstances these travelling technical people are the only technical advice some of these farms actually have, but of course their allegiance is to the company that employs them. Too often their income is also supplemented by commission on sales. On one large independent farm we were tasking through global food safety accreditation we had to give the owner an ultimatum - either he keeps these travelling technical sales people off the farm or we would stop the process of accreditation.

In rural Vietnam, the animal agricultural industries are serviced by veterinary pharmacies who advise farmers and supply medication. Rarely is a farm visit made or a post-mortem done and if it is done it is done by a poorly trained staff member. Supply of medications is done on the symptoms described by the farm manager. The sale of medications is a significant part of the income of these pharmacies. In most countries, the cost of antibiotics is rather cheap and this, combined with their ready availability, makes overuse problematical. In most countries, the farm gate sale of antibiotics is poorly regulated. Some larger farms also have no problems getting around import restrictions and import directly from China. I have seen medication stores on farms filled with pallets of antibiotics imported directly.

In Vietnam, the low cost of antimicrobial products has been given as one reason for the excessive antimicrobial usage (Carrique-Mas et al., 2019). It is my experience also that this is the case throughout the region. The cost of the antimicrobial preparations could also be leading to the misuse of these antimicrobial agents as in many cases the label includes guidelines for both prophylactic and therapeutic use, with the cost of prophylactic use significantly less than the cost of the therapeutic use (Carrique-Mas et al., 2019)). In my experience again most people charged with using the medication do not understand the difference between therapeutic and prophylactic use and will use the lower recommended dose rate as it is cheaper.

b) Regulations: implementation and monitoring

Most countries have inadequate and weak systems for controlling antibiotic use. In a recent publication. Coyne et al. (2019) identified weak or non-existent regulatory framework, suboptimal framework and compliance with existing guidelines as reasons for AMR in Asian countries. In-feed use is probably the exception. Feed sales figures are available from the feed companies and these have been used to estimate the in-feed use. Data on imports of antibiotics is not centrally collected and examined.

In some countries, good regulations are in place; however, it is the implementation of these regulations that is lacking. Whose responsibility is it to monitor the use/misuse of antibiotics? There is a lack of resources to investigate and prosecute breaches of the law and there is a significant lack of understanding of the appropriate regulations. This is not just a problem with agricultural use.

In Vietnam, I once went to a pharmacy for an inner ear infection. I asked for a course of treatment and was given two tablets. My comment was an obvious "No I am after a course of treatment", I was told that it was a course. People come to the pharmacy when they are sick and may buy antibiotics for one day's treatment. If they feel sick the next day, they will get a second lot but of course if they feel better they will not. As 'research' for this paper I went to three separate pharmacies and was able to purchase augmentin, ciprofloxacin and doxycycline. This is despite there being a requirement for a medical prescription and despite the fact that all purchases require documentation in a centralised register. I have been able to purchase what we would consider prescription antibiotics freely in other countries in this region and in one country I saw antibiotics available for sale 'over the counter' at a kiosk in a regional airport.

c) Limited technical and diagnostic understanding and capabilities

Veterinary training varies considerably between countries. In one of the countries I service in this region veterinarians have never heard of histology. There are also a lot of pseudo technical and pseudo veterinarians employed by the vaccine and medication supply companies. These technical people can be the only technical contact a farmer has. There is also a lack of good diagnostic testing in some countries or the diagnostic testing available is quite expensive and is not therefore used. It is a common belief throughout the region that antibiotics can treat viral conditions.

The lack of good diagnostics is not the only problem. In many regions good diagnostic tests are available; however, it is the interpretation of the test results that leads to problems. On one farm, the technical manager took faecal samples from a layer flock because of what he considered to be a change in faecal colour and tested them for clostridia. Clostridia were found (obviously) and necrotic enteritis was 'diagnosed'. Medication was given and was subsequently used for all flocks to treat this necrotic enteritis. Necrotic enteritis is the most over diagnosed condition I find here in East and South East Asia. In may cases, any condition resulting in a change in colour consistency or character of droppings, in litter quality or in gut health is diagnosed as necrotic enteritis and is treated as such. Lack of appropriate diagnostics mean that all mortality conditions can be treated with antibiotics.

On a number of occasions, I have been to look at problems on farms and before I have even entered a shed I have been asked what antibiotic they should be using. Understanding the problem is not important. I have asked then on those occasions what they have been using and have usually been given a list of antibiotics they have tried. I have made the comment that maybe it is the way the antibiotics are being used or that the condition is not even treatable with antibiotics; invariably the answer I have been given has been "Well what else can I use?".

Proprietary feed always comes with antibiotic added. If you do not want an in-feed antibiotic then you need to specifically order feed without it. In most cases, however, the feed companies will not allow changes to proprietary feed because of the issues this creates with production runs at the feedmill. The feed-milling industry is very competitive and to keep costs down, feed mills want long production runs and do not allow any changes to the feed they sell. Many farm managers and even the technical people do not actually know that the feed even contains antibiotic. On many occasions when I have asked what antibiotic is in the feed I have been told that there is not any in the feed. I have had to point out to them the list of ingredients on the feed bag and the antibiotic inclusion. Therefore, the concept of growth promotion using antibiotics is poorly understood. Some published surveys done in the region have reported that no antibiotic is used for growth promotion (WHO, 2018). Whilst that may truly have been the case, in my experience if the feed bags themselves were not actually checked by the authors then the use of antibiotics could have been missed.

Low levels of antibiotic awareness (Coyne et al., 2019) and the lack of understand of how antibiotic resistance occurs also contribute to the difficulty in getting antibiotic stewardship. I have on many occasions, when I have talked about antibiotic resistance, been told that antibiotic resistance is not a problem because farmers follow withholding periods. Most farm managers and staff have not even heard about antibiotic resistance or, if they have, they believe it is a foreign issue. The lack of understanding of how resistance occurs contributes to the misuse of antibiotics. Lack of appropriate awareness and input on how antibiotics should be used results in the use patterns we see. Not many of the farm staff actually charged with administering the antibiotics, know how they should be administered correctly and what happens if they do not administer correctly. Label use recommendations and instructions are rarely used and in my experience are not even consulted. In fact, with a lot of direct imported antibiotics the labels are in a foreign language unfamiliar to the farm manager and staff. I have often been told that antibiotic resistance should not be a problem on this farm because we adhere strictly to withholding periods. On most farms in the region, I find that there is a significant lack of understanding of how antibiotics actually work, even with veterinarians and technical people. Antibiotics are often given for maybe only 2-3 hours a day. Why? Because of other medications that the birds are given during the day or it is just too inconvenient.

The use of flouroquinolones, predominantly enrofloxacin, is common across the region. In Indonesia, it is common that they are used at 2-3 times the usual and published dose rate because ‘they do not work at the recommended dose rate’. When I discuss how antibiotics actually work, and that in most circumstances “more is not better”, I get agreement that the increased really does rate does nothing but no real commitment to change.

d) Lack Of understanding Of Products Used.

I have been onto farms and have asked for a list of the antibiotics used. As part of my farm visits, I always look in the medication store. On a number of occasions, I have seen antibiotic products that have not been included in the list I have been given. On questioning why they were not on the list I have been told they are not antibiotics. In good faith they have purchased various ‘tonics’ without understanding or enquiring about the composition. These tonics often contain low dose antibiotics.

The same thing applies as I have already mentioned with in-feed antibiotics. I find a significant number of farm managers and technical people, whose farms use proprietary feeds, have no or limited understanding of any antibiotics in feed and what they are in there for. In another country an integrator could not get their cholera vaccine on time. I recommended to the veterinarian that he gets some oxytetracycline to have on hand in case we get a break. I

recommended an appropriate dose rate to use. On my next visit I saw that the product the veterinarian had bought was an anti stress product which had oxytetracycline at a very low level and which included various vitamins electrolytes and some amino acids. It was labelled as oxytetracycline anti stress pack. To actually treat a flock with cholera at an appropriate dose rate would have required over 80 kg of the product per day.

e) Counterfeit products, inadequate labelling and quality.

Counterfeit antibiotics are also common. It is estimated that close to 80% of counterfeit drugs available in the world are made in South East and East Asia and that 45% of these medications are consumed here (Delve-Pierre et al., 2012). Recent studies in the Mekong Delta of Vietnam have shown in aquaculture only 8 and 29% of aquaculture antimicrobial products and 28.8% of poultry antimicrobial products had antibiotic active ingredient levels within 10% of the label claim. (Phu et al., 2015; Yen et al., 2019). One study also showed that 65% of antimicrobial preparations for poultry had label recommendations for both therapeutic and prophylactic use, and 5% had no use indications at all; 40% of products had a withdrawal time specified for both egg and meat products, 55% had only a meat withholding period and one product had no recommended withholding period at all (Phu et al., 2015). I personally have seen on farms products with labels totally in Chinese writing.

V.WHAT IS HAPPENING TO ADRESS THE ISSUE OF ANTIBIOTIC USE AND MISUSE AND WHAT SHOULD HAPPEN.

There is much being done in the region through organisations such as WHO, OIE, FAO Asia Pacific Foundation for Infectious Disease (APFID) etc. Most of the recommendations and actions around the programs under the initiatives of these organisations have five main elements (WHO, 2014; Hong-Soong, 2015; WHO, 2015)

1. Improve awareness and understanding of antimicrobial resistance
2. To strengthen knowledge through surveillance and research
3. To reduce the incidence of infection
4. To optimise and promote appropriate use of antibiotics
5. Strengthen national infrastructure.

I am not going to spend any time discussing these regional and national initiatives in any more detail. Their effectiveness varies considerably from country to country. Rather, I will again put my perspective on things and also give some details as to a project of great significance that is happening in the South of Vietnam.

One of the most significant drivers I have seen in the region to address overall issues of food safety and to a lesser extent antibiotic use in animal agriculture is the rise of the middle class. The rise of Asian economies since the end of the Second World War, but more so in the last 20 years, has been spectacular. As an example of how the region has performed, let's quickly look at Vietnam. After 19 years of war, this country was devastated both politically socially and economically. Since the reforms in the mid 1980's, Vietnam has risen from one of the poorest countries in the world to now rank 36th in the world in terms of GDP. 45 million people were lifted out of poverty and now it is estimated that over 25% of the population can be defined as middle class.

Currently, it is estimated that two billion Asians can be defined as middle class which is set to increase to three and a half billion over the next ten years. As has been seen in other countries when there is an increase in prosperity and purchasing power, then there is a subsequent increase in consumer driven activism regarding food safety which includes the issues surrounding antibiotic use in agriculture. I am a member of a number of food blogs in

Asia and it is here that I see active discussion (though not necessarily well informed) concerning agricultural practices. The middle class in Asia tends to not trust Asian produce and its purchasing practices are geared more towards imported foods wherever possible. This is making a difference in agriculture albeit slowly. Food safety auditing programs like Global GAP are becoming more common as a result of consumer pressure, an increase in export orientation as well as the rise in organic produce. The export sector is a significant driver of food safety accreditation and most of these programs have serious relation to antimicrobial stewardship and the prevention of antimicrobial resistance. Dramatic reduction in the use of antimicrobial products is a must in the region. I have already mentioned antibiotic stewardship and antibiotic stewardship is a key driver. We need to use, in my opinion, terminology like antibiotic stewardship, because we still do need antibiotics in animal agriculture in this region. It will be slow process and in the meantime antibiotics are essential. To achieve antibiotic stewardship there are three steps and principles we implement over time:

1. No use of banned or unregistered products e.g. Chloramphenicol, nitrofurans etc
2. Remove any antibiotic deemed by the WHO (and other bodies) as being of critical importance e.g. Cephaloproins, Quinolones, Polymixins etc
3. Remove all prophylactic antibiotics firstly in the water and then (possibly and if considered necessary) in feed ensuring firstly that:
   They are replaced with proven alternative strategies and products (combinations are more effective in the field)
   We have rigid principles of biosecurity in place.
   We have a robust and proven vaccination programme.
   We have a robust structure for diagnosis of mortality and poor performance events.
   We have a pre-approved and pre-planned procedures and products for therapeutic use only when and if necessary.

Many things are needed to be implemented at both regional national local and farm level to drive the concepts of antibiotic stewardship. The following I see as priorities. Some may be controversial and some may disagree with what other have discussed but they are what I see at the ground level.

1. Better educate veterinarians as to the how and why of antimicrobial resistance and the necessity to play a part in antibiotic stewardship. Teach them how to make better choices and give them the sole authority and responsibility to use and prescribe antibiotics. We need to also teach them how to investigate both performance and health issues on farm in a way that identifies root causes.
2. Educate farmers and farm owners to enable them to carry out good husbandry practices that have been proven to result in better performance and flock health.
3. Reduce the availability of antimicrobial products and their cost.
4. Provide the resources to adequately administer and enforce legislation and regulations.
5. Empower consumers with sound knowledge and science regarding how and why antibiotics are used and give them more choice, like audited standards etc.

One of the most exciting projects in the region looking at antimicrobial product use and control is the ViParc project in the Mekong Delta of Vietnam. ViParc stands for Vietnamese Platform for Antimicrobial Reduction in Chicken Production. (www.ViParc.org). It is a collaboration of researchers from the Oxford University Clinical Research Unit and is funded by the Welcome Trust. It specifically targets smaller village type commercial and semi commercial poultry farms in the Mekong Delta and aims at providing scientifically sound interventions to deal with the problem of high antibiotic use. An important component of the project is that it integrates socio-economic analyses of the use of antibiotics to provide insights as to why antibiotics are used and the cost-effectiveness of any proposed interventions (Carriique-Mas 2017).

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A REVIEW OF NOVEL BIOLOGICAL ALTERNATIVES TO ANTIBIOTIC THERAPIES IN POULTRY

A.M LEARY[1] and A. TURNEY[1]

Summary

As poultry producers in Australia continue to limit antibiotic use, increasing numbers of alternative therapies and chemical biosecurity products are being used to overcome intensive farming stressors. These interventions tend to be chemically based and can lead to resistance if used incorrectly. The current paper investigates natural alternatives to some of these interventions and discusses if nature can suggest a more sympathetic solution for the bird and the environment while remaining biologically effective.

I. INTRODUCTION

Antibiotic stewardship and the judicious use of antibiotics as treatment for specific disease occurrences, rather than using sub-therapeutic doses for growth promotion, are well established concepts within the Australian poultry industry (Alfirevich, 2018). Intensive production systems however, are prone to increased stressors and large scale production does not always meet the individual needs of each bird. Optimising production performance within this environment requires maintaining a healthy bird, which in turn is dependent on the bird having a healthy gut and immune system (Kogut et al., 2017).

Gut health is reliant on three critical areas: (i) the chicken's immunity; (ii) the diet fed to the chicken, whether feed meets the chicken's nutritional requirements, and how feed is manufactured, and (iii) the complexity and health of the chicken's commensal bacteria along with the transient and pathogenic bacteria in the gut (Kogut et al., 2017). However, a fourth area is also important – the litter the birds are raised on. Litter is critical because the microflora within the litter influence and are influenced by, the microflora in the chicken's digestive system (Diaz Carrasco et al., 2019). All of these factors will determine if the bird remains healthy. If one or more are disturbed, there is an increasing risk the bird's health will be affected (Kogut et al., 2017).

While the diet has been extensively studied and can be manufactured to meet the changing needs of the growing bird, the same control is not as easily achieved for the host physiology and microbiota. This paper will investigate how natural processes can be utilized in commercial situations to optimise the health of the chicken and reduce the risk of requiring antibiotic intervention.

II. COMPETITIVE EXCLUSION

Our understanding of the gut microbiota in humans and in chickens has increased dramatically in the past decade. We know that only 1% of DNA we carry is human with the rest attributed to the microbiota, now regarded as another organ of the human body (Conlon and Bird, 2015). The contribution of the microbiota and its effect on human, and chicken, physiology and health are only starting to be understood.

There is a vast list of factors that affect the viability and population of the chicken microbiome including age, breed, gut region, material factors, sex, feed, housing, hygiene, medication, temperature, litter and geographical location (Kers et al., 2018). However, the first

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and most critical question for commercial poultry is the origin of microflora within commercially hatched and reared birds?

Current poultry production, regardless of the production system, is a long way removed from what happens naturally (Kubasova et al., 2019). The most obvious example of this is how modern commercial eggs are brooded in sterile hatcheries rather than under the hen. There are many problems associated with the natural brooding process that limit performance of the chicks, leading to poorer welfare outcomes and higher mortality. However, there may also be some positives that can be adapted to commercial operations. For example, exposure to the hen while eggs are brooded, allows the eggs to be exposed to the hen's microflora, which in turn means the hatching chicks are also exposed to that microflora as they pip through the eggs. Ultimately this early exposure to a complex, adult hen microflora allows the chick to quickly develop a healthy gut microbiota encouraging improved gut health and immunity (Kubasova et al., 2019).

Application onto day old chicks of the full caecal microflora from a healthy adult bird (screened for potential pathogens) may be a solution to this problem. Spraying chicks at the hatchery, or on arrival at the farm, while they are preening gives similar exposure as pipping through the eggs in the natural environment. The backyard hens originally selected to donate their caecal contents were healthy, isolated, non-medicated mature hens. Further research has shown that applying the complex caecal microflora to day old chicks results in consistent resistance to colonisation by *Salmonella*, a range of pathogenic *E. coli* and necrotic enteritis (Methner et al., 2017; Ceccarelli et al., 2017; Hofacre et al., 2019). In fact the effectiveness of each batch of this caecal microflora product is tested using a biological model that challenges birds with *Salmonella* and only if a reduction is achieved of at least 99.97% in colonization, compared to a control, is the batch of product released for commercial use (Mead et al., 1989).

III. NUTRITIONAL ADDITIVES

Many options can be considered when optimising gut health, including phytogenics, probiotics, prebiotics, enzymes and acidifiers (Dittoe et al., 2018). Often the acidifiers used are organic acids which include lactic acid, acetic acid, propionic acid, formic acid and their associated salts (Pearlin et al., 2019). Acidification in broilers lowers gut pH resulting in improved gut morphology (villi height and crypt depth), localised antimicrobial effects as pathogenic bacteria tend to prefer neutral pH levels, and improved energy and protein digestibility of the diet. Additionally acidification in poultry has been shown to enhance the immune system (Pearlin et al., 2019).

There are, however, potential risks associated with use of organic acids in feed or water application. Using excessive levels of acids in the diet can decrease palatability of the diet and reduce feed intake. Additionally application of acids in feeding or drinking systems can cause corrosion of steel equipment (Pearlin et al., 2019). Finally natural tolerance or resistance to acidification has been suggested in some pathogens including *E. coli* (Ramirez Cuevas, 2009).

A possible alternative to this application is biological acidification. In this case the animal is given, through drinking water or feed, a specific bacterium, *Pediococcus acidilactici* MA 18/5M, which exclusively and quickly produces a large amount of lactic acid (L+) throughout the digestive system in the chicken (Murray et al., 1984). In this case acidification occurs within the lumen and mucosal brush border where the nutrients are being absorbed. Application with such a bacteria provides all the benefits of acidification, improved feed nutrient digestibility, local immune modulation, gut maturity and gut pH reduction, in a natural way (Larbier, 1997; Temim et al., 2009; Awaad et al, 2005).

IV. DEVELOPING AN ENVIRONMENTAL POSITIVE BIOFILM

Often in poultry production biofilms on housing surfaces or in water systems are discussed due to their negative influence on bird health (Abdelaty et al., 2019). Biofilms form when one or more microorganisms adhere to a surface and begin to colonise. As part of this colonization they produce an extracellular polymeric matrix that protects the microbes and allows them to grow and reproduce. This protection can also be effective against attempts to clean and sanitise surfaces in farm environments (Abdelaty et al., 2019). Studies have shown that, if pathogens are not protected by a biofilm matrix, they are easily susceptible to disinfectants. However, bacteria protected by a biofilm and treated with the same disinfectants can be 10 to 1000 times more resistant to disinfectants and antibiotics than isolated bacteria (Briandet et al., 2012; Cabecca et al., 2012). Commercially, producers attempt to control biofilms by cleaning with detergents and then disinfecting the housing and equipment surfaces. However, this reduces, but does not eliminate biofilms, which will rapidly regrow using remaining bacteria or opportunistic bacteria from within the housing (Abdelaty et al., 2019).

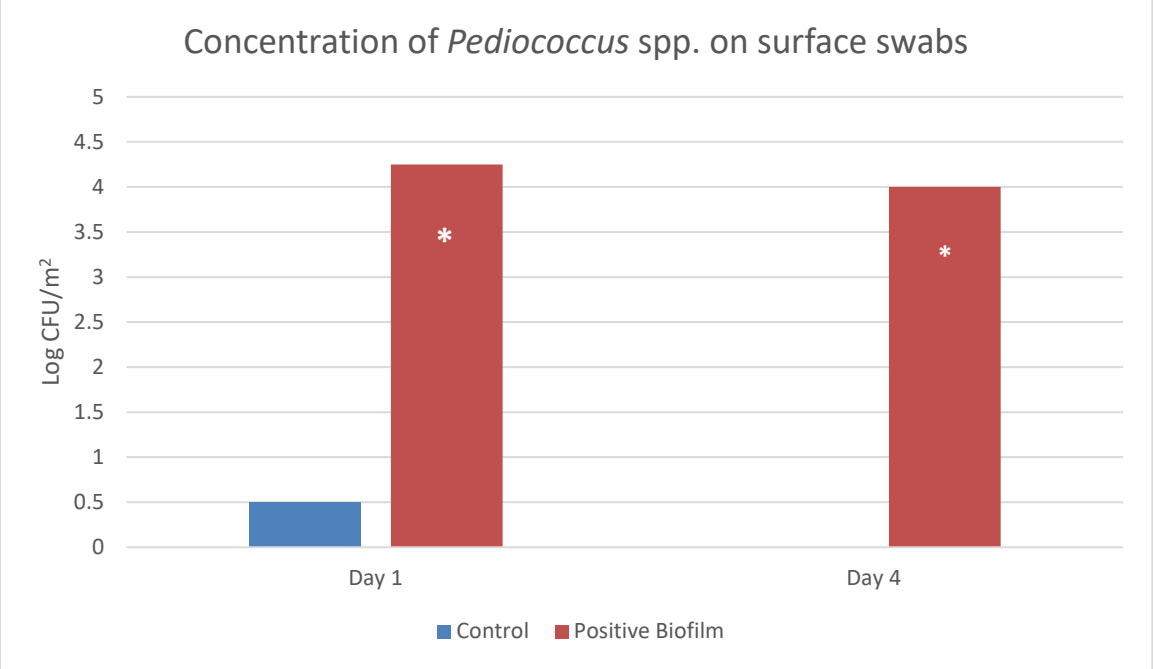

Figure 1- Evolution of beneficial bacteria populations in the sow farrowing room after application with a positive biofilm. Day 0 corresponds to the entry of the sows.

One method used to overcome the challenge associated with unknown biofilms is to populate the housing and equipment with a positive biofilm. By applying a specifically designed positive biofilm developed for its ability to adhere to farm building surfaces it is possible to create a protective biofilm of highly concentrated positive bacteria as selected *Pediococcus* species (Figure 1) (Turney et al., 2018). This will limit the opportunity for pathogenic or opportunistic bacteria to develop through competitive exclusion. While application with positive biofilms may not completely remove any negative bacteria, research has shown it is possible to reduce the areas of high and medium contamination (Figure 2) (Turney et al., 2018).

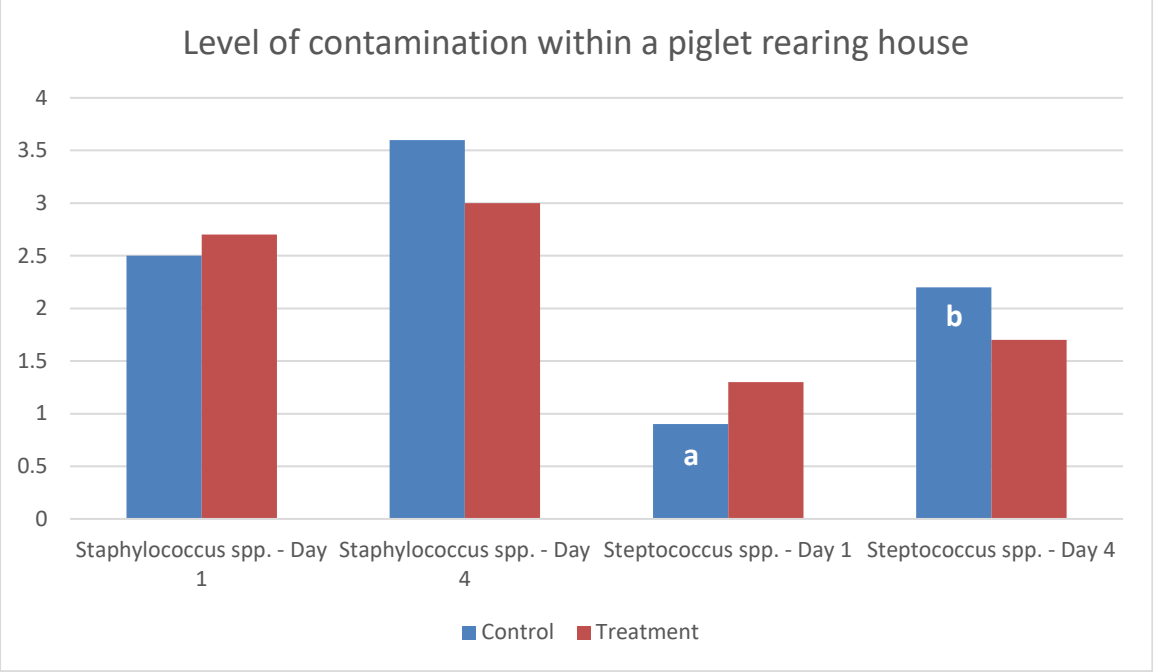

Figure 2 - Evolution of negative bacteria populations in the sow farrowing room after application with a positive biofilm. Day 0 corresponds to the entry of the sows.

V. MANURE MANAGEMENT

Management of litter and manure in poultry sheds is critical to animal health and welfare (Ritz et al., 2005). Indeed some welfare guidelines are very specific about the requirements of litter in broiler sheds to be dry and friable. Maintenance of litter quality in broiler sheds or layers on deep litter reduces humidity in the shed, improves air quality and allows better thermal insulation which ultimately produces higher performance from the birds (Ritz et al., 2005).

Using natural processes to optimize the quality of the litter is a novel solution to improving production performance. Types of actives that could be considered in this situation include a combination of enzymes with a positive bacterial consortium. The enzymes are used to breakdown complex bedding materials into fermentable sugars used as a nutrient source for the bacteria. The bacteria, in turn, positively ferment the organic litter reducing ammonia emissions and therefore improving odour in the shed, and increasing the growth of lactic acid bacteria, which competitively excludes pathogenic bacteria (Internal Lallemand Data, 2008). The result is an environment that is safer and lower in ammonia.

VI. CONCLUSION

Poultry producers have developed environments and processes that place many unnatural challenges on a chicken while still aiming for maximum performance. Stressors come from a variety of avenues and place considerable strain on birds at all life stages. Most conventional therapies revolve around “treatments” or cleaning as the solution and fail to work biologically with the animal in its production environment. There are a range of biological solutions that can be utilised in modern production systems to assist in managing the various stressors upon livestock. These include competitive exclusion, microbiological acidification and manure additives and positive biofilms on environmental surfaces. The pressure from consumers to reduce our reliance on antibiotic treatments and vaccines can be achieved by introducing more biological and natural based solutions to help solve conventional production challenges.

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POTENTIAL OF PLANT EXTRACT TO IMPROVE PERFORMANCE AND INTESTINAL HEALTH IN BROILERS DURING THE ONSET OF CLINICAL NECROTIC ENTERITIS

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Plants extracts (PE) have shown promising effects on performance and intestinal health in broilers. Their potential as antimicrobial (AM) alternatives has been studied for years (Adhikari *et al*., 2020). A feeding study was conducted to examine the effect of a micro-encapsulated product composed of eugenol and garlic tincture on growth performance, mortality, intestinal lesions, jejunal gene expressions, and histology in broilers during the onset of necrotic enteritis (NE). A total of 960 d-old Cobb 500 broiler chicks of mixed sex were assigned to 48-floor pens each stocked with 20 birds. A randomised complete block design was used with 6 treatments replicated 8 times. The treatments were: UC- unchallenged control; CC- challenged control; PE- challenged group plus PE (100 g/t feed); AM- challenged group plus AM (625 g/t; narasin and nicarbazin; 50 ppm); FAP- challenged group plus full dose of AM with PE; HAP- challenged group plus half dose of AM (312.5 g/t; narasin and nicarbazin; 25 ppm) with PE in starter, grower and finisher phases. Diets were based on wheat, sorghum, soybean meal, and meat and bone meal supplemented with phytase. Birds were challenged according to Rodgers *et al*. (2015) by giving 1 mL *per os* field strains of *Eimeria spp*. oocysts consisting of *E. acervulina* (5000), *E. maxima* (5000) and *E. brunetti* (2500) on d 9 and 1 mL *per os* approximately 10^8 CFU/mL of *Clostridium perfringens* (NE-18) on d 14. Mortality caused by NE was determined by necropsy. The sex of the birds was determined by high-resolution melting curve analysis (HRM) using feather crude DNA (England *et al*., 2020). Bird performance was measured from d 9 to 21(corrected to dead birds). Mortality (d 14 to 17), intestinal lesions, jejunal gene expressions (males and females), and histology (males) were measured on d 16. Data were subjected to one-way ANOVA analysis using JMP software.

Increased mortality (17.7%), reduced BWG and feed intake, and impaired FCR and higher intestinal lesions by the challenge ($P < 0.05$) indicate a successful clinical NE challenge. The treatments UC, AM, FAP, and HAP had higher BWG, lower mortality, and FCR, higher villus surface area (VSA), and goblet cell numbers (GCN) compared with CC ($P < 0.05$). Birds supplemented with PE had lower FCR compared with CC ($P < 0.05$). Birds fed PE had lower ileal lesions ($P < 0.05$), tended to lower jejunal lesions ($P = 0.066$) in male birds, and lower mortality (2.9%) compared with the CC group. The expressions of jejunal MUC2, OCLDN, CLDN1 and ZO1 were not different between PE group and different doses of AM groups in male and female birds ($P > 0.05$). Birds in PE group had higher GCN compared with CC ($P < 0.05$) and had similar VSA and GCN compared with HAP group ($P > 0.05$). These findings suggest that different combinations of a PE with antimicrobials were effective in alleviating the impact of clinical NE as indicated by improved performance and health traits. These results also demonstrated that PE supplementation in the diet helps to improve feed efficiency, reduce mortality and intestinal lesions, and increase GCN when birds are infected with NE.

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BUTYRATE FORMULATIONS THAT INCREASE CECAL BUTYRATE CONCENTRATIONS ARE SUPERIOR IN PROTECTING AGAINST SALMONELLA ENTERITIDIS COLONIZATION IN BROILERS

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Summary

The effect of different formulations of butyrate on hindgut butyrate concentration and caecal *Salmonella* load in broilers was investigated. Using commercial products and experimental prototypes, it was demonstrated that supplementing feed with butyrate embedded in a highly protective matrix, such as a wax-based carrier, can increase caecal butyrate concentration and reduce caecal *Salmonella* counts.

I. INTRODUCTION

Butyrate is a molecule that is extensively studied as a feed additive to improve gut health and animal performance. It also has been described to reduce the expression of colonizing genes in *Salmonella* (Gantois et al., 2006). When applied in an *in vivo Salmonella* challenge model, Van Immerseel and co-workers (2005) demonstrated that a fat matrix-protected butyrate, but not an unprotected butyrate, was able to reduce caecal *Salmonella* load and shedding.

Hypothesising that the difference in these effects was due to the distinct butyrate release profiles of both products in the gastro-intestinal tract (GIT) tract of animals, investigations were conducted into the relationships between butyrate formulations, the butyrate delivery characteristics in *in vitro* models of different GIT segments, the effect on caecal butyrate concentration *in vivo*, and the capacity of the different formulations to confer resistance to caecal *Salmonella* colonization *in vivo*.

II. METHOD

Different formulations of butyrate products, each with a distinct expected release profile, were investigated: uncoated sodium butyrate ('SB', absorption in proximal GIT) , tributyrin ('TB', small intestine), 30% fat-protected butyrate ('FPB', Adimix® Precision, Adisseo, France; slow release throughout entire GIT) and two prototypes with supposed superior hindgut release, with butyrate embedded in a micro-crystalline wax matrix: one contained 30% butyrate and 70% wax ('Wax'), while the other was composed of 30% butyrate, 60% wax and 10% soluble potato starch ('Wax+'). Addition of starch to the wax has a disintegrative effect, making the matrix less resistant, thereby influencing the release rate of butyrate.

After differences in butyrate release characteristics were confirmed in the *in vitro* models (data not shown), two *in vivo* trials were conducted. The first trial was set up to screen all the butyrate formulations. One pen of 20 animals was allocated to each of the 6 dietary treatments. The second trial was used to confirm the performance effects of the products that yielded the best results in the first trial. Two pens of 20 animals were allocated to 3 treatments: negative control, FPB, and Wax. These feed additives were included in the diets at 3 g of butyrate per kg of feed.

In each experiment, 17-day-old broilers where orally infected with 10^5 CFU of *S.* Enteritidis phage type 4 strain 147. Four days post-infection, birds were euthanised and caecal

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content was analysed for short-chain fatty acid (SCFA) concentrations and for *Salmonella* load. For SCFA quantification, acetate, propionate and butyrate were extracted with diethyl-ether and analyzed on a gas chromatograph coupled with a flame-ionization detector. Bacterial analysis was performed by homogenising the caecal samples and plating a ten-fold dilution series on streptomycin-supplemented XLD plates. After incubation overnight at 37 °C, the number of colonies was determined and numbers of CFU/g calculated. Samples that were negative after direct plating were enriched in BGT broth at 37 °C overnight and plated. When found to be positive after this step, these samples were presumed to have 83 CFU/g (detection limit of direct plating).

Caecal microbial analysis was performed via 16S rRNA sequencing using MiSeq v2 technology from Illumina. Caecal content was collected from the 21-day-old chickens in the first trial 4 days after *Salmonella* infection. DNA was extracted from the caecal content, and relative abundances were determined.

Data from the first trial were analyzed with a Kruskal–Wallis test. All pairwise differences between the treatments were assessed using Behrens Fisher tests (Munzel & Hothorn, 2001). The data from the second trial and the SCFA measurements were assessed by one-way ANOVA. To determine statistical differences in relative abundances of the bacterial families, non-parametric Kruskal–Wallis test was used.

III. RESULTS

In the first trial, the lowest mean caecal *Salmonella* counts were observed in birds fed the Wax treatment ($P < 0.05$ compared to the control) (Table 1). Birds fed Wax, Wax+ and FPB were also found to be negative for *Salmonella* infection strain, unlike those fed the other dietary treatments (Table 1).

Table 1 – Colonization of caecum by *Salmonella* Enteriditis strain 147 in the first trial

		Control	SB	TB	FPB	Wax+	Wax
% of animals with specific infection level	Neg. after enrichment	0	0	0	16	20	47
	Pos. after enrichment	15	20	40	26	40	21
	$10^2 – 10^4$ log CFU/g	40	50	40	47	0	16
	$> 10^4$ log CFU/g	45	30	40	11	40	16
Mean log CFU/g		3.63^a	3.45^a	2.81^{ab}	2.56^{ab}	2.87^{ab}	1.56^b

Percentage of animals (n=19 for FPB and Wax, n = 20 for other groups) with a specific infection level. Log numbers of CFU per gram caecum content. Mean log CFU/g cecum values are shown at 4 dpi. Significant differences among groups are indicated with different letters.

Similar observations were detected in the second experiment: compared to the control group, Wax-fed birds had a lower mean *Salmonella* count ($P < 0.05$) (Table 2).

Table 2 – Colonization of caecum by *Salmonella* Enteriditis strain 147 in the second trial

		Control	FPB	Wax
% of animals with specific infection level	Neg. after enrichment	0	0	0
	Pos. after enrichment	15	35	68
	$10^2 – 10^4$ log CFU/g	46	55	30
	$> 10^4$ log CFU/g	38	10	3
Mean log CFU/g		3.64^{a}	2.89^{ab}	2.40^{b}

Percentage of animals (n=19 for FPB and Wax, n = 20 for other groups) with a specific infection level. Log numbers of CFU per gram caecum content. Mean log CFU/g cecum values are shown at 4 dpi. Significant differences among groups are indicated with different letters.

In the first experiment, total SCFA and butyrate concentration was numerically higher in birds fed the FPB, Wax+ and Wax treatments compared to those fed the control treatment. The relative abundance of butyrate, as percentage of the total SCFA concentration, was significantly higher in the Wax-fed group ($P < 0.05$), compared to the control (Table 3).

Table 3 – Concentrations of caecal SCFAs in the first trial

	Control	SB	TB	FPB	Wax+	Wax
Total SCFA (mM)	63.18^{ab}	63.34^{ab}	54.15^{b}	71.09^{a}	67.96^{ab}	52.84^{ab}
Butyrate (mM)	9.38	9.22	9.23	13.00	12.37	12.93
% Butyrate/total SCFA	14.95^{c}	14.81^{c}	16.41^{bc}	17.93^{bc}	18.26^{b}	25.15^{a}

Significant differences among groups are indicated with different letters.

SCFA analysis in the second trial yielded similar findings: both the absolute and relative butyrate concentration were higher in the FPB and Wax-fed groups compared to the control fed group (Table 4).

Table 4 – Concentrations of caecal SCFAs in the second trial

	Control	FPB	Wax
Total SCFA (mM)	57.29^{ab}	67.27^{a}	49.67^{b}
Butyrate (mM)	8.48^{a}	12.45^{b}	13.45^{b}
% Butyrate/total SCFA	15.02^{a}	18.39^{b}	27.16^{c}

Significant differences among groups are indicated with different letters.

Interestingly, supplementation of wax- and fat-coated butyrate was linked to changes in caecal microbial composition; for example, an increased prevalence of butyrate-producing Lachnospiraceae and Ruminococcaceae, and a reduced abundance of Enterobacteriaceae and Lactobacillaceae, was detected in birds fed these treatments (Table 5).

Table 5 – Relative abundance of the most prevalent bacterial families

	Control	FPB	Wax
Lachnospiraceae	52.75[a]	50.68[a]	62.48[b]
Ruminococcaceae	18.38[ab]	24.15[b]	13.85[a]
Lachn. + Ruminococc.	*71.13*	*74.83*	*76.33*
Lactobacillaceae	10.22[a]	7.54[ab]	3.82[b]
Streptococcaceae	7.91	7.96	8.34
Vadin BB60	1.14b	2.44[ab]	3.62[a]
Enterobacteriaceae	1.80[a]	0.29[b]	0.54[b]
other	7.80[a]	6.94[b]	7.35[b]

Significant differences among groups are indicated with different letters.

IV. DISCUSSION

These results demonstrate that there is a correlation between delay in butyrate release, increased hindgut butyrate concentrations and reduction in caecal *Salmonella* numbers. More specifically, the data suggest that dietary supplemented butyrate needs to be protected in order to increase caecal butyrate concentrations and protect broilers against *Salmonella* Enteritidis.

Of note, microbial analysis suggests that at least part of the elevated caecal butyrate concentration in the protected butyrate-fed broilers is the result of shifts in caecal microbial composition. Other studies have been published indicating that butyrate supplementation can influence caecal microbiota composition of challenged birds in a way that seems beneficial for health and growth (Zhou *et al*., 2017; Bortoluzzi *et al*., 2018). However, the exact mechanisms underlying these effects remain to be elucidated.

In addition, this study suggests that novel butyrate formulations can be developed with improved efficacy against zoonotic pathogens residing in the hindgut of broilers.

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SUPPLEMENTATION OF A PROTECTED COMPLEX OF BIOFACTORS AND ANTIOXIDANTS ON THE GENE EXPRESSION AND KINOMICS IN BROILER CHICKENS UNDERGOING EARLY LIFE STRESS

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Summary

The objective of this study was to evaluate the dietary supplementation of a protected complex of biofactors and antioxidants P(BF+AOx) on the expression of immune-related genes, and immune and metabolic changes in the intestine and liver of broilers submitted to early life stressors simulated by the combination of a double Infectious Bronchitis (IB) vaccination followed by a temperature stress in their early life. The treatments consisted of feed supplemented with or without P(BF+AOx) from 1 to 14 d of age. Birds were double vaccinated against IB at the hatchery (d 0) and exposed, on d 3, to an acute cold stress with a temperature drop from 32°C to 20-23°C for 48 h. On d 7 and 15, samples of jejunum, ileum, and liver were collected for expression of immune-related genes and kinome array analysis (analysis of kinases). Overall, the supplementation improved growth performance from 1 to 35 d of age. The kinome data functionally agreed with the gene expression and antioxidant results and indicate a general anti-inflammatory and antioxidant response in birds fed the P(BF+AOx) additive. It can be proposed that under early life stress conditions, the supplementation of P(BF+AOx) improves growth performance by modulating the inflammatory and antioxidant response of the host.

I. INTRODUCTION

Early life stress may have a negative impact on the performance of broiler flocks. It has been observed that a severe cold stress (12ºC below the normal temperature) throughout the life cycle of broilers negatively impacts production parameters (Su et al., 2020) and immune system development (Zhao et al., 2013). The use of vitamins and other molecules with antioxidant properties have been investigated in poultry undergoing different challenge models (Ghazi Harsini et al., 2012; Hu et al., 2020). An imbalance between the pro-oxidant and antioxidant systems of the body is likely to drive damage to cellular components (Estévez, 2015), including immune cells, that may impair immune responses. The dietary supplementation of antioxidants and vitamins may improve growth performance and has the function of protecting the cells from reactive oxygen species (ROS; Ghazi Harsini et al., 2012) that are naturally generated due to the immune response or exacerbated in stress situations. Additionally, the supplementation of antioxidant vitamins and biofactors may induce immune-metabolic alterations in different tissues that could help the birds to cope with early life stressors. We hypothesised that the supplementation of different antioxidant molecules through feed would alleviate the negative effects of early life stress in broiler chickens by modulating the immune and antioxidant systems. Therefore, we evaluated the dietary supplementation of a protected complex of biofactors and antioxidants P(BF+AOx), which is a protected source of vitamins

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and fermentation extract, on the expression of immune-related genes, and immunometabolic phenotypic changes in the intestine and liver of broilers submitted to early life stressors simulated by the combination of a double Infectious Bronchitis (IB) vaccination followed by a temperature stress in their early life.

II. MATERIALS AND METHODS

The experiment consisted of two treatments: feed supplemented with or without P(BF+AOx) (Jefo Nutrition Inc., Canada) from 1 to 14 d of age, distributed to a total of 720 one-day old male Ross 308 chickens placed in pens of 30 birds (12 replicates per treatment). When the period of supplementation ended (d 14), all the birds were fed the control diet (without supplementation) until d 35. The P(BF+AOx) is a complex of vitamins and fermentation extract microencapsulated in a matrix of triglycerides from hydrogenated vegetable oil (Jefo Nutrition Inc., Canada). Birds were double vaccinated against IB (MILDVAC-Ma5^{TM}) by spray at the hatchery (d 0) and submitted, on d 3, to an acute cold stress with a temperature drop from 32°C to 20-23°C during 48 h. Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were calculated weekly. On d 7 and 15, one bird/pen was euthanised, and samples of jejunum, ileum, and liver were collected for expression of immune-related genes and kinome array analysis (Kogut and Arsenault, 2015). All the data were analyzed by ANOVA using the software SAS (SAS 9.4; $P < 0.05$).

III. RESULTS AND DISCUSSION

The dietary supplementation of P(BF+AOx) improved the growth performance of the birds on d 21, 28, and 35 (Bortoluzzi et al., 2020). Additionally, on day 7, it was observed that the dietary supplementation upregulated the expression of IL-6 ($P = 0.03$) in the liver. On d 15, the dietary supplementation upregulated the expression of IL-6 in the ileum ($P = 0.04$) and IL-10 in the liver ($P = 0.001$) and tended to upregulate IL-6 in the liver ($P = 0.08$). The results of the kinome peptide array performed on jejunum and liver tissues showed that the treatment had significant effect on oxidative stress resistance on d 15. Catalase was activated via decreased phosphorylation, and the phosphorylation of immunoregulatory or proinflammatory proteins was decreased. We also observed that the supplementation promoted a decrease in the activation of proinflammatory proteins in the FOXO (Forkhead box protein O) pathway, including the transcriptional regulator nuclear factor-kB (NF- kB), and increased phosphorylation of IL-6R and TGF-beta receptor on their active sites. These results, along with increased IL-10 expression in the liver, may be an indication of reduced inflammation or anti-inflammatory responses. In conclusion, the supplementation of P(BF+AOx) improved the growth performance of broiler chickens undergoing early life stress. Further analyses presented herein demonstrated that this novel feed additive was beneficial in modulating the immune and antioxidant defense systems of the birds. Overall, the kinome data functionally agreed with the gene expression and antioxidant results and indicate a general anti-inflammatory and antioxidant response in birds supplemented with P(BF+AOx).

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INTESTINAL HEALTH AND IMMUNE RESPONSE OF BROILER CHICKENS SUPPLEMENTED WITH *BACILLUS SUBTILIS* DSM 29784 IN NECROTIC ENTERITIS EXPERIMENTAL MODEL

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Clostridium perfringens (Cp) strains expressing NetB toxin are the definitive cause of necrotic enteritis (NE) in broiler chickens. Nevertheless, predisposing factors, such as *Eimeria* infection, are necessary to change the environmental conditions leading to increases in the abundance of Cp (Moore, 2016). With the increase in regulations regarding the use of antibiotic growth promoters (AGP), NE became a re-emergent disease. So, the quest for AGP alternative products or approaches has intensified in recent years and probiotics appear to be a reliable solution. The aim of this study was to evaluate the effect of a probiotic strain, *Bacillus subtilis* DSM 29784 (Bs29784), and an AGP (enramycin) on the intestinal mucosa histology and immune response of broilers challenged with Cp and *Eimeria* spp.

A total of 240 male Cobb 500 broilers were allocated in a completely randomized design to 6 treatments (2x3 factorial with 4 replicates of 10 birds per treatment): NC, non-challenged birds and no additive in the feed; NCBS, non-challenged birds receiving Bs29784 in feed; NCAGP, non-challenged birds receiving enramycin in feed; CH, challenged birds and no feed additive in feed; CHBS, challenged birds receiving Bs29784 in feed; CHAGP, challenged birds receiving enramycin in the feed. The challenged birds received 15X the recommended dose of a commercial *Eimeria* vaccine at day 1 (d1) followed by 3 consecutive inoculations by gavage of 10^8 CFU of Cp/mL at d10, d11 and d12. Performance parameters (FI, BWG, FCR) were recorded weekly. At d7, d14 and d21, six birds per treatment were necropsied to collect ileum samples for histopathological and an immunochemistry analysis termed the "I see inside" (ISI) methodology (Kraieski et al., 2017). Data were analyzed using ANOVA and means were compared by Tukey's test at 5% probability.

The challenged birds (CH, CHBS, CHAGP groups) had lower FI at d14 and d21, and lower BWG and FCR at all ages. Higher microscopic alterations were also observed in the ileum for all challenged birds at d7, d14 and d21, represented by a higher ISI score ($P < 0.05$) when comparing to non-challenged groups.

Bs29784 decreased the ISI total score in the ileum of challenged chickens at d7 and d14 ($P < 0.001$) compared to CH and to the enramycin group. At d7, CH had a significantly higher total ISI score (7.62) compared to CHBS (6.47), NC (5.69) and NCBS (5.22) due to a higher lamina propria thickness. This could explain why the non-challenged groups had better BWG than all the other groups. At d14, performance (BWG and FCR) was negatively correlated to ISI score. The presence of *Eimeria* oocysts is expected to be high at d14, correlating with parameters like proliferation of enterocytes, lamina propria thickness, epithelial thickness, inflammatory cell of the lamina propria, and goblet cells proliferation being lower in the challenge groups at this time. No difference of ISI total score was observed between the CH and CHAGP groups ($P > 0.05$). Thus, it is suggested that Bs29784 may enable improved health of meat chickens under NE challenge.

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MOLECULAR DETECTION OF *EIMERIA* SPECIES AND *CLOSTRIDIUM PERFRINGENS* IN POULTRY DUST AND POOLED EXCRETA OF COMMERCIAL BROILER CHICKEN FLOCKS

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Necrotic enteritis (NE) and coccidiosis are the two major economically important enteric diseases of commercial broiler chickens. Coccidiosis predisposes birds to NE by causing physical damage to the gut epithelium and triggering inflammation and immunosuppression which enhances the growth and proliferation of pathogenic *Clostridium perfringens* (Park et al., 2008). This study was designed to investigate the association of flock performance and genome copies (GC) of five *Eimeria* species *(E. acervulina, E. brunetti, E. maxima, E. necatrix* and *E. tenella), C. perfringens* and the associated *netB* toxin plasmid in poultry dust and pooled excreta collected from the floor of commercial broiler flocks. Poultry house dust and pooled excreta from the floor were collected weekly from two Australian integrator companies at days 7, 14, 21, 28 and 35 from 8 farms (n = 2 flocks/farm). Nucleic acids were extracted from dust and excreta using commercial kits with an enhanced lysis step using bead beating to open the oocysts. The farms were ranked as low or high performers based on performance indexes used by the integrator companies. It was hypothesised that (a) *Eimeria* spp, *C. perfringens* and *netB* GC load would be higher in low-performance farms compared to high-performance farms, and (b) The GC levels of *C. perfringens*, *netB* and *Eimeria* spp in poultry dust and pooled excreta would have high agreement. The results showed that there was no association of *Eimeria* spp, *C. perfringens* and *netB* GC load with the production performance contrary to our first hypothesis. This is most likely because the pathogen GC load detected was low to moderate in all studied flocks regardless of ranking. All farms were positive for *C. perfringens, E. acervulina* and *E. maxima* but negative for *E. tenella* and *E. necatrix*. *Eimeria brunetti* was only detected in one low-performance farm while *netB* GC was detected only in one high-performance farm. The agreement of GC levels for dust and pooled excreta was excellent for *E. maxima* [Intraclass correlation (ICC) = 0.99], *E. acervulina* (ICC = 1.00), and *netB* (ICC = 0.98), while there was a poor agreement for *C. perfringens* (ICC = 0.08). In this study, the presence of these pathogens was not directly linked with farm performance suggesting that other factors influenced the production outcomes. Further studies on a larger number of farms are needed to determine whether these population level measurements of key pathogens based on PCR detection of nucleic acids are correlated with performance variables and to investigate other factors associated with production performance in commercial broiler farms.

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GUT HEALTH IN POULTRY PRODUCTION: HOLISTIC APPROACH LEADING TO NOVEL INSIGHTS IN 2021

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Summary

The impressive genetic improvement of broiler growth rate has enabled the poultry industry to meet with a worldwide increased demand for poultry meat in a cost effective and raw material sparing way. The main driver of this improvement is a higher feed intake for a given weight, that allows a faster growth rate, thus reduced slaughter age for a desired weight. This improvement in feed intake behaviour continues to challenge the digestive capacity of the intact gut, although genetic selection has been giving more attention in balancing out growth rates with physiologic capacities, not only on the intestinal level but also on the respiratory and locomotory systems even in challenged environments. Still, these selection programs cannot simulate all field conditions and, in practice, gut health issues in broiler production have become over the last two decades, if seen from a global perspective, the most important disease entity in terms of economic impact.

Therefore, it is essential to create a healthy intestine from the moment of hatch and to maintain optimal gut functions throughout the whole growth period to limit the impact of enteric diseases, such as coccidiosis, viral enteritis (such as avian reovirus ARV) and necrotic enteritis (NE). Any factor that compromises the integrity of the intestinal mucosa and its bacterial community will lead to decreased nutrient absorption. Additionally, activation of immune system and repair processes will cost valuable nutrients and energy that will impair nutrient utilisation. Together, this reduced absorption and utilisation lead to a suboptimal growth rate and deteriorating Feed Conversion Rate (FCR). An altered microbiota-gut-brain axis (MGBA) threshold of negative feedback for feed intake when there is a gut health issue, seems to lead to a continuation of feed intake, even when microbiota and host changes would suggest broilers to reduce their feed intake to avoid a further disruption of gut physiology. Altogether, the occurrence of dysbiosis after the above-mentioned instigators leads to a complex vicious circle of broiler Bacterial Enteritis (BE).

Because of the risk of antimicrobial resistance connected with consumer concern for animal welfare and food safety, pressure on the usage of antimicrobials has caused intensive investigations to find alternative solutions to develop a healthy digestive system in animals without extensive use of ("growth promoting") antibiotics. Management changes and different alternatives for antibiotics such as probiotics, prebiotics, phytogenic products, organic acids and enzymes have been investigated to improve intestinal health and general animal performance. Unfortunately, many of these tools have difficulties to convince general practice as it has proven hard to understand how these tools work, in what conditions, and at what doses, where additive and/or synergistic effects with others can be expected and how the return of investment can be maximized, with antimicrobial growth promotors for many being the reference still.

Diagnosing BE has proven to be a real challenge due to the absence of pathognomonic signs at necropsy but a scoring system based on a-specific signs has been validated by using histologic parameters. Focus is now shifting to (non-invasive) biomarkers and this is expected to further facilitate research on BE.

Recently, models to investigate BE have been developed, and it is expected these models will support the understanding of factors influencing BE and thus facilitate the further finetuning of diagnostics and mitigation strategies.

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A holistic approach to gut health is to look at the whole, not just focussing on enteric diseases, but working on all contributing factors and considering the complex interactions between different drivers of gut health: pathogens, feed, microbiota and host immunity.

I. DEVELOPMENT OF A BALANCED GUT MICROBIOTA ECOSYSTEM AND THE COMPLEX HOST –MICROBIOTA INTERACTION.

In the past, investigation of the intestinal bacterial population has been done with in vitro culture techniques. These techniques are able to assess only those bacterial species that can be grown in different media in laboratory conditions. Modern approaches using molecular techniques were able to show that a significant part of intestinal microbiota was not properly assessed. Any conclusions regarding the composition of intestinal microbiota and its functions must be drawn very carefully. Use of modern molecular techniques has led to a better understanding of the role of microbiota in oral tolerance and physiological functions of a healthy gut and has helped a lot to disqualify simplistic views such as the existing of 'good' *Lactobacillus* spp. and 'bad' *Clostridium* spp. bacteria.

Nowadays, microbiota is considered as a gene toolbox that is complementing the gene pool of the host. The research is focusing on unravelling the complex interactions of what kind of gene pool is linked with good gut health and understanding how genes, both from the gut and its microbiota, can be switched on and off with different diet types, in order to reach the best performance: lowest level of inflammation, best digestive and absorptive properties, What we know, so far, is that a composition of intestinal microbiota is changing throughout the life cycle of an individual, becoming quantitatively and qualitatively more complex with age. Also, environmental factors such as stocking density, diet composition and feeding practice, management, housing conditions, pathogen load in the environment, use of antibiotics can modify intestinal microbiota. Feed withdrawal, especially over a longer time, causes reduction in the number of detected bacterial species. Also, from one segment of the gastro-intestinal tract to another, bacterial populations of the gut vary significantly. In the small intestine of a healthy bird, *Lactobacillaceae spp*. are dominant, whereas in caeca *Clostrideaceae spp.* are prevailing, which is connected with different pH and physiologic functions of these intestinal segments.

There are some members of the mucosa-associated microbial community that are considered to be especially crucial for a healthy status of the gut such as *Ruminococcaceae* and *Lachnospiraceae*. Bacteria producing short chain fatty acids, like acetic, propionic and butyric acid, during the fermentation process of dietary carbohydrates are considered supportive for a good gut health. Production of butyrate near the epithelial cells and in close association with potentially invading and histotoxic pathogens promotes development and recovery of the villi, stimulates the expression of the tight junction proteins, limits invasion of potential pathogens such as *E. coli* and *Salmonella* spp. and further promotes a beneficial microbial ecosystem, which leads to an overall increased tissue health. On the contrary, mucin-desulphating bacteria and sulphate reducers create hydrogen sulphide, which enhances some pathogens and causes tissue damage. Lipopolysaccharide (LPS) containing Enterobacteriaceae are generally considered as linked with negative gut health status.

