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Plenary Session 1:
NUTRITION FOR SUSTAINABILITY IN BROILER, LAYER AND TURKEY PRODUCTION

THE ROLE OF NUTRITION IN SUPPORTING SUSTAINABLE POULTRY PRODUCTION

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Background
Advancing our knowledge of poultry nutrition has been a globally significant research field for approaching a century. This has enabled poultry to become established as the most efficient of all animals as a protein source. From the 1940s to the 1990s, poultry scientists initially established the concept of apparent metabolisable energy as a basis for feed formulation, before systematically working to establish the requirements for vitamins, amino acids and minerals (Elwinger *et al.,* 2016). Through the 1980s the role of exogenous enzymes in enhancing digestion was investigated and quickly adopted universally through a diverse range of enzymes from alpha galactosidases to xylanases (Bedford *et al.,* 2022). The vast knowledge accumulated on how to meet the nutritional requirements of poultry has allowed researchers to evolve towards use of nutrition to achieve the wider aims of global food security. In parallel to the highly focused advances in poultry nutrition, other researchers were grappling with the seemingly intractable conflict between globalized economic growth and accelerating ecological degradation. In 1983, the UN invited former Prime Minister of Norway, Gro Harlem Brundtland to chair an independent commission to explore this conflict and propose solutions. The chief outcome was the reframing of economic development in a new paradigm: sustainable development; defined as "development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (Brundtland, 1987). The concept of sustainable development was explained in depth through the discussion of climate change, economic development, and global goals that should be implemented to achieve sustainable development.
The Brundtland Report delineated how economic growth, social equity and environmental balance are essential to create a sustainable development solutions network – utilizing local, national and global development strategies. From this, sustainable development became based on three fundamental pillars: social, economic and environmental. Sustainability clearly exists to find balance between economic, social, and environmental needs, both now and in the future, which makes the three pillars a logical grouping system. However, sustainable development requires a holistic and systemic approach to the three pillars best visualised as a Venn diagram, shown in figure 1 (Royer, 2019).

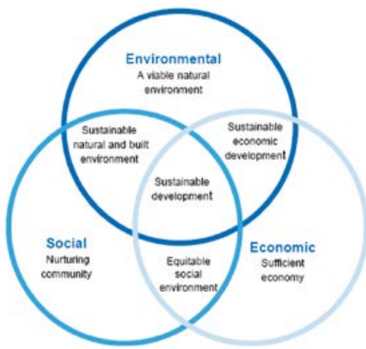

Figure 1: Sustainable Development

THE THREE PILLARS OF SUSTAINABILITY

The close relationship between researchers and practitioners within the poultry sector ensures that economic viability remains at the heart of innovations in poultry nutrition. However, the environmental and societal pillars of sustainable poultry production are complex and often in conflict. Nutrition has a central role in managing these tensions.

All life is ultimately regulated by natural systems, which makes environmental sustainability the first of the three sustainability pillars. Poultry production sometimes takes a narrow approach to environmental sustainability, with a strong focus on finite resources and environmental impact of poultry manure. However, environmental sustainability focuses holistically on the well-being of the environment. Therefore this pillar includes climate change, water supply, and biodiversity; all of which may be influenced by our nutritional strategies for poultry.

Feed is reported to be the major contributor to the carbon cost associated with poultry products (Leinonen *et al.,* 2012[a]; 2012[b]). While many elements of environmental impact resulting from our nutritional strategies lack the models and metrics necessary for quantification, carbon cost, more accurately described as global warming potential (GWP) is quantified in an almost overwhelming plethora of life cycle assessment (LCA) models (Alkhtib *et al.,* 2023). While methodologies and databases for calculating GWP are diverse, LCA provides a common unit; CO_2eq (also written as carbon dioxide equivalent, CO_2 equivalent or CO_2e). As the International Monetary Fund continues to promote carbon taxation policies to support global economic stability (Parry, 2019), nutritional evaluations must also include this unit alongside traditional measures (Burton *et al.,* 2021).

Research for social sustainability has been historically overshadowed by concerns for environmental and economic sustainability (Vallance *et al.,* 2011). Drawing societal sustainability into a poultry context, the UN Sustainable Development Goals (SDGs) while providing an excellent framework for applying nutrition as a tool for sustainable poultry production, fail to consider animal welfare (Messerli *et al.,* 2019). However, within the European Union, the emergence of sustainability as a core policy objective (the 'European Green Deal') is shifting the focus and design of agriculture, food and rural policies to include societal sustainability (European Commission, 2019). In food production sectors as highly evolved as eggs and poultry meat, the lack of established social metrics make it challenging to incorporate this element of sustainability into poultry production (Barral and Detang-Dessendre, 2023). Managing the opposing yet interlocked tensions related to environmental, social, and economic aspects of sustainability is one of the most crucial future challenges in the corporate world (Sasse-Werhahn et al., 2020).

CASE STUDY

Consumption of meat was a critical step in human evolution through provision of nicotinamide to support increasing brain size, but continuing to increase human consumption of meat is no longer an evolutionary advantage (Williams and Hill, 2017). In fact, it is widely stated that meat intake must be limited, as overconsumption in many high- and middle-income countries is associated adverse health outcomes (Willet et al.,2019). While there is a lack of evidence that poultry meat specifically contributes to these health issues, the emerging outcome is that Higher Income Countries are re-considering their relationship with animal protein sources and animals used in food production.

Production systems requiring slower growth of poultry such as the Better Chicken Commitment (BCC, 2019) exemplify tensions between the three pillars. The fixed production constraints of the BCC policy address societal sustainability through improved bird welfare and other, more human-centric parameters. This requires breeders and nutritionists to resolve the apparent economic and environmental imbalances related to lower stocking density and slower growth. The many papers presented at ESPN2023 evaluating non-standard feed demonstrates the research drive to mitigate the increased feed volume required for slower growth systems.

ONE HEALTH

Some of the challenges we may positively influence through nutrition are encapsulated by the UN ONE Health approach. One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems. The term 'One Health' was first used in early 2003 in association with the emergence of severe acute respiratory disease (SARS) and the spread of highly pathogenic avian influenza H5N1. One Health became established conceptually through the 'Manhattan Principles' which clearly recognised the link between human and animal health and the threats that diseases pose to food supplies and economies (Cook *et al*., 2004). The nutritional focus on strategies to enhance poultry gut health to reduce reliance on antibiotics is a powerful example of One Health in practice (La Ragione and Burton, 2023).

NUTRITION IN THE FUTURE

To ensure the poultry sector continues to thrive, nutritionists need to support poultry producers in acting as ambassadors, offering producers new tools to address the requirements of society while continuing to deliver high quality protein with very low environmental impact. As sustainability models progress from a 'cradle to grave' approach to adopt circular and networked economics (a 'cradle to cradle' approach) in managing resources and environmental impact, it is likely nutritionists will have to re-imagine the type of materials we consider for use in feed. As we evaluate each candidate material, nutritionists must keep one eye on the past to learn from historical errors and one eye on the future, to scan ahead for unintended consequences of our decision-making.

An arising skill requirement for poultry nutritionists is application of life cycle analysis to feeding trials and diet formation. Where feed formulation previously reconciled nutrient supply against economic cost, now carbon cost must be considered as a third dimension. A second emerging skill is public communication: nutritionists must be able summarise our sustainability achievements in formats that can be fed into social media streams by those in the poultry supply chain holding consumer relationships. A final competency for nutritionists to maximally support the poultry industry is to maintain awareness of changing societal needs and consider them with a growth mindset (Dweck, 2006); seeking to understand and use nutrition to address underpinning values rather than focusing on the (frequently incorrect) details that accompany these societal messages. In addition to these skills, it is clear the nutrition vanguard must adopt a holistic view of sustainability that considers societal elements alongside environmental and economic in order to realise the enormous potential of poultry nutrition in supporting sustainability development.

CONCLUSION

It is now more important than ever that nutrition continues to facilitate global production of high-quality protein from the lowest possible natural resource use, but we need to work within the context of the three sustainability pillars. The pillars of sustainability help us recognise everything is connected; actions and impacts must be balanced; and no individual, organization, or nation operates by itself. One Health offers a collaborative and multi-disciplinary approach, cutting across boundaries of animal, human, and environmental health, that recognises that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent.

Much of our current nutrition research directly addresses global challenges such as antimicrobial resistance, food safety and promoting the health and integrity of our natural ecosystems. Poultry nutritionists must develop new skills and approaches to maximise the contribution of nutrition to sustainable food production, and ensure the contribution is universally recognised. Using the language of the three sustainability pillars to contextualise sustainable poultry nutrition will allow our stakeholders to better understand the co-benefits, risks, trade-offs and opportunities to advance sustainable nutritional solutions.

REFERENCES

1. Alkhtib, A., Burton, E., Wilson, P.B., Scholey, D. And Bentley, J., (2023). Effect of life cycle inventory choices and nutritional variation on carbon footprint of broiler meat production. Journal of Cleaner Production, 383: 135463. ISSN 0959-6526
2. BCC, 2019 Better Chicken Commitment https://betterchickencommitment.com/policy/ Accessed 2 May 2023. Bedford, M., Gary Partridge, G., Carrie L Walk, C. & Milan Hruby, M. (2022). Enzymes in Farm Animal Nutrition, 3rd Ed. CABI Publishing.
3. Barral, S., & Detang-Dessendre, C. (2023). Reforming the Common Agricultural Policy (2023–2027): multidisciplinary views. Rev Agric Food Environ Stud 104: 47–50. https://doi.org/10.1007/s41130-023-00191-9
4. Brennan, M., Hennessy E., & Meredith D. (2022). Putting social into agricultural sustainability: Integrating assessments of quality of life and wellbeing into farm sustainability indicators. *Sociologica Ruralis* doi.org/10.1111/soru.12417.
5. Brundtland, G.H. (1987) Our Common Future: Report of the World Commission on Environment and Development. Geneva, UN-Dokument A/42/427. http://www.un-documents.net/ocf-ov.htm
6. Burton, E., Scholey, D., Alkhtib, A. & Williams, P. (2021). Use of an ethanol bio-refinery product as a soy bean alternative in diets for fast-growing meat production species: a circular economy approach. Sustainability, 13 (19): 11019. ISSN 2071-1050.
7. Dweck, C. S. (2006). Mindset: The new psychology of success. Random House.
8. Elwinger, K., Fisher, C., Jeroch, H., Sauveur, B., Tiller, H., & Whitehead, C. (2016). A brief history of poultry nutrition over the last hundred years. World's Poultry Science Journal, 72(4), 701-720. doi:10.1017/S004393391600074X
9. European Commission. (2019). Communication from the Commission to the European Parliament, the European Council, the Council, the European Economic and Social Committee and the Committee of the Regions. The European Green Deal. COM (2019) 640 final, 24 p. + Annex. https://eur-lex.europa.eu/resource.html?uri=cellar:b828d165-1c22-11ea-8c1f-01aa75ed71a1.0002.02/DOC_1&format=PDF
10. La Ragione R. & Burton, E. (2023). Moving beyond antibiotic usage to support the health and productivity of commercially reared poultry. In: *Pre and probiotics, nutrition, veterinary and production perspectives.* CABI Publishing.
11. Leinonen I, Williams AG, Wiseman J, Guy J, Kyriazakis I. (2012a) Predicting the environmental impacts of chicken systems in the United Kingdom through a life cycle assessment: egg production systems. Poult Sci. 91:26-40. doi: 10.3382/ps.2011-01635.
12. Leinonen I, Williams AG, Wiseman J, Guy J, Kyriazakis I. (2012b) Predicting the environmental impacts of chicken systems in the United Kingdom through a life cycle assessment: broiler production systems. Poult Sci. 91:8-25. doi: 10.3382/ps.2011-01634.
13. Messerli, P., Murniningtyas, E., Eloundou-Enyegue, P., Foli, E.G., Furman, E., Glassman, A., Hernández Licona, G., Kim, E.M., Lutz, W., Moatti, J.P. and Richardson, K., (2019). Global sustainable development report 2019: the future is now–science for achieving sustainable development.
14. Royer, Pierre. (2019). Circular Economy, utopia or promising new business model? An evaluation of Circular Economy efficiency against environmental challenges. 10.13140/RG.2.2.31586.27842.
15. Sasse-Werhahn, L., Bachmann, C. & Habisch, A. (2020).Managing Tensions in Corporate Sustainability Through a Practical Wisdom Lens. J Bus Ethics 163, 53–66. https://doi.org/10.1007/s10551-018-3994-z
16. Vallance, S, Perkins, H, Dixon, J (2011). What is social sustainability? A clarification of concepts. Geoforum, 42: 342-348 https://doi.org/10.1016/j.geoforum.2011.01.002.
17. William, R., Karesh, B. and Osofsky S. (2004). Wildlife Conservation Society One World-One Health: Building Interdisciplinary Bridges. http://www.oneworldonehealth.org/sept2004/owoh_sept04.html Accessed 05 May 2023.
18. Williams A., Hill L., (2017). Meat and Nicotinamide: A Causal Role in Human Evolution, History, and Demographics. Int J Tryptophan Res. 10:1178646917704661. doi: 10.1177/1178646917704661.

Plenary Session 1:
NUTRITION FOR SUSTAINABILITY IN BROILER, LAYER AND TURKEY PRODUCTION

SUSTAINABILITY AND OPTIMAL NITROGEN NUTRITION

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INTRODUCTION

Poultry meat and eggs currently are the most common animal-based foods worldwide. Production of animal-based food requires land area and water, mainly through the production of feed crops.[1] Hence, arable land and water for feed cropping is expected to become an increasingly scarce resource. Another aspect is the environmental impact of animal farming, which has led to growing concerns over the past decades. Feed production and emissions from excreta represent the major impacts of animal farming in this regard.[2] Major environmentally relevant impacts of feed production include energy consumption of crop farming and transport, emissions from the fields, and the consequences of land-use change when crops are cultivated on converted forests or grasslands. Regarding excreta, the major environmentally relevant impacts include nitrogenous emissions (ammonia, nitrates, nitrous oxide), phosphorus emissions, or fine particles. These emissions contribute to climate change, acidification, eutrophication, and pollution of air and water.

Aims of farm animal nutrition research include to minimise the negative effects of farm animal husbandry on the environment, to ensure animal health and well-being as well as to contribute to global food availability. Investigations identifying requirements for nitrogenous compounds, mainly amino acids (**AA**), and evaluating feed ingredients and feed additives contribute to these aims.[3] Animal nutrition research allows to reduce the needed amount of feed crops and the environmental impact of poultry farming. A key figure to assess the efficiency of the production of animal-based food is the nitrogen (**N**) utilisation efficiency (**NUE**), which is the N accretion in body weight or eggs relative to the N intake. Increasing the NUE results in less feed protein needed and reduced N excretion per produced animal-based food.

This contribution aims to summarise the current knowledge on increasing the NUE in the context of saving feed crops and reducing the environmental impact. A focus is set on an increasingly precise alignment of the supply with and the requirement for digestible AA. Conflict-of-aims among sustainability goals are worked out.

INFLUENCES ON THE NITROGEN UTILISATION EFFICIENCY

The dietary supply below and above the requirement of poultry for digestible AA usually leads to decreased NUE. Consequences of a deficient supply with one or more AA include performance loss, increased degradation of non-limiting AA because they cannot be used for protein synthesis. An excess supply causes decreased NUE because excess AA are degraded by the animal.[4] In both cases, more N is excreted relative to the intake.

Phase-feeding

Phase-feeding is widely applied in practice; thus, it is only mentioned shortly for completeness. Recommended AA concentrations in diets decrease considerably throughout a production period of laying and growing poultry, mainly because of the changing energy requirement. The alignment of recommended and actual dietary AA concentrations is closer the more phases are implemented. This approach reduces surpluses in AA supply and, hence, increases NUE.

Increasing knowledge on amino acid requirements

The requirement for essential AA has been investigated extensively since the middle of the last century. The increasingly advancing knowledge of the requirement for essential AA has enabled the reduction dietary CP, thereby avoiding essential AA deficiency. Current practical recommendations for 1–21-day-old broiler chickens usually range between 21–23% CP[5,6], yielding in about 55% NUE.

Practical feeding experiments show that dietary CP in this age range can be reduced to 19% without performance loss while the estimated NUE increased to more than 60%.[7] Such low CP concentrations are rare in current commercial diets. Cost of free AA contribute to that because the inclusion of free AA increases the lower dietary CP is. Nonetheless, based on current knowledge, dietary CP can be reduced to 15–16% without performance loss in maize/soybean meal-based diets without performance loss.[8] Glycine or serine need to be supplemented to such diets because these AA become growth-limiting when dietary CP is below 19%.[9] In the future, less than 15% of dietary CP may be achievable without performance loss when the next-limiting AA is determined. Although not unequivocally clear, recent experiments indicated that dietary asparagine may limit growth in such diets.[10,11]

The measured NUE determined for broiler chickens fed diets with ~16% CP was 75% to more than 80%.[10,11] Therefore, NUE could be ~20 percentage points (**pp**) higher than the nowadays standard. According to a meta-analysis, decreasing dietary CP from 21% to 16% reduces N excretion by approximately two thirds.[12] The potential of CP reduction to decrease N emissions is higher than the potential to decrease N excretion. The urine-N contributes more to ammonia and nitrous oxide emissions than N excreted via the faeces. When dietary CP is reduced while the requirement for AA is met, urinary N excretion relative to N in faeces becomes lower.

Increasing knowledge on amino acid digestibility

Knowledge of precaecal AA digestibility is a prerequisite for formulating diets according to the requirement of the animals for digestible AA. Increasing AA digestibility increases NUE provided that dietary CP is reduced so that the supply of digestible AA remains unchanged. An increase in AA digestibility without reduction of dietary CP increases N excretion via the urine and decreases N excretion via the faeces. This shift in excretion routes does not affect the total N excretion. Hence, NUE remains unaffected but the higher proportion of N excreted via the urine contributes to higher ammonia and nitrous oxide emissions.

When AA digestibility is varied by an influence not related to the feed ingredients providing AA (such as supplemented enzymes), increasing AA digestibility raises NUE in a linear manner.[13] This increase in NUE is only attributed to the limiting AA in the diet, although supplemented enzymes can increase the digestibility of all AA. An increase in the digestibility of non-limiting AA increases N excretion via the urine, leading to the downsides mentioned earlier in this contribution. This effect can be avoided by using free AA so that supplemented enzymes increase the digestibility of several AA that are provided exactly according to the requirement for digestible AA.

The extent to which AA digestibility influences NUE depends on the ratio of the limiting AA to CP (AA/CP ratio) in a diet. The higher this ratio, the more CP not related to the limiting AA can be spared by increasing the digestibility of the limiting AA. Consequences of increasing the digestibility of lysine as the limiting AA by 1 pp in practical laying hen diets were described in a model calculation.[13] In this model calculation, NUE was increased by 0.36 pp at a lysine/CP ratio of 3.1 and by 0.46 pp at a lysine/CP ratio of 3.9.

Another possibility is to increase AA digestibility by selecting specific variants of a feed ingredient differing in AA digestibility. Consequences on NUE cannot be determined accurately using an equation because AA digestibility and the AA/CP ratio in feed ingredients vary independently. However, the effects of increasing AA digestibility by 1 pp via selection of ingredient variants was approximated in model calculations based on studies on the variation in AA digestibility within ingredients in laying hen diets.[13] Assuming that the considered ingredient contributed 3 g digestible lysine/kg of diet, the increase in NUE per pp higher lysine digestibility was 5–13 and 0–4 pp of NUE for cereal grains and protein-rich ingredients, respectively.

Taking the variation in AA digestibility into account presupposes that AA digestibility of single feedstuff batches is predictable. In the literature, several attempts were made to predict AA digestibility in studies investigating the variation in digestibility within feed ingredients. The methods of prediction included correlations and multiple regressions between AA digestibility and analysed variables of the feed ingredients, and determination of *in vitro* N solubility. Accuracy of such prediction was limited. Some studies found significant correlations, but such results were not consistent among individual AA or results were not verified on independent data sets. Near infrared reflectance spectroscopy (**NIRS**) is an attractive tool to determine characteristics of feed ingredients because measurements are quick, simple, and inexpensive. NIRS calibrations for AA digestibility are commercially available, but the underlying reference measurements are not disclosed. It appears highly unlikely that the reference measurements originate from methodologically uniform experiments, thus decreasing the validity of reference measurements to an unknown extent.

Potential to increase the nitrogen utilisation efficiency in current practical diets

Optimising diets by approximating the requirement for digestible AA to increase NUE involves considering the changing requirement for digestible AA throughout the production period, increasing knowledge of the requirement of animals for single nitrogenous nutrients, and increasing predictability of AA digestibility. Changes in needed AA concentrations throughout the production period have been well-known for years. The limitations for practical application of this knowledge are defined by operational constraints, such as feeding technology and availability of transport and storage capacity for diets with different AA concentrations. Given current inaccuracies in prediction of AA digestibility, formulating diets that meet the requirement of more AA than the current standard (e.g., by inclusion of free AA) appears to be a more promising tool to increase the NUE than attempts to increase AA digestibility in the near future. Rapid analysis tools, such as in-line stream NIRS devices, may prospectively enable to estimate AA concentrations of feed ingredients in real-time and to adjust diet composition automatically to achieve aspired dietary AA concentrations, best on a digestible basis.

CRITICAL EVALUATION OF THE GOAL TO MAXIMISE NITROGEN UTILISATION EFFICIENCY

Minimised nitrogen emissions vs. minimised greenhouse gas emissions

Increasing the NUE results in less N excretion and, hence, less formation of the greenhouse gas (**GHG**) nitrous oxide. Less N excretion further reduces the energy requirement for uric acid formation, which results in less carbon dioxide produced by the metabolism. In addition, less dietary CP and lower energy requirements cause lower GHG emissions from cropping for feed production and transport. However, the need for free AA inclusion to adjust dietary AA concentrations increases the lower dietary CP is and GHG emissions originating from free AA production is a multiple of GHG emissions from plant feedstuff production.[14] Therefore, increasing the NUE by decreasing dietary CP will lead to less GHG emissions related to excreted N but this effect is countered by increasing GHG emissions for free AA production. This results in a conflict-of-aims between minimising GHG emissions and minimising the environmental impact of N emissions and cropping for feed production. A threshold of free AA inclusion for minimised GHG is difficult to identify because of the variation in GHG emissions assigned to feed ingredients. Particularly GHG emissions assigned to free AA differ widely.[15,16] Nonetheless, reducing dietary CP below the current standard should decrease both GHG and N emissions. This is because free AA inclusion in most cases is too low to make the conflict-of-aims between GHG and N emissions relevant at current, unless very high GHG emissions are assigned to free AA production.[16]

Maximised growth vs. maximised nitrogen utilisation efficiency

Prioritised goals of poultry feed formulation may shift in the future. Recently, studies determined an increasing NUE when performance of broiler chickens was reduced as a consequence of AA deficiency.[8,11] This outcome probably is explained by maximised AA utilisation efficiency (increment in

AA accretion per increment in AA intake) at an AA intake level below what is needed for maximised AA accretion. This phenomenon was exemplarily found for methionine[17] and lysine[18] in broiler chickens. Potential explanations for these results include a lower muscle protein turnover in the state of AA deficiency.[19] Increased NUE at an AA supply that does not allow for maximum performance results in a conflict between maximised growth and maximised NUE. At present, high growth performance commonly is considered most important for diet formulation, and reduced nutrient excretion is the next highest priority. However, future economic and ecological constraints may lead to priority changes in the industry. Submaximal growth might be accepted when maximising utilisation of protein sources or minimising emissions emerges as more economically beneficial than maximising growth. Reasons for that may include higher prices of protein sources and increasingly strict fertiliser legislation that determines the animal production volume of a farm.

Maximised global food availability vs. maximised nitrogen utilisation efficiency

A maximised NUE is to be pursued when the major aim of feed formulation is to reduce environmental effects of excreted N. When the major aim of feed formulation is global food availability, a maximised NUE is only beneficial if feed ingredients are used that are not consumed by humans. Lower NUE, for instance in consequence of low AA digestibility, can be accepted for the aim of global food availability when ingredients hardly consumed by humans are used because other components can be consumed directly by humans without transformation losses.

CONCLUSIONS

The NUE is a key figure to assess the sustainability of optimal N nutrition because increasing the NUE implies decreasing environmental issues of N excretion and decreasing field crop production needed to produce animal-based food. However, reduced NUE may be accepted when other sustainability goals are prioritised, like minimising GHG emissions or maximising global food availability. Among the feeding strategies to increase NUE, formulating diets that meet the requirement of more AA than the current standard (e.g., by inclusion of free AA) appears easiest to be put into practice in the near future. Possibilities to increase NUE by considering AA digestibility currently are limited due to low predictability.

REFERENCES

1 Mottet & Tempio. 2017. Worlds Poult Sci J 73: 245–256.
2 Gerber et al. 2013. Rome, FAO, ISBN 9789251079201.
3 Gesellschaft für Ernährungsphysiologie. 2017. Proc Soc Nutr Phys 26: 218–224.
4 Simon. 2020. In: Jeroch et al., eds. ISBN 783825287634.
5 Aviagen. 2019. Ross 308 broiler nutrition specifications.
6 Deutsche Landwirtschafts-Gesellschaft. 2017. DLG Merkblatt 406.
7 Lemme et al. 2019. J Appl Poult Res 28: 867–880.
8 Hofmann et al. 2019. J Nutr 149: 1122–1132.
9 Siegert & Rodehutscord. 2019. Br Poult Sci 60: 579–588.
10 Ibrahim et al. 2023. Proc Soc Nutr Phys 32: 25.
11 Hofmann et al. 2020. Poult Sci 99: 6848–6858.
12 Cappelaere et al. 2021. Front Vet Sci 8: 689259.
13 Siegert. 2022. Habilitation thesis http://opus.uni-hohenheim.de/volltexte/2022/2054
14 Mosnier et al. 2011. Animal 5: 1972–1983.
15 Lammers et al. 2010. J Anim Sci 88: 1204–1212.
16 van Harn et al. 2017. Wageningen Livestock Research Report 1033.
17 Fatufe et al. 2005. Arch Anim Nutr 59: 181–190.
18 Fatufe et al. 2004. Poult Sci 83: 1314–1324.
19 Tesseraud et al. 1992. Reprod Nutr Dev 32: 163–171.

Plenary Session 1:
NUTRITION FOR SUSTAINABILITY IN BROILER, LAYER AND TURKEY PRODUCTION

IMPROVING ENERGY UTILISATION IN POULTRY: CHALLENGES AND OPPORTUNITIES

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INTRODUCTION

Energy is not a nutrient, but a property that some nutrients (carbohydrates, lipids and protein) possess. Dietary energy represents the costliest component in poultry feed formulations and is the first component to be considered when the diets are being balanced. Dietary energy also controls the feed intake, which is the major driver of bird growth. Therefore, an accurate evaluation of the available energy content of ingredients is important for precise and cost-effective feed formulations (Abdollahi et al., 2021).

Different energy systems have been suggested over the years. Apparent metabolisable energy (AME), N-corrected AME (AMEn; Hill and Anderson, 1958), net energy (NE; Swick et al., 2013; Wu et al., 2020), and true ileal digestible energy (TIDE; Khalil et al., 2020, 2022) are the examples of energy systems. Each energy system has its own merits and drawbacks. However, The AMEn is widely used as the default value to express the available energy of feed ingredients and for describing the energy requirements of birds. Energy contents of feed ingredients can be obtained from published table values, prediction equations, or in vivo assays. Predictive equations and in vivo assays are useful and provide a better alternative than tabulated values. Table values are the basis of feed formulation under most practical conditions. However, the wide variability among published data on the energy value of many ingredients limits their applicability. These variabilities are due to the various factors such as different methodologies, age of bird, genotype, feed form, ingredient characteristics, and presence of anti-nutritional factors (ANFs). Therefore, knowledge about these factors could help nutritionist to accurately evaluate the available energy content of ingredients and apply the best strategy to improve energy utilisation. Key strategies to enhance the utilisation of energy from feed ingredients are those ones that can improve carbohydrate, protein and fat digestibility of the diets. Application of feed additives (exogenous enzymes, emulsifiers, prebiotics, and probiotics) have shown to have positive effect on nutrient digestibility.

The aims of the present review are 2-fold: 1) to address the challenges and limitations encountered by the poultry nutritionist when using energy values of feed ingredients, and 2) to suggest the strategies and opportunities that can be applied to improve the energy value of feed ingredients and enhance the precision of feed formulation and production efficiency.

ENERGY SYSTEMS AVAILABLE FOR FEED FORMULATION

The AME system has become the most common system to express the energy requirements for birds and evaluate the available energy of ingredients and diets as it is simple, straightforward and considers most of the energy losses after the digestion and metabolism. Correcting AME values to zero N retention (AMEn) for modern fast-growing broilers penalises the energy value of all feed ingredients that contribute to body protein deposition. The magnitude of decline in energy value due to zero N correction varies depending on the protein content and quality of the ingredient, imposing a heavy penalty to the protein sources with a higher protein quality (Abdollahi et al., 2021). The NE system has received some attention recently as an alternative for the standard AME system in feed formulation for broilers (Swick et al., 2013; Wu et al., 2020). Net energy represents the effective energy for birds used for body maintenance and various forms of production, and it can be defined as the ME minus the energy losses for heat increment, which is the increase in heat production after consumption of food, digestion, metabolism, and excretion of wastes. However, the NE system has been criticised for several reasons.

Most importantly, the NE method relies on the precise measurements of AME, so any error in the AME procedure will be reflected in the NE measurement. Another potential energy system might be ileal digestible energy (IDE). A switch of available energy measurement to IDE will overcome the limitations of AME and also align energy availability with the current trend of using digestible content of nutrients in feed formulations. Researchers at Massey University recently proposed a novel approach to quantify ileal endogenous energy losses in broiler chickens and provided data on the TIDE of some common cereals (Khalil et al., 2020, 2022). Strong positive correlations were observed between the TIDE and ileal digestibility of nutrients than those with the AME or AMEn.

FACTORS INFLUENCING ENERGY CONTRIBUTION OF INGREDIENTS

Age of the birds and genotype

Utilisation of energy by birds of different ages changes according to how ingredients are processed by the digestive enzymes at the different stages of the bird's life. Some of the available tables provide different energy values for birds at different ages (INRA, 2002; CVB, 2016; Feedipedia, 2017; Rostagno et al., 2017); however, other tables do not distinguish between ages (WPSA, 1986; NRC, 1994). In recent studies, Khalil et al. (2021,2022a,b) suggested that, age of broiler has a substantial impact on the AMEn values of cereal grains. These results question the validity of applying a single AME or AMEn value for broilers of different ages, which can under- or over-estimate the energy utilisation. Therefore, to improve the precision of feed formulations and production efficiency, age-dependent AME and AMEn values may need to be considered when formulating broiler diets.

