Precision feeding technologies require multidisciplinary precision nutrition tools to boost efficiency in monogastric animals

M. Cambra-López[1,*], P. J. Marín-García[2] and J. J. Pascual[1]
[1]*Institute of Animal Science and Technology, Universitat Politècnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain*
[2]*Animal Production and Health, Public Health and Food Science and Technology, Veterinary School, Universidad Cardenal Herrera-CEU, 46113 Valencia, Spain*
[*]Corresponding author: María Cambra-López, macamlo@upvnet.upv.es

Abstract

Precision nutrition is an essential part of precision livestock farming, as both pursue enhancing farm profitability, efficiency and sustainability through monitoring animal bioresponses. Precision nutrition in monogastric animals needs tools capable of identifying nutrient imbalances individually over time. Moreover, knowledge of how more efficient ingredients can be synthesized and how feed additives can be intelligently released in the target site within the gastrointestinal tract are also necessary. Our objective was to assess potential tools, combining traditional nutrition with biotechnological, metabolic phenotyping, computational and protein engineering knowledge in a multidisciplinary approach. We evaluated precision nutrition tools based on: (i) metabolic phenotyping and the use of rapid individual biomarkers of amino acid imbalances, (ii) design and modelling of *de novo* proteins that are fully digestible and meet exactly animal requirements and (iii) smart presentation and release of feed additives in the gastrointestinal tract. Each application was illustrated by a case study. Precision nutrition tools addressed in this work are designed to measure and manage dynamic responses and to equal the dietary nutrient supply to the changing nutrient requirements of animals. Therefore, they can be useful in reducing inputs and outputs for efficient and respectful animal nutrition and health. These tools are necessary prerequisites that must be implemented in future automated and tailored feeding technologies.

Keywords: precision nutrition, monogastric, feed efficiency, biotechnology.

Introduction

Precision nutrition strategies are designed to equal the dietary nutrient supply to the nutrient requirements of each animal. These strategies require a well-characterized and accurate nutrient database for each ingredient, together with properly defined animal nutrient requirements (Moss et al., 2021). Precision nutrition is an essential part of precision livestock farming (PLF), as both pursue enhancing farm profitability, efficiency and sustainability (Banhazi et al., 2012) through monitoring animal bioresponses.

By definition, precision nutrition is inherently linked to animal farming practices, and is key to optimizing feed efficiency for maximal economic return and minimum losses. However, the practical implementation of precision nutrition in monogastric animals is not yet entirely achieved, becasue nutritional requirements change quickly over time, and daily variations cannot be easily met with multiphase-feeding only (Hauschild et al.,2015; Warren et al., 2000) or by blending diets (Currie et al., 2006; Moss et al., 2021), for example in broiler production. Moreover, nutritional requirements are commonly set for a population of similar animals. Using the population-feeding approach, individual variations according to body condition, genetics or animal health may be overlooked (Pomar and Remus, 2019).

When feeding systems are not perfectly adjusted to the requirements of each animal, many nutrients reach the distal part of the digestive tract, increasing the risk of digestive disorders and generating losses through the excretion of nutrients into the environment (Gaillard et al., 2020). To improve feed efficiency and reduce

both digestive disorders and the excretion of nutrients into the environment, the precise provision of nutrients and feed additives through the diet is key. For instance, protein over-feeding results in an increase of nitrogen (N) environmental load and ammonia emissions, and causes economic losses (Liu et al., 2021). Moreover, undigested protein and metabolites from protein fermentation (ammonia, amines, p-cresol and indole; Macfarlane and Macfarlane, 2012) can negatively affect intestinal health (Gilbert et al., 2018). Undigested protein in the distal gastrointestinal tract can disrupt gut function and integrity (Celi et al., 2017), and used by undesirable pathogenic bacteria (Bindari and Gerber, 2022). Furthermore, if amino acids exist in excess or are improperly balanced, they need to be catabolized in the liver. The excretion of ammonia is costly for the animal, requiring a supply of energy in the form of adenosine triphosphate (ATP) (McDonald et al., 2011). All this seriously worsens the health and productivity of animals and intensifies the environmental impact of animal production.