When judging feed composition, management actions, additives, anticoccidial, antiviral or other strategies that aim to reduce (the impact of) BE, assessing intestinal microbiome profiling can now be performed more easily for instance by next-generation sequencing of 16S ribosomal DNA. As important, also the host reaction to microbiota changes can now be more easily investigated for instance by evaluating mRNA expression of expression of genes involved in the mitogen-activated protein kinase pathway.

II. VICIOUS CIRCLE OF BACTERIAL ENTERITIS

Since the ban of antimicrobial growth promoters in Europe in 2006, broiler bacterial enteritis (BE) issues have increased in many production units. The aetiology of BE is multifactorial. In modern broiler breeds, selected for maximal growth rate and high feed intake, abundance of non-absorbed nutrients in the gut lumen, in absence of growth promoters with antibacterial properties, causes a chain of events that exacerbates the proliferation of some clusters of bacteria that leads to an inflammatory reaction of the gut wall. This reaction of the gut wall in turn instigates microscopic and macroscopic changes that, as in a vicious circle, will lead to poorer physiologic status of the intestine and to poor digestive and absorptive functions, resulting in even more nutrients in the intestinal lumen, and more substrate for bacterial growth. These macroscopic signs have led to a scoring system that has the advantage it can be used in field conditions with immediate feedback to veterinarians in terms of treatment, versus histology that typically would require a long time for conclusions. On top of this, the macroscopic system for BE can be combined with coccidiosis lesion scoring. Still, innovation on diagnostics is expected to yield within the next couple of years of biomarker-based tests that can support and facilitate diagnosis in field conditions (De Meyer, 2019).

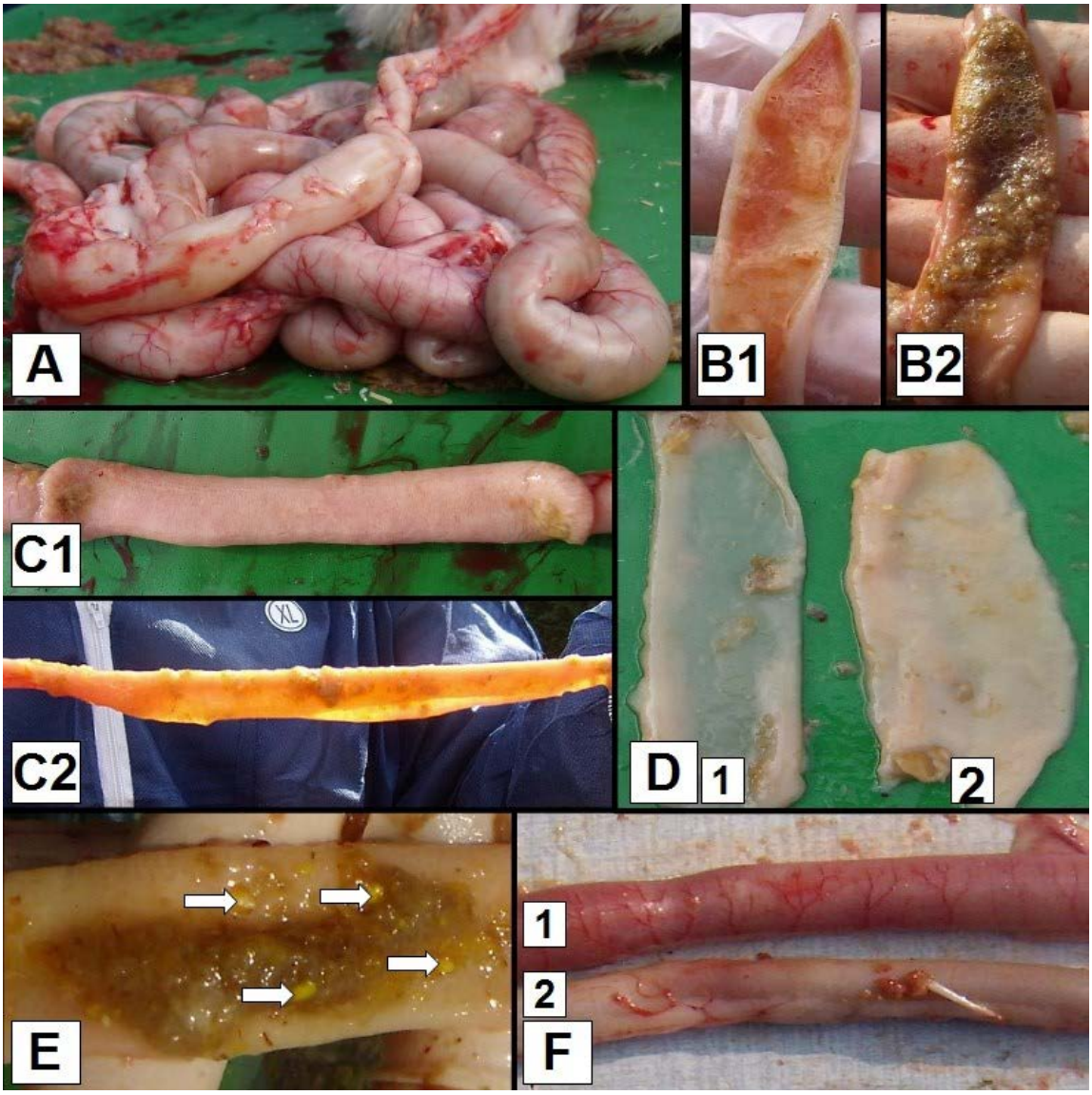

Figure 1 **-** (Maarten De Gussem, 2010). Macroscopic dysbacteriosis score system parameters. A. Overall gut ballooning; B. Content of the intestinal tract, 1. Mucoid, orange intestinal content, 2. Foamy intestinal content; C. Tonus of the intestinal tract, 1. Good tonus, 2. Lack of tonus; D. Macroscopically visible thickness of the intestinal tract, 1. Macroscopically thin intestinal tract, 2. Intestinal tract with normal thickness; E. Undigested particles in the colon (arrows); F. Inflammation of the gut, 1. Inflammation, 2. No inflammation.

The pathogenesis of BE can be described as a vicious circle in 4 steps:
In the first step, the shift of the healthy gut towards BE starts with oversupply of nutrients in the intestinal lumen. In today's broiler, the very high feed intake has accelerated the general feed passage rate in the intestine. So, even minor violations of the digestion and absorption will lead to an increase of the number of nutrients, especially of undigested proteins and high-energy nutrient particles in the hind gut. Among gut damaging factors (often of infectious origin), the so-called BE-instigators (BEI), coccidiosis is considered to be the most important, but also virus infections such as Reo can destroy intestinal epithelia, shorten intestinal villi and lead to poor absorption of the intestine. The stressors of non-infectious origin are dietary changes, nutritional imbalance for instance on crude protein level, soluble non-starch polysaccharides (NSP), enzymatic dysfunctions, mycotoxins and management issues.

As a consequence of the oversupply of nutrients in the intestinal lumen, a shift in proliferation of some clusters of bacteria occurs in the small intestine in step 2 of the vicious circle. The presence of excessive nutritional factors mainly favours the proliferation of *Clostridium perfringens* and *Lactobacilli* and disfavours certain Clostridiales such as Ruminococcaceae and Lachnospiraceae.

In step 3, this disruption of the delicate balance in gut microbial constellation, in an already damaged (by BEI) gut, shifts intestinal immune tolerance towards pathological inflammation reactions and oxidative stress in the gut wall, so morphological and functional alterations in the intestine occur. In case of overgrowth of *Clostridium perfringens spp.* producing Netβ toxin, the two first steps are very similar but these alterations result in necrotic enteritis, with a further toxin - and host related destruction of the intestinal lining.

Step 4 of the vicious circle of BE is characterised by poor digestion of feed and poor absorption of nutrients, as the damaged intestine is not able to fulfil its functions while the feed intake continues to be higher than what can be digested and absorbed due to the poor activation of MGBA negative feedback on feed intake in case of inflammation.

The above described 4 steps of the vicious circle result in a less functioning gut, which in turn leads to further oversupply of nutrients in the intestinal lumen, further reinforcing the vicious circle of BE. On a flock level, there will be flattening of the feed intake, so there will be birds that face a MGBA activation leading to lower feed intake, but that comes usually too late as these birds have already tumbled into the vicious circle. Mostly these flocks will have a further increase of water intake, so this typically leads to increased water/feed ratio (and often related wet litter issues). What makes a vicious circle vicious is the poor response of acting on one of the steps of the circle only. A combined action focusing on breaking the circle at the four steps simultaneously is more successful. A good example is the use of antibiotics (such as AGP or therapeutics) to break the second step. If continuously administered, antibiotics do control BE (in feed AGP) but the moment they are withdrawn, the circle regains immediately traction (therapeutics).

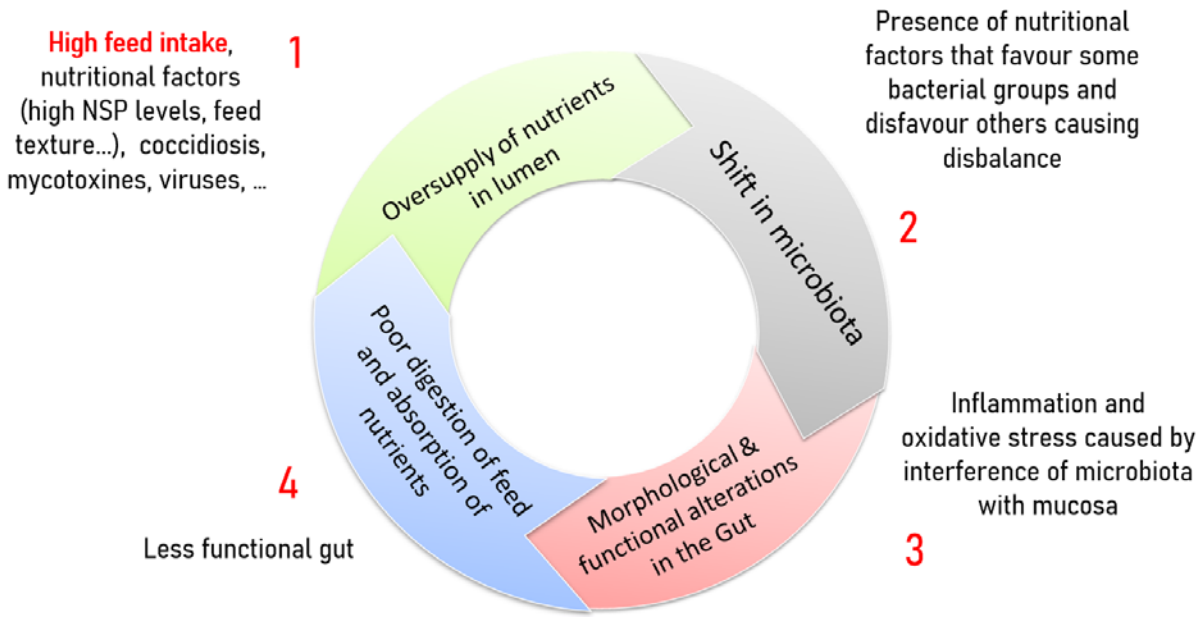

Figure 2- Bacterial Enteritis Vicious Circle (De Gussem, 2010)

III. ROLE OF INSTIGATORS – FROM LOW HANGING FRUIT ON COCCIDIOSIS TO NOVEL INSIGHTS ON VIRAL CHALLENGES

Traditionally, coccidiosis has been the low hanging fruit on preventing BE issues. *Eimeria* spp. infections in broilers have been very well studied and there are a number of novel strategies that have been developed over the last decades in order to cope with the direct and indirect consequences of broiler coccidiosis. In an earlier review (De Gussem, 2007), 0.1€ per 2.5 kg broiler has been put forward as an average loss from subclinical coccidiosis (due to higher FCR and lower ADG). Recently, Blake et al. (2020) calculated an even higher amount but it is clear that together with BE (also estimated at an average 0.1€ cost per bird), coccidiosis is the most costly gut health entity in global poultry production and together these two disease entities are estimated for a loss of over 10B€ yearly.

Novel strategies include restoration of efficacy of in-feed anticoccidials by resting them and rotating to other product and product classes, but also by rotating with live vaccines (consisting of sensitive strains) that have the advantage of accelerating the restoration of sensitivity. The effects on performance but also on BE related treatments have been demonstrated in several trials (Figure 3), confirming the intimate relation between the two diseases.

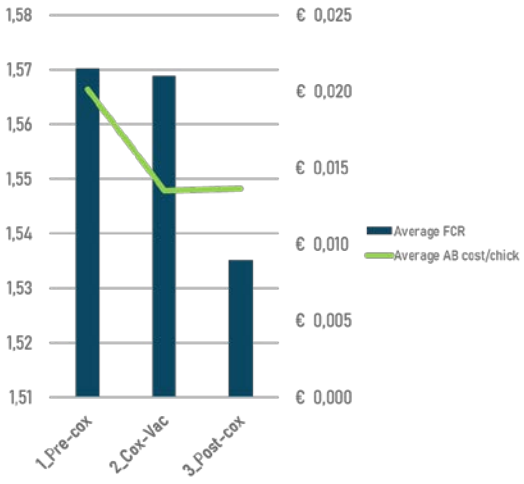

Figure 3 - Impact of live coccidiosis vaccination campaign on performance and Bacterial Enteritis antibiotic use based on data of 113 flocks of 9 different farms.

On the viral side, novel insights are helping to develop new strategies to cope with BE without having to give on performance. In a recent survey (not published) in Belgium, the majority of strains isolated from broiler and breeder farms are categorised after sequencing into Cluster 2 and Cluster 4. Surprisingly the third most abundant cluster retrieved was Cluster 1, although breeder flocks in Belgium are most commonly vaccinated with Cluster 1 vaccines. These findings have led to the insight that vaccines that consist of an older strain isolated in 1973 most probably have led to escape not only of genetically distant strains from other clusters (which would be expected) but also of Cluster 1 strains that are genetically different from the vaccine strains. This kind of antigenic drifting is now leading to redesign of vaccination programs to include also autogenous vaccine strains that are tailor made to the farms concerned. For this strategy, the most pathogenic strains from these farms, as defined in virulence typing work, are included.

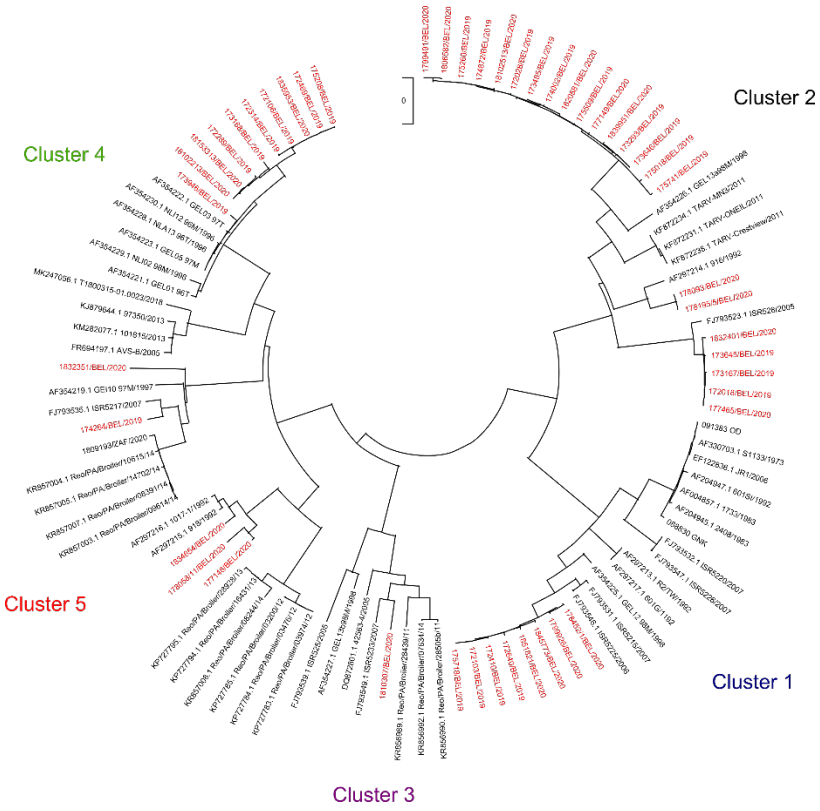

Figure 4 - Cluster analyses of strains isolated in Belgium of ARV in 2019 and 2020 indicated in red.

IV. STRATEGIC USE OF ADDITIVES AND MEDICATION: TRADITIONAL VERSUS ALTERNATIVES?

The choice of best (so called traditional and alternative) solutions should be tailor-made for each operation, looking at the 4 steps of the vicious circle and what contributing factors are causing damage to intestinal health. Solutions work on different parts of the BE circle. Some products have antagonistic, some additive and some have a synergistic effect. Some products will go through the drinking water, some will go through feed or can be sprayed on the chicks or even in the environment.

Examples of traditional additives are antimicrobial growth promoters, anticoccidials, feed enzymes, antibiotics, coccidiosis vaccines, acids used as feed preservative, mycotoxin binders. Examples of alternatives are acids used to steer gut microbiota, probiotics, etheric oils, bacteriophages, beta-glucans. In fact, the differentiation is very artificial, and the ones that are

alternative now will be standard in few years' time. Therefore, they should all just be categorised as gut health support tools.

The objective of the poultry industry is to define what tools are relevant in terms of efficacy and cost for your operation with best return on investment (ROI). It all starts with understanding where the BE circle gets fuelled. Usually, it will be a complex combination of challenges, but with a good starting diagnosis of flocks one will understand where is the low hanging fruit. Coccidiosis for instance is very often the main primary instigator of the BE circle. The levels at a given farm can be very different from neighbouring farms and understanding this can help to define what impact improved coccidiosis control can have on BE or, if well controlled, if resources can be shifted to coping with other challenges such as mycotoxins, microbiota management, inflammation at level of gut, supporting poor absorption due to BE or dealing with litter quality issues.

It is important to understand that, for most production units, if coccidiosis levels are higher than average, it is very unlikely the best return on investment will yield from adding a probiotic in drinking water or working on the 3rd step of the BE circle, by adding anti-inflammatory capacity. Changing the anticoccidial program will commonly give the best results as this will have a direct impact on performance, next to indirectly reducing BE.

So, it all starts with making a good diagnosis of what is going on by using scoring methods to define the levels of gut health challenge; both coccidiosis and BE scores. Next will be listing all what is being done already to support gut health and estimate a cost per kg bird produced for each of them. Other diagnostics typically will involve assessing mycotoxin and viral challenges, next to a nutritional evaluation to assess the complexity (crude protein digestibility, fat quality, anti-nutritional factors, structure, non-starch polysaccharides).

This data is then to be analysed to understand where breaking the BE circle can be most successful and where it is unlikely to be broken, and importantly at what cost. Once this has been defined, choosing gut health support tools becomes a relatively easier task, with usually huge impact on performance and ease of growing birds and very importantly with lower cost of gut health support tools but also lower FCR and higher ADG.

In order to develop and evaluate strategies in a controlled environment, (subclinical) NE models have been often used, but they don't reflect the right level of challenge occurring in the field as subclinical NE is a rare finding in broiler operations, thus often not returning the right dose levels, or dose regimes of additives to cope with BE. Recently, a model to investigate BE has been developed. This model is combining feed complexity, with a mild coccidiosis challenge, a broad-spectrum antibacterial treatment and a bacterial cocktail orally administered. It simulates a mild BE (score 3/10) with a moderate performance impact, that is reflecting the BE status of the majority of operations worldwide. Using a model can accelerate and support findings from in vitro and field trials.

Table 1 - BE in vivo model. Average result of 12 trials (Poulpharm, 2020).

	Difference of IUC with UUC	Average results IUC
Feed conversion ratio (points)	0.11	1.70
Daily weight gain (g/d)	-5.5	54.6
BE lesion score	1.24	3.0

V. CONCLUSIONS

In poultry production, intestinal health is capital for performance. Since the ban of antimicrobial growth promoters more than 10 years ago in EU, research has demonstrated the importance for general health of the intestinal microbial community and the complex interaction with the host. Intestinal health can be achieved by improved feed composition, medication and additives to maintain a rich and diverse microbial community, and to control the host reaction through dietary immunomodulation. Bacterial Enteritis, a typical vicious circle pathology involving a dysbiosis and macroscopic visible signs of inflammation, has been recognised as an important gut health issue, next to coccidiosis. The complexity of BE ant the vicious circle characteristics requires a holistic approach in diagnosis, prevention and treatment of the disease.

Novel insight in coccidiosis control, viral challenges and use of dedicated in vivo models will further boost the understanding and control of BE, allowing the broiler industry to continue to produce birds with faster growth rates thus lower FCR in a sustainable way.

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BALANCING RESEARCH, INNOVATION, AND EXPERIENCE TO MANAGE THE MODERN BROILER BREEDER

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Summary

Broiler breeder management is growing in complexity due to continued changes in bird genetics, a narrowing of the window of optimal management conditions, and the recognition of the need to maintain normal bird behaviour in male:female interactions. Modern broiler breeder strains have a high propensity to deposit muscle tissue. While their lean body condition results in a need to more closely monitor bodyweight and feed allocation, a well-managed flock can still perform as well as previous lower yielding varieties. Although feed restriction is applied to create estimated normal growth and body composition, changes in genetics resulting in lower fat deposition and increased growth potential mean that we have to be prepared to update our management strategies. This is even more pronounced in the broiler breeder male, where low feed requirements have caused competition for feed to complicate our ability to maintain long-term fertility. Having an understanding of 'normal' broiler breeder growth and breeding behaviour could help us to determine if our breeder flock management provides the conditions for reproductive success.

I. INTRODUCTION

The recognition in the late 1960's that excessive growth rates in broiler breeders were negatively impacting rate of lay and increasing production of unsettable eggs (Jaap and Muir, 1968) has been one of the most transformative events in the history of the hatching egg industry. The resultant feed restriction programs during rearing resolved the issue at the time. But continued genetic progress resulted in feed restriction being necessary throughout growth and production. Nutrition and management issues have evolved as genetic growth potential has increased, meaning that the monitoring and management of feed restriction programs have become much more complex.

The increasing degree of feed restriction required to keep broiler breeder pullets and cockerels on the appropriate growth curves have led to sex-separate feeding systems and technological advances in feeding equipment. In the late 1980's, numerous research projects were still comparing ad libitum fed birds to restricted fed birds as a way to characterize the benefits of restricted feeding. Then, in the 1990's there was increased focus on issues related to the onset of egg production. This was a mix of learning about the appropriate body condition and age to commence lay and recognition that slightly aggressive feeding during this period was triggering long-term issues with maintenance of lay (Renema and Robinson, 2004; Eitan and Soller, 2009). Today there is much more research focus on possible ways to relax feed restriction to increase feed intake without hurting egg production and hatchability.

Genetic progress for growth and feed efficiency has reduced fat deposition (Renema and Robinson, 2004). With birds more predisposed to support protein deposition than lipid deposition, the fat depots the birds used to be able to utilize during inadvertent nutrient shortages early in lay are lacking, and young hens must instead rely on the quality of the flock manager and feed delivery system to meet their daily nutrient requirements. This has led to an increase in research on delivering the appropriate nutrients to support key developmental events and daily activities. Issues with overfeeding today are generally due to complications of

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over-fleshing than to reproductive disorders. Emerging with this is an increase in on-farm issues with energy deficiency, particularly during the transition from pullet to hen. Recent research has shown clear links between nutrient delivery in the pullet phase and long-term reproductive success, regardless of what is fed during the laying period (van Emous et al., 2015a) and that feeding decisions in the pullet phase can even impact offspring yield traits (Moraes et al., 2014; van Emous et al., 2015b). The breeding companies have worked to maintain or even increase rates of egg production and hatchability (Laughlin, 2009), but achieving these potential results at the broiler breeder farm level on a consistent basis has been challenging. While these modern flocks have the potential to produce as many chicks as in past years, they are much more likely to falter due to feeding and management errors.

II. FINE-TUNING BROILER BREEDER NUTRIENT DELIVERY

Broiler breeder management has grown more complex as broiler growth efficiency has increased. However, despite the large changes in genetic growth potential of modern broiler breeders, body weight targets have remained relatively constant (Renema et al., 2007a). From a practical standpoint, maintaining this level of feed restriction is similar to needing more and more effort to keep a spring compressed. As the growth potential of broilers continues to increase, the degree of feed restriction required to manage parent stock body weight gains has created a more competitive feeding environment. From the perspective of parent stock managers, modern broiler strains are simply too good at depositing breast muscle. With a propensity to deposit muscle rather than fat, there may not be enough energy stored in the body to mobilize in times of energetic shortage, and as a result, broiler breeder hens may have difficulty with early chick quality and long-term maintenance of lay. Carcass fat in feed restricted birds at sexual maturity averages between 12.5 and 15% of body weight (Renema et al., 2007a, Yu et al., 1992) and has been trending downwards. Apparent reductions in fat content in current stocks are likely a reflection of the increased muscling that has occurred.

While the negative consequences of ad libitum feeding on bird health and welfare are clear (Renema and Robinson, 2004), there are welfare concerns about satiety of feed restricted birds during the rearing phase in particular. This has led to numerous studies on low-density rations, diet dilution, and use of various fiber sources to help the bird feel fuller. However, typical results describe very little difference in feed clean-up time, little effect on body weight uniformity and infrequent significant differences in welfare indicators (Renema et al., 2007a). There is a need for research on feeding preferences as it related to food quantity and quality (D'Eath, 2009) as it relates to satiety.

Bowling et al. (2018) examined the effect of feed restriction on stress and growth of the broiler offspring. They reported that a higher degree of feed restriction increased plasma corticosterone, which appeared to reduce growth of male broiler offspring between 35 and 42 days of age, while a lower degree of feed restriction increased immune response indicators of female offspring. However, these breeder hens were held at specific target weights rather than under conditions similar to what would occur in commercial barns, so care must be taken in applying these results.

Demonstration of effects of what the hen was fed during rearing on broiler offspring traits is a relatively new phenomenon. Van Emous et al. (2015b) reported that hens fed on a higher growth curve during rearing produced broiler offspring early in lay that were heavier at 34 days of age. The 200 g higher growth target at 20 wk of age also resulted in higher fertility and reduced embryonic mortality than hens reared on the standard body weight curve. This group also reported that feeding pullets on a low protein ration during rearing impacted body composition of birds going into production (van Emous et al., 2015a). At 22 wk of age, breast muscle of these pullets was 4% lighter and abdominal fatpad was 97% heavier than that of

birds on the high protein ration. This shift in composition led to hens reared on the low protein ration having increased hatchability during the first of three phases of lay and increased egg production during the second of three phases of lay.

Moraes et al. (2014) studied birds reared on one of two levels of dietary protein and one of three levels of dietary energy, followed by rearing diets with one of two energy levels. They reported that female offspring of 29 wk old breeder hens reared on a lower protein ration were lighter between 22 and 36 days of age. Their high energy pullet ration resulted in fatter hens and fatter broiler progeny. It was noted that if the energy:protein ratio decreased between the rearing and breeding phases, broiler offspring yield was negatively affected. As an example, moving from a higher energy ration in the rearing period to a lower energy ration during the breeder period, which results in a drop in the energy to protein ratio, also hurts broiler offspring breast muscle yield and overall carcass yield by approximately 1% (19.8% vs. 20.9% breast muscle) when compared to treatments where the energy:protein ratio remained the same or increased between the rearing and breeder diets. Weekly pullet growth was more influenced by feed energy than by feed protein. Because weekly body weight gains across treatments correlated much more closely with feed energy, there was a broad range of protein intakes across treatments. This suggests that breeder pullets could be at risk for overfeeding protein if feed mill ration specs include a crude protein buffer to ensure minimum target specs are being met. A test of broiler breeder rations collected from commercial Alberta hatching egg farms demonstrated that the actual crude protein content of these 15% CP diets ranged from 15.8 to 19% (Carney and Renema, unpublished data). Feeding excess protein to birds very adept at growing muscle is a management concern to consider when assessing production problems on commercial hatching egg farms.

To get at the core issues of what causes a broiler breeder hen to allocate nutrients to support growth compared to egg production, there needs to be work with individual birds. This removes feed competition from the assessment. Flock body weight uniformity peaks near the time birds are photostimulated. But from this point, individual body weight will be impacted initially by how long the bird takes to enter lay and subsequently by rate of lay. How much weight a bird gains or loses during lay is impacted by the balance between their energetic efficiency and rate of lay. Within a hen population some hens lose weight in time – often as a result of a high rate of lay, while some gain weight due to a poor rate of lay. But in addition to this, individually-fed birds on the same feed allocation will include a portion of the population that will gain weight while maintaining a high rate of lay, and a portion that will lose weight despite having a low rate of lay (Renema and Zuidhof, unpublished data). Bird:bird weight variability can have a behavioural component, with some birds eating more aggressively than others, as well as energetic efficiency component. Small birds in particular are often found to be less energetically efficient. Less efficient hens have a higher regulatory thermogenesis, resulting in the loss of more energy as heat (Gabarrou et al., 1998). If these less efficient birds also get behind in body weight compared to their flock-mates, they will often also mature later, and with less robust ovarian development than their larger flock-mates.

Maintaining individually-caged birds on non-traditional feed allocation profiles has demonstrated that recent feed allocations can have a larger impact on ovarian morphology parameters than current body weight does (Renema et al., 2007b; Zuidhof et al., 2007). For example, small pullets fed aggressively through the sexual maturation process will lay eggs approximately 2 g heavier than those of large birds fed sparingly through this period. This difference in egg weight is maintained throughout the production period. Thus, there is potential to use modified feeding levels either in groups or in individual birds to manipulate body composition to optimize egg and chick production. The practice of sorting pullets into size groups and re-sorting at set intervals during rearing is an industry practice in some countries that is rooted in this principle at a more moderate scale that puts this into practice.

III. ADJUSTING GROWTH TRAJECTORIES

A common assumption regarding flock body weight management is that productivity will be maximized if body weight uniformity is high – with the ideal case being that all birds had the exact same body weight. To test this, Romero et al. (2009) maintained a group of broiler breeder pullets on either a common feed allocation, or on customized feed allocations for individual birds. Allocation treatments began at 16 wk of age and with birds approaching a common body weight target at 20 wk of age. Body weights of individually managed birds had a very good uniformity (CV=1.9%) between 20 and 60 wk of age compared to the group-fed birds (CV=5.4%).

Romero et al. (2009) reported that reducing body weight variability did not impact ovary weight or follicle numbers at sexual maturity. Furthermore, decreasing body weight of heavier pullets from 16 wk to reach the target weight did not significantly affect their egg production. Care was taken to ensure no birds lost weight during the 4 wk adjustment to individual growth curves. In contrast, however, a very pronounced effect was found when underweight pullets were forced up to the common body weight target. This group produced 14 more eggs in total compared to their low-weight counterparts in the group-fed control treatment. At issue, however, was that 91% of these additional eggs were < 52 g. While likely viable, they are below the lower weight limit accepted by Canadian hatcheries. It is clear that improving the body weight profile of underweight birds has the potential to significantly improve broiler breeder productivity. However, this needs to start earlier in life than 16 wk of age. Breeder recommendations for sorting small birds into a separate pen are to do this closer to 10 wk of age. Companies practicing routine sorting of pullets may start younger than this and resort the flock at approximately 4 wk intervals. Romero et al. (2009) suggested that hens may have different optimal body weights for support of egg production and that forcing them to a common body weight target may have provided insufficient nutrients to hens with more breast muscle or higher rates and energetic demands for maintenance.

Zuidhof followed this work with experiments using an automated precision feeding system from as early as 2 wk of age. By creating conditions of high body weight uniformity as early as possible, this group has been able to assess the possibility of photostimulating birds early (18 vs. 21 wk) (van der Klein et al., 2018) The 18 wk photostimulation age resulted in birds coming into lay later than birds photostimulated at 21 wk of age (182.8 vs. 173.5 d of age). Furthermore, egg production to 55 wk of age for the standard weight birds was 93 compared to 129 for birds maintained on a 22% heavier growth curve (van der Klein et al., 2018). While the authors conclude that current breeder recommended body weight targets may be too low, the egg production profile and carcass traits of their standard control treatment are very indicative of a flock that has been underfed. There appear to be some unintended negative effects of forcing birds onto exactly the same body weight curve. Because previous work with provision of extra feed to underweight birds has yielded positive results, it is surprising that now the standard weight birds have done poorly. It may be that these researchers 'corrected' the growth trajectories too early and that results would have been better if the adjustment to the growth profile had happened closer to when the ovary becomes sensitive to nutrient intake at approximately 10 wk of age. It may also be that birds have a range of normal optimal body weight profiles and that forcing all birds to the same body weight causes unintended damage to their ability to support establishment of a body composition and nutrient allocation system that is conducive to the sustained support of egg production.

Zuidhof's group performed a subsequent study with the precision feeding system where feed restriction was relaxed on one of 10 modified target curves varying by increments of 2.5% (to a maximum of 22.5% higher body weight target) to evaluate if this would impact feed-seeking behavior (Zukiwsky et al., 2020). In contrast to their hypothesis, feeding up to 22.5%

above the standard body weight target did not reduce feeding or feed seeking behavior, and egg production traits were not affected. An impact on egg production traits would have been unexpected considering previous work with birds fed slightly above the target body weight being very tolerant of these conditions. At issue may be the nature of how birds are fed. Rather than in a single feeding, birds are fed small amounts throughout the day. Birds fed in this way will tend to be very lean. With minimal fat stores going into production, these birds may be at a disadvantage when it comes to tolerating any kind of rapid change in energetic requirements associated with ovary development and early lay.

IV. FEED RESTRICTION PROGRAMS TO MAXIMIZE FAT DEPOSITION

How can broiler breeders be grown at an appropriate rate while ensuring carcass stores are present to support long-term egg production traits? With broilers, eggs and chicks are managed with the goal of optimizing breast muscle development and limiting fat deposition. But with broiler breeders we would like the reverse of this. In addition to this there are concerns about the welfare implications of broiler breeder feeding programs during rearing, as demonstrated by high feed seeking behaviour related to a lack of satiety (D'Eath et al., 2009). While some countries have banned non-daily feeding schedules, they are common in North America.

Birds fed every day get just enough nutrients to support target growth. With modern broiler breeders prioritizing protein deposition over fat storage, birds on this treatment would be expected to have the largest breast muscle weight and lowest abdominal fatpad (a key indicator of carcass fat stores). Zuidhof et al. (2015) reported the highest abdominal fatpad weight and lowest breast muscle weight in birds fed on a skip-a-day program. However, these birds also had the lowest ovary weight, large yellow follicle number and weight. Growth hormone will dampen the stimulatory effect of estrogen on the yolk lipid biosynthesis pathway (Walzem, 1996), so this may simply be the result of a redirection of available lipids into storage.

It is suggested that birds on a skip-a-day feeding regimen are metabolically less efficient because of the energetic cost of cycling between nutrient storage and mobilization (de Beer and Coon, 2007). As a result, skip-a-day fed birds will compromise breast muscle growth and divert more energy to storage in the abdominal fat pad. de Beer et al. (2007) reported a 5-fold increase in glycogen and total lipid levels in skip-a-day birds 24 h after refeeding. During fasting, fatty acids are released from adipose tissue, glucose is released from liver glycogen stores, and further energy may be provided through catabolism of muscle protein through gluconeogenesis (de Beer and Coon, 2007; de Beer et al., 2007).

Arrazola et al. (2019) studied the welfare and growth of broiler breeder pullets reared using every day feeding, a diet diluted with oat hulls and calcium propionate, a 4:3 system (4 days on, 3 days off), or a graduated diet where feeding frequency varied with age. They reported that birds in all of the alternatives to every day feeding demonstrated decreased feeding motivation and lower stress. Birds in the every day feeding group also had more feather fault bars (indicator of stress response) and worse feather coverage. Birds on the 4:3 schedule received the highest daily feed allocations which possibly allowed increased lipid storage, thereby allowing them to habituate for off feed days better than the birds on the graduated diet.

V. MANAGING MALE FEEDING

Broiler breeder males have a low nutrient requirement to meet their growth targets because they are so optimized for growth. This continuous increase in broiler breeder growth potential has elevated the importance of sex-separate feeding as a way to limit male protein intake with a low-protein ration to limit breast muscle deposition and support male fertility. Unless a

producer has a reliable supply of spiking males to replace poor-performing males with, extending the duration of the period males are contributing to flock fertility is essential.

Ultimately, male reproductive success results from a mixture of social status and semen production. The job of the manager is to maintain an environment that supports slow, steady growth to maintain testicular condition. This is exceptionally difficult as birds enter the breeder house. Rising testosterone has made them much more competitive, they may be stealing feed from hen feeders, they have a big spike in energy requirements as they learn effective mating behaviour, and they are put into a new environment where there are a limited number of feeders available to them.

Feeder space per bird is a simple and effective management tool available to maximize uniformity of feed consumption by the males in rearing. The goal is to provide enough space that males are not being excluded, while at the same time not having surplus space so the larger and/or more aggressive males have the option of moving to multiple spots to eat before the feed is gone. The heavier males are also the birds with the largest testes and strongest sexual drive. But after peak, male size can start to become an issue due to over-fleshing and development of dexterity issues (Renema and Robinson, 2004). This means that the males that are most valuable to us in a younger breeder flock may end up being the most problematic later in life, when their excessive fleshing is interfering with successful cloacal contact.

While excess weight in male broiler breeders may impact flock fertility for mechanical and behavioral reasons, insufficient weight or nutrient can impact fertility for physiological and behavioral reasons. Cerolini et al. (1995) found evidence that fertility can also be decreased in males due to ME deficiency and that problems would happen more often at the end of the production period. Under conditions of artificial insemination to remove mating behavior effects, they fed Ross broiler breeder males with a standard diet (12% CP and approx. 2,746 kcal ME/kg) from 23 to 54 wk of age with 110, 120, 130 g/bird/d or ad libitum. Body weight ranged by 200 grams among treatments and fertility was increased for males fed increasing amounts of feed (59%, 72%, 79.2% and 79.2% for males fed 110, 120, 130 g/bird/day or ad libitum, respectively. Buckner et al. (1986) found that limiting feed intake decreased body weight, semen volume, number of spermatozoa in the ejaculate, testicle weight, hematocrit and the percentage of males producing semen. They concluded that 113 g/bird/d of their 13.1% CP and 3,167 kcal ME/kg ration was the minimum feed allocation that did not negatively affect the reproductive traits. While recommended composition of modern male diets has changed to include less protein than this, the need to monitor our flocks for overfeeding or underfeeding is still very relevant today. This is all much easier to do when flock body weights are uniform.

In 1987, Wilson et al. demonstrated that feeding caged breeder males 12, 14, 16, or 18% protein isocaloric diets did not affect bird weight, testes weight, or semen quality. However, more males on the 12 or 14% protein diets were able to produce more semen. More recently, Romero-Sanchez et al. (2007) compared 12% and 17% CP male rearing diets fed in a concave or sigmoid pattern to 26 wk of age. The 17 % CP diet increased body weight, but this was limited to the 8 to 32 wk of age period. More importantly, the 12% CP rearing diet improved both weekly and cumulative fertility. The concave feeding program had larger feed increases toward the end of the rearing period. Like the 17% CP diet, this excess nutrition as birds were coming into production led to males that were unable to sustain fertility after 40 wk of age without additional increases in feed allocation. This is a similar effect to what used to happen with overfed hens. The flock would come into lay strongly, there would often be a high peak rate of egg production, and then after 40 to 45 wk of age production would tail off as hens were unable to sustain egg production. Current male stocks appear to still be very responsive to excess nutrition. With their propensity to lay down muscle, this can quickly become an issue as it starts to interfere with fertility due to incomplete matings.

VI. UNDERSTANDING FLOCK MATING DYNAMICS

There has been very little work done in broiler breeders on mating behaviour and how it can impact flock fertility. Modern broiler stocks have been selected for a shorter, wider-legged stance to support rapid broiler growth. In the breeder, shifts in body conformation have the potential to affect how well the male and female are able to make sexual contact during the act of mating in heavy flocks (McGary et al., 2003). The behaviour of these birds suggests they think a complete mating has happened, when no semen transfer occurred. As this likely affects mostly older, heavily muscled males, this could become a criterion for male culling. Unlike underweight males who may express less sexual behavior due to decreased testicular mass and testosterone production, these large males are often still perfectly functional, and only serve to disrupt mating activity of subordinate males. Managing flock fertility requires spending time observing flock mating activity and assessing all males for potential culling. The best males in the younger flock could be the ones causing the most trouble in the older flock if they are not able to complete matings.

Specific recommendations on aligning male and female sexual maturation, modern flock sex-ratios that optimize fertility while reducing issues with aggressive males, use of a male hospital or holding pen in the breeder house, and implementation of a fully separated male feeding area are all examples of current recommendations have been derived from practical field research. Shifts in practice can come quickly due to regulatory changes or from sharing of innovative ideas. For example, biosecurity concerns have limited access to spiking males in some countries. Development of male holding/rest pens at the end of the barn and intra-spiking programs among barns are two techniques that have become much more common. Mphepya et al. (2019) tested exchange of 25, 35, or 45% of males at both 40 and 48 wk of age in a double intra-spiking program. Male competition and flock fertility were increased the most with the 45% exchange of males, with this being the only treatment to return the flock to the breeding company standard. This is a good example of establishment of field practices that are later corroborated in a formal research study.

Understanding the dynamics of rooster and hen sexual behaviour has been enhanced by previous work performed with laying hen stocks on choice testing and male courtship behaviour and, more recently, with in-depth research in wild populations of Red Jungle Fowl. Pizzari et al. (2002) provides an early summary of this ongoing research program. Their work on male:female behavioural interactions during mating has helped increase the understanding of the role of the hen in the acceptance of males and in what attracts roosters to hens. This adds depth to the understanding of normal breeding behaviour. The elements of attraction and rejection among breeding chickens adds a fascinating layer of complexity to flock management. They describe the impact of social status and on male strategies to inseminate sperm of higher or lower quality, as well as female strategies to eject semen or to attract interference to a mating from a male perceived as less desirable. While only part of this may translate to a commercial broiler breeder barn setting, it clearly demonstrates that controlling weight is just one piece of the intricate puzzle leading to the successful production of viable chicks.

In conclusion, as broiler breeders continue to change due to the impact of genetic selection for improved growth efficiency and meat yield, there is value in understanding how our management priorities have changed along with the bird. Both male and female broiler breeders need a positive growth profile in order to maintain reproductive effectiveness, and are now specialized to the point that they are less able to tolerate nutrient deficiencies when demands for growth and reproduction are high. Success in a broiler breeder operation is measured by the production of viable chicks. After providing the best possible conditions, the

managers must leave it up to the bird:bird interactions to sort out the intricacies of dominance and mating behaviours.

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LAYER NUTRITION ASSOCIATED WITH DIFFERENT PRODUCTION SYSTEMS

K. E. ANDERSON[1]

Summary

The egg industry continues to grow; in the past this was in cage production, but today's growth is focused on alternative production systems such as cage-free or range egg production. Contributing to this growth has been intensive egg production that created concerns about the impact of the cage environment on laying hen well-being. Both the commercial egg production sector and small producers using heritage strains of chickens, in flocks ranging in size from 100 to 3,000 hens, are responding by producing eggs in cage-free and range settings. However, one of the current issues is that our knowledge base of how these alternative production methods influence hen nutrition and egg production performance is limited to research studies that were conducted in the late 1940s and early 1950s. This information was collected with specific breeds of hens which no longer exist, and not with modern lines of poultry that have been selected for lower body weights and very high rates of egg production. Therefore, an examination of alterative laying hen nutrition in the context of the current knowledge base would provide beneficial information to identify how feeding practices translate to modern strains of laying hens under cage-free or range production. Research on range or cage-free production done in controlled settings is limited, and additional studies relevant to egg producers wishing to expand cage-free and range egg production are needed.

I. INTRODUCTION

The transition in the layer industry from conventional cages to cage-free and even further into free-range production is rewriting the nutritional requirements of the laying hen. In the last 25 years, the number of eggs a hen can produce has increased by about 2 eggs each year while the amount of feed required to produce these eggs has been reduced by 18.6% in cages (Anderson, 1991; Anderson et al., 2013, Anderson, 2019). The result has been a high value protein source at the low cost the consumers pay for eggs today. During this time, the breeding companies began selecting laying hens that were better adapted to cages. This included smaller body weights, improved feed conversion, increased egg size and quality among a few of the traits selected for. The industry in the US is being pushed to transition from conventional cages to cage-free production systems at a rate which is 3 times faster than was taken to transition to cages originally. The breeding companies are trying to transition the hen into a more productive bird when destined to be in a cage-free setting. Currently in the US, brown egg layers are being used more in the cage free systems and almost exclusively in the free-range systems.

The industry is moving these birds to the cage-free egg production systems while relying on the nutrient requirements that were developed while the birds were housed in cages. Nutritionists are working on developing feed formulation models which will compensate for the increased locomotory movement within these systems while maintaining the same hen day production levels. However, what we are finding is that the variation in energy requirements between individual hens is increasing dramatically due to their activity levels. In addition, there are greater concerns related to egg safety.

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The production systems I will be discussing were derived from industry needs in 2012 and were components of the North Carolina Layer Performance and Management Test (NCLP&MT). The research station transitioned with remodelled facilities from multi-level conventional cage systems that hold relatively small populations of hens in a simple environment with no enrichments. There was a brief period where the industry transitioned to either Enrichable or Enriched Colony Housing Systems. The systems in this comparison were large cages with hen populations ranging in size from 21 to 36 birds. The enrichable Colony Housing Systems was a cage with increased height so the birds would not hit their heads when standing normally with no other enrichments. The Enriched Colony Housing Systems included a nest area 270 in^2 (1742 cm^2) and 96 in (244 cm) of roost space and a scratch area. The Cage-Free Housing System was in a force-ventilated house with a slat litter flooring system. The pens were 12.1 ft x 6.6 ft (4 m x 2 m) density 177 in^2 (1142 cm^2), 1 nest/5 hens and 6 in (15.2 cm) of roost/hen and a dust bathing area. The Free-Range Housing Systems was a standard height curtain ventilated laying house with a slat flooring system with pens 12.1 ft x 6.6 ft (4 m x 2 m) density 177 in^2 (1142 cm^2), 1 nest/5 hens, and 6 in (15.2 cm) of roost/hen. The Veranda 10 ft x15 ft (3.04 m x 4.6 m) of shaded, bare dirt with access to a Paddock 30 ft^2/hen (2.78 m^2/hen) and rotated every 4 wks. This configuration and rotation system allows for a 50% forage cover to be maintained.

For this presentation I went back through the 39th and 40th NCLP&MT Rearing and Single Cycle Reports and used the feed consumption records which included the diets and the amounts fed. These records were maintained for each of the replicates in all of the production systems which consisted of a total of 410 replicates (13,860 hens). All of the birds were hatched on site and reared in the appropriate environments. They were transferred from rearing to the laying phase which commenced at 17 weeks of age. All of the birds were under the same management and dietary protocols with the only caveats being the environments and that each replicate's feed was allocated independently based on feed intake and production. From the calculated nutrient profile of each diet and the feed consumption data, I calculated the nutrients discussed herein, on a hen basis for the 5 production environments. The data were segregated between 11 White egg Strains and 7 Brown egg strains.

II. PULLET REARING

As we shift production systems from cages to extensive systems such as enriched colony, cage-free (aviary) or range we have to start considering how we are rearing the pullets destined for these systems. Pullets should be grown in systems similar to those in which they will be placed for their productive life. This allows them to learn how to use the system and physically develop to better move within them. In order to accomplish this we have to provide them the proper nutrition. In addition, it affords them the opportunity to develop behavior patterns which will minimize floor eggs. In the 39th NCLP&MT, range reared pullets consumed 470 g of protein more than their cage reared hatch mates for both White and Brown egg pullets. Either by genetic selection for extensive production systems, recycling of nutrients, or improved range management by the 40th NCLP&MT, protein consumption via supplemental feed was significantly reduced and was lower than that required by the cage reared pullets. Total energy consumption followed the same patterns as protein consumption. We have no way of accounting for what the pullets consumed in the extensive systems. We have indications that range pullets consume about 11% of their total consumption from the range system on an as fed basis. When the moisture content of that component is accounted for, the birds get only about 3% on a DM basis of their consumption from the range paddock.

III. LAYER PERFORMANCE

Nutrient requirements for the laying hen are highly dependent upon the production characteristics of the hens and how this production varies among the different housing systems. In the 40th NCLP&MT overall, the Brown egg layers consumed more feed than the white egg layers in all of the production systems. Also, both the White and Brown egg layers had increased feed intake in the Colony system and in the range production systems. However, only in the White egg layers was feed conversion depressed in the Colony and Range systems. When comparing the Colony with the Enriched system, Hen-Day production percentage was significantly higher in the Enriched Colony. For each of the production systems the White egg layers produced more eggs than did the Brown egg layer. Mortality was due primarily to trauma. In systems with the greatest ability to move, the result was more broken appendages as well as keel bone damage.

In the 40th NCLP&MT, both White and Brown egg layers had similar egg quality; however, the percentage of checked and loss eggs was greatest in the Colony and Enriched Colony systems with losses ranging from 8.3 to 11.2 %. Cost and income components were relatively consistent across all systems for both egg type layers with the one exception being the White egg layers in the Range system generating the least net income/hen.

IV. LAYER NUTRIENT CONSUMPTION

We have a relatively good estimate of nutrient intake (or feed disappearance) of the laying hen during the production cycle. The White egg layers in a single cycle flock consumed 96 to 108 g of feed/day in both cage and cage free systems. Brown egg layers consumption was 3.3% and 5% higher in cage and cage-free systems, respectively. On range the White egg and Brown egg layers feed consumption increased to 106.8 and 110.9 g/day, respectively. From that, they consume 21 g of protein, of which 57% is dedicated to egg production, 16,6% for body maintenance and growth and the remaining 21.4% for dealing with the environment. Energy consumption is about 300 kcal with 66% used for egg production, 11.3% for growth and body maintenance and the remaining 13.3% for dealing with the environment. If we look at Calcium 75.6% is used for egg production with the rest for skeletal maintenance and that lost to the environment. However, as we move into more extensive production systems, we really have not examined the shift in nutrient partitioning due to the additional needs of the production system. We have to formulate to compensate for the increase in activity levels of the hens, to increase bone density, and to provide the energy required to maintain homeostasis. We also have to deal with the nutrients robbed from the hen by the internal parasite loads such as Heterakis, Roundworms, and Tapeworms.

In all of the extensive production systems, the Brown egg layers consumed significantly more Protein on a daily basis than the White egg layers, except in the conventional cages where the protein consumption was not different. Interestingly, the protein consumed in the enrichable colony system was significantly higher by 7.2% than in the enriched colony system. The protein consumption of the range hens was intermediate to either of the colony housing systems. The energy, Calcium, Available Phosphorus, Lysine and Total Sulphur Amino Acids, as you would conclude, followed similar trends as seen in the protein consumption. However, due to the feeding of individual replicates, we were able to show the influence of environment on White and Brown egg layers.

We do not yet understand the recycling of the nutrients in the cage-free system or the true level of nutrition captured from the range paddocks. We know that additional nutrients are consumed from the range and in the cage-free system through the nutritional makeup of the eggs and yolk color through research at Pennsylvania State University and at North Carolina

State University. However, we need to keep in mind that the hen's consumption rates are higher than in cage–free or the conventional cage systems.

V. CONCLUSION

We have much to learn related to the nutritional needs of the laying hen in extensive production systems. We worked on the nutrition of the hen in cage systems for 75 years so the transition to extensive systems and how we can manage these systems to enhance the nutritional status of the hens is going to be vital. Some research areas are:

1. Understanding what level of nutrition is gained from foraging
2. What is the variation in hen activity levels in the range systems
3. Training hens to minimize floor eggs
4. Impact of internal parasites on performance and health (How to mitigate parasites)

We have a growing world population and, regardless of your viewpoint, there are production issues we have to face if we are going to feed this population. It is a work in progress and will continue long after me.

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PERFORMANCE AND GUT MICROBIAL PROFILE OF BROILERS FED DIETS WITH VARYING LEVELS OF PROTEIN AND ENERGY AND SUPPLEMENTED WITH A PROTEASE COMPLEX

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Nutrients in most feed ingredients are present in a complex matrix. Therefore, it is anticipated that feed enzymes like protease can exert a wide influence on nutrient digestibility beyond their targeted substrates (Cowieson and Bedford, 2009). For instance, the disruption of protein matrix surrounding starch granules due to protease supplementation had been shown to improve energy digestibility in some cereal grains (McAllister et al., 1993). It is hypothesised that protease may benefit broiler performance through improved nutrient digestibility and its concomitant impact on gut microbial profile through substrate availability modifications in the gastrointestinal tract.

A total of 720-one-day-old male broilers (Vencobb 430) were fed for 42 days with either a standard diet or with diets reduced in digestible amino acids (AAs) by 5% and metabolizable energy (ME) by 0.2092 MJ/kg. Both diets were then supplemented with a protease complex. Protease was included at 0, 125, and 200 mg/kg diet. A completely randomized block design with 2 levels of dietary AA (100% or 95% of the standard diet), 2 levels of ME (100% or -0.2092 MJ/kg of the standard diet), and 3 levels of protease (0, 125, or 200 mg/kg diet) in a 2 x 2 x 3 factorial arrangement was used. Data were subjected to ANOVA using a GLM and a polynomial contrast to determine the linearity of dose response to protease. At 42 d, the main effects of dietary AA and ME levels decreased and increased ($P < 0.05$) ADG and FCR, respectively, in broilers fed reduced AA (67.9 vs. 69.6 g and 1.678 vs. 1.640) or ME (67. 8 vs. 69.6 g and 1.691 vs. 1.628) compared to those fed standard diet. On the other hand, the main effect of protease supplementation (0, 125, 200 mg/kg diet) increased ADG (67.7 vs. 69.2 vs. 69.3 g) and tended to improve FCR (1.676 vs. 1.654 vs. 1.649; $P < 0.07$) in a dose dependent linear manner. In terms of gut microbial profile (log_{10} CFU/g), broilers fed reduced dietary AA had increased ($P < 0.05$) ileal *Escherichia coli* (7.83 vs. 7.36), *Salmonella spp.* (2.17 vs. 2.05) and *Clostridium perfringens* (7.00 vs. 6.38), and decreased *Lactobacillus spp.* (7.04 vs. 7.08) compared to those fed standard diet. A similar trend was observed in all bacterial species for broilers fed reduced dietary ME, except for *Lactobacillus* which was increased compared to the standard group. Protease supplementation decreased the counts of all four bacterial species. The nutritional requirements of different bacteria are known to be species-dependent and these could have influenced the observed profile in the gut microbiota. In the reduced AA diet, soybean meal and oil sources were partially replaced by corn and therefore, its starch level was increased in relation to protein. On the other hand, in the reduced ME diet, oil sources were also partially replaced by corn leading to increased dietary starch level in proportion to lipid, while AA levels were maintained. Overall, performance of broilers was negatively affected by reduced dietary nutrient density. Protease supplementation on either standard or nutrient reduced diets improved animal performance and appears to offer benefits with respect to gut health through a reduction in numbers of potential pathogens.

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APPARENT METABOLISABLE ENERGY OF COMMON CEREAL GRAINS FOR BROILER CHICKENS IS INFLUENCED BY BIRD AGE

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Summary

The current study was conducted to investigate the influence of broiler age on the apparent metabolisable energy (AME) and nitrogen-corrected AME (AMEn) of four common cereal grains (wheat, sorghum, barley and maize), measured using the direct method. Six groups of broiler chickens aged 0-7, 7-14, 14-21, 21-28, 28-35 or 35-42 post-hatch, were utilised. Four experimental diets with the same inclusion (957 g/kg) of either of the grains were developed in pellet form. Each diet was randomly allocated to six replicate cages in each age group, and excreta were collected over the last 4 days of each period. Bird age had a significant ($P < 0.001$) effect on AME and AMEn of all cereal grains. The AMEn of wheat declined quadratically ($P < 0.01$) with advancing age, from 14.48 MJ/kg in week 1 to 13.47 MJ/kg in week 2 and then plateaued. The AMEn of sorghum grain declined linearly ($P < 0.001$) with advancing age, from 15.74 MJ/kg in week 1 to 14.88 MJ/kg in week 6. Age quadratically affected the AMEn of barley ($P < 0.001$) and maize ($P < 0.05$), where the AMEn declined by 1.25 MJ/kg in barley and 0.66 MJ/kg in maize from week 1 to week 2 and then increased. In conclusion, the present results showed that broiler age has substantial impact on the AME and AMEn of cereal grains and the effect varied depending on the cereal grain.

