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Amino Acids Were Not All Created Equal

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"The body does not have a store for amino acids. This means that dietary amino acids, in excess of those required for protein synthesis, are rapidly catabolized." (Brosnan, 2003)

Abstract

The contention that amino acids were not all created equal is based on the simple premise that intestinal uptakes of non-bound (synthetic, crystalline, feed-grade) amino acids are more rapid than their protein-bound counterparts. The post-enteral ramifications of this difference in bioequivalence are amplified in broiler chickens given their express growth rates and just one complication is the post-prandial oxidation of amino acids. The lack of bioequivalence between non-bound and protein-bound amino acids is a real obstacle to the development and adoption of reduced-crude protein diets that have the potential to promote sustainable chicken-meat production Thus, the purpose of this paper is to examine our contention that amino acids were no longer created equal with the introduction of non-bound amino acids.

1 Background

Reduced-crude protein (CP) diets have the potential to enhance sustainable chicken-meat production (Selle and Liu, 2020). Typically in reduced-CP diets, feed grain inclusions are increased at the expense of soybean meal but amino acid requirements are still met by increasing inclusions of non-bound (synthetic, crystalline, feed-grade) amino acids. In reality, reduced-CP diets are neither novel nor radical as non-bound methionine, lysine and threonine have been obtainable at feasible prices for decades. Probably without recognition, their availability has already permitted substantial reductions in dietary CP concentrations (Pesti, 2009). However, the current availability of an increasing array of non-bound amino acids provides the opportunity for further reductions in dietary CP levels. This is advantageous in terms of the environment (reduced N excretion and NH_3 emissions), litter quality and bird welfare, flock health and possibly food safety (Greenhalgh et al., 2020a). Moreover, further

reductions in dietary CP would substantially decrease the chicken-meat industry's reliance on (expensive) imported soybean meal in many countries of the world. Indeed, it may be argued that the present Australian dependence on imported soybean meal is simply incompatible with sustainable chicken-meat production.

For example, the transition from 222 to 165 g/kg CP in maize-based diets reduced soybean meal inclusions by 66.2% in Chrystal et al. (2021). This transition generated significant improvements in feed intake and weight gain without compromising FCR. Similarly, the dietary CP reduction from 215 to 195 g/kg in wheat-based diets reduced soybean meal inclusions by 40.5% in Yin et al. (2020) without influencing growth performance. However, the further reduction to 165 g/kg CP significantly compromised FCR. It should be emphasised that the majority of our reduced-CP feeding studies have not yielded equally promising outcomes but this comparison highlights the importance of the feed grain on which reduced-CP diets are based.

2 Amino acid timelines

Amino acids were created shortly after the "big-bang" but it was not until 1806 when the first amino acid, asparagine, was identified and threonine, the last amino acid, was identified by William Cumming Rose in 1935 (Simoni et al., 2002). Methionine was identified in 1922 at Columbia University; however, during the 1940s Dr Werner Schwarze developed the methodology to synthesise *d,l*-methionine efficiently in Germany (Wilke, 2014). Subsequently, Degussa (now Evonik) made *d,l*-methionine available for animals in 1953. Critically, this was the point in time when amino acids were no longer all created equal with the introduction of non-bound amino acids. This is simply because intestinal uptakes of non-bound methionine are more rapid than protein-bound methionine, as demonstrated in rats by Canolty and Nasset (1975). The bioequivalence of non-bound and protein-bound amino acids is considered in more detail in Selle et al. (2022a).

3 Ideal amino acid ratios

Dietary amino acid requirements are usually expressed in relation to lysine (100) as ideal amino acid ratios (IAAR), which are one of the cornerstones of least-cost formulated diets for broiler chickens. "Ideal amino acid ratios" have replaced "ideal protein ratios" to consider both essential and the so-called non-essential amino acids with emphases on glycine and proline (He et al., 2021). Two sets IAAR for broiler chickens from 22 to 42 days post-hatch are shown in Table 1 where amino acid concentrations relative to lysine in whole-body protein, breast muscle and feathers are also tabulated for comparative purposes. A perusal of the IAAR proposed by Wu (2014) and Maharjan et al. (2021) discloses conspicuous differences in leucine, phenylalanine, alanine, cysteine, glycine and proline.

This lack of agreement in standard broiler diets is not ideal but the formulation of reduced-CP broiler diets becomes an even greater challenge. IAAR have been fine-tuned for decades; nevertheless, very few studies have compared different sets of recommendations in broiler chickens. In one such study, Salehifar et al. (2012) reported tangible differences in weight gain and FCR in young broilers offered diets based on six different, but ostensibly similar, IAAR.

Therefore, two sets of recommendations were compared in wheat-based broiler diets containing either 210 or 180 g/kg CP from 14 to 35 days post-hatch in Macelline et al. (2022a). Treatment interactions between dietary CP and amino acid ratios were observed for weight gain (P = 0.039), FCR (P < 0.001) and relative fat-pad weights (P = 0.022) as one IAAR was superior in the standard, 210 g/kg CP diet, but the alternative IAAR was superior in the reduced, 180 g/kg CP diet. For example, Ratio A supported significantly lower FCR by 3.25% (1.579 versus 1.632) in birds offered 180 g/kg CP diets, but Ratio B supported significantly lower FCR by 4.24%% (1.423 versus 1.486) in 210 g/kg CP diets. Therefore, appropriate ideal amino acid ratios are subject to dietary CP concentrations, which is a tangible complicating factor. Interestingly, there was a quadratic relationship (r = 0.863; P < 0.0001) between dietary non-bound amino acids and FCR in the Macelline et al. (2022a) study, which suggests that FCR were compromised once non-bound amino acid inclusions exceeded 14.97 g/kg.