Consequently, in terms of protein use, there is need to adjust the combination of essential amino acids so that they meet exactly animal requirements for protein accretion and maintenance, using the ideal protein profile (Emmert and Baker, 1997); but also, to come up with valid tools that can give real feedback using animal-based biomarkers. Precision nutrition in monogastric animals, particularly in poultry, requires tools capable of identifying nutrient imbalances individually over time. Moreover, knowledge of how more efficient ingredients can be synthesized and how feed additives can be intelligently released in the target site within the gastrointestinal tract are also necessary. This would help reduce the detrimental effects of nutrient over-feeding and/or inaccurate amino acid balancing diets. These strategies are necessary prerequisites that must be implemented in future automated and tailored feeding technologies. In fact, as Moss et al. (2021) stated, the implementation of precision nutrition relies on the ability of the industry to employ precision feeding within its operations, and therefore, precise nutrition strategies must be combined with precise feeding technologies.

Our objective was to assess potential tools, combining traditional nutrition with biotechnological, metabolic phenotyping, computational and protein engineering knowledge in a multidisciplinary approach. We evaluated precision nutrition tools based on: (i) metabolic phenotyping and the use of rapid individual biomarkers of amino acid imbalances, (ii) design and modeling of *de novo* proteins that are fully digestible and meet exactly animal requirements and (iii) smart presentation and release of feed additives in the gastrointestinal tract. Each application was illustrated by a case study with focus on poultry.

Case studies

The tools addressed in the present work are key to formulating an integrated framework for precise nutrition in monogatric animals. Figure 1 illustrates how these tools can fit into a broad scheme, as indispensable to the PLF matrix that combines feeding technologies (including sensors and automatic feeding units), modeling at individual or group levels, and nutritional strategies. The potential use of metabolic phenotyping and the use of individual biomarkers, *de novo* protein desing and smart release of feed additives based on nanotechnology are discussed below.