Digestibility and utilisation of nutrients and energy vary between bird types and are dependent on the strain of bird (Yegani and Korver, 2012). These variabilities may be due to the differences in the developments of the digestive system organs and/or endogenous nutrient or energy losses. This difference in gastrointestinal development may influence nutrient utilisation, which has implications on the formulation of feeds for slow-growing chickens; feeds formulated for slow growers are commonly based on nutritional data derived from fast-growing strains. Most of the current energy requirements listed in the published tables are provided in generic terms (NRC, 1994; Rostagno et al., 2017; Evonik, 2016), or are provided for specific commercial strains of broiler (Aviagen, 2009; Hubbard, 2011). However, there is a lack of information on the influence of poultry genotypes on the AME values of many ingredients.

Feed form

Processing feed affects not only feed intake, but also gastrointestinal tract development, specifically the gizzard, resulting in changes in nutrient utilisation and microbial profile. The effects of pelleting on the energy content of diets are inconsistent and depend on different factors such as ingredient type used in the diet, heat applied and particle size (Mateos et al., 2012). It has been reported that feeding birds on pelleted diets reduces the AME values compared to mash diets and is attributed to the starch overload in the gut and possible starch retrogradation forming resistant starch (Svihus, 2001). Abdollahi et al. (2013) showed that pelleting reduced the AME value of a wheat-based diet by 0.70 MJ/kg compared to the mash form. However, pelleting showed no effect on the AME value for a maize-based diet. Similarly, Amerah et al. (2007) reported that pelleting reduced the AMEn of a wheat-based diet from 12.5 MJ/kg to 11.8 MJ/kg compared to the mash form. In contrast, pelleting might increase the energy utilisation of certain ingredients, as heat and mechanical pressure in the pellet die can disrupt the structure of the cell walls, thus releasing some nutrients such as lipids contained in the oil bodies of oil-containing ingredients (Jiménez-Moreno et al., 2009).

Ingredients type and presence of anti-nutritional factors

Variations in the chemical composition of ingredients can significantly influence the digestibility and availability of nutrients, and hence, these variations can affect the AME values of ingredients. This variation has been explained as the differences in the proportions and the percentages of the major nutrients: protein, fat, and carbohydrates, which are responsible for significant variation in the birds' abilities to utilise energy from different substances (Yegani and Korve, 2012).

There are various types of ANFs in poultry feeds that affect energy utilisation. These ANFs not only affect AME values, but also affect birds' gastrointestinal tract function. Non starch polysaccharides (NSPs) are the main ANFs in cereal sources. They are non-degradable substances and remain undigested by the endogenous enzymes in the gastrointestinal tract of birds. The detrimental and anti-nutritional effects of NSPs on dietary AME are due to their potential to increase the digesta viscosity, hence negatively affecting the digestibility of nutrients and ultimately, energy utilisation (Choct, 2015). The adverse effects of NSPs can be exaggerated by processing, as pelleting at a high temperature can solubilise the insoluble NSP fractions in wheat and barley, leading to higher viscosity of the digesta compared to the unprocessed form of feed, hence reducing digestibility and AME values (Mateos et al., 2019).

Dietary supplementation of exogenous enzymes can reduce the negative effect of ANFs on nutrient digestibility, whilst leaving fewer nutrients to the hindgut, where they would be used by microbiomes to proliferate. It has been reported that enzymes have more pronounced effect in the feed ingredient with high content of ANFs (Yegani and Korver, 2012).

CONCLUSIONS

Although the ME system is currently used as a default method, but NE or TIDE could be used as potential energy systems in feed formulations, as they reported to be more predictive of bird performance and nutrient digestibility than the ME system. However, the suitability of TIDE for application in broiler feed formulations merits further research investment.

The application of age-appropriate AMEn data might enable the poultry industry to improve the precision of feed formulations, broiler performance, profitability and sustainability of poultry production. In commercial practice, pellets are the commonly used feed form in broiler production. Thus, the application of existing AME or AMEn data derived from mash diets to industry situation might result in over- or under-estimation of the available energy content of feed ingredients.

Presence of ANFs in feed ingredients and low secretion of endogenous enzymes can cause low nutrient digestibility and energy utilisation of feed ingredients. Supplementation of diets with feed additives such as exogenous enzymes, probiotics, prebiotics, and emulsifiers have been reported to increase nutrient digestibility and absorption, enhance intestinal health and function, and subsequently increase energy value of the feed ingredients.

REFERENCES

1. Abdollahi MR, Ravindran V, Svihus B. 2013. Animal Feed Science and Technology 186: 193-203.
2. Abdollahi MR, Wiltafsky-Martin M, Ravindran V. 2021. Animals 11(8): 2174.
3. Amerah AM, Ravindran V, Lentle RG, Thomas DG. 2007. Poultry Science 86(12): 2615-2623.
4. Aviagen. 2009. Aviagen, Scotland, UK.
5. Choct M. 2015. Animal Production Science 55(12): 1360-1366.
6. CVB. 2016.
7. Evonik Industries. 2016.
8. Feedipedia. 2017.
9. Hill FW, Anderson DL. 1958. Journal of Nutrition 64: 587-603.
10. Hubbard LLC. 2011.
11. INRA. 2002.
12. Jiménez-Moreno E, González-Alvarado JM, Lázaro R, Mateos GG. 2009. Poultry Science 88(9):1925-1933.
13. Khalil MM, Abdollahi MR, Zaefarian F, Chrystal PV, Ravindran V. 2021. Poultry Science 100(9): 101288.
14. Khalil MM, Abdollahi MR, Zaefarian F, Chrystal PV, Ravindran V. 2022a. Animals 12(2): 183.
15. Khalil MM, Abdollahi MR, Zaefarian F, Chrystal PV, Ravindran V. 2022b. Poultry Science 101(7): 101948.

16. Mateos GG, Cámara L, Fondevila G, Lázaro RP. 2019. Journal of Applied Poultry Research 28(3): 506-525.
17. Mateos, GG, Jiménez-Moreno E, Serrano MP, Lázaro RP. 2012. Journal of Applied Poultry Research 21(1): 156-174.
18. NRC. 1994. National Academies Press.
19. Rostagno HS, Albino LFT, Donzele JL, Gomes PC, Oliveira RF, Lopes DC, ... Euclides RF. 2017.
20. Svihus B. 2001. Animal Feed Science and Technology 92(1-2): 45-49.
21. Swick, RA, Wu SB, Zuo J, Rodgers N, Barekatain MR, Choct M. 2013. Animal Production Science 53(11): 1231-1237.
22. WPSA. 1989.
23. Wu SB, Choct M, Pesti G. 2020. Poultry science 99(1): 385-406.
24. Yegani M, Korver DR. 2012. Canadian Journal of Animal Science 92(3): 261-273.

Plenary Session 1:
NUTRITION FOR SUSTAINABILITY IN BROILER, LAYER AND TURKEY PRODUCTION

SUSTAINABILITY AS RELATED TO OPTIMAL MINERAL NUTRITION

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As we learn more about the negative impacts of calcium (**Ca**) on availability of phosphorus (**P**) and that of other nutrients, it highlights how little we know about Ca requirements, digestibility of Ca in ingredients, and their impacts on P and micromineral digestibility (Angel, 2019). To optimize dietary P, copper (**Cu**) and zinc (**Zn**) utilization from the diet, phytic acid or its salt (phytate) needs to be dephosphorylated, at least partially. As phytate is dephosphorylated, its chelating capacity towards mineral cations greatly decreases (Lonnerdal, 2002). To maximize dephosphorization of dietary phytic acid or its salt (phytate) and minimize potential chelation with mineral cations and other charged nutrients, dietary Ca management must be optimized. By managing Ca supply in the diet, microminerals and P are better digested and absorbed and thus excretion is decreased.

Work on understanding ingredients that supply Ca in the diet (Kim et al., 2019; David et al., 2019; Li et al., 2021) and on Ca requirements of broilers (David et al., 2021, 2022, 2023; Walk et al., 2021, 2022; Angel et al., 2022) has resulted in a clear understanding that if Ca nutrition is optimized, phosphate use can be minimized. Therefore, the standardized ileal digestible (**SID**) of Ca in ingredients (Anwar et al., 2016 a, b, c; Li et al., 2021) is needed to optimize phytase efficacy and P digestibility. In most plant-based broiler diets, limestone (**LS**) contributes between 30 and 75% of the dietary Ca. Because LS are so variable in their characteristics (Galini et al., 2022) and Ca digestibility (Anwar et al., 2016 a, c; Kim et al., 2019; Li et al., 2021), understanding LS Ca digestibility and the factors that influence it (Li et al., 2021, Angel et al., 2022) allows for optimizing the selection of the main Ca source in broiler diets and this in turn will result in improvements in SID P, due partly through better exogenous phytase efficacy (Kim et al., 2019; Li et al., 2021).

Poultry nutritionists have used total Ca to a form of "available" (a) P ratio in the diet with measures of "availability" of P in ingredients being (available, digestible and retainable). However, we continue to use total Ca to formulate poultry diets, define requirements and give matrix values to phytase. Phytase, does not generate additional total Ca to the diet, rather, it catalyzes the stepwise removal of phosphate from phytic acid or its salts (Wyss et al., 1999), releases phosphates and phytate bound Ca and/or microminerals, making the total Ca, Zn, and Cu more "available". To date, the limitations for using a digestible Ca (dCa) system have been: 1) lack of comprehensive ingredient SID Ca database; 2) low robustness in the dataset (SID Ca available in most cases based on 1 value for an ingredient); and more importantly 3) the immense variability in SID Ca from LS (Anwar et al., 2016 a, c; Kim et al., 2018; Angel 2019; Li et al., 2021). The impact of adding LS with different geologies and dynamic (over several time points) solubilities on apparent P digestibility of the diet the LS is added to, was showcased by Kim et al. (2018). These authors showed that changing only the LS added to the same basal broiler diet could change apparent P digestibility from 23.1 to 65.5% in the absence of phytase and from 45.0 to 90.7% when 1000 U of a phytase was added per kg of diet. Understanding the impact of LS source on P digestibility of the diet is the key to be able to formulate to dP requirements of broilers and minimizing P excretions.

In recent years, research has shown that Ca digestibility in broilers varies dramatically depending on the Ca source, solubility and source (geology) of LS, as well as dietary phytate and phytase concentration use in the diet (Anwar et al., 2016c; Angel, 2019; Kim et al., 2019; Li et al., 2021). With the knowledge that Ca digestibility which can be altered by the aforementioned dietary factors can affect P digestibility, the specification of Ca requirements for broilers as total Ca in the diet becomes obsolete, as does the adherence to a fixed ratio of total Ca to dP. Thus, the importance of understanding LS Ca digestibility, and LS Ca interactions with phytate becomes essential if a dCa system is to work.

When comparing LS from the same source, a reduction in particle size from 0.8 mm geometric mean diameter (**GMD**) to 0.15 mm GMD reduced SID Ca of the LS from 49.2% to 38.1% (Li et al., 2021). Importantly, Kim et al. (2019), reported that differences in GMD alone could only explain <40% of the observed differences in Ca digestibility from LS; and differences in LS geology, solubility and physical/chemical characteristics were equally as important in their potential effects on Ca digestibility. Kim et al. (2019) showed that predictions of Ca digestibility could have an r^2 of 0.98. But it is important to note that this was a preliminary study involving only 4 LS and that true validation of the equation with sample data not used to develop the equation was not possible at the time. The authors (Kim et al., 2019) state "the prediction equations should be interpreted with care especially in cases beyond the scope of current study". Additional work has been carried out that now involved more than 51 limestone samples. Data from these studies (7 studies) have been used to develop prediction equations for SID Ca in limestone and SID P in diets the limestones are added to, when diets contain "high" levels of phytase (Angel et al., unpublished). The prediction equations have been used to predict SID Ca and P in data set points not used to develop the prediction equations and compared with *in vivo* results, and errors associated with the predictions are + or - 4% of actual values. These prediction equations now allow us to predict SID Ca in limestones based on laboratory analyzable factors (LS solubility at 5, 15 and 30 min; GMD, Ca and Mg as well as diet phytate concentration). They also allow us to predict the potential impact the LS on the SID P of the diet in the presence or absence of a" high" dose of phytase and thus have a variable phytase matrix that reflects the potential impact of LS characteristics on phytase efficacy (Angel et al., unpublished).

Requirements based on dCa and dP are now available. Although not robust yet, dCa for several ingredients are now available. A dCa system will allow for supplying sufficient quantities of Ca at tissue level to optimize growth and physiological processes. This in turn will result in lower LS in the diet, better dephosphorylation of phytate, less chelation's with mineral cations and thus better digestibility of Ca, P, Zn, Cu among others. Ultimately resulting in a lower excretion of P, Cu and Zn can be expected.

REFERENCES

1. ANGEL, R. (2019) Proc Australian Poult Sci Symp, Sydney, pp 51-56.
2. ANGEL, R., LI, W., Li, and PLUMSTEAD, P.W. (2022) Proc WPC, Paris, pp 324 - 334.
3. ANWAR, N.M., RAVINDRAN, V., MOREL, P., MOREL, C., RAVINDRAN, G. and COWIESON, A.J. (2016a) Anim Feed Sic and Tech 213:142-147.
4. ANWAR, N.M., RAVINDRAN, V., MOREL, P., MOREL, C., RAVINDRAN, G. and COWIESON, A.J. (2016b) Poult Sci 95:70-76.
5. ANWAR, N.M., RAVINDRAN, V., MOREL, P., MOREL, C., RAVINDRAN, G. and COWIESON, A.J. (2016c) Brit Poult Sci 57:707-713.
6. DAVID, L.S., ABDOLLAHI, R., RAVINDRAN, G., WALK, C.L. and RAVINDRAN, V. (2019) Poult Sci 98: 5582-5589.
7. DAVID, L.S., ABDOLLAHI, R., BEDFORD, M.R. and RAVINDRAN, V. (2021) Poult Sci 100:1-19.
8. DAVID, L.S., ABDOLLAHI, R., BEDFORD, M.R. and RAVINDRAN, V. (2022) Poult Sci 101:102135.
9. DAVID, L.S., ABDOLLAHI, R., BEDFORD, M.R. and RAVINDRAN, V. (2023) Poult Sci 102:102492.
10. KIM, S.W., Li, W., ANGEL, R. and PLUMSTEAD, P.W. (2019) Poult Sci 98:6837-6848.

11. LI, W., ANGEL, R., PLUMSTEAD, P.W. and ENTING H. (2021) Poult Sci 100:900-909
12. GALINI, S., MEREU, A., LI, W., PLUMSTEAD, P.W., ANGEL, R., WILKS, G., and DERSJANT-LI, Y. (2022) J Appl Anim Nut 10:19-30.
13. LONNERDAL, B. (2002) Inter J Food Sci and Tech 37:749–758
14. WALK, C.L., WANG, Z., WANG, S., WU, J., SORBARA, J O.B. and ZHANG, J. (2021) Poult Sci 100:101364.
15. WALK, C.L., WANG, Z., WANG, S., WU, J., SORBARA, J.O.B. and ZHANG, J. (2022) Poult Sci 101:1011836.
16. WYSS, M., BRUGGER, R., KRONENBERGER, A., REMY, R., FIMBEL, R., OESTERHELT, G., LEHMANN, M., and VAN LOON, A. P. G. M. (1999) Appl Environ Micro 65:367–373.

Plenary session 2:
NUTRITION INTERVENTION FOR CLIMATE CHANGES

POULTRY PRODUCTION IMPACTS: ENLARGING THE PICTURE

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INTRODUCTION

Minimizing the environmental impact of poultry industry is gaining higher interest due to raising world population and meat demand. The ONE Health approach to animal production (Alders and Tomley, 2022) and the United Nations 17 Sustainable Development Goals (UN, 2015) call for a holistic approach that look at all aspects of sustainability.

Usually, sustainability is depicted as the junction point of three partially intersecting circles: environment, economy, and society (Barbier, 1987)

; however, this representation does not explain any of the cause-effects linkages among the three components. Therefore, we have developed a more logical/consequential sustainability representation based on an Input-State-Output (I-S-O) framework. It sets straight the economy's dependence on societal organization, environmental resources, and ecosystem services (Pulselli et al. 2015) by showing the cause-effect relations, interactions, and feedback among the environment, society and the economy (represented in Figure 1 by arrows going backwards).

The environment is society's input; as for all living systems, consumption derives from the environment and should be under its limits. Society, which is the *state* of our framework, prospers if it succeeds in using in the best possible way the inputs of various kinds that derive from the environment; it organises and generates an *output, the economy,* that produces and distributes the goods and services needed by the society itself.

Qualitatively, feedback between the environment, society, and the economy can be positive (thus contributing to the whole system's health and development) or negative (thus destabilising the system and potentially disrupting its development).

From a quantitative point of view, sustainability is challenging to measure per se: it is an ideal state and propriety of the overall system. What can be measured is the distance from the ideal point of sustainability, i.e. unsustainability, and it can be quantified through systems indicators. A good starting point to try to overcome the sustainability dilemma and have quantitative data is offered by H. Daly's principles of sustainability: 1) resources should be used at a rate that allows their re-formation (sustainable yield); 2) wastes should be produced at a rate which allows the environment to absorb them. It is possible to quantify what is used and/or what is released too fast and too much into the environment (Daly, 1990). These necessary conditions show that unsustainability is related to the total amount of available resources and waste produced (and the speed of their formation/ production). Therefore, any assessment of environmental sustainability must include (at least) two aspects: one related to the use of natural resources (e.g. the renewability of a product extended to the whole value chain), the other to the feedback that the production system drives back to the environment, (e.g. their impacts).

The I-S-O framework can depict the area of interest of different methodologies/indicators, highlight their investigation potentialities and help define what kind of information each of them can provide. Furthermore, using this framework, we can establish overlapping and complementarities among indicators useful to stimulate and optimize efforts in identifying and collecting statistical information and data for policies.

LIFE CYCLE ASSESSMENT

Life Cycle Assessment (LCA) is an environmental systems analysis tool which help provide relevant information on the environmental consequences of different production systems. ISO 14040:2006 and ISO 14044:2006 are the standards that describe the principles and framework for Life Cycle Assessment implementation.

LCA enables the evaluation of products' environmental burdens through their entire life cycle, from cradle to grave: starting from the resources extraction, going through the production of material, product parts and the product itself, until the use phase and end-of-life management (ISO, 2006).

As depicted in Figure 1, LCA interest is in the "technosphere": starts with human intervention and measures its effects (feedbacks) on the environment. It does not account for the resource itself, its availability or renewability. It concentrates on the different impacts a production process will likely have locally and globally. LCA is a perfect candidate for measuring the second of Herman Daly's principles.

EMERGY EVALUATION

Emergy is defined as the solar energy, directly and indirectly, required to produce a product or a service. The unit of solar emergy is the solar emergy Joule (seJ) (Odum , 1996).Emergy evaluation assigns a value to products and services by converting them into solar energy equivalents. This common denominator enables different resources, whether energy or matter, to be measured and compared. The emergy of different products is assessed by multiplying the mass or energy of inputs by a transformation coefficient called *Unit Emergy Value* (UEV). UEV is the solar emergy required, directly or indirectly, to make 1 unit (e.g. J or g) of a product. By definition, the solar emergy *Em* of a product or process is:

$$Em = \sum_{i=1}^{n} E_i \, UEV_i \qquad (1)$$

where E_i is the energy (or mass if UEV_i is in sej/g) content of the *i*th independent input flow to the process, and UEV_i is the unit emergy value of the *i*th input flow.

Emergy evaluation helps quantify Herman Daly's first necessary condition for sustainable development. It can be used to define guidelines for the consumption of resources compatible with their formation times, since it deals with both biosphere and technosphere (Figure 1).

Emergy can be regarded as the solar energy necessary to sustain a system; the greater the total emergy flow required for obtaining a product, the greater the consumption of solar energy necessary for its re-formation once it has been used, and thus the greater its past and present environmental cost.

According to Odum (1996) and based on their provenance and regeneration times, flows of resources can be: a) emergy flows related to local renewable resources (R); b) emergy flows related to local non-renewable resources (N); c) emergy flows related to goods and services purchased outside the study system (F). Besides UEV, other emergy-related indicators may be used to characterize emergy results based on these different emergy flows. To this aim, common indicators considered in the assessment of agricultural and agricultural residues productions are the percentage of renewable inputs (%R), *Emergy Flow Density* (ED) and *Emergy Yield Ratio* (EYR) (Ulgiati et al., 1995). The first indicator highlights the contribution of renewable resources in producing the output considered with respect to the whole emergy supporting flow and is calculated as %R= R/Em. ED accounts for the emergy flow during a specific time interval (usually a year) divided by the area of the production system, thus giving a measure of the concentration of resource use in that area. Finally, the EYR is calculated as Em/F and expresses a process's ability to use local renewable and non-renewable resources by investing a unit of outside resources.

Emergy is a most valuable addition to LCA, that provides a complementary donor-side perspective, a unified measure of the provision of environmental support, and an indication of the work of the environment that would be needed to replace what is consumed (Raugei et al., 2014).

Differences between emergy evaluation and LCA inventories

Setting the boundaries for LCA is regulated by ISO 14040 standards. The "cradle" is typically the

human investment, in terms of energy and material, needed to collect raw materials. The "grave" is typically where the production process assessed ends. In the case of poultry, the analysis often ends at the gate of the farm or the slaughterhouse.
In emergy evaluation, the grave usually is the same as the LCA. Conversely, the cradle is much farther: the solar energy received by all the systems directly and indirectly involved in the production. All the natural processes of agriculture are considered, and inputs such as rain, heat from the ground, water, and soil are included in the inventory per se. Also, human labour is an input of emergy evaluation since many inputs of direct and (especially) indirect solar energy are required to maintain a human life: human work can be substituted by machinery and energy, included in the evaluation. All the inputs carry a certain level of renewability: agricultural crops, since they require renewable inputs (e.g. sunlight and rain) and non-renewable ones (e.g. fossil fuels for the tractors or chemical fertilizers), will have a certain level of renewability resulting from the amount of emergy required from the different inputs.

POULTRY AND ENVIRONMENTAL SUSTAINABILITY ASSESSMENT

LCA applied to poultry shows very well its potentiality: apart from direct ones, chickens' impacts are mostly connected to feed production. Typically impacts on climate (GWP) shows that fodder production is the highest contributors for broilers poultry. Manure is usually the most important item for Acidification Potential and Eutrophication Potential. All these factors can be improved by the adoption of proper diets (Kebreab et al., 2016).
Emergy evaluation has been applied to poultry showing that the efficiency of transforming solar energy from the photosynthesis of the fodder until the final output (inversely proportional to UEVs) is very similar in conventional and organic chicken or in favor of the conventional, depending on the location and technological level of the rearing system. Instead the level of renewability of the whole value chain is quite different: around 15% for conventional and between 38% and 50% for organic.
These results poses a fundamental question also on the reliability of the "functional unit", usually a mass unit of chicken meat. When dividing all the inputs or impacts by the mass of chicken meat we assume that all the chicken meat is equal, implying an equality also of nutrient factors for humans. For example there is evidence that meat from pastured-based poultry may contain additional nutritional benefits such as lower fat content and higher vitamin and mineral contents (Sossidou et al., 2015). Also animal welfare can be very different according to the rearing system adopted.
It can be observed that in assessing agriculture productions, due to its user side perspective, LCA boundaries can be considered from "cradle to gate" where the cradle is the point of human intervention. Neglecting, in this way, the enabling role of the overall context and prerequisite for the production, namely the work of the biosphere. LCA cannot account for the "work" done by ecosystems in providing renewable resources needed for the agriculture value chain (such as sun, rain, wind, and earth heat). Renewable resources sustaining the agricultural production do not produce impact, it is why LCA do not assess their contribution. It can be maintained that LCA treats and assess all the production chains as if they were industrial processes neglecting that agriculture can take place thanks to the fundamental role of ecosystems. In addition, the large majority of LCA takes as a functional unit, the product itself, thus providing results in terms of potential impact based on the productivity for both agriculture in strict sense products (crops, fruit, vegetables) and the livestock (poultry, cattle). This approach leads to results (in the large majority of studies) for the product obtained from conventional practices with lower environmental burdens than the organic ones. In fact, by considering productivity as the guiding principle of production all the remaining aspects (such as quality) cannot be considered. Conventional production chains, in general, produces per unit of area, a larger amount of product and having a high denominator (production) leads to reduce the relevance of the numerator (total emissions).
Moreover, poultry production, like most agricultural and rearing productions, has the main product (chicken meat) and co-products (chicken manure) to be carefully considered during the assessment. The majority of LCA evaluation uses allocation methods to break down input flows among products and co-products, even though the ISO discourages this practice. This fact introduces arbitrariness since the results of assessments can be very different according to the type of allocation adopted (Mondello et al., 2018).
Emergy evaluation, thanks to its donor side perspective, can account for both the biosphere and technosphere contribution for the same product and production chain; On the other side it does not account for the impacts of production systems. Thus, emergy evaluation can be considered as the ideal complement of LCA as sustainability indicator for any nature based production chain, e.g. agriculture, rearing, forestry and all the subsequent production systems. The unique ability of emergy evaluation in considering the contribution of renewable resources gives an insight into the quality of a product and, in particular, of agricultural/livestock products. If an agricultural product results in a high value of renewability it means that the amount of flow that sustains its chain from outside the system (F) is low, and the human contribution is lower compared to the biosphere ones. In addition, emergy evaluation can highlight the role of the context (territorial system) and benefit of the agricultural production by providing the amount of past and present solar energy per unit of area.

CONCLUDING REMARKS

In synthesis, it can be maintained that due to its systems perspective, emergy evaluation assesses the ultimate energy required for the production of agricultural productions enabling a proper and consistent assessment of the biosphere's "work", especially relevant when assessing the sustainability of agriculture/livestock production. On the other side, LCA has the ability to account for drawbacks to the biosphere due to a production chain whatever the nature of the production chain.
Nevertheless, as remarked by Bastianoni et al., (2008), the complexity and multiple nature of a system can be properly assessed and considered only by jointly using indicators that have complementary features. The joint use of emergy evaluation and LCA has been already proven to be a winning solution because the combined reading of their results provides a comprehensive assessment of environmental sustainability of the system under study.

REFERENCES

1. Alders R., F. Tomley, 2022. Animal Board Invited Opinion Paper: Planet, people and poultry - more and better data needed to get the balance right. Animal, 16, 100560.
2. Barbier E., 1987. The concept of sustainable economic development. Environ Conserv 14:101-110.
3. Bastianoni, S., Pulselli, F. M., Focardi, S., Tiezzi, E. B., Gramatica, P., 2008. Correlations and complementarities in data and methods through Principal Components Analysis (PCA) applied to the results of the SPIn-Eco Project. Journal of environmental management, 86(2), 419-426.
4. Daly HE, 1990. Toward some operational principles of sustainable development, Ecol Econ 2: 1-6.
5. ISO 14040:2006 Environmental management - Life cycle assessment - Principles and framework
6. ISO 14044:2006 Environmental management - Life cycle assessment - Requirements and guidelines
7. E. Kebreab, A. Liedke, D. Caro, S. Deimling,M . Binder, M. Finkbeiner, 2016. Environmental impact of using specialty feed ingredients in swine and poultry production: A life cycle assessment. *Journal of Animal Science*, Volume 94, Issue 6, 2664–2681.
8. Mondello G., R. Salomone, E. Neri, N. Patrizi, S. Bastianoni, F. Lanuzza, 2018. Environmental hot-spots and improvement scenarios for Tuscan "Pecorino" cheese using Life Cycle Assessment. Journal of Cleaner Production 195 810-820.
9. Odum, H.T., 1996. Environmental accounting. Emergy and Environmental Decision Making. *John Wiley & Sons, INC*, p.370.

10. Pulselli F. M., L. Coscieme, L. Neri, A. Regoli, P. C. Sutton, A. Lemmi, S. Bastianoni, 2015, The World Economy in a cube: A more rational structural representation of Sustainability, *Glob Environ Chan* 35: 41–51.
11. Raugei, M., Rugani, B., Benetto, E., Ingwersen, W.W., 2014. Integrating emergy into LCA: potential added value and lingering obstacles. Ecological Modelling, 271, 4-9.
12. Sossidou, E.N., Castellini, C., Dal Bosco, A. and Grashorn M.A., 2015, Effects of pasture management on poultry welfare and meat quality in organic poultry production systems. World's Poultry Science Journal, 71, 375-384.
13. United Nations, 2015. Transforming our World: The 2030 Agenda for Sustainable Development
14. Ulgiati S., MT Brown, S Bastianoni, N Marchettini, 1995. Emergy-based indices and ratios to evaluate the sustainable use of resources. Ecological Engineering, 5, 519-531.

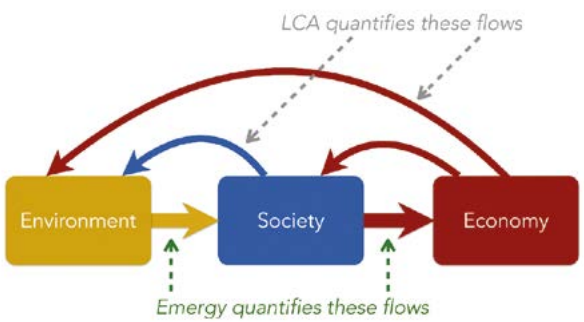

Figure 1. A consequential/logic view of sustainability. Dotted arrows show the area of interest of LCA and emergy evaluation.

Plenary session 2:
NUTRITION INTERVENTION FOR CLIMATE CHANGES

OBSERVATIONS ON WATER QUALITY AND QUANTITY FOR POULTRY

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Water is the single greatest nutrient input in poultry production. Yet due to its relatively inexpensive cost compared to protein or energy requirements, water quality has received little research attention since the introduction of nipple drinkers in the early nineties. Improved efficiencies in genetics, diets and environment plus the dominance of antibiotic free production models are all factors contributing to the need for poultry operations to implement water programs which monitor and control quality and quantity. Identify vulnerable points in water systems such as filters, storage, injection points and develop an action plan tailored to the operation which assures quality and quantity parameters remain acceptable for supporting cost efficient performance. Water management programs should include consistent testing from source to last drinker in the production system to assure water quality starts acceptable and remains acceptable. A thorough understanding of each operations water supplies, both quality parameters and water management tools like distribution pipes, storage tanks and drinker function, are the first steps in assuring water meets acceptable standards. Most water systems require some form of corrective action such as filtration, water softeners, system cleaning or consistent use of sanitizers to prevent factors that are detrimental to quality and quantity. Verification and documentation complete a successful water program. The biggest hurdle for operations with multiple production sites is to not become focused on a “one size fits all solution” but rather accept that all water supplies and systems are unique and efforts should be directed towards identifying and implementing programs that best fit the budget and situation. While solutions, particularly for mineral control or sanitation can be expensive, the costs are more often than not, are recouped by improvements in performance and bird health.

