

Review

Ideal Amino Acid Profiles in Diets for Broiler Chickens Remain Elusive Targets

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Abstract

Amino acids are the ‘building blocks’ of protein, including both feathers and whole-body protein, and serve numerous functional roles, including energy provision. Amino acid requirements for broiler chickens are expressed as ideal amino acid ratios relative to digestible lysine. However, ideal amino acid profiles in diets for broiler chickens are likely to remain elusive targets. This review considers the factors contributing to this situation. Just one complicating factor is that amino acids may be deposited as protein in either feathers or whole-body protein. However, in the partitioning of amino acids, it appears that preference may be given to feathering, which requires clarification. A second complicating factor is the transition of amino acids across enterocytes of the small intestinal gut mucosa, and small intestinal uptake of amino acids may be rate-limiting for growth performance. Protein-bound amino acids may be absorbed as oligopeptides or monomeric amino acids, and non-bound amino acids do not require digestion prior to their intestinal uptake. The transition of amino acids across the gut mucosa is involved because amino acids may be diverted into anabolic and/or catabolic pathways and are thereby denied entry to the portal circulation. The metabolic fate of amino acids in the gut mucosa may be subject to manipulation by nutritional strategies, but our comprehension of the relevant metabolism within avian enterocytes is inadequate. The post-enteral availability of twenty proteinogenic amino acids is complex with important implications for the efficiency of utilisation. Importantly, acceptably accurate amino acid profiles will be advantageous for sustainable chicken-meat production into the future.

Keywords: amino acids; broiler chickens; ideal amino acid ratios; avian nutrition

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1. Introduction

Nearly 80 years ago, Almquist [1,2] assessed the amino acid requirements for broiler chickens, and an almost countless number of studies have since been completed in an ongoing quest to define and meet dietary amino acid requirements for broiler chickens. The inherent problems and pitfalls in meeting this objective, including the use of appropriate statistical approaches, were defined in Baker [3]. One common approach is to evaluate the impact on growth performance responses to inclusions of graded levels of a specific amino acid or combination of amino acids [4]. The graded levels are obtained either

by the dietary addition of non-bound (synthetic, crystalline, feed-grade) amino acids [5] or by the dilution technique [6]. Other approaches have been adopted; nevertheless, the accurate quantification of ideal amino acid profiles in diets for broiler chickens remains elusive, which is not surprising given there are 20 proteinogenic amino acids. Structurally, amino acids are the 'building blocks' of protein; however, amino acids are also functional in that they serve as signalling molecules, neurotransmitters, and regulators of metabolic pathways [7] and also as substrates for energy provision [8]. Additionally, the distinction between essential and non-essential amino acids is becoming increasingly blurred, particularly in the context of reduced-crude protein (CP) diets [9]. Moreover, the case was made that amino acids were not all created equal [10], which questions the bioequivalence of protein-bound versus non-bound amino acids in reduced-CP diets [11].

Somewhat optimistically, more than 60 years ago, Holmes et al. [12] predicted that an enhanced knowledge of the amino acid composition of raw materials and the degree to which the amino acids are available to the bird, coupled with increased production of synthetic amino acids, could lead to the design of commercial diets approaching the ideal amino acid pattern. However, about 30 years ago, David Baker [13] expressed the opinion that an ideal protein does not exist in practice and the concept of an ideal pattern of amino acids is a lofty goal. The use of a constant ideal amino acid profile in poultry nutrition was called into question by Conde-Aguilera et al. [14], and they also suggested that there are limitations in using weight gain as the single criterion to determine amino acid requirements. More recently, Wu and Li [15] argued that the "ideal protein" concept is less than ideal in animal nutrition because optimum ratios should be developed for all proteinogenic amino acids and not a limited number of essential amino acids.

Presently, the lowest-cost formulations of broiler diets are based on ideal amino acid ratios (IAAR) in association with a selected metabolisable or net energy density. This common approach stems from the ideal protein concept in which specifications for targeted amino acids, based on digestible amino acid values, are expressed relative to lysine (100). The rationale for the adoption of IAAR is that ratios of amino acids to lysine remain largely unaffected by the variables stemming from genetic, dietary and environmental factors, and lysine is almost exclusively utilised for whole-body protein accretion rather than feathering or metabolic functions [16]. In one assay, the standardised ileal digestible lysine requirements for 21 to 28 day-old male broiler chickens for body weight gain ranged from 10.7 g/kg to 11.6 g/kg and for FCR from 10.9 g/kg to 12.0 g/kg, depending on whether the data were fitted in a one-slope broken-line or a quadratic line model [17]. In an earlier, similar study [18], the lysine requirements for weight gain ranged from 13.27 to 15.33 g/kg and from 12.89 to 13.99 g/kg for FCR in 1- to 10-day-old male Ross 308 broiler chicks. Moreover, Lee et al. [18] concluded that requirements for lysine *per se* are increasing over time in modern broiler chickens.

Pivotal, it is the functional roles of amino acids in nutrition, metabolism and health [19,20] that complicate the development of accurate dietary IAAR to a greater extent than the classical roles of amino acids as 'building blocks' of protein. Indeed, Battezzati and Riso [21] advised that amino acids should not be viewed simply as building blocks for protein synthesis, but as the most important and diverse nutrient regulators of cell functions. There is also the real challenge for IAAR to keep pace with the rapid genetic progress of broiler chickens, which modifies their nutritional demands [22]. The likelihood is that a perfect ideal amino acid pattern will not emerge; nevertheless, it is imperative that this objective is approached as closely as possible. Therefore, the purpose of this review is to consider the key barriers that are hindering the fine-tuning of ideal dietary amino acid profiles for broiler chickens. Some of the impediments include variations in partitioning of amino acids to structural or functional roles and to feathering, the bioequivalence of amino acids, their intestinal uptake and transition across the gut mucosa, amino acid an-

tagonisms, the catabolism of amino acids and their interactions with macronutrients. Clearly, a better comprehension of the factors that are complicating the fulfilment of the objective can only facilitate the development of more accurate ideal amino acid ratios and more sustainable chicken-meat production.

2. Background

Eight examples of IAAR ranging chronologically from 1965 to 2021 are presented in Table 1. The ratios for aspartic acid and glutamic acid are tabulated; however, the ratios actually represent the sum of asparagine plus aspartate and glutamine plus glutamate, respectively. Moreover, in both humans [23] and broiler chickens [24], the important functional roles of glutamine and glutamate are distinctly different. Valine is probably the fourth limiting amino acid in broiler diets, so its relativity to lysine assumes importance. Across 16 relevant studies summarised in [25], the mean relativity of valine to lysine was 76.9 ± 4.99 , with a range of 64 to 86. In Table 1, the mean value for the relative abundance of valine to lysine is 77 ± 4.704 , with a range of 68 to 82. The extent of both ranges illustrates the inherent problems in determining the relative abundance of 19 proteinogenic amino acids to lysine.

Table 1. Eight examples of ideal amino acid ratios for broiler chickens relative to lysine (100).