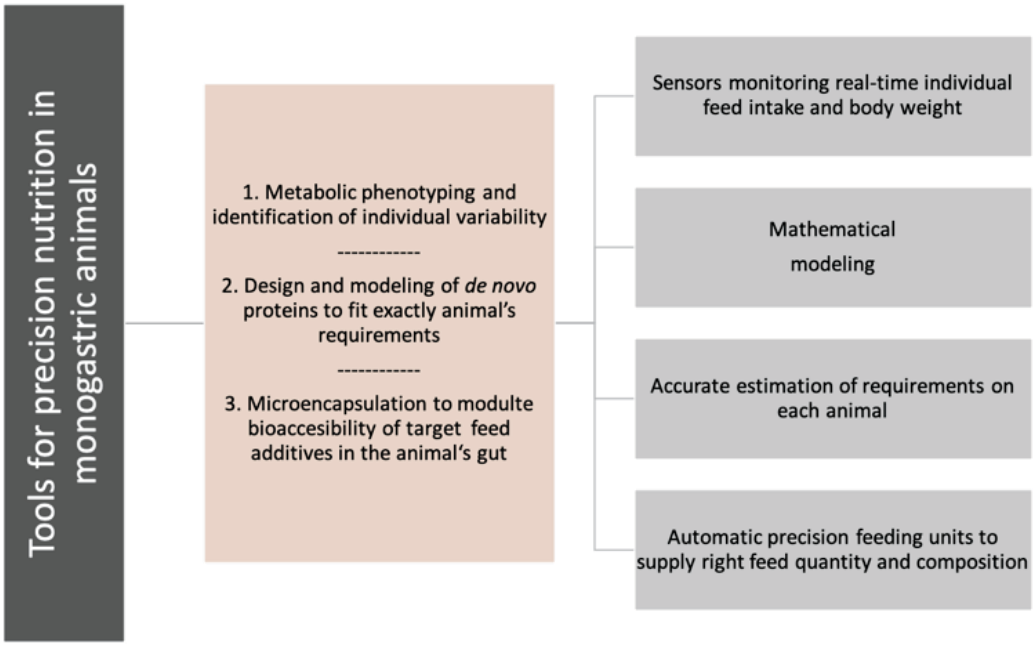


Figure 1: Integrated framework for precise nutrition in monogastric, combining nutritional strategies and feeding technologies. Adapted from Cambra-López et al. (2022).

Case study 1: Unravelling individual variations in amino acid use vs. group data in broilers through metabolic phenotyping

Experimental procedure

This case study illustrates how metabolic phenotyping can be used to detect amino acid imbalances and deficiencies in broilers. Metabolic phenotyping is a promising tool used to verify feed formulations, monitor the ideal balancing of amino acids, and aid in adjusting amino acids to precisely match animal's requirements over time. Serum uric nitrogen (SUN) corresponds to the amount of N in the form of uric acid circulating in the bird's bloodstream. The SUN content can be used as a valid biomarker to this end.

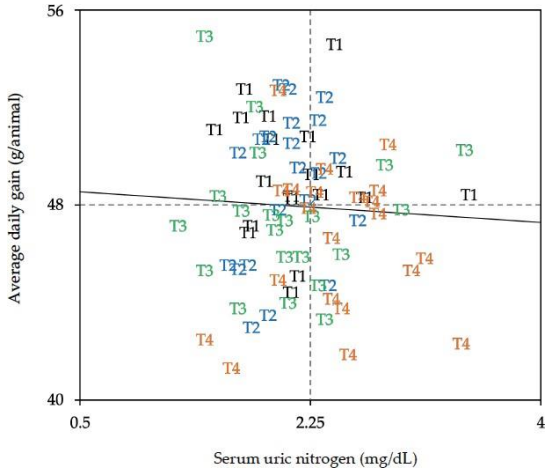
A trial was conducted to determine the effects of reducing dietary protein and adjusting valine and arginine to lysine ratios in broilers. The level of SUN metabolite was used to identify potential amino acid imbalances. The relationship between SUN and performance traits was also evaluated and individual *vs.* group variations were further examined. Three hundred and thirty-six male broilers (Ross 308) were assigned to four dietary treatments from days 14 to 35 of age. Animals were reared in floor pens (12 animals/pen) in an environmentally controlled room. Diets were formulated to meet birds crude protein requirements (20%; in T1) or to be below the crude protein requirements (18%; in T2, T3 and T4) (Belloir et al., 2017), combined with changes in valine (0.70 to 0.80) and arginine (0.90 to 1.05) to lysine ratios. Amino acid changes in dietary treatments were obtained by adding synthetic amino acids to a common basal diet based on corn, wheat and soybean meal. On day 36 of age, blood samples were obtained and SUN biomarker was analyzed as described in Cambra-López et al. (2022).

Results

Average SUN varied from 1.89 ± 0.1 to 2.26 ± 0.1 mg/dL in animals fed the tested diets (data not shown). Animals fed diet T4 showed the highest SUN values (on average +18%; $P < 0.05$) compared with groups T1 to

T3. These results agree with the performance data (weight and average daily gain, ADG), where T4 showed lower values compared with T1 and T2 ($P < 0.05$). On the other hand, SUN concentrations were similar amongst treatments T1 to T3. The final weight and ADG were the highest in animals fed diet T1, medium in diet T2, and the lowest in animals fed diets T3 and T4.

Figure 2 shows there is high individual variability in the ADG and in SUN content amongst animals, even for those within the same dietary treatment. This figure shows that animals fed the diet with the highest protein content (diet T1) are mostly in the upper half (high growth rate), and that in the low-growth and high-SUN quadrant, there are mainly animals fed the diet with a low arginine to lysine ratio (T4). Although more studies are necessary to establish the potential of SUN and other biomarkers (as glutamine or glutamate in the blood) to determine amino acid imbalances in broiler diets, this study highlights the interaction between nutrition and metabolic phenotyping to achieve this goal.