The amino acid requirements for feathering may complicate the accurate definition of IAAR in reduced-CP diets for poultry in comparison to pigs. Leme et al. (2022) completed a detailed investigation into the amino acid composition of feathers which contain in excess of 90% protein and represent 5.1% of bodyweight in broilers at 42 days post-hatch (Sklan and Noy, 1994). Instructively, Leeson and Walsh (2004) noted that diets with less than 160 g/kg CP can trigger poor feathering in young broiler breeder chicks that is not corrected by relatively high dietary non-bound amino acid inclusions. Poor feathering and even feather-pecking was observed in one of our studies (Greenhalgh et al., 2020a) where the transition from 215 to 162.5 g/kg CP in wheat-based diets generated inferior feather scores (3.70 versus 1.80; P = 0.006) which were quadratically associated (r = 0.443; P < 0.005) with retarded weight gains. As tabulated, amino acid profiles of feathers are radically different from whole-body protein and breast muscle as they contain a paucity of lysine but are prolific in serine, proline and glutamic acid. In addition, feather amino acid profiles vary significantly with age, especially from 14 to 42 days post-hatch (Stilborn et al., 1997); moreover, amino acids are partitioned to feathering at the expense of skeletal muscle accretion in turkeys (Wylie et al., 2003) and, presumably, broiler chickens. Thus, partitioning of amino acids for feathering, skeletal muscle

deposition or a range of metabolic functions (Baker, 1991) could confound the accuracy of ideal amino acid ratios.

4 Intestinal amino acid uptake rates in broiler chickens

That intestinal uptakes of non-bound amino acids are more rapid than their protein-bound counterparts in broiler chickens offered standard diets was unequivocally demonstrated by Liu et al. (2013). Sorghum-based diets contained 3.7 g/kg lysine (as lysine HCl), 3.4 g/kg *d,l*-methionine and 1.3 g/kg threonine or a total of 8.4 g/kg non-bound amino acids. It may be deduced from this study that the mean digestion constant of 13 protein-bound amino acids was $2.35*10^{-2}\text{min}^{-1}$ as opposed to $8.78*10^{-2}\text{min}^{-1}$ for non-bound lysine and $8.49*10^{-2}\text{min}^{-1}$ for non-bound methionine. Therefore, intestinal uptakes of non-bound amino acids were nearly four times more rapid than protein-bound amino acids.

The likelihood is that this differential in intestinal amino acid uptake rates triggers post-enteral amino acid imbalances and the catabolism of surplus amino acids. This is not straightforward as amino acids may be denied entry into the portal and, ultimately, systemic circulations because of their entries into either anabolic and/or catabolic pathways in the enterocytes of the gut mucosa (Stoll et al., 1999). Glucose and glutamine are prime energy substrates within enterocytes (Fleming et al., 1997) and it has been suggested that non-bound amino acids are less likely to be catabolised because of their proximal intestinal uptakes where more starch/glucose is available as an alternative energy substrate (Moss et al., 2018). If so, post-enteral amino acid imbalances would be compounded in birds offered reduced-CP diets. Schreurs et al. (1997) argued that amino acids in the systemic circulation are rapidly used for protein synthesis or, if not, undergo metabolic degradation, which led to the concept of post-prandial amino acid oxidation. This is in agreement with the Brosnan (2003) declaration that dietary amino acids in excess of protein synthesis requirements are rapidly catabolised, although this distinction does overlook the multiplicity of functional roles performed by amino acids (Wu, 2010). This proposal was subsequently investigated in several studies including Nolles et al. (2009) in which postprandial oxidation of egg white protein versus a corresponding blend of non-bound amino acids was determined via $^{13}CO_2$ breath tests in rats. After a short adaptation period, postprandial oxidative losses of non-bound leucine were significantly higher than protein-bound leucine by an approximate factor of 1.52 (24.8 versus 16.3%) and by a factor of 1.24 (20.2 versus 16.3%) following a long adaptation period. Thus, non-bound amino acids are more likely to be lost to post-prandial oxidation from post-enteral amino acid imbalances triggered by their more rapid intestinal uptakes than protein-bound amino acids.

5 Catabolism of amino acids and NH$_3$ detoxification

A reduction in the catabolism of amino acids would, if achievable, decrease amino acid requirements (Klasing, 2009). Nevertheless, catabolism of amino acids, either post-enterally or in the gut mucosa, generates NH$_3$ from their deamination which is inherently toxic (Stern and Mozdziak, 2019). There are indications that poultry are more susceptible to NH$_3$ intoxication than mammalian species (Wilson et al., 1968), consequently the failure to detoxify NH$_3$ adequately will compromise broiler performance. Accordingly, elevated plasma NH$_3$ concentrations have been associated with compromised broiler performance in several studies (Namroud et al., 2008; Ospina-Rojas et al., 2013, 2014). Instructively, in one of our unpublished studies, non-bound amino acid inclusions were linearly related (r = 0.546; P = 0.019) to plasma NH$_3$ concentrations which clearly implies that non-bound amino acid are open to post-enteral catabolism.