Amino Acid	Reference								Mean
	Dean and Scott [26]	NRC 1984 ¹	NRC 1994 ¹	Baker and Han [16]	Roth et al. [27]	Tillman and Dozier [28]	Wu [29]	Maharjan et al. [30]	
Arginine	98	120	114	105	108	105	108	98	107
Histidine	27	29	32	37	38		35	39	34
Isoleucine	71	67			63	66	69	71	68
Leucine	107	113	109	111	108		109	126	112
Lysine	100	100	100	100	100	100	100	100	100
Methionine	40	42	46	36	37	45	42	43	41
Phenylalanine	61	60	66	55	62		60	75	63
Threonine	58	67	73	67	67	70	70	67	67
Tryptophan	20	19	18	16	14	16	17	16	17
Valine	73	68	82	77	81	75	80	79	77
Alanine							102	74	88
Aspartic acid							122	152	137
Cysteine	31	36	36	36	33	36	33	25	33
Glutamic acid	1071						306	269	549
Glycine	143						176	61	127
Proline	44		55	44			184	93	84
Serine							69	75	72
Tyrosine	56	52	56	50	59		45		53

¹ data was sourced from Baker and Han [16]. Initially, it was thought that dietary protein requirements would be satisfied when the concentrations of essential amino acids in the diets matched the concentrations in the whole body of broiler chickens. Indeed, it was claimed that the essential amino acid requirements of broiler chickens were related to their proportional incidence in the fat-free carcass [31]. Moreover, Scott and Fisher [31] claimed that the dietary amino acid requirement as a percentage of the diet (y), and its percentage concentration in a 4-week-old carcass, is described by the equation $y = 0.256 - 0.034x + 0.027x^2$. However, if this equation is applied to the amino acid

composition of a 42-day-old carcass [12], only six amino acid concentrations agree with current recommendations, and there are substantial differences for the balance of amino acids.

Decades ago, Sugahara et al. [32] offered chicks 10 experimental diets that were separately formulated to 60% of the requirement for eight essential amino acids and combinations of methionine plus cysteine and phenylalanine plus tyrosine from 8 to 14 days post-hatch. Across the ten treatments, weight gain decreased by an average of 39.3%, feed intake decreased by 18.8%, and FCR increased by 36.4%, relative to the positive control diet. However, the differences between the minimum and maximum responses observed were notable, and the pattern of amino acids involved was inconsistent. The outcomes of [32] illustrate the difficulties in the development of accurate dietary amino acid profiles.

A direct approach to calculate the optimal dietary supply of amino acids for maintenance and growth in broiler chicks was employed by Sklan and Noy [33]. Amino acid intakes and accretion in the carcass were determined from 1 to 42 days post-hatch in Ross 308 birds offered experimental diets with controlled feed intakes. Plotting amino acid intakes per metabolic body weight against accretion amino acids per unit of metabolic body weight resulted in a straight line where the intercept represented maintenance, and the slope represented efficiency of amino acid utilisation for growth. The outcomes of [33] include optimal dietary concentrations for seven amino acids relative to lysine at six weekly intervals as shown in Table 2. There are notable variations in optimal dietary concentrations of amino acids relative to lysine at weekly intervals from 1 to 42 days post-hatch. This was most pronounced for histidine, valine and leucine, and again this study illustrates the difficulties in the development of accurate dietary amino acid profiles.

Table 2. Calculated amino acid intakes of selected amino acids in absolute terms (g/kg) and relative to lysine at six weekly intervals. Adapted from Sklan and Noy [33].

Week	Lys	Arg	His	Ile	Leu	Thr	Phe + Tyr	Val
1	14.1	16.7	4.4	12.0	13.5	9.7	13.8	11.7
		118	31	85	96	69	98	83
2	11.8	13.3	3.4	10.6	12.2	8.6	12.6	10.2
		113	29	90	103	73	107	86
3	9.8	13.0	1.8	8.9	12.1	8.5	11.7	9.8
		133	18	91	123	87	119	100
4	11.3	13.0	3.2	8.7	12.0	8.2	11.2	9.7
		115	28	77	106	73	99	86
5	10.8	13.0	2.8	8.5	12.0	8.1	11.1	9.8
		120	26	79	111	75	103	91
6	9.8	12.5	2.6	8.1	11.1	8.2	11.2	8.9
		128	27	83	113	84	114	91
Mean		121.2	26.5	84.2	108.7	76.8	106.7	89.5
Table 1 ¹		107	34	68	112	67	116	77

¹ this row provides the mean values, relative to lysine, of the relevant amino acids from Table 1.

Non-Bound Amino Acids

The increasing inclusion of non-bound (synthetic, crystalline, feed-grade) amino acids in broiler diets in association with the development of reduced-CP diets [34,35] represents a formidable challenge to the identification of ideal amino acid profiles. It is improbable that non-bound amino acids and amino acids derived from intact protein are bio-equivalent [10,11]. The implications of this inequality are discussed in Section 5.1, and the development of ideal amino acid ratios applicable to reduced-CP diets, in addition to standard diets, may well be required.

The chemical synthetic process to produce *dl*-methionine was developed by Dr Werner Schwarze during the 1940s, and subsequently Degussa (now Evonik) made *dl*-methionine available for poultry and livestock in 1953 [36]. Axiomatically, non-bound amino acids do not require digestion; therefore, they are rapidly absorbed, which was demonstrated with *dl*-methionine in rats [37]. Intestinal uptake of non-bound amino acids is more rapid than their protein-bound counterparts in broiler chickens, as unequivocally demonstrated by Liu et al. [38]. Sorghum-based diets were formulated to contain three non-bound amino acids (3.7 g/kg lysine as lysine HCl, 3.4 g/kg *dl*-methionine and 1.3 g/kg threonine), and the digestion rate constants of 16 amino acids were determined as shown in Table 3. The digestion rate constants of methionine and lysine were 5.51 and 4.14, respectively, as opposed to the average constant for the 13 protein-bound amino acids of $2.35 \text{ } k_{\text{amino acid}} \times 10^{-2} \text{ min}^{-1}$. It may be estimated from this study that the digestion rate constant of non-bound lysine was 8.78 and non-bound methionine was $8.49 \text{ } k_{\text{amino acid}} \times 10^{-2} \text{ min}^{-1}$; therefore, the intestinal uptake of non-bound amino acids was nearly four times more rapid than amino acids derived from intact protein.

Table 3. Digestion rate constants ($k_{\text{amino acid}} \times 10^{-2} \text{ min}^{-1}$) of amino acids in broiler chickens offered a sorghum-based broiler diet supplemented with 3.37 g/kg methionine, 3.74 g/kg lysine and 1.29 g/kg threonine as non-bound amino acids, adapted from Liu et al. [38].

Amino Acid	Constant	Amino Acid	Constant
Arginine	3.25	Valine	2.04
Histidine	2.57	Alanine	2.18
Isoleucine	2.05	Aspartic acid	2.52
Leucine	2.07	Glutamic acid	2.96
Lysine	4.14	Glycine	2.11
Methionine	5.51	Proline	2.28
Phenylalanine	2.39	Serine	2.27
Threonine	2.58	Tyrosine	1.85

Importantly, Baker [39] issued the caveat that there are limits to the extent that non-bound amino acids can replace intact protein without compromising broiler-growth performance. This issue was addressed by Jensen [40], who offered broiler chicks six diets with CP contents ranging from 230 to 180 g/kg and ratios of intact to free protein ranging from 110:1 to 4:1 from 1 to 21 days post-hatch. As the intact-to-free protein ratio declined, there was a quadratic decrease ($r = 0.952$; $p = 0.029$) in weight gain. While this outcome supports the David Baker caveat, the underlying causes have yet to be unequivocally identified.

3. Comparisons of Ideal Amino Acid Ratios

Comparisons of broiler-growth performance supported by isoenergetic diets based on different ideal amino acid ratios should prove instructive, but only a limited number of such direct comparisons have been completed [41–44]. The IAAR of 10 amino acids, methionine plus cysteine, threonine, valine, arginine, tryptophan, isoleucine, histidine, phenylalanine plus tyrosine, relative to lysine, were compared in Taherkhani et al. [41,42], and the outcomes are shown in Table 4. The variations in growth performance supported by the different IAAR are striking, with coefficients of variation for weight gain of 21.1% and 17.3% in the grower and finisher phases, respectively. The corresponding coefficients of variation recorded in FCR were 10.8% and 2.76%.