T1: 20% crude protein content, valine/lysine ratio (0.80) and arginine/lysine ratio (1.05) formulated according to current recommendations; T2: 18% crude protein content and valine/lysine ratio (0.80) and arginine/lysine ratio (1.05) formulated according to current recommendations; T3: 18% crude protein content, below-required valine/lysine ratio (0.70) and arginine/lysine ratio (1.05) formulated according to current recommendations; T4: 18% crude protein content, below-required arginine/lysine ratio (0.92) and valine/lysine ratio (0.80) formulated according to current recommendations.

Figure 2: Relationship between animal's serum uric nitrogen when fed the different experimental diets varying in crude protein, as well as valine and arginine to lysine ratios (T1 to T4); and individual average daily weight gain during the last period of the grower phase (day 28 to 35) in broilers (n = 21 animals per treatment). Adapted from Cambra-López et al. (2022).

Case study 2: Computational and protein engineering to model de novo proteins meeting exactly animal requirements

Experimental procedure

This case study illustrates how *de novo* protein design using computational and protein engineering methods could be used to design a protein sequence and structure that meets the needs of all amino acids (without excesses or defects), and which is fully digested and metabolized by the animal. The obtained protein could be synthesized and used in the future based on protein synthesis biotechnological techniques. A novel stepwise modeling approach to designing an ideal protein prototype is described below.

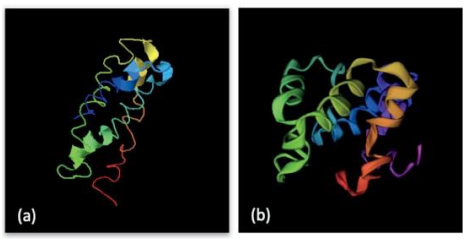
A potential primary polypeptide sequence was designed based on the true ileal amino acid digestible requirements for 21-day-old broilers described in Wu et al. (2014). From the 108 amino acids described in Wu et al. (2014), an initial protein sequence was generated using RandSeq (from the ExPASy online portal, SIB Bioinformatics Resource Portal). Then, several primary structures were designed using Peptide Cutter software's information (ExPASy Bioinformatics Portal, Swiss Institute of Bioinformatics). We chose the shortest protein sequence that fulfilled the following criteria: (i) fully meeting broiler requirements of all

amino acids, while (ii) optimizing digestive enzyme functioning. Finally, we predicted its secondary and tertiary structure and its physicochemical properties using computational methods using two online servers: i-TASSER (Yang and Zhang, 2015) and QUARK (Zhang et al., 2016). Both software were used to predict the folding of sequences.

Results

Figure 3 shows the optimal primary polypeptide sequence obtained following the modelling approach and boundary conditions described above. The resulted sequence was the most digestible sequence based on the action of the chicken digestive enzymes, because it led to a high number of free amino acids after digestion. This sequence was later subjected to manual refinement with the following considerations: (i) prioritizing those amino acids that were a frequent target for digestive enzymes in chickens; (ii) promoting isoleucine addition; (iii) giving special attention to lysine, due to its roles as the first limiting and the reference amino acid; (iv) adding arginine and tryptophan, due to their relevance as limiting amino acids; (v) avoiding cysteine excess (due to the risk of disulfide bridges), which reduces digestive enzymes' efficiency.

The secondary structure presented in Figure 3 was theoretically the most digestible protein based on the hydrolysis degree-secondary structure relationship. Furthermore, it showed an acceptable quality level in terms of reliability in the basis of C-score (accuracy) and TM-score (similarity to native structures). A complete description of quality and reliability criteria of the sequence has been given by Lledó (2020). Its secondary structure contained the lowest percentage of β-sheets (2%), and simultaneously the highest number of α-helices (41%), amongst all tested models. Additionally, it presented one of the lowest percentages of coil regions (on average, 57%).