I. INTRODUCTION

Cereal grains such as wheat, sorghum, barley and maize are commonly used in poultry diets as the main source of energy. Knowledge on the metabolisable energy content of cereal grains is critical for their efficient use and precise poultry feed formulation. Despite several limitations (Mateos et al., 2019; Wu et al., 2020), the AME has been the globally accepted system for describing the dietary energy content for poultry.

Modern, commercial broiler diets are formulated to use AME or AMEn values of feed ingredients from reference tables or equations. Most published data on the AMEn content of feed ingredients have been generated with either adult cockerels or older broilers (typically 5-week old) and are widely used in feed formulations for all the phases of broiler's growth. However, this practice overlooks the potential effect of bird age on the AMEn content of feed ingredients. Furthermore, bird age has been shown to have a substantial effect on the digestion and absorption of energy-yielding nutrients (Bennett et al., 1995; Wiseman, 2006). Birds at different ages have variable ability to digest and metabolise feed ingredients especially those containing anti-nutritive substances such as soluble non-starch polysaccharides (Adeola et al., 2018). Moreover, the capacity of digestive tract to digest and absorb nutrients is limited during the early life of broilers and, there is a consensus that the nutrient digestibility generally increases with advancing age (Brumano et al., 2006; Olukosi et al., 2007).

The relevance of using a single value of AMEn obtained with older birds to all growth phases, especially the early life, of broilers is questionable and highlights the need for age-dependent estimates for use in feed formulations. All previous studies on age-related responses

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have been conducted on grain-based complete diets (Olukosi and Bedford 2019; Yang et al., 2020). To our knowledge, no published data are available on age effects for the AMEn of single cereal grains. Therefore, the aim of this study was to investigate whether the age of broiler chickens has any effect on AMEn of commonly used cereal grains (wheat, sorghum, barley and maize) using the direct method by the total excreta collection (Hill and Anderson, 1958).

II. MATERIALS AND METHODS

Four cereal grains (wheat, sorghum, barley and maize) were obtained from local commercial suppliers. The wheat and sorghum samples were of Australian origin, and maize and barley were sourced from New Zealand. The AME and AMEn of the cereal grains were determined using the direct method. In this method, four basal diets were formulated to contain the same inclusion level (957 g/kg) of each grain, and fortified with macro minerals, vitamin and mineral premixes. Diets were mixed in a paddle mixer and then pelleted.

Day-old male broilers (Ross 308) were obtained from a local hatchery and raised on floor pens until assigned to the experimental treatments weekly. Birds were fed broiler starter mini pellets until day 21 and finisher pellets from day 21 to 35. At the beginning of each week (days 0, 7, 14, 21, 28 and 35), birds were weighed individually and allocated to cages so that the average bird weight per cage was similar at each week. For each cereal grain, the assay diet was fed to six replicate cages of broilers during six periods, namely days 0-7, 7-14, 14-21, 21-28, 28-35 or 35-42. Each replicate cage housed 10 birds during week 1, and 8 birds during weeks 2 to 6 post-hatch. The AME was determined using the total excreta collection procedure (Hill and Anderson, 1958). For each week, diets were fed for 7 days with the first 3 days serving as an adaptation period. The feed intake and total excreta output for each replicate cage were recorded over the last 4 consecutive days of assay. Daily excreta collections were pooled within a replicate cage, mixed in a blender, sub-sampled and lyophilised. Dried excreta samples were ground to pass through a 0.5-mm sieve and, the diets and excreta samples were analysed for dry matter, gross energy (GE) and nitrogen (N). The AMEn was calculated by correction for zero N retention by assuming 36.54 KJ per g N retained in the body as described by Titus et al. (1959). The data were analysed using the General Linear Model procedure of SAS Institute, 2015. Orthogonal polynomial contrasts were performed to determine the linear and quadratic effects of broiler age.

III. RESULTS AND DISCUSSION

Table 1 summarises the effect of age on the AME and AMEn of the cereal grains in broiler chickens. Age had a significant ($P < 0.001$) effect on AME and AMEn of all cereal grains. The AMEn of wheat declined quadratically ($P < 0.01$) with advancing age, from 14.48 MJ/kg in week 1 to 13.47 MJ/kg in week 2 and then plateaued (Fig. 1A). The AMEn of sorghum declined linearly ($P < 0.001$) with advancing age, from 15.74 MJ/kg in week 1 to 14.88 MJ/kg in week 6 (Fig. 1B). The AMEn of barley ($P < 0.001$; Fig. 1C) and maize ($P < 0.05$; Fig. 1D) were reduced with the advancing age of broilers, but the decline was greater between weeks 1 and 2, resulting in a quadratic effect for both grains. The AMEn declined by 1.25 MJ/kg in barley and 0.66 MJ/kg in maize from week 1 to week 2 and then increased.

Table 1- Apparent metabolisable energy (AME; MJ/kg DM basis)[1], and nitrogen-corrected AME (AMEn; MJ/kg DM basis)[1] in cereal grains as influenced by bird age

Age (week)	Wheat		Sorghum		Barley		Maize	
	AME	AMEn	AME	AMEn	AME	AMEn	AME	AMEn
1	14.92	14.48	16.16	15.74	14.19	13.75	15.78	15.48
2	13.69	13.47	15.36	15.12	12.79	12.50	15.01	14.82
3	13.62	13.31	15.40	15.15	13.37	13.04	15.38	15.12
4	13.66	13.37	15.46	15.20	13.24	12.93	15.56	15.31
5	13.81	13.51	15.18	14.92	12.99	12.70	15.66	15.41
6	13.69	13.44	15.13	14.88	13.18	12.89	15.42	15.18
SEM[2]	0.193	0.190	0.110	0.100	0.079	0.073	0.077	0.070
Probabilities, P ≤								
Linear	0.001	0.004	0.001	0.001	0.001	0.001	0.651	0.455
Quadratic	0.001	0.002	0.019	0.072	0.001	0.001	0.036	0.043

[1]Each value represents the mean of six replicates (each replicate cage housing 10 birds [week 1], and 8 birds [weeks 2, 3, 4, 5 and 6]).

[2]Pooled standard error of mean.

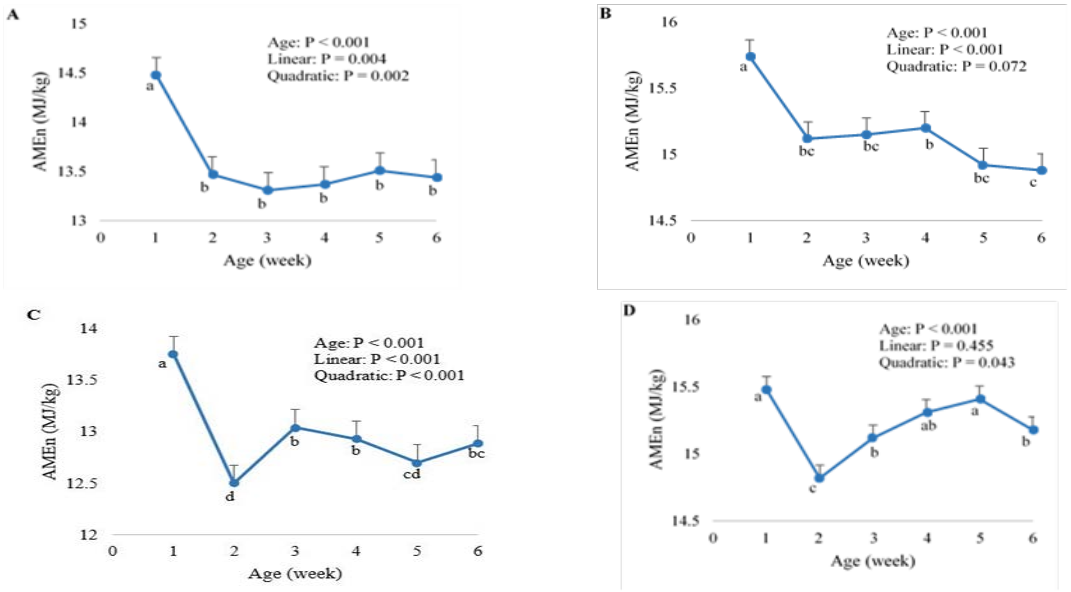

Figure 1- Effect of broiler age on nitrogen-corrected apparent metabolisable energy (AMEn) for wheat (A), sorghum (B), barley (C) and maize (D); mean ± standard deviation. [a-d] Values with different superscripts differ significantly (P < 0.05).

The paramount motive for this study was to investigate whether the age of broilers will (i) influence the metabolisable energy content of common cereal grains, and (ii) whether the effect, if present, is similar for all grains. Predictably, the AME and AMEn values of cereal grains were influenced by bird age, regardless of the cereal type. While the highest AMEn values were observed at the first week for all cereal grains, the AMEn declined, either linearly (sorghum) or quadratically (wheat, barley and maize). It has been documented that, due to the under-developed digestive tract and limitation in digestive enzymes required for nutrient digestion, the utilisation of major nutrients is low in the newly hatched chick but increases with age (Noy and Sklan, 2001). In contrast to the current findings, some studies have shown a lower metabolisable energy during the first few day's post-hatch followed by an increase after the first week (Murakami et al., 1992). Thomas et al. (2008) reported that AMEn of a maize-based diet was higher at day 3 (13.87 MJ/kg), then declined by 1.59 MJ/kg to 12.28 MJ/kg at day 7, and increased to 13.01 MJ/kg by 14 days of age. These researchers reported similar trends for wheat- and sorghum-based diets. However, in agreement with current findings, Moss et al.

(2020) reported higher AME:GE ratios in young broilers (7-9 days) than older broilers (33-34 days) in diets based on wheat (0.799 vs. 0.765), sorghum (0.782 vs. 0.713) or maize (0.796 vs. 0.785).

The higher AMEn during the first week post-hatch may be due partly to the beneficial effect of the yolk sac. During the first week post-hatch, chicks undergo metabolic adaptations while moving from embryonic yolk dependence to rely on obtaining and utilising nutrients from a complex dietary source (Sell, 1996; Noy and Sklan, 2001). Additionally, the digestive tract of the hatchlings is sterile and is rapidly colonised by microflora after hatching. Gut microflora contains various bacterial species that consume energy for their rapid growth and colonisation. These factors may contribute, at least in part, to the subsequent decline in the dietary energy utilisation and is supported by the lower AMEn recorded in week 2 in the present study. After the second week of age, AMEn of ingredients either remained unchanged or increased, the latter of which could be reflective of the increase in digestive and absorptive capacities of the intestine enabling birds to extract more nutrients from feed (Noy and Sklan, 2001).

Overall, the current findings suggest that, regardless of cereal type, age of broilers has substantial impact on AME and AMEn values of cereal grains, and question the application of single value of AME or AMEn for broilers at different ages, which can under- or over-estimate the energy utilisation. Therefore, to enhance the precision of feed formulation and production efficiency, age-dependent AME and AMEn values should be used when formulating broiler diets. Moreover, as the methodology (direct vs. substitution methods) can have an impact on metabolisable energy content of feed ingredients, future studies are warranted to evaluate the AME and AMEn content of cereal grains using the substitution method.

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TRUE ILEAL CALCIUM DIGESTIBILITY IN SOYBEAN MEAL AND CANOLA MEAL, WITHOUT AND WITH MICROBIAL PHYTASE, FOR BROILER STARTER AND FINISHER

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Soybean meal (SBM) and canola meal (CM) are two plant-based protein sources which contain reasonable amounts (2.9 and 6.8 g/kg, respectively) of calcium (Ca). Currently, no published values are available for the Ca digestibility in SBM and limited studies (Anwar et al., 2018; Moss et al., 2018) are available for the Ca digestibility in CM. Microbial phytases are now routinely added in poultry diets to improve the bioavailability of phosphorus (P) bound to phytate (myo-inositol hexaphosphate) and P digestibility. The effect of phytase addition on Ca digestibility, however, is contradictory (Ravindran et al., 2008; Walk et al., 2012). Higher phytase doses, typically over four times the recommended dose and referred to as superdoses, are currently being used by the industry and reported to improve the growth performance and nutrient digestibility (Cowieson et al., 2011). Therefore, two experiments were conducted, with the primary objective of determining the true ileal digestibility of Ca in SBM and CM without and with microbial phytase, during broiler starter (Experiment 1) and finisher (Experiment 2) periods. A secondary objective was to investigate the influence of microbial phytase dose on the true ileal digestibility of Ca in SBM and CM.

Dietary treatments consisted of a 2 × 3 factorial arrangement with ingredient (SBM and CM) and phytase dose (0, 500 and 2000 FTU/kg; Quantum Blue, AB Vista, Marlborough, U.K.) as the main factors. Six experimental diets based on maize-SBM and maize-CM, with three phytase doses, were fed to 6 replicate cages (8 birds per cage) of male broilers (Ross 308) from day 18 to 21 (Experiment 1) or 39 to 42 (Experiment 2) post-hatch. The contribution of Ca from maize was negligible, enabling the use of maize-based diets. A Ca- and P- free diet, with no added phytase, was also developed to determine the endogenous Ca losses. Apparent ileal digestibility was calculated using titanium ratios in the diet and digesta and the true ileal digestibility was calculated by correcting for endogenous Ca losses. Total tract Ca retention was also measured.

Ileal endogenous Ca losses were determined to be 236 and 29 mg/kg of DM intake, respectively, in broiler starter and finisher. True ileal Ca digestibility coefficients of SBM and CM, without added phytase, were determined to be 0.51 and 0.53, respectively, in starter and 0.33 and 0.22, respectively, in finisher. Increasing phytase dose increased ($P < 0.05$) the true ileal Ca digestibility of CM in both starter and finisher, but true ileal Ca digestibility of SBM increased ($P < 0.05$) only at the superdose (2000 FTU/kg) in finisher. Total tract Ca retention ($P < 0.001$) in starter was higher in CM than in SBM and was increased with phytase dose in both ingredients. In broiler finisher, the Ca retention was increased ($P < 0.001$) by both phytase doses in CM, but only by the superdose (2000 FTU/kg) in SBM, resulting in an ingredient × phytase interaction ($P < 0.001$).

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A BOX-BEHNKEN ASSESSMENT OF FISHMEAL AND SORGHUM INCLUSIONS IN BROILER DIETS WITH THREE CRUDE PROTEIN LEVELS ON GROWTH PERFORMANCE FROM 14 to 35 DAYS POST-HATCH

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Summary

The objective of this study was to investigate the impacts of starch and protein digestive dynamics on broiler growth performance using Box-Behnken response surface design. The design consisted of three factors (i) sorghum, (ii) fish meal, (iii) crude protein each at three levels. Thirteen wheat-soybean meal-based diets were offered to 390 off-sex male (Ross 308) broilers from 14 to 35 days post-hatch. The Box-Behnken design established that crude protein and fish meal inclusions influence weight gain and feed intake where maximum weight gain (2157 g/b) and feed intake (3330 g/b) were observed in birds offered 190 g/kg crude protein diets. Fish meal inclusions linearly compromised FCR which was more evident in 190 g/kg CP diets.

I. INTRODUCTION

The inclusion of 175 g/kg fishmeal, essentially at the expense of soybean meal, in sorghum-based broiler diets was shown to improve weight gain by 12.1% (1260 versus 1124; $P < 0.001$) and feed conversion ratio by 8.13% (1.299 versus 1.414; $P < 0.001$) from 15 to 28 days post-hatch (Sydenham et al., 2017). The dietary treatments had an average crude protein (CP) content of 222 g/kg in this study. Slowly digestible starch has been shown to advantage broiler performance (Herwig et al., 2019) and the digestion rate of sorghum starch is very considerably slower than wheat starch under *in vitro* conditions (Giuberti et al., 2012). Differences in starch digestion rates across various feed grains are probably not as pronounced in broiler chickens; nevertheless, starch digestion rates in broiler chickens offered wheat-based diets have been shown to be 56.0% more rapid (0.117 versus 0.075 min^{-1}; $P < 0.025$) than their sorghum-based counterparts where a number of feed grains were compared (Selle et al., 2020a). There is real interest in the development of reduced-CP for broiler chickens as their adoption would promote sustainable chicken-meat production (Selle et al., 2020b). Interestingly, the partial replacement of soybean meal with alternative rapidly digestible sources of amino acids, namely whey protein and non-bound (crystalline, synthetic) amino acids, in wheat-based diets have been shown to enhance broiler performance (Macelline et al., 2020). This, and similar outcomes, emphasise the potential of starch and protein digestive dynamics to manipulate the growth performance of broiler chickens to advantage (Liu and Selle, 2017). Therefore, the objective of the present study was to assess the impacts of fishmeal and sorghum inclusions in diets based on wheat and soybean meal with three dietary CP levels via a Box-Behknen response surface design. The three dietary CP levels were 190, 210 and 230 g/kg, fishmeal was included at 0, 50 and 100 g/kg essentially at the expense of soybean meal and sorghum was included at 0, 150, and 300 g/kg at the expense of wheat.

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 390 off-sex 14-days old male broilers (Ross 308) were randomly distributed into 65 battery cages each of 6 birds (13 treatments × 5 replicates). The cumulative variance of average body weights was maintained at 1.02% between cages. Fishmeal

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(CP = 600 g/kg, Menhaden) and sorghum served as alternative protein and starch sources, respectively, under three dietary protein regimes. The Box-Behnken design comprises

Table 1 Experimental factors and levels used in the Box-Behnken design

Factors	Level (-)	Level (0)	Level (+)
X_1: Sorghum inclusion (g/kg)	0	150	300
X_2: Fish meal inclusion (g/kg)	0	50	100
X_3: Dietary CP (g/kg)	190	210	230

Table 2 Composition and calculated nutrient specification in experimental diets (g/kg)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12	Diet 13
Wheat	392	301	692	608	492	328	392	525	272	276	578	569	502
Sorghum	300	300	-	-	150	150	150	150	300	300	-	-	150
Maize starch	8.69	-	14.9	-	-	150	-	7.50	-	117	-	128	-
Soybean meal	36.1	172	30.7	163	119	20.1	253	91.5	171	59.1	163	52.4	92.9
Canola meal	83.8	100	81.7	100	56.8	100	72.4	100	100	100	100	100	100
Fishmeal	100	-	100	-	100	100	-	-	50	50	50	50	50
Soybean oil	33.6	62.0	36.0	64.7	42.2	52.9	70.0	51.8	58.8	35.2	61.8	37.1	50.2
l-lysine HCl	2.47	3.08	2.48	3.13	0.48	3.30	1.19	5.27	0.73	4.28	0.76	4.32	2.82
d,l-methionine	1.52	2.05	1.25	1.76	1.00	2.25	1.58	2.43	1.27	2.48	0.97	2.23	1.59
l-threonine	1.13	1.06	1.23	1.16	0.33	1.84	0.32	2.06	0.20	1.91	0.29	2.02	1.14
l-tryptophan	-	-	0.02	-	-	0.28	-	0.11	-	0.19	-	0.22	-
l-valine	0.09	0.14	0.27	0.32	-	1.24	-	1.37	-	1.23	-	1.43	0.19
l-arginine	2.66	5.55	2.35	5.16	3.17	3.23	6.11	5.12	4.22	4.36	3.83	4.04	3.75
l-isoleucine	0.25	0.11	0.39	0.26	-	1.27	-	1.31	-	1.23	-	1.40	0.27
l-leucine	-	-	-	-	-	-	-	-	-	-	-	0.79	-
Glycine	4.33	0.47	3.90	0.04	2.48	5.88	-	2.03	0.74	4.19	0.28	3.80	2.18
Salt	-	0.79	-	0.77	0.73	-	1.51	-	1.14	-	1.12	-	0.37
$NaHCO_3$	4.77	4.90	4.68	4.82	3.80	4.78	3.96	5.99	3.73	5.44	3.64	5.34	4.78
Limestone	4.94	1.74	5.04	11.8	4.89	4.78	11.7	12.1	8.01	8.39	8.10	8.47	8.34
MDCP[a]	-	12.4	-	12.3	-	0.36	12.5	13.0	5.44	6.71	5.38	6.68	6.00
Xylanase[b]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Celites	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Sand	-	-	-	-	-	46.8	-	-	-	-	-	-	-
Vitamin mineral px	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Total non-bound AAs	12.5	12.5	11.9	11.8	7.47	19.3	9.20	19.7	7.15	19.9	6.13	20.3	11.9
Nutrient specifications													
ME (MJ/kg)	13.0	13.1	13.1	13.1	13.0	13.1	13.1	13.1	13.1	13.0	13.1	13.0	13.1
Crude protein	210	210	210	210	230	190	230	190	230	190	230	190	210
Starch	440	377	440	377	400	428	340	427	360	462	358	463	406
Starch: protein	2.10	1.80	2.10	1.80	1.74	2.25	1.48	2.25	1.57	2.43	1.57	2.44	1.93
Calcium	8.25	8.25	8.25	8.25	8.25	8.25	8.25	8.25	8.25	8.25	8.25	8.25	8.25
Phosphorous	4.13	4.13	4.13	4.13	4.13	4.13	4.13	4.13	4.13	4.13	4.13	4.13	4.13
Lysine	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Methionine + cysteine	7.40	7.40	7.40	7.40	7.40	7.40	7.40	7.40	7.40	7.40	7.40	7.40	7.40
Threonine	6.70	6.70	6.70	6.70	6.70	6.70	6.70	6.70	6.70	6.70	6.70	6.70	6.70
Tryptophan	1.90	2.14	1.90	2.11	2.20	1.90	2.42	1.90	2.34	1.90	2.32	1.90	2.01
Isoleucine	7.00	7.00	7.00	7.00	7.75	7.00	7.82	7.00	7.85	7.00	7.72	7.00	7.00
Arginine	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4
Valine	8.00	8.00	8.00	8.00	8.00	8.84	8.71	8.00	8.95	8.00	8.79	8.00	8.00
Gly- equivalent	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3
DEB	159	219	150	209	193	135	251	174	226	159	216	150	183

[a]Mono-dicalcium phosphate; [b]Danisco (40000G)

All calculated amino acids are presented in digestible basis

three factors at three levels (Table 1). Thirteen diets based on wheat and soybean meal were formulated to have the same digestible lysine (10 g/kg) and metabolizable energy (13.0 MJ/kg). The diets were cold-pelleted and contained xylanase but not phytase. Dietary compositions and nutrient specifications are shown in Table 2. Birds were offered the same wheat-soybean meal based commercial starter diet (with coccistat, xylanase and phytase) from day 1 to 13 post-hatch and weight gains, feed intakes and FCRs were determined from 14 to 35 days post-hatch. The R 3.5.3 software program was used to plot the surface responses of growth performance parameters, which were fitted by predicted models only with significant terms. JMP Pro 14 was used to perform analyses of variance and to establish correlations when relevant and a 5% probability level was deemed to be significant.

III. RESULTS

The summary of growth performance results is shown in Table 3 and the mortality rate was only 1.28% during the experimental period. Overall performance is comparable to 2019 Ross 308 male performance objectives for weight gain (1912 versus 1849 g/bird) and feed intake (3058 versus 2921 g/bird) and approached the FCR objective (1.601 versus 1.580). The response surface for weight gain is illustrated in Figure 1 where the following equation provides the best fit: $Y = 2981.783 - 43.425X_3 - 31.510X_2$, ($R^2$ = 0.736, P < 0.001). Therefore, both fishmeal and CP had negative impacts on weight gain, irrespective of the inclusion level of sorghum. However, in diets not containing any fishmeal the predicted maximum weight gain of 2157 g/bird was generated by the 190 g/kg CP diets. The response surface for feed intake as influenced by the fishmeal and CP factors is described by following equation, $Y = 13806.511 - 24.72 \times X_2 - 956.755 \times X_3 + 21.334 \times X_3^2$, ($R^2$ = 0.527, P < 0.001). However, dietary CP and sorghum inclusions did not influence FCR and increased fishmeal inclusions linearly increased FCR ($Y = 1.532 + 0.0137 \times X_2$, R^2 = 0.436, P < 0.001). Indeed, the sum of the listed amino acids was quadratically related with FCR, such that feed conversion deteriorated as their concentrations declined as illustrated in Figure 3. Apparent digestibilities of starch, protein and amino acids are being determined to confirm our conclusions.

IV. DISCUSSION

Sorghum inclusions did not influence broiler growth performance; thus, the hypothesis that slowly digestible sorghum starch would improve growth performance was not established. The negative impact of fishmeal on broiler growth performance conflicts with the Sydenham et al. (2017) findings; however, there were very real differences in the basal diets between the two studies such as CP levels, types of feed grain and non-bound amino acid inclusion. In addition, the quality of fishmeal can vary as a result of the rendering process. Interestingly, based on specified values, fishmeal inclusions reduced dietary concentrations of proline, alanine, aspartic acid, glutamic acid and serine in diets. Therefore, it appears that fishmeal inclusions may have triggered deficiencies in certain non-essential amino acids.

Aviagen recommendations for Ross 308 broilers from 14 to 39 days post-hatch is 205 g/kg CP with 10.9 g/kg digestible lysine. Moreover, the transition from 230 g/kg to 190 g/kg CP improved weight gains and feed intakes by 9.5% and 8.2% respectively. This is an outstanding finding because reduced-CP diets will help sustain chicken-meat production. However, fishmeal inclusions in 190 g/kg CP diets had more adverse FCR effects than in the 210 and 230 g/kg CP diets. Significant correlation coefficients for fishmeal inclusions on FCR in 190 g/kg CP diets were r = 0.810 as compared to r = 0.654 and 0.614 in the 210 g/kg and 230 g/kg CP diets, respectively. Reduced-CP diets tend to be inherently deficient in non-essential amino acids; however, fishmeal inclusions may have exacerbated these deficiencies in 190 g/kg CP diets. This may be the possible reason for the pronounced impairment in performance of broiler chickens offered 190 g/kg diets containing fishmeal.

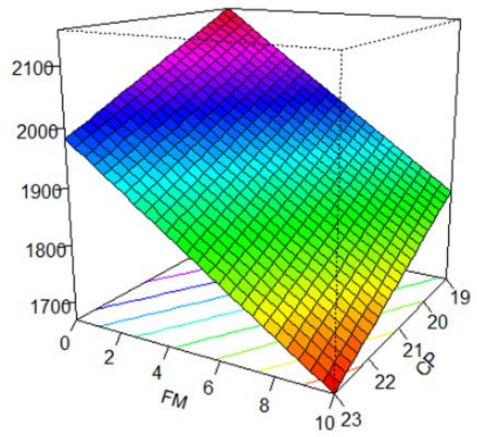

Figure 1 the influence of fishmeal inclusion and dietary CP concentration on weight gain in broiler chickens from 14-35 days post-hatch

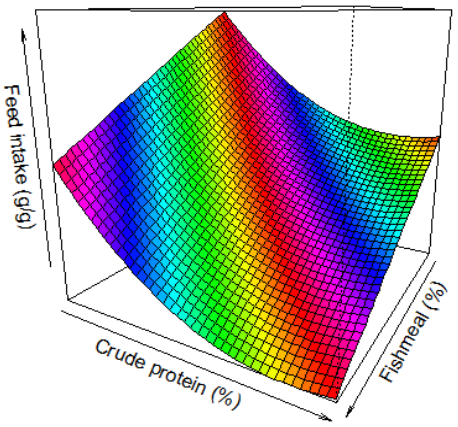

Figure 2 The influence of dietary CP concentration and fishmeal inclusions on feed intake in broiler chickens from 14-35 days post-hatch.

Table 3 Effect of dietary treatments on growth performance

Diet	Feed intake (g)	Weight gain (g)	FCR
1	2883	1764	1.642
2	3160	2053	1.540
3	2783	1682	1.655
4	3144	2063	1.525
5	2889	1678	1.723
6	3158	1875	1.685
7	3128	2002	1.563
8	3270	2140	1.529
9	2934	1800	1.630
10	3129	1953	1.602
11	2901	1857	1.561
12	3267	2064	1.583
13	3024	1925	1.571
SEM	61.5	38.5	0.0278

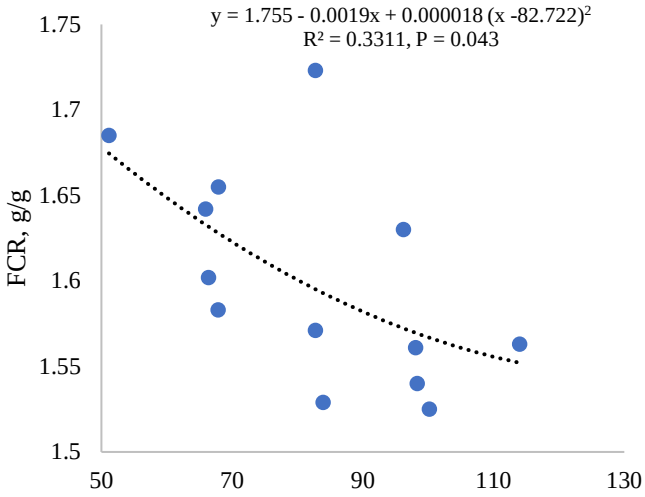

Figure 3. Relationship of dietary non-essential amino acids and FCR from 14-35 days post-hatch

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EFFECTS ON BROILER PERFORMANCE OF INCREASED BRANCHED-CHAIN AMINO ACID INCLUSIONS IN REDUCED-CRUDE PROTEIN, WHEAT-BASED DIETS

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Summary

The objective of this study was to test the hypothesis that elevated leucine inclusions would benefit the performance of broiler chickens offered wheat-based, reduced-crude protein (CP) diets (190 g/kg), provided with concomitant elevations of isoleucine and valine inclusions. The experimental design consisted of a 3 × 3 factorial array of nine dietary treatments comprised of three inclusion levels of digestible leucine (12.71, 15.02 and 17.33 g/kg) and three inclusion levels of combinative, digestible isoleucine and valine (17.21, 20.32 and 23.45 g/kg) which were offered to 378 off-sex male Ross 308 broiler chickens from 7 to 28 days post-hatch. Feed intake increased by 5.23% following the transition from 12.71 to 17.33 g/kg digestible leucine, with a treatment interaction between leucine and isoleucine plus valine observed for weight gain. Isoleucine plus valine concentrations significantly ($P < 0.05$) influenced relative abdominal fat-pad weights with a quadratic relationship ($P = 0.048$) between isoleucine plus valine concentrations. There were no significant differences in FCR and mortality between the dietary treatments.

I. INTRODUCTION

There is widespread interest in the successful development of reduced-crude protein (CP) diets, focusing on alternative dietary strategies to reduce CP levels whilst maintaining acceptable growth performance (Greenhalgh et al., 2020). Reduced CP diets have the potential to provide advantages environmentally by decreasing outputs of nitrogen and ammonia, improving bird welfare by enhancing litter quality and lowering incidences of foot-pad dermatitis and improving flock health by lessening undigested protein entering the large intestine to fuel the proliferation of potential pathogens (Greenhalgh et al., 2020).

Additionally, reduced-CP diets provide economic advantages by lessening feed ingredient costs. Modest reductions in CP are already being realised by inclusions of unbound (synthetic or crystalline) methionine, lysine and threonine, which have been routinely included in poultry diets for decades (Kidd et al., 2013). Of considerable interest are branched-chain amino acids (BCAA); isoleucine, leucine and valine as they are regulators of muscle protein synthesis (Yoshizawa., 2004), accounting for 40% of the dietary essential amino acids in body protein (Ospina-Rojas et al., 2017; Ferrando et al., 1995). Additionally, either isoleucine or valine may be the fourth limiting amino acid in reduced-CP diets, but they may also be antagonised by leucine. BCAA antagonisms in poultry were probably first reported by Mathieu and Scott (1968) and subsequently confirmed by Smith and Austic (1978) and other researchers. While BCAA antagonisms are not thought to be an issue in conventional diets (Waldroup et al., 2002), this may not apply to reduced-CP diets. Characterisation of said BCAA antagonisms include depressed feed intake, poor weight gain and increased FCR (Farran et al., 2003).

The Texas A&M optimal digestible amino acid ratios suggest ratios of 69 isoleucine, 109 leucine and 80 valine relative to lysine (100) (Wu, 2014); however, leucine levels in

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practical broiler diets almost invariably exceed 109 and may be in the order of 130. That requirements for amino acids which are typically present in more than adequate amounts in broiler diets may not be adequately identified was raised by Waldroup (2007) and this caveat certainly applies to leucine.

As an amino acid, leucine is a 'building-block' for protein deposition but when plasma and intracellular levels of leucine exceed the minimum needed for protein accretion, the metabolic roles of this amino acid declare themselves (Harper et al., 1984). Given in sufficient amounts, high concentrations of leucine have been shown to activate mTOR signalling, promoting protein deposition as well as supressing protein degradation in skeletal muscle (Li et al., 2011). Thus, there is the implication that broiler chickens may benefit from dietary leucine inclusions that are higher than standard recommendations. However, it follows that this will probably necessitate higher inclusions of isoleucine and valine to counteract antagonistic BCAA interactions (D'Mello & Lewis, 1970). Therefore, the present study was designed to test the hypothesis that elevated leucine inclusions will benefit the performance of broiler chickens offered wheat-based, reduced-CP diets (190 g/kg) from 7 to 28 days post-hatch provided there are concomitant elevations in isoleucine and valine inclusions.

II. MATERIALS AND METHODS

This study fully complied with the guidelines (2019/1667) specifically approved by the Research Integrity and Ethics Administration of The University of Sydney. The experimental design consisted of a 3 × 3 factorial array of dietary treatments, which were offered to 378 off-sex male Ross 308 broiler chickens from 7 to 28 days post-hatch. The dietary treatments as shown in Table 1 comprised three inclusion levels of digestible leucine (12.71, 15.02 and17.33 g/kg) and three inclusion levels of combinative, digestible isoleucine and valine (g/kg) (17.21, 20.32 and 23.45 g/kg). All nine diets were wheat-based and formulated to contain 190 g/kg crude protein and 11.55 g/kg digestible lysine with an energy density of 12.90 MJ/kg metabolisable energy (ME), and the dietary electrolyte balance was maintained constant at 230 mEq/kg. Weight gain, feed intake, FCR and abdominal fat-pad weights were determined. The experimental data, as a 3 × 3 factorial array, were subject to analyses of variance using the IBM® SPSS® Statistics 24 program (IBM Corporation. Somers, NY). Experimental units were cage means (7 replicate cages of 6 birds per dietary treatment) and probability levels of less than 5% were considered statistically significant by Student's *t*-test.

Table 1- Outline of dietary treatments

Dietary	Digestible concentration (g/kg)				Relative to lysine (100)		
treatment	Leucine	Isoleucine	Valine	Ile + Val	Leucine	Isoleucine	Valine
1A	12.71	7.97	9.24	17.21	110	69	80
2B	12.71	9.41	10.91	20.32	110	82	95
3C	12.71	10.86	12.59	23.45	110	94	109
4D	15.02	7.97	9.24	17.21	130	69	80
5E	15.02	9.41	10.91	20.32	130	82	95
6F	15.02	10.86	12.59	23.45	130	94	109
7G	17.33	7.97	9.24	17.21	150	69	80
8H	17.33	9.41	10.91	20.32	150	82	95
9I	17.33	10.86	12.59	23.45	150	94	109

III. RESULTS AND DISCUSSION

The effects of dietary treatments on growth performance and relative abdominal fat-pad weights are shown in Table 2. A treatment interaction (P = 0.001) between leucine and isoleucine plus valine was observed for weight gain. Weight gains of birds offered diets with digestible leucine contents of 12.71 and 15.02 g/kg declined with increasing isoleucine plus valine concentrations by 8.06% (1311 versus 1426 g/kg; P < 0.001) and 6.82% (1325 versus 1422 g/kg; P = 0.001), respectively. In contrast, at 17.33 g/kg leucine, 23.45 g/kg additional isoleucine plus valine numerically increased weight gains by 2.66% (1427 versus 1390 g/bird P < 0.20), where probability levels are based on pair-wise comparisons. Increasing leucine concentrations significantly (P < 0.02) influenced feed intakes with an increase of 5.23% (2111 versus 2006) following the transition from 12.71 to 17.33 g/kg digestible leucine. These values reflect findings from earlier studies that suggested that inclusions of valine increased efficacy and reduced antagonisms of leucine when included at equally high or higher levels with both leucine and isoleucine as opposed to sub-optimal inclusions. Lower levels of all three BCAA simultaneously have shown no impact, but comparatively fare better than suboptimal inclusions of valine where antagonisms would be more pronounced (D'Mello and Lewis, 1970; Farran and Thomas,1990).

Table 2- Effects of dietary treatments on weight gains, feed intakes, feed conversion ratios (FCR), mortality rates and relative abdominal fat-pad weights from 7 to 28 days post hatch

Treatment		Weight	Feed		Mortality	Relative
Leucine (g/kg)	Iso + Val (g/kg)	gain (g/bird)	Intake (g/bird)	FCR (g/g)	rate (%)	fat-pad weights (g/kg)
12.71	17.21	1426d	2087	1.464	2.39	59.7
	20.32	1364bc	2013	1.476	0.00	48.9
	23.45	1311a	1917	1.461	2.39	50.2
15.02	17.21	1422d	2058	1.447	0.00	58.0
	20.32	1325ab	2028	1.532	4.76	55.1
	23.45	1325ab	2003	1.515	0.00	59.3
17.33	17.21	1390cd	2109	1.512	0.00	55.1
	20.32	1346abc	2047	1.521	2.39	54.4
	23.45	1427d	2178	1.527	0.00	56.9
SEM		18.53	46.30	0.0316	2.100	2.530
Main effects: Leucine						
12.71 g/kg		1367	2006a	1.467	1.59	55.9
15.02 g/kg		1357	2029a	1.498	1.59	59.5
17.33 g/kg		1388	2111b	1.522	0.80	58.4
Isoleucine plus valine						
17.21 g/kg		1413	2085	1.476	0.80	57.5b
20.32 g/kg		1345	2029	1.510	2.38	52.1a
23.45 g/kg		1354	2033	1.501	0.80	55.5ab
Significance (P =)						
Leucine (L)		0.136	0.019	0.130	0.868	0.199
Isoleucine plus valine		< 0.001	0.268	0.429	0.569	0.038
(IV) L × IV interaction		0.001	0.083	0.689	0.416	0.116

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

FCR was not influenced (P > 0.130) by dietary treatments. The low overall mortality rate of 1.32% was not influenced by treatment (P > 0.40). Isoleucine plus valine concentrations significantly (P < 0.05) influenced relative abdominal fat-pad weights. Indeed, a quadratic relationship (r = 0.310; P = 0.048) between isoleucine plus valine concentrations and relative fat-pad weights was found, where:

$y_{(g/kg)} = 240.836 – 18.217 \times (Ile + Val)_{(g/kg)} + 0.4398 \times (Ile + Val)_{(g/kg)}^2$.

It may be deduced from this regression equation that the minimal relative fat-pad weight of 52.2 g/kg would be generated by an isoleucine plus valine concentration of 20.71 g/kg. Reductions in dietary CP often increase bird abdominal fat due to higher starch levels in the diet; however, in a similar study conducted on birds between 21-42 days post hatch, abdominal fat decreased linearly (P< 0.05) with increased valine and leucine (Ospina-Rojas et al., 2017), with leucine decreasing blood triglycerides levels and valine decreasing fatty acid synthesis without stimulating lipid degradation (Ospina-Rojas et al., 2017). The findings of the present study did not meet expected outcomes, with higher inclusions of leucine not altering growth performance in a reduced-CP, wheat-based diet. Further investigation is thus warranted, with adjustments in the inclusion levels of leucine in concomitant with valine and isoleucine to further understand their interactions in the context of reduced-CP diets to improve on broiler performance outcomes.

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SUSTAINABLE CHICKEN MEAT PRODUCTION IS ENHANCED BY TANGIBLY REDUCED CRUDE PROTEIN DIETS

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Summary

In a series of 4 trials using Ross 308 male broilers to 35 days post-hatch, dietary crude protein was, on average, reduced by 39.7 g/kg (from 209.9 to 170.2 g/kg) without compromising bird performance. Notably, in one of the studies broiler performance was maintained with a tangible decrease in dietary crude protein of 57 g/kg and a 66.2% reduction in soyabean meal content, suggesting it is possible to achieve sustainable growth of chicken meat production to meet an estimated 72% growth in demand to 2050. In the context of sustainability, the environmental impact of chicken meat production was assessed using an Australian developed model whereby twelve environmental parameters were included in a single calculated "ecopoint" value. Additionally, as a comparison, six individual environmental parameters utilising European *l'Institut National de Recherche Pour l'Agriculture* (INRA) data, applied to individual feed ingredients were assessed. The environmental impact of soyabean meal is estimated to be approximately three times that of grain and successful implementation of reduced crude protein diets for meat-type chickens supports future sustainability for the poultry sector.

I. INTRODUCTION

Whilst meat-type chicken production compares well with other forms of livestock production systems (Fry et al., 2018) it is nevertheless considered to have a negative environmental impact primarily due to dependence on soyabean meal as the main source of dietary protein (Leinonen and Kyriazakis, 2016; Selle et al., 2020). In a review of comparative life cycle assessment (LCA) de Vries and de Boer (2010) concluded that 1 kg of edible chicken meat production requires between 8.1 and 9.9 m^2 of land, similar to pork (8.9 to 12.1 m^2) and on average 76% less than beef (27-49 m^2). Higher reproduction rates, reduced methane emissions and improved feed efficiencies were identified as the major drivers of these differences. However, reported LCA methodologies are not consistent partly due to the complexity in assigning CO_2 emissions to deforestation for soyabean production, confounded by dynamics of grazing by livestock and logging (Nepstad et al., 2006). More recently, a LCA was conducted by calculating single score "ecopoints" using 12 environmental parameters for Australian chicken meat production on a weighted average annual impact scale of 100 points per person (Bengtsson and Seddon, 2013). These authors combined the life cycle impact of global warming, abiotic resource depletion, land transformation and occupation, water consumption, eutrophication, acidification, eco-toxicity, photochemical smog, ozone depletion, ionizing radiation, human toxicity and respiratory effects into a single score. On average, cooked roast chicken was ascribed 11.9 calculated ecopoints/tonne with feed ingredients identified to contribute 49% (5.83 ecopoints/tonne) towards this value and grains, soyabean meal and meat & bonemeal making up 79% of the feed contribution.

Over the past 10 years approximately 5.6 million hectares of additional land has been required annually to accommodate increasing soyabean production. Furthermore, projected

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increases of 72% in chicken meat production over the next 30 years have been estimated (Alexandratos and Bruinsma, 2012) requiring an extra 121 million hectares globally, based on current levels of dietary soyabean meal inclusion (Chrystal et al., 2020a). Thus, the purpose of this paper is to quantify the differences in ecopoints as described in Bengtsson and Seddon (2013) per tonne of cooked roast chicken between conventional and tangibly reduced crude protein (CP) diets over four broiler studies. Only feeds that resulted in similar broiler performance were selected and assessed for their conduciveness to sustainability of chicken meat production. Additionally, for comparative purposes, individual environmental impact parameters for feed ingredients; cumulative energy demand, phosphorus consumption, climate change (CO_2 emissions) eutrophication and land competition, adapted from INRA data (www.feedtables.com) were assessed.

II. METHODOLOGY

A combined total of 168 off-sex male Ross 308 broilers were used in this comparison from 7 to 35 days post-hatch, comprising 6 birds per replicate treatment over 6, 7 or 8 replicates as described in Chrystal et al. (2020b,c,d,e). Within each study, a conventional (high protein) diet was compared with the lowest selected dietary protein treatment, where broiler performances were statistically similar to the conventional diet. Dietary AME_n averaged 12.90 MJ/kg and digestible lysine 11.15 g/kg across all treatments whilst average CP ranged from 209.9 to 170.2 g/kg (difference of 39.7 g/kg). All diets were steam-pelleted at a conditioning temperature of 80 °C and comprised of either maize-soyabean meal or wheat-soyabean meal, supplemented with non-bound amino acids (AA) to maintain "ideal protein" ratios as recommended by Aviagen, Ross 308 (2019). Concomitant with a reduction in dietary CP, dietary soyabean meal inclusion declined by 44.6% (from 325 to 180 g/kg) and grain inclusion increased by 28.4% (from 549 to 705 g/kg). All feeding studies fully complied with specific guidelines (2016/973) approved by the Animal Ethics Committee of the University of Sydney.

Soyabean meal was allocated 6.7 ecopoints/tonne whilst wheat varied from 0.4 to 0.8 and barley was estimated at 3.1 ecopoints/tonne; surprisingly, canola seed was highest at 8.5, canola meal 5.0 and by deduction, canola oil 3.5 per tonne (Bengtsson and Seddon, 2013). Non-bound, supplemental AA were not individually defined but formed 21% contribution within a normal diet and could thus be proportionately calculated for all diets. Using ecopoints for grain (average 2), soyabean meal and vegetable oil and allocating ecopoints to the balance of the feed, the relative influence of feed differences on ecopoints per tonne of cooked roast chicken was calculated. Thus, the lower the ecopoint value, the lower the total environmental impact. In contrast, applying INRA data and soyabean meal associated with Brazilian deforestation, six environmental impact parameters were individually assessed, emphasising disparity between measures of environmental degradation and depletion of non-renewable resources. Furthermore, INRA assign environmental impact values for only five supplemental AA, requiring assumptions for non-documented dietary AA. Thus, there is a paucity of data on environmental impact of individual AA not commonly used in feeds.

III. RESULTS

Reducing dietary CP resulted in an average reduction of ecopoints/tonne of feed by 21.2% (5.83 versus 4.59 ecopoints/tonne) whilst dietary soyabean meal inclusion declined by 44.6% and grain content increased by 28.4% (Table 1). Interestingly, the largest decrease in calculated ecopoints/tonne (31.4%) was in maize/soyabean-based diets whereby dietary CP was successfully reduced by 57 g/kg (from 222 to 165 g/kg) and supplemented with non-bound AA. In these diets, soyabean meal inclusion declined by 66.2%, from 334 to 113 g/kg and maize inclusion increased by 41.1% from 511 to 721 g/kg (Chrystal et al., 2020b). Average

broiler performance across all treatments compared favourably with Aviagen Ross 308 (2019) performance objectives, whereby weight gain exceeded the breed performance objectives by 7.2% (2067 versus 1929 g) cumulative feed intake increased by 3.1% (3113 versus 3018 g) and feed conversion improved by 3.6% (1.510 versus 1.566 g feed/g of liveweight).

Assuming standard feed contributes 49% towards ecopoints/tonne of roast chicken (Bengtsson and Seddon, 2013), an average reduction in dietary CP by 39.7 g/kg results in a decline of 1.2 ecopoints/tonne, reducing the average environmental impact of roast chicken by 10.1% from 11.9 to 10.7 ecopoints/tonne.

Table 1 - Effects of selected dietary treatments from 4 studies on growth performance from 7 to 35 days post-hatch and calculated ecopoints/tonne of cooked roast chicken

	Diet content (g/kg)				Average growth performance			Ecopoints
Treatment	Grain	Soyabean meal	Vegetable oil	Crude protein	Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Roast chicken (/tonne)
Conventional	549	325	43.0	209.9	2047	3073	1.507	11.9
Reduced CP	705	180	14.9	170.2	2088	3152	1.513	10.7

Surprisingly, when applying INRA data to the diets used in the ecopoints exercise, four out of six calculated environmental impact parameters were higher for reduced CP diets (Table 2). INRA climate change value for L-lysine HCl is 34.7 times higher than maize (10004 versus 288 CO_2 eq/kg) whilst land competition is 377 times higher than maize (0.0377 versus 0.0001 m^2 yr/kg) and further elucidation of these discrepancies is required. Thus, in reduced CP diets, land competition (m^2 yr/kg) was calculated to be 140% higher than standard broiler diets; cumulative energy demand (MJ/kg) 48% higher and similar values of 22% for eutrophication (PO_4 eq/kg) and acidification (H^+ eq/kg). However, on climate change measured as CO_2 eq/kg, reduced CP diets were 11.8% lower and utilised 29.4% less phosphorus (Table 2).

Table 2 – Comparison of individual environmental impact factors on conventional broiler grower diets compared with reduced crude protein diets applying INRA table data (feedtables.com)

Diet parameters		Calculated environmental impact factors					
Treatment	Crude Protein (g/kg)	Cum. energy demand (MJ/kg)	Phosphorus consumption (g/kg)	Climate change (CO_2 eq/kg)	Acidification (H^+ eq/kg)	Eutrophication (PO_4 eq/kg)	Land competition (m^2 yr/kg)
Conventional	209.9	6.407	8.934	941.4	0.01053	43.20	0.0005
Reduced CP	170.2	9.486	6.310	830.4	0.01282	52.49	0.0012

IV. DISCUSSION

Irrespective of the method used to determine impact of chicken meat production on the environment, data reported varies considerably, primarily due to the complexity of calculating relevant data. For example, published values for liveweight broiler emissions range from 2000 to 5480 kg CO_2 equivalent per tonne, with Ingham's average in Australia reported to be 2613 kg CO_2 (Bengtsson and Seddon, 2013), which is in close agreement with 2600 kg CO_2 estimated by Williams et al. (2009). Commonly, feed and the broiler farm are identified as the two main components of the LCA for chicken meat production (Williams et al., 2009; de Vries and de Boer, 2010; Bengtsson and Seddon, 2013; González-García et al., 2014; Leinonen and Kyriazakis, 2016).

In the Australian scenario, based on ecopoints, Bengtsson and Seddon (2013) attributed 29.9% of the environmental impact to cereal grains compared with an equivalent quantity of soyabean meal. In contrast, a Portuguese case study by González-García et al. (2014) regarded both maize and wheat equal and estimated far higher values of 65.4% of the environmental impact of soyabean meal. Presumably geographical distance between country of origin (South

America or USA) and Australia relative to Europe may partly explain these differences. A single ecopoint value is useful in assigning an overall dietary effect on the environment although individual values calculated using INRA data provide absolute measures per LCA category of feed indredients. Importantly, decreasing dietary CP by 30 g/kg is associated with a dietary equivalent CO_2 reduction of 306 kg/tonne of chicken meat (Martin, 2020) and a 14% reduction in N excretion (Kriseldi et al., 2018; van Harn et al., 2019) emphasising the need to include farm effects in environmental LCA of reduced CP diets. Additionally, INRA estimate yields of cereal grains per hectare to be superior to soyabean meal by a factor of 6 with respect to land competition, in close agreement with Williams et al. (2009). This suggests there is less pressure on deforestation with successful development of tangibly reduced CP broiler diets. These data appear to be inadequately assessed in environmental LCA and warrant further consideration. However, despite data inaccuracy, these tools provide new insights into improving sustainability of chicken meat production.

Projected growth in demand for chicken-meat production will increase its environmental impact (Alexandratos and Bruinsma, 2012). However, based on present studies, adopting reduced CP diets with dietary soyabean meal inclusion equivalent to 310 g/kg, without a deterioration in broiler performance, means this demand can be met without reliance on increased soyabean production. Whilst predicted ongoing improvements in efficiency of broiler performance have not been factored into this future scenario, initial outputs from this study suggest that chicken meat is well placed to meet growing demand over the next 30 years in a sustainable manner.

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ALTERNATIVE METHODS OF FEEDING LAYERS

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Summary

The laying hen nutritional requirements not only change significantly as the birds age during egg production, but also from morning during yolk and albumen formation to the outer egg shell deposition in the afternoon and evening. While the conventional supply of a single complete diet during each 10-20 week laying phase is producing very high egg production, there are still concerns about over-consumption of the diet leading to over-sized eggs and also problems with egg-shell quality in late lay. The research outlined in the current paper suggests alternative feeding methods which blend different diets, offer more choice, or probably most importantly split the feed into morning and afternoon diets, improve efficiency, optimize production and significantly increase egg shell quality. The improvement in modern automated feeding technology can potentially be integrated into modern layer operations to lower the cost of egg production and also improve the welfare of the bird.

I. INTRODUCTION

The current system of feeding laying hens with a fully mixed diet, that attempts to meet all the nutrient requirements of the birds, only really developed with the introduction of cage housing facilities after the Second World War. Research on confining laying hens to cages ramped up in the first half of the 20th Century in California but it was not until the late 1940s that the first commercial cage farms started to appear (American Egg Board, 2020). Nutrition research increased during the 1940s with development of the concept of metabolisable energy. Energy requirement and the concept of protein requirement for layers were more fully developed in the 1950s (Elwinger et al, 2016).

Feeding techniques, even for the small flocks of commercial hens, prior to confinement housing, largely involved the hens scavenging around the farmyard, with supplementary feeding comprising scattered grain and some shell grit (American Egg Board, 2020). In British egg farms, as flocks increased in size, hens were confined for the winter and were fed a warm wet mash in the mornings and grains in the afternoon (Henuk and Dingle, 2002). Up to the end of the first half of the 20th century most laying flocks experienced some form of choice feeding.

Once the cage system of feeding became established, fully formulated and processed feeds were the most convenient and consistent way of achieving high production. However, with the re-emergence of alternative housing methods designed to improve bird welfare, attention has again been drawn to providing the hen with more efficient ways of feeding. A number of methods have been tested and all require birds to have some degree of choice over their diet. A further motivation has been to explore alternative methods of calcium delivery to extend the productive life of the flock (Molnar et al, 2017).

II. ALTERNATIVE FEEDING METHODS

Apart from fully formulated mixed diets there are three main options for varying the delivery of nutrients to laying hens. All methods need to consider the provision of a source of calcium (Ca) in the appropriate form at the required time of day.

- Choice feeding involves offering the hens an energy source (typically whole or cracked grain), a protein concentrate and a source of Ca (typically coarse limestone or shell grit). Fine limestone may be included in the protein concentrate.

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- Loose mix feeding is a fully formulated diet where the grain portion of the feed is offered as whole grain. Fine and coarse Ca are supplied as part of the total mixed diet. It may be included in a sequential feeding program.
- Sequential Feeding requires more than one feeding time for the hens - usually early morning and afternoon - such that different nutrients are supplied at different times of the day when the birds require it.

These methods will be considered in more detail with emphasis on their suitability for delivery by mechanised methods.

III. THE IMPORTANCE OF TRAINING THE HENS

A number of studies have reported on the importance of hens getting used to alternative methods of feed presentation. Forbes and Corvasa (1995) reviewed the literature on diet selection, including whole grain feeding, and emphasized that prior experience, training the flock during pullet rearing and social interactions all affect the outcomes of feed choices. Later workers picked up on this and included it in their experimental protocols (Umar-Faruk et al, 2010a).

Because whole grain is visibly different to meals, in mash feed it will be obvious (and attractive) to hens. Hens will naturally pick it out. Commercial experience has shown that naive hens will pick whole particles of grain or grain legume out of mash or pelleted feed to the detriment of production. The same is true for coarse limestone particles; hens pick them out of mash feeds. However, hens have been demonstrated to have a specific appetite for calcium which tends to regulate this feeding behaviour (Classen & Scott 1982; Taher et al, 1984). The essence of whole grain feeding is to get the hens to self-regulate their intake to meet their energy needs without over-consumption.

Broilers take about 10 days to learn to accurately select feeds during choice feeding (Cumming, 1984) so layers will take some time as well. The 2 week pre-lay period has been used for this purpose in some studies (Umar Faruk et al, 2010a).

IV. CHOICE FEEDING

Choice feeding implies offering the hens a choice between an energy source, a protein/mineral concentrate and a source of Ca. Because these have to be offered separately, it also implies the availability of a number of containers or troughs for feeding.

This method was discussed as a possibility for feeding layers before 1920, essentially because it reflected the natural feeding behaviour of chickens. Kempster (1916) published an experiment in which a variety of different feeds were offered to laying hens and the weights of those chosen were recorded. The author noted then the importance of offering the hens limestone or shell grit as a source of Ca. More recent work has shown the economic feasibility of choice feeding on a commercial scale (Leeson and Summers, 1979 Cumming, 1984; Henuk and Dingle, 2002).

The effects of free choice feeding have been reviewed again more recently (Molnar et al, 2018). These authors put together a table summarizing some of the earlier experiments with choice feeding. In all cases egg production was unaffected and feed conversion (FCR) was uniformly improved even though other parameters varied in their response.

The main drawback to commercial implementation is the requirement for multiple feed troughs or containers to keep the offerings separate and allow the hens to choose. A blending system for feeding into a single trough cannot, by definition, support choice feeding so this review will concentrate on those systems that can be delivered in a single trough or pan. Loose Mix feeding and Sequential Feeding satisfy this criterion.

Table 1 Effect of free-choice feeding on performance traits compared to conventional feeding[1].