Table 4. Direct comparisons of the weight gains and FCR supported by four ideal amino acid ratios in male and female broiler chickens from 7 to 21 and 21 to 42 days post-hatch.

Reference Feeding Period	IAAR ¹	Weight Gain (g/bird)			FCR (g/g)		
		Male	Female	Mean	Male	Female	Mean
Takerkhani et al. [41]	A	310	236	273	1.78	2.01	1.90
	B	362	297	330	1.53	1.81	1.67
7 to 21 days	C	340	283	312	1.78	1.87	1.83
post-hatch	D	292	104	198	1.96	2.36	2.16
Grand mean				278 ± 58.6			1.89 ± 0.204
Takerkhani et al. [42]	A	1200	850	1025	2.18	2.22	2.20
	B	1110	840	975	2.22	2.37	2.30
21 to 42 days	C	1130	830	980	2.38	2.31	2.35
post-hatch	D	890	470	680	1.91	2.62	2.27
Grand mean				915 ± 158.3			2.28 ± 0.063

¹ ranges of IAAR-evaluated grower phase: Met + Cys 72–79, Thr 65–73, Val 82–84, Arg 105–117, Trp 18–20, Ile 68–78, His 30–35, Phe + Tyr 105–128. Finisher phase: Met + Cys 72–80, Thr 62–74, Val 80–89, Arg 108–110, Trp 17–19, Ile 69–75, His 32–35, Phe + Tyr 105–128.

Subsequently, two approaches for determining the optimum dietary amino acid ratios for male Cobb 500 broiler chickens over three feeding phases were compared by Dorigam et al. [45]. The first approach was a broken line model with slaughter to determine the ideal dietary amino acid pattern as used in [27], and the second approach involved nitrogen balance studies as described in [46]. The Dorigam et al. [45] study evaluated seven individual amino acids and three combinations (Met + Cys, Phe + Tyr, Gly + Ser) over three feeding phases from 6 to 53 days post-hatch overall, and the outcomes are shown in Table 5. The methodologies followed are outlined in detail in Roth et al. [27], Dorigam et al. [45] and Wecke and Liebert [46]. Overall, across both methodologies, the highest coefficients of variation were observed for isoleucine (12.5%), valine (9.82%), histidine (8.50%) and leucine (8.20%) as shown in parentheses. This suggests that branched-chain amino acid requirements are difficult to assess, which may stem in part from the established antagonism between branched-chain amino acids [47,48].

Table 5. The ideal profile for key amino acids relative to lysine (100) over three feeding phases estimated by two methods, adapted from Dorigam et al. [45].

Phase	Method	Arg	His	Ile	Leu	Met + Cys	Phe + Tyr	Thr	Trp	Val	Gly + Ser
6–21 days	A	109	40	48	119	68	139	70	16	86	156
	B	108	36	67	107	73	115	66	17	77	140
22–37 days	A	116	43	69	130	69	127	68	18	85	158
	B	105	35	67	107	71	114	64	17	76	135
38–53 days	A	98	39	66	118	58	119	61	16	65	150
	B	108	35	68	107	71	115	65	17	76	135
Mean		106.8	38.0	64.2	114.7	68.3	121.5	65.7	16.8	77.5	145.7
Standard dev.		±5.91	±3.23	±7.99	±9.40	±5.35	±9.83	±3.14	±0.75	±7.61	±10.37
C. of Variation		5.53%	8.50%	12.45%	8.20%	7.83%	8.09%	4.78%	4.46%	9.82%	7.12%

A: Broken line model with slaughter technique. B: Goettingen approach with N balance study.

4. Functional Amino Acids

One of the fundamental causes for the elusive nature of the ideal dietary amino acid profiles for broiler chickens is that the physiological roles of amino acids are not confined to serving as ‘building blocks’ of protein but extend to participating in a range of func-

tional duties [19,20,49]. In addition, amino acids also serve as substrates for energy provision in the gut mucosa [50] and via gluconeogenesis in the liver [8,51]. In a review of the functional properties of amino acids in poultry, Lee et al. [52] asserted that the identification of an amino acid requirement becomes increasingly more demanding as the functional roles of an amino acid exceed its synthesis into protein. Therefore, it is instructive to compare the amino acid profile of the 20 proteinogenic amino acids in the whole body of chickens [53] with the IAAR recommendations developed by Texas A&M [29]. The relevant data are shown in Table 6, where the recommended dietary values for glutamine (56%), methionine (36%), cysteine (38%), proline (32%) and glutamate (32%) exceed their whole-body concentrations. Thus, the likelihood is that forecasting the requirements for glutamine and glutamate, methionine plus cysteine and proline will be highly challenging because of their functional roles.

Table 6. Amino acid concentrations in the whole body of 10-day-old broiler chickens and the ratios relative to lysine compared with the Texas A&M ideal amino acid profile in the index column. Adapted from Wu [29] and Wu et al. [53].

Amino Acid	Concentration	Ratio	Texas A&M	Index ¹
Arginine	68.5	111	108	0.97
Histidine	21.1	34	35	1.03
Isoleucine	35.9	58	69	1.19
Leucine	69.2	113	109	0.97
Lysine	61.5	100	100	1.00
Methionine	18.9	31	42	1.36
Phenylalanine	34.8	57	60	1.05
Threonine	36.3	59	70	1.19
Tryptophan	11.6	19	17	0.90
Valine	41.8	68	80	1.18
Alanine	66.3	108	102	0.94
Asparagine	35.5	59	56	0.95
Aspartate	43.1	70	66	0.94
Cysteine	15.0	24	33	1.38
Glutamate	82.9	135	178	1.32
Glutamine	50.5	82	128	1.56
Glycine	115.0	187	176	0.94
Proline	85.3	139	184	1.32
Serine	45.0	73	69	0.95
Tyrosine	26.6	43	45	1.05

¹ the Texas A&M amino acid ratios divided by the amino acid ratios in whole-body protein of broiler chickens.

4.1. Glutamate, Glutamine and Glutamic Acid

Glutamate and glutamine are separate but interconvertible amino acids, and glutamic acid is used to portray glutamate plus glutamine in this review. Glutamate, an abundant amino acid and has been described as “truly functional” [54]. Some fifty years ago, Maruyama et al. [55] questioned whether glutamic acid should be regarded as a nutritionally dispensable amino acid in young chicks and the ‘essentiality’ of glutamine and glutamate has remained a topic of debate [56]. In combination, glutamine and glutamate are vital metabolites and play central roles in cell metabolism and function [23]. They are involved in renal acid-base homeostasis, hepatic and renal gluconeogenesis, oxidative fuel for intestinal cells and inter-organ transport of nitrogen, carbon, and energy. Dietary glutamate and glutamine are abundant in the systemic amino acid pool, although they are

almost entirely catabolised in the intestinal gut mucosa, which means that their systemic presence stems largely from biosynthesis. Glutamine biosynthesis is mainly generated from the condensation of glutamate and ammonia (NH_3) catalysed by glutamine synthetase, a reaction that is pivotal to NH_3 detoxification. Glutaminase catalyses the reciprocal reaction, in which glutamate and NH_3 are converted to glutamine; thus, glutamine and glutamate are interchangeable amino acids. Glutamine also plays a role in immune function, with involvement in lymphocyte proliferation, cytokine production and phagocytosis [57].