Over the years, the Center of Excellence for Poultry Science CORE Analytical Water Lab has analyzed thousands of poultry drinking water samples for mineral, pH and bacteria. Sampling areas have included sources, storage tanks, drinkers, and pre and post treatments. Regarding bird performance and these analyses, the following observations have been noted. Overall, birds are tolerant of average levels of most contaminants, well above the accepted standards. Exceptions to this include sodium and chloride levels which if over 200 ppm or mg/l (each or either) will cause loose droppings and levels greater than 400 ppm will impair weight gains, feed efficiencies and egg production. Sodium and chloride in the water can be compensated for by adjusting dietary sodium and chloride levels. Some breeds are more sensitive than others so work with your genetic company to determine appropriate dietary adjustments. Excessively high levels of salt in the water (>800 ppm) requires corrective action such as reverse osmosis or diluting the water supply with another source. The birds are fairly tolerant of iron, manganese or sulfate in the water but these are a concern due to the pathogenic bacteria which thrive when these nutrients are present in the water supply and allowed to build up in the distribution pipes, storage tanks or filters. The presence of these minerals increases the risk of *pseudomonas, Salmonella, E. Coli* and other pathogens. Along with these mineral contaminants, calcium and magnesium are a concern because of their tendency to create scale or mineral deposits in pipes causing volume restrictions. Scale issues can occur in any number of areas in water systems leading to a pinch point that causes inadequate water volume for the birds. Susceptible scale buildup points are regulators, bends in pipes, pressure reducers, cool cell systems or distribution pipes. Iron, manganese and sulfates can be managed by oxidizing with an oxidizer such as chlorine or chlorine dioxide at the appropriate pH (>7), allowing retention time and then filtering. Hydrogen peroxide is often used as the oxidizer for sulfates because of the higher level required (2.7 to 1). to maximize the oxidation process for this mineral. Hydrogen peroxide does not create bitterness issues that are common with elevated uses of chlorine sources. Other common water issues include acid water. This water has virtually no dissolved minerals and a low pH, usually below 4. Water needs buffering capacity so this can be corrected by adding sodium bicarbonate or soda ash to the water to "neutralize it". A last common challenge is failure to understand the natural buffering capacity of water in the form of how much alkalinity is present and attempting to lower the pH with organic acids that are not designed to decrease pH in the presence of elevated alkalinity. Organic acids are wonderful gut health and antimicrobial tools but they are weak acids which cannot lower the pH of water that has significant alkalinity. Adding more and more organic acid in the attempt to achieve a reduced pH when water has more than 150-200 ppm of alkalinity can lead to wasted product and water that may have an unacceptable taste for the birds.

The best advice about microbial contamination is NEVER assume the quality is acceptable. Verify microbial quality with consistent testing and monitoring. Regarding bacteria, poultry water supplies should have no coliforms or E. coli present and maintaining optimal microbial quality can become more difficult when there are more than 10,000 cfu/ml aerobic plate count bacteria (APC) present. This level of APC alone does not mean that water quality is bad, it's just an indicator that conditions are favorable for exponential increases in the bacteria present, including pathogens like *Bordetalla*, *Staphylococcus*, or P*seudomonas.* Unlike many human water supplies, poultry drinking water systems are more vulnerable to bacterial contamination rapidly increasing (1 E. coli can become trillions in 24 hours at 90 F) due to the presence of the previously mentioned minerals but also because of the slow flows, stagnant flow periods and pinch points that can harbor biofilm.

Our greatest challenge as an industry is critically assessing how or when we add products to water supplies. We must do a better job of understanding the implications of vaccination programs as well as the use of organic acids, probiotics and nutraceuticals. What monitoring tools do we implement to assure these products are not sacrificing water quality leading to microbial blooms that create gut integrity or respiratory issues for vulnerable flocks? All of these factors interact with the naturally occurring water contaminants to create unique conditions that should be monitored for each operation.

Implementing consistent water sanitation has been one of the greatest accomplishments for water management in the industry in the last twenty years. Thorough cleaning of water systems when no birds are present is the critical step in assuring daily water sanitation programs are successful. This requires utilizing products at an appropriate concentration left adequate time to maximize the removal of the biofilm present without damaging the equipment. Inadequate concentration or cleaning time are both failures to the process. To determine what works best, swab water lines pre and post line cleaning (swab different lines) to quantify the presence of biofilm.

In the presence of birds, chlorination is still an excellent tool for controlling microbial contamination in drinking water but it has its limitations. It works best as hypochlorus acid and this form of chlorine is dominant when the water pH is 5-7. If water pH needs to be adjusted, separate acid injector from chlorine injector and assure the first product is thoroughly blended into the water prior to the second injection site. Otherwise, the acid may cause "gassing off" of the chlorine. Chlorine works best when the water is free of turbidity (suspended minerals), the water system is free of biofilm and the chlorine product is fresh and has been properly handled to minimize deterioration of chlorine residual. Adding more chlorine product to achieve a desired residual may result in bitter water that causes birds to drink less so be mindful of how much product is being added into the water, particularly the dry calcium hypochlorite sources that are often allowed

to slowly dissolve into the water stream. Chlorine dioxide works well at a higher pH (8 pH) but chlorine dioxide's sanitizing effect tends to be optimal when it is made onsite by using systems which force blend a clean (contaminant free) inorganic acid with sodium chlorite. This process maximizes the conversion of sodium chlorite to chlorine dioxide (60-80% conversion). Hydrogen peroxide and peracetic acid (PAA) have gained popularity as a daily water sanitizer, particularly the stabilized products which maintain an effective sanitizing residual in the water for extended periods of time, such as days. This is helpful for young flocks who do not move much water through the system. Regardless of what daily water sanitation program is implemented, take time to correlate a sanitizer residual with a confirmed level of microbes present in the water. Two ppm of chlorine means nothing unless there is some confirmation that 2 ppm of chlorine residual in the water is correlated with no bacteria present in the water sample. Then and only then, is the water sanitation monitoring program a success.

A discussion on water systems is not complete without a focus on monitoring water quantity. So many factors can impact how much available water is present for birds and there is no question, if birds do not have enough water, performance will suffer. Monitor throughout the system to assure adequate gallons or liters per minute are available. Confirm that the drinkers are delivering the appropriate amounts throughout the life of the flock. Use drinker manufacturers' guidelines for determining what should be available at each age or stage of production. Since we know one advantage to years of genetic selection is meat birds consume more feed and water faster, confirm that drinker flows are not too restrictive for young birds. Monitor filters to assure mineral buildup does not cause unnecessary reductions in flow. This problem is very common and easily overlooked. If barn filters must be frequently changed each flock, consider investing in filtration technology that can better handle the amount of contaminant and volume of water. Unless farm staff are very diligent in changing dirty filters, filtration upgrades almost always pay for themselves the first year due to better weight gains, feed conversions or egg numbers. Confirm that injection technology is appropriately matched to peak water demand. An undersized injector is an instant restriction point and it doesn't take flocks long to learn they have limited access to water so birds limit feed intake. Implement descaling procedures for water supplies with more than 80-100 ppm of calcium, magnesium, iron, sulfates and/or manganese and natural pH over 7.5. It is very likely this type of water has sediment scale buildup throughout the water distribution system which limits water supply. One last point, assure water temperature is never allowed to become warm. Even new chicks prefer cooler water over water that is similar to their body temperature. Water lines located under heaters may need flushing on a regular basis during the first few days to keep water temperature more palatable.

In conclusion, water is an essential nutrient that cannot be taken for granted when addressing quality and quantity. A consistent and thorough evaluation program is critical for assuring water supports optimal flock performance.

Plenary session 2:
NUTRITION INTERVENTION FOR CLIMATE CHANGES

FEEDING AND NUTRITIONAL STRATEGIES FOR COMMERCIAL POULTRY DEALING WITH CLIMATE CHANGE

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EFFECTS OF CLIMATE CHANGES ON POULTRY HEALTH AND PRODUCTION

The earth's average temperature has risen 1.1 to 1.6°F over the past century and is projected to rise another 0.5 to 8.6°F over the next hundred years (Lenssen et al., 2019). Climate change is a global issue affecting livestock production, including poultry. Climate change directly affects poultry production through heat stress and indirectly by promoting an environment for diseases. Among the livestock species, poultry is more prone to heat stress because of higher body temperature, the presence of feathers, and the absence of sweat glands. Heat stress is one of the major environmental stressors in the poultry industry, resulting in a substantial economic loss by altering the poultry's health and production. Due to a higher metabolic rate, they produce more body heat and cellular free radicals and are prone to heat stress. Heat stress significantly decreases feed intake and body weight and increases the feed conversion ratio (FCR) in broilers (Wasti et al., 2021). Heat stress negatively affects feed intake, body weight, and egg production in laying hens (Wasti et al., 2020). Hens under heat stress had higher cortisol levels, reduced estradiol and progesterone levels, and induced ovarian follicular atresia, reducing ovulatory follicles and egg production (Li et al., 2020). Hens under heat stress also displayed panting to reduce body temperature resulting in respiratory alkalosis. The elevated blood pH causes poor bioavailability of calcium for eggshell biomineralization, resulting in poor eggshell quality. Heat stress negatively affects gut health, including altering the morphometric of the gastrointestinal tract, cecal volatile fatty acid production, and expression of heat sock, antioxidant, immune, and tight junction-related genes (Wasti et al., 2021). Exposure to a high temperature negatively impacts the poultry's intestinal microbial ecology, specifically by decreasing the species' abundance (Rostagno, 2020; Wasti et al., 2021), affecting poultry performance.

STRATEGIES TO MITIGATE THE ADVERSE EFFECTS OF HEAT STRESS

Climate change due to global warming is a threat to the poultry industry. Because of heat stress, the poultry industry faces increased production costs, severely altering the meat and egg quality. Many studies have revealed that high environmental temperature causes oxidative stress by producing excessive reactive oxygen species (ROS) and decreasing cellular antioxidants (Mishra and Jha, 2019). Previous studies in poultry have shown that heat stress is associated with cellular oxidative stress. The oxidative stress in poultry is associated with severe health disorders, poor growth performances, and economic losses. As oxidative stress is downstream of heat stress on poultry health and production, several feeding and nutritional strategies have been implemented to mitigate heat stress-induced oxidative stress in poultry.

Feeding strategies for mitigating heat stress

Feeding strategies like restricted feeding, wet or dual feeding, and adding fat to diets have been widely studied and found to reduce the deleterious effects of heat stress (Wasti et al., 2020). Restricted feeding during the hotter period of the day reduces the metabolic rate of birds. Feed restriction reduces rectal temperature, minimizes mortality, and decreases abdominal fat in heat-stressed broilers and layers. As this approach results in a reduced growth rate and delayed marketing age of the birds, it is not widely practiced in the poultry industry. Feed restriction results in overcrowding and rush at a re-feeding time, resulting in additional mortality. Thus, the dual feeding regime ensures that birds can feed throughout the day. The thermic effects of proteins are higher than carbohydrates and produce higher metabolic heat. Therefore, the protein-rich diet is provided during the cooler period, and the energy-rich diet during the warmer period to reduce body temperature and mortality in the heat-stressed broilers.

Due to heat stress, birds lose water through respiration, and water intake is markedly increased to restore thermoregulatory balance (Wasti et al., 2020). Wet feed also reduces viscosity in the gut resulting in the faster passage of the feed. Wet feeding promotes digestion, improves the absorption of nutrients from the gut, and accelerates the action of the digestive enzyme on the feed. In broilers, wet feeding enhanced the feed intake, body weight, and weight of the gastrointestinal tract (GIT). In laying hens, wet feeding increases dry matter intake, egg weight, and egg production (Li et al., 2020). Although this approach is beneficial in managing heat stress, it is less common among poultry producers due to the risk of fungal growth in the wet feed.

Higher energy diets (fats) produce lower heat increment during metabolism than protein and carbohydrates. Fat supplementation in the poultry diet increases nutrient utilization in the GIT and also helps to increase the energy value of the other feed constituents and increase feed intake in heat-stressed broilers and layers. Essential oil supplementation in the diet relieved the negative effects of chronic heat stress on broiler and layer's performance.

Nutritional strategies in mitigating heat stress

Supplementation of Electrolytes and Osmolytes

Panting in heat-stressed birds alters the blood plasma's acid-base balance, ultimately leading to respiratory alkalosis. In heat-stressed birds, sodium and potassium supplementation is favored to increase the blood pH and HCO_3^-. Supplementation of $NaHCO_3$ improved eggshell quality in layers and growth performances in broilers during heat stress. Besides including these salts in the diet, supplementation of 0.2% NH_4Cl or 0.15% KCl, 0.6% KCl, 0.2% $NaHCO_3$, and carbonated water in drinking water also improved the performance of the heat-stressed broilers.

Osmolytes such as Betaine and Taurine are widely used in the poultry diet to alleviate the heat stress effects. Betaine plays a vital role in regulating the cellular osmotic environment, preventing dehydration by increasing the water-holding capacity of the cell. During heat stress, betaine supplementation improved poultry's feed intake, carcass trait, and egg production parameters. In laying hens, supplementing betaine and vitamin C improved laying performance during chronic heat stress. In roosters, betaine supplementation improved sperm concentration and livability, seminal plasma total antioxidant capacity, fertility, and welfare under heat stress.

Taurine plays a role in antioxidant action, bile acid conjugation, maintenance of calcium homeostasis, osmoregulation, and membrane stabilization. Supplementation of taurine in the drinking water demonstrated significant improvement in the final body weight of chronic heat-stressed broilers. Supplementation of taurine in broilers under heat stress improved jejunal morphology, decreased serum ghrelin concentrations, increased somatostatin, and peptide concentrations YY in the duodenum, and increased the expression of appetite-related genes. In addition, taurine supplementation in the laying hen enhanced oviductal health.

Supplementation of Vitamins and Minerals

Vitamins are essential to the poultry diet due to their health benefits, including growth performance, enhancing immunity, and alleviating oxidative stress. During heat stress, birds cannot synthesize enough vitamins. Therefore, supplementation of specific vitamins (vitamin -E, -A, and -C) as an antioxidant in poultry diet is beneficial in mitigating heat stress effects on broilers' and layers' health and performances. Vitamin A is the most effective antioxidant at low oxygen tensions, which is found to reduce singlet oxygen, neutralize thiyl radicals, and combine with and stabilize peroxyl radicals. Vitamin E is a fat-soluble vitamin with antioxidant activity and helps scavenge free radicals produced inside the cell. Vitamin E regulates inflammatory signaling, and the production of prostaglandins, cytokines, and leukotrienes and improves the phagocytic activity of macrophages in broiler chickens. Vitamin C protects against oxidative stress by scavenging ROS and protecting cells from oxidative damage. Vitamin C also improves immunity by enhancing the differentiation and proliferation of T and B cells. One of the major consequences of heat stress is an increase in mineral excretion and a lowering of these key nutrient levels in the circulating bloodstream and liver. Supplementation of Zinc, Chromium, and Selenium in poultry diet is highly beneficial in maintaining the redox system, birds' performance, and product quality.

Supplementation of Phytochemicals

Phytogenic feed additives have been reported to alleviate the negative consequences of heat stress. Phytochemicals, such as Lycopene, Resveratrol, Epigallocatechin gallate (EGCG), Curcumin, dried plum (DP), and microalgae, have been studied in poultry under heat stress (Wasti et al., 2020; Mullenix et al., 2021). Supplementation of lycopene in heat-stressed broilers improved the cumulative feed intake, body weight, and FCR. Lycopene improved the level of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in broilers. In laying hens, dietary lycopene supplementation improved oxidative status, enhanced egg vitamin levels, and improved the egg's oxidative stability and yolk color.

Supplementation of resveratrol improved the average daily gain, decreased the rectal temperature, and lowered the level of corticosterone, adrenocorticotropin hormone, cholesterol, and MDA in yellow-feather broilers under heat stress. Resveratrol also improved gut health parameters such as microbial profile, villus-crypt structure, and expression of the tight junction and adherence junction-related genes in the heat-stressed broilers. In laying hens, resveratrol supplementation improved egg production, reduced the total serum cholesterol and triglycerides, reduced egg cholesterol content, improved antioxidant activity, and improved egg sensory scores. The EGCG is the polyphenol in green tea extract with high antioxidant and anti-inflammatory properties. EGCG supplementation increased body weight, feed intake, serum total protein level, glucose, and alkaline phosphatase activity in heat-stressed birds. Also, dietary inclusion of EGCG improved body weight and antioxidant enzymes (GSH-Px, SOD, and catalase) in the liver and serum of heat-stressed broiler birds.

Curcumin is the primary polyphenol extracted from turmeric and possesses antioxidant and anti-inflammatory properties. Curcumin supplementation improves the growth performance of heat-stressed broiler chickens. Curcumins fortification reduced the mitochondrial MDA level; reduced the ROS production by increasing the activity of Mn-SOD, GSH-Px, Glutathione S-transferase and increased gene expression of thioredoxin-2 and peroxiredoxin-3 during heat stress in broilers. In laying hens, dietary curcumin supplementation improved the laying performance, egg quality, antioxidant enzyme activity, and immune function during heat stress.

Dried plum contains a higher amount of antioxidants (fat-soluble carotenoids, alpha-tocopherol, etc.), polyphenolic compounds (chlorogenic acids, proanthocyanidins, etc.), sorbitol, and fibers. DP is a good source of several vitamins (vitamins A, C, K1, B1, B2, and niacin) and minerals (Ca, K, Mg, Se, and Zn). Besides its antioxidant role, DP has several beneficial effects on gut health, calcium metabolism, and immune function. The dietary supplementation of DP significantly improved the body weight, average daily gain, average feed intake, FCR, ileum histomorphology, cecal VFA production, and expression of heat sock, antioxidant, immune, and tight junction related genes in the heat-stressed birds. Moreover, DP also improved the relative abundance of beneficial bacteria in the chicken gut (Wasti et al., 2021). Thus, considering the benefits, the dietary supplementation of DP can be considered as a potential strategy to mitigate the adverse effects of heat stress in poultry.

Microalgae (*Spirulina platensis*) is rich in antioxidants and have several health benefits. The dietary supplementation of microalgae significantly improved the body weight, average daily gain, average feed intake, FCR, ileum histomorphology, cecal VFA production, and expression of heat sock, antioxidant, immune, and tight junction-related genes in the heat-stressed birds (Mullenix et al., 2021, unpublished data from our lab). Gut microbial metagenomics showed a higher alpha diversity in the heat-stressed birds supplemented with microalgae (unpublished data from our lab).

CONCLUSION AND FUTURE DIRECTION

Because of climate change, the detrimental consequences of heat stress on poultry health and production will likely continue. Commercial poultry breeds cannot withstand heat stress, resulting in substantial economic losses to the industry, which triggers food security issues. Therefore, there is a critical need to develop heat-tolerant breeds and feeding and nutritional strategies to minimize the adverse effects of heat stress on poultry production.

REFERENCES

1. Lenssen, N, Schmidt G, Hansen J, Menne M, Persin A, Ruedy R, and Zyss D, (2019). Improvements in the GISTEMP uncertainty model. J*ournal Of Geophysical Research-Atmospheres*124 (12) 6307-6326.
2. Li GM, Liu LP, Yin B, Liu YY, Dong WW, Gong S, Zhang J, Tan JH. Heat stress decreases egg production of laying hens by inducing apoptosis of follicular cells via activating the FasL/Fas and TNF-α systems (2020). *Poultry Science* 99(11):6084-6093.
3. Mishra B, and Jha R. (2019). Oxidative stress in the poultry gut: Potential challenges and intervention. Frontiers in Veterinary Science, section Animal Nutrition and Metabolism. *Frontiers in veterinary science in Animal Nutrition and Metabolism section*; 6: 60.
4. Mullenix GJ, Greene ES, Emami NK, Tellez-Isaias G, Bottje WG, Erf GF, Kidd MT and Dridi S (2021). Spirulina platensis inclusion reverses circulating pro-inflammatory (Chemo)cytokine profiles in broilers fed low-protein diets. *Frontiers in Veterinary Science* 8:640968.
5. Rostagno MH. Effects of heat stress on the gut health of poultry. *Journal of Anim Science*, 98(4).
6. Wasti S, Sah N, and Mishra B. (2020). Impact of heat stress on poultry health and performances, and potential mitigation strategies. *Animals*, 10, 1266.
7. Wasti S, Sah N, Singh AK, Lee CN, Jha R, and Mishra B. (2021). Dietary supplementation of alpha-lipoic acid mitigates the negative effects of heat stress in broilers. *PLOS ONE 16(7)*: e0254936.
8. Wasti S, Sah N, Singh AK, Lee CN, Jha R, and Mishra B. (2021). Dietary supplementation of dried plum: A novel strategy to mitigate heat stress in broiler chickens. *Journal of Animal Science and Biotechnology* 12:58.

Plenary Session 3:
NUTRITION AND MICROBIOME UNDER CHALLENGING CONDITIONS

NUTRITIONAL AND FEEDING STRATEGIES FOR ANTIBIOTIC-FREE POULTRY PRODUCTION

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INTRODUCTION

Antibiotic-free (ABF) poultry production has multiplied worldwide. The motives are different in each country or region. They can vary between governmental regulations as a precaution for antimicrobial resistance, local consumer pressure, or the exportation of poultry products to countries with regulations for foodstuffs free of antibiotics or its metabolites. In some areas of the world, ABF is still difficult to accept for some poultry producers, technical personnel, and farmers. But some poultry companies in many countries voluntarily decided to produce without antibiotic growth-promotors (AGPs) two decades ago. Several producers can already claim successful ABF production. However, reaching productivity, health, livability, food safety, and environmental impacts similar to conventional systems with AGPs is still a challenge for many. Data from AgriStats (the largest benchmarking company in the USA) indicates a clear gap in productivity aspects between ABF programs and traditional production systems.

On the other hand, it is also important to remember that AGPs have not always been effective in preventing diseases, even when applying high dosage rates of AGPs and ionophores or adding combinations of multiple products. In some farms, gut and respiratory health issues are frequent independently of using AGPs, ionophores, chemical coccidiostats, or alternative feed additives. The evidence indicates that the feed additive *per se* is not the solution to some potential performance and health issues primarily related to feed and water quality, housing infrastructure, environmental stress, and management. Bacterial and mycoplasma resistance to certain antibiotics (Nhung *et al*., 2017) and *Eimeria* resistance to some coccidiostats (Chapman and Rathinam, 2022) were already evident in many areas of the world decades before ABF became popular. Consequently, transitioning to ABF systems could also be considered an alternative to more sustainable and efficient production for those who have not yet done so.

There are several categories of ABF production around the world. A standard label Certification used by many poultry producers worldwide is raised without antibiotics (RWA) because birds may receive antibiotics *in ovo* or at hatch with vaccines. In the USA, ionophore coccidiostats are classified as AGP, and consequently, not even those ionophore products are used as feed additives in some of these ABF programs. The American industry has voluntarily chosen to grow more than half of the broilers in systems targeting the requirements of the "No-Antibiotics-Ever" (NAE) label description regulated by the US Department of Agriculture (USDA). Nevertheless, when diseases happen, and birds need to be treated, these flocks are not sold with this label. The NAE production is more challenging to maintain, and total production costs are higher than ABF. However, these NAE systems keep growing in the USA (Figure 1) in the total number of chickens produced (Rennier Associates Inc., 2022; AgriStats, 2023). However, there is some seasonality in the number of flocks that finish in this NAE program.

ACHIEVING SUSTAINABLE ABF PRODUCTION

Changing to ABF programs depend on a decision related to the withdrawal of feed additives. But it is already proven by experience in many companies that maintaining similar productivity, poultry health and welfare, and food safety requires multiple interventions in the production system (Smith, 2011, 2019). This presentation is focused on nutritional and feeding strategies. However, the first take-home message is that, independently of all improvements we can make in nutrition, feed manufacturing, and feeding, they will not be enough to achieve optimal ABF production. Breeder intestinal health and enhanced maternal immunity, optimum incubation practices, improved housing infrastructure, proper ventilation, optimum house environmental control, management, frequent disposition of mortality, biosecurity, effective vaccination programs, and water quality are also fundamental to achieving sustainable ABF systems.

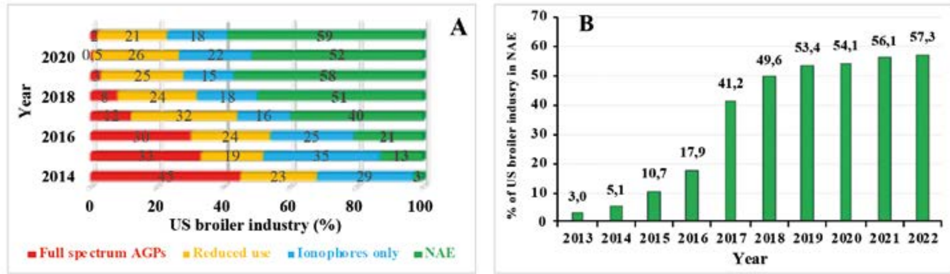

Figure 1A Distribution of the US broiler industry according to marketing program with either full use of all antibiotic growth-promoters (AGPs), reduced use, use of only ionophores or Non-Antibiotics Ever (NAE), (**Source:** Rennier Associates Inc., May 2022). **B.** Percentage of the US broiler industry production raising under NAE programs (**Source:** AgriStats Inc. 6510 Mutual Dr, Fort Wayne, IN 46825).

The reduction in AGP use is easy to obtain by withdrawing or restricting the use of specific products. Still, many times fails to minimize the use of molecules that could increase multi-antimicrobial resistance. For example, Nunan (2022) reported that in the United Kingdom, the British Poultry Council reported that the use of medically-important antibiotics reduced by 85% between 2013 and 2017 from 94 tons to 14 tons. In contrast, the use of ionophores increased from 209 tons to 281 tons in the same period, causing the overall antibiotic use to barely change in the same period. Higher use of ionophores in the UK (265 tons in 2019) is related to a higher incidence of gut health problems, necrotic enteritis, and coccidiosis. The lack of AGPs did not cause these issues. They are due to prevalent feed, housing, biosecurity, and litter management problems that should be controlled with other strategies (Smith, 2011, 2019; Cervantes and McDougald, 2023)

It is observed worldwide that despite having the best housing infrastructure and management practices, there is a seasonality in poultry health issues and productivity. The seasonal effect could depend on the weather but may be related to higher stocking density forced by market conditions or chicks of lower quality from eggs stored for a long time. In the USA, AgriStats reports indicate that between January and March, flock livability decreases. The calorie conversion to meat worsens, and the days to reach the target weight for processing increase in the same period. The seasonality is similar in traditional, ABF, or NAE programs, but the harmful effects are generally worst in NAE flocks. This data and observations worldwide provide clear evidence that nutrition and feeding cannot always mitigate these problems. But, nutritional and feeding strategies should consider seasonalities.

On the other hand, water, the most essential nutrient, can modify the animal response to any diet. The nutritionist should keep in mind the effects of water availability, temperature, physicochemical properties, and microbiological quality. In specific farms and regions, water factors could be the leading cause of eventual gut health issues, and no feed intervention can solve them completely. Adding probiotics, prebiotics, essential oils, organic acids, electrolytes, minerals like copper sulfate, and vitamins to the water lines for several days may cause clogging, increased algae and bacteria, and damage water equipment. These products should be included in the feed.

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NUTRITIONAL AND FEEDING STRATEGIES

Poultry flocks raised in ABF programs have potentially more microbial and immunological challenges, dysbiosis, and mucosal inflammation. The protein turnover and energy needs are potentially higher. Therefore, from the nutritional standpoint, it is vital to enhance gizzard and intestinal development early in life, stimulate an early development of a healthy gut microbiome, and minimize contamination and development of pathogenic bacteria and protozoa. The following are critical nutritional and feeding points to consider in ABF production.

FEED FORMULATION

- Design a feeding program with more dietary phases but avoiding changes near the peaks of coccidia infection or age periods with the most common management stress or dysbiosis events.
- Select ingredients with higher digestibility, a lower potential to increase gut viscosity, and minor risks of Clostridial or Salmonella contamination, especially in the pre-starter and starter diets. This generally means lower inclusion of animal-origin feedstuffs and more vegetarian diets.
- Avoid protein excess, but guarantee optimum levels of essential amino acids by using more crystalline sources.
- Include descriptive fiber parameters like ADF, insoluble, and soluble fiber in the feed formulation matrix. Implement a minimum level of ADF or insoluble dietary fiber (minimum of 2% and maximum of 3%) and a maximum of soluble fiber (no more than 20 to 30% of total dietary fiber). The ADF/NDF ratio in the feed should be kept at a minimum of 0.37 to achieve optimum gut development, nutrient digestibility, and performance (Nursiam *et al*., 2022).
- Account for all calcium sources in the diet and limit its inclusion to requirement levels.
- Keep a dietary electrolyte balance (Na+K – Cl) between 200 and 250 mEq/kg and adjust depending on water mineral content.

FEEDSTUFF QUALITY

- Improve description of feedstuff energy values, nutrient digestibility, and nutrient and antinutrient contents using rapid methods like the NIRS calibration models available and identify its variability.
- Control fungi and mycotoxin contamination and insect infestation in the whole system.
- If possible, use fat sources with better digestibility rich in medium-chain fatty acids (lauric, caprylic, and capric) like palm kernel oil, coconut oil, or even insect oil.
- Avoid rancidity in all ingredients rich in fatty acids (peroxide values ideally max. 5-10 mEq O_2/kg, TBARS 2-3 mg MDA/kg, and p-Anisidine value below 5).
- Limit the inclusion of soybean sources with trypsin inhibitor levels higher than 4.5 mg/g, and avoid overprocessed soy products.
- Select or request soybean meal with a geometric mean particle size (d_{gw}) of a minimum of 900 mm (minimize particles lower than 250 mm or higher than 1,700 mm)
- Choose trace minerals with higher bioavailability. Organic Zn, Cu, and Mn bring health benefits.

FEED ADDITIVE SELECTION

- Seed the gut with probiotics and prebiotics, including these products in pre-starter and starter diets.
- Include exogenous enzymes according to substrate concentration to improve digestibility or minimize antinutrient effects. All diets may benefit from phytase at higher levels and xylanase.
- Use additives to modulate gut microbiota and have anti-inflammatory properties like plant extracts, essential oils, polyphenols, and higher levels of vitamins A, E, D, and C, especially in the seasons with a higher incidence of health outbreaks.
- Provide gut mucosa integrity with butyric acid, mycotoxin binders, or detoxifiers.
- Emulsifiers, lipotropic, and bile salts may play a role in protecting liver function.
- Establish an effective coccidia control program according to the ABF program selected (Smith, 2019; Cervantes and McDougald, 2023).

FEED MANUFACTURING

- Grind ingredients coarse targeting a minimum d_{gw} of 700 mm for starter diets, 850 mm for grower diets, 1,000 for finisher diets, and at least 1,250 for breeders and layers. But, minimize variability (S_{gw}) in particle distribution and particles smaller than 250 mm for broilers and 500 mm for hens.
- Try the inclusion of whole grains. Positive results have been observed, including between 5 to 15% whole wheat (Sadati *et al*., 2022) and 2.5 to 5% whole corn (Ovi *et al*., 2021) in pelleted diets.
- Use physical or chemical methods to decrease bacterial load in feed (Huss *et al*., 2018).
- Avoid pelleting temperatures higher than 90ºC to minimize deleterious Maillard reactions.