One-letter amino acid code: A—alanine, C—cysteine, D—aspartic acid, E—glutamic acid, F—phenylalanine, G—glycine, H—histidine, I—isoleucine, K—lysine, L—leucine, M—methionine, N—asparagine, P—proline, Q—glutamine, R—arginine, S—serine, T—threonine, V—valine, W—tryptophan, Y—tyrosine.

Round 3.1: M-G-A-E-Y-S-K-A-Q-P-L-Q-I-S-P-F-Q-R-M-S-R-G-E-P-F-E-P-W-N-K-Y-A-E-P-L-D-P-L-T-R-M-G-V-G-V-Q-P-F-G-Q-K-G-N-R-E-K-Q-R-C-K-A-N-I-T-I-N-P-F-G-E-K-G-D-P-L-A-E-R-H-D-I-G-D-P-L-E-T-I-V-H-G-G-S-K-E-R-V-E-R-A-C-P-L-Q-Y-T-P-L-E-W-V-Q

Figure 3: Refined sequence with 112 amino acids modeled for complete digestion (Round 3.1) and protein 3D structure model using (a) the I-TASSER model and (b) the QUARK model. Source: Adapted from Lledó (2020).

Through the procedure followed in this work, we obtained a prototype that meets most of the conditions that a synthetic protein should have, such as being completely digestible, not generating an excess supply of amino acids (since it is ideally adjusted to the requirements of the targeted animal), and therefore coming as close as possible to the concept of an ideal protein.

Case study 3: Smart delivery of feed additives based on nanotechnology

Experimental procedure

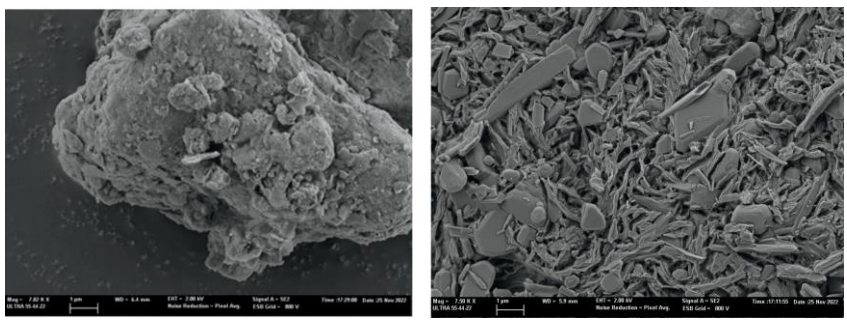
This case study describes how specific microencapsulation technologies can be used to modulate the bioavailability of target feed additives and/or bioactive molecules along the animal's gut. Its application in animal feed would contribute to achieve a controlled release of such compounds, maximizing their biological stability, protecting sensitive molecules, preserving them from environmental stress during digestion, and enhancing their effect at the target site in the animal's gut.

We examined the potential use of low-cost siliceous materials that can entrap and adsorb butyric acid and deliver it homogeneously throughout the gut. Butyric acid can contribute to intestinal mucosa integrity, being an attractive feed additive for broilers, piglets and weaning rabbits (Grilli et al., 2016). A total of five inorganic siliceous materials were used (authorized feed additives such as montmorillonite, sepiolite, bentonite, kaolinite and illite) together with a positive control – commercially available protected butyric acid. Developed delivery systems were characterized by instrumental techniques to ensure adequate butyrate encapsulation and morphological properties using Field Emission Scanning Electron Microscopy (FESEM).

Butyrate release kinetics from each delivery system was evaluated after an *in vitro* digestion assay (Pascual et al., 2000) and at certain times, simulating different digestion areas. At different moments, aliquots were taken and analyzed through gas chromatography (GC-MS) to determine butyric acid release.