Although the two amino acids are separate entities, in practice, dietary concentrations of glutamine and glutamate are usually expressed on a combined basis as glutamic acid in poultry nutrition [24]. This is because glutamine is converted to glutamate by acidic extractions in standard amino acid assays. Clearly, specific glutamine and glutamate analyses would be preferable and may be completed following enzymatic hydrolysis by reverse-phase, high-performance liquid chromatography [58]. There is a paucity of recommended ideal ratios for glutamic acid relative to lysine; however, Wu [29] proposed ratios of 178 for glutamate and 128 for glutamine or a sum of 306 for glutamic acid.

The outcomes of 20 feeding studies in which glutamine was evaluated in broilers kept under thermoneutral conditions were summarised in [24]. An inclusion of 10 g/kg glutamine was evaluated in 16 of these studies in which positive weight gain responses were observed in 12 studies and positive FCR responses in 13 studies. The mean positive weight gain response was 5.62% (range: 0.39 to 10.6%), and FCR response was 2.93% (range: 0.13 to 6.57%). The most instructive study was completed by Fasina et al. [59]. Glutamine (10 g/kg) was included in a 13.6 MJ/kg, 220 g/kg CP, maize-soy diet, which was offered to straight-run Ross broiler chicks. The calculated concentrations of glutamine and lysine increased from 12.4 and 33.3 g/kg in the control diet to 12.7 and 41.9 g/kg in the treatment diet. Thus, the glutamine to lysine ratio was elevated from 269 to 330, which was associated with an increase in weight gains of 10.3% (325 versus 319 g/bird) and a numerical improvement in FCR of 2.93% (1.256 versus 1.293) from 1 to 14 days post-hatch. This outcome suggests that the rate of glutamine biosynthesis may not be adequate in rapidly growing broilers and that the glutamine to lysine ratio should be more than 300, particularly in reduced-CP broiler diets.

4.2. Methionine and Cysteine

Methionine and cysteine are sulphur-containing, proteinogenic amino acids, and methionine is usually the first limiting amino acid in broiler diets. In addition to being an essential amino acid for protein synthesis, methionine is a methyl donor in DNA methylation and a precursor of cysteine and carnosine. Methionine maintains antioxidant defence via glutathione and taurine pathways and is also involved in polyamine synthesis and as a sulphur donor [60,61].

Feathers contain an abundance of cysteine (75.3 versus 7.1 mg/g) relative to methionine [62], and methionine may be converted to cysteine via the transsulphuration pathway in the liver and in feather follicles [63]. The interrelationship between methionine and cysteine is important, and Sasse and Baker [64] suggested that cysteine should constitute between 48.5% and 52.7% of the total sulphur amino acids (TSAA). More recently, Kalinowski et al. [65] proposed that cysteine should represent 44% of TSAA in diets for slow-feathering birds and 47% for fast-feathering birds. This difference reflects the impact feathering has on amino acid requirements for ideal amino acid profiles for broiler chickens.

In practice, in reduced-CP diets, TSAA requirements are often met by additional methionine supplementation because monomeric cysteine is far more expensive. However, the implicit assumption is that methionine is converted to cysteine at sufficiently adequate rates, but this approach also depresses the cysteine proportion of TSAA. For example,

pursuant to dietary CP reductions from 222 to 165 g/kg in [66], *d,l*-methionine inclusions were increased from 2.67 to 4.53 g/kg. This approach decreased the specified proportion of cysteine in TSAA from 53.3% to 32.4%, which is well below the recommended proportions discussed [64,65] and may have compromised broiler performance.

The optimal concentration of digestible methionine plus cysteine dietary concentrations for broiler chicken performance was recently estimated using two statistical models [5]. In the grower phase (21 to 35 days post-hatch), optimal weight gain was supported by an average of 9.7 g/kg TSAA and in the finisher phase (36 to 42 days post-hatch), 8.20 g/kg TSAA supported optimal weight gain. Calculated digestible lysine levels were 10.0 g/kg in the grower and 8.9 g/kg in the finisher diets; therefore, the TSAA to lysine ratios were 97 and 92, respectively. These ratios are noticeably higher than the mean ratio of 74 in Table 1, which ranged from 70 to 82.

4.3. Proline

Proline is deemed to be a non-essential amino acid because proline may be synthesised from arginine in the avian liver by quite complex pathways [67]. However, Graber and Baker [68] reckoned that proline has an ‘essential nature’ in growing chickens because proline was essential for maximal performance. For example, weight gains were compromised by 9.72% from 1 to 35 days post-hatch in broilers offered diets from which proline had been eliminated. These researchers concluded that the growing broiler chickens could synthesise approximately 90% of their proline requirements. More recently, the roles of dietary proline, hydroxyproline and glycine in collagen synthesis and animal performance were reviewed [69]. Collagen is the most abundant protein in the body and collectively proline, hydroxyproline and glycine constitute 57% of the total amino acids in collagen. Birds can synthesise proline from arginine, hydroxyproline from proline residues in collagen and glycine from threonine, serine and choline. However, Li and Wu [69] concluded that the endogenous synthesis of these three amino acids is inadequate for maximal growth, collagen production, or feed efficiency in chickens.

Proline constitutes 8.83% of whole-body protein in chickens [53]. In addition, proline is an abundant amino acid in feathers, constituting 9.67% of feather protein (Table 7). It has been estimated that 19.3% of dietary proline is deposited in feathers [70,71]. The impact of feathering on the estimation of ideal amino acid profiles is considered more fully in Section 6.

Table 7. Average concentration of amino acids in feathers from four studies ¹, their share of total amino acid concentrations, and their relation to lysine.

Amino Acid	Mean (g/kg)	Share (%)	Ratio to Lys	Amino Acid	Mean (g/kg)	Share (%)	Ratio to Lys
Arginine	67.3	6.92	354	Alanine	44.3	4.55	233
Histidine	6.1	0.63	32	Aspartic acid	62.2	6.39	327
Isoleucine	46.4	4.77	244	Cysteine	71.3	7.33	375
Leucine	79.8	8.20	420	Glutamic acid	103.1	10.60	543
Lysine	19.0	1.95	100	Glycine	69.5	7.14	366
Methionine	5.5	0.57	29	Proline	94.1	9.67	495
Phenylalanine	46.5	4.78	245	Serine	113.8	11.70	599
Threonine	43.5	4.47	229	Tyrosine	27.0	2.77	142
Tryptophan	5.4	0.56	28	Total AA	972.9	100.0	
Valine	68.1	7.01	358	Crude protein	959.7		

¹ data adapted from Stilborn et al. [72], Adler et al. [62], Wecke et al. [73], and Greenhalgh et al. [70].

5. Bioequivalence of Amino Acids

There appears to be the tacit assumption across avian nutrition that the bioequivalence of non-bound and protein-bound amino acids in poultry is identical. However, the case has been made that amino acids were not all created equal [10]. The likelihood is that differences in intestinal uptake and post-enteral metabolism mean that the bioequivalence of non-bound amino acids versus amino acids derived from intact protein is not identical [11]. This fundamental dichotomy is almost certainly thwarting the development of ideal amino acid profiles, only more so in the context of reduced-CP diets.

5.1. Intestinal Uptake of Amino Acids

The contention advanced by Croom et al. [74] was that intensive genetic selection for enhanced growth performance in broiler chickens has resulted in intestinal absorption becoming rate-limiting for the full phenotypic expression of these production traits. The probability is that uptake rates of peptides and amino acids into the small intestinal enterocytes are more influential for growth performance than rates of enzymatic digestion of protein along the digestive tract [75]. Protein digestion is initiated in the proventriculus of poultry, where secretions of pepsin and HCl convert proteins into polypeptides, which are, in turn, degraded to short peptide fragments pursuant to the secretion of several pancreatic enzymes (trypsin, chymotrypsin, carboxypeptidase, elastase) into the duodenum. The peptide fragments are then broken down to di- and tri-peptides, or oligopeptides, by amino peptidases and dipeptidases in the apical membrane of enterocytes [76].