FEEDING PRACTICES

- Try to increase feeding phases to meet nutrient needs closely and avoid nutrient intake excess.
- Maximize feed intake during the first hours post-placement using additional lights or higher intensity near feeders, paper on the floor, and supplemental feeders.
- Consider using control feeding programs or intermittent lighting programs to slow down feed intake and improve crop retention time and foregut digestion.
- Control house feed inventories to avoid periods of feed withdrawal.

MISSING KNOWLEDGE The nutritional concepts needed to improve poultry nutrition in general but essential to improve ABF production are 1) the kinetics of starch and protein digestibility; values of rapid and slow starch digestion, resistant starch, damaged starch, and amylose-to-amylopectin ratios to apply them in feed formulation; 2) Optimum values of starch and fiber parameters; 3) Net energy values for each feed ingredient and feeding phase; 4) Digestible calcium values; 5) More accurate NIRS curves to predict digestibility and energy values; and 6) True protein for feed formulation.

CONCLUSIONS

The nutritional and feeding strategies can be planned to address the challenges observed in ABF and NAE production. However, breeder, hatchery, infrastructure, and husbandry interventions are necessary. Diet formulation, feedstuff quality, additive selection, and feed manufacturing should be targeted to maximize gizzard and intestinal development, stimulate a healthy microbiome, minimize contamination and infection, and maintain gut physiology. Feeding practices can aid in minimizing issues of early feed intake and fast feed intake later on.

REFERENCES

1. Cervantes, H.M., and L.R. McDougald, 2023. Raising broiler chickens without ionophore anticoccidials. J. Appl. Poult. Res. 100347. doi.org/10.1016/j.japr.2023.100347
2. Chapman, H.D., and T. Rathinam. 2022. Focused review: The role of drug combinations for the control of coccidiosis in commercially reared chickens. Int. J. Parasitol.: Drugs Drug Resist. doi.org/10.1016/j.ijpddr.2022.01.001
3. Huss, A., R. Cochrane, C. Jones, and G. G. Atungulu. 2017. Physical and chemical methods for the reduction of biological hazards in animal feeds. Food and Feed Safety Systems and Analysis, pp. 83–95. doi: 10.1016/B978-0-12-811835-1.00005-1
4. Nhung, N.T., N. Chansiripornchai, and J. J. Carrique-Mas. 2017. Antimicrobial resistance in bacterial poultry pathogens: a review. Front. Vet. Sci. 4: 126. doi.org/10.3389/fvets.2017.00126

5. Nunan, C., 2022. Ending routine farm antibiotic use in Europe. Achieving responsible farm antibiotic use through improving animal health and welfare in pig and poultry production. https://www.saveourantibiotics.org/media/2019/report_ending-routine-farm-antibiotic-use-in-europe_final_january2022-1.pdf
6. Ovi, F. K., C. Bortoluzzi, T. J. Applegate, C. R. Starkey, K. S. Macklin, A. Morey, and W. J. Pacheco. 2021. Effects of prepelleting whole corn inclusion on broiler performance, intestinal microbiota, and carcass characteristics. J. Appl. Poult. Res. 30(2): 100114. doi.org/10.1016/j.japr.2020.100114
7. Sadati, M. M., V. Rezaeipour, and R. Abdullahpour. 2022. Efficacy of whole wheat grain in combination with acidified drinking water on growth performance, gizzard development, intestinal morphology, and microbial population of broiler chickens. Livest. Sci. 259: 104911. doi.org/10.1016/j.livsci.2022.104911
8. Smith, J. A., 2011. Experiences with drug-free broiler production. Poult. Sci. 90(11): 2670-2678. doi.org/10.3382/ps.2010-01032
9. Smith, J. A. 2019. Broiler production without antibiotics: United States field perspectives. Anim. Feed Sci. Technol 250: 93-98. doi.org/10.1016/j.anifeedsci.2018.04.027

Plenary Session 3:

NUTRITION AND MICROBIOME UNDER CHALLENGING CONDITIONS

DISENTANGLING THE EFFECTS OF FEED COMPOSITION ON THE CONSEQUENCES OF COCCIDIA INFECTION IN BROILERS

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Concerns about the sustainable control of coccidiosis in broilers have renewed efforts towards understanding the effect of nutrient resources on the consequences of the infection. Currently, however, there is some confusion about how to feed infected birds during the course of (subclinical) infection and what to expect from it. It is suggested that this confusion arises from the lack of consideration of the time scale of the infection and its consequences. We have found it useful to investigate these effects by dividing the infection into its **prepatent, acute and recovery phases**. During the **prepatent phase** of infection (first 3-5 days) infected and uninfected birds respond identically to feed composition. The main effects of coccidiosis occur during the **acute phase** which lasts approximately for 7 days. During this phase feed intake is reduced (anorexia), and nutrient resource digestibility and utilization decline. Investigations into the effects of nutrient resources (feed energy and protein contents) during this phase suggest that through the reductions in feed intake, birds seem to target a nutrient resource intake, which appears to be beneficial for infection outcomes, while at the same time they avoid excess protein intake. The consequence of this is that through dietary manipulation the extent of anorexia can also be manipulated and resistance to infection can be enhanced, as the number of coccidia oocyst excreted can be reduced. Finally, there is consistent evidence that during the **recovery phase** of the infection, birds benefit from feeds of high nutrient content, such as CP or amino acids, which allow for catch up growth. Depending on feed composition the catch up growth can be complete or incomplete It is suggested that by looking at the different phases of the coccidia infection, the effects of feed composition on the infection and its consequences can be disentangled.

Plenary Session 3:
NUTRITION AND MICROBIOME UNDER CHALLENGING CONDITIONS

FEEDING THE MICROBIOME

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INTRODUCTION

Bacteria have been on this planet for millions of years before the vertebrates evolved. Thus vertebrates emerged in a world that was already conquered by the microbes. When considering the animals microbiome, we are thus looking at millions of years of coevolution. It is therefore not surprising that the interactions between both kingdoms are extremely complex. When considering, however, that the building blocks of a bacterial cell (i.e. amino acids, nucleic acids, lipids, trace minerals, etc) are remarkably similar to the building blocks of an animal or a human cell, it can be inferred that both bacteria and higher organisms compete for the same nutrients. In order to avoid such competition, absorption of nutrients in higher organisms occurs in a secluded space, namely the intestinal tract. Indeed, in the small intestine, where the actual digestion and absorption takes place, a large spectrum of control mechanisms keeps the density of bacteria remarkably low, mostly below 10^3 per gram. In the caeca of birds and in the caecum and colon of mammals, however, bacteria are not only tolerated but even they are supported and fed by the host, through the secretion of large amounts of mucins. These mucins are used as nutrient source by specialized bacterial species such as *Akkermansia muciniphila*, which cross-feed to other bacterial species in order to preserve the individuals core microbiome during episodes of starvation (Such et al., 2019). When feed is available, most of the digestible fraction in absorbed in the small intestine, thus leaving only the undigestible fraction for the microbes that live in the lower intestinal tract. Expansion of the microbial population in the small intestine, especially in the duodenum and jejunum, is inhibited by powerful host antibacterial defense mechanisms which include not only specific anti-bacterial peptides and proteins, but also non-specific inhibiting substances such as the acid produced in the proventriculus. The latter can be bactericidal but it can also induce the 'viable-but-non-culturable-state' (Geirnaert et al., 2014), allowing bacteria to pass through the small intestine in a metabolically inactive state.

MICROBIOTA OF THE LOWER INTESTINAL TRACT AND THEIR NATURAL SUBSTRATES

In the lower intestinal tract of poultry, there appears to be a specific spatial distribution of microbial families and functions, with members of the Lactobacillaceae family dominating in the ileum at densities up to 10^9 active bacteria per gram, and the Oscillisporaceae (formerly: Ruminococcaceae) and Lachnospiraceae families making up the majority of the bacteria in the caeca, which has an even denser population of 10^{11} bacteria per gram (Rychlik, 2020). Members of the Lactobacillaceae and Oscillospiraceae families and of the Bacteroidota phylum are involved in the primary degradation of the undigestible fraction of the feed, which is largely composed of non-starch polysaccharides (NSP) from the cell walls of plant cells. The NSP fraction of feed has been neglected for a long time in animal nutrition research, first of all because it is undigestible and thus often considered as a 'filler', and secondly because the analytical techniques for accurately determining their composition are lacking. This has changed in the last decades (Knudsen, 2001). The NSP fraction of most chicken feed formulas is composed of complex arabinoxylans, beta-glucans, mannans and cellulose, which often form dense and intertwined networks of mostly insoluble fiber. It is therefore not surprising that the breakdown of these fibers for use as microbial nutrients requires the concerted actions of many different microbes. Additional substrates for the microbiota in the caeca are proteins from the host (from secreted enzymes and detached cells) and uric acid (by antiperistalic waves ascending from the cloaca) which constitute important nitrogen sources. Many of the intermediate metabolites and even more so the end metabolites of microbial fermentation are taken up by the host mucosa through a receptor-mediated process. They constitute a source of energy (such as the short chain fatty acids) and a source of signals informing the host about the prevailing conditions in the intestinal tract. They are also an source of B-vitamins. Positive signals such as butyrate and propionate will have beneficial effects on the intestinal mucosal architecture and function, whereas negative signals such as hydrogen sulfide will cause epithelial damage.

DYSBIOSIS

Todays feed formulas for broilers are characterized by a very high energy and protein density, which is needed to fulfill the ever increasing requirements of broiler breeds which have been selected since more than half a century for high feed intake. The ingredients used in these formulas are derived from high yielding crops with a relatively low and variable NSP content. By feeding broilers in this way, we unintentionally starve the microbiome of their lower intestinal tract. In addition, high performing broilers require feed with a high density of essential nutrients such as essential amino acids. Since an 'all vegetable' diet usually has a certain degree of amino acid imbalance, the feed formulas usually contain excess protein used to compensate for certain essential amino acid deficits. This may lead to incomplete digestion and absorption of the protein fraction, with ensuing excess protein entering the caeca. This is one of the main triggers of a shift in the caecal microbiota composition, commonly denominated dysbiosis. Dysbiosis is worse when pathogens, such as *Eimeria acervulina* or *Eimeria maxima* disturb the digestion and absorption of nutrients in the small intestine. It can get worse also in case of feed formulas containing poorly digestible ingredients or in case of poorly available nutrients due to the 'caging' effect. Dysbiosis is characterized not only by a shift in the microbiota, but also by increased permeability of the intestinal barrier and inflammation (Ducatelle et al., 2023). The microbiota shift typically shows a dramatic reduction in butyrate producers, usually accompanied by an expansion of the phylum of the Proteobacteria. The inflammation which accompanies the dysbiosis triggers further damage to the mucosal barrier, thus initiating a vicious circle of mucosal leakage, inflammation and microbial imbalance, which ultimately may increase the moisture content of faeces and cause wet litter. When the situation has degraded to that point, however, it is difficult to revert it and performance losses can be considerable. Therefore, efforts are made to support the microbiota in the lower intestinal tract of broilers from the start. Hereafter, an overview is given of the currently available tools to support (feed) the microbiome.

FEEDING THE MICROBIOME THROUGH INGREDIENT CHOICE

The undigestible (NSP) fraction of the raw materials used to formulate the feed is the natural substrate for the microbiota in the lower intestinal tract. It is difficult, however, to steer the microbiota through selecting specific ingredients for the feed formula, because the NSP fraction in the ingredients is variable and the analytical techniques available for routine analyses of NSP are insufficiently refined to allow prediction of the effects on the microbiota. Moreover, ingredient choice is meant to cover the primary nutritional needs of the birds and the NSP fraction just comes with it.

FEEDING THE MICROBIOME USING ENZYME SUPPLEMENTS

The immature microbiome of young broilers usually is established rather slowly and late during the rearing process (Rychlik, 2020). Especially many of the primary degraders of fiber (e.g. Bacteroidota) may be lacking. This is the main reason why the industry has invested in the design of fiber-degrading enzymes as feed supplements. There is a choice of fiber-degrading NSP-ases available today, including xylanases, arabino-furanosidases, mannanases, cellulales, glucuronidases, all of which can take care of the primary degradation of different types of NSPs (Derqaiou et al., 2022). For several of these, a beneficial effect on the caecal microbiota has been reported (Ducatelle et al., 2022) It appears to be preferable for the enzymes to only partially degrade the fiber and leave the final steps of degradation to the microbiota, to the point that enzymatic degradation down to the monosaccharide level may even be a disadvantage, since the monosaccharides appear to reduce the growth rate of the birds (Peng et al., 2004). It has been shown indeed that xylanase degradation of the arabinoxylan fraction of wheat to a degree of polymerization of around 30 provides an optimal substrate for the caecal microbiota of young broilers (Yacoubi et al., 2018). Currently studies are ongoing to investigate the effects of combining different NSPases. Preliminary results indicate that an enzyme targeting one type of NSP may improve the availability of another type of NSP, thus underling the importance of untangling the complex NSP network of the plant cell walls.

PREBIOTICS FOR FEEDING THE MICROBIOME

Partially degraded NSPs are most commonly used as prebiotics. They can be directly supplied to the birds in order to provide a readily available source of nutrients for the microbiome. In this context it was shown already some years ago that directly fed xylooligosaccharides support the growth of Lactobacillaceae which cross-feed to the Lachnospiraceae (De Maesschalck et al., 2015). In some cases even the mere purification of single NSP without degradation may even be sufficient to trigger a prebiotic effect, as has been shown for pure amorphous cellulose in young broiler chicks (De Maesschalck et al., 2019).

CONCLUSION

A range of different feeding strategies are currently available which allow to steer and support the beneficial gut microbiota in chickens. Some may be complementary, supporting different microbial metabolic pathways, why others are not. The challenge for the nutritionists and veterinarians is to make rational choices in order to improve the health and performance of the birds.

REFERENCES

1. De Maesschalck C, Eeckhaut V, Maertens L, De Lange L, Marschal L, Daube G, et al. (2019). Amorphous cellulose feed supplement alter the broiler caecal microbiome. **Poultry Science** 98, 3811-3817.
2. De Maesschalck C, Eeckhaut V, Maertens L, De Lange L, Marschal L, Nezer C, et al. (2015). The effects of xylo-oligosaccharides on performance and microbiota in broiler chickens. **Applied & Environmental Microbiology** 81, 5880-5888.
3. Derqaiou S, Oukessou M & Nassik S (2022). NSPases: can those exogenous enzymes really constitute natural growth cofactors in broiler chickens? **International Journal of Advanced Research** 10, 450-464.
4. Ducatelle R, Goossens E, Eeckhaut V & Van Immerseel F (2023). Poultry gut health and beyond. **Animal Nutrition** (in press).
5. Ducatelle R, Van Immerseel F, Eeckhaut V & Goossens E (2022). Enzymes and the microbiome in the post-antibiotic era. In: Enzymes in farm animal nutrition. 3rd ed. M. Bedford, G.G. Partridge, M. Hruby & C.L. Walk, eds CAB International, 254-264.
6. Geirnaert A, Steyaert A, Eeckhaut V, Debruyne B, Arends JBA, Van Immerseel F et al. (2014). *Butyricicoccus pullicaecorum*, a butyrate producer with probiotic potential, is intrinsically tolerant to stomach and small intestine conditions. **Anaerobe** 30, 70-74.

7. Knudsen KEB (2001). The nutritional significance of “dietary fibre” analysis. **Animal Feed Science and Technology** 90, 3-20.
8. Peng YL, Guo YM & Yuan JM (2004). Effect of feeding xylose on the growth of broilers and nutrient digestibility as well as absorption of xylose in the portal-drained viscera. **Asian-Australasian Journal of Animal Sciences** 17, 1123-1130.
9. Rychlik I (2020). Composition and function of chicken gut microbiota. **Animals** 10, 103.
10. Such N, Farkas V, Koltay IA, Rawash MA, Dublecz K & Molar A (2019). Wheat bran supplementation increases the abundance of *Akkermansia muciniphila* whereas single strain probiotic *Clostridium butyricum* fails to influence caecal microflora composition of broiler chickens. **Proceeding of the 18th BOKU symposium in animal nutrition**, 189-192.
11. Yacoubi N, Saulnier L, Bonnin E, Devillard E, Eeckhaut V, Rhayat L, et al. (2018). Short chain arabinoxylans prepared from enzymatically treated wheat grain exert prebiotic effects during the broiler starter period. **Poultry Science** 97, 412-424.

Plenary Session 4:
SOLUTIONS TO IMPROVE THE NUTRITIONAL VALUE OF FEED

THE EFFECT OF FIBRES IN FEEDSTUFFS ON NUTRITIONAL VALUE AND HOW TO DETERMINE IT

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INTRODUCTION

The nutritional value of a feed refers to "the amount of nutrients contained in a feed that can be utilized by the animal" and its estimation consequently involve how well the nutrients are digested and utilized by the animals. In poultry nutrition, it is well known that content and composition of fibre (non-starch polysaccharides (NSP) and lignin) have a profound influence on nutrient digestibility and utilisation as it represents the largest part of the feed that cannot be digested by endogenous enzymes. Fibre can also influence nutrient digestibility due to its viscosity associated with soluble NSP and the encapsulation of important nutrients in cell walls (CW) rich in insoluble NSP. However, insoluble NSP may help develop the gizzard and other parts of the gastrointestinal tract and thereby improve the digestibility of nutrients.
The main purpose of the present paper is to review the interplay between **NSP** and **fibre** and the digestion processes. Since cereal grains constitute the largest part of the NSP present in the diets for broilers most emphasis will be devoted on cereal NSP.

HOW TO DETERMINE FIBRE?

A broad variety of analytical methods have been used for the analysis of fibre in feeds. The crude fibre method is the oldest fibre methods and still used in the proximate analysis of feeds. The detergent methods were developed by Van Soest and co-workers and were originally developed for the analysis of fibre rich feedstuffs (roughages) but has latter on been applied also to concentrate feeds. More recently the enzymatic-gravimetric AOAC (Association of Official Analytical Chemists) procedures and the enzymatic-chemical Englyst and Uppsala procedures for the analysis of fibre (dietary fibre) have been developed. These latter methods were originally developed for foods but have also been used when analysing feedstuffs. For further information see [1].

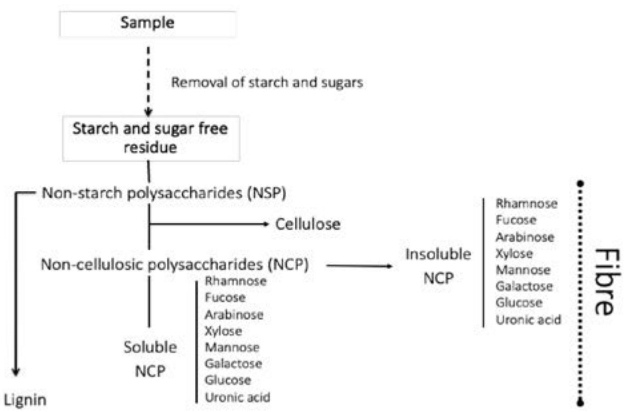

Figure 1. Determination of fibre as non-starch polysaccharides and Klason lignin

The method illustrated in Figure 1 enables separate estimations of cellulose, soluble NCP and insoluble NCP and lignin (Klason) using enzymatic-chemical-gravimetric principles. The method also provides detailed information about the monomeric composition of the NCP fraction. Information concerning polymers making up the NCP fraction can be depicted from the monomeric sugar residues, i.e., NCP arabinose and xylose are markers for arabinoxylan (AX) and NCP glucose for mixed linkage β-glucan in cereals, uronic acids for the backbone in pectin, and arabinose, galactose etc. markers of the side groups in pectic polysaccharides. A comparison of results obtained when analysing the same feedstuffs by the different analytical methods show distinct differences; crude fibre values are much lower than values obtained by the neutral detergent fibre method that is somewhat lower than those obtained by the enzymatic-chemical methods.

EXAMPLES OF FIBRE COMPOSITION OF DIFFERENT FEEDSTUFFS

The NSP and lignin content of feedstuffs varies widely. Cereals have in general a lower concentration of NSP and lignin than protein rich crops whereas co-products from cereals and products from the agro and food industries have higher concentration (Table 1).

Table 1. Non-starch polysaccharide and lignin content (g/kg dry matter) of selected feedstuffs

		Non-cellulosic polysaccharides									
	Cell	Ara	Xyl	Man	Gal	Glc	UA	NCP	NSP	KL	Fibre
Cereals											
Brown rice	1	5	4	1	1	8	2	21 (2)	22 (2)	13	35
Maize	22	22	30	3	5	10	7	75 (9)	97 (9)	11	108
Sorghum	15	17	13	1	3	10	4	51 (4)	66 (4)	16	83
Wheat	20	29	47	3	4	11	5	99 (25)	119 (25)	10	138
Rye	16	36	61	5	5	26	4	136 (42)	152 (42)	21	174
Barley	43	28	56	4	3	47	6	143 (56)	186 (56)	35	221
Oats	82	18	80	3	7	33	10	150 (40)	232 (40)	66	298
Cereal co-products											
Maize bran	83	72	116	4	20	16	44	271 (32)	354 (32)	25	379
Maize feed meal	33	33	48	2	9	10	20	123 (10)	156 (10)	18	174
Maize DDGS	58	52	71	19	13	21	16	192 (34)	250 (34)	39	289
Wheat bran	72	90	148	5	8	35	15	302 (29)	374 (29)	75	449
Wheat middling	19	48	67	8	7	33	10	171 (71)	190 (71)	11	201
Wheat DDGS	50	57	86	16	11	33	8	212 (67)	262 (67)	66	328
Rye bran	39	78	213	3	12	66	10	383 (63)	422 (63)	68	490
Barley hull meal	192	51	184	3	6	25	18	286 (20)	478 (20)	115	594
Oat hull meal	196	28	212	2	9	20	36	309 (13)	505 (13)	148	653
Protein rich feedstuffs											
Soybean meal	62	26	19	13	41	7	48	155 (63)	217 (63)	16	233
Rapeseed meal	52	43	17	6	19	21	61	168 (55)	220 (55)	134	354
Peas	53	26	13	2	7	36	32	127 (52)	180 (52)	12	192
Fava beans	81	24	12	2	6	32	33	109 (50)	190 (50)	20	210
Lupin	131	43	36	9	141	2	39	274 (134)	405 (134)	12	416
Palm cake	73	12	1	309	15	7	19	393 (32)	466 (32)	136	602
Sunflower cake	123	31	59	12	13	17	67	192 (57)	315 (57)	133	448

NCP, non-cellulosic polysaccharides; NSP, non-starch polysaccharides; Cell, cellulose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acids; KL, Klason lignin; DDGS, distillers dry grains with soluble. Values in parentheses are soluble NCP/NSP.
Data from own database.

While cellulose is present in all plant CW at different concentration, there is a wide variation in the composition of the non-cellulosic polysaccharides among the feedstuffs. β-Glucan (NCP-glucose) is responsible for the relatively high concentration of soluble NCP in barley and oats and soluble AX (arabinose + xylose) responsible for most of soluble NCP in wheat and rye. In legumes, protein crops and feedstuffs, pectin substances expressed by uronic acids, galactose and arabinose, xyloglucans expressed by xylose and glucose, galactans expressed by galactose in lupin and mannans expressed by mannose in palm cake are the main non-cellulosic polysaccharides responsible for the relative high levels of soluble NCP in these feedstuffs.

NUTRITIONAL EFFECTS OF FIBRE

Diets for broiler chickens have traditionally been based on relatively few feedstuffs like maize and soybean meal. Today, however, several other less expensive energy and protein crops and co-products are frequently being used. Common for the latter group of feedstuffs is that they have a higher content of NSP, are more viscous and have a lower nutrient digestibility and utilisation. The lower nutrient digestibility and utilisation is a consequence of the higher NSP content and is due to the following:

1. The NSP fraction represent the largest part of the feed dry matter not digested by the bird's endogenous enzymes.
2. The viscous nature of some soluble NSP may interfere with the enzymatic digestion processes making them less efficient.
3. The insoluble NSP in some CW structures may encapsulate potentially available nutrients.

NSP is the diet factor with the largest negative effect on nutrient digestibility. In broilers, the digestibility of NSP is influenced by age and the NSP composition but is generally lower in younger than older poultry. In 5-10 d old birds, it has been found that soluble and insoluble AX with low arabinose to xylose ratios were able to enter the caecum but were not extensively fermented by the caecal microbiota, whereas the establishment of a mature microbial community from 21 d of age resulted in an increase in both solubilization of insoluble AX and fermentation of soluble AX at ileum and caecum [2]. Despite low NSP degradation in poultry, the microbiota will nevertheless provide energy to the host. It has been estimated that the upper capacity of NSP fermentation contributed 3-4% of metabolizable energy (ME) and the excretion of organic acids (mainly lactic acid and acetic acid) accounted for up to 2% of ME [3]. Measurable quantities of H_2 was also produced whereas no CH_4 could be detected indicating lack of methanogenic bacteria. Other studies have also shown that variation in the NSP content within individual raw material to a large extent account for the variation in ME content as found for wheat varieties in several studies [4].

The soluble and hydrophilic nature of AX and β-glucan enables the two polymers to disperse relatively easily in the gastrointestinal tract and to increase intestinal viscosity with negative effects on nutrient digestibility and animal performance [5]. The mechanism behind is thought to be inhibition of nutrient digestion by impeding the diffusion of digestive enzymes and their substrate and products. An associated effect is a reduced ability of the gut to physically mix the contents. This has been found to implicate particularly fat digestion and more for saturated than unsaturated fat as vigorous mixing is required for good emulsification, which is a prerequisite for efficient fat digestion. The study of Steenfeldt [6] identified a significant negative relationship between the concentration of AX in wheat varieties and the apparent fat digestibility coefficient measured in excreta. The negative effect of viscosity on nutrient digestibility was further illustrated in the model study of Choct & Annison [7] where a significant negative effect of concentrated wheat AX (pentosans) on the ileal digestibility of starch, protein and lipid was seen. Age plays a role for the negative effect of viscosity on nutrient digestibility; while substantial in broiler chickens up to 21-28 d of age, the effects in older birds are less presumably because in older birds the microbiota can digest and utilize some of the viscous polysaccharides [2].

It has long been known that some CW structures may encapsulate potentially available nutrients and thereby reduce their digestibility [8]. Examples of such CW structures are the aleurone and endosperm cells of cereals and the cotyledon CW of legumes. The aleurone layer is a specific tissue of the cereal endosperm, one layer thick in wheat and rye and three layer thick in barley. The aleurone AX across cereals is largely insoluble and with a lower A/X ratio than in the starchy endosperm in both wheat and barley. The AX of the aleurone cells is also more heavily esterified with ferulic acid dehydrodiferulic acids along with *p*-coumaric acid than the AX of the starchy endosperm. The insoluble nature of the aleurone CW makes them resistant to digestion by endogenous enzymes and intact aleurone cells have been identified in excreta from chickens causing a concomitant lower digestibility of protein.

Cotyledon CW of peas, fava beans and other beans can limit the access of endogenous enzymes to starch and protein [9]. The CW of the cotyledon is hydrophilic and primarily composed of pectic polysaccharides, xyloglucans and cellulose and although the NSP in the cotyledon analytically behaves like soluble NSP, it is mostly insoluble under the conditions that persist in the gastrointestinal tract. These properties of legume CW have been found to control *in vitro* rate and extent of starch digestion in peas, common beans, and fava beans. The extent of starch digestion in legumes is far lower than in cereals and particularly in tapioca [10].

In addition to the viscous properties of barley β-glucan, the endosperm CW of barley may also act as a physical barrier to endogenous enzymes, and this may delay the digestion of starch in the small intestine [11]. Partly degradation of the β-glucan via enzyme addition resulted in a more anterior disappearance of starch in the small intestine [11]. There can also be an interaction between the endosperm CW and the insoluble protein matrix as indicated in a study with sorghum and barley. It was found that the rate of starch hydrolysis was significantly higher in barley than sorghum when milled to varying fragment sizes, presumably reflecting a reduced access of α-amylase to the starch granule because of the insoluble protein matrix and the surrounding CW [10]. Thus, endosperm CW from cereals may influence the digestion of starch in some cereals caused by the interaction with the insoluble protein matrix and in others with the viscosity properties of AX and β-glucan.

CONCLUSION

Fibres represent the part of the feed that cannot be digested by endogenous enzymes and has therefore a significant and negative effect on the nutritional value of the feed. Different methods are available for its determination but today it is common to use enzymatic-chemical-gravimetric methods which in addition to the total content also give information of its solubility and, for some type of feedstuffs, the main polysaccharides.

REFERENCES

1. Bach Knudsen, K.E. and H.N. Lærke, *Carbohydrates and lignin in the feed – from sugars to complex composed fibres*, in *Feed Evaluation Science* P. Moughan and W. Hendriks, Editors. 2018, Wageningen Academic Publisher: Wageningen. p. 109-140.
2. Bautil, A., et al., *Age-related arabinoxylan hydrolysis and fermentation in the gastrointestinal tract of broilers fed wheat-based diets.* Poult Sci, 2019. 98(10): p. 4606-4621.
3. Jørgensen, H., et al., *The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens.* Br J Nutr, 1996. **75**(3): p. 379-95.
4. Annison, G., *Relationship between the levels of soluble nonstarch polysaccharides and the apparent metabolizable energy of wheats assayed in broiler chickens.* J Agric Food Chem, 1991. 39: p. 1252-1256.
5. Pettersson, D. and P. Åman, *Enzyme supplementation of a poultry diet containing rye and wheat.* British Journal of Nutrition, 1989. 62: p. 139-149.

6. Steenfeldt, S., *The dietary effect of different wheat cultivars for broiler chickens.* Br Poult Sci, 2001. 42(5): p. 595-609.
7. Choct, M. and G. Annison, *The inhibition of nutrient digestion by wheat pentosans.* Br J Nutr, 1992. 67(1): p. 123-32.
8. Classen, H., *Cereal grain starch and exogenous enzymes in poultry diets.* Anim Feed Sci Technol, 1996. 62(1): p. 21-27.
9. Dhital, S., et al., *Intactness of cell wall structure controls the in vitro digestion of starch in legumes.* Food Function, 2016. 7(3): p. 1367-79.
10. Weurding, R.E., et al., *Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs.* J Nutr, 2001. 131(9): p. 2329-35.
11. Hesselman, K. and P. Åman, *The effect of beta-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low or high viscosity.* Anim. Feed Sci. Technol., 1986. 15: p. 83-93.

Plenary Session 4:
SOLUTIONS TO IMPROVE THE NUTRITIONAL VALUE OF FEED

IMPROVING THE NUTRITIONAL VALUE OF INGREDIENTS THROUGH THE USE OF FEED ENZYMES

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One hundred years ago the broiler chicken industry as we know it today did not exist. Birds were grown in small flocks on small holdings and fed whatever grains and protein sources were at hand, with the result that the diet they received was based on many ingredients, most of which rarely exceeded 20% of the total ration. Modern-day poultry rations are quite different, often being based upon two or three major ingredients making up 70% or more of the total diet. Thus the anti-nutritional factors (ANF) present in an ingredient employed could now present itself at levels hitherto unseen in the past. The majority of ANFs that enzymes address today are fibre, phytate and to a lesser extent proteinaceous ANF's, but for the sake of this short paper the focus will be on the major cereal NSP and phytic acid.