Excreta NH$_3$ concentrations from birds offered maize-soy diets containing 163, 147 and 132 g/kg CP with four levels of glycine equivalents from 7 to 21 days post-hatch were determined by Hofmann et al. (2019). Dietary inclusions of non-bound amino acids ranged from 17.5 to 67.3 g/kg across twelve dietary treatments. Excreta NH$_3$ concentrations were indicative in this study as they were negatively correlated with weight gain (r = -0.761; P = 0.004), feed intake (r = -0.754; P = 0.005) and gain to feed (r = -0.753; P = 0.005). Moreover, a quadratic relationship (r = 0.978; P < 0.0001) may be derived between dietary non-bound amino acid inclusions and excreta NH$_3$ concentrations, as shown in Figure 1. The regression equation predicts that excreta NH$_3$ concentrations increases once dietary non-bound amino acid inclusions exceed 31.1 g/kg. These outcomes indicate that non-bound amino acid inclusions may become excessive such that NH$_3$ is not adequately detoxified and that the resultant 'NH$_3$ overload' compromises growth performance.

Ammonia detoxification is achieved by the condensation of NH$_3$ with glutamic acid to generate glutamine which enters the Krebs uric acid cycle and NH$_3$-N is ultimately excreted as uric acid-N. This process demands energy and glycine is a prerequisite for the Krebs uric acid cycle as one mole of glycine is required for every mole of uric acid excreted (Stern and Mozdziak, 2019; Salway, 2019). From the Chrystal et al. (2021) study, it was retrospectively estimated that the proportion of dietary glycine required for the Krebs uric acid cycle ranged from 25.0% to 80.9% about a mean of a 49.2% (Selle et al., 2021a). However, glycine and serine are interconvertible (Sugahara and Kandatsu, 1976) and when expressed as glycine equivalents [one glycine equivalent (g/kg) = glycine + 0.7143*serine], then the mean entry level of dietary glycine equivalents into the urea cycle was 27.5%, ranging from 14.0% to 45.4%. Therefore, it is not surprising that glycine (and

serine) concentrations in reduced-CP diets have received considerable and merited attention (Dean et al., 2006; Siegert et al., 2016; Siegert and Rodehutscord, 2019). Indeed, Siegert and Rodehutscord (2019) concluded that from 11 to 20 g/kg glycine equivalents is the dietary requirement for young broilers.

Free amino acid concentrations in portal (anterior mesenteric vein) and systemic (brachial vein) plasma in birds offered 215 and 165 g/kg CP, wheat-based diets were reported by Yin et al. (2020). Portal concentrations were higher than systemic concentrations, but the variations followed similar patterns which indicate that amino acids in the gut mucosa originate from both the gut lumen and the arterial circulation (Newsholme and Carrié, 1994). Pursuant to the dietary CP reduction, glutamic acid decreased by an average of 11.5% and glutamine concentrations increased 24.1%, which is consistent with more condensation reactions converting glutamic acid plus NH_3 into glutamine. Average glycine and serine concentrations decreased by 28.3% and 22.3%, respectively, which reflects their increased entries into the Krebs uric acid cycle to complete NH_3 detoxification. Conversely, average threonine concentrations increased by 27.7% which suggests that threonine was not serving as a glycine precursor. Indeed, elevated free threonine plasma concentrations in chickens offered reduced-crude protein diets is a consistent outcome, which may be triggered by inhibition of threonine dehydrogenase activity (Selle et al., 2021b).

6 Branched-chain amino acids

In particular, the branched-chain amino acids (BCAA), isoleucine, leucine and valine, demonstrate inequalities between non-bound and protein-bound amino acids probably because BCAA derived from intact protein have relatively slow rates of intestinal uptakes. However, leucine derived from intact protein is better utilised for human whole body protein synthesis than non-bound leucine because of increased oxidation of the free form (Metges et al., 2000). Also, BCAA undergo catabolism in porcine enterocytes (Chen et al., 2009), but it is not known if this applies to poultry. In Greenhalgh et al. (2020b), broilers were offered nutritionally equivalent wheat- or sorghum-based 187.5 g/kg CP diets from 7 to 28 days post-hatch. These dietary treatments contained either standard, elevated leucine or elevated BCAA concentrations relative to lysine. Elevated BCAA levels in sorghum-based diets significantly improved weight gain by 9.26% (1451 versus 1328 g/bird) and numerically improved FCR by 0.86% (1.378 versus 1.390). In direct contrast, elevated BCAA levels significantly depressed weight gain by 9.49% (1288 versus 1423 g/bird) and significantly compromised FCR by 8.33% (1.537 versus 1.665) in wheat-based diets. Non-bound leucine represented 55.6% of the analysed leucine concentration in wheat-based diet with elevated BCAA

levels, whereas the proportion was 24.2% in sorghum-based diets. The corresponding differences were 31.6% and 53.2% for isoleucine and 29.3% and 47.1% for valine; non-bound BCAA proportions were substantially higher in wheat-based diets. It is noteworthy that there were quadratic relationships between the proportion of non-bound BCAA of analysed BCAA concentrations with weight gain (r = 0.700; P < 0.0001) and FCR (r = 0.803; P < 0.0001) in Greenhalgh et al. (2022b). The regression equations predict that weight gain is depressed once the proportion of 29.2% is exceeded and FCR deteriorates when the exceeded proportion is 17.3%.