The intestinal uptake of the end-products of enzymatic protein digestion in broiler chickens occurs as di- and tri-peptides (oligopeptides) via the peptide transporter, Pept-1 [77], or as monomeric amino acids via an array of sodium Na^+ -dependent and Na^+ -independent transport systems [78]. Moreover, Miska and Fetterer [78] compared gene expression for small intestinal amino acid and sugar transporters in rapidly and slowly growing broilers and found they could be correlated to growth. This is supportive of the Croom et al. [75] hypothesis that the intestinal absorption capacity is rate-limiting for broiler-growth performance. Non-bound amino acids do not require digestion and are rapidly absorbed and generate higher amino acid concentrations in systemic plasma than protein-bound amino acids [19]. Pept-1 was identified in 1994 and is responsible for intestinal uptake of more than 400 different dipeptides and some 8000 tripeptides in humans [79]. Moreover, 'protein' is predominantly absorbed as peptides rather than as monomeric amino acids [80]; indeed, Krehbiel and Matthews [81] asserted that 70 to 85% of amino acids are absorbed as oligopeptides via the peptide transporter, Pept-1. The activity of Pept-1 is totally dependent on NHE for proton donations with which to co-absorb peptides; therefore, the functions of Pept-1 and NHE are inextricably linked [82]. An avian Na^+/H^+ exchanger (NHE) has been identified [83]. Intact protein must be degraded to peptides or monomeric amino acids prior to absorption, but then the intestinal uptake of amino acids as peptides via Pept-1 is more rapid and energetically efficient than uptake of monomeric amino acids via Na^+ -dependent and Na^+ -independent transporters [84]. Pept-1 mRNA expression occurs mainly in the small intestine, and peptide transport via Pept-1 is regulated by factors including amino acids, peptide substrates, insulin, and growth factors in broiler chickens [85].

The duodenal infusion of equivalent amino acid blends consisting of either free amino acids or small peptides in pigs was investigated by R  rat et al. [86]. These researchers reported marked differences in the pattern of immediate amino acid absorption, as amino acids infused as peptides, particularly arginine, histidine, lysine, phenylalanine, threonine, proline and tyrosine, were more rapidly absorbed in the first hour. Also, the uptake of amino acids from the systemic circulation by the gut mucosa was greater fol-

lowing free amino acid infusion, including the uptake of isoleucine, leucine and valine, than following infusions of peptides.

Intestinal uptake of monomeric amino acids, including those derived from intact protein or included in diets as non-bound amino acids, is conducted via an array of some thirteen Na⁺-dependent and nine Na⁺-independent transport systems with overlapping affinities [87]. Because non-bound amino acids do not have to be digested prior to absorption, it is usually accepted that their intestinal uptake is complete and that their digestibility is complete. Indeed, Lemme et al. [88] concluded that the standardised ileal digestibility of non-bound amino acids was 100%. However, in [88] the experimental diets were atypical in that they were devoid of di- and tri-peptides and intact proteins, so the outcomes may be misleading.

Intestinal uptake of glucose predominantly takes place via the sodium-glucose co-transporter 1 or SGLT-1, where glucose is co-absorbed with sodium [89]. Thus, monomeric amino acids and glucose effectively compete for co-absorption with sodium (Na) via their respective Na⁺-dependent transport systems. This implies that there could be mutual inhibition of intestinal uptake of amino acids and glucose in broiler chickens; a possibility is supported in several studies [90–93], although not specific to poultry. However, dietary starch was shown to influence the digestibility of amino acids in broiler chickens and offered six low-protein, maize-based diets from 7 to 28 days post-hatch in Moss et al.'s research [94]. Across six dietary treatments, the mean apparent proximal ileal starch digestibility coefficient was 0.942, ranging from 0.832 to 0.968. However, there were significant, negative correlations between starch and the proximal ileal digestibility coefficient of 12 amino acids, as only alanine, aspartate, cysteine and proline were not statistically correlated. The most strongly correlated were phenylalanine (-0.507 ; $p < 0.001$), arginine (-0.502 ; $p < 0.001$) and isoleucine (-0.500 ; $p < 0.001$). More recently, Riedel et al. [95] investigated intestinal interactions between glucose and alanine in broiler chickens offered low-protein, wheat-based diets. These researchers reported that 20 mM glucose nearly completely inhibited the Na⁺-dependent portion of alanine absorption and concluded that there is a bidirectional interaction of amino acid and glucose in jejunal intestinal uptake. This may be due to subapical Na accumulation in enterocytes and may partially explain reduced growth performance in broilers fed low-protein wheat-based diets as reported in [66].

5.2. Apparent Digestibility Coefficients of Amino Acids

Eight amino acid digestibility assays involving reduced-CP, maize- and wheat-based diets completed by the Poultry Research Foundation were reviewed [96]. Dietary CP concentrations were reduced by an average of 44.2 g/kg, from 214.1 to 169.9 g/kg. Mean percentage responses to this CP reduction ranged from a decrease of 8.21% to an increase of 29.4% in distal jejunal digestibility coefficients and from a decrease of 8.36% to an increase of 7.43% in the distal ileum. These aberrations in the digestibility of amino acids are clearly a challenge to the acceptance of reduced-CP broiler diets and have obvious implications for the precision of ideal amino acid profiles in reduced-CP diets. Increases in the digestibility of amino acids could stem from dietary inclusions of non-bound amino acids because of their notional 100% digestibility [88]. Decreases in digestibility could stem from competition for intestinal uptake between amino acids themselves [97] and between amino acids and glucose [93].

An instructive study was completed by Macelline et al. [98] in which graded inclusions of *l*-methionine (1.38, 2.76, 4.14 g/kg) were added to a standard wheat-based diet with specified digestible levels of 3.07 g/kg methionine and 3.00 g/kg cysteine, which were offered to broiler chickens from 1 to 21 days post-hatch. However, additional *l*-methionine quadratically ($r = 0.841$; $p < 0.001$) increased apparent ileal digestibility coefficients of me-

thionine *per se* by up to 5.19% (0.933 versus 0.887). In contrast, additional *l*-methionine significantly depressed digestibility coefficients of the balance of 15 amino acids by an average of −5.04% (0.754 versus 0.794). Aspartic acid (−7.96%), serine (−7.24%) and tyrosine (−7.04%) were the most influenced. Methionine has been shown to inhibit intestinal uptake of alanine and glycine in poultry [97]. Thus, it is consistent that additional methionine depressed digestibility of alanine by up to 5.35% (0.726 versus 0.767) and glycine by up to 6.49% (0.677 versus 0.724) in the Macelline et al. [98] study. The fact that graded inclusions of *l*-methionine significantly depressed the apparent ileal digestibility of the entire balance of the 15 assayed amino acids has obvious implications for any similar studies. Based on the data of Hyde et al. [87], methionine shares overlapping affinities with 11 neutral amino acids via Na⁺-dependent transport systems and with seven neutral amino acids via Na⁺-independent transport systems.