	Performance traits							
Reference	**Egg prod.**	**Egg weight**	**Egg mass**	**Feed intake**	**Energy intake**	**Protein intake**	**Ca intake**	**FCR**
Karunajeewa (1978)		↑		↓				↓
Leeson and Summers (1979)	=	↑		↓	↓	↓	↑	
Farrell *et al.* (1981)	=	=		↓	↓	=		↓
Tauson and Elwinger (1986)	=	↑	↑					
Chah and Moran (1985)	=	=		=	↓	↓	↓	
Olver and Malan (2000)	=	↑		=	↓	↓	↑	↓

↓: represents a significant decrease ↑: represents a significant increase
=: no significant difference, no sign: parameter is not reported

V. LOOSE MIX FEEDING

As a result of the success of feeding whole grain to broilers in northern Europe, the possibility of including whole grain into layer feed was also considered. The reason for this was to lower production costs because the grain did not have to be rolled or ground. Loose mix feeding was considered as an alternative to choice feeding because it could all be done in one feed trough and was adaptable to all conventional houses (Noiret et al, 1998).

Published examples on loose mix feeding are limited but one early study (Blair et al, 1973) showed that hens reduced energy and protein intake but maintained similar egg production and egg weight when a mixture of whole wheat, whole barley and kibble maize was fed. Later studies found contrary results. Bennett & Classen (2003) fed 2 strains of hens a mash diet with barley as the sole grain, either in ground or whole form and with or without insoluble grit. In the birds fed whole barley, egg production was reduced, feed intake was increased, egg specific gravity fell and body weight gain was increased. There was no difference in response to feeding insoluble grit and no difference between the 2 strains of hens. One reason for the decline in egg production is the absence of an appropriate beta glucanase enzyme to reduce the gut viscosity in the whole barely.

Oaurt et al (1986) fed various particle size wheat and corn to white leghorn hens and showed egg production was independent of grain type or particle size. However, in treatments with large particle size corn or with whole wheat, egg specific gravity was reduced. The authors speculated that this was due to the hens selecting the large grain particles in preference to the fine meal which contains the calcium source. The particle size of the limestone was not specified. One factor that could have impacted this experiment is that there was no mention of training the birds by exposing them to large particle size corn or whole wheat.

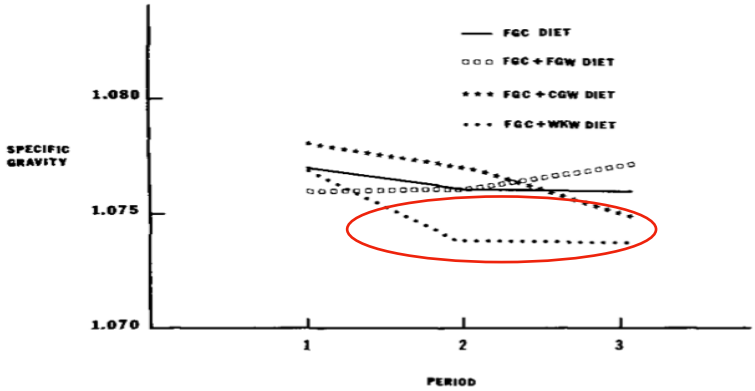

FIG. 1. Period × diet interaction means for specific gravity. Diets are: FGC = 100% fine ground corn; FGC + FGW = 49% fine ground corn plus 51% fine ground wheat; FGC + CGW = 49% fine ground corn plus 51% coarse ground corn; FGC + WKW = 49% fine ground wheat plus 51% whole kernel wheat.

Figure 1 - Reduced egg specific gravity when fed whole wheat. Ouart et al (1986)

VI. BLEND FEEDING

Larger egg layer operations typically offer 2 to 3 diet regimes. Ideally the diets offered are an early layer, peak layer diet and a late lay diet (Hyline, 2018). The diets vary in energy (AME), essential amino acids and calcium/mineral content, depending on the birds age and feed intake, to meet the birds changing demands.

Each diet change is significant in the specifications and also raw material content, and can potentially disrupt feed intake and health health/microflora profiles. An experiment was designed to investigate the difference between the normal three diet program, changing at a specific time, compared with gradually blending in the diets to provide a more gradual change in dietary specifications and contents, which aimed to match the birds gradually changing metabolic and maintenance demands.

The study was conducted at the SCOLEXIA SCARF Attwood research centre (Melbourne Australia) in 2012, compromising 172 Hyline hens starting at 25 weeks of age with an average egg production of 97% (Scott, unpublished). Birds were housed in individual cages in an environmentally controlled shed. The control birds were offered an early layer diet for 8 weeks (change over at 33 weeks of age), mid or peak lay diet for 8 weeks and late lay diet until the study end with the birds at 55 weeks of age. The birds on the blended diets (conducted automatically by a Feedlogic Standard mixing and delivery unit) received a blending diet change every 10 days, blending two diets at the one time to more accurately match the birds' requirements. As the birds on the blending diets aged, the blends of the different diets changed, to where for the last 10 days the birds received just the late lay diet.

Individual bird weight was unaffected by the treatment. There was no significant difference in egg production; however the birds on the blended diet exhibited numerically higher egg numbers of 6 eggs per bird. The number of cracked or damaged eggs was reduced from 572 in the control birds down to 222 egg for layers on the blending treatment ($P<0.001$) and this correlated with an increase in egg shell thickness ($P=0.024$). There was also a significant reduction in egg weight of 2 grams per egg ($P=0.041$) for birds on the blending regime.

It was concluded that the more precise delivery of dietary energy, amino acid and mineral requirements enables the bird to produce a significantly better quality, smaller egg. The researchers

suggested in non-blended diets (control), there was a over consumption of essential protein and energy, which produced a larger, potentially more fragile egg. The reduction in protein and energy consumption in the blended diets was evident due to a significant reduction in the amount of the early lay diet for birds on the blended diets, which also reduced the diet cost by 5% (19 cents per bird). Therefore, the blending of the different diets improved production measures and also reduced the cost of production of eggs.

VII. SEQUENTIAL FEEDING OR AM/PM FEEDING

Sequential feeding, Split feeding or AM/PM involves feeding a different diet in the morning to that fed in the afternoon. It was found that white leghorn hens fed a low (13%) protein corn/soy diet in the morning (0800-1400) and a high (16%) protein diet in the afternoon (1400-0800) had similar egg production and a similar egg size to hens fed the 16% protein diet alone (Penz and Jensen, 1991). This led to the conclusion that more protein is required when the birds are synthesizing albumen than at oviposition. Further, it provided an advantage over feeding a high protein diet for the whole day because the cost of the overall daily feed intake was lower. In other reports, energy, protein and Ca supply were varied during the day. De Los Mozos et al. (2012a) looked at the effect of feeding a diet high in ME (2900 kcal/kg) and protein (18.5%) and low in Ca (1.6%) in the morning from 2 hr before egg laying until 2hr, 4hr, 6hr, 8hr & 10hr after. From that time until 2hr before oviposition the next day, the hens were fed a diet low in ME and protein (2323 kcal; 13.3% CP) and high in Ca (4.5%). In hens fed the high Ca/low protein/low energy diet from 6 hr after oviposition compared to the control, hens on the split feeding regime consumed less energy (5%), less protein (13%) and less Ca (10%) but overall feed intake was similar. Further, despite consuming less Ca, eggshell quality parameters and egg weights were not affected. In a second experiment, this group looked at further reducing the crude protein of the afternoon diet (De Los Mozos et al., 2012b). Results showed that, while a smaller reduction of crude protein from 17% to 15.5% improved FCR, it had no effect on egg production. However, further reducing the crude protein of the afternoon feed to 14% protein made FCR significantly worse and numerically reduced egg weight.

Other approaches involved testing sequential feeding and feeding loose mix feed in the one experiment under different housing conditions (Umar Faruk et al., 2010a, 2010b). It is important to note that this experiment included a 2 wk feeding period before point of lay to allow the birds to get used to whole grain in their diet. During this period, the feed intake for the 3 feeding systems was about the same. In the first experiment, hens housed in groups of 5 birds per cage were fed either a complete mash feed or a protein/mineral/grain concentrate plus whole grain, either in a loose mix or sequentially as shown in Figure 2.

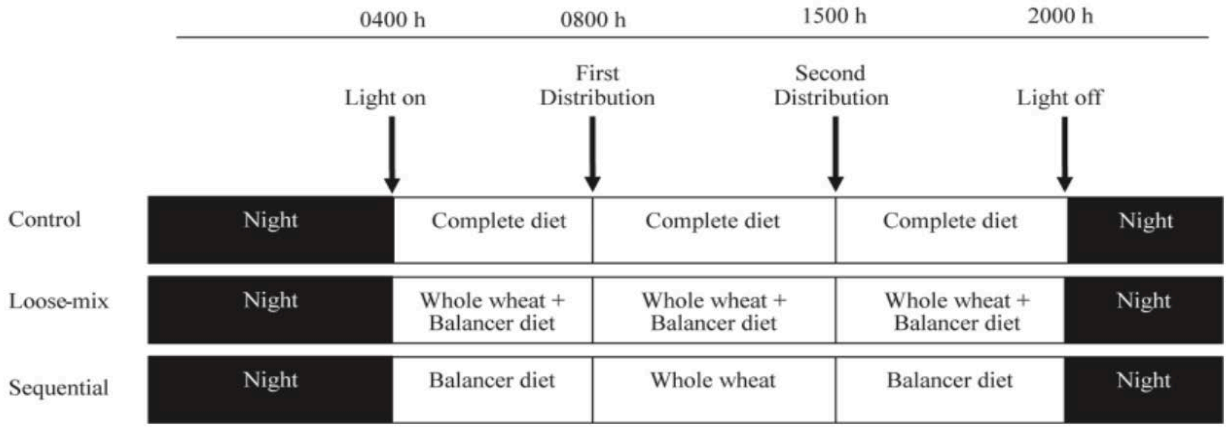

Figure 2 - Feeding and lighting regime from Umar Faruk et al (2010a). Whole wheat made up 50% of the grain portion of the loose-mix and sequentially fed diets

In the laying phase, which ran from 18 wk - 46 wk of age, the sequentially fed birds responded differently to the control group and to the birds fed the loose mix diet. The control diet (a complete mixed mash: 2,750 kcal of ME/kg and 17.5% CP) was offered in 2 equal portions of 60.5g per bird. What was not eaten was cleaned out of the feeders and weighed just before the afternoon feed and the same thing was done before the next morning feed. The loose mix feed, with whole wheat as 50% of the diet, was offered in a similar way to the control: 2 equal portions am and pm with feed remaining weighed each time. The sequential feeding was completely different: the whole wheat was offered in the morning and any remaining removed and weighed before the afternoon feed, which consisted entirely of Balancer (2,380 kcal of ME/kg, 23% CP, and 7.2% Ca). The results were analysed over 3 periods; Pre-Peak 19-26 wk; Peak 27-37 wk; Post-Peak 38-46 wk. The highlights were:

- Total feed intake was significantly lower in the Sequential feeding treatment
- Whole wheat intake was significantly lower in the Sequential compared with the Loose Mix treatment.
- As a result, FCR was significantly improved in the Sequential treatment compared to Control and Loose Mix treatments.
- There were no differences between treatments in Egg Production, Egg Weight or Egg Mass.
- Birds were small group housed (5 birds per cage).

In a second experiment (Umar Faruk et al., 2010b) these authors introduced a treatment where birds were offered (in Loose Mix or Sequential Fed) a diet designed to contain 25% whole wheat plus a balancer supplying the other 75% of the nutrients. Further variations were that the birds were housed in single bird cages and the diets fed ad libitum. In this experiment, the egg laying performance was recorded over a 23 wk period from 19-42 wk of age. In the 3 wk pre-lay period (16-18 wk) 2 groups of birds were habituated to whole wheat by sequential feeding of whole wheat (morning) and a concentrate balancer (afternoon) or ad libitum feeding of a Loose Mix of 35% whole wheat: 65% balancer. A control group was fed a conventional completely mixed mash diet (16% protein, 11.7 mj/kg ME, 1.2% Ca). All groups were fed ad libitum by increasing the time of daily access to feed to allow intake to increase.

From 19-42 wk the birds were assigned to 5 treatments:

1. Control: conventional layer diet ad libitum fed (17.5% protein, 11.5 mj/kg ME, 3.61% Ca).
2. Loose Mix 25 (25% whole wheat: 75% balancer concentrate).
3. Loose Mix 50 (50% whole wheat: 50% balancer concentrate).
4. Sequential 25 (25% whole wheat, fed morning: 75% balancer concentrate, fed afternoon).
5. Sequential 50 (50% whole wheat, fed morning: 50% balancer concentrate, fed afternoon).

In the habituation period feed intakes and body weight gains were about the same for all groups. The Sequential fed birds increased their daily whole wheat intake from 16g/d in wk 16 to 40 g/d in wk 18, demonstrating their acclimatization to whole wheat feeding. Balancer intake decreased across the same period from 51.6g/d to 40 g/d indicating the birds were able to balance their diet successfully.
In the experimental period significant effects were:

- Feed intake was not different from Control (C) diet for Sequential fed (SF) birds.
- Feed intake for Loose Mix fed birds was significantly lower that SF birds ($P<0.01$).
- Whole wheat intake was significantly higher in Loose Mix fed birds than SF birds ($P<0.01$)
- Egg production was significantly higher in Sequential fed birds than Loose Mix fed birds ($P<0.05$) and not different from the control (94.8% SF: 94.6% C).
- Egg weight was significantly higher by 2.3g in SF birds than Loose Mix fed birds ($P<0.05$) and numerically higher but not different from the control (58.3g SF: 57.3g C).
- There was no significant difference in body weight gain between Control and SF birds but both gained more weight than the Loose Mix fed birds ($P<0.01$).
- There was no difference in FCE (g egg/g/feed) between treatments.

The implication of these two experiments is that the birds fed in groups (albeit small groups) did better on Sequential feeding that birds housed singly. This would certainly suit non-cage housing systems but would equally work in cage systems with multiple birds per cage.

VIII. SEQUENTIAL FEEDING FOR OLDER HENS

A consequence of changing welfare regulations on commercial egg production is the likelihood that force molting will not be allowed in future. In response to this, the major breed suppliers have selected for birds that will produce in a single cycle to 100 wk of age (Hy-Line 2018). It is well established that feeding hens coarse limestone in conventional diets leads to better shell strength (Koreleski & Swiatkiewicz, 2004) and improved bone strength late in lay (Saunders-Blades et al, 2009).

The effect of sequential feeding of different ratios of fine and coarse limestone was tested on egg shell strength and bone integrity in old laying hens (Molnar et al, 2017). These authors fed Lohmann Brown hens, aged 72-83 wk and housed in cages (7 birds/cage). A 2 wk pre-experimental feeding period was also employed in this work when the hens were fed a corn/soy standard laying diet containing 4.25% Ca with ratio of 30:70 fine to coarse particles. Internal and external egg quality was recorded to provide a baseline for later treatments. Treatments with a range of limestone particle size ratios were offered to groups of hens.

The light period was from 04:30 to 20:30 and the morning feed was distributed at 07:30 and the afternoon feed at 14:30. All birds were left with the afternoon feed until the morning distribution was made at 07:30. The ‘C’ treatments were offered all their feed in the morning (126g/bird) with the ratios of fine to coarse limestone shown in Table 2. The ’S’ treatments were offered 58 g/bird at the morning feed of a basal diet supplemented with 20% whole wheat. In the afternoon, each diet fed had a different ratio of fine to coarse limestone as shown in Table 2. It is important to note the morning diet for the ’S’ treatment was a low Ca diet (0.73%) so these birds are taking in the bulk of their Ca in the afternoon feed.

Calcium intake in this experiment is very interesting and different between the birds fed the conventional ‘C’ diets and the sequential fed ’S’ treatments (Table 2).

Table 2 - Daily Ca and P intake of laying hens (73 to 83 wk) per treatment. Adapted from Molnar et al, (2017).

Feed Distribution	Coarse to Fine Limestone ratio	Ca Intake (g/d)	Phosphorus intake (g/d)
C	50	5.44^{e}	0.648^{e}
C	70	6.00^{d}	0.741^{d}
C	100	6.06^{c}	0.705^{c}
S	50	6.52^{a}	0.712^{b}
S	70	6.53^{a}	0.698^{d}
S	100	6.18^{b}	0.694^{d}
	Pooled SEM	0.011	0.001
	P-value	<0.001	<0.001

Columns with different superscripts are significantly different (P<0.05).

In conventionally fed birds, Ca intake increased with the inclusion rate of coarse limestone. In the birds fed sequentially, Ca intake was maximized at 50% coarse limestone but was also significantly higher than the Ca intake of the conventionally fed birds. The ’S’ 70% coarse lime treatment had the lowest cracked egg percentage over the experimental period but the crack percentage of groups before the trial started was not reported. Cracked egg percentages were variable and not consistent between treatments. The treatments with the lowest cracked egg percentages over the whole trial were ‘C’ 50CL and ‘S’ 70CL. Why this was the case was not apparent and was not commented on by the authors. Importantly, over the whole experimental period, birds from the ’S’ groups ate significantly less feed than the conventionally fed birds (Figure 3).

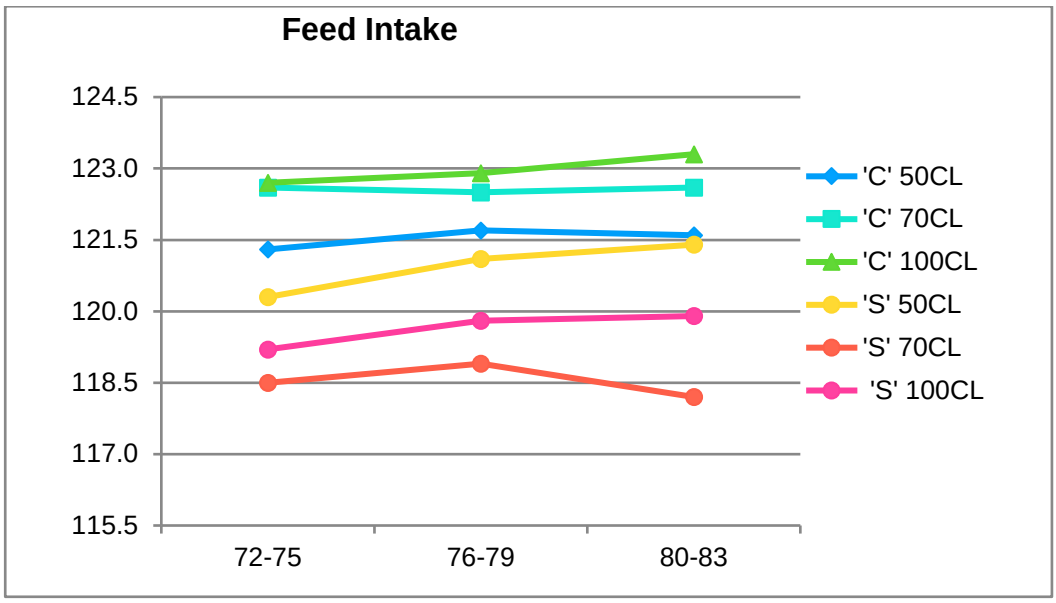

Figure 3 - Daily feed intake (Adapted from Molnar et al, 2017).

Morning and afternoon feed intakes were not reported directly but it was mentioned that 'S' treatment hens consumed about half their feed in each feeding period whereas the 'C' treatment hens consumed more feed in the morning.

Rate of lay declined in all treatments and, although there were some significant differences within each time period, when looked at over the whole period of the experiment, it was hard to see any trends that may have favoured any treatment or feeding system (Figure 4). The one treatment that seemed to come out worst was 'S' CL100. This treatment also had the highest overall incidence of cracked eggs and was significantly worse in all 3 periods than most other treatments. The coarse limestone was uniformly coarse with only about 6% of particles less than 1mm.

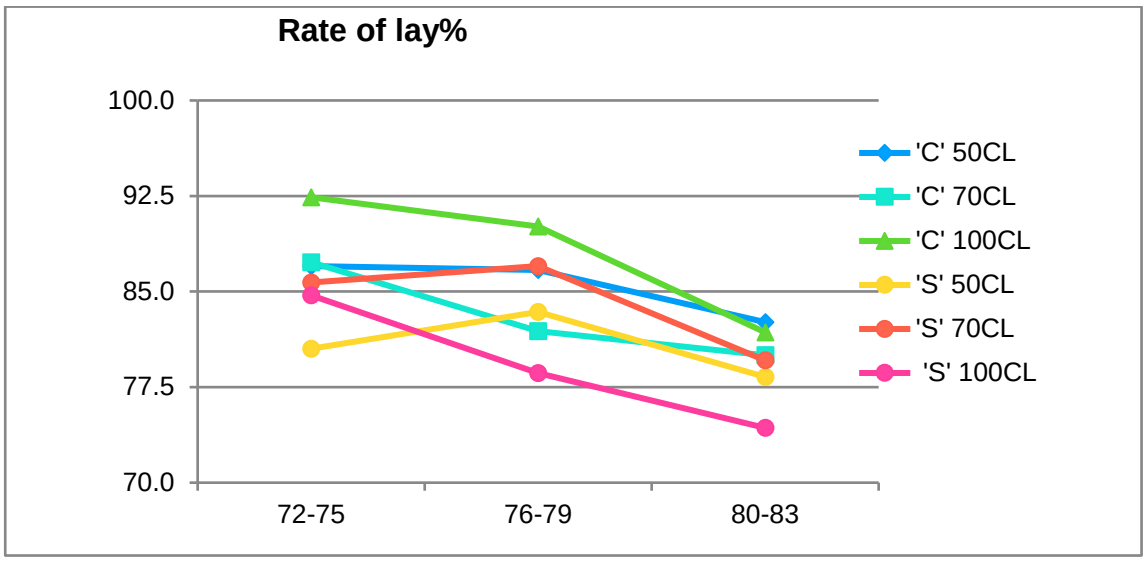

Figure 4 - Rate of lay (Adapted from Molnar et al, 2017).

In a series of studies by Trouw Nutrition in Belgium between 2015 and 2018 that involved 2,800 broiler breeders (https://www.feedstuffs.com/nutrition-health/split-feeding-scheme-broiler), researchers compared the reproductive and physical performance breeders receiving a split-feeding program in the morning and afternoon to a group of commercial hens fed a regular broiler breeder diet to hens. The split-feeding or AM/PM program was designed to provide a more accurate nutrient

supply, according to the egg formation need of breeders. This dietary strategy is similar to that tested in the previous mentioned studies in egg layers, which provided less crude protein (CP), apparent metabolizable energy (AME) poultry, calcium and digestible phosphorous compared to the control diet. The key findings of the research by the Trouw Nutrition group studies include:

- Increased egg production in birds fed a split-feeding regimen compared to the control group, resulting in higher total and hatching eggs, as well as higher chick quality and subsequent growth.
- A significantly lower feed cost for birds fed the sequential or split-feeding system compared to birds receiving the control diet, and
- Birds receiving the split-feeding program demonstrated improved feathering, reduced pecking and showed fewer behaviors indicative of hunger.

This research was further supported by three large commercial evaluations involving 122,600 breeders, using both Ross and Cobb genetics. This demonstrates the advantages of sequential or split feeding across all layer and broiler breeders as well as commercial egg laying flocks.

IX. COMMERCIAL IMPLICATIONS AND SOLUTIONS

Of the 3 systems that involve some form of choice feeding, only Loose Mix and Sequential feeding make sense if applying them to existing sheds. They could both be adapted to all current Australian housing systems: cage, barn and free range, aviary or flat floor. Both should work with trough or pan feeding. Although feeding whole grain was considered in most of the experiments reported, it is not a pre-requisite. However, if it significantly contributed to the economics of production, there is ample evidence that current laying strains can be readily adapted to it.

Taken together, the experiments above indicate that SF is certainly feasible but its major drawback remains the accurate distribution of feed in the morning and afternoon. That is where an automated feeding system capable of accurately weighing feed is essential to make it work. Industry experience indicates that a number of egg producers have been interested in it over the years but did not have the means to make SF work. As the data presented by Molnar et al. (2017) indicated that sequential fed birds ate about 50% of their feed in the morning and 50% in the afternoon, it is relatively easy to allocate feed, given that the producer has good flock feed intake data for the sheds. Most of the bigger players have those data. The setting of the feeding times is also important and 07:30 to 08:00 for distribution of the morning feed and 14:30 to 15:00 for the afternoon feed would fit in with the work practices on most farms.

Any investment must return a profit, so quantifying the advantages of SF is necessary to convince producers to invest in new equipment. The improvement in feed intake is likely to be the best way to demonstrate a payback. In the experiment reported by Umar Faruk et al. (2010a), feed intake was reduced by 7g/bid per day over the course of the experiment for the same egg production, egg weight and mortality. The advantage of SF over conventional feeding is that, with the latter, the birds need to over-consume feed to match the metabolic needs of albumen formation and shell formation which occur at different times of the day.

To make it easy to visualize the economic advantage, an average feed cost of $510/t was assumed. The savings over laying periods of 62 wk (18-80 wk) and 82 wk (18-100 wk) are shown in Table 3.

Table 3 - Feed cost savings for flocks of various sizes for a weighted average feed cost of AUD$510/t.

	Feed intake/bird g/d	Flock longevity (weeks)	Feed/bird/period kg	Flock size 15,000	25,000	50,000
Conventional	115	62	49.9	749	1248	2496
Sequential	108	62	46.9	703	1172	2344
Difference	7		3.0	46	76	152
Conventional	115	82	66.0	990	1650	3301
Sequential	108	82	62.0	930	1550	3100
Difference	7		4.0	60	100	201
At $510/T average feed cost		62		$23,241	$38,051	$77,469
		82		$30,738	$51,230	$102,459

Feed savings per flock are potentially substantial depending on the reduction in feed intake actually achieved in practice. However, the projections shown should be attractive to farmers with rapid paybacks available, depending on the price of the blending equipment.

The other important element of this discussion is how to best formulate and implement a feeding program that will reproduce the feed savings shown in the research situations. It is clear that two bins with different feeds that can be delivered to the birds in the morning and afternoon will be necessary. The normal situation is one feed bin per house. However, if houses are close together, it may be possible to place 2 bins, each holding a different feed, between 2 houses, meaning that extra bins may not be required but some will have to be moved. Of course, if houses are spread out this might not be possible. Many older farms have houses relatively close together so extra bins might not be a major disincentive on these farms.

Formulation approaches can be flexible. As most diets currently have a mix of grains, due to the price structure of the current market, a whole grain morning: concentrate afternoon approach might not be the best way to go. It is not clear how the birds would take to a mix of whole grains and whether one would be preferred and the other rejected. However, a mix of coarse cracked grains in the morning with a concentrate in the afternoon might work. The research reviewed above supports using a mix of coarse and fine limestone and this is compatible with all mash feeding systems.

The other complication that needs to be addressed is the variation in the nutrient requirements of hens over the production cycle. Young birds with low feed intake will require a feed with a higher concentration of energy and amino acids than birds at the end of the program. With a 2 bin feeding system it may possible to run the afternoon feed with high calcium as a constant formulation whilst varying the nutrient density of the morning feed.

On balance, it is suggested that the approach of De Los Mozos (2012a) is worth considering. In this experiment the morning feed was high in ME and protein and low in Ca compared to the afternoon feed. Because it will be required to start this program at 16 wk of age with newly placed pullets, making the morning feed as a high nutrient density diet with Pre-Layer levels of Ca and available phosphorus would work well until first egg. In the Pre-lay period (16-18) wk of age the same diet can be fed morning and afternoon to both to train the birds and meet their nutrient needs. Equal portions of feed can be offered morning and afternoon and this period can also be used to learn the flock's consumption pattern. At first egg the afternoon feed can be changed to the high Ca balance with lower ME and amino acids.

XI. CONCLUSIONS

There are significant advantages of SF, choice feeding or blending of current mash diets compared to the current strategy of providing one specific diet to the bird at time. While commercial diets in mash form do offer some choice to pick out individual components, the alternative feeding methods mentioned in this paper, SF and feed blending in particular, have been shown to reduce the over-consumption of protein and energy and producing more ideal Ca intake through the layers life. This, in turn, improves feed efficiency and increases egg quality.

Choice feeding will be difficult to implement into the commercial shed designs; however SF or the blending of diets can be incorporated into new buildings or retro-fitted using the current feeding lines and pans. The performance benefits potentially create a rapid rate of return on the installation of alternative automatic feeding systems.

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MATERNAL STRESS, THE POTENTIAL IMPACT ON BROILER BREEDERS AND SUBSEQUENT CHICK DEVELOPMENT

R.E.A FORDER[1] and M. LANE[1]

Summary

Managing stress in broiler breeders has been a continual topic of discussion since the early 1980's, but our understanding of stress and its influence on gastrointestinal health and reproductive performance in poultry has only recently received attention. Evidence from both mammalian and ecology literature, in regards to maternal stress and developmental programming, highlights the need for further investigation into the physiology and behaviour of hens during lay; to find novel strategies to alleviate stress and in turn potentially improve welfare and production outcomes of both the hen and her progeny.

I. INTRODUCTION

The chicken meat industry has made tremendous production gains through advanced utilisation of genetic selection and nutritional understanding to ensure that chicken meat remains a low cost, desirable product that consumers continuously demand (Zuidhof et al., 2015). The industry now requires new approaches to further advance efficiency and production, with developmental programming at the forefront of industry development (Hynd et al., 2016). Developmental programming is defined as "alterations in the *in ovo* environment, induced by the maternal environment, resulting in developmental adaptations that permanently change the structure, physiology, metabolism, health and production of the offspring".

Environmental conditions provided during gestation/egg formation, primarily involving stress and nutrition, have the capacity to contribute, enhance or disrupt programming of physiological mechanisms regulating progeny growth, health, behaviour and production (Reynolds et al., 2010). Developmental programming can be affected by environmental factors, such as stress and nutrition, which compromise the maternal environment experienced by developing offspring, resulting in permanent physiological alterations to offspring phenotype, further impacting their health and productive performance (Hynd et al., 2016). Physiological alterations can be induced directly through altered organ development and disrupted endocrine axes development (Henriksen, et al., 2011b), which may be mediated through epigenetic effects, with potential trans-generational impacts (Chango and Pogribny, 2015). Additionally, the microbial environment housed by the mother is generating enormous interest through its potential to contribute to environmental factors influencing the maternal environment, via the brain-gut-microbiota axis and its potential influence on developmental programming mechanisms.

Progeny exposure to maternal stress during early development can change an organism's microbiota composition, and the microbiota can alter the organism's ability to respond to stress after birth (Hechler, et al., 2019; Jasarevic, et al., 2017). Recent findings from our group have demonstrated that maternal stress in broilers can have significant negative effects on progeny body weight, stress-linked behaviour, immune response (Bowling, et al., 2018) and body composition (Angove, et al., 2020). The extent to which alterations to the intestinal environment, including microbiota, affects the programming of gut-brain signalling pathways of both the hen and her progeny, is an area of great interest. Considering commercial chicken meat birds now spend ~40% of their life *in-ovo*, the influence of breeder hen rearing

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practices on progeny development, especially through maternal stress, may provide a pathway to improve production aspects through nutritional manipulations of already well-established industry protocols. Thus, there is substantial opportunity to optimise breeder hen health, reproductive capacity and welfare standards, all of which will improve industry profitability, as well as community perception of the chicken meat industry.

II. BRAIN-GUT-MICROBIOTA AXIS

The intimate interaction between the central nervous system, the gastrointestinal tract and the residing microbiota, coined the *brain-gut-microbiota axis* has been extensively reviewed in the literature (Al-Asmakh, et al., 2012; Carabotti, et al., 2015; Kelly, et al., 2016b; Powell, et al., 2017). Bi-directional communication exists between the gastrointestinal tract and the central nervous system that not only ensures the proper maintenance of gastrointestinal homeostasis and digestion but is likely to have multiple effects on higher cognitive functions (Mayer, 2011). Studies on GF animals have demonstrated that the gut microbiota can modulate brain development, function and behaviour, (Cryan and Dinan, 2012; Kelly, et al., 2016a; Stilling, et al., 2014), and that brain function or behaviour can affect the microbiota composition and gastrointestinal function (Demaude, et al., 2006; Galley, et al., 2014; Park, et al., 2009). Evidence supporting such modulation in chickens, however, is still elusive. A study by Calefi, et al. (2016) demonstrated that *C. perfringens* infection was able evoke behavioural changes in chickens; increasing the frequency of sleep-like behaviour and decreased feeding, walking, feather pecking, and standing behaviours, when birds were exposed to heat stress. Their results demonstrated a direct relationship between heat stress and C. perfringens-induced effects on gut inflammation, corticosterone serum levels and immune reactive neurons, which are related to HPA-axis activity, providing some insight into the importance of gut-brain interactions in maintaining intestinal homeostasis in birds.

Communication between the brain and gut, including microbiota, occurs via complex neural (CNS, autonomic and enteric nervous system), endocrine (specifically the hypothalamo-pituitary-adrenal (HPA) axis) and immune signalling pathways. (Fung et al, 2017; Powell, et al., 2017; Figure 1). Disturbances to this axis, have been implicated in a wide range of disorders, including functional and inflammatory gastrointestinal disorders, such as IBD and IBS (Jones, et al., 2006) as well as extra-intestinal disorders such as allergy, obesity, cardiovascular disease and neurological disorders, such as anxiety and depression. (Carding, et al., 2015; Kelly et al., 2016a)

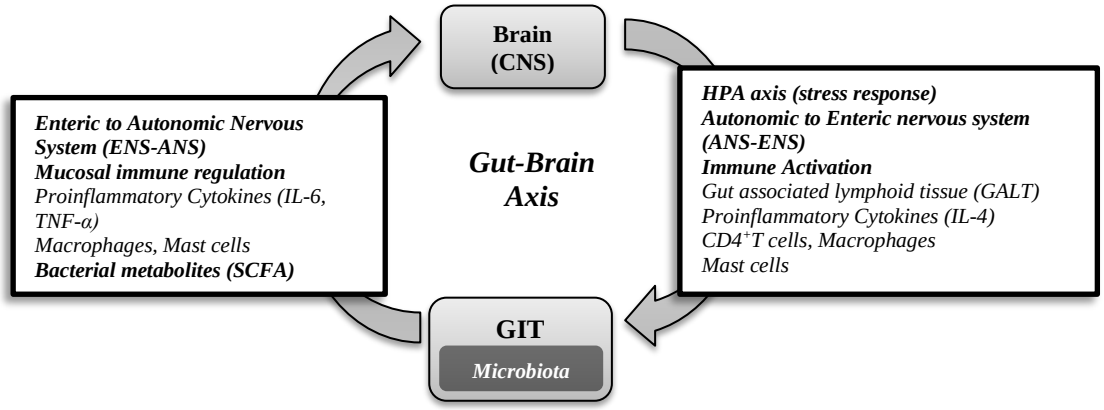

Figure 1- Bidirectional communication between the gut microbiota and central nervous system (CNS). Interactions between the intestinal microbiota, immune system and CNS are essential for the maintenance of host health.

III. GUT DYSBIOSIS

Gut dysbiosis occurs when there is a disruption in the gut-brain axis, and consequent disruption to the symbiotic relationship between gut microbial population and the intestinal mucosa. It has been speculated that intestinal inflammation develops as the result of an imbalance in the maintenance of homeostasis between intestinal commensals and immunity to pathogens, with an impairment of intestinal barrier function and increased intestinal permeability (Demaude et al., 2006). In addition, there is also a decrease in metabolic function including the production of essential host nutrients such as short chain fatty acids (SCFA) and vitamins (Carding et al., 2015). Dysbiosis or dysbacteriosis has been characterised in broiler chickens with non-infectious factors, including nutritional and management stressors being key causes (Teirlynck, et al., 2011).

Of growing interest, is the relationship between stressor-induced alterations to the maternal brain-gut-microbiota axis, and the impact on progeny development. Primary mediators of the stress response, including corticosterone (HPA axis) and the sympathetic nervous system (SNS), can regulate multiple aspects of immune function and, in return, immune mediators trigger the stress response and modulate its effects on the gastrointestinal tract, including the growth and type of commensal and pathogenic organisms (Fung, et al., 2017; Galley et al., 2016; Powell et al., 2017) Such axes modulation via stress factors can cause alteration to intestinal permeability, motility and changes in mucus production, causing an inflammatory response (Powell et al., 2017). Consequently, increased permeability of the intestinal barrier and microbial driven inflammation can then exert influence back on the HPA-axis. Continuous stress-induced impairment of the intestinal barrier creates a positive feedback-like situation whereby inflammatory cytokines persistently activate the SNS and HPA-axis resulting in barrier disruption, increased endotoxin translocation and a low-grade inflammatory state. (de Punder and Pruimboom, 2015). Interestingly, this continual low-grade chronic inflammation has been reported to affect fertility and subsequent embryo development in rodent models of obesity. Investigators were able to draw correlations between obesity-mediated gut dysbiosis and markers of ovarian inflammatory responses and oocyte gene expression (Xie, et al., 2016). Further investigation into linking stress-mediated gut dysbiosis and reproductive performance, through such mechanisms, would be of great interest not only in clinical application but also for commercial poultry production, where the health and performance of their breeding stock is paramount.

While it is becoming increasingly evident that stressor-induced alterations in the microbiota of adult animals can significantly impact host physiology and associated disorders, there is also evidence to suggest that the consequences of these alternations can have long-term negative implications in regards to both pre-natal and post-natal development of their progeny (Gur and Bailey, 2016; Veiga-Fernandes and Pachnis, 2017). There are two possible potential mechanisms: as mentioned before, microbial communities have been implicated in altering neuroendocrine axis, therefore changing the maternal profile of hormones and other signalling molecules that are exposed to the developing foetus and chick (Sudo, et al., 2004). Another possibility is that, in many animals, it is well established that bacterial sources for the newborn are derived from the maternal microbiota, either through the placenta, uterus and vagina, and recently discovered through the oviduct to the egg of poultry (Ding, et al., 2017). This initial colonisation of the gut by maternally-derived microbes can ultimately "reset" neuroendocrine axes and have a profound influence on growth, health and development of progeny (Al-Asmakh et al., 2012).

IV. MATERNAL STRESS AND PROGENY DEVELOPMENT

Maternal stress is well documented to influence progeny growth rate in various species and is a pressing issue in the broiler breeder industry due to current feed restriction practises (Lu et al., 2003). Feed restriction allows hens to maintain an optimum body weight and reproductive capacity; however it may result in hens experiencing chronic stress due to prolonged hunger (De Jong and Guemene, 2011) with reported increase in gastrointestinal inflammation and permeability (Bentley, et al., 2020; Kuttappan, et al., 2015). Corticosterone depositions in the yolk of the egg have been identified, potentially providing a link between maternal stress and progeny development in egg laying species, similar to that of placental transfer in mammals (Henriksen, et al., 2011a; Seckl, 2004).

Gestational stress in mammalian species is linked with reductions in birthweight (Sloboda, et al., 2005), permanent hypertension (Nuyt, 2008; O'Regan, et al., 2001), hyperglycaemia/hyperinsulinemia (O'Regan et al., 2001), behavioural alterations, as well as reduced immunocompetence and endocrine axis disruption (Henriksen et al., 2011b; Hyatt, et al., 2007). Similar physiological impacts have been noted in avian species in response to feed restriction, malnourishment and environmental conditions, such as cognitive disruption, anxiety and aggressive behaviours (Ahmed, et al., 2014; Lindqvist, et al., 2007), delayed sexual maturation (Henriksen et al., 2011b), compromised T-cell and B-cell mediated immunity (Love et al., 2005) and elevated baseline testosterone concentration (Henriksen, et al., 2013). Decreased progeny growth rate and hatchling mass have also been reported in response to maternal stress, or *in-ovo* administration of synthetic glucocorticoids to mimic the effects of maternal stress, although with limited consistency (Ahmed, et al., 2016; Hayward and Wingfield, 2004; Love, et al., 2005).

Interestingly, animal studies altering the maternal environment, either through nutritional or environmental factors, have highlighted significant phenotypic variations between male and female progeny, identifying sex-dependent changes in body composition, hormonal profiles, muscle mass and growth in response to stress (Angove and Forder, 2020; Angove et al., 2020). Thus, maternal stress has implications for a wide variety of physiological functions in both male and female progeny (Hayward et al., 2004), which is immensely important when considering mixed flock performance and flock uniformity.

Studies investigating nutritional means to reduce stress in hens found that reducing caloric density but increasing dietary fibre was shown to decrease chronic hunger behaviours (Hocking et al., 2004). On a low caloric density diet, hens were also shown to achieve significantly higher rates of lay and higher egg weights, with the percentage of fertile eggs remaining the same (Enting, et al., 2007b). The resulting progeny were heavier at hatch and 38 days old compared to progeny from hens on a standard commercial diet and also exhibited lower mortality rates (Enting et al., 2007a). Such dietary strategies, including qualitative feed restriction, have been recently studied in broiler breeder pullets (Tahamtani et al., 2020) and ultimately demonstrate how improving stress and considering hen welfare could have a significant impact on progeny development, and commercial productivity.

V. CONCLUSION

Taking advantage of the potential transgenerational maternal effects within the pyramid breeding system may enable a targeted approach in regards to nutritional or other supplement interventions at significant physiological time points, such as point of lay. Optimising gut health and reproductive output though implementation of novel breeder management strategies will positively influence all facets of production, including improved behaviour, immunity, growth and efficiency of subsequent generations. However, more research is needed to disentangle the mechanisms underpinning maternal stress, gut health and developmental programming in commercial poultry production.

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BARLEY IN PELLETED BROILER STARTER DIETS: EFFECTS OF CARBOHYDRASE SUPPLEMENTATION AND STEAM-CONDITIONING TEMPERATURE

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Summary

The effect of pelleting barley-based diets on bird performance and nutrient utilisation depends, in part, on the conditioning temperature (CT) used. The influence of supplemental carbohydrase (Carb) and CT on growth performance and nutrient utilisation of broilers (d 1-21) fed barley-based diets was examined in a 2 × 3 factorial arrangement of treatments, evaluating two levels of Carb (0 and 0.15 g/kg of feed) and three CT (60, 74 and 88°C). There was no significant interaction between Carb and CT for any tested parameter. Supplemental Carb increased the weight gain (WG; $P < 0.05$) and reduced the feed conversion ratio (FCR; $P < 0.001$) by 30 g/bird and 6.5 points, respectively. Birds fed diets conditioned at 60 and 74°C had a similar WG, but higher ($P < 0.05$) than those fed diets conditioned at 88°C. Conditioning diets at 88°C increased ($P < 0.05$) the FCR compared to those conditioned at 60 and 74°C. Regardless of CT, Carb enhanced the digestibility of starch ($P < 0.01$) by 1.2%. Compared to diets conditioned at 60 and 74°C, conditioning diets at 88°C increased ($P < 0.05$) the jejunal digesta viscosity and reduced nitrogen digestibility ($P < 0.01$). Diets conditioned at 88°C impaired ($P < 0.05$) starch digestibility compared to those conditioned at 60°C. Overall, Carb improved the WG, FCR, and starch utilisation whilst conditioning diets at 88°C negatively influenced the WG, FCR and, ileal digestibility of nitrogen and starch. The lack of significant interaction between Carb and CT suggests that the negative impacts of high CT on bird performance and nutrient utilisation were not alleviated by supplemental Carb.

I. INTRODUCTION

With the recognition that pelleting can enhance the feeding value of barley in poultry diets, mostly through the break-down of cell wall matrix resulting a greater accessibility of encapsulated nutrients to digestive enzymes (Abdollahi and Ravindran, 2019), barley has been successfully used in pelleted broiler diets (Perera et al., 2019b; Perera et al., 2020). While the optimum inclusion level (Perera et al., 2019b) and particle size (Perera et al., 2020) for barley in pelleted broiler diets have been evaluated, the optimum conditioning temperature (CT) for pelleting barley-based diets remains unexplored. High CT (> 80°C) are commonly employed by feed manufacturers to obtain superior pellet quality and feed hygiene. However, the resultant nutritional losses (Papadopoulos, 1989) and viscosity-induced interference to nutrient absorption (Almirall et al., 1995) due to high CT lead to impaired nutrient utilisation by birds. On the other hand, lower CT can hinder the inactivation of anti-nutritive factors and result in insufficient starch gelatinisation, protein denaturation and poor pellet quality (Abdollahi et al., 2013). As effects of CT also vary depending on the grain type (Abdollahi et al., 2010), the importance of determining the optimum CT for each grain type used is highlighted. Studies evaluating the influence of CT on barley-based diets are limited (Samarasinghe et al., 2000).

Supplementation of non-starch polysaccharide (NSP)-degrading enzymes is a routine practice in poultry diets based on viscous grains such as wheat and barley to overcome the adverse effects of NSP, mainly the high digesta viscosity in birds fed barley-based diets. As

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high CT during the pelleting process may exacerbate the adverse effects of viscosity, through solubilising the insoluble NSP (Cowieson et al., 2005), the use of exogenous enzymes becomes even more critical in pelleted diets. A better understanding of possible interactions between NSP-degrading enzyme and CT would allow the poultry industry to optimise the use and potential of barley in poultry diets. Accordingly, the present study was conducted to evaluate the effect of a supplemental carbohydrase (Carb), and CT on growth performance and nutrient utilisation in broilers fed barley-based starter diets.

II. MATERIALS AND METHODS

Normal-starch hulled barley (cultivar, Fortitude) was ground in a hammer mill to pass through the screen size of 8.0 mm. Nutrient composition, nitrogen (N)-corrected apparent metabolisable energy and standardised digestible amino acid contents of barley determined in a previous study (Perera et al., 2019a) were used in formulating a basal diet that was then used to develop two feed batches, without and with a supplemental Carb (Ronozyme® Multigrain; 0 and 0.15 g/kg of feed). Each diet was then divided into three equal batches and conditioned at three different temperatures (60, 74 and 88°C) by adjusting the steam flow rate. Mash diets were steam-conditioned for 30 s and the CT was measured continuously at the conditioner outlet (close to the exit point). Following conditioning, all diets were pelleted using a pellet mill equipped with a die ring with 3.0 mm holes and 35 mm thickness. The diets contained 5.0 g/kg of titanium dioxide as an indigestible marker. Each of the six dietary treatments was offered ad libitum to six replicate cages (eight birds per cage). Body weights and feed intake (FI) were recorded at weekly intervals throughout the 21-d trial. On d 21, ileal digesta were collected for determination of the coefficient of apparent ileal digestibility (CAID) of N and starch. Jejunal digesta were collected for determination of intestinal digesta viscosity.

III. RESULTS AND DISCUSSION

As there was no significant ($P > 0.05$) interaction between Carb and CT for any tested parameter, only the main effects of enzyme addition and CT on growth performance and nutrient utilisation are summarised in Table 1. Addition of Carb increased the weight gain (WG; $P < 0.05$) and reduced the feed conversion ratio (FCR; $P < 0.001$) by 30 g/bird and 6.5 points, respectively. Owing to the absence of Carb effect on jejunal digesta viscosity, the improvement in WG and FCR due to supplemental Carb might be attributed to the degradation of endosperm cell walls by added Carb, and possible generation of prebiotic oligosaccharides (Bedford, 2018). Birds fed diets conditioned at 60 and 74°C had similar ($P > 0.05$) WG, but greater ($P < 0.05$) than those fed the diets conditioned at 88°C. Compared to diets conditioned at 60 and 74°C, those conditioned at 88°C impaired the WG by 62 and 85 g/bird, respectively. Conditioning the diets at 88°C tended ($P = 0.054$) to lower the FI by 29 g/bird compared to CT at 60°C, due possibly to the slower feed passage associated with greater digesta viscosity (Almirall et al., 1995). Conditioning at 88°C increased ($P < 0.05$) the FCR compared to those conditioned at 60 and 74°C. Supporting the fact that elevated digesta viscosity is primarily responsible for the poorer performance of birds fed high-temperature conditioned diets (Cowieson et al., 2005), FCR of birds in the current study was impaired by 2.4 points per 0.1 cP increase in jejunal digesta viscosity in response to the increasing CT from 60 to 88°C.

It has been suggested that WG and FI responses of broilers fed diets pelleted at different CT represent the balance between the negative effect of high CT on nutrient availability and the positive effect of high CT on pellet quality (Abdollahi et al., 2013). In the current study, the superior durability of pellets conditioned at 88°C ($P < 0.05$) compared to those conditioned at 60°C (66.4 vs. 62.2%), however, was insufficient to overcome the adverse effects of high CT on nutrient utilisation and could not support the growth performance of birds.

No interaction between supplemental Carb and CT was observed for CAID of N or starch (Table 1). Regardless of the CT, supplemental Carb enhanced starch digestibility by 1.2%. The enhanced starch digestibility, and the lack of Carb effect on jejunal digesta viscosity, implies the action of Carb on hydrolysing the cell wall matrix (Bedford, 2018) to release encapsulated starch granules, leading to better interactions with digestive enzymes.

Diets conditioned at 88°C resulted in 1.4% lower ($P < 0.05$) starch digestibility than those conditioned at 60°C, due probably to the interference caused by elevated intestinal digesta viscosity and the formation of resistant starch. Digestibility of N was influenced ($P < 0.001$) by the CT, where diets conditioned at 88°C had 5.3% lower N digestibility compared to those conditioned at 60°C. Increasing the CT to a certain extent benefits the protein digestibility by inactivating proteinaceous enzyme inhibitors and denaturing proteins to expose more sites for enzyme attack (Abdollahi et al., 2013). However, extreme CT can reduce N digestibility by degradation of heat-labile amino acids (Papadopoulos, 1989).

Supplemental Carb and CT did not interact ($P > 0.05$) to influence the viscosity of jejunal digesta. Jejunal digesta viscosity was significantly ($P < 0.05$) influenced by the CT, as the diet conditioned at 88°C resulted in 10.1% (0.32 cP) greater digesta viscosity compared to those conditioned at 60 and 74°C. The viscosity of feed and intestinal digesta can be elevated by an increased release of encapsulated NSP, increasing solubilisation of NSP (Cowieson et al., 2005), presence of NSP with greater molecular weights due to less depolymerisation of carbohydrates (Abdollahi et al., 2013) or destruction of enzymes (Silversides and Bedford, 1999), during the application of high CT.

In conclusion, steam-conditioning barley-based diets at 88°C negatively influenced the WG, feed efficiency, and ileal digestibility of N and starch. Despite the more durable pellets obtained in diets conditioned at 88°C, feed efficiency and nutrient utilisation was severely compromised, most likely due to the increased digesta viscosity. The lack of interactions between supplemental Carb and CT indicated that the exogenous enzyme had similar efficacy at each CT in improving the WG, feed efficiency and starch digestibility in broiler starters.

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Table 1- Influence of carbohydrase enzyme addition and conditioning temperature (CT) on weight gain (WG; g/bird)[1], feed intake (FI; g/bird)[1] and feed conversion ratio (FCR)[1], coefficient of apparent ileal digestibility (CAID)[2] of nitrogen (N), starch and jejunal digesta

	CT (°C)	WG	FI	FCR	CAID		Jejunal digesta viscosity
					N	Starch	
Main effects							
Enzyme addition							
-		1001b	1380	1.391a	0.788	0.956b	3.27
+		1031a	1366	1.326b	0.800	0.967a	3.24
CT, (°C)							
	60	1052a	1387	1.327b	0.805a	0.968a	3.13b
	74	1029a	1373	1.341b	0.815a	0.963ab	3.17b
	88	967b	1358	1.408a	0.762b	0.954b	3.47a
Probabilities, P ≤							
Enzyme addition		0.011	0.122	0.001	0.310	0.007	0.806
CT		0.001	0.054	0.002	0.001	0.021	0.032
Enzyme addition × CT		0.175	0.272	0.355	0.347	0.705	0.494

Means in a column not sharing common letters (a,b) are different (P < 0.05).
[1]Each value represents the mean of six replicates (eight birds per replicate), measured from d 1-21.
[2]Each value represents the mean of six replicates (six birds per replicate).
[3]Each value represents the mean of six replicates (two birds per replicate).

A RATIONALE FOR ELEVATED FREE THREONINE PLASMA CONCENTRATIONS IN CHICKENS OFFERED REDUCED-CRUDE PROTEIN DIETS

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Summary

Pronounced elevations in free threonine plasma concentrations have been consistently observed pursuant to crude protein reductions in broiler diets. However, the magnitude of these threonine spikes appears related to inferior feed conversion ratios. Therefore, this paper seeks to develop a rationale for the mechanisms underlying elevated threonine plasma levels.

I. INTRODUCTION

Threonine is the most recently discovered amino acid but is also the third limiting amino acid in diets for broiler chickens (Kidd, 2000). There is considerable interest in reducing crude protein (CP) contents in broiler diets because their successful development would generate several advantages (Greenhalgh et al., 2020a) including a reduced dependence on imported soybean meal (Selle et al., 2020). Decades ago, Fancher and Jensen (1989) reported that free threonine concentrations in systemic plasma spiked by 87% (1635 versus 876 nmol/L) in female birds at 42 days post-hatch following a reduction in dietary CP from 183 to 159 g/kg. Moreover, we have consistently observed similar outcomes including a threonine spike of 116% (1093 versus 505 μmol/L) in systemic plasma following a 200 to 156 g/kg CP reduction in diets offered to male birds at 35 days post-hatch (Chrystal et al., 2020a). There was a linear relationship ($r = 0.723$; $P < 0.0001$) between increasing threonine plasma levels and deteriorating FCRs across four dietary CP levels in this study. Thus, questions are raised as to the genesis and relevance of free threonine spikes in plasma concentrations in chickens offered reduced-crude protein diets.

II. RATIONALE

Threonine is an essential amino acid; therefore, the likelihood is that elevated threonine plasma concentrations are due to a down-regulation of hepatic enzymes with the potential capacity to catabolise threonine. Threonine is converted to acetaldehyde and glycine by threonine aldolase (TA), to α-ketobutyrate by threonine dehydratase (TH) and to acetyl-CoA and glycine by threonine-3-dehydrogenase (TDH) (Davis and Austic, 1982). However, Akagi et al. (2004) found that hepatic TDH activity (88%) is dominant in avian species (Japanese quail); whereas, TH (93%) is dominant in rats. As TDH metabolises threonine to acetyl-CoA, these researchers concluded that threonine is a ketogenic amino acid in birds, as opposed to mammals, where it is regarded as almost an exclusively glucogenic amino acid (D'Andrea, 2000). Consequently, it follows that the genesis of elevated free threonine plasma concentration is the down-regulation of hepatic TDH activity. Theoretically, threonine is a glycine precursor, but this would not be the case if TDH activity is being down-regulated. In Chrystal et al. (2020a) increasing threonine plasma concentrations were linearly associated ($r = -0.608$; $P < 0.001$) with decreasing glycine concentrations, which indicates that threonine was not being catalysed into glycine.

Several papers (Davis and Austic, 1994; 1997; Yuan et al., 2000; Yuan and Austic, 2001) have investigated the impacts of dietary levels of protein, amino acids and threonine on TDH activity but they have not been conclusive. This is reflected in the Davis and Austic (1997) suggestion that hepatic TDH activity is influenced by dietary protein levels or other amino acids more so than by threonine itself. Threonine is the dominant amino acid in avian mucin (Fang et al.,

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1993) and is present in other endogenous secretions into the gut. Consequently, in the order of 30% dietary threonine is utilised by the gut mucosa and is denied entry into the portal circulation and threonine is also required for feathering and to maintain immuno-competence in addition to being incorporated into protein (Kidd, 2000). Nevertheless, the reasons for elevated free threonine plasma levels from reduced-CP diets remain obscure.

The catabolism of threonine by TDH generates acetyl-CoA which is a central metabolic intermediate capable of influencing the activity of multiple enzymes (Pietrocola et al., 2015). Interestingly, Guerranti et al. (2001) investigated the inhibition of hepatic TDH activity in rats with a focus on fatty acids but their conclusion was that TDH is the target of selective feedback inhibition by all compounds derived from its major end-product, acetyl-CoA. However, glucose can be metabolised to generate acetyl-CoA (Kaempfer et al., 1991; Shi and Tu, 2015), which may be pivotal. Reduced-CP diets typically contain more feed grain, more starch and elevated starch:protein ratios; therefore, reduced-CP diets have the potential to generate more glucose than conventional diets. Thus, the rationale is that increased acetyl-CoA concentrations derived from relatively high starch/glucose levels in birds offered reduced-CP diets down-regulate TDH activity via feedback inhibition to generate spikes in free threonine plasma concentrations.