FIBRE

The definition of fibre and the methods used to analyse fibre have evolved significantly and will likely evolve further as more is understood of this largely ignored fraction of a monogastric's diet (Asp, 1987; Bach Knudsen, 2018). The monogastric nutritionist views fibre as a composite entity which can fit into one of three categories; antinutritional (eg viscous soluble polysaccharides, intact fibrous cell walls which encapsulate nutrient contents), beneficial (functional insoluble fibre which stimulates gizzard development, fermentable oligosaccharides,) or nutrient diluent (inert non-functional insoluble fibre). We still lack the ability to routinely measure these categories with the current methods at hand due to a lack of information on three-dimensional structures, linkages, size and susceptibility to fermentation. Regardless, it is clear that benefit is derived if the fibre present in the feed ingredients offered is depolymerised, as this reduces the antinutritional and diluent effects of fibre and simultaneously creates more fermentable fibre as a result. Thus, increasing fibre "digestibility" should be the goal of any nutritionist employing a fibre-targeting enzyme.

Table 1. Selected NSP and cellulose content of major feed materials

		Total (g/kg)			Soluble (% of total)			g/kg	g/kg
	Sample n	A+X[1]	B-glucans	Mannan	A+X	B-glucans	Mannan	Uronic acids	Cellulose
Barley	8	77	47	4	13%	52%	30%	4	28
Rye	20	85	20	5	36%	33%	43%	3	12
Triticale	20	55	7	4	23%	14%	30%	3	19
Wheat	20	64	6	2	22%	33%	25%	3	14
Maize	3	52	1	3	10%	10%	67%	7	22
SBM	6	45		13	24%		38%	48	62
RSM	4	60		6	27%		17%	61	52

Adapted from (Rodehutscord *et al.*, 2016) and (Knudsen, 1997).

[1]Arabinose + xylose residues

The traditional approach to treating fibrous ingredients with enzymes to increase fibre "digestion" is to analyse the NSP sugar components of the fibre and apply enzymes capable of attacking the presumed substrate. Table 1 lists the arabinose + xylose (A+X), Β glucan and mannose contents of several typical ingredients used in poultry nutrition and what is clear is that the relative preponderance of A+X is high in all ingredients, including the oilseed meals. However, such an analysis does not definitively identify the fibre type from which the sugars were derived. A+X, for example, is likely mostly derived from arabinoxylans in cereals but in oilseed meals there are significant amounts of arabinans and xyloglucans which contribute to this A+X fraction. Thus, targeting the fibre components of an ingredient needs structural as well as compositional knowledge.

A secondary consideration is the solubility of the fibre fraction. Insoluble fractions, if they remain insoluble throughout the intestinal tract do not take part in the digestion or fermentation process directly but can influence nutrient density of the diet by

1. Binding nutrients
2. Acting as a diluent
3. Speeding up passage rate
4. Stimulating gizzard development

The first 3 activities could be detrimental whereas the 4th would likely be beneficial given that increased gizzard activity is known to improve the digestibility of the entire diet.

Many NSP'ases can degrade and thereby dissolve variable proportions of ingredient insoluble fibre such that it can be further depolymerised and subsequently fermented or excreted. The extent to which this is accomplished depends upon the feed ingredient. For example the more complex substitutions of the xylan backbone in maize compared with wheat make it less susceptible to the endo-xylanase attack (Dale *et al.*, 2022). The specificity of the enzyme also plays a significant role here as well (Kluepfel *et al.*, 1992). Regardless, NSPase application routinely increases the "digestibility" of the insoluble NSP of an ingredient and diet. Whilst this may reduce the influence the insoluble fibre has on the first 3 properties noted above, it is debatable whether the dissolution of the insoluble fibre by action of an NSPase will significantly influence gizzard development. The pH optima of most NSPase on the market are such that the activity of these enzymes is confined to the crop, small intestine and caeca. Since much of the diet bypasses the crop in the *ad libitum* fed broiler the likelihood of significant activity prior to the gizzard is therefore low and hence the ability of the NSPase to reduce the gizzard stimulating effects of the fibre is limited.

The soluble fibre fraction can influence several facets of the digestive/fermentative process including;

1. Increasing intestinal viscosity (higher molecular weight fractions only)
2. Excessive supply can stimulate bacterial overgrowth and dysbacteriosis
3. Providing fuel for beneficial bacteria (prebiotic effect)
4. Signalling bacteria to produce their own fibre degrading enzymes (stimbiotic effect)
5. Directly influencing the immune system
6. Indirectly influencing the immune system through changing bacterial populations and community structure by all mechanisms noted above

Progressive depolymerisation of soluble fibre can have profound implications for all 6 effects noted above. Provided the provision of smaller, more fermentable fibre structures is not excessive then improvements in nutrient digestion, energy recovery from fibre fermentation and subsequent improvements in intestinal structure if specific volatile fatty acids (eg butyrate) production is enhanced would be expected.

INGREDIENT RESPONSES TO NSPASES

The question at hand is how the addition of specific exogenous fibre degrading enzymes influences the effects of both the insoluble and soluble fibre components arising from the most common feed ingredients. Variation in fibre structure between and within ingredients results in variable rates of dissolution of insoluble and depolymerisation of soluble NSP and hence subsequent performance of the bird. This is just one of the reasons why the response to exogenous NSPases are varied between studies and presents the industry with a conundrum with regards how such products should be evaluated. Regardless of the ingredient, the benefit noted on NSPase use is far greater than the apparent energy value of the fibre digested, suggesting several mechanisms are involved in the response. It is clear from table 1 that the fibre content (soluble and insoluble) of some ingredients, such as maize, is much lower than that in wheat and in particular rye or barley. The implications of this are that the initial anti-nutrient effects of fibre in maize are clearly less than those of wheat, triticale, barley and rye (in ascending order) and as a result, the benefits achieved on use of an NSPase will be more constrained. This is not only because there is less of an ANF effect to overcome, but as a consequence this means that the benefits that accrue from moving insoluble and soluble, viscous NSP towards fermentable oligosaccharides will be more limited as well. In addition to this, the structure of the fibre in maize and particularly sorghum is more complex and substituted than that in other cereals, further limiting the value extracted (Bach Knudsen, 2014). Further evolution of fibre degrading enzymes is necessary if the full value is to be extracted from these more intransigent but commonplace cereals. Whereas the focus of this section has been on the cereal fibre components, equal consideration should also be given to that in the oilseed meal components of the diet but space precludes any coverage in this paper.

PHYTATE

Phytic acid is not only a source of "unavailable" phosphorus but also a significant antinutrient due to its ability to bind to minerals, proteins and any positively charged nutrients and in doing so reduce their digestibility as the complex cannot be absorbed. Thus, the use of a phytase to remove the phosphates from the inositol ring not only presents the animal with an available phosphorus source but also simultaneously reduces the antinutrient capacity of the molecule. In typical monogastric diets the cereal and oilseed meals contribute relatively similar proportions of phytic acid to the total found in the diet. Although phytate, unlike fibre, is clearly chemically defined, its location can vary between ingredients. In maize it is concentrated in the germ, in small grain cereals it is in the aleurone layer and in oilseed meals it is usually located in protein bodies in the cotyledons. Thus, although a phytase identifies phytic acid as its substrate regardless of where it is derived, in its native form in a given ingredient the associated minerals, proteins and physical location may limit its accessibility and thus susceptibility to this enzyme. As a result, the responsiveness of an ingredient (and thus by extension the diet) to added phytase may vary. Furthermore, variation in phytic acid concentration due to genotypic and phenotypic variation (Godoy *et al.*, 2005) means that care must be taken when using higher doses of phytase to ensure that the expected P release does not exceed the phytic acid content of the diet.

Whereas low levels of phytase are capable of releasing significant amounts of phosphate which then becomes available for the host animal to utilise, it will not quantitatively rid the diet of antinutritive phytic acid (InsP6) and indeed will create a whole series of lower ester phosphates (InsP5, 4, 3 and 2). Although these are less antinutritive than InsP6, they are all antinutrients in their own right and as a result the removal of all antinutritive effects of phytic acid by ever increasing doses of phytase is a continuous event, and is only complete when all lower esters are removed. Such an event requires orders of magnitude more phytase than is currently employed commercially which would not be economic today. Nevertheless, as the benefits of a more complete ester removal are understood (Kriseldi *et al.*, 2021), the dose employed commercially has risen considerably with time. Some recent findings suggest that more comprehensive phytate destruction and provision of inositol can reduce the incidence of some metabolic diseases such as woody breast and femoral head necrosis (Greene *et al.*, 2020). More complete phytate destruction is not only dependent upon enzyme dose or ingredient location. Since phytic acid is more soluble and thus more susceptible to hydrolysis at low pH, any ingredient that increases crop or gizzard retention would be expected to increase the extent of phytate hydrolysis. Such effects have been seen for fibre (cellulose, oat hulls), added fat and coarse grinding of materials (Ballam *et al.*, 1984). However, the higher the dose of phytase employed, the less likely ingredient source, fibre content, particle size, dietary Ca and P level and age of the bird (Beaulac, 2015) is to have on the response noted . Consequently, results become more consistent and predictable as dosage increases, regardless of the ingredients employed. It is envisaged that phytase dosages will increase further over time, enabling more efficient use of resources and more sustainable poultry production which may remove the concern of variable phytate "availability" between ingredients.

REFERENCES

1. Asp, N.-G. (1987). Definition and analysis of dietary fibre. *Scandinavian Journal of Gastroenterology,* 22, 16-20. doi: 10.3109/00365528709095845
2. Bach Knudsen, K.E. (2014). Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. *Poultry Science,* 93, 2380-2393. doi: http://dx.doi.org/10.3382/ps.2014-03902
3. Bach Knudsen, K.E. (2018). Dietary fibre analyses in a nutritional and physiological context - past and present. *Proceedings of the Society of Nutrition and Physiology,* 27, 189-192.
4. Ballam, G.C., Nelson, T.S. & Kirby, L.K. (1984). Effect of fiber and phytate source and of calcium and phosphorus level on phytate hydrolysis in the chick. *Poultry Science,* 63, 333-338.
5. Beaulac, K. (2015). The effects of graded levels of phytase in broiler diets on growth, intestinal development, and nutrient digestibility. In: University of Saskatchewan.
6. Dale, T., Bedford, M.R., Tucker, G.A., Brameld, J.M. & Parr, T. (2022). The effects of exogenous fibrolytic enzymes on the in vitro generation of xylooligosaccharides and monosaccharides is dependent upon cereal type. *Animal Feed Science and Technology,* 292, 115406. doi: https://doi.org/10.1016/j.anifeedsci.2022.115406
7. Godoy, S., Chicco, C., Meschy, F. & Requena, F. (2005). Phytic phosphorus and phytase activity of animal feed ingredients. *Interciencia,* 30, 24-28.
8. Greene, E., Cauble, R., Dhamad, A.E., Kidd, M.T., Kong, B., Howard, S.M., et al. (2020). Muscle Metabolome Profiles in Woody Breast-(un)Affected Broilers: Effects of Quantum Blue Phytase-Enriched Diet. *Frontiers in Veterinary Science,* 7. doi: 10.3389/fvets.2020.00458
9. Kluepfel, D., Daigneault, N., Morosoli, R. & Shareck, F. (1992). Purification and characterization of a new xylanase (xylanase C) produced by Streptomyces lividans 66. *Applied Microbiology and Biotechnology,* 36, 626-631. doi: 10.1007/BF00183240
10. Knudsen, K.E.B. (1997). Carbohydrate and lignin contents of plant materials used in animal feeding. *Animal Feed Science and Technology,* 67, 319-338. doi: https://doi.org/10.1016/S0377-8401(97)00009-6
11. Kriseldi, R., Walk, C.L., Bedford, M.R. & Dozier, W.A. (2021). Inositol and gradient phytase supplementation in broiler diets during a 6-week production period: 2. Effects on phytate degradation and inositol liberation in gizzard and ileal digesta contents1. *Poultry Science,* 100, 100899. doi: https://doi.org/10.1016/j.psj.2020.11.068
12. Rodehutscord, M., Ruckert, C., Maurer, H.P., Schenkel, H., Schipprack, W., Bach Knudsen, K.E., et al. (2016). Variation in chemical composition and physical characteristics of cereal grains from different genotypes. *Archives of Animal Nutrition,* 70, 87-107.

Plenary Session 4:
SOLUTIONS TO IMPROVE THE NUTRITIONAL VALUE OF FEED

THE IMPORTANCE OF DIGESTIVE DYNAMICS IN BROILER CHICKEN NUTRITION

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DIGESTIVE DYNAMICS MATTER

In typical broiler diets, protein and amino acids are primarily the building blocks of protein and starch and glucose are the principal source of energy. Both are required for optimal growth performance and an appropriate balance of amino acids and glucose should be made available at sites of protein synthesis to generate efficient protein accretion and growth. The various body proteins require a specific profile of amino acids and each gram of protein synthesised demands an energy input of 5.35 kJ for in broiler chickens (Aoyagi *et al.,* 1988). This is the fundamental construct of digestive dynamics. Conventionally, nutrient utilisation in chickens is determined from total tract nutrient digestion and retention or apparent digestibility coefficients based on digesta taken from the terminal ileum. While the later eliminates the confounding effects of hindgut fermentation it overlooks the fact that digestion of protein and starch, absorption of amino acids and glucose principally occurs in duodenum and jejunum (Hurwitz *et al.*, 1972, Riesenfeld *et al.*, 1980). Moreover, the digestion of starch is more rapid than protein. Sorghum is considered to be a slowly digestible source of starch (Giuberti *et al.*, 2012); however, starch digestion rates were nearly 40% faster than protein (3.38 versus 2.46 $\times 10^{-2}$ min^{-1}, Figure 1) in birds offered conventional, sorghum-based diets in Liu et al. (2013c). Digestive dynamics consider the extent, rate and site of nutrient digestion along the small intestine and the bilateral, post-enteral bioavailability of amino acids and glucose. The fundamental premise is that balanced quantities of amino acids and glucose should be made available at the sites of protein synthesis to drive efficient protein deposition and growth performance (Liu and Selle, 2017).

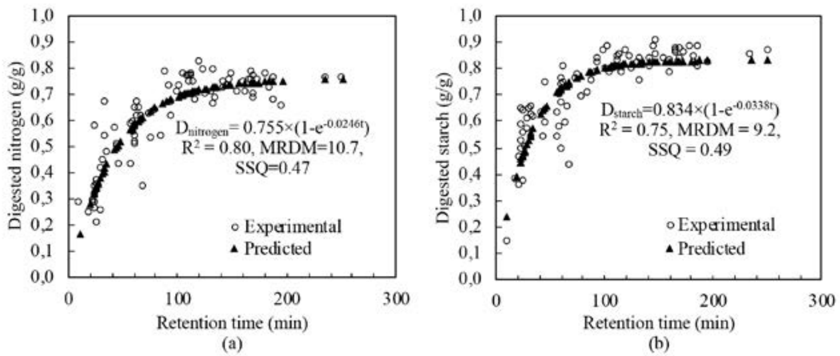

Figure 1 Protein (N) and starch digestion in sorghum-based broiler diets (Liu *et al.*, 2013c)

The 20 proteinogenic amino acids have different rates of absorption. Amino acids with larger nonpolar side chains, such as methionine, isoleucine, valine, leucine, tryptophan and phenylalanine are absorbed rapidly, whereas amino acids with polar side chains, such as glutamic acid, aspartic acid and arginine, and glycine, are absorbed slowly (Tasaki and Takahashi, 1966). Melnick *et al.* (1946) suggested that for optimum utilisation of dietary proteins, all essential amino acids must be liberated and absorbed as a result of digestion at rates permitting mutual supplementation. Subsequently, Gupta *et al.* (1958) compared digestion of different proteins and amino acid mixtures and confirmed that essential amino acids must be present simultaneously at sites of protein synthesis for efficient utilisation of dietary protein. Liu *et al.* (2013d) reported negative correlation between rates of starch digestion and retention of N, where rapid starch digestion rates depressed N retention. It was concluded by Liu and Selle (2015) digestion rates of starch and protein are correlated with feed conversion efficiency in sorghum-based broiler diets and rapidly digestible protein or slowly digestible starch benefits feed efficiency. Subsequently, correlations between growth performance and jejunal and ileal starch:protein disappearance rate ratios were reported in several studies (Truong *et al.*, 2015, 2016, Sydenham *et al.*, 2017).

RECENT UPDATES AND REMAINING CHALLENGES

Digestion rate in feed formulation, is it possible?

Since the correlation between FCR, nitrogen retention and digestion rates of starch and protein were established in sorghum-based diets (Liu and Selle, 2015), the relevance of digestive dynamics was confirmed in diets based on wheat and maize (Liu *et al.*, 2014). Determinations of digestion rates in a range of feed ingredients have been completed (Bryan *et al.*, 2019a, 2019b, Macelline *et al.*, 2020b, Toghyani *et al.*, 2020, Selle *et al.*, 2021). Furthermore, Liu *et al.* (2020) formulated diets containing six ratios of starch to protein digestion rates ranging from 1.567 to 2.453 (Table 1), but with similar amino acid profiles and energy densities. The experimental diets were offered to broiler chickens from 7 to 35 days post-hatch. Quadratic relationships ($P < 0.01$) between starch and protein digestion rates, and their ratios, with feed conversion ratio (FCR) were detected. The predicted optimal FCR of 1.446 was achieved from a starch digestion rate of 3.31 min^{-1}. An optimal FCR of 1.450 was predicted with a protein digestion rate of 2.02 min^{-1} or with a starch:protein digestion rate ratio of 1.663. Increasing protein digestion rates, or decreasing starch digestion rates, or narrowing starch and protein digestion rate ratios tended to improve feed conversion efficiency. This outcome is consistent with earlier concepts of starch and protein digestion rates and demonstrated the feasibility of incorporating starch and protein digestion rates into nutrient matrices for least-cost feed formulation. However, this study had some limitations as there were no significant differences in growth performance across the six experimental diets despite quadratic relationships between formulated rate ratios and feed conversion ratio. This suggests the extent of starch and protein digestion is critical and digestive dynamics will further optimise performance. Secondly, there was only one feeding phase from 7-35 days post-hatch and this may have confounded outcomes. Thirdly, the number of feed ingredients tested for establishing matrices for diet formulation was limited and the relationship of digestion rates between feed ingredients may or may not be additive in complete diets. Nevertheless, this study is the only one in the literature which demonstrates the possibility of formulating broiler diets on the basis of starch and protein digestion rates. More research is required to verify current findings and to apply rates of digestion in practical feed formulation.

PROTEIN-BOUND AND NON-BOUND AMINO ACIDS

The balance between protein-bound amino acid and non-bound amino acid (NBAA) has gained more recent attention from the formulation of low protein diets (Liu and Selle, 2017). Macelline *et al.* (2020a) used a triangular response surface design to compare three diets rich in soybean meal, whey protein concentrate or NBAA to determine the optimal level of NBAA inclusion in wheat-based diets. Superior weight gain and FCR was found in broiler chickens offered an equal blend of soybean meal and whey

protein diets. This diet was constituted of 84.3 g/kg whey protein concentrate and 13.4 g/kg NBAA. This finding is curious because the diet containing the highest NBAA inclusions generated relatively inferior growth performance. Liu *et al.* (2013b) reported methionine and lysine had higher rates of digestion in standard broiler diets which contained some synthetic methionine and crystalline lysine (Table 1). Apparent amino acid digestibility coefficients are largely influenced by endogenous flows of amino acids. In Liu *et al.* (2013b), threonine had the lowest ileal digestibility coefficient (0.744) and methionine had the highest (0.947), which consistent with their relative contributions to endogenous protein. Hence, the balance between intestinal uptakes of amino acid and endogenous amino acid flows is a challenges for future research. Matrix values based on amino acid digestion rates, rather than protein, may be more useful for least-cost feed formulation, especially in low protein diets.

Table 1 Diet formulations included starch and protein digestion rates (g/kg) (Liu *et al.*, 2020)

Diet	1	2	3	4	5	6
Wheat	411	262	200	200	200	200
Red sorghum	100	276	385	435	479	510
Canola seed	50.0	50.0	50.0	30.0	30.0	30.0
Soybean meal	243	234	272	257	213	165
Canola Meal	100	100	19	0	0	0
Soybean oil	35.6	27.2	19.2	18.0	11.3	10.0
L-Lys HCl	2.21	2.51	2.81	3.80	5.09	6.54
DL - Met	2.14	2.27	2.78	3.19	3.56	4.01
L-Thr	0.97	0.99	1.17	1.61	2.16	2.81
L-Trp	0.00	0.00	0.00	0.00	0.00	0.01
L-Val	0.10	0.04	0.22	0.73	1.39	2.19
L-Arg	0.00	0.00	0.02	0.92	2.16	3.57
L-Ile	0.05	0.00	0.00	0.40	1.06	1.85
L-His	0.00	0.00	0.00	0.00	0.00	0.32
Salt	1.91	1.61	1.66	0.47	0.00	0.00
Sodium bicarbonate	2.03	2.50	2.68	4.47	5.17	5.19
Potassium bicarbonate	0.00	0.00	0.00	0.00	1.55	3.93
Limestone	11.1	11.2	11.7	12.0	12.1	12.3
Di-calcium phosphate	7.20	7.21	8.19	8.69	9.09	9.57
Xylanase[1]	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[2]	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.80	0.80	0.80	0.80	0.80	0.80
Celite™	20.0	20.0	20.0	20.0	20.0	20.0
Sand	9.89	0.00	0.00	0.00	0.00	9.77
Vit-mineral Premix[3]	2.00	2.00	2.00	2.00	2.00	2.00
Calculated nutrient specifications						
Starch digestion rate	3.419	3.129	3.169	3.394	3.590	3.728
Protein digestion rate	2.181	2.107	2.219	2.083	1.817	1.519
Starch:protein digestion rate ratio	1.567	1.485	1.428	1.629	1.976	2.453

[1]Danisco 40,000 G; [2]Axtra PHY TPT 10,000

[3]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

Table 2 Amino acid digestion rates (×10^{-2} min^{-1}) in typical broiler diets (Liu *et al.*, 2013b)

Amino acid	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
Rate	3.25	2.57	2.05	2.07	4.14	5.51	2.39	2.58	2.04	2.18	2.52	2.96	2.11	2.28	2.27	1.85

RAPID MEASUREMENT? IS IT POSSIBLE?

The measurement of digestion rate involves determination of apparent digestibility coefficients at various sites of small intestine and estimation of retention time by digesta content and dietary marker concentration (Weurding *et al.*, 2001). This process is expensive and time-consuming. A few studies have reported the potential of *in vitro* assessments (Bryan *et al.*, 2019a, Bryan and Classen, 2020), where they concluded *in vitro* methods do not fully replicate normal *in vivo* digestive conditions and cannot mimic numerous biological factors involved in *in vivo* digestion and the complex interaction between different nutrients. However, in addition to ileal digestible amino acids, it is possible to include jejunal digestibilities as a quick indicator of rate of digestion (Figure 2). Figure 2 shows the more pronounced correlation between FCR and jejunal digestibilities in comparison to ileal digestibilities.

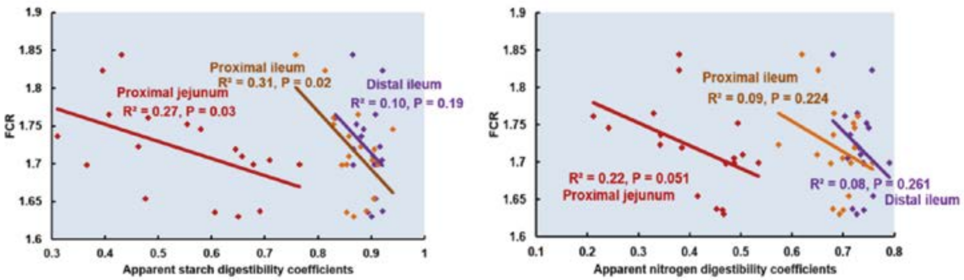

Fig. 2. Correlations between FCR and protein (nitrogen) and starch digestibilities in small intestine (Liu *et al.*, 2013a)

Other challenges

The transfer of amino acids absorbed into enterocytes along the small intestine into the portal and, ultimately, the systemic circulation requires more research. The gastrointestinal tract consumes approximately 20% of all incoming energy (Cant *et al.*, 1996) and both amino acids and glucose are oxidised in the gut mucosa to meet this demand. Moreover, post-enteral amino acid metabolism and deamination of amino acids are reviewed in Selle *et al.* (2022) which could also impact on digestive dynamics.

REFERENCES

1. Aoyagi Y, Tasaki I, Okumura J, Muramatsu T (1988) *Comparative Biochemistry and Physiology* **91A,** 165–168.
2. Bryan D, Abbott DA, Classen HL (2019a) *Animal Feed Science and Technology* **248,** 106-113.
3. Bryan D, Abbott DA, Van Kessel AG, Classen HL (2019b) *Poultry Science* **98,** 3313-3325.
4. Bryan D, Classen HL (2020) *Animals* **10**.
5. Cant JP, McBride BW, Croom WJ (1996) *Journal of Animal Science* **74,** 2541-2553.
6. Giuberti G, Gallo A, Cerioli C, Masoero F (2012) *Animal Feed Science and Technology* **174,** 163-173.

7. Gupta JD, Dakroury AM, Harper AE (1958) *Journal of Nutrition* **64,** 447-456.
8. Hurwitz S, Shamir N, Bar A (1972) *American Journal of Clinical Nutrition* **25,** 311-&.
9. Liu S, Selle P, Gill R, Cowieson A (2013a)**,** In: European Symposium on Poultry Nutrition, Potsdam, Germany.
10. Liu SY, Selle PH, Court SG, Cowieson AJ (2013b) *Animal Feed Science and Technology* **183,** 175-183.
11. Liu SY, Selle PH, Cowieson AJ (2013c) *Animal Feed Science and Technology* **185,** 85-93.
12. Liu SY, Selle PH, Cowieson AJ (2013d) *Animal Production Science* **53,** 1033-1040.
13. Liu SY, Cadogan DJ, Péron A, Truong HH, Selle PH (2014) *Animal Feed Science and Technology* **197,** 164-175.
14. Liu SY, Selle PH (2015) *Worlds Poultry Science Journal* **71,** 297-310.
15. Liu SY, Selle PH (2017) *Animal Production Science* **57,** 2250-2256.
16. Liu SY, Chrystal PV, Selle PH (2020) *Proceedings, Australian Poultry Science Symposium* **31,** 90 - 94.
17. Macelline SP, Chrystal PV, Toghyani M, Greenhalgh S, Selle PH, Liu SY (2020a) *Proceedings, Australian Poultry Science Symposium* **31,** 95-98.
18. Macelline SP, McQuade L, McInerney BV, Moss AF, Selle PH, Liu SY (2020b) *Animal Nutrition* **6,** 521 - 528.
19. Melnick D, Oser BL, Weiss S (1946) *Science* **103,** 326-329.
20. Riesenfeld G, Sklan D, Bar A, Eisner U, Hurwitz S (1980) *Journal of Nutrition* **110,** 117-121.
21. Selle PH, Moss AF, Khoddami A, Chrystal PV, Liu SY (2021) *Animal Nutrition* **7,** 450-459.
22. Selle PH, Macelline SP, Chrystal PV, Liu SY (2022) *Frontiers in Bioscience-Landmark* **27,** 126.
23. Sydenham CJ, Truong HH, Moss AF, Selle PH, Liu SY (2017) *Animal Feed Science and Technology* **227,** 32 - 41.
24. Tasaki I, Takahash N (1966) *Journal of Nutrition* **88,** 359-364.
25. Toghyani M, McQuade LR, McInerney BV, Moss AF, Selle PH, Liu SY (2020) *Plos One* **15,** e0239156.
26. Truong HH, Neilson KA, McInerney BV, Khoddami A, Roberts TH, Liu SY, Selle PH (2015) *Animal Nutrition* **1,** 220 - 228.
27. Truong HH, Neilson KA, McInerney BV, Khoddami A, Roberts TH, Liu SY, Selle PH (2016) *Animal Feed Science and Technology* **219,** 159-174.
28. Weurding RE, Veldman A, Veen WAG, van der Aar PJ, Verstegen MWA (2001) *Journal of Nutrition* **131,** 2329-2335.

Plenary Session 5:
NON-SOY PROTEIN SOURCES

PERSPECTIVE AND LIMITS IN THE USE OF ALGAE AND FREE AMINO ACIDS IN LOW PROTEIN DIETS

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INTRODUCTION

Meeting amino acid needs of commercial broilers relies heavily on the dietary inclusion of soybean meal to supply the major portion of protein needs. Although soybean oil demands in the US are increasing, human protein consumption of poultry products in China, Asia, Africa, and Middle Eastern countries is resulting in substantial growth in broiler production in many countries. Soybean meal and soybean products will continue to meet protein needs of broiler diets in emerging markets, as well as in the Americas, but tight supplies may warrant heightened use of feed grade amino acids and alternative protein meals (e.g., *Arthrospira platensis*).

FEED GRADE AMINO ACID USE IN BROILER DIETS

As amino acids were being discovered in the 1800's it was widely known that protein denaturation through acid boiling provided compounds in crystalline forms. A review has been written on the synergistic use of feed grade amino acids (i.e., methionine) in broiler diets coupled with the adoption of linear programming-least cost diet formulation (Kidd et al., 2013) which began in the 1950's. At this time, it was known that amino acids could be produced through ingredient extraction procedures. However, in the 1950's a group of Japanese researchers of Kyowa Hakko Kogyo derived a fermentation method to yield L-form amino acids by using available sugar sources and ammonia (Kinoshita et al., 1957). The fermentation method encompassed the use of *Corynebacterium glutamicum* beginning with glutamate production (Kinoshita et al., 1957), and led to industrial production of lysine, arginine, histidine, valine, isoleucine, etc. (Ikeda, 2003). It can be argued that commercial production of L-lysine via fermentation revolutionized protein nutrition of poultry (Wittmann and Becker, 2007) and paved the way to the concept of "low protein diets".

The inclusion of each sequential limiting feed grade amino acid in formulation results in a decrease of intact protein contributing ingredients (Table 1). Challenges for nutritionists represent many, of which some include: 1) understanding non-essential amino acid supply as CP decreases; 2) understanding the implications of increased leucine; and 3) understanding nutritional needs of essential, but less defined, amino acids (i.e., arginine, isoleucine, proline, histidine, and phenylalanine). Considering leucine, feed grade amino acid use or L-valine or L-isoleucine may increase if nutrient needs of the former are adjusted upwards to account for a practical antagonism. Further, an arginine to lysine minimum of 1.05 versus 1.10 completely changes diet makeup as satisfying the former necessitates increased soybean meal or L-arginine. Recent work on the branched-chain amino acids and histidine needs of broilers will be presented, in addition to gut health implications of low CP diets, as noted in a subsequent section.