Similarly, the dietary inclusion of 1.8 g/kg lysine, as lysine monohydrochloride, significantly increased the ileal digestibility of lysine *per se* by 5.42% (0.837 versus 0.794) in [99]. However, the additional lysine significantly increased the ileal digestibility of seven amino acids (isoleucine, methionine, phenylalanine, valine, aspartic acid, glutamic acid, tyrosine) by an average of 3.66% (0.822 versus 0.771), which is in complete contrast to the negative impact of additional methionine. That additional lysine increased the ileal digestibility of lysine *per se* was attributed to the up-regulation of two amino acid transport systems identified in [100]. These researchers reported that increasing the dietary lysine concentration by 4.0 g/kg, from 9.6 to 13.6 g/kg, significantly increased lysine transport across the jejunal brush border membrane in chickens via two particular b^{0,+}-like and y⁺-like, Na⁺-independent transport systems. Subsequently, Torras-Llort et al. [101] described the b^{0,+}-like transporter as having a broad specificity incorporating cationic and neutral amino acids, behaving as an obligatory amino acid exchanger and playing a key role in the intestinal absorption of amino acids. Thus, it follows that when the b^{0,+}-like transporter is up-regulated by dietary lysine enrichment, there is the real possibility that absorption of certain other amino acids will be positively influenced.

5.3. Transition Across Gut Mucosa

The transition of amino acids across enterocytes of the gut mucosa along the small intestine is not straightforward. This is because amino acids may be diverted into catabolic or anabolic pathways and are denied entry into the portal circulation. However, the catabolism of amino acids in enterocytes is considered in Section 7.1. The entry of amino acids into anabolic pathways is considerable; the gut mucosa contributes substantially to protein synthesis in humans [102]. This protein synthesis is necessary to maintain gut integrity and serve as precursors for mucin, numerous digestive enzymes, nucleotides, polyamines and amino acids [19]. Porcine mucin has a CP content of 343 g/kg and remains essentially undigested along the small intestine to contribute to endogenous amino acid flows [103]. Therefore, the pattern of amino acids in portal venous blood differs markedly from that in the diet because of the diversion of amino acids into catabolic or anabolic pathways in the gut mucosa, which negates their availability to extraintestinal tissues [50].

5.4. Post-Enteral Metabolism

The kinetics of radio-actively labelled, free or protein-bound leucine have been investigated in humans [104]. It was found that the form in which leucine is consumed influences its immediate metabolic fate and retention in the body. The more rapid intestinal uptake of non-bound amino acids is fundamental to the differences in bioavailability between non-bound and protein-bound amino acids because of their asynchronous entry into the systemic pool. The likelihood is that these asynchronous entries generate post-enteral amino acid imbalances. Amino acid imbalances have been defined as changes in

proportions of amino acids resulting in adverse effects [105], and the likelihood is that they would be more in evidence in birds offered low protein diets [106]. The adverse impacts of broiler diets deliberately formulated to include amino acid imbalances were demonstrated by Swatson et al. [107], where the imbalanced, 200 g/kg CP amino acid diet compromised weight gain by 43.0% (17.0 versus 29.8 g/bird/day) from 10 to 24 days post-hatch in comparison to the control diet

The concept of post-prandial amino acid oxidation was proposed by Schreurs et al. [108]. Subsequently, the postprandial oxidation of egg white protein was compared with a corresponding non-bound amino acid blend via $^{13}\text{CO}_2$ breath tests in rats [109]. Post-prandial oxidative losses of non-bound leucine (22.5%) were significantly higher than leucine from intact protein (16.3%). This suggests that non-bound amino acids are more likely to undergo post-prandial oxidation than their protein-bound counterparts, presumably from their more rapid systemic entries.

5.5. Amino Acid Antagonisms

Antagonistic interactions between amino acids will impinge on their bioavailability, and the antagonisms between lysine and arginine [110,111] and the branched-chain amino acids, isoleucine, leucine and valine [112,113] are recognised in poultry. However, lysine–arginine antagonism became less of an issue when the formulation of broiler diets became based on amino acid concentrations rather than crude protein levels [114]. Nevertheless, the lysine–arginine antagonism was evaluated in reduced-CP broiler diets by Schedle et al. [115]. Antagonism was not detected in growth performance parameters, although antagonism was evident with respect to breast-meat yield and carcass weight.

The antagonism across the branched-chain amino acids was considered a re-emerging concern by Maynard and Kidd [114] and more likely to be declared in reduced-CP diets. Branched-chain amino acid metabolism and antagonism are highly complicated, as reflected in the Harper et al. [116] review. Fundamentally, the antagonism stems from the fact that their degrading enzymes, firstly branched-chain aminotransferase and secondly branched-chain α -keto acid dehydrogenase, are commonly shared. Classically, high leucine levels will trigger these degrading enzymes, which would also increase the degradation of valine and isoleucine. [117]. Wheat- and sorghum-based diets with CP contents of 187.5 g/kg and three branched-chain amino acid inclusion regimes were offered to male broiler chicks from 7 to 28 days post-hatch in Greenhalgh et al. [118]. The first or standard tier of inclusion regimes relative to lysine were leucine 105, isoleucine 65 and valine 75, and the third or high tier inclusions were leucine 150, isoleucine 75 and valine 85. With the wheat-based diets, the transition from the standard to high branched-chain amino acid inclusion regime significantly depressed growth rates by 9.49% (1288 versus 1423 g/bird) and compromised FCR by 8.33% (1.665 versus 1.537). In contrast, with the sorghum-based diet, the same transition significantly increased weight gains by 9.26% (1451 versus 1328 g/bird), while fractionally improving FCR from 1.390 to 1.378. This extraordinary outcome illustrates both the complexity of branched-chain amino acids and the importance of the different properties of feed grains in poultry.

A novel proposition for branched-chain amino acid antagonism in broiler chickens was recently advanced by Wang et al. [119]. These researchers reckoned that antagonism triggered by high leucine levels could be alleviated by either valine addition or isoleucine restriction, while making the case that the function of insulin was mainly responsible for this alleviation. These researchers concluded that isoleucine and valine modulated antagonism through influencing the insulin signalling pathway and that this outcome offers a strategy to ensure adequate amino acid nutrition in low-protein diets. Avian species are usually considered to be both hyperglycaemic and resistant to insulin [120]; consequently, insulin is generally overlooked in broiler chickens. In humans, links between leucine and

insulin are recognised [121,122] as leucine regulates several cellular processes within pancreatic β -cells, including insulin secretion. However, it does appear that embryonic and young broiler chicks are in fact sensitive to insulin and insulin resistance develops with age [123,124]. Indeed, the perception has been advanced that the feed grain basis (maize versus wheat) of reduced-CP diets modifies the anabolic impact of insulin on the growth performance of broiler chickens [125]. Also, Wang et al. [126] contended that insulin is involved in differences in growth performance and fat deposition in broiler chickens offered reduced-CP diets based on either maize or wheat. Interestingly, branched-chain amino acids have been shown to increase the sensitivity of muscle protein synthesis to insulin in rats [127]. The suggestion by Wang et al. [119] that insulin is involved in branched-chain amino acid antagonism in broiler chickens is certainly of interest but should be treated with caution as broilers are usually considered to be resistant to insulin.

6. Feathering

It is generally accepted that the capacity of broiler chickens to accommodate reductions in dietary CP with uncompromised growth performance [96,128] is inferior to that of pigs [129]. Feathering may be a fundamental reason for this curious difference. It was suggested some sixty years ago that the increasing contribution of feather protein to total protein was the only variation in the otherwise constant amino acid composition of the fat-free broiler carcass [12]. Obviously, meeting the amino acid requirements for feathering is a challenge faced only by poultry nutritionists and is not a complicating factor in swine. The nutritional regulation and molecular signalling of feather growth in poultry was considered in Chen et al. [130]. As outlined in [130], the sulphur-containing amino acids, methionine and cysteine, are considered the first-limiting amino acids in most practical poultry diets and play an important role in feather follicle development and feather growth. The branched-chain amino acids (isoleucine, leucine, valine) also influence feather growth and their deficiencies can trigger feathering abnormalities. Collectively, the branched-chain amino acids constitute 17.52% of feather amino acids (Table 7).