Results

Figure 4 shows the morphology of two nanomaterials with butyric acid using the vaporization technique (encapsulated at 40°C) following two clay:butiric acid ratios (1:1 and 1:2).



(a) (b)

Figure 4: Photomicrographs of montmorillonite inorganic siliceous material (a) and commercially available protected butyric acid (b) obtained by field emission scanning electron microscopy. Authors (2023).

Additionally, preliminary *in vitro* results indicate butyrate release kinetics differs according to microencpasulation method and substrate. Figure 5 shows an example for two different siliceous materials and depicts butyric acid release in time.

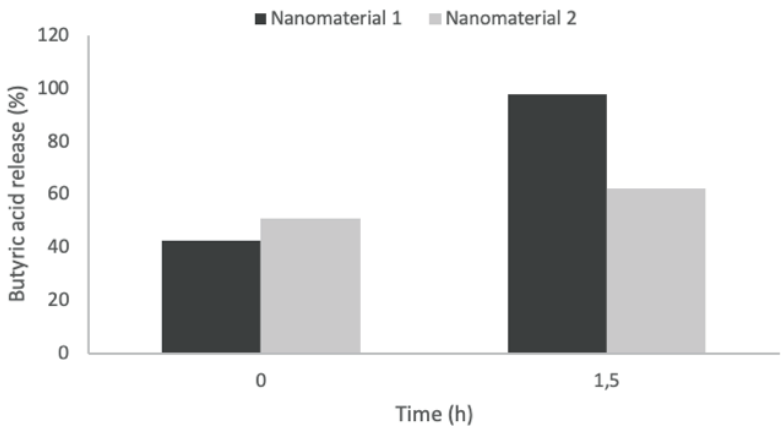


Figure 5: An example of in vitro butyric acid release kinetics of two microencapsulated materials after stomach digestion (1.5 h).

Bioactive delivery systems based on nanotechnology have been stressed as a very valuable approach for the prevention and treatment of different health disorders in humans (Martínez-Ballesta et al., 2018). The development of complex feed formulations including smart delivery systems for feed ingredients and feed additives could be effective to manage the health status of group-housed animals, that would be less dependent on the use of antibiotics. This would contribute to achieving a controlled release of substances at the site of interest avoiding over-feeding.

Conclusions

Precision nutrition tools addressed in this work are designed to measure and manage dynamic responses and to equal the dietary nutrient supply to the nutrient requirements of animals. Therefore, they can be useful in reducing inputs and outputs for efficient and respectful animal nutrition and health. These tools are necessary prerequisites that must be implemented in future automated and tailored feeding technologies.

Acknowledgments

This project was funded by SMART-RAB project AICO/2021/256 (Consellería de Innovación, Universidades, Ciencia y Sociedad Digital, Generalitat Valenciana).

References

Banhazi, T., Babinszky, L., Halas, V. and Tscharke, M. (2012) Precision Livestock Farming: Precision feeding technologies and sustainable animal production. *International Journal of Agricultural and Biological Engineering* 5, 54–61.

Belloir, P., Méda, B., Lambert, W., Corrent, E., Juin, H., Lessire, M. and Tesseraud, S. (2017) Reducing the cp content in broiler feeds: Impact on animal performance, meat quality and nitrogen utilization. *Animal* 11, 1881–1889.

Bindari, Y.R. and Gerber, P.F. (2022) Centennial Review: Factors affecting the chicken gastrointestinal microbial composition and their association with gut health and productive performance. *Poultry Science* 101, 101612.

Cambra-López, M., Marín-García, P.J., Lledó, C., Cerisuelo, A. and Pascual, J.J. (2022) Biomarkers and de novo protein design can improve precise amino acid nutrition in broilers. *Animals* 12(7), 935.

Celi, P., Cowieson, A.J., Fru-Nji, F., Steinert, R.E., Kluenter, A.-M. and Verlhac, V. (2017) Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production. *Animal Feed Science and Technology* 234, 88–100.