7 Is there a ceiling on non-bound amino acid inclusions?

Instructively, Baker (2009) suggested that there are limits to the extent that intact protein can be replaced by non-bound amino acids in terms of achieving maximal weight gain and feed efficiency. Certainly, the capacity of broiler chickens to accommodate substantial non-bound amino acid dietary inclusions appears finite, although this ceiling is likely to be highly variable. Broilers were offered wheat-based diets in an equilateral triangle response surface design feeding study in Macelline et al. (2022b). The three apical diets contained 203 g/kg true protein, but this protein was largely derived from either soybean meal, non-bound amino acids or whey protein and non-bound amino acid inclusions ranged from 6.75 to 66.9 and 19.4 g/kg, respectively. Maximum weight gain (2089 g/bird) and minimum FCR (1.401) from 14 to 35 days post-hatch were supported by an equal blend of the soybean meal and whey protein diets which contained 13.1 g/kg non-bound amino acids. In comparison, the diet containing 66.9 g/kg non-bound amino acids generated inferior weight gain by 9.05% (1900 versus 2089 g/bird) and FCR by 4.43% (1.463 versus 1.401). In an earlier study (Chrystal et al., 2021), broiler chickens were offered maize-based diets, ground wheat-based diets and wheat-based diets with 15% whole wheat with CP levels of 222, 193 and 165 g/kg in a 3 x 3 factorial design from 7 to 35 days post-hatch. Non-bound amino acid inclusions ranged from 7.23 to 49.39 g/kg and as shown in Figures 2 to 4, there were quadratic relationships between non-bound amino acid inclusions and weight gain (r = 0.891; P < 0.0001), feed intake (r = 0.796; P < 0.0001) and FCR (r = 0.872; P < 0.0001). Collectively, the three regression equations indicate that the optimal non-bound amino acid inclusion level was 19.23 g/kg and growth performance deteriorates when this level was exceeded. Thus, the Chrystal et al. (2021) study suggests that while moderate non-bound amino acid inclusions are advantageous, a ceiling exists when higher levels become deleterious. Clearly, the challenge is to develop a better understanding of the mechanisms that impose this ceiling on non-bound amino acid inclusions if it is to be counteracted.

8 Future directions

The strategy that is central to this paper and obviously worth investigating is protected or "delayed-release" amino acids with retarded intestinal uptake rates more closely aligned with protein-bound amino acids. Advanced oral delivery technologies for the delayed absorption of amino acids and peptide molecules are under development (Choonara et al., 2014). Microencapsulated lysine has shown some promise in grower-finisher pigs (Prandini et al., 2013). Also, it has been reported that lipid-encapsulated lysine and methionine were both more effectively utilised in broiler chickens than as non-bound entities (Sun et al., 2020). The economic feasibility of protected amino acids may be an issue; nevertheless, they should permit higher inclusions of supplemental amino acids to meet target specifications in reduced-CP diets.

This is not to imply that the future development of reduced-CP diets should be restricted to protected amino acids. Additional strategies that should be explored include the capping of dietary starch:protein ratios. An initial evaluation in wheat-based diets showed some promise (Greenhalgh et al., 2020b) and a second evaluation has been completed in maize-based diets (Greenhalgh et al., 2022b). Capping starch:protein ratios in 175 g/kg CP, maize-based diets improved weight gain by 3.45% (2398 versus 2318 g/bird; P = 0.012), FCR by 3.75% (1.360 versus 1.413; P < 0.001) with lighter relative fat-pad weights by 10.3% (11.47 versus 12.78 g/kg; P = 0.050). Thus, the strategy again showed promise and there is the distinct possibility that high starch concentrations in reduced-CP diets, especially wheat starch, influence the metabolism of amino acids.

The feed grain on which a reduced-CP diet is based has an enormous impact on growth performance and lipid deposition. From direct comparisons and indirect evidence, we have consistently found wheat to be variably inferior to maize (and sorghum) in growth performance; paradoxically, wheat promotes less fat deposition. Numerous factors are involved, including starch-protein digestive dynamics (Liu and Selle, 2017); however, wheat-based diets invariably contain higher non-bound amino acid inclusions (and less intact soy protein) than maize- or sorghum-based diets because wheat has higher protein contents. Therefore, the lack of amino acid bioequivalency is a greater impediment to growth performance in broilers offered wheat-based, reduced-CP broiler diets. However, other factors inherent in wheat may be contributing, including soluble non-starch polysaccharides, amylase-trypsin inhibitors and gluten (Selle et al., 2022b). That wheat supports lighter relative abdominal fat-pad weights may stem from the fact that wheat starch digestion rates are more rapid than starch of maize and sorghum (Selle et al., 2021c). It has been suggested that rapidly digestible wheat starch is being directly oxidised whereas glucose from slowly digestible

starch is being converted to glycogen and then fat via *de novo* lipogenesis to greater extents (Selle et al., 2022c). Maize is practically unavailable in Australia; therefore, it may be better to base reduced-CP diets on sorghum-wheat blends to retard starch digestion rates and lower non-bound amino acid inclusions; both strategies should prove advantageous.

References

Baker DH (1991) Partitioning of nutrients for growth and other metabolic functions: efficiency and priority considerations. *Poultry Science* **70**, 1797-1805.

Brosnan JT (2003) Interorgan amino acid transport and its regulation. *Journal of Nutrition* **133**, 2068S-2072S.

Canolty NL, Nasset ES (1975) Intestinal absorption of free and protein-bound dietary methionine in the rat. *Journal of Nutrition* **105**, 867-877.

Chen L, Li P, Wang J, Li X, Gao H, Yin Y, Hou Y, Wu G (2009) Catabolism of nutritionally essential amino acids in developing porcine enterocytes. *Amino Acids* **37**, 143-152.

Choonara BF, Choonara YE, Kumar P, Bijukumar D, du Toit LC, Pillay V (2014) A review of advanced oral drug delivery technologies facilitating the protection and absorption of protein and peptide molecules. *Biotechnology Advances* **32**, 1269–1282.

Chrystal PV, Greenhalgh S, McInerney BV, McQuade LR, Selle PH, Liu SY (2021) Maize-based diets are more conducive to crude protein reductions than wheat-based diets for broiler chickens. *Animal Feed Science and Technology* **275**, 114867.