The crude protein content of feathers of broiler chickens exceeds 950 g/kg (Table 7). Interestingly, an elevation in dietary CP from 170 to 250 g/kg increased feather weights from 5.97 to 9.30 g/bird and from 1.23% to 1.40% as a percentage of body weight [131]. The amino acid composition of the feathers of broiler chickens is shown in Table 7, where the values have been averaged from four studies [62,70,72,73]. As tabulated, serine, glutamic acid and proline are dominant and constitute 32.0% of the total amino acids in feathers. In one study [72], amino acid contents of feathers were determined in birds at various ages, and significant differences were recorded for all 18 amino acids assessed. For example, from 14 to 56 days post-hatch, concentrations of serine and proline increased as a percentage of feather protein. It is pertinent that feathers contain an abundance of cysteine relative to methionine by a 13-fold factor (71.3 versus 5.5 g/kg). The proportions of six digestible dietary amino acids deposited in feathers of broiler chickens between 24 and 39 days of age were estimated by Leeson [132]. Values ranged from 52% for cysteine, 18% for valine, 14% for arginine, 8% for threonine, and 5% for methionine and lysine. Using a similar approach, proportions of eighteen dietary amino acids deposited in feathers of 39-day-old chickens were estimated in [71], based on the data in [70], as shown in Table 8. Clearly, substantial proportions of dietary cysteine (54.0%) and serine (28.8%) are incorporated into feathers, in sharp contrast to lysine (3.0%) and methionine (2.9%).

Table 8. Estimated proportion of dietary amino acids deposited as feathers (ranked in descending order).

Amino Acid	Estimate (%)	Amino Acid	Estimate (%)
Cysteine	54.0	Leucine	12.6
Serine	28.8	Tyrosine	11.7
Histidine	20.1	Alanine	11.6
Glycine	19.6	Phenylalanine	11.5
Proline	19.3	Aspartic acid	7.8
Valine	19.2	Glutamic acid	5.7
Threonine	14.1	Tryptophan	4.9
Arginine	13.1	Lysine	3.0
Isoleucine	12.8	Methionine	2.9

Adapted from Selle et al. [71] and Greenhalgh et al. [70].

Feathers as a percentage of body weight increased from 0.9% at hatch, to 2.4% at 14 days, 4.7% at 21 days, 3.9% at 28 days and 5.1% at 42 days post-hatch. This was recorded in male Ross chickens in the Sklan and Noy [33] study to determine optimal amino acid intakes for maintenance and growth. It may be deduced from the data that feathers as a percentage of body weight increased in a linear manner ($r = 0.903$; $p = 0.036$), which lends support to the Holmes et al. [12] postulate. In a survey of male and female Cobb and Ross birds conducted by Vargas et al. [133], feathers constituted an average of 3.84% (107.5 from 2801 g/bird) of body weight at 42 days post-hatch. In male Ross birds specifically, at 42 days, feathers constituted 3.78% (116.0 from 3065 g/bird) of body weight.

Poor feathering was observed in a feeding study involving reduced-CP, wheat-based diets [70]. The reduction in dietary CP from 198.5 to 180.0 and 162.5 g/kg was associated with a significant ($p = 0.031$) deterioration in feather scores from 1.9 to 2.9 and 3.1; moreover, feather scores were related to declining weight gains in a quadratic ($r = 0.443$; $p < 0.005$) manner. In this study, a positive control diet had a CP content of 215 g/kg, and it is instructive to compare the analysed dietary amino acid concentrations of the positive control diet with the two 162.5 g/kg CP diets. Concentrations of serine declined by 35.8% (7.0 versus 11.7 g/kg), glutamic acid by 28.6% (32.75 versus 45.9 g/kg), proline by 25.7% (10.25 versus 13.8 g/kg), and glycine by 34.2% (6.25 versus 9.5 g/kg). Cysteine concentrations were not determined; nevertheless, the above four amino acids constitute 39.1% of total amino acids in feathers (Table 3). Therefore, it is plausible that dietary concentrations of these notionally non-essential amino acids were not sufficient to support adequate feathering and growth performance. It is relevant that Leeson and Walsh [134] concluded that in young broiler breeder chicks, diets with less than 160 g/kg CP can trigger poor feathering that is not corrected by relatively high dietary inclusions of non-bound amino acids.

6.1. Partitioning of Amino Acids

Partitioning of nutrients for growth and other metabolic functions and their priorities were examined by Baker [39]. However, the partitioning of amino acids to protein synthesis as either feathers or muscle tissue was not considered. The vast difference between amino acid compositions of carcass and feathers of broiler chickens is evident in the data generated by Wang et al. [135] in Table 9. For instance, the proportion of cysteine in feathers exceeds that in the carcass by a factor of 7.4, whereas the proportion of methionine in the carcass exceeds that in feathers by a factor of 5.6. Thus, the allocation of priorities for the partitioning of amino acids, especially methionine and cysteine, for protein deposition is an issue.

Table 9. Relative carcass and feather amino acid compositions (proportion of protein) in broilers at 15 to 28 days post-hatch, ranked in descending order, adapted from Wang et al. [135].

Amino Acid	Carcass (%)	Feathers (%)	Factor
Cysteine	0.80	5.90	7.38
Serine	3.75	10.39	2.77
Proline	4.38	8.44	1.93
Phenylalanine	3.75	4.59	1.22
Valine	5.38	6.54	1.22
Threonine	3.98	4.48	1.13
Leucine	7.12	7.76	1.09
Isoleucine	4.20	4.42	1.05
Arginine	6.90	6.88	1.00
Glycine	7.10	6.76	0.95
Glutamic acid	13.15	10.53	0.80
Aspartic acid	8.30	6.49	0.78
Tyrosine	2.05	1.60	0.78
Alanine	6.18	4.15	0.67
Histidine	2.34	0.79	0.34
Lysine	6.85	2.13	0.31
Methionine	2.53	0.45	0.18

It appears that the likelihood is that the partitioning of amino acids towards feathering is given priority over skeletal and smooth muscle protein deposition. Conde-Aguilera et al. [136] asserted that given a methionine deficiency, feather formation is given priority in broiler chickens. In support, Wecke et al. [73] stated that feather protein synthesis is given priority in the event of dietary amino acid inadequacies. The efficiency of methionine and cysteine utilisation for feathering surpassed that for feather-free body protein deposition in a study, which used stable, labelled methionine and cysteine isotopes [137]. This outcome supports the proposition that preference is given to feathering in the partitioning of amino acids. Moreover, albeit in turkeys, Wylie et al. [138] concluded that feather growth was maintained as much as possible at the expense of body growth when the amino acid concentration of the ration was less than that required to maximise body and muscle weight gain. Interestingly, these researchers found dietary crude protein was preferentially partitioned to feather growth rather than muscle growth in modern turkeys (British United Turkeys) in contrast to traditional turkeys (Nebraska Spot) in which the growth of feathers and muscle were affected equally. These researchers also found that arginine and methionine supported increased feather growth rather than muscle growth, which was not the case for tyrosine and valine.

Whether amino acids are preferentially partitioned to feathers or to whole-body protein—especially skeletal protein—is certainly a pivotal issue. If, in fact, feathering is given priority for amino acids, it then becomes imperative to pay more attention to the amino acid profiles of feathers in the formulation of diets for broiler chickens.