III. DISCUSSION

A series of five reduced-CP diet feeding studies have been completed in which average CP contents were reduced in a step-wise manner from 213.5 to 166.5 g/kg CP (Moss et al., 2018; Chrystal et al., 2020a,b,c,d). There is a quadratic relationship ($r = 0.702$; $P < 0.005$) between dietary CP and threonine plasma levels across these five studies where threonine levels escalate once CP is reduced below 204.4 g/kg, as shown in Figure 1. It may be deduced from the relevant quadratic regression equation that a reduction in dietary CP from 210 to 160 g/kg would generate an 87.9% (1090 versus 580 μmol/L) spike in free threonine plasma concentrations. There is also a quadratic relationship ($r = 0.841$; $P < 0.0001$) between threonine plasma levels and FCR where high threonine levels are associated with inferior feed conversion efficiencies as shown in Figure 2. In addition, there are significant linear relationships between analysed dietary starch concentrations ($r = 0.522$; $P < 0.025$) and analysed dietary starch:protein ratios ($r = 0.623$; $P < 0.005$) with threonine plasma levels. Finally,
these relationships do not validate the rationale; however, they are entirely consistent with the premise that acetyl-CoA derived from starch and glucose is down-regulating TDH activity and generating elevated threonine plasma levels. That threonine spikes are associated with deteriorating FCR is probably not a direct “cause and effect” but may reflect an underlying physiological disturbance.

It appears that one strategy that might diminish elevated plasma threonine concentrations would be to limit increases in starch concentrations and starch:protein ratios in reduced-CP diets. The strategy of ‘capping’ dietary starch:protein ratios approach has been evaluated and displayed some promise (Greenhalgh et al., 2020b). Reducing analysed dietary starch:proten ratios from 1.68 to 1.41 in 205 g/kg CP, wheat-based diets improved weight gain by 10.37% (2161 versus 1958 g/bird), feed intake by 3.10% (3492 versus 3387 g/bird) and FCR by 4.04% (1.616 versus 1.684) at 35 days post-hatch. By notionally relacing soybean meal (475 g/kg) with full-fat soy (360 g/kg) to reduce dietary CP in this study, wheat inclusions were reduced from 607 to 502 g/kg, analysed starch concentrations from 347 to 288 g/kg while CP was maintained at 206 and 205 g/kg.

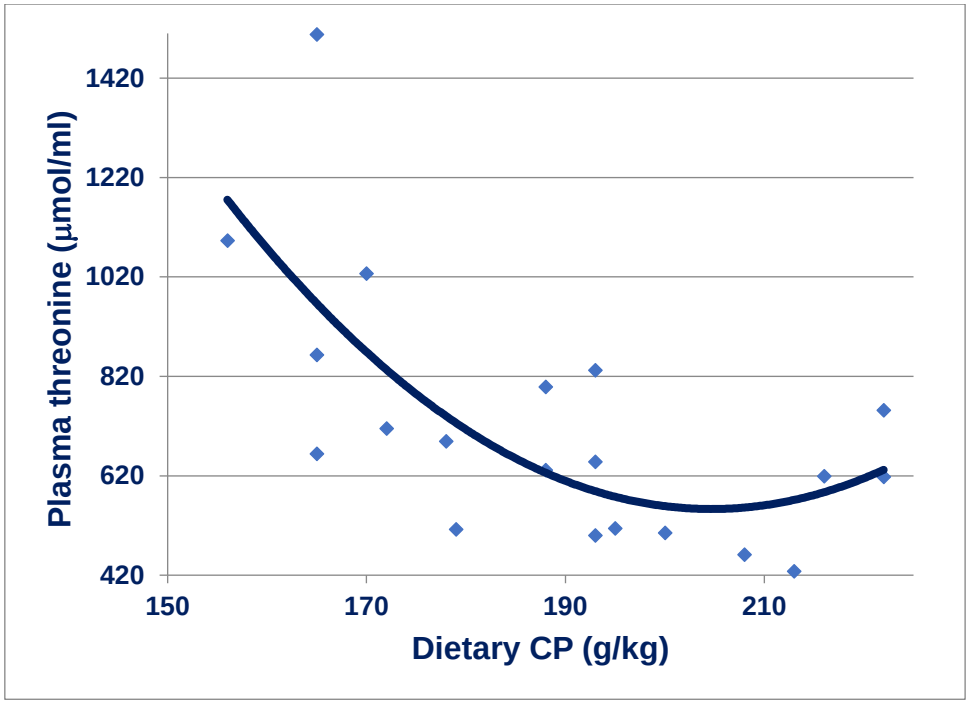

Figure 1 - Quadratic relationship (r = 0.702; P = 0.003) between dietary CP concentrations and free threonine plasma levels across five studies where y = 11557 - 107.5*CP + 0.263*CP^2**.** Adapted from Moss et al. (2018); Chrystal et al. (2020a,b,c,d)

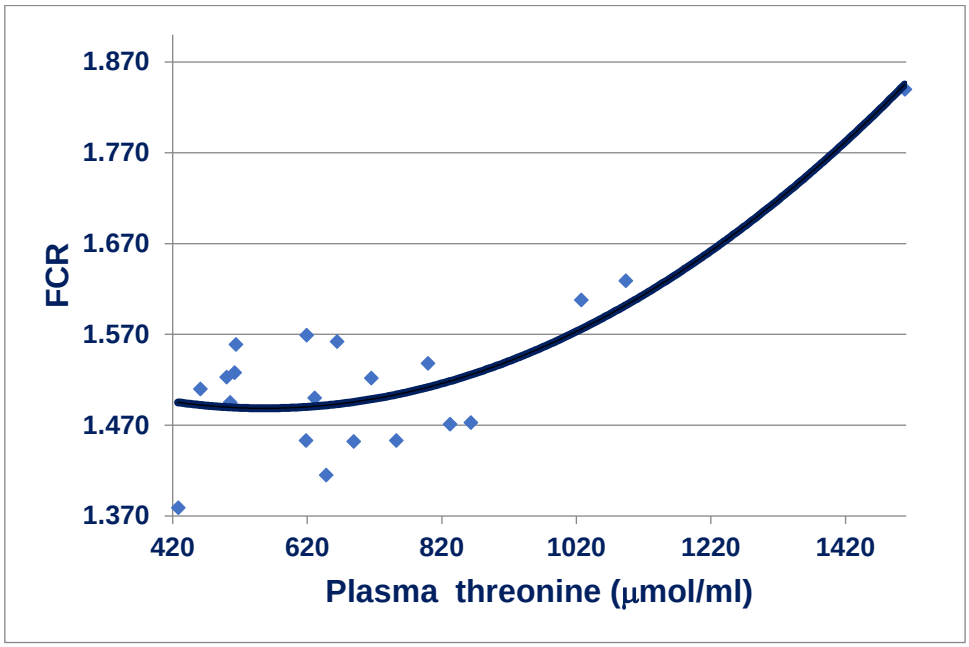

Figure 2 - Quadratic relationship (r = 0.841; P = 0.00003) between free threonine plasma levels and FCR across five studies where y = 1.611 – 0.004*thr + 0.0000004*thr^2. Adapted from Moss et al. (2018); Chrystal et al., (2020a,b,c,d)

Free threonine plasma concentrations is an intriguing subject. In rats offered a diet in which amino acids were derived from casein, threonine plasma concentrations increased by 36.0% (424.4 versus 312.0 µmol/L) over a post-prandial period of 3 hours (Daenzer et al., 2001). However, when a corresponding blend of 'free' amino acids was offered, threonine concentrations increased by a more robust 60.5% (760.4 versus 473.7 µmol/L). This equates to a difference of 38.3% and implies that inclusions of non-bound (synthetic, crystalline) amino acids in broiler diets may be contributing to elevated threonine plasma levels in some way. Our contention is that the strategy

of reducing dietary starch:protein ratios should be pursued in the development of reduced-CP diets. This strategy may enhance growth performance and curb increased fat deposition in broilers offered tangibly reduced-CP diets. Given such an outcome, it is possible that elevations in threonine plasma levels will be diminished, almost as an indication of acceptable performance in this context.

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FORMULATING LAYER DIETS BEYOND THE LEAST-COST MODEL

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Due to the high cost of feed for poultry, there is continuous pressure to formulate 'least-cost' diets that meet nutritional requirements. However, the main aim of any commercial enterprise is usually to maximise profits with the resources or inputs available. The increasingly dynamic nature of egg supply and volatility of egg price in Australia has reinforced the need for a more sophisticated approach than the total reliance on least-cost ration formulation alone for the evolution of the sector. Increased flexibility during uncertain times may give the layer industry greater opportunity and capacity to cope with market fluctuations. Thus, least-cost and max-profit feed formulation models were compared in a practical simulation to demonstrate the differences between these feed formulation strategies. The requirements for amino acids are an expensive constituent of diets and hold important implications for egg size; thus this example will focus on methionine (Met) levels in layer diets.

A feed formulation exercise was completed using example data sourced from industry and that published within the literature. The response of feed intake, egg weight and percentage production and egg mass of caged white egg layers 52-58 weeks of age to diets containing five graded true digestible Met levels (0.6, 0.48, 0.37, 0.25, 0.13%) was sourced from Bregendahl et al. (2008); with 0.48% dietary Met level standard for industry diets formulated to nutrient requirements via least-cost. These data were used to model the response of layers to Met over the six week period (52-58 weeks of age). Economic data was sourced from industry (4th quarter 2019, $AUD). Diets were formulated using EFG Software (2020) and data were modelled in Microsoft® Office Excel (2016). All diets were iso-energetic and formulated to the same digestible lysine concentration (0.91%), keeping all other amino acids (except Met) constant in a ratio to digestible lysine. Profit over the six week simulation was calculated via the following equation;

Profit = Egg sale + Spent hen sale – diet cost – packaging cost – pullet cost – other cost

The greatest profit of $34,830 over the 6 week simulation may be achieved with the 0.6% dietary Met level with a diet cost of $465 per tonne. The 0.6% dietary Met level cost $4 more per tonne than the standard 0.48% dietary Met level, but generated $186 more profit due to the reduced feed intake compared to the 0.48% methionine diet (egg production of hens offered 0.6 and 0.48% dietary Met was equal).

Present least-cost feed formulation uses requirements which are based on optimal biological performance, and may not necessarily optimise profits. This restricts the options that nutritionists and poultry managers have to navigate difficult economic times. Therefore, max-profit approaches use production and market data in addition to least-cost feed formulation to formulate diets by more economically sustainable means; giving increased flexibility, opportunity and capacity for the Australian poultry industry to cope and thrive under market challenges.

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A NOVEL PHYTASE ENHANCES GROWTH PERFORMANCE IN BROILER CHICKENS OFFERED REDUCED CRUDE PROTEIN DIETS

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Summary

The objective of this study was to evaluate the efficacy of a novel consensus bacterial 6-phytase variant (PhyG) dosed at 1500 FTU/kg on growth performance in broiler chickens offered diets with three different crude protein (CP) concentrations (Low, Medium and High). The maize-soy experimental diets were offered to 720 off-sex male Ross 308 broiler chickens from 1-10 (starter), 11-28 (grower) and 29-41 (finisher) days post-hatch. Each of the six treatments was offered to eight replicates with 15 birds per replicate. No interaction was found between phytase and dietary CP. Phytase significantly enhanced feed intake and weight gain during the starter phase ($P < 0.001$). During the grower phase, phytase significantly enhanced weight gain ($P = 0.001$) and reduced FCR ($P = 0.023$). Cumulatively, reducing dietary CP from High to Medium did not compromise weight gain but increased weigh-corrected FCR ($P < 0.001$); whereas phytase significantly enhanced weight gain ($P < 0.001$) and improved weight-corrected FCR of broiler chickens ($P < 0.001$) from 0-41 days post-hatch. The present study confirmed the benefit of supplementing exogenous phytase in diets containing reduced CP content where soybean meal was partially substituted by crystalline or synthetic amino acids.

I INTRODUCTION

Phytate is ubiquitous in plant-based feed ingredients and phytase is routinely supplemented in poultry diets. Moreover, there is considerable interest within the chicken-meat industry in increasing dietary inclusion rates of crystalline or synthetic amino acids to develop reduced protein diets. An increasing array of both essential and nonessential non-bound amino acids is becoming commercially available at inclusion costs that are becoming increasingly feasible. Reduced CP diets reduces the industry's dependency on imported soybean meals and was reported to have less nitrogen excretion (Nahm, 2007), enhance litter quality and reduce the incidence of footpad lesions. It is straightforward that non-bound amino acids partially replace soybean meal in reduced CP diets which leads to the lower concentration of phytate in the diets. Therefore, the intention of the present study is to evaluation the efficacy of a novel consensus bacterial 6-phytase variant in broiler diets containing conventional and reduced CP levels.

II MATERIALS AND METHODS

The feeding study complied with specific guidelines approved by the Animal Ethics Committee of The University of Sydney. A novel consensus bacterial 6-phytase variant (PhyG, Axtra® PHY GOLD, DuPont Animal Nutrition) was supplemented at 1500 FTU/kg to diets with three different CP concentrations (235, 215, 195 g/kg for starter; 215, 195, 175 g/kg for grower; 195, 175, 155 g/kg for finisher). For each phase, the six experimental diets were formulated to be iso-energetic and contained the same dietary levels of digestible lysine (12.8 g/kg for starter, 11.5 g/kg for grower and 10.2 g/kg for finisher), total sulphur amino acids (TSAA), threonine,

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and valine within each phase. The phytase supplemented diets were formulated with reduction of avP (1.95 g/kg), Ca (2.1 g/kg) and Na (0.45 g/kg). Starter, grower, and finisher diets were fed from 0 to 10 days, 11 to 28 days, and 29 to 41 days, respectively. The average analysed phytase activities in the control and supplemented diets were 200 and 1926 FTU/kg, respectively. All diets contained similar ideal protein ratios and were cold-pelleted. Starter diets were offered as crumble, whereas subsequent diets were in pellet form. Each of the six dietary treatments was offered to 8 replicate floor pens (15 birds per pen) or a total of 720 off-sex male Ross 308 chicks (parent line). Chickens had *ad libitum* access to feed and water. Initial and final body weights were determined, and feed intakes were recorded from which feed conversion ratios (FCR) were calculated. The incidence of dead or culled birds was recorded daily and their body-weights used to adjust FCR calculations. ANOVA and linear and quadratic correlation were performed using JMP® 13.0.0 and significance was determined at $P < 0.05$ by Student *t*-test.

Table 1 - Dietary compositions and calculated nutrient specifications in control diets

	Starter			Grower			Finisher		
Item (g/kg)	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3
Maize	513	583	653	549	628	708	558	636	715
Canola seed	30	30	30	40	40	40	50	50	50
Soybean meal	361	282	203	309	228	147	262	181	100
Canola meal	30	30	30	30	30	30	30	30	30
Soybean oil	21.4	10.7	0	31.9	18.2	4.6	43.5	30.2	17
L-lysine HCl	2.4	4.7	7.0	2.1	4.4	6.8	1.8	4.1	6.5
DL-methionine	3.4	4.0	4.6	2.9	3.5	4.1	2.5	3.1	3.8
L-threonine	1.2	2.2	3.3	1.1	2.1	3.2	0.8	1.9	2.9
L-tryptophan	0.0	0.2	0.4	0.0	0.3	0.5	0.0	0.3	0.6
L-valine	0.7	2.0	3.3	0.5	1.8	3.2	0.3	1.6	3.0
L-arginine	0.0	1.9	3.7	0.0	2.0	3.9	0.0	2.0	4.0
L-iso-leucine	0.2	1.5	2.8	0.3	1.6	2.9	0.3	1.6	2.9
L-leucine	0.0	0.4	0.7	0.0	0.2	0.5	0.0	0.2	0.3
L-histidine	0.0	0.1	0.2	0.0	0.1	0.2	0.0	0.1	0.2
Glycine	0.4	1.5	2.7	0.4	1.5	2.6	0.3	1.5	2.6
L-serine	1.0	2.4	3.7	0.9	2.2	3.5	0.8	2.1	3.4
Other ingredients	35.6	43.6	51.5	32.0	35.5	39.2	50.0	54.1	58.3
Nutrient Specifications									
AMEn(MJ/kg)	12.3	12.3	12.3	12.9	12.9	12.9	13.1	13.1	13.1
Crude protein	235	215	195	215	195	175	195	175	155
Lys[4]	12.8	12.8	12.8	11.5	11.5	11.5	10.2	10.2	10.2
Met + Cys	9.3	9.3	9.3	8.5	8.5	8.5	7.8	7.8	7.8
Thr	8.4	8.4	8.4	7.7	7.7	7.7	6.8	6.8	6.8
Ile	8.7	8.7	8.7	8.1	8.1	8.1	7.2	7.2	7.2
Val	10.1	10.1	10.1	9.2	9.2	9.2	8.2	8.2	8.2
Gly_eq	15.9	15.9	15.9	14.5	14.5	14.5	13.2	13.2	13.2
Ca	9.6	9.6	9.6	8.3	8.3	8.3	7.3	7.3	7.3
Total P	7.4	7.3	7.1	6.9	6.7	6.6	6.5	6.3	6.1
Available P	4.8	4.8	4.8	4.4	4.4	4.4	4.1	4.1	4.1
Na	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
DEB	247	238	230	230	230	230	230	230	230

[1]Phytase matrix (g/kg): Total P, 1.955; available P, 1.955; Calcium, 2.077; Sodium, 0.452; The phytase supplemented diets were formulated with reduction of avP (1.95 g/kg), Ca (2.1 g/kg) and Na (0.45 g/kg) [2]All amino acids are digestible basis; [3]Other ingredients include salt, sodium bicarbonate, potassium bicarbonate, limestone, mono-dicalcium phosphate, choline chloride 60%, Celite, premix and phytase.

Table 2 - Growth performance in broiler chickens from 0-10, 0-28 and 0-41 days post-hatch

Diet	Crude protein	Phytase	Starter (0-10 d)			0-28 days post-hatch				0-41 days post-hatch			
			Feed intake (g/bird)	Weight gain (g/bird)	FCR (g/g)	Feed intake (g/bird)	Weight gain (g/bird)	FCR (g/g)	Mort (%)	Feed intake (g/bird)	Weight gain (g/bird)	FCR (g/g)	Mort (%)
1	High	Nil	273	263	1.038	2321	1751	1.326	0.83	5005	3487	1.436	0.83
2	Medium	Nil	277	257	1.077	2298	1708	1.347	0.83	4959	3393	1.462	0.83
3	Low	Nil	267	240	1.113	2279	1684	1.354	0.83	4889	3241	1.508	1.67
4	High	Plus	278	269	1.036	2344	1818	1.29	0.83	5065	3521	1.439	1.67
5	Medium	Plus	279	263	1.063	2326	1778	1.309	0.00	5038	3497	1.441	0.00
6	Low	Plus	280	256	1.097	2309	1720	1.343	0.83	4984	3321	1.501	1.67
		SEM	3.5	4.0	0.0082	22.2	18.3	0.0102	0.761	42.7	31.7	0.0092	1.045
Main effects, Crude protein													
		High	276	266[a]	1.037[c]	2333	1784[a]	1.308[b]	0.83	5035	3504[a]	1.437[b]	1.25
		Medium	278	260[a]	1.070[b]	2312	1743[b]	1.328[b]	0.42	4999	3445[a]	1.452[b]	0.42
		Low	274	248[b]	1.105[a]	2294	1702[c]	1.348[a]	0.83	4937	3281[b]	1.505[a]	1.67
	Phytase												
		Nil	272[b]	253[b]	1.076	2300	1714[b]	1.342[a]	0.83	4951[b]	3374[b]	1.469	1.11
		Plus	279[a]	262[a]	1.066	2327	1772[a]	1.314[b]	0.56	5029[a]	3446[a]	1.46	1.11
Significance, P-value													
	Crude protein		0.500	<0.001	<0.001	0.236	<0.001	0.001	0.820	0.077	<0.001	<0.001	0.482
	Phytase		0.017	0.009	0.131	0.143	<0.001	0.002	0.657	0.031	0.008	0.261	1.000
	Interaction		0.287	0.346	0.634	0.989	0.585	0.335	0.820	0.919	0.533	0.437	0.729

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

III RESULTS AND DISCUSSION

There was no interaction between phytase and dietary CP on growth performance ($P > 0.05$). Predictably, reducing dietary CP depressed FCR for all the three growing phases ($P < 0.05$) and moderate dietary CP reduction did not influence weight gain from 0-10 and 0-41 days post-hatch ($P > 0.05$). Collectively, phytase supplementation improved weight gain and feed conversion regardless of the dietary CP levels. For instance, from 0-41 days, phytase supplementations improved weight corrected FCR by 0.56%, 3.68% and 2.14% in diets containing High, Medium and Low CP, respectively. It also increased weight gain by 0.98%, 3.07% and 2.47% in High, Medium and Low CP diets, respectively. It is encouraging phytase had more pronounced impact on growth performance in reduced CP diets compared to conventional diets, this may be attributed to increasing phytate to intact protein ratios (Table 3). Consistently, Liu and Selle (2017) reported increasing dietary phytate-P concentration from 2.6 g/kg to 3.0 g/kg in a diet containing higher concentration of crystalline amino acids reduced weight gain by 16% (1628 versus 1374 g/bird respectively), in comparison with a 5% reduction in weight gain when the same levels of phytate-P were added to a conventional diet; similar impact were observed on FCR where increasing phytate-P in high non-bound amino acid diet compromised FCR by 22% (1.130 versus 1.374 g/g respectively), but did not influence FCR in conventional diets. Phytate may influence protein digestion and utilization by forming binary or ternary phytate-protein complexes depending on the isoelectric points of the ingredients and environment (Selle *et al.*, 2012). In the present study, the High, Medium and Low CP diets were estimated to contain 39.2, 42.8 and 47.7 g phytate relative to 1 kg of intact protein, respectively. Another possibility is phytate may have larger negative impact on amino acid and glucose absorption in reduced CP diets. Eighty per cent of dietary glucose is actively absorbed by Na^+-dependent transport systems and phytate has been shown to decrease sodium-pump activity and glucose absorption in rats (Dilworth *et al.*, 2005). The present study focused on growth performance only and further research is required to understand the impact of phytate on nutrient absorption and the benefit of phytase on nutrient utilizations in reduced CP diets.

Table 3 - Estimated phytate: intact protein ratios in grower diets

	Dietary inclusion (g/kg)			Intact protein concentrations (g/kg)				Phytate (g/kg)			
Diets	1	2	3	CP ingredients	1	2	3	Phytate ingredients	1	2	3
Maize	549	628	708	91	50.0	57.2	64.4	6	3.29	3.77	4.25
Canola seed	40	40	40	210	8.4	8.4	8.4	12.4	0.5	0.5	0.5
SBM	309	228	147	475	146.8	108.3	69.8	13.1	4.05	2.99	1.93
Canola meal	30	30	30	349	10.5	10.5	10.5	20.6	0.62	0.62	0.62
Sum					215.6	184.3	153.1		8.5	7.9	7.3
Phytate :intact protein ratios (g/kg)									39.2	42.8	47.7

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INTERACTIVE EFFECTS OF A NOVEL CONSENSUS BACTERIAL 6-PHYTASE VARIANT WITH DIETARY PHYTATE ON DIGESTIBILITY OF MACRO MINERALS AND AMINO ACIDS IN BROILER CHICKENS

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Summary

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) in broiler diets, formulated to contain either low (2.45 g/kg), medium (2.95 g/kg) or high (3.45 g/kg) phytate phosphorus (PP), on nutrient digestibility. 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 starter diets (2.77 g/kg AvP and 7.6 g/kg Ca) for the first 10 days and then offered the grower diets (2.25 g/kg AvP and 6.4 g/kg Ca) until day 21. On day 21, six birds per cage were euthanised to collect distal ileum content. Samples were pooled per replicate cage and analyzed for Ca, P, Na and amino acids (AA) to calculate ileal digestibility. Increasing dietary PP decreased ($P < 0.001$) the average ileal digestibility of all the AA tested, by 3.8 and 4.0 % in medium and high PP groups compared to low PP. Phytase inclusion improved ($P < 0.001$) the ileal digestibility of all AA, except methionine, regardless of PP levels. Fitting exponential models predicted the digestibility coefficients (total AA) of 0.830, 0.797 and 0.789 in low, medium and high PP groups with no phytase, respectively, which increased to 0.875, 0.830 and 0.831 in response to the highest phytase inclusion. Ileal digestible Ca (g/kg) decreased from 4.12 in low PP diets to 3.65 and 3.40 in medium and high PP diets, respectively ($P < 0.001$), but was not affected by phytase. Increasing PP decreased ($P < 0.001$) ileal digestible Na (g/kg) by 36.9 and 71.4 % in medium and high PP compared to low PP diets. Exponential models predicted a maximum improvement of 70% (low PP), 55% (medium PP) and 48 % (high PP) in Na digestibility in response to the highest phytase inclusion. Phytase inclusion increased ($P < 0.001$) ileal digestible P (g/kg), the improvement in response to each phytase dose was higher in birds offered the high PP diets, than medium and low PP diets resulting in a significant phytate × phytase interaction. Exponential models predicted digestible P levels of 2.27, 2.00 and 1.71 g/kg in low, medium and high PP diets without added phytase, respectively, increasing to 3.72, 3.77 and 4.41 g/kg in response to 4000 FTU/kg phytase supplementation.

I. INTRODUCTION

Phytic acid salts or phytate constitutes up to 70 % of the phosphorous (P) stored in cereal grains and oilseed meals and this P is poorly utilized by poultry due to limited endogenous phytase secretion (Ravindran, 1995). Therefore, exogenous phytase is routinely used in poultry feed worldwide to address the issue of optimizing P utilization and mitigating the negative impact of dietary phytate on bioavailability of other nutrients i.e. minerals and AA (Selle et al., 2000). Phytate, a negatively charged molecule, can electrostatically bind up other cations and

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positively charged nutrients such as proteins rendering them unavailable for further digestion and absorption (Selle and Ravindran, 2007). Although phytate exerts its negative effect over a wide pH range, its anti-nutritional effect is exacerbated as the pH of the gastrointestinal tract increases post-gizzard (Selle and Ravindran, 2007). Higher pH in the small intestine in the presence of phytate may precipitate negatively charged proteins via tertiary complexes with cations, forming a bridge between the two molecules. Such complexes are highly insoluble and more resistant to hydrolysis by proteases reducing the availability of both proteins and minerals (Kies et al., 2006). Thus, new generation microbial phytases have been developed to act more rapidly and completely to hydrolyze phytate, particularly in the upper digestive tract to avoid formation of phytate-mineral-protein complexes in the small intestine.

The current research trial was performed to compare the responses of incremental inclusion of a novel microbial phytase to different dietary phytate concentrations, measuring distal ileal digestibility of P, Ca, Na and AA as response criteria.

II. MATERIALS AND METHODS

The study procedures were reviewed and approved by the University of Sydney Animal Ethics Committee. A total of 1800 off-sex male chicks (Ross 308) were obtained from a local hatchery (Aviagen, Goulburn, NSW, Australia). Upon arrival, birds were group weighed and distributed to 90 battery cages with 6 replicate cages of 20 birds per treatment. A total of 15 dietary treatments were generated by factorially arranging 3 concentrations of dietary phytate (2.45, 2.95 or 3.45 g/kg) and 5 dietary inclusion levels of a novel consensus bacterial 6-phytase variant (PhyG), 0, 500, 1000, 2000 or 4000 FTU/kg. The starter and grower basal diets were formulated to contain 2.77 and 2.25 g/kg AvP and 7.6 and 6.4 g/kg Ca, respectively.

At 21 days post-hatch, 6 birds per replicate cage were euthanised by an intravenous injection of sodium pentobarbitone. The small intestine was removed and digesta was gently expressed from the distal half of the ileum and pooled by cage, homogenised, and freeze dried to determine the apparent digestibility coefficients of Ca, P, Na and AA. The obtained digestibility coefficients and analyzed dietary Ca, P and Na content were used to calculate the distal ileal digestible content (g/kg) of the above minerals.

Data were subjected to statistical analysis using 2-way ANOVA, GLM procedure (JMP Pro 14.0, SAS Institute, Cary, NC) to assess the main effects of dietary phytate, phytase inclusion, and their interaction. Significant treatment effects were subjected to Least Significant Differences tests for pairwise comparisons ($P < 0.05$). Applying exponential models, minimum and maximum responses to phytase inclusion were determined for each phytate level.

III. RESULTS

The effects of dietary treatments on ileal digestible Ca, P and Na (g/kg) and total AA ileal digestibility coefficients are presented in Table 1. Increasing dietary phytate decreased ($P < 0.001$) ileal digestibility of all AA (data not presented), Ca and Na. Regardless of phytate levels, phytase inclusion improved ($P < 0.001$) the digestibility of Na and all the AA tested, except methionine but had no effect ($P > 0.05$) on Ca digestibility. Ileal digestible P improved ($P < 0.001$) with increasing phytase inclusion. The improvement in digestible P associated with increasing phytase inclusion was more pronounced in high phytate diets resulting in a phytate level × phytase inclusion interaction ($P < 0.001$).

At the highest inclusion of 4000 FTU/kg phytase dietary ileal digestibility coefficient of total AA, using exponential models improved by 5.40, 4.14 and 5.32 % in low, medium and high PP diets, respectively (Table 2).

Table 1 - Total amino acids digestibility coefficient (TAA), dietary Ca, P and Na digested at distal ileum (g/kg) in response to dietary treatments

Treatments		Digested at distal ileum (g/kg)			Digestibility %
Phytate	Phytase (FTU)	Ca	P	Na	TAA
Low	0	4.38	2.26	-1.59	0.833
Low	500	4.16	2.94	-1.09	0.836
Low	1000	3.90	3.30	-0.67	0.844
Low	2000	4.00	3.50	-0.61	0.867
Low	4000	4.20	3.81	-0.43	0.873
Medium	0	3.60	1.99	-1.89	0.796
Medium	500	3.82	2.93	-1.30	0.819
Medium	1000	3.92	3.61	-1.07	0.825
Medium	2000	3.53	3.70	-0.68	0.833
Medium	4000	3.60	3.71	-0.98	0.828
High	0	3.20	1.80	-2.14	0.791
High	500	3.27	3.05	-1.48	0.817
High	1000	3.07	3.18	-1.40	0.815
High	2000	3.48	4.11	-1.34	0.826
High	4000	3.92	4.46	-1.02	0.835
	SEM	0.197	0.151	0.171	0.010
Main effects					
Phytate					
Low		4.12^{a}	3.17^{b}	-0.84^{a}	0.850^{a}
Medium		3.65^{b}	3.18^{b}	-1.15^{b}	0.819^{b}
High		3.40^{c}	3.36^{a}	-1.44^{c}	0.817^{b}
	SEM	0.081	0.061	0.069	0.004
Phytase (FTU)					
0		3.72	2.02^{d}	-1.88^{c}	0.807^{d}
500		3.75	2.97^{c}	-1.29^{b}	0.824^{c}
1000		3.63	3.38^{b}	-1.05^{ab}	0.828^{bc}
2000		3.67	3.77^{a}	-0.88^{a}	0.842^{ab}
4000		3.91	3.99^{a}	-0.81^{a}	0.845^{a}
	SEM	0.114	0.087	0.098	0.006
Source of variation (*P*-value)					
Phytate		<.001	0.055	<.001	<.001
Phytase		0.561	<.001	<.001	<.001
Phytate x Phytase		0.120	0.001	0.894	0.942

$^{a-c}$ values in a column with no common superscripts differ significantly ($P \leq 0.05$) – LSD Test

The predicted increase in Na digestibility in response to the highest dose of phytase was 70.5, 55.3, and 48.0 % in low, medium and high PP diets, respectively. The exponential models predicted an improvement of 63.7 % in digestible P at low PP, which increased to 88.0 % at medium PP and over 158 % at high PP content of the diets.

IV. DISCUSSION AND CONCLUSION

The improvement in AA digestibility was remarkably consistent at different phytate levels, averaging around 5.0 %. Similarly, in a systematic review Cowieson et al. (2017) reported a mean response of 4.0 % uplift in AA digestibility with phytase supplementation. The authors suggested that the pattern of response across all AA indicates that much of the beneficial effect stems from a reduction in the loss of endogenous protein from the intestine. Phytate has been shown to increase nutrients endogenous losses by interacting with endogenous enzymes or

gastrointestinal mucin, increasing the excretion of endogenous AA and minerals (Cowieson et al., 2004).

Table 2 - Exponential models fit to predict coefficient of digestibility (%) of total amino acids (TAA), digestible (DIG) Na and P (g/kg) at distal ileum

Model	Nutrient	Regression equation	Min Response	Max Response	P- value	R^2
Low PP	TAA	Y = 0.891 – 0.06811 × EXP(–0.0003X)	0.830	0.875	<.0001	0.35
Medium PP	TAA	Y = 0.830 – 0.06255 × EXP(–0.0017X)	0.797	0.830	<.0001	0.22
High PP	TAA	Y = 0.831 – 0.07096 × EXP(–0.0014X)	0.789	0.831	<.0001	0.25
Low PP	DIG Na	Y = -0.466 – 1.82220 × EXP(–0.0013X)	-1.596	-0.471	<.0001	0.58
Medium PP	DIG Na	Y = -0.840 – 1.73389 × EXP(–0.0014X)	-1.855	-0.843	<.0001	0.43
High PP	DIG Na	Y = -1.175 – 2.35284 × EXP(–0.0003X)	-2.259	-1.175	<.0001	0.40
Low PP	DIG P	Y = 3.738 – 2.22379 × EXP(–0.0011X)	2.276	3.726	<.0001	0.74
Medium PP	DIG P	Y = 3.770 – 2.94913 × EXP(–0.0014X)	2.006	3.771	<.0001	0.70
High PP	DIG P	Y = 4.453 – 3.99839 × EXP(–0.0010X)	1.707	4.415	<.0001	0.89

Exponential asymptotic: Y=A+B × EXP (C × X), where Y is the response variable, X is the growth rate, A is the response variable predicted at the highest phytase inclusion, B is the slope of the exponential curve, C is phytase inclusion level. Maximum and minimum responses were calculated based on actual data input at 4400 and 355 FTU phytase analyzed in the diets using the regression model at each PP level.

The formation of indigestible protein-phytate complexes may lead to hypersecretion of both pepsin and hydrochloric acid, as a compensatory mechanism to rectify protein digestion. In response and as a protective measure to buffer intestine pH, pancreatic secretion of Na bicarbonate and gut mucous production increases, resulting in further endogenous loss of Na and the AA present in mucin. Furthermore, Na recovery due to phytase supplementation may promote Na^+-dependent transporters activity and thereby increase the intestinal uptake of both glucose and AA. The results observed on ileal P digestibility suggest that at high dietary PP a higher phytase inclusion (> 2000 FTU/kg) is required to maximize P utilization by increasing phytate hydrolyzation. However, aligning P digestibility with Na and AA, as phytase doses are elevated beyond 2000 FTU suggests that whilst P continues to be liberated, from PP, this may not be the case and/or to the same extent for other nutrients, particularly AA. Further analysis of phytate destruction in the proximal gastrointestinal tract and small intestine should provide a clearer insight into the possible mechanisms through which phytase has improved AA, Na and P digestibility.

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RESPONSE OF BROILERS TO DIETARY INCLUSIONS OF DIFFERENT INSOLUBLE FIBRE SOURCES IN A REDUCED CRUDE PROTEIN DIET

N.K. SHARMA[1], S.K. KHERAVII[1], K. GURNEY[2], M. CHOCT[1] and S.-B. WU [1]

Recent studies have shown that the decreased performance of broilers associated with feeding a reduced crude protein (RCP) diet cannot be fully recovered by supplementing essential amino acids (Hilliar et al., 2019). We hypothesized that the dietary inclusion of moderate amounts of insoluble fibre would improve gizzard function and protein/amino acid digestibility that could help to restore the performance loss associated with feeding a RCP diet. This study investigated the effects of oat hulls, soy hulls, sugarcane bagasse or lignocellulose based product as insoluble fibre sources in a RCP diet fed to broilers.

A total of 672 d-old Ross 308 male parental birds were fed a common starter diet until 10 d of age. On d 10, birds were assigned into 6 treatments with 8 replicates of 14 birds per pen. The treatments were: a normal protein diet (NP, grower 211 g/kg CP, finisher 195 g/kg CP), a RCP diet (CP reduced by 20 g/kg in grower and finisher phases) and RP diets formulated with either sugarcane bagasse at 20 g/kg, lignocellulose based product at 10 g/kg, oat hulls at 30 g/kg, or soy hulls at 30 g/kg. The basal diet of fibre treatments was the same and the formulations were adjusted by adding Celite, an indigestible component as a filler. The basal diet contained wheat, sorghum and soybean meal as major ingredients and was supplemented with xylanase and phytase. The diets met Ross 308 nutrient specifications with digestible lysine levels of 11.5 g/kg and 10.2 g/kg in grower (10 to 24 d) and finisher (24-35 d) phases, respectively. Feed intake, weight gain, and FCR were determined from d 10 to 35. Carcass parameters were measured on d 35. Data were subjected to one way analysis of variance using SPSS v. 22 and significance was determined at $P < 0.05$ using Duncan's multiple range test.

During d 0 to 35, dietary treatments had a significant effect on feed intake ($P < 0.001$), weight gain ($P < 0.001$), FCR ($P < 0.01$), as well as relative weights of abdominal fat pad ($P = 0.0501$), and gizzard ($P < 0.001$). The birds fed the RP diet with soy hulls or bagasse had a lower FCR compared to those fed RP diet without fibre and similar FCR to those fed NP diet (RP- 1.460, RP + bagasse- 1.443, RP + soy hulls- 1.448, and NP- 1.439). The birds fed RP diet with soy hulls had higher body weight gain compared to those fed RP diet without fibre and similar body weight gains to RP + bagasse and NP treatments (RP- 2190 g, RP + bagasse- 2227 g, RP + soy hulls- 2263 g, and NP- 2298 g). Adding insoluble fibre sources to the RP diet had no effect on relative fat pad weight. The birds that received the RP diet with oat hulls or bagasse had higher relative gizzard weight compared to those that received RP and NP diets (NP- 11.49, RP- 11.47, RP + oat hulls- 13.24, and RP + bagasse- 12.56 g/kg).

These findings suggest that performance loss in broilers associated with 20 g/kg CP reduction in a wheat, sorghum and soybean meal based diet can be fully restored for FCR and partly restored for body weight by including either 20 g/kg sugarcane bagasse or 30 g/kg soy hulls in the diets. Further research is warranted to examine the dose response of selected fibres and the effect of particle size distribution of fibres on performance of broilers offered reduced crude protein diets.

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EFFECT OF XYLO-OLIGOSACCHARIDES, XYLANASE AND WHEAT BRAN IN SORGHUM-BASED DIETS FED TO YOUNG BROILERS

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There is continued interest in using sorghum as an alternative to wheat in Australian poultry diets, but there is concern about the comparatively lower digestibility of sorghum-based diets. This study examined if it is possible to accelerate sorghum digestion in young birds by targeting fermentation of the xylan in its endosperm cell walls. The hypothesis was that supplementing sorghum-soybean meal based diets with a combination of xylo-oligosaccharides (XOS), xylanase and wheat bran would establish xylan-degrading bacteria in the bird's microbiota, thus improving utilization of the nutrients in sorghum. To verify this, 970 mixed-sex Cobb 500 birds were fed one of 12 sorghum-based dietary treatments, with 8 replicates per treatment and approximately 10 birds per replicate. For half of the treatments, 10% of the sorghum in the diet was directly replaced with 10% wheat bran. The diets were supplemented with combinations of xylanase (16,000 BXU Econase XT 5P) and XOS at either 50g/t or 2kg/t. Body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio corrected for mortality (cFCR) at d0-12 were determined per pen (Table 1). All data was analysed as a 2 x 2 x 3 factorial using IBM SPSS Statistics 25, with pen means as the experimental unit and percentage males per pen as a covariate. Differences were considered significant at $P < 0.05$.

Table 1- Effect of XOS, xylanase and wheat bran in sorghum based diets on individual feed intake (FI), body weight gain (BWG) and feed conversion ratio corrected for mortality (cFCR) at age d0-12

Wheat Bran (%)	Xylanase (BXU/kg)	XOS (g/t)	FI (g)	BWG (g)	cFCR	BW d12
0	0	0	528.83^{a}	398.34^{ab}	1.33^{abc}	443.57^{ab}
0	0	50	507.99^{ab}	379.80^{abc}	1.33^{ab}	425.56^{abc}
0	0	2000	468.59^{bcde}	356.87^{cd}	1.32^{abcd}	401.91^{cd}
0	16,000	0	446.99^{e}	374.68^{bcd}	1.20^{d}	420.19^{bcd}
0	16,000	50	465.92^{cde}	373.67^{bcd}	1.25^{bcd}	419.23^{bcd}
0	16,000	2000	478.62^{bcde}	364.67^{cd}	1.31^{abcd}	411.15^{cd}
10	0	0	487.23^{bcd}	349.31^{d}	1.39^{a}	394.35^{d}
10	0	50	458.16^{cde}	383.33^{abc}	1.20^{d}	428.48^{abc}
10	0	2000	463.25^{cde}	378.57^{abcd}	1.23^{bcd}	423.98^{abc}
10	16,000	0	452.28^{de}	362.51^{cd}	1.26^{bcd}	407.14^{cd}
10	16,000	50	469.62^{bcde}	370.44^{bcd}	1.28^{abcd}	416.34^{bcd}
10	16,000	2000	492.70^{bc}	406.89^{a}	1.21^{cd}	451.60^{a}
P-values						
Wheat Bran			0.209	0.996	0.355	0.942
Xylanase			0.033	0.857	0.056	0.832
XOS			0.895	0.691	0.532	0.647
Wheat Bran x Xylanase			0.031	0.148	0.498	0.160
Wheat Bran x XOS			0.330	<0.001	0.048	<0.001
Xylanase x XOS			0.002	0.124	0.065	0.116
Wheat Bran x Xylanase x XOS			0.638	0.371	0.349	0.396

Significant interactions between wheat bran and XOS were observed for BW, BWG and cFCR, indicating that in the presence of wheat bran performance improved with increasing XOS level, but in the absence of wheat bran the opposite was true. Feed intake was greatest in birds fed the control diet, demonstrating the advantages of XOS and xylanase application in sorghum-based diets. BW and BWG was highest in birds fed wheat bran, xylanase and 2kg/t XOS, confirming that the beneficial effects of these combined supplements on microbiota transpire into improved performance. Lack of dose effect of XOS on FCR indicates XOS induces positive effects on the microbiota even at very low levels. In conclusion, it appears that sorghum digestibility is improved by supplementing sorghum-based with a fermentable fibre source, xylanase and XOS.

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SUPPLEMENTATION OF BROILER DIETS WITH A MULTI-PROTEASE ENZYME INCREASES GROWTH PERFORMANCE AND NUTRIENT DIGESTION OF BROILER CHICKENS

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Summary

The present study investigates the effect of protease (Kemzyme® Protease) on the productivity of broilers under reduced crude protein (CP) and amino acid (AA) diet conditions. A total of 504 one-day-old Ross 308 broiler chicks were allocated into nine treatments with eight replicates per treatment. Basal diets 1-3 weeks and 4-5 weeks included; positive control (PC), negative control one (NC1) with 0.5% lower CP and 2% lower AA and negative control two (NC2) with 1.0% lower CP and 4% lower AA compared to PC diets. Amino acids reduced in NC1 and NC2 diets were dig Lys, dig M+C and dig Thr compared to PC. These basal diets were used to create six more treatments by adding protease on top. Protease was included at 150g/ton and 300g/ton for NC1 and at 150g/ton, 200g/ton and 300g/ton of protease for NC2 diets. All treatment diets were provided ad-libitum in a mash form until of age. Production performance, carcass traits, carcass composition and production indices were analyzed at 21 and 35 days. Data were analyzed using one-way ANOVA in SPSS. Results showed that the supplementation of protease ($P < 0.05$) improved the daily gain and breast meat yield in positive and negative control groups. In conclusion, the results indicated that the supplementation of protease into the reduced CP and AA diets can improve the growth performance of broiler chickens.

I. INTRODUCTION

Soybean meal (SBM) is the primary protein source in broiler diets. Although SBM is highly digestible, it contains glycinin, protease inhibitors, and antigenic proteins that are indigestible and can cause intestinal damage and impair immune functions resulting in suboptimal growth performance (Pan et al., 2016). With the development of commercially available protease, protein and amino digestibility is greatly improved allowing the control of anti-nutritional factors in SBM and enables the use of alternative protein sources in formulations. This leads to lower formula cost and better growth performance (Rooke et al., 1998; Piao et al., 1999; Yu et al., 2007; Romero et al., 2013). Reduction of nitrogen emissions in livestock production is also a growing concern because of its effect on the environment and human health (He et al., 2006; Chen et al., 2014). An effective way to reduce nitrogen pollution is by enhancing nitrogen digestibility and reducing nitrogen excretion in animal production. Thus, it is important to explore effective proteases to improve the digestibility of protein and thus decrease nitrogen excretion for successful and sustainable animal production (Brotzge et al., 2014). Therefore, this study was undertaken to determine whether the supplementation of protease in lowered CP and amino acid broiler diets can deliver the same or better performance compared to a standard diet. It is hypothesized that feed protease can help improve feed deficiency, lower crude protein level in basal diets and reduce nitrogen excretion.

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II. MATERIALS & METHODS

This experiment was carried out at the Department of Animal Science and Biotechnology Chungnam National University. A total of 504 one-day-old Ross 308 broiler chicks were allocated into nine treatments with eight replicates per treatment using a completely randomized design. Treatments were made by creating basal diets 1 - 21 days and 28 to 35 days: positive control (PC), negative control One (NC1) with 0.5% lower CP and 2% lower amino acids and negative control Two (NC2) with 1.0% lower CP and 4% lower amino acids. Amino acids reduced were dig Lys, dig M+C and dig Thr compared to PC. These basal diets were used to create six more treatments by adding a protease (Kemzyme® Protease, containing acidic, neutral and alkaline proteases produced by *Aspergillus niger*, *Bacillus subtilis* and *Bacillus licheniformis*, respectively) on top. Addition of 150g/ton and 300g/ton of protease for NC1 and 150g/ton, 200g/ton and 300g/ton of protease for NC2 diets. All treatment diets were provided *ad libitum* in a mash form until 35 days of age. The AA contents in the diets were also measured according to a method described by Cohen (2001) to countercheck formulated from actual amino acids in the produced diets. Cr_2O_3 (Chromium oxide powder, > 99.9% purity, Sigma-Aldrich, USA) was added as an internal indigestible marker for digestibility analysis in a proportion of 0.3% to all experimental diets. Average daily gain (ADG), average daily feed intake, and feed conversion ratio were calculated from weekly body weights and feed consumption. Feed cost was measured by calculating raw material costs.

Digesta samples from the ileum were also collected and pre-dried at 55 ºC for 24 h for analysis of dry matter, crude protein [Macro-Kjeldahl, N × 6.25 (1/0.16 = 6.25) to convert nitrogen content into protein content], ether extract, and gross energy according to the methodologies of AOAC, 2005. The digestibility coefficient of nutrients was calculated as described by Huang et al. 2005.

Data were analyzed using the general linear model (GLM) procedure of one-way ANOVA of SPSS software (Version 21; IBM SPSS 2012). Mean differences were considered significant at $P<0.05$. When treatment effects were significant ($P< 0.05$), means were separated using Turkey's multiple range test procedures of SPSS software.

III. RESULTS AND DISCUSSION

The effect of supplementation of protease on growth performance in the broiler for 1-21 days; 28-35 days and 1-35 days performance is presented in Table 1. The PC Diets (PC and PC-150 had significantly higher ($P < 0.05$) ADG compared to NC2 and NC2-150 but no significant difference in ADG with NC1 diets (NC1, NC1-150 and NC1- 300); NC2-200 and NC2-300 fed birds in week 28–35 days. This may indicate that the 150g/ton inclusion of the protease is not enough to compensate for the lowering of CP by 1% and amino acids by 4% in 28–35 days. Although the ADG in 1-21 days was not significantly different in all treatments, it is possible that the lowered ADG at 28–35 days for NC2 and NC2-150 is the effect of the numerically lower ADG at 1-21 days. The total gain of the birds from PC and PC-150 at 1–35 days was also significantly higher than the NC2 and NC2-150 fed birds. The protease could compensate for the lowered CP (0.5% reduction) and AA (2% reduction) in NC2 diets at 200g/ton. Angel et al. (2011) also demonstrated that birds offered reduced CP diets supplemented with protease at doses of at least 200 mg/ton have comparable growth performance to birds offered standard CP diets.

The effect of supplementation of protease on ileal digestibility in broilers after 21 days and 35 days are presented in Table 2. There were significant improvements observed in the ileal digestibility of dry matter, CP, and energy with supplementation of protease ($P < 0.05$). The protease improved ($P < 0.05$) energy digestibility as a cumulative effect of increased

protein digestibility. In both experiments, the addition of protease had significant benefits on the performance of the animals, mainly by increasing the digestibility of CP, and consequently, digestible energy. The activity of the exogenous protease can complement that of the endogenous protease. In diets with deficient AA, the effect of the protease seems to be more pronounced.

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Table 1 - Effect of protease on growth performance of broiler[1] for Week 1-3, Week 4-5 and Week 1-5.

	1-21 D			28-35D			1-35D				
	ADFI (g/bird)	ADG (g/bird)	FCR (g/g)	ADFI (g/bird)	ADG (g/bird)	FCR (g/g)	ADFI (g/bird)	TFI (g)	ADG (g/bird)	Total gain	FCR (g/g)
PC	54.2	39.7	1.3	149.1	93.95^{a}	1.6	92.2	3,226	69.8	2,148^{a}	1.4
PC-150	52	39.9	1.3	144.1	96.06^{a}	1.5	88.9	3,111	70.9	2,182^{a}	1.4
NC1	54	39.3	1.4	137.6	90.47^{abc}	1.5	87.5	3,061	68	2,092^{abc}	1.5
NC1-150	54.2	39.5	1.3	145	92.07^{ab}	1.6	90.6	3,169	68.8	2,117^{abc}	1.4
NC1-300	52.9	39.9	1.3	152.6	93.72^{a}	1.6	92.8	3,246	69.8	2,149^{a}	1.4
NC2	53.9	38.9	1.4	149	86.11^{c}	1.7	91.9	3,218	65.7	2,022^{c}	1.5
NC2-150	54.5	39.2	1.4	148.1	87.11^{bc}	1.7	91.9	3,218	66.4	2,043^{bc}	1.5
NC2-200	51.8	39.4	1.3	150	92.19^{ab}	1.6	91.1	3,188	68.8	2,117^{abc}	1.4
NC2-300	52.4	39.6	1.3	153.1	92.52^{ab}	1.7	92.7	3,244	69.1	2,126^{ab}	1.5
SEM[3]	0.57	0.19	0.01	1.83	0.67	0.02	0.91	31.91	0.82	10.86	0.01
P-value	0.94	0.97	0.16	0.66	0.01	0.03	0.91	0.91	0.9	0.01	0.09

[1]Dietary treatments were: positive control with standard diet (PC), PC + protease (Kemin; 150g/ton; PC-150), negative control with CP 0.5% and AA 2% down from PC (NC1), NC1 + protease (150g/ton; NC1-150), NC1 + protease (300g/ton; NC1-300), negative control with CP 1.0% and AA 4% down from PC (NC2), NC + protease (150g/ton; NC2-150), NC2 + protease (300g/ton; NC2-300).
[2]List of amino acid: lysine, methionine, threonine and methionine + cystine
[3]TFI: Total Feed Intake.
[4]a b c; means within columns not sharing a common suffix are significantly different at the 5% level of probability

Table 2 - Effect of protease in diet on ileal digestibility co-efficient of dry matter, crude protein and energy in broilers[1]

	PC	PC-150	NC1	NC1-150	NC1-300	NC2	NC2-150	NC2-200	NC2-300	SEM[3]	P-value
Crude protein, %	-	-	-0.5	-0.5	-0.5	-1	-1	-1	-1		
Amino acid[2], %	-	-	-2	-2	-2	-4	-4	-4	-4		
21D											
Dry matter	0.79^{ab}	0.80^{a}	0.78^{bc}	0.80^{abc}	0.81^{ab}	0.77^{c}	0.78^{bc}	0.78^{bc}	0.79^{bc}	0.002	<0.001
Crude protein	0.70^{bc}	0.73^{a}	0.67^{de}	0.70^{bc}	0.71^{ab}	0.66^{e}	0.68^{cde}	0.69^{bcde}	0.69^{bcd}	0.003	<0.001
Energy	0.79^{ab}	0.80^{a}	0.78^{b}	0.79^{ab}	0.79^{ab}	0.77^{b}	0.77^{b}	0.78^{ab}	0.78^{ab}	0.003	0.03
35 D											
Dry matter	0.81^{bc}	0.82^{a}	0.80^{cd}	0.81^{ab}	0.81^{a}	0.80^{d}	0.80^{cd}	0.80^{bcd}	0.81^{bc}	0.001	0.005
Crude protein	0.81^{b}	0.82^{a}	0.79^{bc}	0.80^{bc}	0.81^{b}	0.78^{c}	0.79^{bc}	0.79^{bc}	0.79^{c}	0.002	<0.001
Energy	0.80^{ab}	0.81^{a}	0.79^{bc}	0.80^{ab}	0.80^{ab}	0.78^{c}	0.78^{c}	0.79^{bc}	0.79^{bc}	0.002	0.002

[1]Dietary treatments were: positive control with standard diet (PC), PC + protease (Kemin; 150g/ton; PC-150), negative control with CP 0.5% and AA 2% down from PC (NC1), NC1 + protease (150g/ton; NC1-150), NC1 + protease (300g/ton; NC1-300), negative control with CP [1].0% and AA 4% down from PC (NC2), NC + protease (150g/ton; NC2-150), NC2 + protease (300g/ton; NC2-300).
[2]List of amino acid: lysine, methionine, threonine and methionine + cystine

HYDROXY-SELENOMETHIONINE SUSTAINS EGG PRODUCTION AND IMPROVES EGG SELENIUM DEPOSITION IN AGED LAYING HENS UNDER HEAT STRESS

C.P. JUZAITIS-BOELTER[1], A.J. DAVIS[1], M. DE MARCO[2], J.K. BRACKENRIDGE[3] and R.B. SHIRLEY[3]

Under heat-stress conditions (i.e. high heat and humidity) and as the hen ages, oxidative stress increases due to the synthesis of reactive oxygen species (ROS). When the hen is unable to neutralize the various ROS molecules that are generated, the hen's normal metabolism, physiology and production are negatively affected (Fouad et al., 2016). To reduce oxidative stress, selenium (Se) is often supplemented into poultry diets because of its specific and integral role in the synthesis of selenoproteins. Selenoproteins are directly involved in thyroid hormone activation/metabolism and several antioxidant defenses, which include reducing ROS, reducing and reactivating oxidized amino acids, and modulating various immune responses (Surai, 2018). Regardless of the Se source, the maximum amount of supplemental Se that can be added to animal diets is normally limited to 0.3 ppm. The supplementation of Se typically is mediated via inorganic sources such as sodium selenite (SS). Contemporary research has demonstrated however, that supplementing with organic selenium sources, such as Se-Yeast (SY) or pure chemically synthetized Se forms, like hydroxy-selenomethionine (OH-SeMet), enhances Se absorption, retention in body tissues and production under stressful conditions compared to SS. The aim of this study was to determine if the production of ageing layers under heat stress conditions would be impacted when fed different Se sources. In this trial, HyLine WD-36 laying hens were fed a corn-SBM-DDGS layer diet that was supplemented with 0.3 ppm Se from either SS, SY or OH-SeMet, from 41 to 71 weeks-of-age. Each dietary treatment was provided to 12 blocks of cages, with 10 cages/block and 2 hens/cage (120 cages/treatment; 240 birds/treatment). The ambient, average daily hen house temperature was 30.1°C, and the average humidity was 52.3% (range 38.6 to 66.8%) throughout the experiment. Hen body weights and feed consumption were not different throughout the experiment between the three dietary treatments ($P > 0.119$). On a per-hen-basis, over the 30-wk experimental period, hens fed the SS, SY and OH-SeMet produced a total of 179, 182 and 184 eggs, respectively ($P = 0.036$), and 177, 180 and 182 total marketable eggs/hen, respectively ($P = 0.022$). Egg weight and specific gravity were not different in the eggs produced from the hens fed the different Se sources. Comparing the feed consumption per dozen eggs over the 30-week period, feeding SS, SY and OH-SeMet resulted in the respective feed conversions of 1.656, 1.641 ($P > 0.05$), and 1.603 ($P < 0.012$). Evaluating the Se content of whole eggs from 47 to 71 weeks, there was no difference in Se concentration between the SS and SY fed hens (1.11 vs. 1.16 ug/g DM; $P > 0.05$); however, there was a significant increase is Se concentration when OH-SeMet was fed (1.72 ug/g DM; $P < 0.0001$). In conclusion, this study indicates that feeding organic selenium in the form of OH-SeMet, significantly enhances Se transfer into the egg and appears to be a promising solution that alleviates the detrimental effects of heat stress upon production in ageing laying hens.