ALGAE USE IN BROILER DIETS

The cost-effective nature and well-balanced amino acid profile of soybean meal dictates that

alternative protein feed sources will be difficult to introduce into practical broiler diets. Many dietary protein alternatives are region specific and often are by-products of another industry (i.e., other oil seed meals from oil extraction, distillers dried grains with solubles from ethanol production, and animal meals from commercial production). More novel dietary animal dietary protein sources, such as single cell proteins, have gained scientific interest because of their nutrient profile, biological activity, and the potential role they could play in sustainable animal production (Suman et al., 2015). Certain single cell proteins, such as microalgae, can be produced with minimal water usage and its production methods have a distinct advantage over terrestrial crops in that they're less impacted by unpredictable weather, diseases, pests, weeds, and variable soil quality (FAO, 2008; Hartman et al., 2011).
Arthrospira platensis, or commonly referred to as *Spirulina*, is a ubiquitous photosynthetic cyanobacteria and is prized as a nutrient dense microalga. *Spirulina* is high in protein (55-70%), minerals, vitamins, pigments, and essential fatty acid content (Henrikson, 1994). Many of these nutrients provide health promoting effects and *Spirulina* has proven to be beneficial in weight loss, diabetes, hypertension, viral protection, anticancer properties, cholesterol management, anemia, cardiovascular health, antioxidation, and anti-inflammation (Bishop and Zubeck, 2012). These health promoting benefits and an amino acid profile that can easily be incorporated in broiler diets make *Spirulina* an appealing alternative dietary protein source. However, *Spirulina's* limited production, costly mediums, and demand for its byproducts equate to a product that is still well above consideration for use in most commercial broiler diets.

LIMITS IN RESPONSES

Establishment of low crude protein test diets was done so after *Spirulina* analyses (Table 2) with the addition of feed grade Met, Lys, Thr, Arg, Val, Ile, and Trp sources in a diet reduced from 20 to 17% CP (Mullenix et al., 2022). The test diet was then formulated with 10% Spirulina resulting in soybean meal inclusion being reduced from 22.2 to 10.5% of diet. Diets reduced in CP resulted in 35-day old female birds (Experiment 1) and 37-day old male birds (Experiment 2) exhibiting significantly increased feed conversion. *Spirulina*, however, restored feed conversion in Experiment 2, but not Experiment 1 (Mullenix et al., 2022). Subsequent research (Mullenix, 2021) revealed that lower additions of *Sprirulina*, up to 5%, may prove efficacious in terms of meeting the amino acid needs of broilers.

Additional testing of plasma from broilers fed the former mentioned three test diets revealed that pro-inflammatory circulating cytokines (IL-3,4,6, and 18) were elevating as CP was reduced, but this inflammatory response was reduced equal to the 20% CP control when *Spirulina* was added (Mullenix et al., 2021). Further testing by Mullenix (2021) indicated that the culprit behind increased inflammation as CP is reduced may be leaky gut syndrome. Indeed, the research of Mullenix (2021) revealed numerous benefits from reductions in dietary CP (i.e., reduced water intake and foot pad lesions), but future research addressing diet amino acid supply for optimal gut health in low CP diets is warranted.

CONCLUSIONS

Reductions in dietary CP will continue to be achieved as feed grade amino acids become available. However, balancing diet needs for birds' intestinal health may dictate dietary amino acid supply. As alternative protein sources are commercialized, *Spirulina* has promise as an upcoming feed ingredient for broilers.

REFERENCES

1. Bishop, W.M. and H.M. Zubeck. 2012. Evaluation of Microalgae for use as Nutraceuticals and Nutritional Supplements. Journal of Nutrition and Food Sciences 2:147.
2. FAO. 2008. A review on culture, production, and use of Spirulina as food for humans and feeds for domestic animals and fish. Fisheries and Aquaculture Circular. No. 1034, Food and Agriculture Organization of the United Nations, Rome, Italy.
3. Hartman, G.L., E.D. West, and T.K. Herman. 2011. Crops that feed the World 2. Soybean worldwide production, use, and constraints caused by pathogens and pests. Food. Sec. 3:5-17.
4. Henrikson, R. 1994. "Superfood Spirulina microalgae future", Microalgae Spirulina, superalimento del futuro. 2nd ed. Ronore Enterprises; 222 p
5. Ikeda, M. 2003. The amino acid production process. In: Faurie, R., Thommel, J. (eds.) Adv. Biochem. Eng. Biotech. 79:1-36.
6. Kidd, M. T., P. B. Tillman, P. W. Waldroup, and W. Holder, 2013. Feed-grade amino acid use in the United States: The synergetic inclusion history with linear programming. J. Applied Poult. Res. 22:583-590.
7. Kinoshita, S., S. Udaka, and M. Shimono, 1957. Studies of the amino acid fermentation, part I. Production of L-glutamic acid by various microorganisms. J. Gen. Appl. Microbiol. 3:193-205.
8. Mullenix, G. J. 2021. Efficacy of Spirulina (Arthrospira platensis) Utilization in Broiler Diets. Dissertation, University of Arkansas, Fayetteville.
9. Mullenix, G. J., C. J. Maynard, C. M., Owens, S. J., Rochell, W. G., Bottje, R. D., Brister, M. T., Kidd 2022. Spirulina platensis meal inclusion effects on broilers fed a reduced protein diet. Journal of Applied Poultry Research, 31, 100199.
10. Mullenix, G. J., E. S. Greene, N. K., Emami, G., Tellez-Isaias, W. G., Bottje, G. F., Erf, M. T., Kidd, and S. Dridi. 2021. Spirulina platensis inclusion reverses circulating pro-inflammatory (Chemo)cytokine profiles in broilers fed low-protein diets. Front. Vet. Sci., 8, 640968.
11. Suman, G., M. Nupur, S. Anuradha, and B. Pradeep. 2015. Single Cell Protein Production: A Review. International Journal of Current Microbiology and Applied Sciences, 4(9): 251-262.
12. Wittmann, C. and J. Becker, 2007. The L-lysine story: From metabolic pathways to industrial production. In. Wendisch V. F. (ed.) Amino acid biosynthesis-pathways, regulation and metabolic engineering. 39-70.

Table 1. Broiler diet changes in response to feed grade amino acid source offerings[1]

Ingredients	Resultant diet CP level and ingredient inclusions (%)					
	24.7	24.3	22.2	20.0	20.0	17.4
Corn	43.0	44.4	57.7	53.4	61.8	73.1
Soybean meal	38.1	36.9	35.1	28.8	21.2	10.6
Distiller's grains	10.0	10.0	3.6	10.0	10.0	10.0
Methionine	0.23	0.24	0.31	0.30	0.36	0.44
Lysine[2]	0.01	0.04	0.15	0.28	0.51	0.83
Threonine	0.03	0.05	0.12	0.15	0.25	0.39
Isoleucine		0.02	0.11	0.13	0.24	0.39
Valine			0.09	0.11	0.22	0.37
Arginine				0.10	0.35	0.59
Tryptophan					0.03	0.08
Histidine[2]						0.11

Table 2. Amino acid analyses of soybean meal and Spirulina relative to crude protein (CP)[1,2]

[1]Digestible lysine (1.22% of diet) and subsequent ratios in the formulation exercise were: methionine + cystine 0.75; threonine 0.68; arginine 1.05; valine 0.77; isoleucine 0.66; tryptophan 0.16; and histidine 0.35.

[2]Sources of Cl salts.

Analysis	Soybean Meal[3]	Spirulina[4] A	B	C	D	Spirulina SID[5]
Met	1.4	2.5	2.3	2.3	2.5	86.8
Cys	1.5	0.9	0.9	0.9	1.0	86.5
Lys	6.2	4.5	4.6	4.7	4.3	86.1
Thr	3.9	4.9	4.9	4.9	4.8	85.2
Val	4.7	5.6	5.6	5.3	5.7	83.2
Ile	4.5	5.4	5.4	5.3	5.5	83.9
Arg	7.3	6.3	6.2	6.4	6.0	86.4

[1]Amino acids represented total analyzed levels relative to ingredient CP. Adapted from Mullenix (2021).

[2] Funding for some of the research presented herein was provided by USDA-NIFA SAS (#2019 69012-29905) and the Division of Agriculture, University of Arkansas, Fayetteville AR 72701.

[3]NRC (1994).

[4]Represents analyses from four separate *Spirulina* batches.

[5]Represents standardized ileal digestibility from batch A.

Plenary Session 5:
NON-SOY PROTEIN SOURCES

NUTRITIONAL CONFLICTS IN USING PROCESSED ANIMAL PROTEINS: NEW PERSPECTIVE ON THEIR USE

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The protein supply for European poultry compound feeds is highly dependent on soybean import, which is close to 17 million metric tons per year. Local production of soybean tripled in the last years, while 3 million metric tons were harvested last year. However, it is still far from meeting the market demand. On the other hand, on average 30 to 48% of animal by-products are coming each year from animal industry depending on the animal species and post-slaughter values, generating a source of the protein and fat in recycling. In this scenario PAPs may play an important role while according to the European Fat Processors and Renderers Association (EFPRA) 18.6 MT of different animal origin raw by-products on over 400 production lines are converted into 3 million tons of fat and 4.1 million tons of animal protein classified into 3 different categories. It should be stressed here that in poultry nutrition only category 3 (CAT3) can be used, which originates from different sources such as slaughter animal by-products whether suitable or not for human consumption provided they pose no risk to animals, humans and the environment, as well as domestic catering waste. While the currently binding legislation regulating PAPs in the EU allows to use only porcine proteins in poultry nutrition (cross-feeding), it should be pointed out that out of 2.85 million tons of CAT3 only approximately 0.45 MT is porcine PAPs, including blood meal and its fractions.
In 2022 in the European poultry industrial feed sector production was reduced by 3.4% compared to the previous year. The main reason is connected with the occurrence of avian influenza across Europe as well as increased production costs, resulting from feed, energy, labor and inflation. However, still more than 50 million metric tons of compound feed are produced per year. Assuming that porcine PAPs would be present in only 3% in all poultry feeds produced by the EU feed industry, 1.5 MT is needed only to cover domestic market needs. However, despite the fact that in September 2021 amendments in Annex IV of EU Regulation (EC) No. 999/2001 were passed, allowing the application of PAPs in poultry production, the availability and application of PAPs are limited and consequently few feed producers have started to use PAPs again. While animal by-products are still present, the question arises what has changed in the last 20 years, and who is the main consumer of PAPs in Europe.
The first not only nutritional, but also economic conflict, which should be listed when talking about the use of processed animal proteins is the pet food industry. Dogs (93 million) and cats (113 million) are the pets present in 46% of European homes and consuming annually over 10 million metric tons of various pet food diets. Today companion animals, particularly dogs and cats, are considered family members. The main trend in the recent years in the pet food industry is connected with humanization of companion animals resulting in the growth of premium brands, particularly in the market share of products claimed as natural and organic. Therefore, one of the outcomes of this humanization trend is the greater demand for meat type products and the presence of grain-free, gluten-free and fresh meat based diets. The PAP producers dynamically followed the pet food industry and today according to EFPRA 2.15 MT out of 2.85 MT of PAPs (CAT3) produced in the EU are used in dog and cat nutrition. Moreover, application of different meat and bone meal, feather or blood meals is not as restricted as in poultry nutrition, while no cross-feeding procedure is needed, so different PAPs can be mixed in one formulation, including multispecies meals. Finally the pet food industry is still growing by approximately 3.5% annually, in terms of willingness to pay more for different types of PAPs than any other feed sector and even buying human grade products, particularly meat and fats. Therefore one of the key factors affecting usage of PAPs in the EU is currently their price and availability driven by pet food. Today, most rendered animal by-products are simply too expensive in the least cost formulation systems used in poultry nutrition. Moreover, the growing usage in the pet food industry will continue and likely PAP prices will also increase. This situation will not change in the coming years, while dogs and particularly cats need animal origin protein, and they owners called today "petsumers" are willing to pay much higher prices for petfood, expecting that it is human grade and based on quality proteins. This is hard competition for the poultry feed industry and we should not expect changes increasing PAP availability for poultry.
Since 2001 PAP usage has been limited and most of the EU raised fowl became vegetarians. Therefore maybe we should post the question if we need PAPs in chicken or turkey diets. Looking at modern poultry meat and egg production it seems that we forgot many evolutionary adaptations present in birds in the natural environment. Chickens, turkeys and quail belong to the Galliformes, but this is a much larger order representing over 200 species, including pheasants, quails, guinea fowl and partridges. In natural conditions all of those species have different nutritional strategies and should be characterized as omnivores rather than granivores. This is not only reflected in the composition of their diets, nutritional requirements, but also anatomy and functions of their gastrointestinal tract. However, one common feature should be indicated here, which is an evolutionary adaptation to flight. Birds tend to choose feed with the highest concentration of digestible nutrients. Therefore even herbivorous birds select plant parts that are relatively high in protein and low in fiber, such as rhizomes, buds, flowers, catkins, very young leaves, and young grasses. Modern broiler chickens originate from the Red Jungle Fowl (Gallus gallus), originating in Southeast Asia and in wild populations their diet is in 60% based on invertebrates, mainly termites and their eggs. Studies conducted on turkeys in natural conditions also demonstrate the importance of diet diversification, while in 524 turkey crops and gizzards from Virginia 350 plant species and 300 invertebrate species were identified. Therefore from the evolutionary point of view, in most of the Galliformes animal origin proteins play an important role and were present long before the introduction of selective breeding and compound feed production. In contrast, commercial production of poultry meat demonstrates that usually birds perform very well on vegetarian diets. On the other hand, many metabolic disorders resulting in wet litter as well as higher water consumption are also linked to antinutritional factors found in plant material. Therefore replacing a part of soybean meal with PAPs can improve performance of turkey poults in the first stage of rearing. For years PAPs, particularly meat and bone meals, have been considered not only as a source of protein, but also energy derived from fat, as well as different minerals, particularly organic phosphorus, which is not associated with phytic acid, thus considered as more available than P present in plant origin raw materials. Therefore some research suggests that incorporation of 5% meat and bone meal (MBM) is sufficient to eliminate inorganic P reducing feed costs. In contrast, true ileal P digestibility in broiler chickens fed soybean base diets was estimated at 74-83%, while in the case of MBM true ileal P digestibility ranged from 42 to 69%.
Another potential conflict in the application of PAPs, particularly MBM and fishmeal, is their effect on gastrointestinal microbiota. It is well demonstrated that the dietary protein source (animal vs. plant) play an important role in the development of *Clostridium perfringens* populations. While a significant correlation ($P < 0.05$) between the level of dietary glycine and the numbers of *C. perfringens* in the ileum and cecum, suggesting that animal origin proteins stimulate proliferation of *C. perfringens* mainly due to 2-4 higher levels of glycine in amino acid profiles. Therefore for many years fishmeal was considered as an important factor contributing to the development of necrotic enteritis (NE) in studies on broiler chickens and most of the infection models are based on 2-5% fishmeal inclusion in the diets. Regarding MBM and its possible role in proliferation of *C. perfringens* and/or NE not much data is available. In recent years much attention has been paid to alternative proteins in animal nutrition, in the case of PAPs not many new products have been launched on the market with one exception, i.e. insect biomass. However, we should pose the

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question whether this is a real alternative and something totally new or rather a natural choice for birds, applied in their natural nutritional strategies for over 60 million years? In contrast to other PAPs, insects contain two functional fractions such as chitin and antimicrobial peptides (AMPs). According to the Antimicrobial Database (aps.unmc.edu), to date 326 AMPs have been identified from insects. At present two main insect species are produced for animal nutrition: the black soldier fly (*H. illucens*) and mealworms (*T. molitor*), with both containing AMPs. The inhibition activities of AMPs extracted from *H. illucens* have been proven against gram-negative bacteria, *E. coli, E. coli serotype O157:H7, Salmonella pullorum, S. typhimurium, S. enteritidis, Enterobacter aerogenes,* and *Pseudomonas aeruginosa;* gram-positive bacteria: *Staphylococcus aureus, methicillin-resistant S. aureus* (MRSA), *S. epidermidis, Streptococcus suis, Listeria ivanovii, Bacillus subtilis,* and *Micrococcus luteus*; as well as fungi, *Rhizoctonia solani, Sclerotinia sclerotiorum*, and *Candida albicans.* Therefore, besides nutritional properties of insect proteins and fats there could be important effects on gut health, which maybe already is used by birds in natural nutritional strategies. However, similarly to other PAPs we observe a conflict in their availability and economy. Insect industrial production is a new branch of agriculture, which is developing dynamically in many EU countries, although the currently produced amounts are limited and mostly used in the pet food and aquaculture sectors.

Finally, one important factor should be mentioned here in connection with all PAPs, i.e. energy. Rendering is a process consuming gas and electricity at many steps of pasteurization, drying and milling, therefore PAP prices are greatly dependent on energy cost and supply. Therefore, in the coming years European poultry companies will be dependent on fluctuations of energy costs not only in terms of animal rearing or feed production, but also indirectly on raw materials. Prospects on the production and usage of new PAPs are improving for instance with every new insect protein factory being opened; however, it will take several years to meet the market demand.

Plenary Session 5:
NON-SOY PROTEIN SOURCES

INSECTS IN POULTRY FEEDING: REGULATORY ISSUES AND PRODUCTIVE UPDATES

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POULTRY PRODUCTION AND SUSTAINABILITY

Poultry production, which contributes more than 30% of protein for human consumption globally through meat and eggs due to its high nutritional value and affordability, is expected to grow further. This increased demand for poultry products implies an increase in demand for poultry feed. To improve the sustainability of the poultry value chain, sustainability aspects related to the feed ingredients used in poultry feed must be considered and finding sustainable feed ingredients would be an important step toward more sustainable poultry production. The substitution of soybean meal by locally derived protein sources will most likely result in increased sustainability in terms of land use, energy use, and emissions. Insect farming, as a sustainable, environmentally friendly agricultural practice, can avoid overexploitation of natural resources by converting organic side-streams and biowaste into high-quality insect products. Producing insect protein requires a fraction of the space required to produce the same amount of protein from soybeans. Housefly (HF, *Musca domestica*) and black soldier fly (BSF, *Hermetia illucens*) rearing on 1 hectare of land, for example, could produce at least 150 tons of insect protein per year, compared to less than a ton of soybeans protein for the same area (Dunkel and Payne, 2016). Furthermore, the use of organic side-streams and biowaste from various agricultural processes reduces the amount of land used for insect feed production. Compared to other agricultural activities, insect farming has a low water footprint, with the most footprint attributed to insect feed (Miglietta et al., 2015). Oonincx (2021) reviewed the first studies on the environmental impact of insect farming, which revealed that land use associated with insect production was generally low in comparison to conventional feed ingredients. When insects are reared in temperate climates, which require extensive climate control, the energy use of insect production is often high compared to conventional products. Aside from the energy consumed during the rearing process, the commercial allowed feedstock accounts for a significant portion of the environmental impact. This effect can be mitigated by using low impact feedstock, assuming that feed can be used efficiently, lowering the environmental impact of insect production. As the sector develops, increased efficiency and thus reduced environmental impact are expected. Based on the foregoing, insect farming has the potential to be a sustainable and environmentally friendly agri-food sector producing more sustainable alternative ingredients for the feed sector. Replacing conventional ingredients with insect products in poultry feed could reduce the environmental impact of chicken meat and egg.

INSECT PRODUCTIVE UPDATE

For the implementation of insect products in poultry feed, it is required to deliver large volumes with a consistent nutritional composition and quality. Currently, insect production is taking place at different scales globally. Small-scale farmers can benefit greatly from the production of insects for feed (Barragán-Fonseca et al., 2020). Most producers begin on a small-scale to learn how to optimize conditions for the insects in terms of substrate preparation, handling larvae and adults, egg production, and harvesting time. After a successful start, they may eventually scale up to moderate or even large-scale production units (van Huis and Tomberlin, 2017). Small-scale producers can form organized cooperatives to centralize processing, reducing the need for higher investment and costs while meeting feed mills' constant, high-volume demand. In addition to small-scale producers, insect products may need to be produced on a large and highly automated scale while adhering to strict quality and safety standards. If these new products are included in feed formulations for various animals, feed manufacturers must ensure that the necessary quantity, quality, and supply are available in the various insect-derived products. Establishing a full-scale insect rearing unit could cost several million dollars. However, these units are adapted to global market needs, dealing with higher production capabilities, product quality demands, and feed safety regulations that small scale producers find more difficult to meet. Currently most of the insect farms are SMEs (e.g. small enterprises, with 10 to 50 employees or medium enterprises, with 50 to 250 employees). Micro enterprises (1 to 10 employees) also represent more than 40% of the companies active in feed production. By the end of the decade, it is expected that almost 1 out of 2 insect farms will be a large enterprise – with over 250 employees (Figure 1). Total production of the European insect sector may reach 1 million tonnes of insect meal by 2030 (IPIFF, 2021).

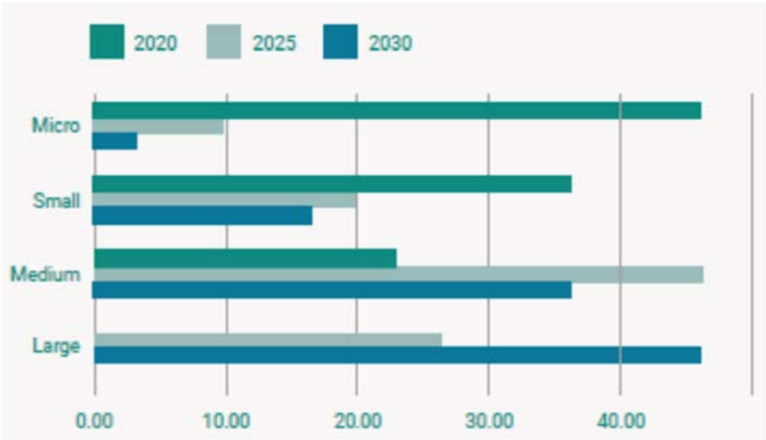

Figure 1. Relative share of insect feed business operators by company size in total production (IPIFF, 2021)

As is currently the case in agriculture and livestock production in general, future insect producers may opt for a decentralized system with many producers specializing in every stage of the insect production chain (e.g., breeding, rearing, and processing). Insect production can benefit from existing knowledge in other livestock sectors, such as breeders, fatteners, and processors.

FACTORS AFFECTING THE NUTRITIONAL COMPOSITION OF INSECTS

The nutrient composition of insects may vary depending on life stage (Kipkoech et al., 2017), sex (Meyer-Rochow et al., 2021), processing methods (Oonincx and Finke, 2021), and substrate, also called feedstock (Naser El Deen et al., 2023). For instance fatty acid and mineral composition of yellow mealworm (YMW, *Tenebrio molitor*), house cricket (HC, *Acheta domesticus*), lesser mealworm (LMW, *Alphitobius diaperinus*), and BSF can be altered by substrate (Dreassi et al., 2017, Oonincx et al., 2020, Spranghers et al., 2017). Aside from the nutritional properties of insects, the substrate can have a significant impact on their nutraceutical properties, such as the antioxidant activity of the larvae (Andreadis et al., 2022) and antimicrobial peptides (AMPs) in larvae (Vogel et al., 2018). These bioactive compounds could contribute to the immune modulation and the health promoting effects that have been observed by including insect products in poultry (de Souza Vilela et al., 2021, Facey et al., 2023). Including insect products in the diet of broilers can enhance immune response during microbial infections and increase survivability of broilers (Lee et al., 2018). Considering the antimicrobial and antibacterial activity of several insect peptides (Veldkamp et al., 2022), it is clear that there is still much to learn about modulating insect composition via the feeding substrate, not only to produce insects of high nutritional quality, but also insects that could promote animal health when used as feed ingredients. Depending on the producer, insect products can range from fresh and frozen larval biomass as final products to processed products (larval puree, insect meals, and insect oils), or highly purified bio-components. The low consistency of insect products can be attributed to the various substrates used by producers, which are frequently made up of a mixture of several side-streams. Biomass collection, delivery, and processing can have a significant impact on production costs, so producers must ensure its availability and, ideally, stability throughout the year. Research is now focusing on the safety of using substrates that are currently not yet legally allowed to grow insects, which may contribute to increased insect production capabilities. Large quantities of substrates with consistent quality, frequent delivery, and a reasonable price are required for the widespread use of insect products in poultry feed. As a result, further scaling up of the insect chain is required, and a consistent supply of raw materials is critical.

INSECT PRODUCTS IN POULTRY FEED

There are numerous applications for insect products in poultry feed. Whole insects can be processed, and the nutrient composition of the derived products (meals and oils) can change as a result. The insect industry relies on dry fractionation, which produces partially defatted meals with a crude protein (CP) content of 50-65% (DM) and an ether extract (EE) content of 8-10% (DM). The defatting process can be improved further by using solvents, resulting in highly defatted meals containing approximately 58-70% CP and 4-6% EE (DM) (Ravi et al., 2021). Compared to full-fat meals, defatted meals are more digestible by monogastrics (Schiavone et al., 2019), and are also more stable, richer in proteins, and employable at higher rates in diets for poultry. Wet fractionation is also possible by steam-blanching the larvae, which can be chemically or enzymatically pre-treated with protease or chitinase enzymes. They are then processed into a chitin-rich press cake (similar to a partially defatted meal) and a juice that, when centrifuged further, yields four distinct fractions with high CP (aqueous and solid fractions) and EE contents (lipid and cream fractions) (Ravi et al., 2021). This alternative processing method also provides significant benefits to the insect industry, as product diversification allows for increased yields in proteins, lipids, and chitin, as well as providing the market with products that can be easily incorporated into various feed formulations (Ravi et al., 2021). Insects have an excellent nutrient profile, particularly in terms of CP content and amino acid (AA) profile. Insects with the highest potential as poultry feed ingredients, such as BSF, HF, and YMW, contain 35% to 60% CP (as whole and expressed in dry matter, DM), which is typically higher than SBM (DiGiacomo and Leury, 2019, Koutsos et al., 2019). When comparing protein composition data, pay close attention to the nitrogen-to-protein (N-P) conversion factor used to calculate protein content. Indeed, the conventional N-P factor of 6.25 overestimates the true content, and more appropriate N-P factors have been proposed (Janssen et al., 2017). Because of their higher content in some essential AAs (mainly lysine, valine, and – for some insect species – methionine), BSF, HF, and YMW have a better amino acid (AA) profile than SBM (DiGiacomo and Leury, 2019, Hong et al., 2020). The CP and AA digestibility in insect meals is generally good to high, with apparent digestibility coefficients ranging from 51 to 90% (Lee et al., 2022) and from 42 to 99% (Oonincx and Finke, 2021), respectively. In addition to protein and AAs, insects are a good source of lipids (10-30%), which are also highly digestible by monogastrics, with EE apparent digestibility coefficients ranging from 78 to 99% (Gasco et al., 2022, Lee et al., 2022), and minerals such as calcium (DiGiacomo and Leury, 2019).

EU LEGISLATION AND REGULATION

IPIFF's website (https://ipiff.org/insects-eu-legislation/) contains European legislative and regulatory documents. In accordance with EU regulations in the areas of food and feed hygiene, insect producers have to adhere to best hygiene practices, comply with traceability requirements and follow appropriate mitigation and risk management procedures in case of a safety incident. Operators of insect feed businesses must implement a permanent procedure or procedures based on HACCP principles. As a resource for insect producers, IPIFF has created a good practices guide (IPIFF, 2022) with the aim to support the effective implementation of EU food and feed safety legislation. With a few exceptions, farmed insects in the European Union can only be fed vegetal materials (EU, 2011). Research on not yet allowed feed side-streams is ongoing on the bio-conversion properties and safety of insects which can upcycle these otherwise unsuitable materials into suitable feed materials (FEFAC, 2022). Feed safety should be prioritized, so expanding the substrate spectrum should be accompanied by a safety evaluation. The Regulation (EU) 2017/893 authorised the use of PAPs derived from insects and compound feed containing such processed animal protein for feeding aquaculture animals in 2017. The legislative breakthrough of 2017 was followed by a milestone in 2021, when the authorized use of insect processed animal proteins (PAPs) was extended to the pig and poultry sectors (EU, 2021).