Dean DW, Bidner TD, Southern LL (2006) Glycine supplementation to low protein, amino acid-supplemented diets supports optimal performance of broiler chicks. *Poultry Science* **85**, 288-296.

Fleming SE, Zambell KL, Fitch MD (1997) Glucose and glutamine provide similar proportions of energy to mucosal cells of rat small intestine. *American Journal of Physiology (Gastrointestinal and Liver Physiology)* **273,** G968-G978.

Greenhalgh S, Chrystal PV, Selle PH, Liu SY (2020a) Reduced-crude protein diets in chicken-meat production: Justification for an imperative. *World's Poultry Science Journal* **76**, 537-548.

Greenhalgh S, McInerney BV, McQuade LR, Chrystal PV, Khoddami A, Zhuang MAM, Liu SY, Selle PH, (2020b) Capping dietary starch:protein ratios in moderately reduced crude protein, wheat-based diets showed promise but further reductions generated inferior growth performance in broiler chickens. *Animal Nutrition* **6**, 168-178.

Greenhalgh S, Chrystal PV, Lemme A, Dorigam JCdeP, Macelline SP, Liu SY, Selle PH (2022a) Capping dietary starch:protein ratios enhances performance of broiler chickens offered reduced-crude protein, maize-based diets. *Animal Feed Science and Technology* (submitted for publication)

Greenhalgh S, Macelline SP, Chrystal PV, Liu SY, Selle PH (2022b) Elevated branched-chain amino acid inclusions generate distinctly divergent growth performance responses in broiler chickens offered wheat- and/or sorghum-based, reduced-crude protein diets. *Animal Feed Science and Technology* (submitted for publication)

Hamm D (1981) Amino acid composition of breast and thigh meat from broilers produced in four locations of the United States. *Journal of Food Science* **46**, 1122-1124.

He W, Li P, Wu G (2021) Amino acid nutrition and metabolism in chickens. In: Advances in Experimental Medicine and Biology 1285 (Guoyao Wu, Editor) pp. 109-131. Springer Nature Switzerland AG.

Hofmann P, Siegert W, Kenéz Á, D Naranjo VD, Rodehutscord M (2019) Very low crude protein and varying glycine concentrations in the diet affect growth performance, characteristics of nitrogen excretion, and the blood metabolome of broiler chickens. *Journal of Nutrition* **149**, 1122-1132.

Klasing KC (2009) Minimizing amino acid catabolism decreases amino acid requirements. *Journal of Nutrition* **139**, 11-12.

Leeson S, Walsh T (2004) Feathering in commercial poultry II. Factors influencing feather growth and feather loss. *World's Poultry Science Journal* **60**, 52-63.

Leme BB, Sakomura NK, Vargas L, Nascimento CCN, Antayhua FAP, Dorigam JC, Macari M, Gous RM (2022) Composition of feathers and pulp of two broiler genotypes. *British Poultry Science* DOI: 10.1080/00071668.2022.2041185.

Liu SY, Selle PH, Court SG, Cowieson AJ (2013) Protease supplementation of sorghum-based broiler diets enhances amino acid digestibility coefficients in four small intestinal sites and accelerates their rates of digestion. *Animal Feed Science and Technology* **183**, 175-183.

Liu SY, Selle PH (2017) Starch and protein digestive dynamics in low-protein diets supplemented with crystalline amino acids. *Animal Production Science* **57**, 2250-2256.

Macelline SP, Kidd MT, Chrystal PV, Toghyani M, Greenhalgh S, Selle PH, Liu SY (2022a) Optimal ideal amino acid ratios for growth and carcass yield depend on dietary crude protein concentrations in broiler chickens. *Animal Feed Science and Technology* (submitted for publication).

Macelline SP, Chrystal PV, Selle PH, Liu SY (2022b) Protein sources and starch protein digestive dynamics manipulate growth performance in broiler chickens defined by an equilateral triangle response surface design. *Animal Nutrition* **9**, 204-213.

Maharjan P, Martinez DA, Weil J, Suesuttajit N, Umberson C, Mullenix G, Hilton KM, Beitia A, Coon CN (2021) Review: Physiological growth trend of current meat broilers and dietary protein and energy management approaches for sustainable broiler production. *Animal* **15** (Supplement 1), 100284.

Metges CC, El-Khoury AE, Selvaraj AB, Tsay RH, Atkinson A, Regan MM, Bequette BJ, Young VR (2000) Kinetics of L-[1-^{13}C]leucine when ingested with free amino acids, unlabeled or intrinsically labeled casein. *American Journal of Physiology (Endocrinology and Metabolism)* **278**, E1000-E1009.

Moss AF, Sydenham CJ, Khoddami A, Naranjo VD, Liu SY, Selle PH (2018) Dietary starch influences growth performance, nutrient utilisation and digestive dynamics of protein and amino acids in broiler chickens offered low-protein diets. *Animal Feed Science and Technology* **237**, 55-67.

Namroud NF, Shivazad M, Zaghari M (2008) Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. *Poultry Science* **87**, 2250-2258.

Newsholme EA, Carrié A-L (1994) Quantitive aspects of glucose and glutamine metabolism by intestinal cells *Gut* **35**, (Supplement 1) S13-S17.

Nolles JA, Verreijen AM, Koopmanschap RE, Verstegen MWA, Schreurs VVAM (2009) Postprandial oxidative losses of free and protein-bound amino acids in the diet: interactions and adaptation. *Journal of Animal Physiology and Animal Nutrition* **93**, 431-438.