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COMPARATIVE SUSTAINABILITY OF DIFFERING DIETARY AMINO ACIDS AND ENERGY REGIMES FOR INDIVIDUAL LAYING HENS AT PEAK PRODUCTION

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Summary

Poultry production has been identified as an efficient source of terrestrial, farmed animal protein. However, in tandem with all food production, sustainability into the future will be a fundamental requirement. Animal nutrition of farmed animals is central to all aspects of sustainability. The more efficient animals are, the more financially viable production operations become, with a reduced environmental impact and in many cases improved bird welfare. However, little work on the impact of nutrition on sustainability of egg production has been published. This paper reports an evaluation of diets and and egg output using field performance data from individually housed Hy-Line Brown hens, calculated by applying six environmental parameters assessed for individual feed ingredients by *l'Institut National de Recherche Pour l'Agriculture* (INRA). A modelling exercise allowed for the determination of the environmental impact of layer diets.

I. INTRODUCTION

True sustainability was defined by the World Commission on Environment and Development (Brundtland Commission, 1987) as *"the ability to meet the needs of the present without compromising the ability of future generations to meet their own needs"*. Sustainability is a concept with multiple facets including, environmental (which includes both the demand for resources and environmental pollution), ethical (welfare and social conscience), economic and enforcement (often described as four E's of sustainability) (FAO, 2012). There are interdependencies between the different elements of sustainability, and often progress in one area has negative consequences on another (European Union, 2001). Measuring sustainability is complex as the entire value chain ought to be considered. Environmental and economic components are included in a complete lifecycle assessment (LCA) (Pelletier, 2015). In a complete LCA of poultry in the United Kingdom Leinonen and Kyriazakis (2016) applied the BSI (2011) PAS 2050:2011 carbon footprint standards and identified feed components as the largest contributor towards global warming potential. de Vries and de Boer (2010) calculated production of a kg of egg protein was similar to a kg of broiler protein, requiring between 41 and 48 m^2 of land resources. Poultry is the most environmentally friendly of farmed terrestial and aquatic animal production per unit of edible product (Fry et al., 2018). However, opportunities exist to improve efficiencies through genetic selection, improvements in housing, energy usage, manure management and a change in feeding strategies (de Vries and de Boer, 2010; Pelletier 2015; Leinonen and Kyriazakis, 2016).

Research explicitly addressing the environmental impact of differing feeding strategies on egg production is rare. However, nitrogen emissions from monogastric animals have received considerable attention (Klein Goldewijk et al., 2005: Pelletier et al., 2014). The optimum levels of dietary energy and balanced protein requirements of laying hens are ultimately a financial decision (Gous and Kleyn, 1989; Kumar et al., 2018; Spangler et al., 2019; Kleyn et al., 2020). However, an economic evaluation on its own provides no indication of whether a feeding system is sustainable or not, and it is this aspect that is investigated in this paper.

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II. METHODOLOGY

The response of individually housed Hy-Line Brown hens, aged 27 to 30 post-hatch, was used as the basis of this study. Maize-based diets were formulated to contain 11.00, 11.75 or 12.50 MJ/kg dietary apparent metabolisable energy levels, adjusted for endogenous nitrogen loss (AME_n) and 6, 7, 8 or 9 g/kg standardised ileal digestible lysine (SID Lys), as a proxy for balanced protein. All diets were formulated to contain 3.5 g/kg non-phytate phosphorus and 35 g/kg calcium. Full details of the experimental 4 × 3 randomised factorial design and the diets used were reported by Kleyn et al. (2020). Data from INRA (2020) (www.feedtables.com) was used for each of the six environmental parameters considered: phosphorus consumption; cumulative energy demand; climate change (carbon footprint); acidification; eutrophication and land competition. Values were ascribed to each feed ingredient used and by calculation, the environmental impact for each of the diets and per gram of egg output was determined. This feeding study was approved by the Animal Ethics Committee of the University of KwaZulu Natal and birds were handled within the 2018 South African Poultry Association's code of conduct.

III. RESULTS

Increasing dietary AME_n from 11.0 to 12.50 MJ/kg linearly decreased feed intake by 10.3% (117.6 versus 105.5 g/day; $P < 0.001$), improved feed conversion ratio (FCR) by 12.2% (2.137 versus 1.876 g feed/g egg day^{-1}; $P < 0.001$) and changed balanced protein intake as dietary SID Lys levels increased from 6 to 9 g/kg. Increasing dietary SID Lys intake increased egg weight by 3.4% (56.88 versus 58.82 g/egg; $P < 0.05$) but had no significant impact on either feed intake or hen day production. Layer performance for this trial was reported in detail in Kleyn et al. (2020).

A summary of the estimated environmental impact of each diet, together with the estimated environmental cost per gram of egg produced is shown in Table 1. High-protein diets increased egg output but led to inefficient protein utilisation and an increase of 18% in carbon footprint per gram of egg produced (1.278 verus. 1.578 g CO_2eq/g egg). Increasing dietary AME_n increased the carbon footprint by 6.8% (1.510 versus 1.613 g CO_2eq/g egg). Additionally, average cumulative energy demand increased by 29.5% with an increase in dietary AME_n or SID Lys (6.1 versus 4.7 MJ/kg). An apparent anomaly is that reduced protein diets (6.0 g/kg digestible lysine) had a higher acidification potential per gramme of egg, which can be explained by the relatively high acidification impact of maize, higher maize content of these diets and a lower egg weight output per day.

IV. DISCUSSION

The ingredients used in the manufacture of poultry diets are a vital aspect of sustainability. Feed has the largest impact on the overall LCA of egg production and whilst specific emissions from the hen associated with different feeds are acknowledged, they are beyond the scope of this paper and therefore were not assessed. The production of grain and soybeans in particular, are associated with environmental degradation often including lengthy transport chains that both harm sustainability (Leinonen and Kyriazakis, 2016). Importantly, not all ingredients should be viewed in the same light when considering their environmental impact. Production methods, such as precision farming and no-till conservation tillage, play a role in reducing inputs. The genotype and yields of the cultivars used impact on efficiency. Furthermore, the land-use changes (LUC) all need to be considered (Nepstad et al., 2006). LUC is used to describe practices such as deforestation, which has a tremendous impact; alternatively, the re-

deployment of this "set aside" land for agronomy has a minimal effect on the carbon footprint Leinonen and Kyriazakis (2016). Products associated with deforestation such as soyabean meal have a higher environmental impact than crops not associated with deforestation (INRA) illustrating how difficult it is to determine the environmental impact of a specific ingredient accurately. In this evaluation, it was assumed that soybean meal was indeed sourced from previously forested areas because this will be the reality as demand for the crop increases.

Formulating diets for poultry has moved on from the production of least-cost diets and nutritionists and producers currently focus on maximising returns of the feeding operation. In future, they will also need to consider all aspects of the sustainability issue. Adding values for environmental impact to a formulation system is a useful starting point, such as the INRA (2020) data used here, will allow nutritionists to begin to assess the environmental impact of diet formulation. It will be possible to optimise poultry production systems, but also consider environmental aspects.

Whilst this paper singles out a segment of environmental impact, it is acknowledged that a complete LCA requires all aspects to be considered. However, the many different LCA data reported in the published literature attest to the complexity of defining the total impact on sustainable poultry production. In broad terms, the use of high protein, high energy diets increases the carbon footprint of egg production. However, the use of low protein diets also leads to reduced egg size, implying that sustainably aware consumers should consider buying only small eggs.

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Table 1 - Calculated environmental contamination coefficients utilising six environmental parameters from *l'Institut National de Recherche Pour l'Agriculture* (INRA) for layer feed and estimated levels to produce one gram of egg output.

Diet, production and egg parameters	SID Lysine Comparison				AME_n Comparison		
AME_n (MJ/kg)	11.75	11.75	11.75	11.75	11.00	11.75	12.50
SID lysine (g/kg)	6.0	7.0	8.0	9.0	8.0	8.0	8.0
Feed cost ($/ton)[1]	294	311	327	346	311	327	362
Acidification (mol H^+ eq/ton)[2]	9.54	9.62	9.71	9.82	8.51	9.71	10.11
Carbon footprint (CO_2 eq g/kg)	624.5	698.7	772.9	805.2	710.1	772.9	846.8
Cumulative energy demand (MJ/kg)	4.50	4.96	5.40	6.07	4.94	5.40	6.15
Eutrophication potential (gPO_4 eq/kg)	40.08	40.04	40.00	40.26	34.92	40.00	41.35
Land competition (1000 m^2 yr/ton)	0.27	0.30	0.33	0.43	0.29	0.33	0.43
Phosphorus consumption (g P/kg)	10.92	11.47	12.01	12.02	10.93	12.01	12.80
Hen day production (%)	97.04	97.62	96.97	97.25	96.09	97.55	98.04
Egg size (g)3	56.88	58.05	58.59	58.82	57.55	59.10	56.50
Egg output (g/d)	55.20	56.67	56.81	57.20	55.30	57.65	55.39
Feed intake (g/day)	113.0	113.1	112.9	109.9	117.6	113.3	105.5
Feed conversion ratio (g feed/g egg/day)	2.047	1.996	1.987	1.921	2.127	1.965	1.905
Acidification (mol H+eq/g egg)	0.0195	0.0192	0.0193	0.0189	0.0181	0.0191	0.0193
Carbon footprint (CO2 eq/g egg)	1.278	1.395	1.536	1.547	1.510	1.519	1.613
Cumulative energy demand (kJ/g egg)	9.22	9.89	10.74	11.66	10.50	10.62	11.71
Eutrophication potential (gPO4 eq/g egg)	0.0820	0.0799	0.0795	0.0773	0.0743	0.0786	0.0788
Land competition (1000 m^2 yr/g egg)	0.0005	0.0006	0.0007	0.0008	0.0006	0.0006	0.0008
Phosphorus consumption (g P/g egg)	0.0224	0.0229	0.0239	0.0231	0.0232	0.0236	0.0244

[1]Diets formulated to meet the specifications used in Kleyn et al. (2020). The ingredient costs are for illustrative purposes only.
[2]All environmental data derived from INRA www.feedtables.com and expressed per kg of feed with the exception of land competition provided in metric tons.
[3]Hen performance data derived from Kleyn et al. (2020) and environmental data calculated by applying INRA calculated feed values to egg output.

ARTIFICAL STRUCTURES MITIGATE SIMULATED ADVERSE POPHOLE CONDITIONS

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There is some evidence that commercial meat chickens prefer to access the range through 'favoured' pop-holes (Taylor et al., 2017); however what makes pop-holes attractive or aversive is unknown. Therefore, we aimed to i) determine the most aversive stimuli associated with pop-holes and ii) identify the most effective artificial structure to minimise aversion. We hypothesised that the simulation of wind, UV light and high light intensity at an artificial pop-hole would be aversive for meat chickens and predicted that barriers, shade cloth and tunnel structures would reduce aversion and increase the transition of birds through pop-holes.

We used one hundred 14-day old mixed sex Cobb 500^{TM} meat chickens. The birds were trained to walk through an artificial pop-hole (60 x 40 cm) to obtain live mealworms, referred to hereafter as the learning/habituation round. Chickens were placed in a start box (1700 × 1700 × 800 mm) with visual access to an adjacent area (1700 × 1700 × 500 mm) with a green bowl containing five mealworms accessible via the artificial pop-hole. The time taken to exit the pop-hole and reach the bowl was recorded. If a chicken did not access the bowl of mealworms within eight minutes the chicken was carried to the bowl and left with the assessor out of sight for 2-3 minutes or until the meal worms were consumed. The learning/habitation round was repeated three times and any chicken that did not cross the pop-hole was excluded from further testing. Each chicken was randomly allocated to one of four structural treatments groups; barrier, tunnel, tall shade or control treatment. The control group had no artificial structure surrounding the pop-hole. The barrier treatment included two wooden structures (400 × 400 × 2 mm) extending out from the pop-hole wall and a Perspex barrier (800 x 25 mm x 2mm) positioned parallel 0.5m from the pop-hole. The tunnel treatment was an 800 mm long (600 mm high) perpendicular tunnel extending from the pop-hole into the arena, covered in shade cloth (50 – 90 % UV). The tall shade treatment was a wooden rectangular structure (800 × 700 × 750 mm) covered with 70% UV shade cloth. After three rounds of learning/habituation aversive stimuli were added; either simulated wind at a speed of 3.0 m/s (Dynabreeze 600 mm industrial drum fan., NZ), UV light (2 × UV 200 UV light globes, Exo Terra, CA) or high light intensity (LED light, 54,000 lux at chicken height, Hi-Bay 200W, Cetnaj, NSW). Each chicken was exposed to each aversive stimulus individually and all stimuli together once in a randomly assigned order over four consecutive days. The time taken to step, cross through the pop-hole and to peck the mealworm bowl was recorded. Data were analysed with a repeated measure mixed model. Preliminary analysis showed that there was no interaction between pop-hole structure and aversive stimuli ($p = 0.705$). However, chickens took longer to pass through the pop-hole when provided with a barrier structure (103 ± 20.7 s) compared to chickens without a structure (25.1 ± 21.5 s; $p = 0.037$) or tunnel (26.4 ± 23.9 s $p = 0.041$) and when high light intensity was applied (intensity 113.1 ± 21.4 s) compared to wind (77.9 ± 21.3 s; $p = 0.11$).

We provide some insight into the perception of pop-hole environments by meat chickens and the aversive stimuli that may prevent or slow transitions from shed to range environments. Further studies will investigate the impact of pop-hole structures in commercial conditions.

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INCLUSION OF BLACK SOLDIER FLY LARVAE IN A MEAT CHICKEN DIET HAS MINOR EFFECT ON CAECA MICROBIOTA

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The caecal microbiota composition of commercial broilers is essential to the poultry industry as it can affect the broiler's health and performance (Pandit et al., 2018). Bioactive components of the Black Soldier Fly larvae (BSFL) include antimicrobial peptides, chitin, and lauric acid. They are known to modulate the immune system of broiler chickens (De Souza-Vilela et al., 2020). This study aimed to investigate the impact of BSFL on the diversity, structure, and composition of the caecal microbiota of broilers fed up to 20% BSFL, partially replacing soybean meal and soybean oil in a commercial diet. A total of 400 Ross 308 male broilers were randomly assigned to 5 dietary treatments with 8 replicates in each treatment diet divided into 3 phases: starter (day 2 to 10), grower (day 11 to 21), and finisher (day 22 to 42). The inclusion levels of BSFL were 0, 2.5, 5, 7.5, and 10% in the starter diet followed by 0, 5, 10, 15, and 20% in the grower and finisher diets.

The caecal content of one broiler per cage (n = 40 cages) on day 21 and day 42 was used to extract the DNA using the DNeasy PowerSoil Pro kit (Qiagen, Inc., Doncaster, VIC, Australia) with some modifications. The V3-V4 region of the 16S rRNA gene was sequenced with Illumina MiSeq platform resulting 2×300 bp paired-end reads. The DNA sequence reads were analysed with QIIME2 using DADA2 plugin for quality control and denoising. The downstream analysis of the resultant feature table with frequency was done using Calypso.
Up to 20% BSFL dietary inclusion did not significantly alter alpha diversity measured with Shannon index or beta diversity measured with weighted and unweighted UniFrac both at day 21 and day 42. Shannon index was not significantly different either at amplicon sequence variants (ASV) or genus level at day 21 or day 42. Microbial diversity measured with other common alpha and beta diversity indicators were also similar between treatments.

The abundance of sequence variants representing *Enterococcus* and unclassified Christensenellaceae decreased at day 21 ($P = 0.048$) and ($P = 0.025$), respectively, compared to the control group when the BSFL were added into the broiler diets at 20%. However, at day 42, the sequence variants representing the *Dehalobacterium* increased in the 20% BSFL group compared to the control group ($P = 0.027$). Li et al. (2018) reported improvement in hens' intestinal function accompanied by a *Dehalobacterium* decrease. In conclusion, BSFL inclusion in broiler diets improved the broiler's performance, but had negligible effects on the diversity of caeca microbiota in broilers at days 21 and 42, while 20% inclusion appears to affect the abundance of some bacterial groups. Therefore, BSFL can be used in broiler diets without detrimental effects on the caecal microbiota.

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NO EVIDENCE OF LEVAMISOLE RESISTANCE IN *ASCARIDIA GALLI* ON A FREE-RANGE EGG FARM IN AUSTRALIA

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With the growing popularity of free range production systems, the incidence of helminth infections has increased in commercial poultry farms in Australia. Adequate control can only be maintained by regular application of commercial anthelmintics. Until very recently, levamisole (LEV) and piperazine (PIP) were the only registered chemicals to treat nematode infections in chickens with no published appraisal of their efficacy status since registration. We report the first formal investigation into the efficacy of commercial anthelmintics against chicken ascaridiasis in Australia.

A controlled experiment following international guidelines (Yazwinski et al., 2003) was conducted to evaluate the efficacy of LEV, PIP and fenbendazole (FBZ) plus levamisole-piperazine combination (LEV-PIP), administered via drinking water or as a single oral drench, against an experimental *A. galli* infection in chickens. The *A. galli* isolate used in this study was isolated from naturally infected free-range laying hens from a private commercial farm with a history of regular application of LEV. Adult female worms were recovered from hens killed at the time of scheduled depopulation three weeks post flock deworming with LEV and used as source of infective eggs for artificial infection. We therefore defined this isolate as 'a suspected case of resistance to LEV'. A total of 108 *A. galli* artificially infected cockerels were randomized into nine experimental groups of 12 birds each, with each treatment administered as a single individual oral dose or in drinking water delivered via nipple drinkers with drip trays over 8 hours each day for 1, 2 and 5 days respectively for LEV, PIP and FBZ (Panacur 25® Sheep drench). Chickens received label-recommended doses of LEV (28 mg/kg), PIP (100 mg/kg) or LEV-PIP co-administered at their full individual dose rates while FBZ was tested off-label at two dose rates (10 mg/kg as a single oral drench or 5 mg/kg in drinking water). Anthelmintic efficacies were estimated by both worm count reduction (WCR %), and excreta egg count reduction (EECR %). Values below 90 and 95% respectively were considered as indicative of loss of efficacy for WCR % and EECR %.

Ten days post treatment, the untreated control birds harboured significantly higher ($P < 0.0001$) worm burdens (7.67±0.91) and excreta egg counts (440±122.2) than all treatment groups with individual oral treatment causing a greater ($P = 0.03$) reduction than medication via drinking water. The WCR efficacies for the single oral drenches were 97, 89, 94 and 100% respectively for LEV, PIP, FBZ and LEV-PIP, whereas efficacy values of 92, 82, 72 and 93% respectively were recorded for the corresponding treatments applied in drinking water. The EECR values were largely consistent with WCR %. An overall lower efficacy was observed when the drugs were administered via drinking water. Put together, contrary to our suspicion, this study revealed no evidence of LEV resistance against the test *A. galli* isolate but a depressed efficacy of PIP which may signal the onset of resistance. The LEV-PIP combination, however, exhibited superior efficacy and may provide an option for control of chicken ascaridiasis. FBZ provided optimum efficacy (94%) as a single oral drench but this was an off-usage for target species, dose rate and route of administration for the product used.

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COMPARISON OF THE MINI-FLOTAC AND MODIFIED Mc MASTER METHODS FOR ENUMERATION OF ASCARIDIA GALLI EGGS IN CHICKEN EXCRETA

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Excreta egg counting techniques can provide valuable information for assessing flock infection levels, selecting nematode resistant chicken breeds and for determining anthelmintic efficacy. Although the Modified McMaster (MDM) method has been used for a long time, it is generally considered to have low sensitivity and precision (Das *et al*., 2020). The Mini-FLOTAC (MF) is a more recently developed commercial flotation method for excreta which could be a good alternative to replace MDM. The aim of this study was to compare the MF to the traditional MDM for their sensitivity, accuracy and precision using egg spiked chicken excreta samples. Time spent on sample processing and operator factors was also evaluated.

The diagnostic performance of the methods was compared using chicken excreta spiked with *Ascaridia galli* in a 2 x 2 x 3 x 4 factorial experiment testing the effects of excreta egg counting method (MDM with analytical sensitivity of 40 EPG, and MF with analytical sensitivity of 5EPG), person preparing the sample (preparer A and B), excreta egg counts (EPG, eggs/g) level (5, 50, 500 EPG) and person doing the egg counting (1, 2, 3, 4). EPG value was tested for normality and transformed by cube root prior to data analysis to meet the best assumption of analysis of variance. EPG and times were subjected to analysis of variance fitting the four effects tested in the model and interactions up to 3-way using JMP 14. The sensitivity of each technique was calculated as follows: [(total number of observed positive samples (true positive)/the total number observation for each EPG level and method)*100]. Moreover, precision (i.e. how close measurements are to each other) was calculated by subtractions of coefficient variation (CV) from 100 (precision = 100- CV).

The overall sensitivity of MF and MDM at detecting positive samples across all EPG levels was 79.2% and 68.3%, respectively. However, MF was significantly ($P = 0.0467$) more sensitive than MDM at the 50 EPG level. The overall mean egg recovery rates of MF and MDM were 24.6 and 35.9 EPG, respectively ($P = 0.08$). However, the mean egg count between EPG levels was significantly different ($P < 0.0001$). Furthermore, the logistic interaction between excreta egg counting methods and EPG level was significantly different ($P = 0.0026$), whereas the two and three ways logistic interaction between methods, preparers and observers did not have a significant effect on mean egg recovery of spiked eggs ($P = 0.42$-0.97). The average precision of MDM across all EPG levels was 72.6%, whereas it was 77.7% for MF. There were no significant differences between observers and preparers for mean egg count with respective EPG levels and methods; however, operator factors can generally cause an unexplained source of variation. The total time taken for sample preparation and egg counting (slide reading) was significantly lower with MDM (5 min) than with MF (22 min) ($P < 0.0001$). In conclusion, MDM was relatively more accurate but less sensitive and precise than MF. Taken as a whole, our observation suggests that the MDM method appears to be more appropriate for rapid diagnosis of chicken nematodes in the field. Our finding is in line with a recent study (Das *et al*., 2020) who concluded that MDM is faster, relatively more accurate but less precise than Mini-FLOTAC.

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EFFICACY OF NICARBAZIN/MONENSIN A NOVEL COCCIDIOSTAT COMBINATION PRODUCT IN THE CONTROL OF COCCIDIOSIS IN BROILERS

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Summary

The efficacy of nicarbazin/monensin at a concentration of 40mg/kg (40ppm) nicarbazin and 40mg/kg (40ppm) monensin, administered in feed was assessed in broilers after an experimental coccidiosis challenge under battery cage conditions. The efficacy of nicarbazin/monensin for the prevention of coccidiosis in broilers was demonstrated based on significantly improved growth rates and feed efficiency in the nicarbazin/monensin treated birds than in the untreated control group.

I. INTRODUCTION

Anticoccidials remain an essential tool in the prevention and control of coccidiosis, which is still one of the most important diseases contributing to gut health disorders. Many anticoccidials have been available for use for considerable periods of time. Monimax® (nicarbazin/monensin) is a novel coccidiostat for coccidiosis control in broilers.

II. METHOD

The efficacy of nicarbazin/monensin (Monimax® supplied by Huvepharma®, Belgium) at a concentration of 40mg/kg (40ppm) nicarbazin and 40mg/kg (40ppm) monensin, administered in feed was assessed in broilers after an experimental coccidiosis challenge under battery cage conditions.

Birds were reared without coccidiostats until 14 days of age when they were allocated to the different groups. Nicarbazin/monensin treated birds were compared to i) infected untreated control (IUC) and ii) uninfected untreated control (UUC) groups. Each group consisted of 7 replicate cages each containing 5 Ross 308 males.

At 17 days of age all birds in the IUC and nicarbazin/monensin groups were inoculated with a mixture of *Eimeria acervulina, E. maxima* and *E. tenella* (all of European origin). The UUC birds were sham inoculated.

Daily weight gain (DWG), feed conversion ratio (FCR) and intestinal lesion scores (ILS) were compared. Statistical analysis was performed in accordance with those outlined in the WAAVP guidelines for evaluating anticoccidial drugs in chickens and turkeys (Holdsworth et al., 2004). All tests were 2-sided and the level of significance was set at 5%.

III. RESULTS

The challenge was successful as shown by the significantly lower DWG, increased FCR and significantly higher average ILS (total ILS and *E. tenella* ILS) in the IUC compared to the UUC.

Administration of nicarbazin/monensin was able to significantly improve performance (DWG and FCR) in the acute infection phase (D17-D23).

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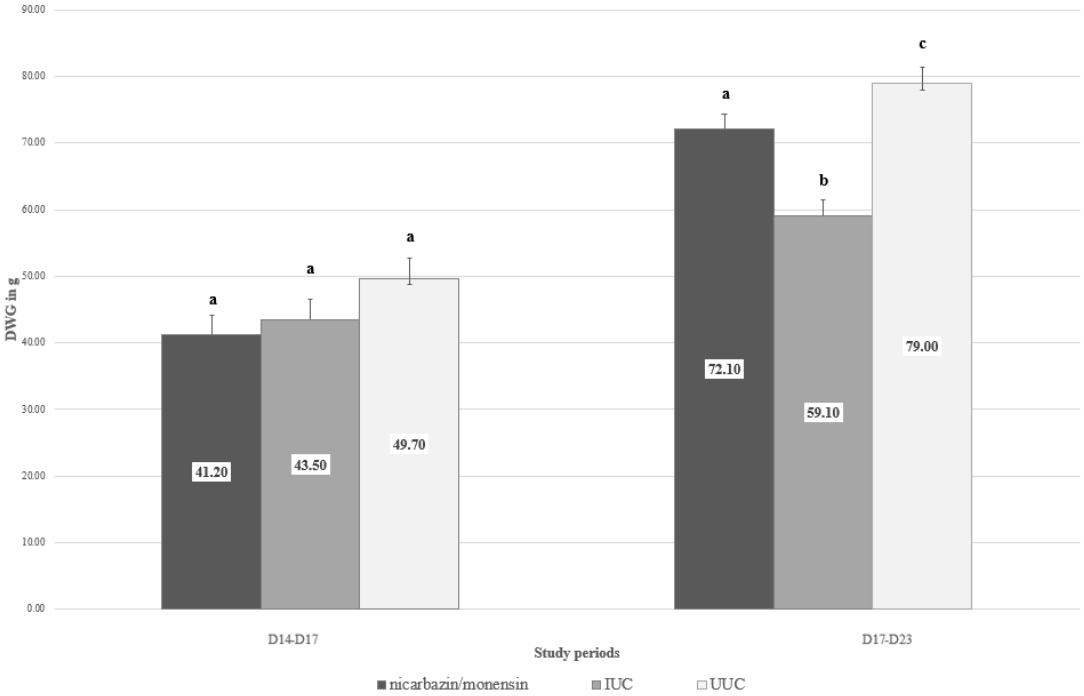
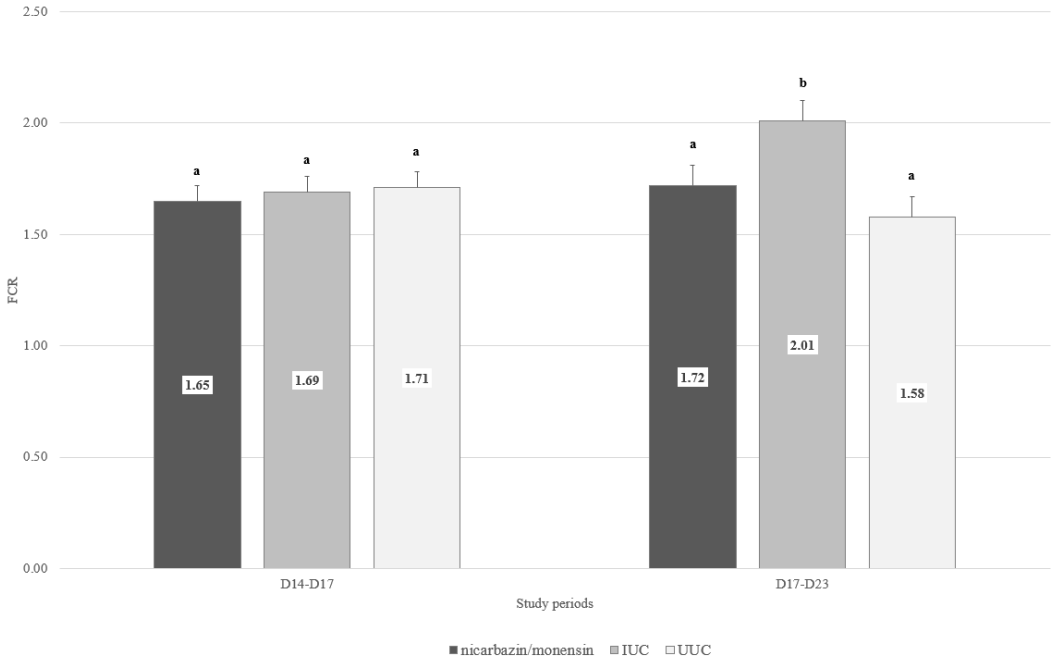


Within the two time periods bars with different superscripts differ significantly in body weight ($P < 0.05$).

Figure 1 - Daily Weight Gain (DWG) in g per day per animal for the different study periods



Within the two time periods bars with different superscripts differ significantly in FCR ($P < 0.05$).

Figure 2 – Feed conversion ratio (FCR) for the different study periods

Although not statistically significant, the total ILS at D23 tended to be lower in the nicarbazin/monensin supplemented group in comparison to the IUC.

Table 1 - Average lesion scores (LS) on study D23 per treatment group for the species *Eimeria acervulina, E. maxima, E. tenella* and for the total lesion score.*

	Average Lesion Score (LS) on D23			
Treatment	*E. acervulina*	*E. maxima*	*E. tenella*	Total
Nicarbazin/monensin	0.6 a	0.6 a	1.7 b	2.9 b
IUC	0.7 a	0.9 a	2.2 b	3.8 b
UUC	0.3 a	0.4 a	0.1 a	0.7 a

Different letters indicate significant differences at $P < 0.05$.

IV. DISCUSSION

The results from this trial demonstrate the efficacy of nicarbazin/monensin in reducing the impact of coccidiosis in broilers. Under the present study conditions, we can conclude, based on significantly better growth rate and feed efficiency performance, that the administration of nicarbazin/monensin is efficacious in reducing the impact of coccidiosis infection in broilers. The number of animals included in the study appeared to be too small to draw conclusions on the effect of nicarbazin/monensin on lesion scores.

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RESPONSE OF BROILERS TO EARLY STAGE FEEDING OF SPRAY DRIED PORCINE PLASMA IN PRESENCE OF NECROTIC ENTERITIS CHALLENGE

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Necrotic enteritis (NE) is an enteric disease of poultry caused mainly by a spore-forming gram-positive bacterium, *Clostridium perfringens*, resulting in decreased growth, high mortality, and increased veterinary costs (Wade and Keyburn, 2015). While supplementing poultry diets with antibiotics can control NE, emerging antibiotic-resistant microbes and transfer of such resistance factors into human medicine have resulted in restriction or ban of antibiotic usage across the world. Inclusion of plasma proteins in feed may show potential for poultry health in a post-antibiotic era. Plasma proteins contain immunoglobulins and other components that may benefit both healthy and NE affected birds. The current study was carried out to examine the effects of spray-dried porcine plasma (SDPP) on growth performance and immunological parameters in broilers under NE challenge.

A total of 720 day-old Ross 308 male parental line were randomly assigned to four treatments with 12 replications in a 2 × 2 factorial arrangement. The factors included NE challenge (no, yes) and SDPP (0 or 20 g/kg in starter phase, 0 to 10 d). Challenged birds were gavaged with 1 ml *Eimeria* vaccine (*E. acervulina, E. brunetti and E. maxima*) on d 9 and *C. perfringens* strain NE-18 on d 14. Feed intake, body weight gain (BWG), and FCR were determined on d 8 and 29. *Eimeria* oocysts were counted in the excreta on d 14 and 16. On day 16, three birds from each pen were sampled to examine lesion scores, organ weights, and gut leakage by determining passage of gavaged FITC-d into blood. Jejunal tissues were collected for histological examinations. Serum samples were collected to measure immunoglobulins (IgA, IgM, IgG), α-1 acid glycoprotein (αAGP), ovotransferrin and interleukin-6 (IL-6).

The NE challenge was successful as shown by a lower feed intake, reduced body weight gain, higher FCR ($P < 0.001$) and higher lesion scores ($P < 0.01$) in duodenum, jejunum and ileum than the unchallenged birds. Challenged birds had lower villus height to crypt depth ratio, and higher FITC-d concentrations in blood serum than the unchallenged birds ($P < 0.001$). Early feeding of SDPP (0 to 10 d) decreased FCR by 4.5 points before NE challenge (i.e. from d 0 to 8, $P < 0.001$). During the overall period of 0 to 29 d, dietary SDPP decreased ($P < 0.01$) FCR by 1.5 points and tended ($P = 0.07$) to increase BWG. Dietary SDPP did not affect lesion scores in the gut, oocyst counts in the excreta, and villus height to crypt depth ratio ($P > 0.05$). An interaction was observed ($P < 0.05$) that SDPP lowered ($P < 0.01$) serum FITC-d concentration only in the challenged birds compared to those without SDPP, but not in non-challenged birds. Dietary SDPP increased ($P < 0.05$) the relative weight of bursa on d 16. There was no interaction between SDPP and NE challenge on performance parameters, lesion scores, and oocyst counts in the excreta. Challenge increased the levels of IgA, IgM and αAGP levels in the serum on day 16. Dietary SDPP decreased ($P < 0.05$) the level of αAGP and IL-6 in serum on d 16. These results indicate that early inclusion of SDPP enhances performance possibly through reduced inflammation in the NE infected or uninfected birds. Further work should examine shorter periods of SDPP inclusion at higher doses.

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SUPPLEMENTATION OF BROILER DIETS WITH INTRISCALLY HEAT STABLE XYLANASE TO INCREASE GROWTH PERFORMANCE AND PROMOTE GUT HEALTH OF BROILER CHICKENS

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Summary

Xylanase is commonly used as a feed additive to reduce cost, decrease viscosity of the digesta and improve non-starch polysaccharide and protein digestibility. The supplementation of xylanase has also been shown to have a prebiotic effect by promoting gut health. In this study we investigated in a broiler challenge trial the dose-related effect of the supplementation of an intrinsically heat stable xylanase to a corn-soy diet typical of South East Asia. The results indicated that xylanase supplementation could compensate down specifications of 100kcal apparent metabolizable energy and 2% crude protein and digestible amino acids in term of growth performance. Additionally, the study showed the dose-dependent effect of xylanase on gut health with changes in intestinal microbiota, an increase in short chain fatty acids content and positive changes in gut morphology.

I. INTRODUCTION

Xylans, together with β-glucans and cellulose constitute non-starch polysaccharides (NSP) that cannot be fully degraded by monogastric animals (Cowieson at al., 2008). NSPs have an anti-nutritive effect by increasing the viscosity of the intestinal digesta (Choct and Annison, 1992; Craig et al., 2020). This increase in digesta viscosity causes an impairment of nutrient bioavailability and a decrease in metabolizable energy, lowering the overall performance of animals (Bedford and Morgan, 1996). Poultry do not produce endogenous carbohydrases capable of hydrolysing the pentosan NSPs, such as the arabinoxylans, present in cereals. Addition of enzymes such as xylanases, β-glucanases, and cellulases to the feed reduce the antinutritional effect of NSPs. It is believed that these enzymes degrade polysaccharide cage structures around proteins and reduce the viscosity of the intestinal contents of the animals. Therefore, enzymes are widely used in animal nutrition, especially for poultry and pigs, for more complete utilization of feed components originating from plants. Supplementing feed with xylanases has been shown to improve nutrient digestibility and help maintain good gut health (Walsh et al., 1993; Singh et al., 2012; Craig et al., 2020). In this study we investigated the dose-related effect of supplementation of an intrinsically heat stable xylanase on performance and gut health of broilers fed with a corn-soy broiler diet.

II. MATERIALS & METHODS

A total of 1,000 broiler chickens (Ross 308) were allocated to five dietary treatments comprising 10 pens each (20 chickens per pen) for 1 to 35 d of age. The treatments were: standard corn soybean diet (PC), diet with 100 kcal/kg reduction in metabolizable energy and 2% reduction in crude protein and digestible amino acids (NC), NC with 10g/T of xylanase (T1), NC with 15g/T of xylanase (T2) and NC with 30g/T of xylanase (T3). The feed was pelleted at 80°C for 30 seconds. The activity of xylanase recovered after pelleting was greater than 80%.

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Bird weight and feed intake were measured on a pen basis up to 35 days. Caeca of 36 broilers were collected at 35 d for the quantitative analysis of microflora. *Lactobacillus spp.* and *Escherichia coli* enumerations were done on MacConkey agar and eosin methylene blue agar, respectively. Intestinal segments (duodenum, jejunum and ileum) were collected from 36 broilers at the end of the experiment to determine intestinal morphology. Tissue samples were washed then fixed in 10% formaldehyde solution. The villus length (VH) was defined from the villus tip to the villus-crypt junction between each villus, while the crypt depth (CD) was measured from the top to the bottom of villi between adjacent villi and the villus width. Caecal digesta samples from 6 birds in each treatment were collected at the end of the experiment for the measurement of volatile fatty acid (VFAs) content. VFAs were quantified according to Apajalahti et al. (2019).

The effect of dietary treatments on performance parameters of broilers in the present study was analyzed with one-way ANOVA using SAS 9.0. The GLM procedure was used to quantify significant difference among treatments for all measurement by Duncan's new multiple range test. The average values including the standard error of the mean were calculated for every examined parameter. The level of significance was set at $P<0.05$.

Table 1 - Main ingredients of the basal diets for each growing phase

Ingredient (%)	Day 0 to Day 14		Day 15 to Day 35	
	PC	NC	PC	NC
Corn	40.93	41.39	43.43	45.42
Rice bran	10.00	12.36	15.00	17.94
Extruded soybean meal	15.00	15.00	15.00	15.00
Dehulled soybean meal	26.79	26.13	19.71	16.79
Rice bran oil	3.42	1.26	3.50	1.30
Dicalcium phosphate	1.60	1.64	1.30	1.42
Limestone	1.30	1.26	1.25	1.18
Sodium chloride	0.25	0.25	0.25	0.25
Choline chloride	0.05	0.05	0.05	0.04
DL-methionine	0.40	0.40	0.25	0.40
Phytase	0.01	0.01	0.01	0.01
Vitamin-mineral premix	0.25	0.25	0.25	0.25
Total	100	100	100	100
Calculated value				
AMEn, kcal/kg	3,150	3,050	3,200	3,100
CP, %	23.00	23.00	20.00	20.00
Ca, %	0.96	0.96	0.84	0.84
Available P, %	0.47	0.47	0.42	0.42
Digestible Lys, %	1.31	1.31	1.11	1.11
Digestible Met + Cys, %	0.95	0.95	0.74	0.74
Digestible Thr, %	0.87	0.87	0.76	0.76
Ash, %	2.55	2.55	3.14	3.14
Fiber, %	4.03	4.03	4.82	4.82
Linoleic acid, %	3.26	3.26	3.35	3.35

III. RESULTS AND DISCUSSION

A dose response was observed for body weight and feed conversion ratio (Table 2) after 35 days with 10 g/T xylanase and 15 g/T able to compensate the down specifications in term of growth performances and 30g/T yielding a statistically significant difference compared with 10 g/T for both body weight and FCR.

Table 2 - Body weight at Day 35 and FCR over 35 days.

	PC	NC	T1	T2	T3
Body weight, g	2219^{ab}	1977^{c}	2145^{b}	2190^{ab}	2289^{a}
FCR (feed/g)	1.50^{bc}	1.76^{a}	1.56^{b}	1.53^{bc}	1.47^{c}

^{a,b,c} Means without a common superscript within a column significantly differ ($P < 0.05$).

The study also showed the effect of xylanase on the gut. Caecal samples were collected and *Lactobacillus spp* and *Escherichia coli* were counted (Table 3). Counts of *Lactobacillus spp* tended to increase with the addition of 10g/T and 15g/T and the change was statistically significant at 30g/T. The opposite effect was observed for *Escherichia coli* with a count decrease observed upon xylanase supplementation (Table 3).

Table 3 - Body weight at Day 35 and FCR over 35 days.

	PC	NC	T1	T2	T3
Log10 cfu/g content					
Lactobacillus spp	4.47^{b}	4.62^{b}	5.86^{ab}	5.59^{ab}	6.92^{a}
Escherichia coli	5.27^{ab}	5.86^{a}	4.18^{b}	4.41^{ab}	3.71^{b}

^{a,b,c} Means without a common superscript within a column significantly differ ($P < 0.05$).

Caecal volatile fatty acids of the broiler chickens at 35 days were measured and showed that the concentration of butyrate was greater in xylanase supplemented groups (Table 4) compared to PC and NC ($P < 0.05$). The dose response was only a trend but all treated groups were statistically higher than PC and NC. Acetate was unaffected by xylanase supplementation but propionate levels in PC were recovered in the xylanase groups ($P < 0.05$).

Table 4 - Treatment effects on caecal volatile fatty acids content (mmol/g digesta)

	PC	NC	T1	T2	T3
Acetate	25.1	25.34	25.63	28.58	27.33
Propionate	7.48^{bc}	6.76^{c}	9.12^{ab}	8.97^{ab}	9.36^{a}
Butyrate	11.54^{b}	8.38^{c}	13.04^{a}	15.94^{a}	17.08^{a}
BCFAs	1.29^{ab}	0.78^{b}	1.21^{ab}	1.58^{ab}	1.69^{a}
Total VFAs	45.41^{bc}	41.27^{c}	49.00^{b}	55.08^{a}	55.46^{a}

VFAs = acetic acid + propionic acid + butyric acid + branched-chain fatty acids.
^{a,b,c}Means without a common superscript within a column significantly differ ($P < 0.05$).

The morphologies of duodenum, jejunum, and ileum were measured through villus height and crypt depth. There was no significant effect on the crypt depth of the broiler chickens at 35 days of age. However, the villus height in the duodenum and in the ileum changed upon xylanase treatment; ileal villus height, and the VH:CD ratio in the duodenum and jejunum were significantly ($P < 0.05$) affected by the xylanase supplementation compared with the NC group. The dose response with T1, T2 and T3 was only numerical.

Change in bacterial population, increase in amounts of VFA's and increase of VH:CD ratio constitute an array of data suggesting that the supplementation of Xylanase had a beneficial effect on the gut health of the animal. This has been previously demonstrated in other study (Liu and Kim, 2017).

The study presented here demonstrates that supplementation of xylanase at 10g/T enabled recovery of the growth performance obtained with the PC in a corn soy diet. Further supplementation of xylanase tended to result in additional improvements.

The data collected demonstrate that dietary supplementation of xylanase even at the lowest dose of 10 g/T of feed can result in significant improvements of broiler performance, nutrient utilization, beneficial microbial growth, and VFAs concentration.

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NUTRITIONAL PROFILING OF SOYBEAN MEAL USING IN VIVO BASED NIR MODELS ON A LARGE NUMBER OF INDUSTRY SAMPLES

L.H. ZHANG[1], L.W.O. SOUZA[1], and Y.G. LIU[1]

Summary

This paper compares the nutrition profiles of soybean meal (SBM) from three different places of origin: USA, Brazil and Argentina. The study found i) heterogeneous data reflect the diversity of SBM qualities used by feed mills; (ii) individual SBM samples play a higher role on their nutritional variation than origin of production; (iii) working on analyses of real SBM using *in vivo* based NIR models is more time-efficient compared to traditional lab methods; iv) DAAs, AME or AMEn have high impact on SBM value, price and cost of final feeds. The study concluded that, by selecting the best AMEn and DAA profile in the present dataset, one could save up to 1.29% of feed production costs.

I. INTRODUCTION

Soybean meal (SBM) remains a standard protein source for feed producers worldwide. Seed genetics (Palacios et al., 2004), growing environment and processing technique (Karr-Lilienthal et al., 2005) increase variation in SBM quality and difficulty in SBM procurement. One common question for purchasers is which origin of SBM is better. To help feed producers better assess the quality of SBM, we have compared the nutritional profiles of SBM samples from three different origins analyzed by Asia Pacific (APAC) feed mills. Predicted nutritional parameters included proximate nutrients (PROX), total amino acids (TAA), standardised ileal digestibility of amino acids (SID AA), and apparent metabolizable energy corrected for nitrogen (AMEn) or not (AME) using Near Infrared Reflectance Spectroscopy (NIRs). Calibration models were derived from already existing chemical analyses and *in vivo* determinations. The objective of this study was to check the most discriminating nutrient variables of SBM and their economic impact on feed formulation.

II. MATERIAL & METHOD

In total, 3621 SBM samples originating from Brazil, USA and Argentina were submitted to the Precise Nutrition Evaluation (PNE) platform during the year 2019. All SBM samples were scanned on Adisseo standardized and validated NIR instruments at the feed mill level located in APAC. The origins of these SBM samples were declared in the platform by PNE users. Samples were predicted for their concentrations in PROX: dry matter (DM), crude protein (CP), ash, crude fiber (CF) and fat, TAA and SID AA, digestible amino acids for poultry (DAA), AME and AMEn. Predictions employed Adisseo's NIRs predictive equations derived from *in vivo* digestibility tests (Tang et al., 2008). Digestibility coefficients for SBM were determined *in vivo*, using the model of adult caecectomised cockerels (Green et al., 1987). The AME and AMEn calibration for SBM were obtained through *in vivo* measurements using 3-week-old male broilers (Bourdillon et al., 1990) with *ad libitum* feeding and total excreta collection.

By fixing AMEn or DLys:CP at two different levels, four SBM profiles (SBM 1, 2, 3 and 4) were selected using Argentina originated SBM data set. SBM1 and SBM2 had the same AMEn level at 8.79 MJ/kg as fed, but with different DLys:CP levels (0.053 for SBM1 and 0.057 for SBM2). SBM3 and SBM4 had the same AMEn at a higher level of 9.20 MJ/kg as

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fed, with different DLys:CP levels (0.053 for SBM3 and 0.057 for SBM4). Nutrient values of the 4 SBM profiles were used for broiler grower phase (21-35d industry specifications) formulation on a digestible amino acids basis. Missing nutritional data were included from a standard 460 g/kg CP SBM (Rostagno et al., 2017), with same values for all studied profiles. All diets contained 13.39 MJ/kg AMEn, 226.0 g/kg CP, 12.3 g/kg DLys, 9.1 g/kg DMet+DCys, 8.1 g/kg DThr and 2.5 g/kg DTrp as fed basis.

III. NIRS PREDICTION FOR NUTRIENTS IN SBM

Substantial variations were observed for all parameters. The proximate nutrients and total essential amino acids of SBM samples were summarized in Table 1 on a dry matter (DM) basis. SBM originating from Brazil showed higher average CP level (537.0 g/kg DM) compared to USA origin (524.7 g/kg DM) and Argentina origin (518.1 g/kg DM). Regarding the total AA contents, Brazil SBM ranked the highest average values. By comparing the standard deviation values, Brazil SBM showed the highest variation for both proximate and TAA contents.

Table 1 – Proximate compositions and total amino acid levels of SBM from Argentina, Brazil and USA.

Origins	Argentina				Brazil				USA			
Dry Matter	Mean	Std[#]	Min	Max	Mean	Std	Min	Max	Mean	Std	Min	Max
PROX (g/kg)	N = 1532				N = 1396				N = 693			
CP	518.1[a]	7.2	490.0	549.6	537.0[c]	12.7	494.4	571.5	524.7[b]	8.1	492.8	548.5
ASH	74.4[a]	2.7	54.1	82.9	72.4[c]	3.0	51.9	81.1	73.2[b]	3.3	59.0	78.6
FAT	20.4[a]	3.8	5.4	34.8	21.4[b]	4.3	5.22	34.5	21.5[b]	4.3	10.7	36.3
CF	41.4[a]	5.1	27.3	65.0	44.0[c]	6.2	27.5	68.8	42.5[b]	4.6	27.5	63.1
TAA (g/kg)	N = 1511				N = 1396				N = 692			
Lysine	32.9[a]	0.7	30	35.8	33.7[c]	0.9	30.3	36.2	33.3[b]	0.6	30.8	34.9
Methionine	7.2[a]	0.1	6.7	8.1	7.2[a]	0.2	6.3	8	7.1[b]	0.2	6.4	7.7
Cystine	7.6[b]	0.2	6.5	8.8	7.8[c]	0.2	6.8	8.4	7.5[a]	0.4	6.6	8.1
Threonine	21.2[b]	0.4	19.8	22.8	21.6[c]	0.6	19	22.9	21.1[a]	0.5	19.5	22.3
Tryptophan	7.6[a]	0.2	7	8.3	7.8[c]	0.2	7.2	8.5	7.6[b]	0.2	6.8	8.2
Valine	26.0[a]	0.5	24.2	28.5	26.8[c]	0.8	23.9	28.4	26.2[b]	0.5	24.3	27.8
Isoleucine	25.3[a]	0.5	23.5	27.2	26.2[c]	0.8	22.8	27.7	25.5[b]	0.5	23.8	27.6
Leucine	40.2[a]	1.0	37	43.7	42.0[c]	1.4	36.5	44.7	40.6[b]	0.7	37.0	43.7
Histidine	13.2[a]	0.3	12.1	14.4	13.8[c]	0.4	12.2	14.7	13.3[b]	0.2	12.1	14.4
Arginine	37.1[a]	1.2	33.1	41.8	39.8[c]	1.6	34.9	43	38.5[b]	1.0	35.0	41.9

[a-c] within a row, means without a common letter are significantly different ($P < 0.05$).
[#] std = standard deviation.

As shown in Table 2, Brazil SBM showed the highest SID for all essential amino acids, followed by Argentina SBM. USA SBM showed the lowest SID AA levels. The average SID Lys for all these three origins (86.37% for Argentina, 88.46% for Brazil, and 86.11% for USA) fall below the published value of 90% (Bryden et al., 2009), which is expected if we consider other effects on DAA by processing conditions. Average AMEn for SBM originating from Argentina, Brazil and USA are 10.09, 10.03 and 10.41 MJ/kg DM, respectively. The ranking of SBM quality is USA > Argentina > Brazil in terms of AMEn average values. Argentina SBM showed the highest variation with a standard deviation of 0.32 MJ/kg DM. These results reflect the diversity among SBM sources and reveal the true parameters of nutrients such as the content of available energy.

Beyond average values, individual variation shows higher amplitude on their nutrient values. For example, SID Lys of Brazil SBM ranges between 81.70 to 93.60%, while SID Lys of Argentina SBM ranges from 80.00 to 95.20%. Moreover, AMEn of Brazil SBM ranges from 9.21 to 11.07 MJ/kg DM, while USA SBM changes between 9.70 to 11.07 MJ/kg DM. Argentina SBM gives the widest AMEn range which is between 9.15 to 11.15 MJ/kg DM. As shown in Figure 1, there is a large number of samples having higher or lower AMEn or SID Lys than average values. Individual SBM sample with higher average values observed for each particular country does not necessarily have higher nutritional value. The reason can be processing conditions (Karr-Lilienthal et al., 2005) that can change the final SBM quality dramatically. Working on analysis of specific SBM sample with PNE is more efficient.

Table 2 –Contents of SID essential amino acids and AME of SBM from Argentina, Brazil and USA.

Origins	Argentina				Brazil				USA			
Dry Matter	Mean	Std	Min	Max	Mean	Std	Min	Max	Mean	Std	Min	Max
SID AA (%)		N = 1509				N = 1427				N = 692		
Lysine	86.37^{b}	2.34	80.00	95.20	88.46^{c}	2.23	81.70	93.60	86.11^{a}	1.95	80.00	92.50
Methionine	91.33^{b}	2.00	86.10	99.20	92.62^{c}	2.09	87.80	97.00	90.68^{a}	2.04	85.50	97.90
Cystine	80.40^{b}	3.49	71.70	93.40	82.57^{c}	2.78	70.80	89.40	79.33^{a}	3.43	69.80	87.80
Threonine	83.62^{b}	1.75	80.00	91.40	85.25^{c}	1.73	79.70	89.30	83.37^{a}	1.52	76.80	89.30
Tryptophan	87.70^{b}	2.96	82.00	99.30	89.76^{c}	2.45	80.10	96.00	86.92^{a}	2.89	79.90	96.10
Valine	83.61^{b}	1.68	77.50	92.60	85.15^{c}	1.67	80.60	90.40	83.00^{a}	1.87	77.20	90.00
Isoleucine	88.79^{b}	1.68	85.30	96.40	90.43^{c}	1.51	85.80	94.40	88.21^{a}	1.59	84.20	94.30
Leucine	89.29^{b}	1.34	85.20	95.30	90.27^{c}	1.40	87.00	93.80	88.80^{a}	1.58	84.50	93.50
Histidine	89.16^{b}	1.83	85.10	99.00	90.39^{c}	1.84	85.50	95.70	88.60^{a}	1.52	85.30	95.40
Arginine	92.81^{b}	1.04	90.40	98.10	93.71^{c}	1.18	90.70	96.80	92.35^{a}	1.00	89.00	97.20
Energy (MJ/kg)		N = 1211				N = 1340				N = 328		
AME	10.93^{a}	0.34	9.89	12.09	10.92^{a}	0.27	10.01	11.91	11.28^{b}	0.27	10.48	11.91
AMEn	10.09^{b}	0.32	9.15	11.15	10.03^{a}	0.24	9.21	11.07	10.41^{c}	0.25	9.70	11.07

$^{a-c}$ within a row, means without a common letter are significantly different (P < 0.05).

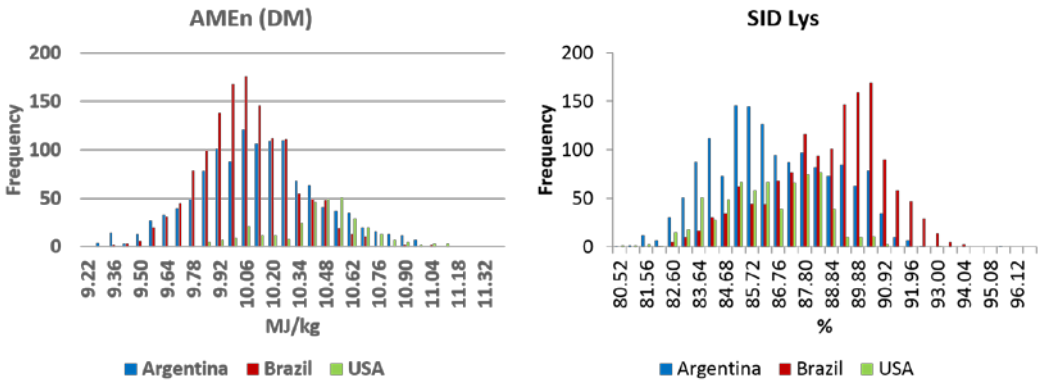

Figure 1 – Histogram distribution of AMEn and SID Lys for SBM originated from Argentina, Brazil and USA.

As shown in Figure 2, an increment of 0.004 of DLys:CP could decrease formulation cost between 1.69 to 1.93 USD/MT feed, depending on AMEn levels. Meanwhile, if digestible amino acids are kept constant, a difference of 0.41 MJ/kg AMEn of SBM could impact the

formulation cost by 2.39 to 2.63 USD/MT feed. This calculation confirms that, not only DAA, but also AMEn plays an important role on SBM value. Comparing to SBM4, using the lower nutritional SBM profiles (SBM1, SBM2 and SBM3) to maintain the same formulation cost, their prices should be 11.6, 6.4 and 4.5 USD/MT lower, respectively.