REFERENCES

1. ANDREADIS, S. S., PANTELI, N., MASTORAKI, M., RIZOU, E., STEFANOU, V., TZENTILASVILI, S., SARROU, E., CHATZIFOTIS, S., KRIGAS, N. and ANTONOPOULOU, E. (2022) Towards functional insect feeds: Agri-food by-products enriched with post-distillation residues of medicinal aromatic plants in *tenebrio molitor* (coleoptera: Tenebrionidae) breeding. *Antioxidants* 11: 68.
2. BARRAGÁN-FONSECA, K. Y., BARRAGÁN-FONSECA, K. B., VERSCHOOR, G., VAN LOON, J. J. A. and DICKE, M. (2020) Insects for peace. *Current Opinion in Insect Science* 40: 85-93.
3. DE SOUZA VILELA, J., ANDRONICOS, N. M., KOLAKSHYAPATI, M., HILLIAR, M., SIBANDA, T. Z., ANDREW, N. R., SWICK, R. A., WILKINSON, S. and RUHNKE, I. (2021) Black soldier fly larvae in broiler diets improve broiler performance and modulate the immune system. *Animal nutrition* **7:** 695-706.
4. DIGIACOMO, K. and LEURY, B. J. (2019) Review: Insect meal: A future source of protein feed for pigs? *Animal* 13: 3022-3030.
5. DREASSI, E., CITO, A., ZANFINI, A., MATEROZZI, L., BOTTA, M. and FRANCARDI, V**.** (2017) Dietary fatty acids influence the growth and fatty acid composition of the yellow mealworm *tenebrio molitor* (coleoptera: Tenebrionidae). *Lipids* 52: 285-294.
6. DUNKEL, F. V. and PAYNE, C. (2016) Chapter 1 - introduction to edible insects, in: DOSSEY, A. T., MORALES-RAMOS, J. A. & ROJAS, M. G. (Eds) Journal, pp. 1-27 (San Diego, Academic Press).
7. EU (2011) Regulation (eu) no. 142/2011 of 25 february 2011 implementing regulation (ec) no 1069/2009 of the european parliament and of the council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing council directive 97/78/ec as regards certain samples and items exempt from veterinary checks at the border under that directive, Journal, (Brussels, Belgium).
8. EU (2021) Commission regulation (eu) 2021/1372 of 17 august 2021 amending annex iv to regulation (ec) no 999/2001 of the european parliament and of the council as regards the prohibition to feed non-ruminant farmed animals, other than fur animals, with protein derived from animals., Journal, pp. 1-17. (Official Journal).
9. FACEY, H., KITHAMA, M., MOHAMMADIGHEISAR, M., HUBER, L.-A., SHOVELLER, A. K. and KIARIE, E. G. (2023) Complete replacement of soybean meal with black soldier fly larvae meal in feeding program for broiler chickens from placement through to 49 days of age reduced growth performance and altered organs morphology. *Poultry science* 102: 102293.
10. FEFAC (2022) Fefac circular factsheet "circular feed – optimised nutrient recovery through animal nutrition". 21.
11. GASCO, L., CAIMI, C., TROCINO, A., LUSSIANA, C., ODDON, S. B., MALFATTO, V., ANEDDA, R., SERRA, G., BIASATO, I., SCHIAVONE, A., GAI, F. and RENNA, M. (2022) Digestibility of defatted insect meals for rainbow trout aquafeeds. *Journal of Insects as Food and Feed* 8: 1385-1399.
12. HONG, J., HAN, T. and KIM, Y. Y. (2020) Mealworm (*tenebrio molitor* larvae) as an alternative protein source for monogastric animal: A review. *Animals* 10: 2068.
13. IPIFF (2021) Ipiff factsheet "an overview of the european market of insects as feed", Journal.
14. IPIFF (2022) Ipiff guide on good hygiene practices for european union producers of insects as food and feed – updated september 2022, Journal, (Brussels, Belgium).
15. JANSSEN, R. H., VINCKEN, J.-P., VAN DEN BROEK, L. A. M., FOGLIANO, V. and LAKEMOND, C. M. M. (2017) Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*, *alphitobius diaperinus*, and *hermetia illucens*. *Journal of Agricultural and Food Chemistry* 65: 2275-2278.
16. KIPKOECH, C., KINYURU, J. N., IMATHIU, S. and ROOS, N. (2017) Use of house cricket to address food security in kenya: Nutrient and chitin composition of farmed crickets as influenced by age. *African Journal of Agricultural Research* 12: 3189-3197.
17. KOUTSOS, L., MCCOMB, A. and FINKE, M. (2019) Insect composition and uses in animal feeding applications: A brief review. *Annals of the Entomological Society of America* 112: 544-551.
18. LEE, J.-H., KIM, T.-K., CHA, J. Y., JANG, H. W., YONG, H. I. and CHOI, Y.-S. (2022) How to develop strategies to use insects as animal feed: Digestibility, functionality, safety, and regulation. *Journal of Animal Science and Technology* 64: 409-431.
19. LEE, J., KIM, Y. M., PARK, Y. K., YANG, Y. C., JUNG, B. G. and LEE, B. J. (2018) Black soldier fly (*hermetia illucens*) larvae enhances immune activities and increases survivability of broiler chicks against experimental infection of salmonella gallinarum. J Vet Med Sci 80: 736-740.
20. MEYER-ROCHOW, V. B., GAHUKAR, R. T., GHOSH, S. and JUNG, C. (2021) Chemical composition, nutrient quality and acceptability of edible insects are affected by species, developmental stage, gender, diet, and processing method. *Foods* 10: 1036.
21. MIGLIETTA, P. P., DE LEO, F., RUBERTI, M. and MASSARI, S. (2015) Mealworms for food: A water footprint perspective. *Water* **7:** 6190-6203.
22. NASER EL DEEN, S., VAN ROZEN, K., ELISSEN, H., VAN WIKSELAAR, P., FODOR, I., VAN DER WEIDE, R., HOEK-VAN DEN HIL, E. F., REZAEI FAR, A. and VELDKAMP, T. (2023) Bioconversion of different waste streams of animal and vegetal origin and manure by black soldier fly larvae *hermetia illucens* l. (diptera: Stratiomyidae). *Insects* 14**:** 204.
23. OONINCX, D. G. (2021) Environmental impact of insect rearing (CABI International).

24. OONINCX, D. G. A. B. and FINKE, M. D. (2021) Nutritional value of insects and ways to manipulate their composition. *Journal of Insects as Food and Feed* 7: 639-659.
25. OONINCX, D. G. A. B., LAURENT, S., VEENENBOS, M. E. and VAN LOON, J. J. A. (2020) Dietary enrichment of edible insects with omega 3 fatty acids. *Insect Science* 27: 500-509.
26. RAVI, H. K., GUIDOU, C., COSTIL, J., TRESPEUCH, C., CHEMAT, F. and VIAN, M. A. (2021) Novel insights on the sustainable wet mode fractionation of black soldier fly larvae (*hermetia illucens*) into lipids, proteins and chitin. *Processes* 9: 1888.
27. SCHIAVONE, A., DABBOU, S., PETRACCI, M., ZAMPIGA, M., SIRRI, F., BIASATO, I., GAI, F. and GASCO, L. (2019) Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on carcass traits, breast meat quality and safety. *Animal* 13: 2397-2405.
28. SPRANGHERS, T., OTTOBONI, M., KLOOTWIJK, C., OVYN, A., DEBOOSERE, S., DE MEULENAER, B., MICHIELS, J., EECKHOUT, M., DE CLERCQ, P. and DE SMET, S. (2017) Nutritional composition of black soldier fly (*hermetia illucens*) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture* 97: 2594-2600.
29. VAN HUIS, A. and TOMBERLIN, J. K. (2017) Insects as food and feed: From production to consumption.
30. VELDKAMP, T., DONG, L., PAUL, A. and GOVERS, C. (2022) Bioactive properties of insect products for monogastric animals – a review. *Journal of Insects as Food and Feed* 8: 1027-1040.
31. VOGEL, H., MÜLLER, A., HECKEL, D. G., GUTZEIT, H. and VILCINSKAS, A. (2018) Nutritional immunology: Diversification and diet-dependent expression of antimicrobial peptides in the black soldier fly *hermetia illucens*. *Developmental & Comparative Immunology* 78: 141-148.

Plenary Session 5:
NON-SOY PROTEIN SOURCES

INSECTS IN POULTRY FEEDING: TRANSLATING RESEARCH OUTCOMES INTO PRACTICE

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The consumer's demand for animal products still increases, and further increase is expected determined by the global population growth, therefore the food industry and the farmers face a great challenge (Avendaño, Sánchez, and Valenzuela 2020; WHO 2007; Govorushko 2019). Different aspects of the productive chain need to be further optimised and improved, since the consumer becomes more demanding and selective too. The increase production of animal origin foods generates a huge pressure in the environment, the water sources, and the biodiversity, with consequential climate changes (Avendaño, Sánchez, and Valenzuela 2020).
The major cost in a farm production cycle is represented by the feed, for instance in broilers, around 60 % of cost engravement is due to the feed needed to produce 1 kg of meat (Centro Ricerche Produzioni Animali, 2011). Besides, the feed ingredients used and how these are processed engrave not only the monetary aspect, but also the bird's performance (Avendaño, Sánchez, and Valenzuela 2020). High protein components are necessary for the bird's growth and for a high productivity (Sverguzova et al. 2021). Plant proteins represent the main supply source, and soybean meal is the most used in the poultry feed industry, mainly due to a balanced profile in essential amino acids (Beski, Swick, and Iji 2015; Ravindran 2013). However, this is not the rule in other plant sources, which in most cases lack a balanced essential amino acids profile. Contrarily, proteins of animal origin offer a high biological value protein, even if these are often more expensive (Saima et al. 2008; Beski, Swick, and Iji 2015). The resources needed to produce conventional protein feedstuffs are considerable, becoming economically unsustainable (Gasco et al. 2019), therefore new strategies and feedstuffs are being studied and progressively becoming more tested and adopted (Avendaño, Sánchez, and Valenzuela 2020; Bellezza Oddon et al. 2021; Colombino et al. 2021). The use of insects in poultry feed has received huge interest as a potential solution to improve sustainability of poultry diets. Insects are proficient in converting agricultural and biological residues in high qualitative nutrients, reducing drastically gas emissions and waste mass (Avendaño, Sánchez, and Valenzuela 2020; T. Veldkamp et al. 2022). Insects are high in protein and fat content, and provide other nutrients like macro and micro minerals, vitamins, etc. Insects contain bioactive compounds such as antimicrobial peptides, fatty acids, and polysaccharides, which might give protection against oxidative tissue damage and defend against microbial threats (Veldkamp et al. 2022). A wide range of insects are potential candidates as protein source, and the most studied and used insects in poultry feeding are the black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*) and mealworm (*Tenebrio molitor*) (Avendaño, Sánchez, and Valenzuela 2020). Insects are also versatile, being offered in different forms as meals, oils, dehydrated or live larvae (Veldkamp and van Niekerk 2019; Gasco et al. 2019).

A higher importance should be given to the knowledge of the natural behaviour of birds, to keep farm birds under conditions able to satisfy the animal's welfare. Farming conditions should be able to give birds the possibility to express as much as possible their natural behaviours. Fortunately, an increasing concern on the farm animal's welfare has been developed not only by the farmers, but by the consumers also, and the use of live insects in the bird's diet may highly contribute to this purpose. In this sense, few studies have been focused on testing the bird's response and beneficial effects the dietary inclusion of live insects may produce (Biasato et al. 2022; Tahamtani et al. 2021; Colombino et al. 2021; Bellezza Oddon et al. 2021; Star et al. 2020; Ipema et al. 2020; Veldkamp and van Niekerk 2019; Bongiorno et al. 2022; Ipema et al. 2022). However, scientists have realised the importance of this practice, and most probably in the near future an increasingly number of papers will be published, analysing several aspects which need to be considered, for instance: a) the bird species and their physiological state and age; b) edible insect species used and which developmental stage to use; c) time, amount, frequency and mode of provision of the insects.

Under natural conditions, poultry reveals their feeding behaviour, eating a wide-ranging diet, which includes ingredients differing for their nutritional value, and from which the bird may choose to satisfy its daily nutritional requirements. Throughout the daily routine, the two important meals occur at dawn and at dusk, since the bird needs to refill its crop after depleted overnight, and to refill it for the night (Moreby, Aebischer, and Southway 2006; Mench 2009). However, a great part of the everyday the bird forages for feed. During the foraging, the bird pecks and scratches the ground, and eats. In the case of ducks, they use their bills to sort out food items from the water (Moreby, Aebischer, and Southway 2006; Coppinger 1970; Mench 2009) or when ducks find hard, unusual or an unknown tasty food, they use to soak it into water to render it "eatable". The time dedicated to feeding might be influenced by the type of feed the bird is able to find and consume, to satisfy its needs. In the case of edible invertebrates, some authors associated the availability of this feed source to the bird's preference, however, other elements are determinant in the bird's choice, for instance colour, size, movement, etc. (Green 1984). In grey partridge chicks, observations reported that when dead insects were offered, their preference was for those larger in size and green-yellow coloured (Moreby, Aebischer, and Southway 2006). Between red-black or green-yellow ant pupae, a preference towards the last two colours was observed (Moreby, Aebischer, and Southway 2006; Potts 1986). Quails prevent red coloured insects, choosing green or black arthropods (Moreby, Aebischer, and Southway 2006). Still in grey partridge chicks, certain dead insect species (*Lasius sp.*, *Bembidion lampros.* and *Lema melanophera*) were rejected, while the same living species were quickly eaten. Larger live insects (*Lygus lineolaris*, *Adelphocoris lineolatus*) were preferred by pheasants, rather than small or very large (moths, grasshoppers) ones (Whitmore, Pruess, and Gold 1986). As describe in previous reports, the prey handling may contribute to the bird's choice, for instance chicks to eat a whole mealworm need to accomplish a complex sequence of actions rather than adequate chick-bill-size preys that might be entirely eaten at once (Moreby, Aebischer, and Southway 2006). Studies have shown that novel food items are often aversive, and that the degree of rejection is related to the previous experience of the bird and how the food differs from the one the bird is used to (Coppinger 1970).

Table 1: Live or whole dried larvae inclusion in poultry diets: inclusion modality, quantity, and related effects on bird.

		bird's age (d)	insect inclusion	daily insect distribution	effects on bird	reference
Broiler chicken	*Hermetia illucens*[1]	1 to 35	8 % TDDM[2]	4 times	Increased bird's activity; increased daily body weight gain; higher final body weight and lower total dry matter intake; better litter quality; food pad dermatitis was less severe	(Ipema et al. 2022)
		1 to 42	10 % FEDI[3]	4 times	increased bird's activity; improved leg health; no significant effect on performance	(Ipema et al. 2020)
		4 to 38	5 % FEDI[3]	once	no effects on growth and health status; improve caecal microbiota; no effect on mucin composition or local immune response	(Colombino et al. 2021; Bellezza Oddon et al. 2021)
					increased foraging; birds more active; reduction of behaviours attributable to frustration; no effect on plumage status, leg health, excreta corticosterone metabolites	(Biasato et al. 2022)
		1 to 42	Basal diet *ad libitum* + 10%, 20% or 30% defrozen larvae	once	no adverse effect on growth and feed conversion ratio with up to 20%, higher levels associated with lower protein utilization	(Seyedalmoosavi et al. 2022)
	Tenebrio molitor[1]	4 to 38	5 % FEDI[3]	once	no effects on growth and health status; improved feed conversion ratio; improve caecal microbiota; no effect on mucin composition or local immune response	(Bellezza Oddon et al. 2021; Colombino et al. 2021)
					increased foraging; birds more active; reduction of behaviours attributable to frustration; no effect on plumage status, leg health, excreta corticosterone metabolites	(Biasato et al. 2022)
	Alphitobius diaperinus[1]	3 to 8	7 to 13 mm length larvae on litter, *ad libitum*	only larvae	chicks had no access to feed, eating exclusively larvae, which causes distress and no weight gain	(Despins and Axtell 1995)
		2 to 9	7 to 13 mm length larvae on litter, *ad libitum*	feed + larvae	less feed consumption, no effect on weight gain, from 10% to 35% of protein intake from larvae	
	dried maggots	1 to 35	5%, 10%, 15% or 20% OFB[4]	with feed	improved feed conversion ratio and live body weight	(Hwangbo et al. 2009)
Slow growing chickens	*Hermetia illucens*[1]	28 to 82	10 % FEDI[3]	once	no negative effects on growth and slaughtering performance; immune organ activity might be improved	(Bongiorno et al. 2022)
Laying hen	*Hermetia illucens*[1]	469 to 546	12.5 g/ hen	6 h period	positive effects on feather condition; no effect on performance and egg quality	(Star et al. 2020)
		126 to 210	ad libitum (aprox. 52 % OEDMI[5])	163 g	decrease feed consumption; increased body weight	(Tahamtani et al. 2021)
			ad libitum or 10 % or 20 % FEDI	once	no effect on egg production, egg weight, shell thickness; no effect on hen behaviour towards a novel food	
Local chickens	*Hermetia illucens*[1]	14 until first laid egg	30 - 50 g	three times	improved egg weight, clutch size, hatched eggs, chick weight	(Dankwa et al. 2004)
	dried maggots	126 to 168	30 min each meal	three times	approx. consumption: 4.68 g (am) 5.23 g (noon) 4.6 g (pm); no effect on egg parameters; maggots seem to be preferred at noon	(Traoré et al. 2020)
Turkey	*Hermetia illucens*[1]	0 to 35	10 % FEDI[3]	once	increased daily feed intake and body weight gain; lower feed conversion ratio; reduced aggressive pecking; a tendency of lowered incidence of feather and skin damage	(Veldkamp and van Niekerk 2019)
Guinea fowl	dried maggots	308 to 357	50 g	three times	preference for cereal grains rather than dried maggots	(Traore et al. 2020)

[1]Live larvae; [2]total dietary dry matter; [3]feed estimated daily intake; [4]on feed bases; [5]on estimated dry matter intake.

Since insects are already part of the natural diet of poultry and as shown by the numerous papers published in the past ten years regarding the benefits of the use of dietary insect meals (Veldkamp et al. 2022; Gasco et al. 2019; Elahi et al. 2022), the use of live and/or dried whole insects is highly recommended and should be further studied (Table 1).

Apparently, the substitution at different levels of the bird's diet with live larvae of *Hermetia illucens* or *Tenebrio molitor* might bring wellness to the bird; their foraging behaviour is stimulated so birds become more active, the aggressive pecking is reduced, and good feathers condition is preserved. The productivity in broilers, laying hens and turkeys might be improved and no negative effects have been yet reported. At present, dietary live insect employment might be suitable in organic or free-range productive systems, while the feasibility in intensive productive systems, is reasonable difficult to be adopted, mainly for the huge bird numbers managed in these systems, consequently huge economic and logistic efforts are needed. On the other hand, the highest bird's welfare benefit may be obtained in intensive systems. Hence, it is desirable that soon, with an increasingly number of companies producing live insects, such a practice may become more approachable by all productive systems.

Considering what has been studied until now, further research is recommended, in particular on how the dietary live insect inclusion might influence the quality of life as perceived by the bird, keeping in mind that the animal's welfare is related to how the bird is inclined to behave under natural conditions.

REFERENCES

1. Avendaño, C., M. Sánchez, and C. Valenzuela. 2020. "Insectos: Son Realmente Una Alternativa Para La Alimentación de Animales y Humanos." *Revista Chilena de Nutrición* 47 (6). https://doi.org/10.4067/s0717-75182020000601029.
2. Bellezza Oddon, S., I. Biasato, A. Imarisio, M. Pipan, D. Dekleva, E. Colombino, M. T. Capucchio, et al. 2021. "Black Soldier Fly and Yellow Mealworm Live Larvae for Broiler Chickens: Effects on Bird Performance and Health Status." *Journal of Animal Physiology and Animal Nutrition* 105 (S1). https://doi.org/10.1111/jpn.13567.
3. Biasato, I., S. Bellezza Oddon, G. Chemello, M. Gariglio, E. Fiorilla, S. Dabbou, M. Pipan, et al. 2022. "Welfare Implications for Broiler Chickens Reared in an Insect Larvae-Enriched Environment: Focus on Bird Behaviour, Plumage Status, Leg Health, and Excreta Corticosterone." *Frontiers in Physiology* 13 (August). https://doi.org/10.3389/fphys.2022.930158.
4. Bongiorno, V., M. Gariglio, V. Zambotto, E.E. Cappone, I. Biasato, M. Renna, C. Forte, et al. 2022. "Black Soldier Fly Larvae Used for Environmental Enrichment Purposes: Can They Affect the Growth, Slaughter Performance, and Blood Chemistry of Medium-Growing Chickens?" *Frontiers in Veterinary Science* 9 (December). https://doi.org/10.3389/fvets.2022.1064017.
5. Centro Ricerche Produzioni Animali. 2011. "Avicultura Italiana e Costi Di Produzione."
6. Colombino, E., I. Biasato, I. Ferrocino, S. Bellezza Oddon, C. Caimi, M. Gariglio, S. Dabbou, et al. 2021. "Effect of Insect Live Larvae as Environmental Enrichment on Poultry Gut Health: Gut Mucin Composition, Microbiota and Local Immune Response Evaluation." *Animals* 11 (10). https://doi.org/10.3390/ani11102819.
7. Coppinger, R.P. 1970. "The Effect of Experience and Novelty on Avian Feeding Behavior with Reference to the Evolution of Warning Coloration in Butterflies. II. Reactions of Naive Birds to Novel Insects." *The American Naturalist* 104 (938). https://doi.org/10.1086/282666.
8. Dankwa, D., F.S. Nelson, E.O.K. Oddoye, and J.L. Duncan. 2004. "Housefly Larvae as a Feed Supplement for Rural Poultry - Research and Development Notes." *Ghana Journal of Agricultural Science* 35 (1). https://doi.org/10.4314/gjas.v35i1.1859.
9. Despins, Joseph L., and Richard C. Axtell. 1995. "Feeding Behavior and Growth of Broiler Chicks Fed Larvae of the Darkling Beetle, *Alphitobius* diaperinus." Poultry Science 74 (2): 331–36. https://doi.org/10.3382/ps.0740331.

10. Elahi, U. C.C. Xu, J. Wang, J. Lin, S.G. Wu, H.J. Zhang, and G.H. Qi. 2022. "Insect Meal as a Feed Ingredient for Poultry." *Animal Bioscience*. https://doi.org/10.5713/ab.21.0435.
11. Gasco, L., I. Biasato, S. Dabbou, A. Schiavone, and F. Gai. 2019. "Animals Fed Insect-Based Diets: State-of-the-Art on Digestibility, Performance and Product Quality." *Animals*. https://doi.org/10.3390/ani9040170.
12. Govorushko, S. 2019. "Global Status of Insects as Food and Feed Source: A Review." *Trends in Food Science and Technology*. https://doi.org/10.1016/j.tifs.2019.07.032.
13. Green, R. E. 1984. "The Feeding Ecology and Survival of Partridge Chicks (*Alectoris rufa* and *Perdix perdix*) on Arable Farmland in East Anglia." *The Journal of Applied Ecology* 21 (3). https://doi.org/10.2307/2405049.
14. Hwangbo, J., E. C. Hong, A. Jang, H. K. Kang, J. S. Oh, B. W. Kim, and B. S. Park. 2009. "Utilization of House Fly-Maggots, a Feed Supplement in the Production of Broiler Chickens." *Journal of Environmental Biology* 30 (4).
15. Ipema, A.F., E.A.M. Bokkers, Walter J.J. Gerrits, Bas Kemp, and J. Elizabeth Bolhuis. 2022. "Provision of Black Soldier Fly Larvae (*Hermetia illucens*) in Different Ways Benefits Broiler Welfare and Performance, with Largest Effects of Scattering Live Larvae." *Physiology & Behavior* 257 (December): 113999. https://doi.org/10.1016/j.physbeh.2022.113999.
16. Ipema, A. F., W.J.J. Gerrits, E.A.M. Bokkers, B. Kemp, and J. E. Bolhuis. 2020. "Provisioning of Live Black Soldier Fly Larvae (*Hermetia illucens*) Benefits Broiler Activity and Leg Health in a Frequency- and Dose-Dependent Manner." *Applied Animal Behaviour Science* 230. https://doi.org/10.1016/j.applanim.2020.105082.
17. Mench, J. A. 2009. "Behaviour of Fowl and Other Domesticated Birds." In *The Ethology of Domestic Animals: An Introductory Text, Modular Texts*. https://doi.org/10.1079/9781845935368.0121.
18. Moreby, S. J., N. J. Aebischer, and S. Southway. 2006. "Food Preferences of Grey Partridge Chicks, *Perdix perdix*, in Relation to Size, Colour and Movement of Insect Prey." *Animal Behaviour* 71 (4). https://doi.org/10.1016/j.anbehav.2005.06.017.
19. Potts, G. R. 1986. *The Partridge: Pesticides, Predation and Conservation.* Edited by Collins. First edition. London: Sheridan House Inc.
20. Ravindran, V. 2013. "Poultry Development Review: Poultry Feed Availability and Nutrition in Developing Countries - Advances in Poultry Nutrition." *Poultry Development Review*.
21. Saima, M. Akhter, M. Z.U. Khan, M. I. Anjum, S. Ahmed, M. Rizwan, and M. Ijaz. 2008. "Investigation on the Availability of Amino Acids from Different Animal Protein Sources in Golden Cockerels." *Journal of Animal and Plant Sciences* 18 (2–3).
22. Seyedalmoosavi, M. M., M. Mielenz, S. Görs, P. Wolf, G. Daş, and C. C. Metges. 2022. "Effects of Increasing Levels of Whole Black Soldier Fly (*Hermetia illucens*) Larvae in Broiler Rations on Acceptance, Nutrient and Energy Intakes and Utilization, and Growth Performance of Broilers." Poultry Science 101 (12): 102202. https://doi.org/10.1016/J.PSJ.2022.102202.
23. Star, L., T. Arsiwalla, F. Molist, R. Leushuis, M. Dalim, and A. Paul. 2020. "Gradual Provision of Live Black Soldier Fly (*Hermetia illucens*) Larvae to Older Laying Hens: Effect on Production Performance, Egg Quality, Feather Condition and Behavior." *Animals* 10 (2). https://doi.org/10.3390/ani10020216.
24. Sverguzova, S. v., I.H. Shaikhiev, Z.A. Sapronova, E.v. Fomina, and Y.L. Makridina. 2021. "Use of Fly Larvae *Hermetia illucens* in Poultry Feeding: A Review Paper." *Journal of Water and Land Development*. https://doi.org/10.24425/jwld.2021.137101.
25. Tahamtani, F. M., E. Ivarsson, V. Wiklicky, C. Lalander, H. Wall, T. Bas Rodenburg, F. A.M. Tuyttens, and C.E. Hernandez. 2021. "Feeding Live Black Soldier Fly Larvae (*Hermetia illucens*) to Laying Hens: Effects on Feed Consumption, Hen Health, Hen Behavior, and Egg Quality." *Poultry Science* 100 (10). https://doi.org/10.1016/j.psj.2021.101400.
26. Traoré, I., S. Pousga, F. Sankara, Z.G. Zongo, K. Coulibaly, J-P. Nacoulma, M. Kenis, and G.A. Ouédraogo. 2020. "Influence Des Larves Séchées de Mouches Domestiques (*Musca domestica*,

L.) Sur La Prise Alimentaire Du Poulet Local (*Gallus domesticus*, L.) Au Burkina Faso." *Journal of Animal & Plant Sciences* 45 (2). https://doi.org/10.35759/janmplsci.v45-2.2.
27. Traoré, I., S. Pousga, F. Sankara, K. Coulibaly, J-P. Nacoulma, M. Kenis, G. A. Mensah, and G. A. Ouédraogo. 2020. "Étude Du Comportement Alimentaire de La Pintade Locale (*Numida meleagris*, L.) à l'Ouest Du Burkina-Faso." *International Journal of Biological and Chemical Sciences* 14 (1). https://doi.org/10.4314/ijbcs.v14i1.13.
28. Veldkamp, T., L. Dong, A. Paul, and C. Govers. 2022. "Bioactive Properties of Insect Products for Monogastric Animals – a Review." *Journal of Insects as Food and Feed* 8 (9). https://doi.org/10.3920/JIFF2021.0031.
29. Veldkamp, T., and T. G.C.M. van Niekerk. 2019. "Live Black Soldier Fly Larvae (*Hermetia illucens*) for Turkey Poults." *Journal of Insects as Food and Feed* 5 (4). https://doi.org/10.3920/JIFF2018.0031.
30. Whitmore, R. W., K. P. Pruess, and R. E. Gold. 1986. "Insect Food Selection by 2-Week-Old Ring-Necked Pheasant Chicks." *The Journal of Wildlife Management* 50 (2). https://doi.org/10.2307/3801902.
31. WHO. 2007. "Protein and Amino Acid Requirements in Human Nutrition. Report of a Joint WHO/FAO/UNU Expert Consultation." *WHO Technical Report Series*.

Plenary Session 6:
UNSOLVED ISSUES IN POULTRY NUTRITION

"OXIDATIVE STRESS IN POULTRY: A HOLISTIC OVERVIEW FROM THE ANIMAL TO THE FINAL CONSUMER"

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INTRODUCTION

Recent history in poultry production illustrates the extent to which decisions made at the farm level have a crucial impact on meat quality. This presentation provides an updated review on the main sources of oxidative stress (OS) to domestic birds, its consequences and the application of innovative means of antioxidant protection. We emphasize strategies and interventions designed to not only improve animal health and meat quality, but also consumers' health and well-being.

ROLE OF CHIKEN MEAT IN HUMAN NUTRITION

Globally, the world faces two major issues in regards to foods and nutrition. One of them is hunger. Limited (or no access) to nutritious foods, which leads to undernutrition, stunting and eventually, death, is suffered by an estimated 8% of world population (more than 600 million people)[1]. Globally, one out of three women suffer anemia, and in Spain, 1 out of 3 children are at risk of food poverty[2]. The other issue is the first cause of death caused by a non-communicable disease: cardiovascular diseases typically linked to obesity and Type 2 diabetes mellitus (T2DM). In this case, the driving cause is unlimited access (and sustained consumption) of refined carbs and ultraprocessed foods[3]. Meat and other animal-based foods are not only responsible of these two situations but they could, in fact, play a major role in the solution. The FAO[1] has recurrently reported by the hunger is to be battled by providing nutrient-dense foods and animal-based foods (meat, dairy, fish…) are particularly lacking in underdeveloped countries and among poor population. On the other hand, it is well-known that T2DM is caused by chronic exposure to sugars and carbs which leads to insulin resistance, chronic hyperglycemia and metabolic syndrome[3]. Meat, as well as other animal products, lack such nutrients, do not induce insulin secretion and in fact, a low-carb high-quality protein diet is known to reverse T2DM condition in specific patients. Despite the many educational, academic and scientific reports claiming for cuts in meat production and consumption, the truth is that meat remains an essential component of a balanced diet[4]. Chicken in particular, remains as the most consumed type of meat worldwide and its popularity relies on its relatively low price, no religious/moral restrictions, ease to prepare and cook, and its pleasant sensory properties.

ROLE OF ANIMAL BACKGROUND (GENETICS/FEEDING/HANDLING) ON MEAT QUALITY: MYOPATHIES AS EXAMPLE

Designing the composition and quality traits in chicken meat starts at the farm. Decisions made at the farm level have crucial consequences in terms of meat quality and safety. The high incidence of chicken breast myopathies in the last two centuries is an example of such connection between the two extremes of the meat production chain. The priority of the chicken meat sector in producing fast, and efficiently, high-yield broilers may not respond only to economic reasons: the necessity of producing chicken meat for a growing population and hence, satisfy the demand of consumers worldwide, requires intensive production systems. The remarkable progress made in the poultry sector in terms of genetic selection, optimized feed formulations and proper handling led to produce 2 kg live weight broilers in less than 42 days. However, this proficient production system appeared to have caused certain pathophysiological processes in the animals that led to diverse myopathies, identified as white-striping (WS), wooden breast (WB) and spaghetti meat (SM)[5]. While the causes and quality features of these abnormal breasts may vary, the underlying mechanisms seems to be related to the onset of OS due to mitochondrial disturbance and generation of reactive-oxygen species that leads to tissue generation[5]. This is just one remarkable example on how the onset of OS is linked to animal performance and health and responsible for the poor quality of the meat. OS is in fact related to many pathophysiological conditions in animals and humans such as inflammatory processes, chronic diseases and aging[6]. Animals suffering from OS may also be more susceptible to suffer infectious diseases. It is hence, important to identify the sources of OS for animals so that we can minimize this condition and hence, avoid its negative consequences in terms of animal performance and meat quality.