Ospina-Rojas IC, Murakami AE, Moreira I, Picoli KP, Rodrigueiro RJB, Furlan AC (2013) Dietary glycine+serine responses of male broilers given low-protein diets with different concentrations of threonine. *British Poultry Science* **54**, 486-493.

Ospina-Rojas IC, Murakami AE, Duarte CRA Eyng C, Oliveira CAL, Janeiro V (2014) Valine, isoleucine, arginine and glycine supplementation of low-protein diets for broiler chickens during the starter and grower phases. *British Poultry Science* **55**, 766-773.

Pesti GM (2009) Impact of dietary amino acid and crude protein levels in broiler feeds on biological performance. *Journal of Applied Poultry Research* **18**, 477-486.

Prandini A, Sigolo S, Morlacchini M, Grilli E, Fiorentini L (2013) Microencapsulated lysine and low-protein diets: Effects on performance, carcass characteristics and nitrogen excretion in heavy growing–finishing pigs. *Journal of Animal Science* **91**, 4226-4234.

Salehifar E, Shivazad M, Foroudi F, Chamani M, Bahari Kashani R (2012) Reevaluation of digestible amino acid requirements of male and female broilers based on different ideal amino acids ratios in starter period. *Livestock Science* **147**, 154-158.

Salway JG (2018) The Krebs uric acid cycle: A forgotten Krebs cycle. *Trends in Biochemical Sciences* **43**, 847-849,

Schreurs VVAM, Koopmanschap RE, Boekholt HA (1997) Short-term dynamics in protein and amino acid metabolism. *Zeitschrift für Ernährungswissenschaft* **36**, 336-339.

Selle PH, Liu SY (2020) Towards sustainable chicken-meat production. *Proceedings, Arkansas Nutrition Conference.* The Poultry Federation. Little Rock, AK.

Selle PH, Cantor DI, McQuade LR, McInerney BV, Dorigam JCdeP, Macelline SP, Chrystal PV, Liu SY (2021a) Implications of excreta uric acid concentrations in broiler chickens offered reduced-crude protein, maize- and wheat-based diets and dietary glycine requirements for uric acid synthesis. *Animal Nutrition* **7**, 939-946.

Selle PH, Macelline SP, Chrystal PV, Liu SY (2021b) A rationale for elevated free threonine plasma concentrations in chickens offered reduced-crude protein diets. *Proceedings, Australian Poultry Science Symposium* **32**, 121-124.

Selle PH, Moss AF, Khoddami A, Chrystal PV, Liu SY (2021c) Starch digestion rates in multiple samples of commonly used feed grains in diets for broiler chickens. *Animal Nutrition* **7**, 450-459.

Selle PH, Macelline SP, Chrystal PV, Liu SY (2022a) The impact of digestive dynamics on the bioequivalence of amino acids in broiler chickens. *Frontiers in Bioscience – Landmark* **27**, 126.

Selle PH, Macelline SP, Greenhalgh S, Chrystal PV, Liu SY (2022b) Identifying the shortfalls of wheat-based, reduced-crude protein diets. *Animal Nutrition* (submitted for publication)

Selle PH, Macelline SP, Chrystal PV, Liu SY (2022c) Fat deposition in broiler chickens offered reduced-crude protein diets. *Proceedings, Australian Poultry Science Symposium* **33**, (accepted for publication).

Siegert W, Wild KJ, Schollenberger M, Helmbrecht A, Rodehutscord M (2016) Effect of glycine supplementation in low protein diets with amino acids from soy protein isolate or free amino acids on broiler growth and nitrogen utilisation. *British Poultry Science* **57**, 424-434.

Siegert W, Rodehutscord M (2019) The relevance of glycine and serine in poultry nutrition: a review. *British Poultry Science* **60**, 579-588.

Simoni RD, Hill RL Vaughan M (2002) The discovery of the amino acid threonine: the work of Willliam C. Rose. *Journal of Biological Chemistry* 2**77**, 56-58.

Sklan D, Noy Y (2004) Catabolism and deposition of amino acids in growing chicks: Effects of dietary supply. *Poultry Science* **83**, 952-961.

Stern RA, Mozdziak PE (2019) Differential ammonia metabolism and toxicity between avian and mammalian species, and effect of ammonia on skeletal muscle: A comparative review. *Journal of Animal Physiology and Animal Nutrition* **103**, 774–785.

Stilborn HL, Moran ET, Gous RM, Harrison MD (1997) Effect of age on feather amino acid content in two broiler strain crosses and sexes. *Journal of Applied Poultry Research* **6**, 205-209.

Stoll B, Burrin DJ, Henry J, Yu H, Jahoor F, Reeds PJ (1999) Substrate oxidation by the portal drained viscera of fed piglets. *American Journal of Physiology (Endocrinology and Metabolism 40)* **277**, E168-E175.

Sugahara M, Kandatsu M (1976) Glycine serine interconversion in the rooster. *Agricultural and Biological Chemistry* **40**, 833-837.

Sun M, Jiao H, Wang X, Uyanga VA, Zhao J, Lin H (2020) Encapsulated crystalline lysine and DL-methionine have higher efficiency than the crystalline form in broilers. *Poultry Science* **99**,

Wilke T (2014) Methionine production — a critical review. *Applied Microbiology and Technology* **98**, 9893-9914.

Wilson R, Muhrer M, Bloomfield R (1968) Comparative ammonia toxicity. *Comparative Biochemistry and Physiology* **25**, 295–301.

Wu G (2010) Functional amino acids in growth, reproduction, and health. *Advances in Nutrition* **1**, 31-37.

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.

Wylie LM, Robertson GW, Hocking PM (2003) Effects of dietary protein concentration and specific amino acids on body weight, body composition and feather growth in young turkeys. *British Poultry Science* **44**, 75-87.