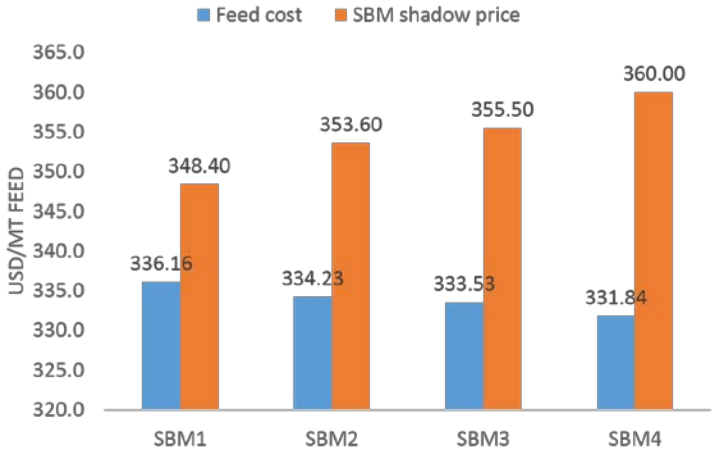

Figure 2 – Formulation costs and shadow prices for 4 selected SBM profiles.

IV. CONCLUSIONS

In conclusion, this study reveals that working on analysis of the specific sample is more valuable and realistic than considering the origin of SBM and historical average values for the particular country for ingredient quality control and formulation. Apart from the origin, there are many other elements affecting the final SBM quality. Predicting from the *in vivo* referenced NIR models, digestibility parameters can be routinely applied to quality control at feed mill level to monitor SBM quality and nutritive value. The formulation exercises suggest that both digestible amino acids and metabolizable energy contents of SBM have paramount impact upon their shadow prices.

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EFFECT OF MULTI-XYLANASE SYSTEM ON GROWTH PERFORMANCE AND GUT HEALTH IN BROILERS

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Summary

Non-starch polysaccharides (NSP), particularly arabinoxylans (AX), with complex biochemical structures are one of the main causative factors for impaired growth performance and poor gut health in broilers. Use of multi-xylanase systems, that target both the xylose backbone and short side chains attached to xylan, can synergistically improve the digestibility of AX-rich plant-derived feed raw materials (Cozannet et al., 2017). In this review, we demonstrated that a multi-enzyme system, containing mainly xylanase and arabinofuranosidase, can be used to improve digestibility of feed ingredients, with resulting impact on growth performance and key gut health indicators in broilers.

I. INTRODUCTION

NSP is one of the key anti-nutritional factors in monogastric animal diets. NSP are present at high levels in both corn and wheat, as well as in plant-based crude protein sources, such as soybean meal and corn distillers dried grains with solubles (DDGS). NSP polymers are categorized as cellulose, hemi-cellulose (AX and beta-glucans) and pectin, which are mainly present in the cell walls of the endosperm and in the bran. AX, which is the predominant NSP, varies in level and biochemical structure between different plant-based feed raw materials. AX consists of a linear backbone of β-(1-4)-xylopyranosyl units (X) that are substituted with short side chains, including L-arabinose (A), ferulic acid, and Oacetyl. AX cannot be readily digested in the small intestine of monogastric animals, resulting in viscous chyme, dilution of nutrients and physical barriers in the gastrointestinal tract. The consequence of this is impaired growth performance and gut health problems, such as proliferation of pathogenic bacteria, intestinal inflammation, weakened intestinal barrier function, and intestinal lesions. Supplementation of AX-degrading enzymes in feed mitigates the adverse effects of AX. Given the complex structure of AX, it is possible that it may be advantageous to use a multi-xylanase system, instead of just single xylanase, in an effort to target both the xylose backbone (such as endo-1,4-b-xylanase, Xyl) and the short side chains (such as arabinofuranosidase, Abf). It is predicted that this will deliver significant improvements in feed digestibility, resulting in enhanced animal performance and gut health status in broilers (Kiarie et al., 2013). To examine this hypothesis, both *in vitro* and *in vivo* models were used to assess the efficacy of multi-enzyme preparation Rovabio (MEP, Adisseo), as well as Xyl and Abf singly or in combination.

II. METHOD

a) In vitro trial

Enzyme preparations of Xyl (GH11 family) and Abf (GH51) were isolated from *Talaromyces versatilis* and expressed in *Pichia pastoris*. These enzymes were evaluated for their efficacy to digest both corn and wheat flours individually and in combination (Xyl; Abf; Xyl+Abf).

The *in vitro* incubation method used in this study was the NSP analysis procedure described by Boisen and Fernandez (1997) with exclusion of the third step. The method is a multi-

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enzymatic hydrolytic process. The wheat/corn flour samples were incubated successively with 1) pepsin, pancreatin for 3h, and 2) with and without the exogenous enzyme (Xyl, Abf or Xyl+Abf) for 4h. The *in vitro* digestibility coefficient of dry matter (dig DM) was calculated for each incubation according to the calculation used in *in vivo* model: digDM = (feed DM−fecal DM)/feed DM where feed DM and fecal DM correspond to the dry matter weight in the feed and in the unhydrolyzed residue. Dinitrosalicylic acid (DNS) assay was conducted to measure the reducing ends released from the polysaccharides (Miller, 1959).

b) <u>In vivo trial</u>

The Rovabio MEP was produced by the fermentation of *Talaromyces versatilis sp.nov.* and provided in liquid form. It contained xylanase (5500 U/mL), glucanase (7500 U/mL) and arabinofuranosidase (23000 U/mL).

A total of 288 one-day-old Ross PM3 male broilers was divided into 3 groups of 96 animals, i.e. one non-supplemented control (NC) and 2 treatments comprising the two types of water-soluble wheat fractions (WF) at 0.1% on top of the basal diets, designated as positive control (PC) and MEP, respectively. Each treatment group comprised 8 pens of 12 animals. The same basal experimental wheat-based diet was given to all the birds during the entire trial (0-14d). Briefly, the WF supplemented in MEP group was incubated with Rovabio whilst in PC group the WF was untreated. The characterization of both WFs was shown in Table 1. The procedure to prepare the WFs was described in detail by Yacoubi et. al., (2017). Each treatment group was comprised of 8 pens of 12 animals.

Table 1 - Characterization of water-soluble wheat fractions added to PC group (untreated wheat-based broiler diet) and MEP group (wheat-based broiler diet with additional multi-enzymes containing xylanase, glucanase and arabinofuranosidase), n=6

	PC	MEP	P-value
Protein (% dry weight)	$14.0^{b} \pm 0.2$	$21.0^{a} \pm 0.1$	<0.001
Sugars (% dry weight)			
Arabinose	19.1 ± 1.6	19.3 ± 1.7	0.291
Xylose	32.2 ± 1.7	34.5 ± 2.1	0.104
Mannose	0.2 ± 0.1	0.5 ± 0.1	0.709
Galactose	3.2 ± 0.4	4.4 ± 0.3	0.971
Glucose	5.2 ± 0.5	5.4 ± 0.6	0.382
Degree of polymerization	$270^{b} \pm 13$	$54^{a} \pm 7$	<0.001
Molecular weight (kDa)	178.6	49.6	-
Intrinsic viscosity (mL/g)	215.7	54.1	-

At d14, the animals were weighed individually and feed intake (FI) measured per pen, to calculate feed conversion ratio (FCR) and body weight gain (BWG). Three animals per pen, 24 animals per treatment, with average body weight were euthanised by CO_2 asphyxiation. The caecum digesta content was weighed, immediately mixed with distilled water at a ratio of 2 mL/g and stored at −20◦C for SCFA and lactic acid analyses. Approximately 1cm of tissue from the distal end of the cecum and from the ileum at the Meckel's diverticulum were collected immediately post euthanasia, placed in RNALater (Sigma-Aldrich, St, Louis, MO) and stored at −20°C. To quantify the infiltration of T-lymphocytes (a sign of inflammation level) in the caecum and ileum, immunohistochemistry was performed as described previously by Van Immerseel et al. (2002), using a monoclonal antibody targeting CD3 (Dako, Glostrup, Denmark). A polyclonal rabbit antihuman GLP-2 antibody (Phoenix Pharmaceuticals, Inc., Burlingame, CA 94010. USA) was used to detect L-cells (lymphocytes) through immunohistochemical staining.

III. RESULTS AND DISCUSSION

a) In vitro trial

The A/X ratios differed between the wheat (0.65) and corn (0.73), consistent with previous report by Bach Knudsen (2014). DM digestibility and DNS obtained either in the presence or absence of Xyl and Abf added, alone and in combination, are presented in Table 2. The highest DM digestibility and DNS values were observed when Abf alone, and Xyl and Abf together were fed in combination ($P < 0.001$). It is therefore indicated that multi-xylanase system can significantly improve digestibility of feed raw materials, especially those with more complex AX biochemical structures, such as wheat. We also noticed that feeding Abf alone showed similar improvement as in the group treated with Xyl and Abf together. We hypothesized that it may due to the limited complexity of the *in vitro* model to show the further improvement from the combination of Xyl and Abf. The animal trial can be a better model to demonstrate the effect of multi-enzymes with effect on both xylose and its short side chains compared with single enzymes.

Table 2 - Impact of exogenous enzyme on dry matter digestibility (dig DM) and Dinitrosalicylic acid (DNS) content in control groups (pure wheat or corn flours), Xyl group (wheat or corn flours treated with xylanase), Abf group (wheat or corn flours treated with abrabinofuranosidase) and Xyl+Abf group (wheat or corn flours treated with xylanase and arabinofuranosidate), n=6

	Control	Xyl	Abf	Xyl+Abf	P-values
dig DM, %					
Wheat	72.5^{c}	75.4^{b}	78.2^{a}	79.6^{a}	<0.001
Corn	49.8^{b}	48.8^{b}	52.8^{a}	52.0^{a}	<0.001
DNS					
Wheat	1.728^{b}	1.717^{b}	2.783^{a}	2.803^{a}	<0.001
Corn	2.470^{c}	2.509^{b}	3.650^{a}	3.884^{a}	<0.001

b) In vivo trial

As shown in Table 3, the addition of WF treated with MEP in the basal diet significantly increased feed intake and body weight gain. However, dietary treatment had no impact on FCR.

Table 3 - Effect of dietary supplementation of 0.1% water-soluble wheat fractions (WF) treated with multi-enzyme (MEP) or without multi-enzyme (PC), compared a diet without WF (NC), on feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) at age d0-14

Parameters (0-14d)	NC	PC	MEP	P-values
FI (g/bird)	547^{b} ± 29	559^{b} ± 33	591^{a} ± 31	<0.001
BWG (g/bird)	425^{b} ± 26	430^{b} ± 25	458^{a} ± 26	<0.001
FCR	1.29 ± 0.18	1.30 ± 0.19	1.29 ± 0.18	0.397

Chickens receiving the diet supplemented with MEP-treated WF presented higher density of L-cells in the ileal crypt and villus epithelium and lower infiltration of T-lymphocyte in the ileum and cecum compared with those fed the PC and NC treatment (Table 4). This result suggested that treating the WF with MEP resulted in a stronger intestinal barrier function in broilers, resulting in better growth performance as shown above.

Table 4 also illustrates that total SCFA, acetate and butyrate concentration in the ceca were significantly increased in the group fed the MEP- treated WF compared with those fed the NC and PC treatments (Table 4), likely due to greater abundance of oligosaccharides produced for beneficial bacteria to utilize to produce SCFAs.

Table 4 - Effect of dietary supplementation of 0.1% WFs treated with (MEP) or without (PC) Rovbio on L-cell density in the ileum, T-cells in the ileal and cecal mucosa, and total short chain fatty acids (SCFAs), acetate, butyrate and lactate concentration (in µM) in the cecal content of 14-day-old broilers. NC: non-supplemented control.

Parameters		NC	PC	MEP	P-values
L-cells (Cells/mm^2)	In crypt	$1.1^{b} \pm 0.1$	$2.2^{b} \pm 0.3$	$7.8^{a} \pm 0.2$	<0.001
	In villi	$15.9^{c} \pm 1.1$	$25.2^{b} \pm 2.8$	$79.9^{a} \pm 2.6$	<0.001
T-cells (% labeled area)	Ileum	$16.1^{b} \pm 1.1$	$15.9^{b} \pm 0.6$	$8.1^{a} \pm 0.6$	<0.001
	Cecum	$16.8^{b} \pm 0.9$	$15.7^{b} \pm 0.7$	$7.0^{a} \pm 0.6$	<0.001
Total SCFAs	Cecal content	$43.6^{b} \pm 3.4$	$39.4^{b} \pm 2.5$	$65.6^{a} \pm 5.6$	<0.001
Acetate		$33.4^{b} \pm 2.8$	$30.6^{b} \pm 2.1$	$49.7^{a} \pm 4.9$	<0.001
Butyrate		$6.6^{b} \pm 0.6$	$5.3^{b} \pm 0.6$	$10.2^{a} \pm 1.0$	<0.001
Lactate		$2.9^{b} \pm 0.3$	$2.5^{b} \pm 0.3$	$3.7^{a} \pm 0.5$	<0.001

IV. CONCLUSION

The *in vitro* study showed that Xyl efficiency might be increased by Abf supplementation, to enhance the degradation of fibre-rich cell wall constituents in plant-derived feed raw materials. Consistent with the *in vitro* results, in the *in vivo* trial, treatment of water-soluble wheat fractions with MEP resulted in increased BWG of young broilers without affecting FCR. These effects may be attributed to enhanced immunity, and improved gut integrity due to increased SCFAs in the hindgut.

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DIETARY SOLUBLE NON-STARCH POLYSACCHARIDE LEVEL INFLUENCES BROILER PERFORMANCE AND EXCRETA DRY MATTER

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Despite extensive research presenting both the benefits and detriments of soluble non-starch polysaccharides (sNSP) in poultry diets, its concentration and composition in feed ingredients is not commonly considered during commercial feed formulation. Consequently, a study was conducted in which birds were fed commercial-type diets formulated to Cobb recommendations (barley, corn, sorghum or wheat-based) formulated to contain similar protein levels but differing soluble NSP levels (Low, Medium or High), resulting in 12 dietary treatments. sNSP level was determined in the feed ingredients and final diets by measuring the constituent sugars using gas chromatography. These diets were fed to 1110 mixed-sex Cobb 500 birds, distributed into 108 pens, with 9 replicates per dietary treatment and approximately 10 birds per replicate. Body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio corrected for mortality

Table 1- Effect of sNSP level and grain type on individual feed intake (FI), body weight gain (BWG) and feed conversion ratio corrected for mortality (cFCR) at age d0-31 and excreta dry matter (DM) content at d31

		Diet sNSP Level (g/kg DM)						
Grain	sNSP Level	Starter	Grower	Finisher	FI (g)	BWG (g)	cFCR	Excreta DM (%)
	Low	14.12	19.85	24.57	2727	2005	1.36^{b}	18.04^{ab}
Barley	Medium	15.17	22.95	26.20	2740	1970	1.39^{ab}	18.59^{ab}
	High	15.57	28.24	28.31	2727	1973	1.38^{ab}	17.86^{ab}
	Low	9.27	20.09	23.45	2810	2001	1.40^{ab}	16.81^{b}
Corn	Medium	10.81	22.02	25.66	2871	2010	1.43^{a}	17.30^{b}
	High	11.80	25.41	27.94	2789	2013	1.39^{ab}	17.64^{ab}
	Low	10.75	20.03	20.01	2876	2045	1.41^{ab}	18.94^{ab}
Sorghum	Medium	13.30	21.92	21.06	2880	2028	1.42^{ab}	19.72^{a}
	High	21.36	25.39	22.79	2817	2000	1.41^{ab}	17.63^{ab}
	Low	12.70	24.08	20.94	2740	1995	1.38^{ab}	18.92^{ab}
Wheat	Medium	13.48	27.86	22.15	2753	1974	1.40^{ab}	17.99^{ab}
	High	13.59	32.11	23.33	2750	1974	1.39^{ab}	18.21^{ab}
Barley		14.95	23.68	26.36	2732^{c}	1983	1.38	18.18
Corn		10.63	22.51	25.68	2823^{ab}	2008	1.41	17.26
Sorghum		15.14	22.45	21.29	2858^{a}	2024	1.41	18.67
Wheat		13.26	28.02	22.14	2748^{bc}	1981	1.39	18.36
P-values								
Grain					0.001	0.264	0.013	0.004
NSP					0.404	0.600	0.073	0.176
Grain x NSP					0.938	0.977	0.047	0.045

(cFCR) at d0-31 was determined per pen. Additionally, fresh excreta samples were collected per pen and dry matter content measured (Table 1). Data was analysed as a 4 x 3 factorial arrangement using IBM SPSS Statistics 25, with differences considered significant at $P < 0.05$.

Generally, corn is thought to be a superior grain for broilers, and only the anti-nutritional effects of sNSP are considered, but in this study birds fed the corn-based diet with low sNSP presented the highest cFCR and lowest excreta DM content. This highlights that sNSP is required in poultry diets, due to its role in maintaining digesta passage rate, water absorption and as a fuel for beneficial microbiota. Also, barley was shown to be efficacious when the dietary sNSP level was low. This suggests it is advantageous to consider sNSP during feed formulation, to ensure there is sufficient, but not excessive, quantities in the diet to achieve optimal performance and litter quality.

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BIO-EFFICACY OF ORGANIC SELENIUM COMPOUNDS IN BROILER CHICKENS

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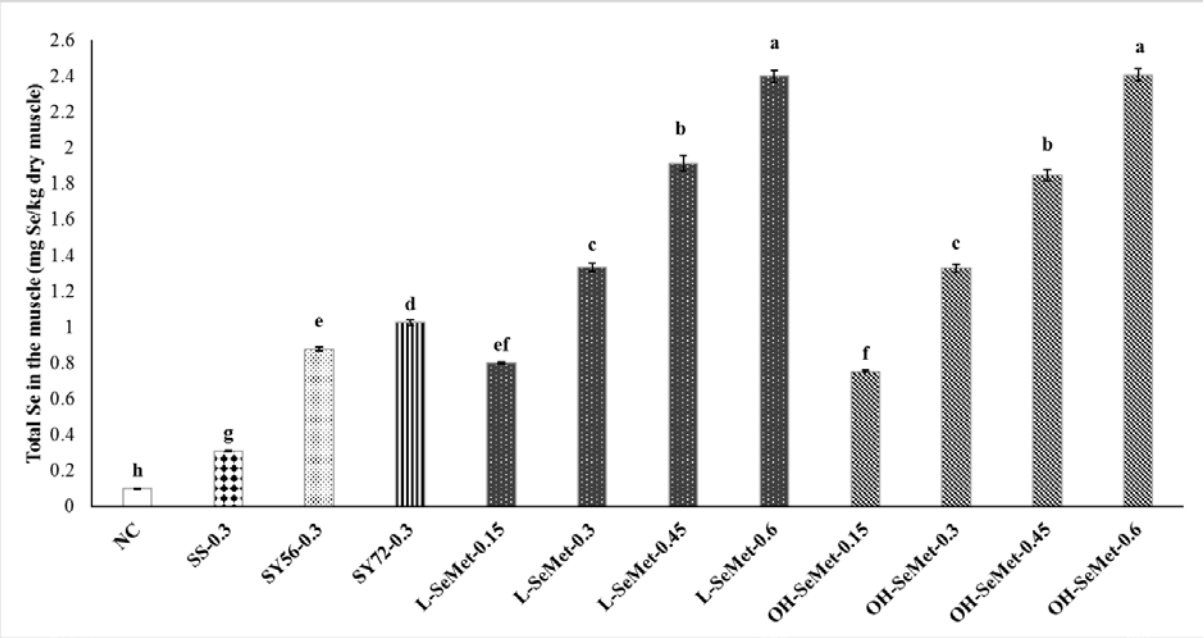
Selenium (Se) is an essential micronutrient added to livestock diets in either inorganic form (sodium selenite, SS) or organic forms, such as Se-yeast (SY) or as pure chemically synthetized Se forms, like hydroxy-selenomethionine (OH-SeMet) or L-selenomethionine (L-SeMet). Se compounds can be distinguished on the basis of their bio-efficacy, one way method of which can be assessed by measuring the Se deposition in muscle tissue. The present study was aimed to compare the bio-efficacy of different Se sources in broiler chickens. A total of 432 d-old male broilers (Ross 308) were assigned to 12 treatments (3 replicates). The birds were fed for 14 d a negative control diet (NC; no supplemental Se), a NC diet supplemented with SS, or two different SY (56% or 72% of Se as SeMet; SY56, SY72), L-SeMet or OH-SeMet. Se sources were supplemented at 0.3 mg Se/kg, except L-SeMet and OH-SeMet, which were supplemented at 0.15, 0.3, 0.45 and 0.6 mg Se/kg. On d 14, 9 birds/treatment were sampled for determination of total Se concentration in breast muscles by inductively coupled mass spectrometry. Differences were tested by means of one-way ANOVA ($P < 0.05$).

Figure 1 – Selenium (mean ± SD) in the muscle of broiler fed different selenium sources and levels.



The Se sources significantly affected the Se deposition (mg Se/kg of DM) in the breast muscles ($P < 0.01$; Fig. 1). Statistics confirmed the higher efficacy of the organic forms (SY56, SY72, L-SeMet and OH-SeMet) than SS and the higher efficacy of both L-SeMet and OH-SeMet than SY56 and SY72 ($P < 0.01$). Increasing the dose from 0.15 to 0.6 mg Se/kg led to a significant dose dependent Se deposition for both L-SeMet and OH-SeMet ($P < 0.01$). Both L-SeMet and OH-SeMet showed an equivalent bio-efficacy ($P > 0.05$) regardless of the dose used. This study has confirmed the higher efficacy of organic Se sources for tissue Se transfer than SS. Moreover, L-SeMet and OH-SeMet were equivalent and both exhibited a higher efficacy than both SY at 0.3 mg Se/kg supplementation level.

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VARIABILITY IN NUTRIENT MEASUREMENT OF FEED INGREDIENTS COMPROMISES PROFITABILITY IN THE AUSTRALIAN POULTRY INDUSTRY

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Feed accounts for more than 65% of poultry production costs; thus, accurate feed formulation is vital to ensure poultry are receiving an optimal diet and nutrients are not in under- or over-supply. However, this is difficult when the nutrient specifications of feed ingredients are highly variable. In order to help reduce this variability, appropriate sampling methodology is critical. Nevertheless, recommended methodology and depth of detail within technical articles varies greatly and does not always reflect the recommendations of the AOAC, a non-profit scientific association that publishes standardised analytical methods. This has been an ongoing issue for some time; 45 years ago Lerman and Bie (1975) concluded that improper sampling technique is a major component of ingredient variability. Nevertheless, few animal nutrition studies report the sampling technique used, nor is the potential economic cost of variability often discussed. Protein is an expensive and crucial macronutrient component of poultry diets; thus, the extent that variation in protein in feed ingredients affects expected performance and profits for the Australian poultry industry was modelled.

Standard Australian wheat-SBM-canola meal-based starter, grower, finisher and withdrawal diets were formulated and profitability modelled using EFG Broiler Model software (EFG Software, 2020). The variability (coefficient of variation; CV) in crude protein of the components of Australian poultry diets were estimated from Moss (2020), and simulations were performed to estimate the likelihood a diet would fall below nutrient recommendations using Excel 2016, NORMINV function (10,000 simulations/diet). All prices are in $AUD. CV's grew larger in finisher and withdrawal diets compared to starter and grower diets, and wheat was the single greatest source of variability in crude protein content of diets. Within withdrawal diets formulated to 19.2 g/kg crude protein from book values, there is approximately a 10% probability (or one in 10 diets) that it will fall below 182 g/kg CP, and diets may fall as low as 162 g/kg CP; which was modelled to lower the gross margin from $21.26/m^2 ($1.417/bird/cycle) to 7.88/m^2 ($0.525/bird/cycle) − a reduction in profits of 63%. Therefore, it is possible to incur a difference of up to $26,753 in gross margin from one cycle of 30,000 broilers by simply overestimating the nutrient content of feedstuffs. Hence, it is clear that identifying the most accurate way to sample, and improving the understanding and implementation of proper sampling methodology, is of great importance for the Australian poultry industry.

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METHIONINE SUPPLEMENTATION REDUCES PLASMA AMINO ACID CIRCULATION AND ENHANCED APPARENT DIGESTIBILITY IN BROILER CHICKENS

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Summary

The objective of this study was to evaluate the impact of methionine supplementation on amino acid concentration in systemic plasma, apparent amino acid digestibilities and their relevance to growth performance in broiler chickens from 0-21 days post-hatch. Four experimental diets based on wheat, soybean meal and canola meal were supplemented with 0, 1.38, 2.76 and 4.14 g/kg *l*-methionine which reflected total sulphur amino acid (TSAA) to lysine (Lys) ratios of 50, 60, 70 and 80, respectively. Each of the four treatments was offered to 6 replicates with 8 birds per replicate or total 192 off-sex male Ross 308 chicks (parent line). There was quadratic correlation between TSAA:Lys ratios and growth performance ($P < 0.05$). At day 20, increasing dietary TSAA:Lys ratios linearly increased systemic plasma concentrations of Met ($r = 0.841$, $P < 0.001$) but decreased concentrations of His, Ser, Gly, Thr, Lys and Val ($P < 0.05$). In contrast, increasing dietary TSAA:Lys ratios from 50 to 70 and 80 increased apparent digestibility coefficients of 16 analysed amino acids ($P < 0.001$). The present study emphasised the importance of amino acid balance on protein utilisation and deposition; additionally it suggested the additional value of increasing dietary TSAA:Lys ratios from 70 to 80.

I INTRODUCTION

Methionine (Met) is often considered to be the first limiting amino acid in modern broiler diets. Synthetic Met has been commercially available for animal feed since the 1950s and is routinely included in modern poultry diets. Whilst studies on Met requirements, based on growth performance parameters are not novel, understanding the impact of dietary Met supplementations on apparent digestibility and plasma concentrations of amino acid is important in conventional diets but particularly in reduced crude protein diets. A preliminary study by Truong *et al.* (2017) reported that synthetic amino acid inclusions altered free amino acid concentrations individually and collectively in both portal and systemic blood plasma despite there being no correlation between growth performance and plasma amino acid concentrations. The present study continued the investigation, examining the impact of Met supplementation on amino acid concentration in systemic plasma, apparent amino acid digestibilities and their relevance to growth performance in broiler chickens.

II MATERIALS AND METHODS

The feeding study complied with specific guidelines approved by the Animal Ethics Committee of The University of Sydney. Four experimental diets based on wheat, soybean meal and canola meal were supplemented with 0, 1.38, 2.76 and 4.14 g/kg *l*-methionine which reflected total sulphur amino acid (TSAA) to lysine (Lys) ratios of 50, 60, 70 and 80, respectively. Each of the four treatments were offered to 6 replicates with 8 birds per replicate or total 192 off-sex male Ross 308 chicks (parent line) from 0-21 days post-hatch. The four experimental diets were formulated to be iso-energetic (12.34 MJ/kg) and contained the same dietary levels of

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digestible lysine (13.0 g/kg) as shown in Table 1. All diets contained commercially relevant inclusions of phytase and xylanase and diets were steam-pelleted at 80 ˚C then crumbled. Diets were initially offered to birds as crumble from 0-14 days and subsequently in pellet form. Chickens had *ad libitum* access to feed and water. Initial and final body weights were determined, and feed intakes were recorded to calculate feed conversion ratios (FCR). The incidence of dead or culled birds was recorded daily and their body-weights used to adjust FCR calculations. During 18 to 20 days post-hatch, feed intake and excreta output were recorded to determine apparent metabolizable energy (AME), N corrected apparent metabolizable energy (AMEn) and N retention. At day 20, three birds at random were selected from each cage and blood samples were taken from the brachial vein. Blood samples were then centrifuged and the decanted plasma samples were stored at −80˚C prior to analysis. Concentrations of twenty proteinogenic amino acids in plasma were determined using precolumn derivatisation amino acid analysis with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC; Waters™ AccQTag Ultra; www.waters.com) followed by separation of the derivatives and quantification by reversed phase ultra-performance liquid chromatography (Cohen, 2001). At day 21, all birds were euthanized and digesta samples were collected from the distal ileum and pooled from the same cage to determine the apparent digestibility of protein and amino acids. ANOVA and linear and quadratic correlations were performed using JMP® 13.0.0 and significance was determined at $P < 0.05$ by Student's *t*-test.

Table 1-Composition and nutrient specifications of control diet

Feed ingredient	(g/kg)	Calculated specification	(g/kg)
Wheat	495	Metabolisable energy (MJ/kg)	12.34
Soybean meal	315	Crude protein	236.9
Canola meal	75.0	Calcium	10.50
Soy oil	50.0	Total phosphorus	6.46
l-lysine HCl	3.21	Phytate phosphorus	2.49
l-threonine	1.61	Non-phytate phosphorus	3.97
l-valine	0.90	Sodium	1.80
l-arginine	0.74	Potassium	10.03
l-isoleucine	0.39	Chloride	2.50
Sodium chloride	2.17	Dietary electrolyte balance (mEq/kg)	264
Sodium bicarbonate	1.66	Fat	73.5
Limestone	11.14	Fibre	26.9
Dicalcium phosphate	15.38	Digestible lysine	13.0
Xylanase	0.05	Digestible methionine	3.07
Phytase	0.05	Digestible threonine	8.58
Choline chloride 60%	1.00	Digestible tryptophan	2.68
Celite	20.0	Digestible isoleucine	8.84
Sand[1]	4.14	Digestible leucine	14.78
Vitamin-mineral premix[2]	3.00	Digestible valine	10.27

[1]*l*-methionine was incorporated into the basal, control diet at the expense of sand at appropriate inclusion rates to generate the balance of dietary treatments

[2]The vitamin-mineral premix supplied per tonne of feed: [MIU] retinol 12, cholecalciferol 5, [g] tocopherol 50, menadione 3, thiamine 3, riboflavin 9, pyridoxine 5, cobalamin 0.025, niacin 50, pantothenate 18, folate 2, biotin 0.2, copper 20, iron 40, manganese 110, cobalt 0.25, iodine 1, molybdenum 2, zinc 90, selenium 0.3.

III RESULTS AND DISCUSSION

The effects of dietary treatments on growth performance and nutrient utilisation are shown in Table 2. Increasing TSAA:Lys ratios from 50 to 60 significantly improved weight gain, FCR and N retention. There was quadratic correlation between TSAA:Lys ratios and growth performance (Figure 1). Based on the regression equation, the optimal weight gain of 1036 g/bird was predicted when TSAA:Lys ratio equalled 74.9 whereas the optimal FCR of 1.198

was predicted to be reached when TSAA: Lys ratio equalled 74.2. Various targeted parameters may lead to different amino acid requirements; for instance, Liu *et al*. (2019) reported digestible lysine requirement for FCR was higher than that for weight gain (12.3 *versus* 11.3 g/kg). Figure 2 illustrates the influence of TSAA:Lys ratios on apparent digestibilities of amino acids in the distal ileum. Supplementation of Met linearly increased apparent digestibility of Met. Dietary TSAA:Lys ratios significantly influenced apparent digestibility of all amino acids and increasing TSAA:Lys ratios from 50 to 80 significantly increased apparent digestibility of Gly, Pro, Val, Ile, Leu and Phe. The significant improvement in apparent digestibility of amino acids and the overall N retention emphasized the importance of dietary amino acid balance. Moreover, increasing TSAA:Lys ratios linearly decreased systemic plasma concentrations of His, Ser, Gly, Thr, Lys and Val but linearly increased systemic plasma concentrations of Met ($P < 0.05$, Figure 3). Interestingly, weight gain was negatively correlated with plasma concentrations of His ($r = -0.532$, $P = 0.008$), Ser ($r = -0.514$, $P = 0.010$), Thr ($r = -0.528$, $P = 0.008$), Lys ($r = -0.623$, $P = 0.001$) and positively correlated with Met concentration ($r = 0.427$, $P = 0.038$). Similarly, FCR was correlated with His ($r = 0.728$, $P < 0.001$), Ser ($r = 0.629$, $P = 0.010$), Gly ($r = 0.597$, $P = 0.002$), Thr ($r = 0.646$, $P < 0.001$), Pro ($r = 0.475$, $P = 0.020$), Lys ($r = 0.787$, $P < 0.0001$), Met ($r = -0.547$, $P = 0.006$) and Val ($r = 0.551$, $P = 0.005$). The reduction of amino acids circulating in the plasma by supplementing Met and their relevance to improved growth performance may indicate improved amino acid utilization.

Table 2- Effects of dietary treatments on growth performance from 0-21 days and nutrient utilisation in broiler chickens from 18-20 days post-hatch

TSAA:Lys	L-met (g/kg)	Feed intake (g/bird)	Weight gain (g/bird)	FCR	AME (MJ/kg)	AMEn (MJ/kg)	N retention (%)
50	0	1175	911[a]	1.291[a]	12.59	11.31	66.04[b]
60	1.38	1237	1007[b]	1.229[b]	12.43	11.03	70.33[a]
70	2.76	1222	1017[b]	1.202[b]	12.39	11.04	71.08[a]
80	4.14	1246	1036[b]	1.204[b]	12.14	10.77	70.08[a]
SEM		*22.8*	*21.8*	*0.013*	*0.123*	*0.135*	*0.988*
ANOVA	P-value	0.164	0.003	<0.001	0.105	0.071	0.008
Linear	r =	0.38	0.627	-0.698	-0.492	-0.506	0.486
	P-value	0.067	0.001	<0.001	0.015	0.012	0.016

[a,b] Means within columns not sharing a common superscript differ at the 5% level of probability.

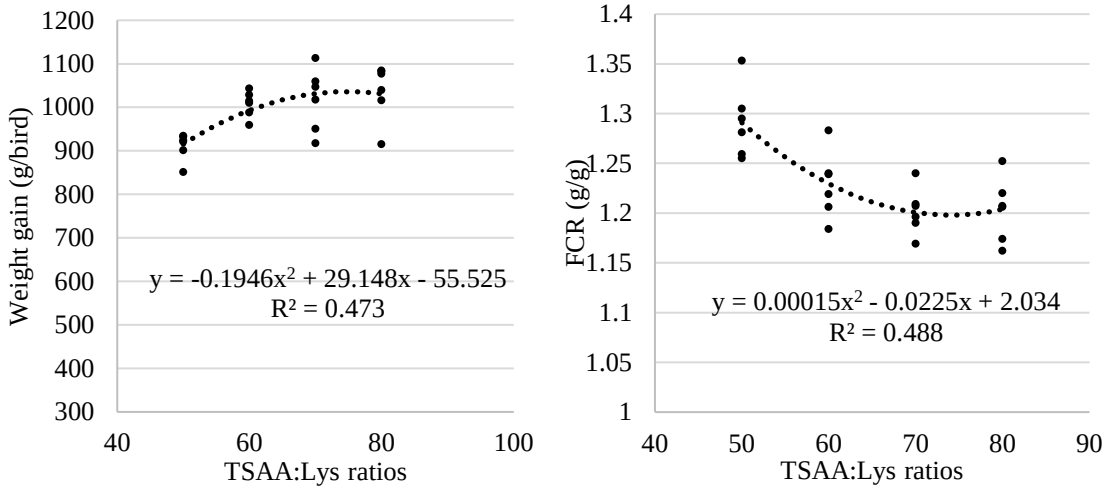

Figure 1- Quadratic influence of Digestible TSAA:Lys ratios on weight gain ($P = 0.001$) and FCR ($P < 0.0001$) in broiler chickens from 0-21 days

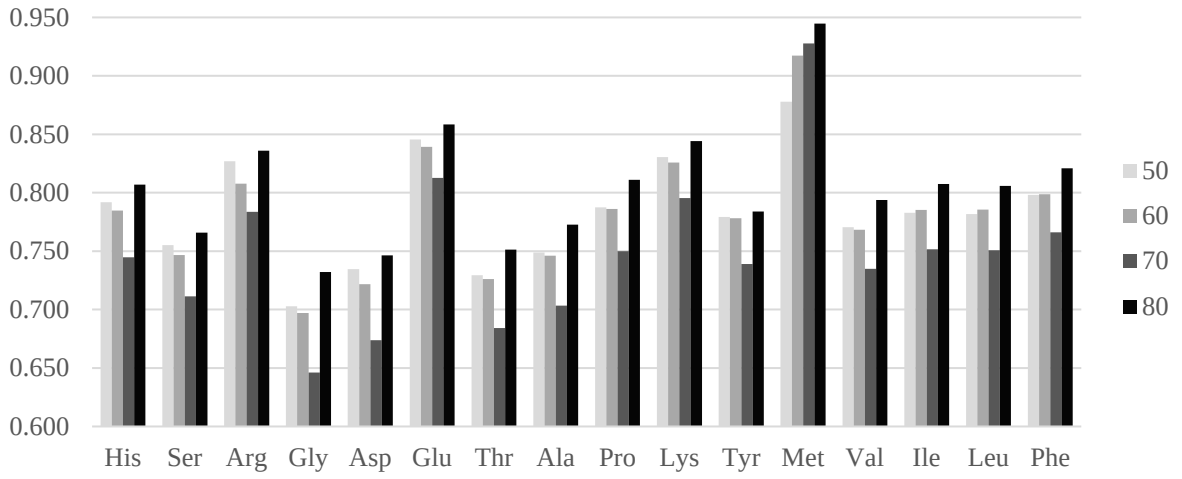

Figure 2- Significant influence of TSAA:Lys ratios on apparent digestibilities of amino acids in the distal ileum ($P < 0.05$)

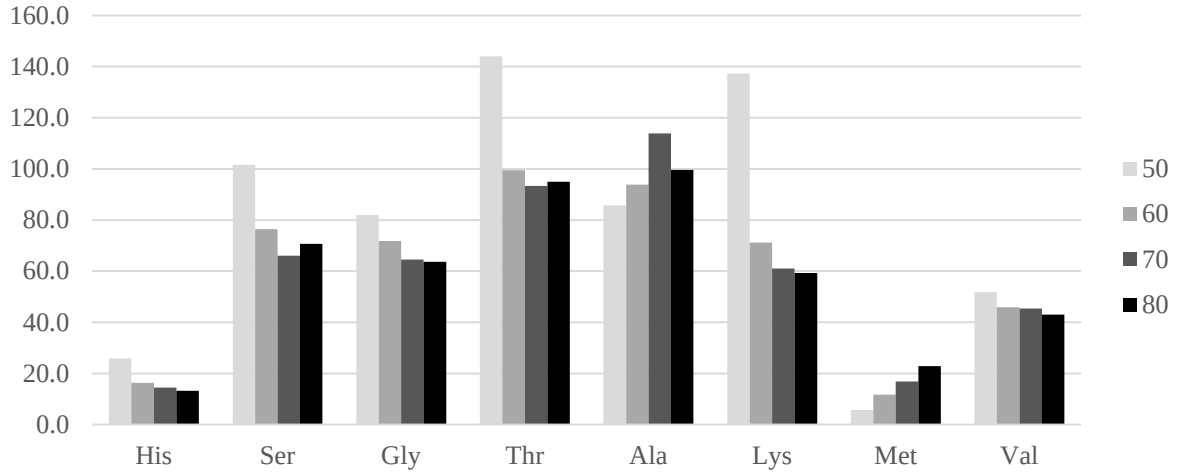

Figure 3- Effects of dietary treatments on free amino acid concentrations (µg/mL) in systemic plasma at 20 days post-hatch (significant effects only)

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DOES DIET NUTRIENT DENSITY AND HEN SIZE IMPACT HEN PRODUCTIVITY AND EGG QUALITY AT 50 WEEKS OF AGE?

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Summary

This study evaluated the effect of diet nutrient density, specifically a higher nutrient density (HND) and lower nutrient density (LND) diet fed during early lay on ISA Brown hen production and egg quality. At 18 weeks of age (woa) pullets were allocated to either a breed standard weight (BSW) or lighter weight (LW) group. Within each weight group birds were then allocated to either the HND or LND dietary treatments, which were fed from 18 to 24 woa. At 25 woa hens fed the HND diet were placed on the LND. All hens remained on a LND diet until 50 woa. Hen performance was measured through to 50 woa and egg quality was evaluated at 50 woa. BSW at 18 woa resulted in significantly higher body weight, daily average and cumulative feed intake and egg weight at 50 woa compared to the LW birds. The LND diet increased yolk colour at 50 weeks. There were treatment interactions for Haugh units and cumulative feed conversion ratio (CFCR) at 50 woa. The BSW birds fed the HND diet had the highest mean Haugh unit whereas the LW birds on the HND had the lowest mean Haugh unit. For CFCR, the LW birds fed the HND diet during early lay had the lowest CFCR which was significantly lower than the CFCR of LW birds on the LND and the CFCR for all BSW birds to 50 woa.

I. INTRODUCTION

Optimum BW is an important consideration in achieving early maturity, high productivity and uniformity in laying hens (Lacin et al., 2009). Parkinson et al. (2015) noted that it was common for Australian flocks to have an average body weight (BW) 100-300 grams above the breed standard weight (BSW). This higher BW is indicative of bird obesity and is associated with the production of excessively large eggs with consequently lower eggshell quality and poorer persistency of lay compared to the smaller sized birds. Parkinson et al. (2015) suggested that aligning BW more closely to BSW or even a slightly lighter weight could significantly improve egg production and laying persistency.

Increasing the diet nutrient density for birds at the beginning of their laying period and as they progress towards peak production could be beneficial as it may facilitate consumption of the required nutrients but within a smaller quantity of feed. This may achieve both immediate and longer term benefits for smaller sized hens with innately lower feed intake (FI) compared to larger birds. Understanding whether a higher nutrient density (HND) diet fed to late stage pullets of different weights could improve their persistency of lay, eggshell quality and feed efficiency through to mid lay compared to a lower nutrient density (LND) diet could offer growers options in managing hens as they transition from point of lay to peak lay.

Therefore, this study was designed to compare the performance of BSW and LW ISA Brown hens when fed either a HND or LND diet from point of lay for 6 weeks, on their egg production, FCR and egg quality at 50 woa.

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II. MATERIALS AND METHODS

This study was a 2 × 2 factorial arrangement consisting of 2 nutrient densities (HND and LND) and 2, 18 woa bird weight groups: mean weight 1.58 kg (BSW) and 1.40 kg (LW). When 16 woa, 240 ISA Brown commercial strain pullets were purchased from a certified grower, transported to University of Sydney, Camden and housed individually in 25 × 50 × 50 cm cages with individual feeders and waters within a high rise layer shed. They were fed the LND diet *ad libitum* and allowed to acclimate for a 2-week period. At 18 woa all birds were weighed and 120 pullets allocated to both weight groups (BSW or LW). Sixty pullets from each weight group were then randomly allocated to either a HND (formulated on 90g FI/day; 2900 kcal/kg, 0.83% SID.Lys) or LND (formulated to 110g FI/day; 2725 kcal/kg; 0.74% SID.Lys) diet. All hens were fed the experimental diets from 18 to 24 woa. At 25 woa hens on HND diet were consuming at least 100g FI/day and so all were moved to the LND diet. The dietary nutrient specifications meet the recommended requirements of the ISA Brown to 50 woa. From 18-50 woa individual hen egg production (EP), egg weight (EW) and FI were measured to allow weekly determination of FCR. Cumulative measures of production were then also calculated. At 50 woa 10 eggs/treatment group were assessed for egg quality. Data was analysed using a factorial ANOVA with 18 woa BW and diet nutrient density as the main effects.

III. RESULTS AND DISCUSSION

At 16 woa pullets at the rearing facility were above BSW and hence the slightly heavier weights of these birds from the start of the study. Hen production is presented in Table 1. At 50 woa average BW, average daily FI and cumulative FI (CFI) of BSW birds were significantly higher ($P < 0.05$) than the LW birds. Birds of the 18 woa BSW group consumed on average 12% more feed from 18-50 woa than the LW hens. Similarly unpublished findings of Christmas and Harms referred to in Harms et al. (1982) report up to a 12% difference in FI between the heaviest and lightest strains of birds. There was no effect of treatments on percent egg production (EP), but BSW birds had notably higher cumulative egg mass (CEM) (P=0.06) at 50 woa. The latter is in agreement with Leeson and Summers (1987) where higher BW birds produced significantly greater EM compared to LW birds.

Birds have been seen to adjust their FI based on the nutrient density of the diet (Morris, 1968) but this is unlikely to be in perfect alignment (Leeson et al., 2001). In this study significant differences in FI were associated with bird weight only, not dietary treatment. This positive association of bird weight on FI has also been observed in brown egg-laying hens by Perez-Bonilla et al. (2012), but the reasons diet density had no significant effect on FI in this study are not immediately clear.

There was an interaction between 18 woa BW and diet nutrient density on CFCR. LW birds fed the HND diet until 24 woa had the lowest CFCRC, being significantly lower than LW birds fed the LND diet. The CFCR for both BSW groups was significantly higher than both of the LW groups. This demonstrates a clear benefit of the HND diet for the LW birds through to 50 woa.

The effects of BW and diet nutrient density on egg quality at 50 woa is presented in Table 2.

Table 1 - Hen production from 18 to 50 weeks of age.

	BW (Kg)^	FI (g/d)	CFI (Kg)	EP (%)	EW (g)	CEM (Kg)	CFCR
BW (18woa)							
BSW	2.09	116	26.1	97.6	59.4	12.8	2.06
LW	1.88	108	23.3	96.3	58.1	12.3	1.90
DND							
HND[#]	1.98	111	24.4	97.2	58.9	12.4	1.97
LND	1.99	112	25.0	96.7	58.6	12.6	1.99
Interaction							
BSW*HND	2.09	115	26.0	97.7	59.5	12.5	2.08[a]
BSW*LND	2.09	116	26.3	97.4	59.2	12.9	2.03[a]
LW*HND	1.87	107	22.8	96.7	58.3	12.3	1.86[c]
LW*LND	1.89	108	23.8	95.9	57.9	12.3	1.94[b]
P- Values							
BW	< 0.001	0.0001	< 0.001	0.25	0.006	0.06	< 0.001
DND	0.66	0.47	0.19	0.62	0.44	0.43	0.43
BW*DND	0.78	0.75	0.40	0.84	0.96	0.29	0.003

BW(18woa): 18 weeks of age body weight; BSW: Breed standard 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 at 50 weeks of age; FI: average feed intake; CFI: Cumulative feed intake; EP(%): average percent egg production; EW: average egg weight; CEM: Cumulative egg mass; CFCR: Cumulative feed conversion ratio. #HND diet was fed up to the end of 24 weeks of age and then replaced with the LND diet. [abc] Means within column not sharing a common suffix are significantly different at $P < 0.05$.

There were no treatment effects on yolk % - YP (yolk weight as % egg weight), shell %- SP (shell weight as % egg weight) nor shell thickness (ST), however there is a tendency ($P = 0.06$) for higher shell thickness in the LW birds that received the HND diet compared to all BSW birds. Mean EW was significantly higher ($P= 0.05$) in BSW birds irrespective of diet. There was a treatment interaction for Haugh units ($P = 0.01$) where BSW birds that had received the HND diet had the highest Haugh units and LW birds on the HND diet had the lowest Haugh units. Haugh units of the LW birds on the LND were not different to the BSW birds that had received the HND diet. The yolk colour score was highest for birds on the LND diet ($P= 0.001$) compared to HND diet.

Our observed production of lighter weight eggs by LW birds compared to BSW birds of the same flock has been reported by others (Leeson et al., 1997). The variation in yolk colour associated with diet density in this study could be a characteristic of individual hens or due to differences in FI and therefore pigment intake (Karunajeewa et al., 1984). In this regard birds fed the LND diet had a higher CFI to 50 woa, though that increase was not statistically significant. The egg quality observations presented here are from a single assessment on 10 eggs per treatment group. Interestingly combined egg quality assessment across several weeks leading up to 50 woa (i.e. from 45-50 woa) have also demonstrated a tendency ($P= 0.08$) to darker yolk colour in hens fed the LND diet (data not shown).

Table 2 - Egg quality at 50 weeks of age.

Treatment	50 weeks of age					
	EW (g)	HU	YP (%)	YC	SP (%)	ST (mm)
BW(18woa)						
BSW	62.1	96.5	26.8	12.9	10.4	0.39
LW	59.8	95.4	26.6	12.6	10.5	0.39
DND						
#HND	60.9	95.3	26.3	12.3	10.5	0.40
LND	61.0	96.4	27.0	13.3	10.4	0.39
Interaction						
BSW*HND	62.7	98.4[a]	26.2	12.4	10.4	0.39
BSW*LND	61.5	94.6[ab]	27.3	13.5	10.4	0.39
LW*HND	60.0	92.1[b]	26.4	12.2	10.6	0.41
LW*LND	60.5	98.5[a]	26.8	13.1	10.3	0.38
P-Value						
BW	0.05	0.42	0.69	0.28	0.80	0.74
DND	0.90	0.35	0.15	0.001	0.50	0.17
BW*DND	0.23	0.01	0.47	0.71	0.77	0.06

BW: 18 weeks of age body weight; BSW: Breed standard 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); EW: Egg weight; HU: Haugh unit; YP: Yolk weight % of egg weight; YC: Yolk colour; SP: Shell weight % of egg weight; ST: Shell thickness. #HND diet was fed up to the end of 24 weeks of age and then replaced with the LND diet. [abc] Means within column not sharing a common suffix are significantly different at $P < 0.05$.

While BSW birds have the highest EW and cumulative egg mass at 50 woa the benefit of the HND diet for LW bird on shell thickness and CFCR is of particular interest. This study is continuing until birds are 90 woa allowing for ongoing evaluation of egg production and quality due to BW and diet nutrient density through to very late lay.

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A DELETERIOUS GENE MUTATION AND THE DOWNREGULATED EXPRESSION OF THE GLUCOSE TRANSPORTER SYSTEM (GLUT) IN THE GUT IS ASSOCIATED WITH POOR GROWTH IN BROILER CHICKENS

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Understanding the mechanisms underpinning individual variation in growth is an important question to address uniformity and overall performance in broiler chickens. The robust association between feed intake and growth has been well established, and energy is one of the main drivers of feed intake (Roura and Navarro, 2018). However, the specific differences in feed intake mechanisms between slow- and fast-growing chickens remain poorly understood. The universal energy source in cell metabolism is glucose. We hypothesized that broiler chickens with low compared to high feed intake and growth are less efficient in utilizing dietary glucose.

A total of 580 day-old male chickens (Ross 308) were transferred to the Poultry Unit facilities of the University of Queensland (Gatton, Australia) and reared on floor pens. Chickens were provided with *ad libitum* water and standard feed commercial (Starter: 25.1% CP, 12.65MJ/kg; Grower: 21.6%CP, 13.2MJ/kg; Finisher: 19.29CP, 13.3MJ/kg). Performance parameters were measured weekly. After 3 weeks, all the birds were weighed, and the 48 heaviest and the 48 lightest were selected as fast-growing and slow-growing chickens, respectively. They were placed in individual cages to measure individual feed intake and body weight until week 6. At the end of week 6, we selected the six heaviest (3598g, FCR=1.81) and the six lightest birds (2828g, FCR=1.61) and tissues samples from the proventriculus and duodenum were collected. Transport and metabolism of glucose in the gut were compared between slow- and fast-growing chickens using transcriptomic (RNAseq), proteomic, and genomic analyses.

Results showed that the two biological pathways identified as ‘translocation of glucose transporter in membrane’ and ‘glycolysis’ were significantly ($P<0.05$) downregulated in slow-growing chickens. In addition, protein abundance of glucose transporter 2 (GLUT2), together with the gene expression of glucose transporters GLUT1 and GLUT10 were all downregulated in slow-growing chickens ($P<0.05$). Furthermore, the SLC2A4RG, a transcription factor regulating the gene expression of glucose transporters (Oshel et al., 2000), carried a deleterious mutation only in slow-growing chickens. In conclusion, a deficiency in the cellular glucose uptake system and metabolism in proventriculus and duodenum may partially explain poor growth rate in broiler chickens.

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RESPONSE OF BROILERS TO DIETARY INCLUSIONS OF SUGARCANE BAGASSE AND PROTEASE IN A REDUCED CRUDE PROTEIN DIET

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Nutritional strategies to improve performance of broilers offered reduced crude protein (RCP) diets supplemented with crystalline amino acids are of interest to the poultry industry. We hypothesized that the dietary inclusion of moderate amounts of insoluble fibre would stimulate gizzard function and increase the retention time of digesta in the foregut allowing more time for exogenous protease to act on their substrates leading to greater digestibility of protein/amino acids and better performance of birds offered RCP diets. This study investigated the effectiveness of dietary sugarcane bagasse as an insoluble fibre source with or without an exogenous protease in RCP diets fed to broilers.

A total of 672 d-old Ross 308 male parent-line birds were fed a common starter diet until 10 d of age. On d 10, birds were assigned to 8 treatments, each replicated 6 times with 14 birds per pen in a 2 × 2 × 2 factorial arrangement of treatments with dietary crude protein (CP)- normal (NCP, 213 g/kg CP for 10 to 24 d in grower phase and 195 g/kg CP for 24 to 35 d in finisher phase) or reduced by 25 g/kg CP in both the grower and finisher phases, bagasse included in the diets at 0 or 20 g/kg and protease added over the top of the diets at 0 or 0.2 g/kg. The diets were cold-pelleted and contained wheat, sorghum and soybean meal as major ingredients. All diets were supplemented with xylanase and phytase and were formulated to meet Ross 308 nutrient specifications. Feed intake, weight gain, and FCR were determined from d 10 to 35. Carcass parameters were measured on d 35 and 42. JMP Pro 14 was used to perform analyses of variance and significance was determined at $P < 0.05$ using Tukey's HSD test.

The reduction in dietary CP decreased feed intake ($P < 0.001$) by 3.66% (3361 versus 3238 g), weight gain ($P < 0.001$) by 5.78% (2284 versus 2152 g) and increased FCR ($P < 0.001$) by 3.2 points (1.476 versus 1.508) during d 10 to 35. Protease or bagasse had no effect ($P > 0.05$) on feed intake during the experimental periods. A 3-way CP × bagasse × protease interaction was observed for weight gain during d 10 to 24 ($P < 0.05$) and for FCR during d 10 to 24 and d 10 to 35 ($P < 0.01$). During d 10 to 24, weight gain was increased by 4.37% (1030 versus 1075 g) when protease was added to the NCP diet without bagasse but not when it was added to the RCP diet without bagasse. Meanwhile, FCR was decreased by protease in all except when bagasse was added to the NCP diet. On the other hand, dietary inclusion of bagasse only reduced FCR when no protease was added in the NCP diet (1.354 versus 1.327 during d 10 to 24 and 1.476 versus 1.459 during d 10 to 35) but not in any other circumstance. There were no further improvements in weight gain and FCR when bagasse and protease were added in tandem in the NCP diet. Bagasse increased ($P < 0.01$) relative gizzard weight by 8.47% on d 35 and 9.52% on d 42. Reduction in CP decreased ($P < 0.01$) relative pancreas weight by 14.01% and tended to decrease ($P = 0.057$) breast meat yield (181 versus 176 g/kg) on d 42. A 3-way CP × bagasse × protease interaction ($P < 0.01$) was observed for the relative abdominal fat pad weight on d 42. The relative abdominal fat pad weight decreased when protease or bagasse alone was added to the NCP diet but increased when added to the RCP diet ($P < 0.01$). No changes were observed when they were added in tandem.

This study showed that dietary supplementation of bagasse or protease alone improved performance of broilers offered a NCP diet. There were no further improvements on performance when they were added in tandem. The addition of protease alone in the RCP diet improved FCR. The RCP diet in this study may have been marginal in some other amino acids, possibly glycine which led to the lack of response of bagasse addition in the RCP treatment.

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WATER INTAKE IN BROILERS AS AFFECTED BY INSOLUBLE FIBRES AND EXOGENOUS PROTEASE IN A REDUCED CRUDE PROTEIN DIET

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Water is important for the overall health and performance of birds, yet less focus has been on water compared to the other nutrients. Increase or decrease in water to feed intake ratio (WI:FI) indicates issues related to nutrition, health or management of the shed particularly in broilers due to their fast growth. An automated water measurement system was developed at the University of New England to measure water intake in broilers. This system was used to measure water intake in two experiments to determine the effects of dietary inclusion of insoluble fibre and exogenous protease in a reduced crude protein (RP) diet. The hypothesis was that the reduction in dietary crude protein would decrease water intake and adding insoluble fibre or protease in RP diets would not affect water intake of birds beyond the effect produced by the RP diets. In both the experiments, day-old Ross 308 male parental birds were used and fed a common starter diet until d 10. The birds were reared in floor pens with wood shavings as bedding material.