OXIDATIVE STRESS (OS) AS A SOURCE OF DECREASED ANIMAL PERFORMANCE AND IMPAIRED MEAT QUALITY

OS can be defined as the imbalance between pro-oxidant factors (mainly ROS and free radicals) and the endogenous antioxidant defenses so that the latter cannot counteract/neutralize the oxidizing potential of the former[6]. Under these circumstances, ROS and radicals oxidize molecules of biological relevance (membrane lipids, proteins, DNA) which is manifested, depending on the severity of the oxidative damage and its duration, to pathological conditions (inflammation, susceptibility to infections, tumors…). Yet, under physiological conditions the endogenous antioxidant defenses is well equiped with molecules (tocopherols, ascorbic acid, carotenoids, glutathione…) and enzymes (catalase, peroxidases, dismutases…) which are able to scavenge the pro-oxidant species[7]. It is worth to highlight that as well as the immune system can be strengthened via vaccination, the endogenous antioxidant system can also be reinforced by dietary means[8]. In fact, most of the elements of such systems are essential nutrients or are synthesis in our tissues using essential nutrients and therefore, an adequate supply of certain nutrients is essential to keep a good redox balance in our cells. As far as domestic birds is concerned, these are particularly susceptible to suffer OS given the high energetic demand in skeletal muscles from animals genetically selected to synthesize large amounts of meat protein in a short period of time[6]. The energy (ATP) required for such accelerated anabolism is preferentially obtained from the degradation of carbohydrates in the presence of oxygen. The final stage of such metabolic route (oxidative phosphorylation) takes place in mitochondrial membranes and the formation of ROS in an unavoidable consequence of such metabolism. While these ROS are neutralized under homeostasis, if the production of ROS is elevated and surpass the endogenous antioxidant defenses, OS may occur. While apparently contradictory, situations of hypoxia/anoxia, such as those occurred in the onset of myopathies, facilitate ROS formation and OS, as mitochondrial activity is not efficient at low oxygen concentrations. The high demand of oxygen to produce ATP may not be fulfilled in cells when energy demand is high and oxygen supply is reduced due to impaired supportive circulatory system (i.e. myopathies) or any other circumstances (respiratory diseases, poor air circulation…)[9]. OS can also be facilitated under conditions of physical stress (cortisol release), heat stress (increased energetic demand) or dietary OS (~ feeding animals with oxidized oils/fats)[6,9]. The consequences of OS are well known in terms of animal production parameters and meat quality traits[6]. Fortunately, laying hens, chicks and broilers can be protected from OS by fortifying their endogenous antioxidant defenses as explained in the following lines.

NUTRITIONAL INTERVENTIONS TO AVOID OXIDATIVE STRESS

We can modulate the susceptibility of poultry to suffer OS through dietary means. First relevant issue to take into account is the fat quality of the feeds in terms of composition (avoid highly susceptible polyunsaturated fatty acids) and degree of oxidation (as intake of oxidized lipids leads to OS). Then, we can reinforce the endogenous antioxidant defenses by enriching broiler feeds with tocopherol, ascorbic acid/carotenoids, organic selenium and assorted forms of methionine including selenomethionine[8]. Other elements currently used in animal nutrition such as phytogenic and probiotics, display, in my opinion, the most remarkable potential, not only for protecting animals (and humans) against OS but also to improve overall well-being. Phytochemicals (used as such or in plant-based materials) are known to display assorted benefits including antioxidant, antimicrobial, anti-inflammatory, anti-proliferative and antidiabetic effects, among others[10]. Interestingly, some oxidized forms of such phytochemicals (phenolic/quinone forms) may induce a mild extent of OS in cells from the animal, inducing, as a result, the strengthening of the endogenous antioxidant defenses[11]. These species may act as "vaccines" for the antioxidant defenses in cells as they would prepare tissues to an upcoming and most severe oxidative insult. Additionally, phytochemicals act as prebiotics, promoting microbiota homeostasis and hence, intestinal health. Yet, current research in human and animal medicine explores the ability of microbiota to modulate function of distant internal organs such as central nervous system (SNC) (Brain-gut axis)[12]. It is proven that some microbial metabolites from phytochemicals act as neurotransmitters promoting assorted health benefits, including mental health and overall well-being[13]. While this is much less studies in animals, recent studies emphasize the occurrence of similar mechanisms in farm animals that worth be explored further.

CONCLUSION

This paper provides an updated review on the main sources of OS to domestic birds, its consequences and the application of innovative means of antioxidant protection. The ultimate purpose of this paper is bringing awareness of the role that animal nutritionists and meat producers have in feeding a world suffering from malnutrition and hunger. Producing enough meat to fulfil global demands is as relevant as assuring its nutritional quality, sensory properties and safety, and animal nutritionists play a major role in this relevant mission. While formulations should still be based on optimizing growth and carcass yield, feeds should also include elements that provide benefits to the animal in terms of health and well-being and to the final consumer, as he/she should be offered safe, nutritious and appealing chicken meat.

REFERENCES

1. FAO, (2022). In Brief to The State of Food Security and Nutrition in the World 2022. Repurposing food and agricultural policies to make healthy diets more affordable. Rome, FAO. https://doi.org/10.4060/cc0640en
2. Save the Children (2023). Retreive from (28/03/2023): https://www.savethechildren.es/trabajo-ong/pobreza-infantil/pobreza-infantil-en-espana
3. Willet et al., (2002); https://doi.org/10.1093/ajcn/76.1.274S
4. Klurfled (2018); https://doi.org/10.1093/af/vfy009
5. Petracci et al. (2019). https://doi.org/10.1111/1541-4337.12431
6. Estévez (2015). https://doi.org/10.3382/ps/pev094
7. Surai et al. (2019). https://doi.org/10.3390/antiox8070235
8. Surai et al. (2020). https://doi.org/10.3390/antiox9020105
9. Chen et al. (2022). https://doi.org/10.5713/ab.22.0019
10. Abdeli et al. (2021). https://doi.org/10.3390/ani11123471
11. Speisky et al. (2022). https://doi.org/10.3390/antiox11010133
12. Cao et al. (2021). https://doi.org/10.3389/fphys.2021.752265
13. Banc et al. (2023). https://doi.org/10.3390/foods12020270

Plenary Session 6:
UNSOLVED ISSUES IN POULTRY NUTRITION

NUTRITION OF LAYING HENS FOR AN EXTENDED LAYING CYCLE

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INTRODUCTION

Improvements in genetic selection have made it possible to extend the laying cycle of hens from 68 to 80-90 weeks or even to more than 100 weeks since the 1970's. During this period about 500 eggs are produced per laying hen (Van Sambeek, 2011). Extending the laying period has both financial and environmental benefits. In a longer laying cycle, the costs of egg production (e.g.: pullet purchase price, feed) are balanced by the earnings of a longer productivity period and a reduced amount of resources that are needed in the long term, as replacement of hens, depopulation and cleaning of stables would be less often required. In fact, if commercial layer flocks are replaced less often, less pullets would have to be reared, which would also have an impact on the breeder flock size. All in all, the main goal of extended laying cycles is to produce more eggs with less birds.

However, extending the production period of laying hens still faces several bottlenecks, such as the declining laying persistency and egg and bone quality, health and welfare status (Bain et al., 2016). To tackle these problems a multifactorial approach is needed: not only breeding programs have to focus on improving these traits, but also nutrition and management have to be optimized to be able to exploit the genetic potential of highly prolific layer hybrids during extended production cycles. Further, scarce information is available on the nutritional requirements of aged laying hens (>70 weeks). Considering the growing tendency towards the extension of production cycles, gaining insights in these demands is of utmost importance. Feeding laying hens according to their needs is essential to fully exploit their genetic production potential while maintaining a good birds' health status. The role of nutrition to tackle the bottlenecks of extending the laying cycle will be discussed.

SPLIT FEEDING STRATEGY

In general, laying hens are fed one single diet throughout the day, and its composition is adjusted based on the age of the birds: in each consecutive phase energy, protein, Ca and P levels are adjusted towards the end of the laying cycle. The length of each feeding phase should be defined by the performance of each specific flock and cannot be generalized (Leeson and Summers, 2009).
Alternative feeding systems for laying hens have been developed to achieve an efficient resource use. In these systems diet formulation is based on (1) birds' specific appetite for certain nutrients throughout the day (Holcombe *et al.*, 1976), (2) their ability to regulate nutrient intake based on the daily egg formation cycle (Chah and Moran, 1985) and (3) their preferences and ability to select feed ingredients according to their particle size (Bennett and Classen, 2003). Different known feeding systems are free-choice feeding, loose-mix feeding, sequential feeding and split feeding**,** i.e. feeding different diets throughout the day, with different nutritional compositions, matched to the stage of egg formation (Keshavarz, 1998). In this way, during the first half of the day (first 5-6 h after ovulation) the feed will have higher levels of essential amino acids, needed for albumen synthesis and shell membrane formation. As the egg enters the shell gland, where shell formation occurs, Ca requirement increases. A diet richer in Ca is therefore be needed during the afternoon and the evening.

Some of the studies focusing on split feeding systems tried to improve egg quality of younger hens by adjusting Ca and P levels in the morning and afternoon diet according to the egg formation cycle (Ahmad and Balander, 2003). Others focused on reducing the birds' need for specific nutrients by only supplying these (amino acids, P and Ca) when required for egg formation (De los Mozos, 2012). De los Mozos et al. (2012) found that hens aged 65 to 80 weeks can maintain a similar performance when fed 17.0% protein in the morning and 15.5% in the afternoon compared to hens fed a constant 17.0% protein level. Zhang and coon (1997) indicated that not only the level of Ca is important in the diet but also the particle size of its source. It has been suggested that incorporation of coarse Ca source particles is beneficial when these particles have low in vitro solubility, suggesting a longer retention time in the gizzard and higher availability for absorption (Zhang and Coon, 1997). Because the eggshell is formed mostly during the dark hours, providing more course limestone particles as Ca source during the afternoon could improve the utilization of this mineral and possibly the quality of the eggshell, thus reducing the amount of Ca mobilized from bone reserves. Several trials were performed by Molnar et al. (2016, 2017, 2018 a, b) investigating the effect of different ratios of fine (F) and coarse (C) limestone (L) (0FL:100CL, 30FL:70CL, 50FL:50CL) in conventional and split feeding systems for aged brown and white laying hens (> 70 weeks). Although the positive effects on performance, egg and bone quality were very small and in most cases non-significant there might be a potential for split feeding to reduce the occurrence of cracked eggs in the last phase of the production cycle. For brown laying hens 30 FL in the morning and 70 CL in the afternoon seemed to be the best strategy to maintain egg production and quality. However, for white hen providing constant levels as 50 FL in the first half and 50 CL in the second half of the day proved more beneficial. The different responses in white and brown hens to the same diets suggest that it might be necessary to apply different nutritional strategies for white and brown laying hens.

CALCIUM AND PHOSPHORUS LEVELS FOR SENIOR LAYING HENS

In order to fulfill the Ca and P requirements of several biological processes, these minerals have to be available from the blood plasma. The supply of the minerals into the plasma pools occurs by three controlled systems, i.e., the absorption of dietary Ca and P, mobilization from the medullary bone and resorption in the kidney. Phosphorus requirements are constant throughout the day, leading to small changes in the plasma pool compared to the changes that occur for the Ca plasma pool due to fluctuating Ca requirements throughout the day, mostly linked to the eggshell formation. Changes in the Ca plasma pool are regulated by the Ca regulated hormones parathyroid hormone (PTH), calcitonin and 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) (Bar, 2008). While Ca level in layer diets is increased throughout the production period, P level is reduced in each consecutive dietary phase as hens become older (Keshavarz, 2000)20 to 36 wk of age; Phase 2, 36 to 54 wk of age; and Phase 3,52 to 64 wk of age. The treatments (T. Although the Ca and P metabolism of laying hens is frequently studied, the nutritional requirements of laying hens past 70 weeks of age are still unknown. Therefore, our research group conducted two experiments to determine the available P (avP) requirement and the effect of additional phytase using diets with different feedstuffs. Wheat-based diets varying from 0.30 avP to 0.11 avP mg/kg diet with a gradually reduction of 0.05, with or without phytase supplementation, were fed to Lohmann Brown hens (90 weeks of age). No effect on performance, egg and bone quality was observed, indicating that avP can be reduced to 0.11. In a second experiment, Isa Brown hens and Dekalb White hens from 70 to 80 weeks of age, were fed a maize- or wheat-based diet with avP levels of 0.45 or 0.11. No phytase was supplemented to the diets. Breed and feed ingredients used significantly affected performance and egg quality, with egg quality of white laying hens being decreased, and this effect being more pronounced for the maize-based diet. From these two experiments, it is clear that P levels can be reduced in diets of laying hens older than 70 weeks, but to what extent this is possible, depends on the breed and feed ingredients used, indicating the importance of the feed ingredient endogenic phytase activity and of the different Ca-P metabolism of white and brown laying hens.

ENERGY/ CRUDE PROTEIN

Determining the energy (E) and crude protein (CP) needs of senior laying hens is of high importance,

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as this is linked to feed costs and environmental impact of the production system. It is generally assumed that E and protein demands of aged laying hens decreases, nevertheless specific guidelines are lacking. Our research group conducted another experiment to determine whether there is a need to reduce the feed E and/or protein level for laying hens with 74 to 91 weeks of age. Hens were distributed over 4 dietary treatments: 1) a standard laying hen diet containing 2759 kcal ME/kg and 15.5% CP, 2) a reduced CP-diet, with 10% reduction of levels of essential amino-acids Met, Cys, Lys and Thr, 3) a reduced E-diet by 5%, based on reduction of starch levels and 4) a reduced E and CP-diet, (as well aslimiting AA's). In all four diets, crude fat was maintained constant at 5.60% , and all diets were maize-soy based. Both E and CP levels did not significantly influence laying hen performance, egg production or quality. Also, no significant differences were found for liver health associated parameters such as lactate dehydrogenase, aspartate transaminase, liver weight, abdominal fat content, liver hemorrhagic and color scores. Plumage condition was also not affected by the feed E and/or CP level. A follow-up trial was performed to observe if further reductions, namely of 15% for CP and of 20% for E would also be possible. Results indicate that it is possible to reduce E by 1% and CP by 10% in feed of senior laying hens, without compromising performance or egg quality. Reductions of 15% in CP and of 20% for E did affect performance and egg quality: hens receiving the lower E diet showed an increased feed intake and a lower laying percentage, whereas hens receiving the lower CP diet, had a lower egg and yolk weight, and a lower liver health status.

PRE-LAY PERIOD

When laying hens are kept in production for extended periods, the contribution of the rearing and pre-laying period become more important and should not be forgotten. Optimal pullet development and nutrition is a prerequisite for a successful extended production cycle. Growth during rearing and the early laying period determine for a great part, how a flock will perform and how long it can be kept in production. Kwakkel et al. (2017) highlighted that during the relatively short period of rearing - 20 weeks compared to a laying cycle of 80 weeks that follows - young birds face several challenges such as dehydration during the first few days and stressful vaccinations. At the same time, their feed intake capacity has to be increased. With the goal of extending the laying cycle, perhaps not only laying hen nutrition has to be revised, but the feeding system of the rearing period also has to be re-examined.

In an innovative approach in broiler nutrition a wet, porridge-type diet was offered as a choice next to a dry feed. Birds choose consistently the wet diet, and at 21d of age, broilers consuming the wet diet achieved a higher body weight (+10%) compared to broilers eating the dry diet (Kwakkel et al., 2017). This approach could be applicable during pullet rearing to prevent dehydration, ensure a good start and so an optimal growth. It is well known that changes or deviations from the optimal growth curve will have an effect of egg production, egg weight and overall performance of the hen. Indeed, initial pullet weight at the onset of lay is related to total egg output, and the growth during this period determines how egg size will increase during the start of lay.

Another approach to ensure a good onset of lay and a high laying percentage in extended production cycles is to offer the young hen a high energetic feed to optimize nutritional uptake, or feed the laying hens a structure rich-feed to stimulate development of the gut. Both interventions were shown to affect feed intake, development of the gut and egg quality. However, the observed effects were short term, as differences were leveled out by 54 weeks of age. As in extended production cycles hens will maintain peak performance for longer, shifts between diet phases might have been too fast in this trial.

CONCLUSION

Split feeding has proved beneficial for laying hens in extended production cycles, however **further optimization** of this feeding concept is necessary. Nutritional needs for energy, crude protein and minerals of the senior laying hens (> 70 weeks) are lower compared to their younger counterparts, and diets can be adapted to these different needs without negatively affecting performance, egg- and bone quality. However, **more experiments with aged laying hens** (>70 weeks) should be conducted to gain insights into the true nutritional requirements of these senior hens. In addition, these nutritional requirements should be established also taking into account the breed metabolic differences. Revisiting pullet nutrition in the frame of extended production cycles would also contribute to a more sustainable and economically feasible extension of the production cycle.

REFERENCES

1. Ahmad, H. A., and R. J. Balander. 2003. Alternative feedig regimen of Calcium Source and Phosphorus Level for Better Eggshell Quality in Commercial Layers. J. Appl. Poult. Res. 12 (4): 509-514.
2. Bain, M. M., Y. Nys, and I. C. Dunn. 2016. Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? Br. Poult. Sci. 57:330–338
3. Bar, A. 2008. Calcium homeostasis and vitamin D metabolism and expression in strongly calcifying laying birds. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 151:477–90
4. Bennett, C. D., and H. L. Classen. 2003. Performance of two strains of laying hens fed ground and whole barley with and without access to insoluble grit. Poult. Sci. 82:147–149
5. Chah, C. C., and E. T. Moran. 1985. Egg characteristics of high performance of hens at the end of lay hen given cafeteria access to energy, protein and calcium. Poult. Sci. 64:1696–1712.
6. Dunn, I. C. 2013. Long Life Layer; genetic and physiological limitations to extend the laying period.Pages 124–129 in 19th European Symposium on Poultry Nutrition. Potsdam.
7. Holcombe, D. J., D. a Roland, and R. H. Harms. 1976. The ability of hens to regulate protein intake when offered a choice of diets containing different levels of protein. Poult. Sci. 55:1731–1737
8. Keshavarz, K. 2000. Nonphytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. Poult. Sci. 79:748–763.
9. Kwakkel, R., M. L. Elling-Staats, and M. M. Van Krimpen. 2017. How to survive a challenging childhood: rearing the laying hen.in XXIst European Symposium on Poultry Nutrition. Salou, Spain.
10. Leeson, S., and J. D. Summers. 2009. Commercial Poultry Nutrition.
11. Mozos de los, J., A. Gutierrez del Alamo, T. v. Gerwe, A. Sacranie and P. Perez de Ayala (2012a). "Oviposition feeding compared to normal feeding: effect on performance and egg shell quality." Proceedings of the 23rd Annual Australian Poultry Science Symposium, Sydney, New South Wales, Australia, 19-22 February 2012, Poultry Research Foundation.
12. Sambeek, F. Van. 2011. Breeding for 500 eggs in 100 weeks. World. Poultr.:14-16.
13. Molnar, A., Maertens, L., Ampe, B., Buyse, J., Kempen, I., Zoons, J., Delezie, E. 2016. Changes in egg quality traits during the last phase of production: is there potential for an extended laying cycle? British Poultry Science, 57 (6): 842-847.
14. Molnár, A., L. Maertens, B. Ampe, J. Buyse, J. Zoons, and E. Delezie. 2017. Supplementation of fine and coarse limestone in different ratios in a split feeding system: effects on performance, egg quality and bone strength in old laying hens. Poultry Science, 96 (6): 1659–1671.
15. Molnár, A., L. Maertens, B. Ampe, J. Buyse, J. Zoons, and E. Delezie**.** Maertens. 2018. Effect of different split-feeding treatments on performance, egg quality, and bone quality of individually housed aged laying hens. Poultry science. 97, 1, blz. 88-101
16. Molnár, A., I. Kempen, N. Sleeckx, J. Zoons, L. Maertens, B. Ampe, J. Buyse, and E. Delezie. 2018. Effects of Split Feeding on Performance, Egg Quality and Bone Strength in Brown Laying Hens in Aviary System. Journal of Applied Poultry Research, 27 (3): 401-415.
17. Zhang, B., and C. N. Coon. 1997. The relationship of calcium intake, source, size, solubility in vitro and in vivo, and gizzard limestone retention in laying hens. Poult. Sci. 76:1702–1706.

Plenary Session 6:
UNSOLVED ISSUES IN POULTRY NUTRITION

ECONOMICAL EFFICIENCY IN TURKEY NUTRITION

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INTRODUCTION

Economical feed efficiency is critical in terms of meeting the financial objectives of the turkey enterprise. This has never been more important given the volatility of the raw material market and lack of consistent supply of materials. In recent years feed cost has increased, as a proportion of total cost to the enterprise, from 65% to 70% of the total cost of turkey production.

In such situations, it is critical that nutritionists can make informed decisions in order to choose the most efficient feeding programme for different economic circumstances. This must be considered within the context of an ever-changing growing environment where the turkey's response to a given feeding regime is influenced by health challenges, environmental constraints, and increased removal of antibiotics and, in some cases, removal of coccidiostats.

REVENUE DRIVERS

Any feeding programme needs to take into account the revenue drivers of the operation. The majority of turkeys grown in Europe are processed into portions. Breast meat, in the majority of countries, is the most valuable part of the turkey carcass (Noll, 2001). While breast meat only contributes 26-28% of the weight of the bird, it represents 60-70% of the income from the carcass in most European countries (Hammond, 2006). There have been significant advances in breast meat development as selection by breeders for this trait has intensified. Typical breast meat improvement over the last 10 years is estimated at 1.6% (Noirault, 2014) and the annual rate of improvement is increasing and now estimated to be approximately +0.2% per annum. It is of economic importance to exploit this genetic potential and maximize the growth of breast meat in terms of the weight of breast meat per bird and the percentage that is takes up in the carcass (Noirault, 2015). An economic feeding programme must minimise feed cost but at the same time maximise output as efficiently as possible for both a farm situation and end processed product perspective.

NUTRIENT DENSITY RESPONSES

Economic assessments have been conducted at varying nutrient levels in different studies. Lemme *et al*, 2005, assessed the impact of different levels of essential amino acids on heavy strain turkeys in different phases of the growing period. Birds fed lower amino acids up to 9 weeks of age had lower bodyweights than those fed higher amino acid levels. Final bodyweights were not as influenced by dietary amino acid levels as much as breast meat yield, which was higher in those birds fed higher amino acid levels. Optimal economic performance (income over feed cost/bird and breast) was achieved in those birds fed higher amino density diets in the early phases, control levels of amino acids in the intermediate phase and lower amino acid levels in the later phases. Hammond (2006), fed diets based on breed recommended amino acid levels and lower amino acid levels at different phases through the growing period; 0-6, 6-12 and 12-18 weeks of age. Highest liveweight was achieved in birds fed the breed recommended amino acid levels in the last phase of growth. Highest breast meat yield was achieved at the higher amino acid density levels fed throughout all phases. The most influential phase of growth, in terms of breast meat yield, was the last phase between 12 to 18 weeks of age. In a similarly designed study (Kenny, 2018) diets varying in amino acid density relative to a breed standard, were provided in a continuous or phased regime to heavy strain male turkeys. Birds fed intermediate amino acid density diets showed a trend towards increased bodyweight relative to those fed lower amino acid density diets while breast meat yield was highest in those birds fed the highest amino acid density diets. Optimal farm margin (income over feed cost/bird) was achieved at lower amino acid densities while optimal processing margin (income over feed cost/breast meat) was achieved at intermediate to higher amino acid levels.

A further evaluation (Aviagen Turkeys Inc. unpublished data) assessed the impact of increased balanced protein (crude protein and amino acid levels) on performance of heavy strain male turkeys relative to a breed standard nutrient recommendation (Aviagen Turkeys Inc.). The evaluation showed that males fed the higher density diet gained a higher bodyweight and higher breast meat yield. Feed cost was higher for those birds fed the high-density diets relative to those fed the lower density feed. However, those birds fed the higher density feed yielded more revenue from meat, consequently those birds fed the higher density diet achieved higher margin.

The above evaluations assessed the impact of varying amino acid densities while energy density levels remained constant between treatments. Amprou (2108) assessed the effect of two digestible lysine levels and two energy levels on heavy strain turkeys fed between 62 to 124 days of age. Reducing the energy content of the diets had no impact on growth but resulted in poorer FCR. Increased digestible lysine improved growth but had no effect on FCR. A financial assessment based on mass production cost showed the lower energy and higher digestible lysine regime resulted in the lowest cost relative to all other treatments. In a series of trials, Kenny (2023) examined the impact of various energy and amino acid density diets on the performance of heavy strain male turkeys. Performance data from these trials were collated to assess the response of turkeys over a range of nutrient densities. Figure.1a,b shows liveweight and feed conversion ratio (FCR) responses to altering nutrient density. Both liveweight and FCR were responsive to amino acid and energy density, both of which were optimised at the highest nutrient densities. The data also suggests that energy density appears to impact positively on the response to increasing amino acid concentration suggesting that diet energy density should be considered when feeding higher amino acid densities.

Figure 1a,b: Liveweight and FCR responses to a range of amino acid and energy densities.

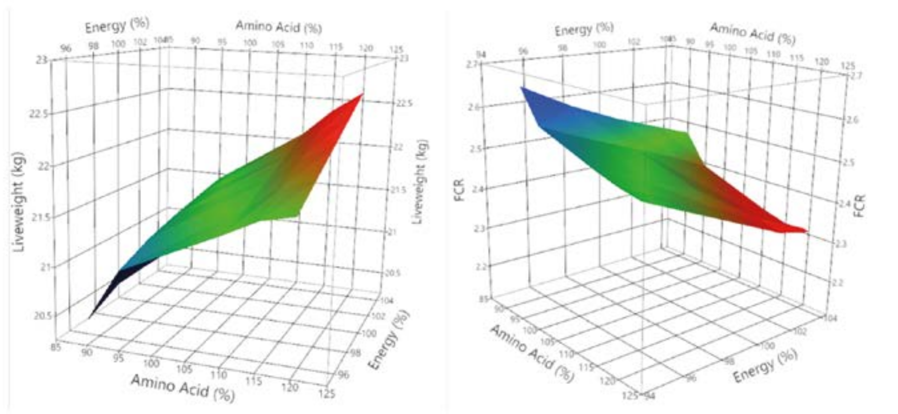


Fig 1a: Liveweight (kg) at 20.5 weeks **Fig 1b:** FCR at 20.5 weeks

Processing traits were also assessed, as with liveweight and FCR responses, increasing nutrient density had a positive impact on breast meat yield (see figure 2). At lower energy densities breast meat yield (BMY) showed a curvilinear response to increasing amino acid density. However, BMY continued to respond to increasing amino acid concentration at higher energy densities, this reinforces the importance of considering both nutrients particularly when optimising processing yield. This is a similar observation to that made by Lemme et al. 2005 who proposed that there are interactions between nutrient and energy intake with respect to their effectiveness of utilisation for meat deposition.

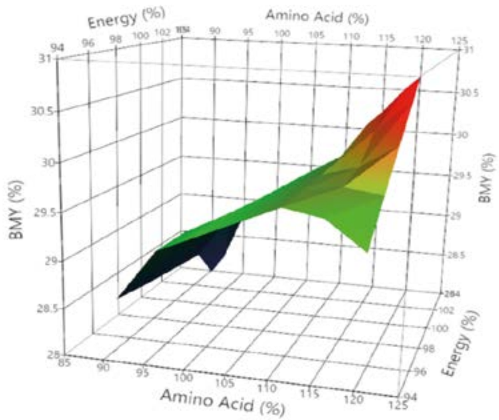


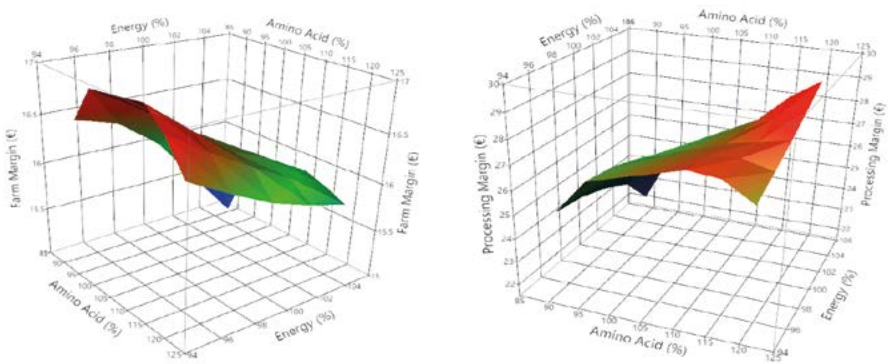
Figure 2: 20.5 week breast meat yield response to a range of amino acid and energy densities (% relative to standard).

ECONOMIC RESPONSES

The data set derived by Kenny (2023) was used to estimate economic responses, margin (after feed cost) was calculated by estimating the difference between revenue (value of live bird or meat) and feed cost at each nutrient density. Examining the profile of the surface plot graph (figure 3a) the effect of nutrient density on farm margin can be established. The same approach was taken to derive an estimate of margin for processed products, margin was estimated at each nutrient density (figure 3b) based on revenue (the value of breast meat yield) minus feed cost per bird.

Figure 3a,b: Farm and processing margin (Euro/bird) at differing amino acid and energy densities.

3a: Farm margin[1] (Euro/bird) after feed cost   3b: Processing margin[2] (Euro/bird) after feed cost



[1]*revenue based on Euro 1.71/kg liveweight*   [2]*revenue based on Euro 7.45/kg breast meat*

Optimal farm margin was achieved at an amino acid density above the standard (105% of standard) and at the lowest energy density (95% of standard). Energy density has a much greater influence on margin than amino acid density and reflects the impact of the recent increase in the cost of dietary energy. The lowest farm margin was at the highest energy (105%) and lowest amino acid concentration (90%). Based on existing raw material costs, higher margin is achieved at higher amino acid density and lower energy density than the existing standard. Higher energy densities may achieve higher biological performance however this is outweighed by increased feed cost per bird. Contrary to farm margin optimal processing margin was achieved at both the highest amino acid and energy density. This reflected the response of breast meat yield to both nutrients and the higher revenue associated with processed products relative to liveweight. Increasing amino acid density without a concomitant increase in energy results in lower margin, lowest margin was at the lower amino acid densities.

CONCLUSION

The modern turkey responds to a wide variety of nutrient levels and the choice of nutrient density is principally an economic one. The most economically efficient nutrient density is also dependent on the objectives of the enterprise, based on current European raw material costs optimal farm (bird) economic performance is achieved at lower energy densities and higher amino acid concentrations whereas optimal processing (breast meat) is achieved at higher amino acid and energy density.

REFERENCES

1. Amprou, E. 2018. Study of the response of male heavy turkeys to lysine and ideal protein intake from 0 to 48 days and energy and lysine intake from 62 to 124 day. Proc. of the 12th Turkey Science and Production Conference. 64-69.
2. Hammond, L. 2006. The influence of diet on breast meat yield. Proc. of the 29th Technical Turkey Conference: 29-32.
3. Kenny, M., 2018. Nutritional developments in Turkeys. Proc. of the 12th Turkey Science and Production Conference: 70-77.
4. Kenny, M., 2023. Feeding strategies for commercial turkeys. Proc. of the 15th Turkey Science and Production Conference.
5. Lemme, A., Petri, A., Frackenpohl, U. and Meyer, H. 2005. Response of male BUT Big 6 turkeys to varying amino acid feeding programs.
6. Noirault, J. 2014. CP01 Factors Affecting Breast Meat Yield in Turkeys. Aviagen Management Article.
7. Noirault, J. 2015. Turkey breast meat development and maximizing the genetic potential. Proc. of the 9th Turkey Science and Production Conference: 25-28.
8. Noll, S. 2001. Nutrition and management effects on growth and breast meat yield. Proc. of the 24th Technical Turkey Conference: 47-51.