Yin D, Chrystal PV, Moss AF, Liu SY, Selle PH (2020) Effects of reduced crude protein and whole grain feeding on performance and amino acid metabolism in broiler chickens offered wheat-based diets. *Animal Feed Science and Technology* **260**, 114386.

Table 1   Two ideal amino acid ratios and amino acid concentrations in whole-body protein, breast muscle and feathers expressed relative to lysine

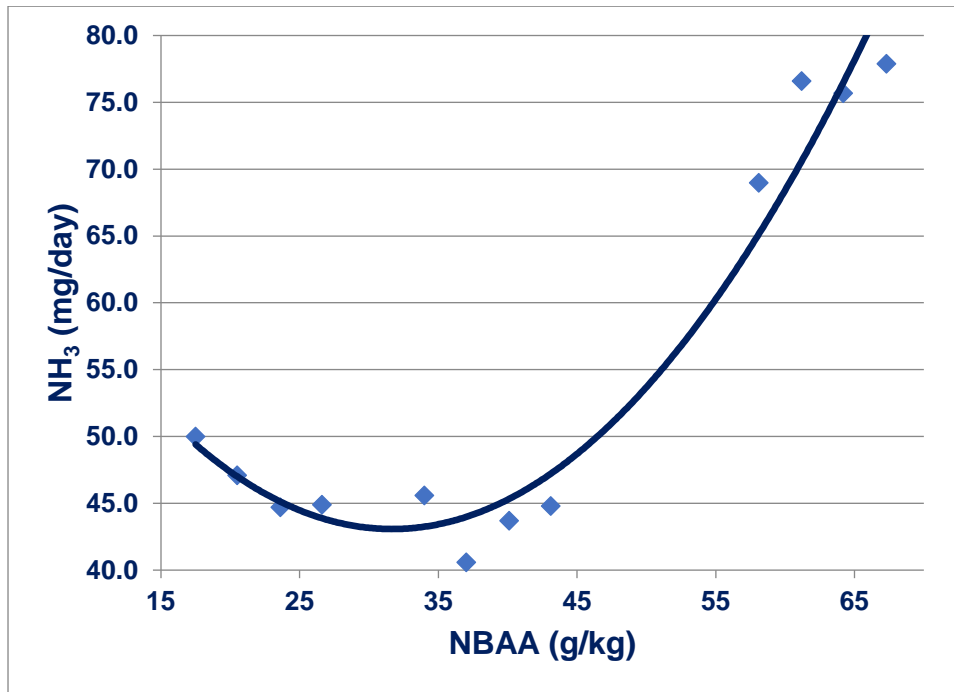| Amino acid	Ideal amino acid ratios		Whole-body protein Wu (2014)	Breast Hamm (1981)	Feathers Greenhalgh et al. (2020b)
	Wu (2014)	Maharjan et al. (2021)			
Arg	108	98	111	84	488
His	35	39	34	49	32
Ile	69	71	58	55	336
Leu	109	126	113	102	571
Lys	100	100	100	100	100
Met	42	46	31	36	29
Phe	60	71	57	47	339
Thr	70	67	59	56	335
Trp	17	16	19	-	33
Val	80	79	68	61	549
Ala	102	74	108	49	280
Asn/Asp	122	150	129	116	439
Cys	33	25	24	13	526
Gln/Glu	306	270	217	179	79
Gly	176	61	187	59	507
Pro	184	89	139	48	727
Ser	69	78	57	58	855
Tyr	45	-	43	55	170

Figure 1   Quadratic relationship (r = 0.978; P < 0.0001) between dietary non-bound amino acid (NBAA) inclusions (g/kg) and excreta ammonia (NH_3) concentrations (mg/day) from 7 to 21 days post-hatch, where y = 74.63 + 0.032*$NBAA^2$ – 1.993*NBAA. Adapted from Hofmann et al. (2019)
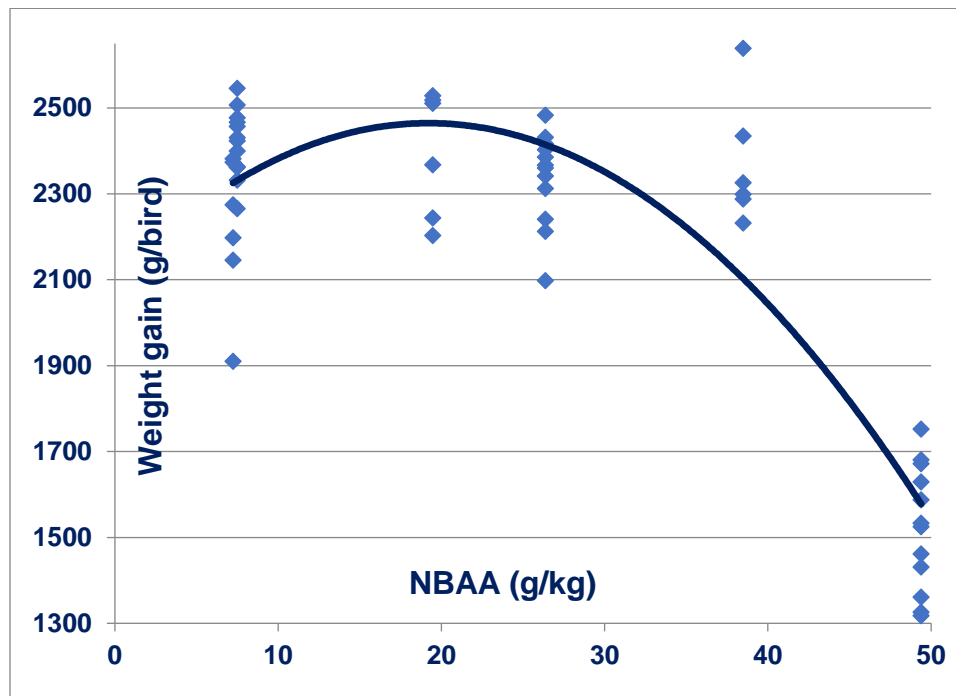
Figure 2   Quadratic relationship (r = 0.891; P < 0.0001) between non-bound amino acid NBAA) inclusions and weight gain where