The first experiment consisted of 8 treatments with 6 replicate pens each with 14 birds and arranged in a 2 × 2 × 2 factorial with dietary crude protein (CP) - normal (NP, 213 g/kg CP in grower and 195 g/kg CP in finisher diets) or reduced by 25 g/kg CP (RP), sugarcane bagasse- 0 or 20 g/kg and protease- 0 or 0.2 g/kg. Water intake was measured at two time points, 16 to 20 d and 20 to 24 d. In the second experiment, birds were assigned to 6 treatments with 8 replicate pens of 14 birds each. The treatments were NP diet (grower 211 g/kg CP, finisher- 195 g/kg CP), RP (reduced by 20 g/kg CP), and RP diets formulated in with sugarcane bagasse at 20 g/kg, lignocellulose based product at 10 g/kg, oat hulls at 30 g/kg, or soy hulls at 30 g/kg. The basal diet of the fibre treatments was same and the formulations were adjusted by adding Celite, an indigestible component as filler. Water intake was measured from d 10 to 35. In both the experiments, the reduction in dietary CP decreased ($P < 0.001$) water intake and WI:FI. The inclusions of 20 g/kg sugarcane bagasse had no effect ($P > 0.05$) on water intake and WI:FI. The addition of 0.2 g/kg protease had no effect ($P > 0.05$) on water intake but increased ($P < 0.05$) WI:FI. The inclusions of insoluble fibres in the RP diet had no effect ($P > 0.05$) on water intake and WI:FI. These experiments demonstrated that the automated system can be used to measure water intake in nutrition research. The findings showed that water intake can be decreased by lowering dietary crude protein level and the inclusion of insoluble fibres at moderate amounts in reduced crude protein diet will not affect water intake of birds.

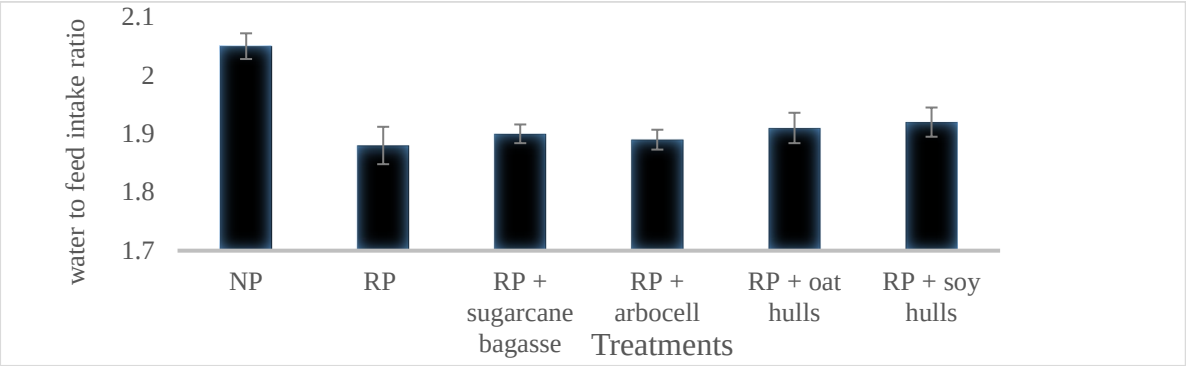

Figure 1 - Effect of reduced crude protein diet and insoluble fibres on water to feed intake ratio (d 10 to 35)

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INCREASING ENERGY AND AMINO ACID LEVELS IN LAYER DIETS IMPROVED LAYING PERFORMANCE BUT NOT EGG QUALITY IN RANGING FREE-RANGE HENS

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It has been proposed that the metabolic energy requirements of free-ranging hens are up to 15% higher compared to caged hens due to the increased metabolic activity required for locomotion and thermoregulation (GfE 1999; Tiller 2001; Aerni et al. 2005). The aim of the study was to investigate the impact of various feed strategies on laying performance and egg quality of free-range laying hens. A total of 9,375 hens, placed amongst 5 flocks of 40,000 hens each were selected according to their range usage (hens accessed the range at least 70% of available days at 18-22 weeks of age). For each of the flocks, these rangers (outdoor preferring hens) were randomly separated into 3 treatment groups of 625 hens per replicate/flock housed in 3 identical partitions amongst their flock mates. Each of the three treatment groups was fed either a standard commercial diet (T1), allowed access to an outdoor feeder filled with the standard commercial diet (T2), or were fed with a diet containing + 10% Metabolisable Energy (ME) and +10% increased amino acids compared to the standard commercial diet (T3). All in-shed diets (T1, T2, T3) were delivered via a feeding chain, the additional outdoor feeder (T2) was available *ad libitum* during pop hole opening times (9am-8pm). A mixed restricted estimate of maximum likelihood (REML) model with the flock as a random factor, and treatment group, hen age and their interactions as fixed effects was used to analyse laying performance, egg quality and body weight. Feeding T3 resulted in significantly higher laying performance at 52, 62 and 72 weeks of age compared to hens that were fed T1 and T2. Albumen height decreased with the age of the hens ($P < 0.001$) but adding an extra 10 % ME and inclusion of the outdoor feeder had no effect on the albumen height ($P = 0.56$). Age of the hen, treatment and their interaction had an effect on the yolk colour ($P < 0.001$) where the addition of extra of 10% ME had effect of yolk colour at 42 weeks of age only. The rangers fed with extra 10 % ME (T3) had a darker yolk colour compared to all other treatment groups. There was no significant difference in body weight between all treatments at 16, 22, and 74 weeks of age ($P>0.05$). In conclusion, increasing energy and protein levels of the diet increased laying performance significantly at end of lay with minor impact on egg quality. This may be relevant when considering extending flock use to 100 weeks of age and beyond.

Table 1 - The difference in egg laying performance between hens fed a conventional diet, hens using an outdoor feeder, and hens with a diet with extra 10% ME and 10% CP.

Age of hens	Week 22	Week 32	Week 42	Week 52	Week 62	Week 72
Control group (T1)	89.5 ± 2.8^{a}	90.3 ± 0.2^{a}	90.0 ± 1.5^{a}	77.9 ± 3.7^{b}	75.3 ± 4.7^{b}	71.6 ± 1.1^{c}
Access to outdoor feeder (T2)	89.9 ± 3.4^{a}	97.2 ± 0.9^{a}	90.7 ± 1.4^{a}	79.0 ± 4.2^{b}	81.1 ± 3.7^{b}	77.7 ± 1.5^{b}
+10% ME; +10 % AA (T3)	86.7 ± 3.7^{a}	98.7 ± 2.7^{a}	94.9 ± 2.6^{a}	92.0 ± 3.8^{a}	90.3 ± 1.5^{a}	83.2 ± 3.8^{a}
P-value	0.839	0.149	0.357	0.006	0.012	0.003

Numbers with different superscripts in the column represent significant difference ($P < 0.05$).

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IDEAL PROTEIN RATIOS AND DIETARY CRUDE PROTEIN CONTENTS INTERACT IN BROILER CHICKENS FROM 14 TO 35 DAYS POST-HATCH

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Summary

The aim of this study was to evaluate two ideal protein ratios (A and B) and two dietary crude protein (CP) levels (180 and 210 g/kg) in wheat-based diets offered to a total of 420 broiler chickens from 14 to 35 days post-hatch as a 2 × 2 factorial treatment array. Significant interactions for weight gain, FCR and relative fat-pad weights were observed, and it was concluded that the precise ideal protein ratio should be adjusted depending on the dietary CP level. In 180 g/kg CP diets, ideal protein ratio A was superior, and consideration is given to the likely underlying mechanisms.

I. INTRODUCTION

Broiler diets are formulated on the basis of ideal protein ratios (IPR) where selected amino acid requirements are expressed relative to lysine (100). However, IPR have been developed over decades for standard diets rather than diets with reduced-CP. This has led to the possibility that reduced-CP diets require modified IPR. For this reason, two sets of ratios were evaluated in diets with 210 and 180 g/kg CP in a 2 × 2 factorial design.

II. MATERIAL AND METHODS

This feeding study was conducted in compliance with the guidelines of the Animal Ethics Committee of The University of Sydney. Two dietary CP levels (210 and 180 g/kg) were tested under two ideal protein ratios (A and B) as shown in Table 1. All diets were formulated to have an energy density of 13.0 MJ/kg and 11 g/kg of digestible lysine (Table 2). The diets were cold-pelleted and contained xylanase enzyme. A total of 420 off-sex male Ross 308 broilers at 14 days of post-hatch were distributed to 28 floor pens (15 birds/pen) to have even distribution of average body weights and each of the four diet had seven replications. Weight gain, feed intake and FCRs were determined from 14 to 35 days post-hatch and carcass parameters were measured on 35 days post-hatch. JMP Pro 14 was used to perform analyses of variance and significance was determined at $P < 0.05$ using LSD student *t*-test.

Table 1 - Ideal protein ratios (IPR) used in the study

IPR	Lys	TSAA	Thr	Trp	Arg	Ile	Leu	Val	His	P + T*	Pro
A	100	74	64	16	104	69	107	79	33	116	-
B	100	75	66	17	110	67	110	77	35	102	140

*P + T: phenylalanine + tyrosine

III. RESULTS

Growth performance from 14 to 35 days post-hatch and carcass parameters in broilers on 35 days post-hatch are shown in Table 3 and mortality rate was recorded as 2.4 % and not relevant to treatments. Overall weight

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gain was comparable to the Ross 308 male performance objectives (1853 versus 1849 g/bird) but feed conversion exceeded by 3.10% (1.531 versus 1.580) and reduced the feed intake by 3.3% (2826 versus 2921 g/bird). Significant interactions of dietary CP and ideal protein ratios were observed for weight gain (P = 0.039), FCR (P = 0.007) and relative fat pad weights (P = 0.022).

Table 2 - Composition and calculated nutrient specification in experimental diets (g/kg)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
	A*	B*	A	B
Wheat	638	655	792	810
Canola seed	60.0	60.0	60.0	60.0
Soybean meal	200	176	29.6	
Canola meal				
Soybean oil	33.4	29.9	6.94	3.70
l-lysine HCl	4.24	4.96	9.19	10.1
d,*l*-methionine	2.59	2.88	3.73	4.08
l-threonine	1.78	1.99	3.92	4.21
l-tryptophan			0.47	0.68
l-valine	0.94	1.00	3.55	3.72
l-arginine	0.80	2.14	5.43	6.95
l-tyrosine	1.32		6.50	1.79
l-isoleucine	0.68	0.74	3.27	3.43
l-phenylalanine				1.70
l-leucine		0.39	3.66	4.81
l-histidine			0.80	1.31
Glycine	0.40	0.73	2.57	2.99
l-serine	0.31	0.71	2.93	3.44
l-proline		6.68		8.46
Salt				2.72
$NaHCO_3$	5.54	5.53	5.40	1.43
K_2CO_3	0.39	1.55	8.22	12.9
Limestone	12.0	12.1	12.7	12.8
1,2-$Ca_3(PO_4)_2$	14.5	14.7	15.7	15.9
Xylanase[b]	0.20	0.20	0.20	0.20
Choline chloride	0.90	0.90	0.90	0.90
Celite	20.0	20.0	20.0	20.0
Vitamin mineral px	2.00	2.00	2.00	2.00
Total non-bound AAs	13.1	22.2	46.0	57.7
Calculated nutrition composition				
ME, MJ/kg	13.0	13.0	13.0	13.0
Crude protein	210	210	180	180
True protein	192	192	169	170
Starch	399	409	493	504
Lysine	11.0	11.0	11.0	11.0
Methionine	5.12	5.31	5.57	5.79
Methionine + cysteine	8.14	8.25	8.14	8.25
Threonine	7.37	7.26	7.37	7.26
Tryptophan	2.13	2.01	1.82	1.87
Isoleucine	7.70	7.37	7.70	7.37
Leucine	12.4	12.1	11.8	12.1
Arginine	11.4	12.1	11.4	12.1
Valine	8.80	8.47	8.80	8.47
Histidine	4.28	4.07	3.63	3.85
Phenylalanine	8.34	7.90	5.47	6.60
Tyrosine	6.81	5.17	9.67	4.62
Proline	14.0	20.2	12.3	20.2
Glycine equivalent	13.3	13.3	13.3	13.3

*Ideal protein ratio; [b]Danisco (40000G); Calculated amino acids were presented as digestible basis

Table 3 - Growth performance from 14 to 35 days post-hatch and relative carcass parameters in broilers on 35 days post-hatch

Treatment		Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Fat pads (g/kg)	[1]Breast (g/kg)	[2]Leg quarters (g/kg)
CP (g/kg)	IPR						
210	A	1942a	2887	1.486c	8.94c	205	197
	B	1986a	2829	1.424d	8.00c	206	194
180	A	1781b	2814	1.580b	10.97b	191	195
	B	1703b	2777	1.632a	12.30a	186	198
SEM		27.7	49.1	0.0147	0.430	2.4	0.9
Main effects							
Ideal protein ratio (IPR)							
A		1862	2851	1.533	9.95	198	147
B		1844	2803	1.528	10.51	194	136
Crude protein level (CP)							
210 g/kg		1964	2858	1.455	8.47	206b	196
180 g/kg		1742	2795	1.606	11.74	187a	197
Significance (P =)							
IPR		0.541	0.213	0.747	0.669	0.407	0.999
CP		< 0.001	0.339	< 0.001	< 0.001	< 0.001	0.629
Interaction IPR × CP		0.039	0.831	0.007	0.022	0.314	0.241

[1]Pectoral major + Pectoral minor; [2]Skin-off and bone in

Broilers offered ideal protein ratio B improved weight gain by 2.27% (1942 versus 1986 g) in comparison to ratio A in 210 g/kg CP diets and reduced weight gain by 4.58% (1781 versus 1703 g) in 180 g/kg CP diets. Ideal protein ratio B improved FCR by 4.35% in comparison to ratio A (1.424 versus 1.486) in 210 g/kg CP diets; whereas, ratio B compromised FCR by 3.29% (1.632versus 1.580) in 180 g/kg CP diets. Ideal protein ratios did not influence the relative fat pad weights in 210 g/kg CP diets, but ideal ratio B generated a 12.1% (12.30 versus 10.97 g/kg) increment in 180 CP diets. Broilers offered 210 g/kg CP diets showed a 10.2% higher ($P < 0.001$) breast muscle yield than those offered 180 CP diets (206 versus 187 g/kg) regardless of ideal protein ratios.

III. DISCUSSION

Treatment interactions between dietary crude protein level and ideal protein ratio were observed for weight gain ($P < 0.05$), FCR ($P < 0.01$) and relative abdominal fat-pad weights ($P < 0.05$). This indicates that IPR A was superior to B when birds were offered 180 g/kg CP diets by 4.58%, 3.29% and 10.8%, for weight gain, FCR, and relative fat-pad weights respectively. The situation was reversed when birds were offered 210 g/kg CP diets. Therefore, the proposition that appropriate IPRs are dependent on dietary CP levels was validated. From Table 1, it is evident that the only real discrepancy between the two ideal protein ratios for the nominated amino acids was for phenylalanine plus tyrosine at 116 and 102, relative to lysine. This meant, based on calculated nutrient specifications (Table 2), that 180 g/kg CP IPR A diets contained 15.14 g/kg phenylalanine plus tyrosine (138 relatives to lysine) as opposed to 11.22 g/kg (102 relatives to lysine) in IPR B diets.

Phenylalanine and tyrosine are interrelated as phenylalanine may be efficiently converted to tyrosine in poultry (Sasse and Baker, 1972). In addition to being incorporated into protein, both amino acids are related to circulating thyroid hormone levels in poultry (Elkin et al., 1980) and the synthesis of catecholamines, including dopamine, in brain tissue (Fernstrom and Fernstrom, 2017). Numerous recommendations have been put forward; however, Dorigam et al. (2013) concluded that the ideal ratio for phenylalanine plus tyrosine should be 115, relative to lysine. This suggests that the ratio of 102 in the IPR B reduced-CP diets may have been inadequate in comparison to the 138 ratio in IPR A diets in the present

study. The analysed dietary amino acid concentrations should be more instructive when they come to hand. Nevertheless, the likelihood is that this differential contributed to the superiority of IPR A in reduced-CP diets.

While speculative, there is another factor that may have contributed to the superiority of IPR A in reduced-CP diets. The 180 g/kg CP diets contained concentrations of either 46.0 g/kg (IPR A) or 57.7 g/kg (IPR B) non-bound amino acids. In Chrystal et al. (2020), 165 g/kg CP diets based on maize contained 38.5 g/kg as opposed to 49.4 g/kg non-bound amino acids in the corresponding wheat-based diets and the performance of the birds offered maize-based diets was clearly superior. However, across all diets in this study there was a highly significant quadratic relationship ($r = 0.933$; $P < 0.0001$), which indicated that FCR began to deteriorate once a concentration of 17.0 g/kg non-bound amino acids was exceeded. Moreover, in the present study there was a quadratic relationship ($r = 0.861$; $P < 0.0001$) between FCR and concentrations of non-bound amino acids (NBAA) in birds offered diets 1 to 4, inclusive, where:

$$y_{(FCR)} = 1.353 + 0.00391*NBAA_{(g/kg)} + 0.0001306*NBAA_{(g/kg)}^2.$$

As shown in Figure 1, this indicates that FCR started to deteriorate when a concentration of 14.97 g/kg NBAA was exceeded.

These relationships may not be meaningful, but they could indicate that broiler chickens may not be able to accommodate high non-bound amino acid dietary inclusions. This contention was advanced by Pinchasov et al. (1990) who argued that there may be a need for a minimum quantity of 'intact' protein because intestinal uptakes of oligopeptides and non-bound amino acids are independent, but oligopeptides are absorbed more rapidly (Matthews and Abidi, 1976). Pinchasov et al. (1990) also raised the possibility that the relevant transport systems for non-bound amino acids may become saturated and that the difference in rates of intestinal uptakes may lead to amino acid imbalances at sites of protein synthesis. This is a real possibility as the digestive dynamics of non-bound versus protein-bound amino acids are inherently different (Liu and Selle, 2017).

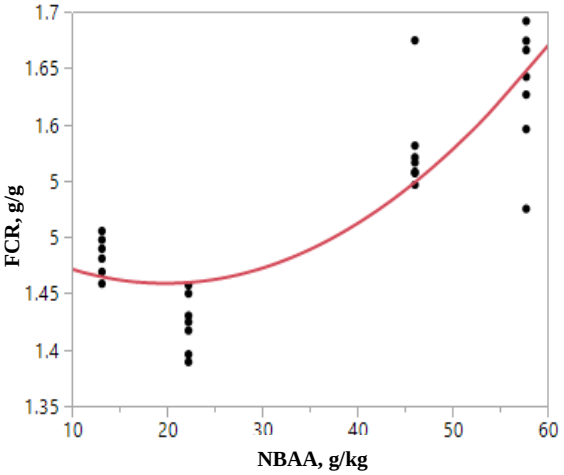

Figure 1 - Quadratic relationship ($r = 0.861$; $P < 0.0001$) between concentrations of non-bound amino acids (NBAA) and FCR in birds offered diet 2 to 4, inclusive.

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A DOSE RESPONSE OF A HEAT STABLE PHYTASE ON BROILER PERFORMANCE AND NUTRIENT DIGESTIBILITY

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Summary

A total of 2160 male broilers were distributed over 60 pens to host 5 treatments. A starter feed (day 1 to 21) and finisher feed (day 22-35) were used (both pelleted). A negative control (NC) feed was reformulated by reducing a positive control (PC) feed by 0.2 percentage point protein, 0.02 percentage point dig. Lys (and other essential amino acids keeping a constant ratio to Lys), 0.18 percentage point Ca and P, and 0.09 MJ/kg ME. The PC starter feed contained 202 g/kg crude protein (CP), 11.5 g/kg dig Lys, 12.04 MJ/kg ME broiler, 8.0 g/kg Ca, 6.8 g/kg P, and 4.0 g/kg available P (aP). The PC finisher feed contained 176 g/kg CP, 9.5 g/kg dig. Lys, 12.67 MJ/kg ME broiler, 7.0 g/kg Ca, 5.9 g/kg P and 3.4 g/kg aP. To the NC feeds 0, 250, 500 or 1000 FTU of phytase was added per kg of feed and performance was measured per feeding phase. At 21 days, bone ash was determined on 3 birds per pen. At the end of the trial, 10 birds per pen were killed and ileal samples was collected to determine P, Ca and CP in order to calculate their digestibility.

Results indicated that the phytase at 250 FTU/kg could compensate for the performance loss due to the feeding the NC, bringing body weight back to the PC level (2445 g/b vs 2428 g/b and 2396 g/b for 250 FTU/kg for the PC and the NC, respectively). The final body weight reached 2447 g/b and 2518 g/b for the 500 FTU and 1000 FTU/kg treatments. Feed conversion (FCR) was significantly increased in the NC (1.600 vs 1.572 for the PC; $P < 0.05$) while the FCR was 1.575 and 1.580 for the 250 FTU/kg and 500 FTU/kg treatment (not significant versus the PC nor NC). The FCR at a dose of 1000 FTU/kg (1.572) was significantly different from the NC ($P< 0.05$). Bone ash increased in a dose responsive way whereby the 1000 FTU/kg treatment reached nearly the value of the bone ash of the PC (39.2 % vs 39.7 % respectively).

The digestibility of P increased linearly with increasing levels of phytase addition (71.3, 76.9 and 78.4 % for 250, 500 and 1000 FTU/kg versus 46.3 and 48.7 % for the PC and the NC respectively). This effect was significant for all phytase vs PC and NC while the 1000 FTU/kg treatment was also significantly different from the 250 FTU/kg treatment ($P < 0.05$). Crude protein digestibility increased with phytase addition, however no significant differences between treatments could be detected (73.3, 73.4, 75.5, 76.4 and 76.3 % for PC, NC, 250 FTU/kg, 500 FTU/kg and 1000 FTU/kg, respectively). Ca digestibility was significantly higher for the NC and the phytase supplemented feeds (30.6, 48.2, 49.8, 50.5 and 54.3 %, respectively). Based on the digestibility values and the P, Ca and CP levels in the feed, it was calculated that the phytase at 250, 500 and 1000 FTU/kg resulted in improvements of 1.11, 1.38 and 1.45 g/kg dig P, of 3.95, 5.67 and 5.49 g/kg digestible protein and of 0.11, 0.15 and 0.40 % dig. Ca per kg feed respectively.

I. INTRODUCTION

Phytases have been heavily researched for decades and been used commercially since the early 1990s). Phytase from microbial origin is therefore added to monogastric diets as it can reduce the incorporation of inorganic P sources in the feed, reducing feed cost and P excretion in the environment. Phytate is the main storage form of phosphate in plant matter and in vegetable feed ingredients where it can normally be found in concentrations of 5 to 25 g/kg (CVB, 2018).

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Phytate has anti-nutritive effects in poultry because it binds phosphorous and other nutrients and decreases their availability (Beeson et al., 2017). Therefore, phytase can also lead to improved animal performance. The aim of this trials was to evaluate the effect of a novel, intrinsic heat stable phytase on animal performance and nutrient digestibility.

II. MATERIAL AND METHODS

A total of 2160 male broilers (DOC MB202 Platinum) were distributed over 60 pens (12 pens per treatment and 36 broilers per pen) for 35 days. A starter feed (day 1 to 21) and finisher feed (day 22-35) were used (both pelleted). A positive control feed was formulated to meet all animal requirements including P and Ca. A negative control (NC) feed was reformulated to reduce this positive control (PC) by 2 g/kg protein, 0.2 g/kg dig. lys, 1.8 g/kg Ca and available P (aP), and 0.09 MJ/kg ME (Table 1). The reduction in dig. lysine was applied to all other essential amino acids in order to maintain the same ratio to lysine as in the PC.

Table 1 - Feed composition and calculated composition for starter and finisher (g/kg)

	Starter (1-21 d)		**Finisher (22-35 d)**	
Ingredient	**Pos. control**	**Neg. control**	**Pos. control**	**Neg. control**
Corn	566.0	567.5	638.2	639.1
DDGS USA	50.0	50.0	50.0	50.0
Wheat Bran Pellet	48.3	75.4	32.2	58.5
Soybean meal HiPro	271.8	259.1	212.9	201.4
Palm Olein	20.0	15.0	30.0	25.0
Limestone	13.42	12.02	12.12	10.71
MCP	12.66	4.13	10.40	1.86
Salt	1.01	0.94	0.89	0.71
L - Lysine HCl	3.32	3.30	2.65	2.65
DL - Meth	3.47	3.32	2.45	2.32
L - Threonine	1.38	1.33	0.93	0.88
L - Valine	0.64	0.56	0.24	0.18
Sodium Bicarbonate	4.40	3.75	3.50	3.00
Trace Mineral Mix*	0.65	0.65	0.60	0.60
Choline-Cl 60%	0.49	0.49	0.57	0.57
Vitamin Mix + Hostazym® X	2.5	2.5	2.5	2.5
Nutrient				
Crude protein	201.8	199.7	176.0	174.5
Dig. Lysine	11.50	11.27	9.50	9.31
Ca	8.00	6.20	7.00	5.20
P tot	6.78	5.04	5.94	4.19
Available P	4.00	2.20	3.40	1.60
ME Broiler (MJ/kg)	12.04	11.95	12.67	12.58

*finisher feed containing TiO_2 at 4 g/kg

The NC was supplemented with a novel, intrinsic thermostable phytase (OptiPhos® Plus, Huvepharma) to reach 0, 250, 500 and 1000 FTU/kg of feed. To all feed an NSPase (Hostazym® X, Huvepharma) was added. The finisher feed contained TiO_2 as a marker at 4 g/kg.

Body weight gain, daily growth rate, feed intake and feed conversion were determined for every feeding phase. Bone ash was determined on a pooled sample of 3 birds per pen. The bones were dried overnight at 100ºC, defatted in ether for 6 h, and burnt to ash in a muffle furnace. At the end of the trial, 10 birds per replicate were euthanised and ileal samples was collected according to the methodology of Shastak et al. (2012). Total P, Ca, CP and TiO_2 were analysed in order to calculate their digestibility using TiO_2 as digestibility marker. Phytase was analysed in all feeds by ISO30024 (2009) to verify good incorporation.

III. RESULT AND DISCUSSION

Phytase levels detected in the phytase supplemented feed were in line with the aimed doses (260, 510 and 875 FTU/kg for starter and 270, 670 and 1010 FTU/kg in finisher for 250, 500 and 1000 FTU/kg respectively). Phytase levels in all PC and NC feeds were not detectable (< 50 FTU/kg).

Despite the reduction in nutrients, in particular the strong reduction in Ca and P level, there was only a limited effect on end weight (2396 vs 2428 g for the NC and the PC respectively), and a small, but statistically significant, effect on feed conversion ratio (FCR; 1.600 vs 1.572 respectively; $P < 0.05$; Fig. 1). Adding the phytase at 250 FTU/kg already brought FCR back to the level of the PC feed, while end weight increased to 2445 g. Dosing higher levels of the phytase did not have a supplemental effect on FCR; however at 1000 FTU/kg the final end weight was 90 g higher than the end weight of the PC birds.

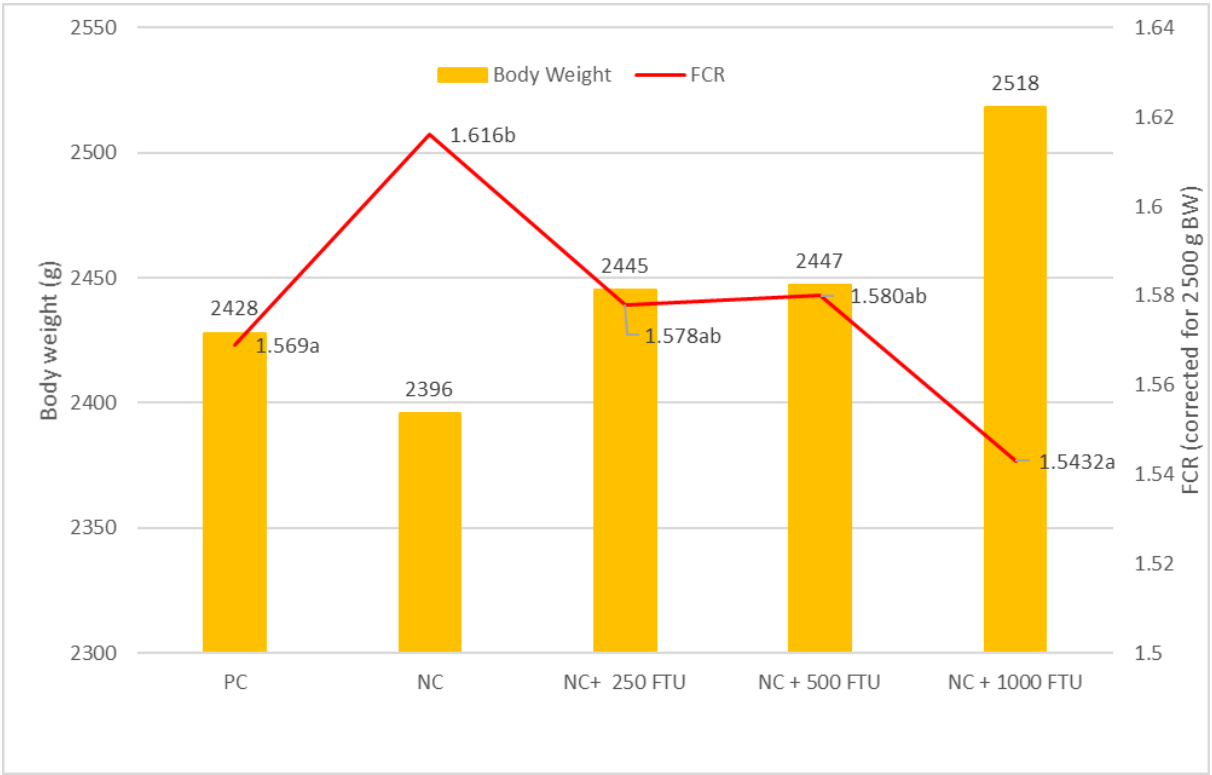

Figure 1- Effect on technical performance at the end of the trial (a,b: values followed by a different superscript are sign. different $P < 0.05$)

Addition of the phytase had a dose-response effect on bone ash percentage (Table 2). The inclusion of 1000 FTU/kg could bring back the bone ash level to that of the PC level, indicating that it can compensate almost completely for the 1.5 g/kg reduction in aP in the feed.

Table 2 – Effect of phytase addition on bone ash

Treatment	Bone ash (%)
PC	39.7[a]
NC	36.9[c]
NC + 250 FTU	37.1[c]
NC + 500 FTU	38.0[bc]
NC + 1000 FTU	39.2[ab]

a,c Values within a row with different superscript are significantly different at P<0.05

The digestibility of P increased linearly with increasing levels of phytase addition (71.3, 76.9 and 78.4 % for 250, 500 and 1000 FTU/kg versus 46.3 and 48.7 % for the PC and the NC respectively; Table 3). This effect was significant for all phytase versus PC and NC while also the 1000 FTU/kg level gave significant higher P digestibility vs the 250 FTU/kg inclusion level. Crude protein digestibility tended to increase with phytase addition. Ca digestibility was significantly higher for the NC and the phytase supplemented feeds (30.6 %, 48.2 %, 49.8%; 50.5 % and 54.3 % respectively).

Based on these digestibility data and knowing the P, Ca and CP level in the feed, it could be calculated that the phytase at 250, 500 and 1000 FTU/kg gave improvements of 1.11, 1.38 and 1.45 g/kg dig. P, 3.95, 5.67 and 5.49 g digestible protein, and of 0.11, 0.15 and 0.40 g dig. Ca per kg feed respectively.

Table 3 – Improvement in ileal digestibility (%)

	PC	NC	NC + 250 FTU	NC + 500 FTU	NC + 1000 FTU
Ca	30.6[b]	48.2[a]	49.8[a]	50.5[a]	54.3[a]
P	46.3[c]	48.7[c]	71.3[b]	76.9[ab]	78.4[a]
Crude protein	73.3	73.4	75.5	76.4	76.3

a,c Values within a row with different superscript are significantly different at P<0.05

IV. CONCLUSIONS

It can be concluded from this trial that this novel, intrinsic heat stable phytase has the potential to compensate for a reduction of 0.2 % protein, 0.02 % dig. Lys, 0.18 % Ca and P and 0.09 MJ/kg ME in feed while improving nutrient digestibility. The increase of 1.11, 1.38 and 1.45 g dig. P per kg of feed at the inclusion level of 250, 500 and 1000 FTU/kg is comparable with the standard matrix values of commercially available phytases.

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A REEXAMINATION OF THE OPTIMUM DIETARY NON-PHYTATE PHOSPHORUS FOR BROILER CHICKENS

U. AFTAB[1]

Summary

Phosphorus (P) is the third most expensive nutrient in commercial broiler diets. In addition to its cost implications, optimizing dietary P is crucial from a standpoint of environmental-safety (eutrophication) and sustainability. Published estimates of P requirements of broiler chickens vary between 20 and 43% within a given phase. Two experiments were conducted to reexamine the optimum dietary P for broiler chickens. In these experiments, a 'standard' plan – based on the P levels currently used in commercial feed industry - was compared with a 'test' plan. Results of these studies suggested that non-phytate phosphorus/calcium (NPP/Ca) levels of 4.0/8.0 g/kg, 3.5/7.0 g/kg and 3.0/6.0 g/kg respectively for 1-10 d, 11-25 d, and 26-35 or 42 d were sufficient to support the optimal growth performance (Trials 1 and 2) and bone mineralisation (Trial 1) of broiler chickens.

I. INTRODUCTION

Phosphorus (P) is a critical nutrient for growth and structural integrity of growing animals. Nutritionists generally tend to use a significant margin of safety while formulating commercial diets to avoid any deficiency symptoms. A large variation appears to exist in P requirement estimates/recommendations for broiler chickens across different sources; e.g. summarizing some selected references, Table 1 shows the highest vs. lowest estimates to differ by 0.8 to 1.3 g/kg available P (AP), or a variance of 20-43 % for a given phase.

Table 1 – Available phosphorus (P) recommendations for straight-run broilers by source

	Starter	Grower	Finisher
Source	Dietary available P* (g/kg)		
Creswell (2012)	4.0	3.5	3.0
Ross 308 (2019)	4.8	4.4	4.1
Cobb 500 (2015)	4.5	4.2	3.8
Rostagno (2017)	4.8	4.3	3.7
CVB (2018)**	4.0	3.1	2.8

*The definition of available phosphorus (P) may differ from source to source; **Digestible P (Wageningen Livestock Research, The Netherlands).

The factors likely to contribute to reported variation include lack of universally accepted definitions of AP (Shastak and Rodehutscord, 2013), variation in the relative bioavailability of P among different sources of inorganic phosphates detailed in Sauvant et al., (2004) and by complex interactions between dietary P, Ca, phytase, and vitamin D3 (Mohammad et al., 1991; Tamim et al., 2004).

II. MATERIALS AND METHODS

Commercial-type broiler diets (maize-soybean meal) were formulated to supply energy and nutrients as per industry practice. The test variable was the dietary AP (and Ca) which was represented as 'standard' and 'test' plans. The 'standard' plans were based on the P/Ca levels currently in use by the commercial industry and were closer to those recommended by the

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primary breeders (Table 1), while the test plan was based on the recommendations published by Creswell (2012). For the purpose of these studies, non-phytate P (NPP) was used for AP and was defined as the sum of the total P from inorganic phosphate (mono-di-calcium phosphate and mono-calcium phosphate, respectively in trial 1 and 2) and non-phytate P from the remaining constituent ingredients derived from Sauvant et al. (2004). No phytase was used in trial 1 while 1000 FTU of an enhanced *Escherichia coli* phytase (Quantum Blue – AB Vista UK) formulated with the mineral contribution as per supplier's matrix (1.95 g/kg AP and 2.15 g/kg Ca) was included in trial 2. In trial 1, feed samples were analyzed for proximate, total P and Ca by Association of Official Agricultural Chemists (AOAC, 2015) methods. Milled sample of test feeds were scanned using a FOSS NIR spectrophotometer for the prediction of phytate-P (Enzyme Services and Consultancy, UK). Dietary NPP was calculated as the difference between total and phytate P. A three-phase feeding program was employed in both trials with feed offered as crumbles (1-10 days post-hatch) and pellets (11 to 42 or 35 days post-hatch in the first and second trial respectively). Each diet was fed to 16 replicates of 30 chicks (Lohman Indian River, Trial 1) and 10 replicates of 20 chicks (Ross 308, Trial 2). Pen feed intake (FI) and body weight (BW) were recorded at the end of every feeding phase, and feed conversion ratio (FCR) corrected for mortality was calculated. In Trial 1, at day 25, two birds (one male and one female) close to the pen average weight from each pen were chosen for the measurement of toe ash. BW, FI, FCR, mortality and toe ash were calculated and 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.

III. RESULTS AND DISCUSSION

a. Trial 1

Table 2 shows the NPP and Ca levels used in standard and test plans. The test plan resulted in higher feed intake at 10 and 25 days post-hatch and higher body weight at 10 days while no treatment effect was observed for the performance at day 42 post-hatch (Table 3). Toe ash, (total and %) measured at 25 days did not differ across treatments (351 vs. 345 mg or 12.6 vs. 13.0%, respectively for standard and test plans).

Table 2 – Formulated dietary levels of non-phytate phosphorus (NPP) and calcium (Ca), Trial 1

Program	NPP/Ca (g/kg)*		
	1-10 d	11-25 d	26-42 d
Standard**	4.5/9.0	4.0/8.0	3.5/7.0
Test***	4.0/8.0	3.5/7.0	3.0/6.0

*INRA, (2004); **Analyzed NPP (total P – phytate P)/Ca; 4.2/8.3, 3.7/7.6, and 3.5/6.9, g/kg respectively for 1-10, 11-25, and 26-42 days; ***Analyzed NPP (total P – phytate P)/Ca; 4.1/7.4, 3.3/6.6, and 3.0/6.1 g/kg respectively for 1-10, 11-25, and 26-42 days

Table 3 - Growth Performance of broilers, Trial 1

Diet	Body weight (g)	Feed intake (g)	Feed conversion ratio (g/g)
		1-10 days	
Standard	320[a]	317[a]	0.990
Test	330[b]	330[b]	0.998
		1-25 days	
Standard	1441[a]	1913	1.318
Test	1468[b]	1949	1.316
		1-42 days	
Standard	3080	5003	1.607
Test	3142	5136	1.591

[a-b] Means within the same column with no common superscript differ significantly ($P < 0.05$)

b. Trial 2

Table 4 shows the NPP and Ca levels used in standard and test plans. The test plan resulted in better FCR compared with the standard plan at 10 days post-hatch; no treatment effect was observed for the growth performance at 25- or 35-days (Table 5).

Table 4 – Formulated dietary level of non-phytate phosphorus (NPP) and calcium (Ca), Trial 2

Program	NPP/Ca (g/kg)*		
	1-10 d	11-25 d	26-35 d
Standard**	4.2/8.4	4.0/8.0	3.8/7.6
Test***	4.0/8.0	3.5/7.0	3.0/6.0

*INRA (2004) tables plus a contribution (virtual) 1.95 g/kg NPP and 2.15 g/kg Ca by phytase

Table 5 - Growth Performance of broilers, Trial 2

Diet	Body weight (g)	Feed intake (g)	Corr. FCR
		1-10 days	
Standard	333	327	0.983[b]
Test	331	320	0.966[a]
		1-25 days	
Standard	1424	1823	1.281
Test	1404	1799	1.281
		1-35 days	
Standard	2408	3399	1.412
Test	2372	3365	1.419

[a-b] Means within the same column with no common superscript differ significantly ($P < 0.05$)

IV. CONCLUSIONS

Data from the above studies suggest that NPP/Ca level of 4.0/8.0, 3.5/7.0 and 3.0/6.0 g/kg respectively for 1-10, 11-25 and 26-35 or 42 days post-hatch was sufficient to support the optimal growth performance (Trials 1 and 2) and bone mineralization (Trial 1) of commercial broiler.

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ESTIMATION OF LYSINE INTAKE FOR BREEDING JAPANESE QUAIL

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Summary

The objective of the study was to estimate the optimal intake of Lysine (Lys) for egg mass production of breeding egg-type Japanese quail, using the dilution technique. The study was conducted using a completely randomized design, with seven levels of Lys (14.9, 11.9, 10.4, 7.4, 5.9, 4.4 and 2.9 g / kg) and seven replicates of one bird per experimental unit. The experiment started at 14 weeks of age, at peak production, and lasted 22 days. The variables collected were: leftover feed, body weight, egg production, and egg weight. The variables analyzed were Lys intake (mg/Bird. d) and egg output (EO, g/g) and broken line, linear-plateau and quadratic-plateau models were fitted to the data. The abscissa value corresponding to the first interception of the quadratic curve of the linear-plateau model provided the estimate for lysine requirement. Responses to the different levels of Lys were significant ($p< 0.05$), for all the studied variables, The requirement for optimal intake of Lys for egg production in layer breeders was estimated at 218 mg/bird.d.

I. INTRODUCTION

Breeding egg type Japanese quail are responsible for the production of fertile eggs upon which the improved Japanese quail multiplication system for commercial egg production is based. The nutrition of these birds needs to be focused on the production and quality of eggs that are intended for artificial incubation. Maternal nutrition affects egg composition and should provide optimal conditions for embryonic development (Li et al., 2019). This sector has been consolidating as a business activity and, to support this growth, scientific development is needed (Minvielle, 2004), especially in nutrition, which substantially impacts the cost of production.

In this context, lysine (Lys) plays an important role in the protein metabolism of the bird, being considered as a reference amino acid to establish the ideal relation between the essential amino acids, in the concept of ideal protein (Emmert and Baker, 1997). The Lys recommendation values available in the literature have been established for commercial egg producing quail and, to date, no published study has yet demonstrated a difference in Lys requirement between layers and breeders. However, breeding Japanese quail present differences in egg production from quail used for egg production, especially in the sequence of laying and length of pauses, which result in a shorter laying cycle duration. Considering the above, the objective of the study was to estimate the ideal intake of Lys for egg mass production of breeding Japanese quail, using the dilution technique.

II. METHOD

The study was conducted at the Poultry Science Laboratory of the Animal Science Department of the College of Agricultural and Veterinary Sciences of the University Estadual Paulista, Campus of Jaboticabal, São Paulo, Brazil. The Animal Use Ethics Committee approved this study under protocol 012203/17.

Forty-nine breeding Japanese quail females (14 weeks old) in peak health, were housed in galvanized wire cages equipped with trough-type feeders and nipple-type drinkers, with ad

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libitum water supply. The average temperature during the whole experimental period was 24.5 ºC and the relative humidity was 45% and the light program was 16 hours (h) of light and 8 hours (h) of darkness. A completely randomized design was used, with seven replications and seven treatments of a bird per experimental unit.

Two experimental diets were formulated, one with a high protein content (HPC), with a relative deficiency of 50% Lys, and an excess of 50% concerning the other essential amino acids. The protein-free diet (PFD) was formulated to meet the same nutritional levels as HPC except for crude protein and amino acids. For the formulation of experimental diets, the dilution technique was used (Fischer and Morris, 1970). The intermediate levels were obtained with the dilution between the two diets HPC and PFD, according to the following proportions: HPC: PFD, 100:0; 70:30; 50.1:49.9; 40:60; 30:70; 20.1:79.9; 10:90; thus obtaining Lys concentrations: 14.9, 11.9, 10.4, 7.4, 5.9, 4.4 and 2.9 g / kg, respectively. The trial lasted 22 days, with seven days of adaptation to the facilities and experimental diets and 15 days of data collection. The feed supply was provided according to the maximum expression of the genetic potential, corrected weekly, considering as a basis the metabolic weight of the population and egg production of the respective treatment. The variables collected were: leftover feed, body weight, egg production and egg weight. The variables analysed and the calculated measurements ingestion of Lys and egg output were subjected to orthogonal contrast analysis for the linear and quadratic effect of Lys levels and when the effect was detected (significance level 0.05), the broken line, linear-plateau (BL) models were adjusted and quadratic-plateau (BLq), described, respectively: $\gamma = \varphi + \alpha [\lambda - \delta]$ and $\gamma = \xi + \beta [\tau - \delta]^2$, where $(\lambda-\delta)$ and $(\tau-\delta)$ is 0 for values of $\delta > \lambda$ and $\delta > \tau$. γ is the answer; δ is the regressor variable; φ and ξ is the answer corresponding to λ and τ, respectively on the ordinate axis; λ and τ is the point indicating a change in the path of γ corresponding to the abscissa axis; α and β represent the slope, according to Robbins et al. (2006). To determine the ideal Lys intake, the abscissa value corresponding to the first intercept of the quadratic curve was calculated using the linear-plateau model: $\delta = -(-\beta\lambda + \text{sqrt}(\beta\varphi - \beta\lambda)) / \beta$. The parameters were estimated using SAS 9.4 software (SAS Institute Inc., 2014).

III. RESULTS

Lys levels affected ($p < 0.05$) the analyzed variables (Table 1). There were significant linear and quadratic effects of Lys level on all of the analyzed variables. The lower Lys level (0.298%), showed a 60% reduction in feed intake compared to 1.043% of Lys in the diet, which showed higher feed intake. The reduction in Lys intake was 87%, which resulted in a 74% reduction in egg production. Egg production increased up to 1.192% of dietary Lys. The differences between the production variables and egg weight consequently affected egg mass, which was reduced by 79% compared to the lower level of Lys in the diet of 0.298%.

To interpret the linear and quadratic effects of Lys levels, the linear-plateau and quadratic-plateau models were tested (Figure 1). The two equations gave R^2 values of 0.73 and 0.75 and BIC values of 151 and 223, respectively, indicating that the linear-plateau model provided the best fit for the data. Lys intake estimates were 200 mg/bird d and 283 mg/bird d by the linear-plateau and quadratic-plateau models, respectively. The ideal intake of Lys was calculated at 218 mg/bird d, considering the first interception of the quadratic model in the plateau of the linear model.

Table 1 - Average responses to the dietary levels of lysine

Levels	Feed intake	Lysine intake	Rate of lay	Egg weight	Egg output
0.298	15.64	46.62	23.75	8.44	2.01
0.447	18.65	83.38	44.64	8.54	3.81
0.596	22.34	133.15	73.96	10.00	7.34
0.745	23.22	172.96	75.00	10.70	8.04
1.043	25.07	261.50	86.61	10.79	9.32
1.192	23.89	284.78	90.62	10.46	9.47
1.489	23.51	350.11	84.82	11.21	9.50
p-Value					
Treatment	<.0001	<.0001	<.0001	<.0001	<.0001
Linear	<.0001	<.0001	<.0001	<.0001	<.0001
Quadratic	<.0001	0.0051	0.0002	0.0032	0.0003

Feed intake (g/day); Lysine intake (mg/day); Rate of lay (%); Egg weight (g); Egg output (g/day d)

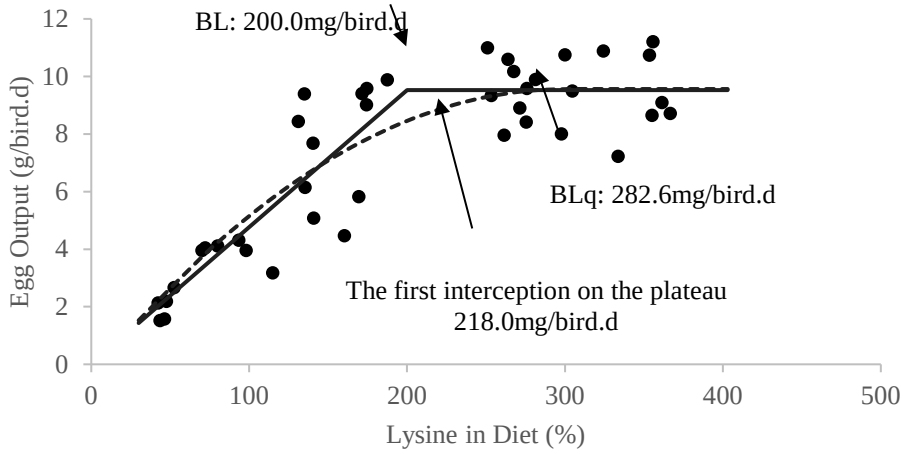

Figure 1 - Relation between lysine intake (mg/bird.d) and egg output (g/bird.d). ● Observed values egg output . ——————, Predicted values egg output by broken-line model (BL). – – – – , Predicted values egg output by quadratic broken-line model (BLq). Calculated value the first intercept of BLq on the plateau of the BL.

IV. DISCUSSION

The optimal dietary level of Lys of 218 mg/bird d, obtained in this study as the intermediate value between the estimates from the two mathematical models used, can be considered as a robust estimate due to the close fit to the data and the considerable increase in response in egg mass to increase in dietary lysine. (Figure 1).

This study is the first to propose a recommendation for optimal dietary levels of Lys for breeding egg-type Japanese quail. The value of 218 mg/bird d is considerably lower than those documented in the literature, ranging from 292 mg/bird d (Costa et al., 2008) to 321 mg/bird d (Ribeiro et al., 2003). The difference between these studies justifies the conduct of this research and supports the understanding that breeding Japanese quail have differences in the requirement of Lys from commercial egg producing quail. A common practice among poultry nutritionists has been to use recommendations obtained from studies with commercial layer quail for breeders. Based on this study, in addition to offering excess dietary lysine, it is very possible that such diets also contain an excess of other amino acids, due to the fact that

lysine is the reference for establishing requirements for the other amino acids. The possible excess of Lys and other amino acids are converted into ammonia, later into uric acid, which has in its synthesis an expensive expenditure of chemical energy (Wu et al., 2013). The greater energy partitioned to eliminate the excess nitrogen can reduce the energy partitioned for egg production.

Increased intake of Lys increased egg weight (Table 1). Increased egg weight for breeding birds invariably affects the hatchability of eggs due to reduced shell thickness (Fouad et al. (2017). A relationship between increased intake of Lys and increased egg weight was observed in duck breeders (Fouad et al., 2017). Although care with egg weight control for breeder eggs is necessary, the values obtained in this research are within the egg weight limit used by the industry, which is between 11 and 12 g.; therefore, the increase described did not provide loss in the hatchability of eggs.

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GLYCINE SUPPLEMENTATION IMPROVES BODY WEIGHT GAIN IN SLOW-GROWING BROILER CHICKENS

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Nutrient-specific appetite has been defined as a dietary selection developed as a response to a specific nutritional requirement (Niknafs and Roura 2018). In chickens, specific appetites offer the opportunity to determine individual differences in nutrient requirements (Roura and Navarro 2018). In a double-choice feed model, slow compared to fast-growing chicks showed a high preference for feed supplemented with Glycine (Gly) versus an iso-energetic balanced diet (Roura and Navarro 2019). Based on this specific amino acid preference, a 96 floor-pen experiment with 576 day-old chickens (Ross 308) was performed at the Queensland Animal Science Precinct (University of Queensland-Gatton, Australia). Chicks were tagged and sexed by extracting DNA from individual feather tips. At three weeks old, chickens were re-grouped into 8 categories based on body weight (BW) and assigned 1 of the 4 experimental treatments (standard diet (ST) + 1% ideal protein -caseinate-; ST + 1% Gly, ST + 1% Asp, and ST + 0.5% Gly + 0.5% Asp) following a randomized block design. ANOVA analysis was used to generate the least-squares means for each treatment with a covariate representing the sex ratio in each pen. Homogeneity was defined as all pens having a similar BW at day 42, with a regression line of chick performance at 42 days relative to BW at day 21. Pens supplemented with Gly 1% had consistently higher intercepts and lower slope values for BW ($P=0.014$), ADG ($P=0.011$) and ADFI ($P<0.001$) compared with the control or the other supplemented diets Regarding BW, the slope for the glycine group (diet 3) was 0.890 while for diets 1 (control), 2 (aspartic) and 4 (glycine + aspartic) were 1.834, 2.295, and 1.923, respectively. The higher the slope the poorer the homogeneity. Gly supplementation (1%) improved the uniformity of BW at 42 days of age in broiler chickens by improving the growth in the poor-growing categories.

Gly has a pivotal role in amino acid metabolism sitting in the center of several pathways involving essential (especially sulphur amino acids, threonine, and arginine) and non-essential (serine, alanine, glutamic acid, and glutamine) amino acids (Li and Wu 2018). Our results indicate that a higher level of dietary Gly has a growth-boosting effect in slow-growing birds. These results agree with previous literature supporting conditional essentiality of Gly in chicken diets (Dean, et al., 2006). A cost-effectiveness analysis of Gly supplementation should be addressed to incorporate higher levels of Glycine in broiler diets.

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SUPPLEMENTATION OF FIBRE-DEGRADING ENZYMES TO WHEAT- OR MAIZE-BASED DIETS OFFERED TO BROILER CHICKENS UNDER NECROTIC ENTERITIS

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A study was conducted to investigate whether the functional oligosaccharides released *in situ* as a consequence of fibre-degrading enzyme application ameliorate the severity of necrotic enteritis (NE) in broilers offered wheat- or maize-based diets. Day-old Cobb 500 mixed-sex broilers (n = 1536) were assigned to a 2 × 2 × 4 factorial arrangement of treatments, with six replicates per treatment, for 21 days. Factors were cereal type (wheat or maize), NE challenge (without or with), and four fibre-degrading enzyme treatments (no enzymes, family 10 xylanase, family 11 xylanase or mannanase). The NE challenge was induced by orally inoculating birds with 1 mL of *Eimeria* spp. on d 9, followed by oral inoculation of 1 mL *C. perfringens* on d 14 and 15. Body weight and feed intake were measured on d 0 and 21, and feed conversion ratio (FCR; corrected for mortality) was calculated. Normal distribution of the data was confirmed and a generalised mixed model used to test main effects, 2- and 3-way interactions at P<0.05, using IBM SPSS Statistics 25. Tukey's HSD test was used to separate means. Percentage of male birds in each pen was used as a covariate.

A three-way interaction was observed for d 0-21 weight gain (P=0.026) and FCR (P=0.001). Enzyme supplementation improved weight gain and FCR in birds fed the wheat-based diet, regardless of NE challenge. Unchallenged birds offered the maize-based diet with supplemental enzymes presented improved weight gain and FCR compared to those offered the maize-based diet without enzymes. However, NE challenged birds offered the maize-based diet supplemented with either family 10 or 11 xylanases presented poorer FCR compared to those offered the maize-based diet without enzymes.

This study presented evidence that, in the absence of NE challenge, xylanase and mannanase induced positive effects on bird performance, regardless of diet type. However, in the presence of NE challenge, xylanase exacerbated the negative impact of NE on weight gain and feed efficiency, but only in birds fed maize-based diets. A possible explanation is that the xylanase solubilised the insoluble NSP in maize at the beginning of the gastrointestinal tract, providing fuel for the pathogenic bacteria. This effect was not observed with wheat, possibly due to its higher soluble NSP composition, and therefore greater oligomer production in the presence of xylanase. Further research is warranted to examine this theory and assess oligosaccharide production in different dietary combinations and environments.

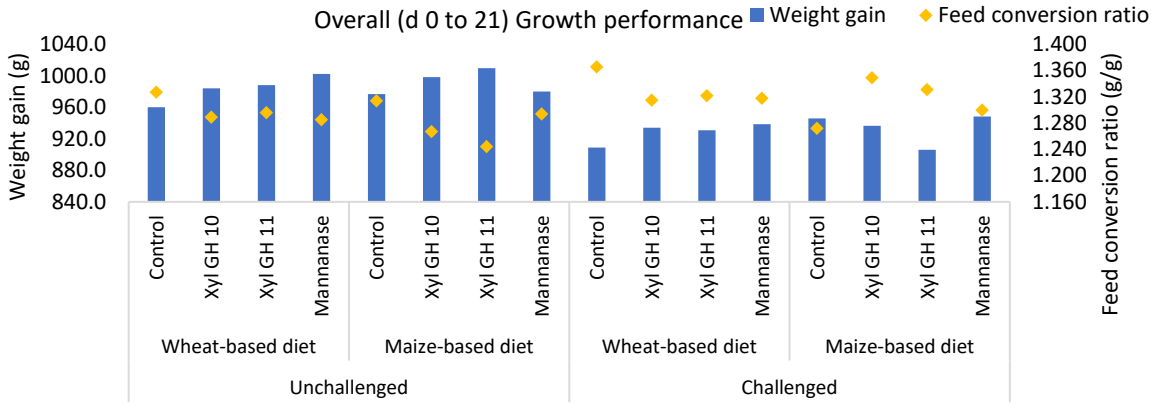

Figure 1 – Necrotic enteritis challenge × grain type × enzyme three-way interactions for overall (d 0 – 21) weight gain (P=0.026) and feed conversion ratio (p=0.001).

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