Currie, R., MacIsaac, J. and Rathgeber, B. (2006) Increasing the number of phases fed to broiler chickens by blending diets. *Poultry Science* 85, 52–52.

Emmert, J.L. and Baker, D.H. (1997) Use of the ideal protein concept for precision formulation of amino acid levels in broiler diets. *Journal of Applied Poultry Research* 6, 462–470.

Gaillard, G., Brossard, L. and Dourmad, J-Y. (2020) Improvement of feed and nutrient efficiency in pig production through precision feeding. *Animal Feed Science and Technology* 268, 114611.

Gilbert, M.S., Ijssennagger, N., Kies, A.K. and van Mil, S.W.C. (2018) Protein fermentation in the gut; implications for intestinal dys-function in humans, pigs, and poultry. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 315, G159–G170.

Grilli, E., Tugnoli, B., Foerster, C.J. and Piva, A. (2016) Butyrate modulates inflammatory cytokines and tight junctions components along the gut of weaned pigs. *Journal of Animal Science* 94 (3), 433-436.

Hauschild, L., Bueno, C.F.D., Remus, A., Gobi, J.D.P., Isola, R.D.G. and Sakomura, N.K. (2015) Multiphase feeding program for broilers can replace traditional system. *Scientia Agricola* 72, 210–214.

Liu, S.Y., Macelline, S.P., Chrystal, P.V. and Selle, P.H. (2021) Progress towards reduced-crude protein diets for broiler chickens and sustainable chicken-meat production. *Journal of Animal Science and Biotechnology* 12, 20.

Lledó, C. (2020) De novo design of an ideal protein for feeding meat chickens from 0 to 21 days. Bachelor's Thesis, Universitat Politècnica de València, Spain.
Macfarlane, G.T. and Macfarlane, S. (2012) Bacteria, colonic fermentation, and gastrointestinal health. *Journal of AOAC International* 95, 50–60.
Martínez-Ballesta, M., Gil-Izquierdo, Á., García-Viguera, C. and Domínguez-Perles, R. (2018) Nanoparticles and controlled delivery for bioactive compounds: Outlining challenges for new "smart-foods" for health. *Foods* 7, 72.
McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A. and Wilkinson, L.G. (2011) *Animal Nutrition*, 7th ed.; Pearson, Prentice Hall: Hoboken, NJ, USA.
Moss, A.F., Chrystal, P.V., Cadogan, D.J., Wilkinson, S.J., Crowley, T.M. and Choct, M. (2021) Precision feeding and precision nutrition: A paradigm shift in broiler feed formulation? *Animal Biosciences* 34, 354–362.
Pascual, J.J., Cervera, C. and Fernández-Carmona, J. (2000) Comparison of different in vitro digestibility methods for nutritive evaluation of rabbit diets. *World Rabbit Science* 8(2), 93-97.
Pomar, C. and Remus, A. (2019) Precision pig feeding: A breakthrough toward sustainability. *Animal Frontiers* 9, 52–59.
Warren, W.A. and Emmert, J.L. (2000) Efficacy of phase-feeding in supporting growth performance of broiler chicks during the starter and finisher phases. *Poultry Science* 79, 764–770.
Wu, G. (2014) Dietary requirements of synthesizable amino acids by animals: A paradigm shift in protein nutrition. *Journal of Animal Science and Biotechnology* 5, 34.
Yang, J. and Zhang, Y. (2015) Protein structure and function prediction using I-TASSER. *Current Protocols in Bioinformatics* 52, 5.8.1-5.8.15.
Zhang, W., Yang, J., He, B., Walker, S.E., Zhang, H., Govindarajoo, B., Virtanen, J., Xue, Z., Shen, H.-B. and Zhang, Y. (2016) Integration of QUARK and I-TASSER for ab initio protein structure prediction in CASP11: ab initio structure prediction in CASP11. *Proteins* 84, 76–86.