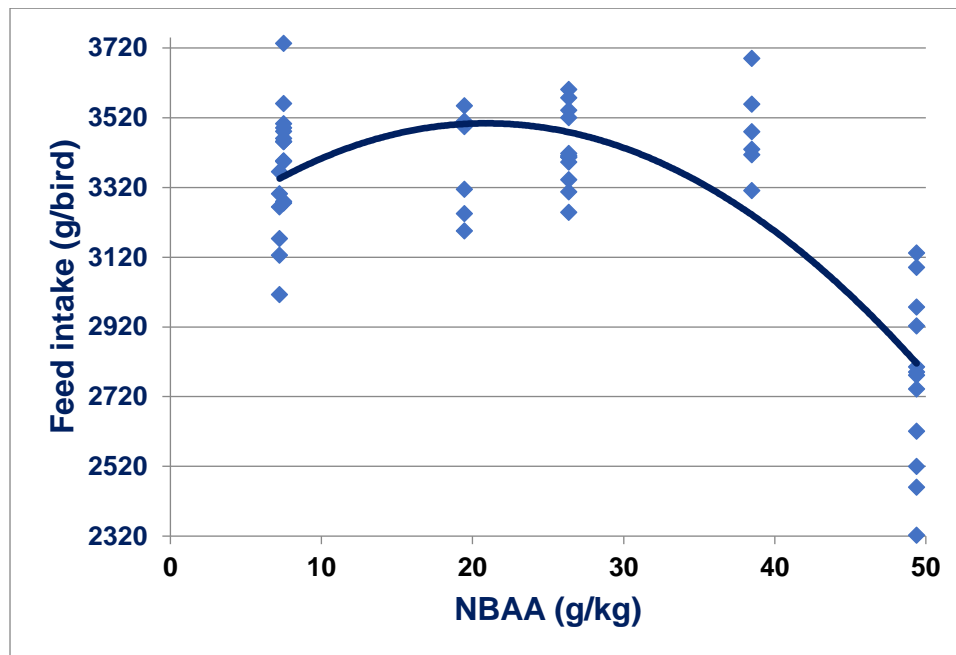y = 2106 + 37.376*NBAA − 0.973*NBAA^2 (adapted from Chrystal et al. 2021)

Figure 3   Quadratic relationship (r = 0.796; P < 0.0001) between non-bound amino acid NBAA) inclusions and feed intake where

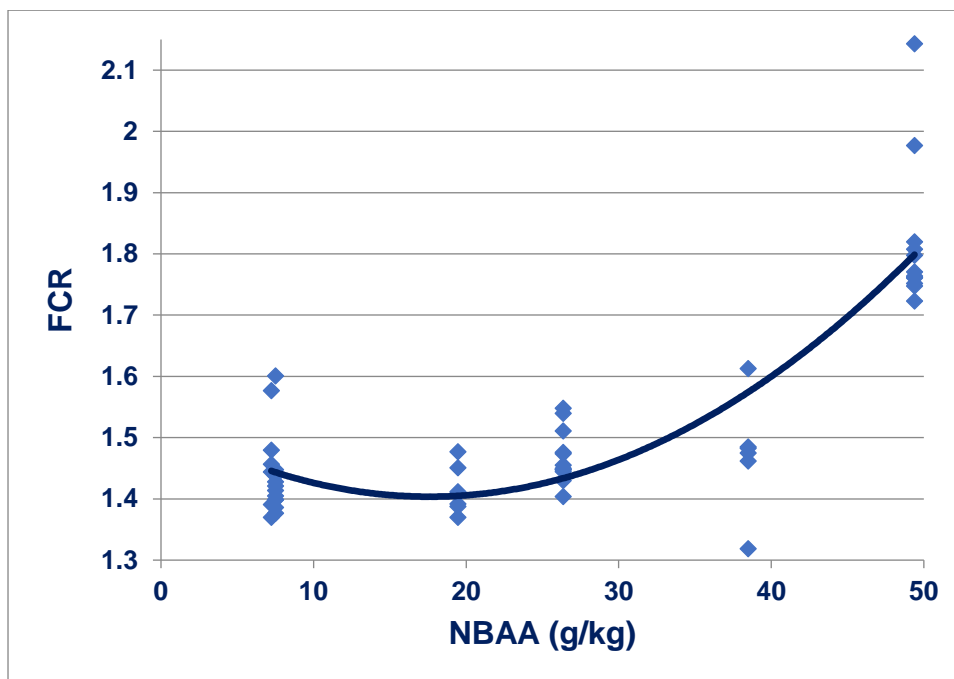y = 3133 + 35.467*NBAA – 0.848*NBAA^2 (adapted from Chrystal et al. 2021)

Figure 4   Quadratic relationship (r = 0.872; P < 0.0001) between non-bound amino acid NBAA) inclusions and FCR where

$$y = 1.525 - 0.013749*NBAA - 0.000391*NBAA^2$$

(adapted from Chrystal et al. 2021)