6.2. Slow Versus Fast Feathering

Presently, broiler chickens are either fast-feathering or slow-feathering birds. Fast-feathering is controlled by a recessive sex-linked gene (k^+), whereas the dominant allele (K) produces slow-feathering [139]. These researchers investigated but did not observe any relationship between feathering rate and body weight in broiler chickens more than 60 years ago. More recently, the effects of late- or early-feathering genotypes on performance and carcass traits of broiler chickens were investigated in [140]. Higher weight gains and feed conversion ratios were observed in late-feathering birds, and the weights

of the carcass, heart, spleen and liver were also higher. Interestingly, the addition of 1.8 g/kg *L*-threonine to a threonine-deficient diet (5.6 g/kg threonine, 9.5 g/kg lysine) was shown to increase weight gain of fast-feathering birds by 7.06% (91.0 versus 85.0 g/bird/day) from 42 to 56 days post-hatch [141]. In contrast, the weight gain (88.8 versus 87.5 g/bird/day) of slow-feathering birds was not influenced by additional threonine. As threonine constitutes 4.47% of total amino acids in feather protein (Table 6), the fact that there were differences in weight gain responses to threonine in fast- and slow-feathering broiler chickens emphasises the impact feathering has on amino acid requirements. Also, as noted, it appears that fast-feathering birds need more cysteine relative to methionine (47 versus 44%) than slow-feathering birds to meet requirements [65]. Decades earlier, Moran [142] reasoned that differences in feathering rates because of strain, sex, and age could explain a large part of the variability in determined methionine plus cysteine requirements.

Broiler chickens are commonly reared on a straight-run or mixed-sex basis. In theory, single-sex or separate-sex rearing would permit more precise nutritional regimes by recognising the known effects sex has on lean tissue deposition and amino acid requirements [143]. The influence of mixed versus single-sex rearing in relation to performance, coefficient of variation, flock uniformity and research settings was considered by England et al. [144,145]. These researchers issued the caveat that the difference in rearing options would only be feasible provided quick, practical and cost-effective methods of sexing birds are available. However, automatic feather-sexing technologies have now been developed. It has been reported that one machine is up to 98% accurate and can process up to 160,000 birds per hour depending on configuration [146]. The introduction of this new technology may well increase the acceptance of slow-feathering broiler chickens because they can be automatically feather-sexed, which would facilitate the transition to separate-sex rearing in the future.

7. Catabolism of Amino Acids

The utilisation of amino acids for energy provision rather than functional roles or protein accretion appears counterproductive; nevertheless, it has been estimated that 40.8% of digestible, proteinogenic amino acids are catabolised to carbon dioxide in broiler chickens [7]. The average metabolisable energy value of 20 non-bound, proteinogenic amino acids was 20.3 MJ/kg $\pm$ 5.74, ranging from 11.9 MJ/kg (aspartate) to 29.0 MJ/kg (phenylalanine) as reported in [147]. The catabolism of amino acids for energy provision has been considered more fully in [8].

7.1. Catabolism in Enterocytes

As discussed, the transition of amino acids across enterocytes of the gut mucosa along the small intestine is not straightforward. The gastrointestinal tract consumes approximately 20% of incoming energy to drive digestive processes [148], and much of this demand is met by the catabolism of amino acids in the gut mucosa [149]. The catabolism of glutamate and aspartate of dietary origin and glutamine of systemic origin accounts for most of this energy provision in mammalian species [150]. Investigations into this phenomenon in broiler chickens are limited [151,152], which is unfortunate because it may be possible to ameliorate amino acid catabolism by nutritional strategies. However, the oxidation of amino acids, glucose, and fatty acids as metabolic fuels in enterocytes of broiler chickens was investigated by He et al. [153]. These researchers concluded that glutamate, followed by glucose, was the major energy source for avian enterocytes and the oxidation of the other amino acids assessed, glutamine, aspartate, and alanine, was limited. Also, avian enterocytes had a limited ability to degrade long- and short-chain fatty acids. The contention of He et al. [153] was that dietary glutamate is essential for the integrity and

function of the small intestine because chicken enterocytes do not take up glutamate from the arterial blood. Data generated in weaner pigs suggest that about 18% of dietary amino acids enter catabolic pathways in the gut mucosa and that glutamate, in particular glutamine and glucose, meet three-quarters of the energy demands of the portal-drained viscera in weaner pigs [154].

In rats, glucose and glutamine provide similar proportions of energy to the gut mucosa mucosal cells of rats' small intestines [155]. This suggests there may be a 'catabolic ratio' between glucose and amino acids for energy provision to enterocytes, which could differ across animal species but may be subject to manipulation by dietary strategies. For example, it has been suggested that the dietary provision of slowly digestible starch may spare amino acids from catabolism in the avian gut mucosa [156]. It follows that slowly digestible starch would provide a more persistent alternative energy substrate along the small intestine. However, the utilisation of substrates by enterocytes for energy provision was specifically reviewed by Duée et al. [157], and fuel selection in intestinal cells is complicated. The high turnover rates of enterocyte and intracellular protein synthesis drive the high energy demand of the gut, but the source of this energy may change depending on whether the animal is fed or fasted.

7.2. Systemic Catabolism

Both amino acid imbalances and protein turnover will lead to the systemic catabolism of amino acids. Amino acids, other than branched-chain amino acids, are mainly catabolised in the liver, where the α -amino groups of amino acids are lost by oxidative deamination or transamination. The carbon skeletons are either converted to glucose via gluconeogenesis or oxidised for ATP synthesis [158]. For adult humans in nitrogen balance, a quantity of amino acids almost equal to the dietary intake can be considered as surplus in that they will be catabolised [159]. As the body does not have a store for amino acids, dietary amino acids surplus to requirements for protein synthesis in humans are rapidly catabolised [160]. In broiler chickens, with positive nitrogen balances, any dietary imbalances will result in the catabolism of amino acids that are in excess [161].

Protein turnover is an ongoing, dynamic balance between protein deposition and protein degradation. The absolute synthesis and breakdown rates of protein were determined in *Pectoralis major* in 21-day-old broiler chickens by Urdaneta-Rincon and Leeson [162]. Absolute protein synthesis averaged 626 mg/day and protein breakdown averaged 321 mg/day; thus, net protein synthesis or protein accretion was 305 mg/day. Superficially, protein degradation appears counter-productive; however, it is required to remove misfolded, aged, or damaged proteins [163]. Interestingly, amino acids rendered redundant by protein turnover are used primarily for energy production rather than *de novo* protein synthesis [164].

7.3. Ammonia and Uric Acid Synthesis

The catabolism of amino acids generates ammonia (NH_3), which is inherently toxic, and the intravenous LD_{50} of ammonium acetate in broiler chickens is about half that of mice [165]. Several broiler studies have associated elevated plasma NH_3 concentrations with compromised growth performance [166–169]. Therefore, it is imperative that NH_3 is rapidly detoxified, which involves the condensation of NH_3 with glutamic acid to generate glutamine in a reaction catalysed by glutamine synthetase [170]. Glutamine enters the Krebs cycle, where it is converted to and excreted via the kidneys as uric acid [171]. The conversion of four NH_3 molecules into uric acid in the avian liver requires two molecules of glutamine and one molecule of glycine and aspartate [172]. Also, there is a minimal energy cost of 64.7 kJ/g N excreted as uric acid in [173]. The genesis of intrinsic NH_3 and

its impacts on the growth performance of broiler chickens is considered in more detail in Selle et al. [174].

8. Summary

An abundance of variables is impacting on the bioavailability of twenty proteino-genic amino acids to complicate estimations of ideal amino acid profiles, as discussed in the foregoing sections. Moreover, our knowledge of these variables is largely incomplete; consequently, it is difficult to rank the relative importance of these contributing factors. A schematic diagram (Figure 1) illustrates some of the factors that impact the accuracy of ideal amino acid ratios in broiler chickens. Arguably, the most challenging factor is the partitioning of amino acids to either a plethora of functional roles or to becoming ‘building blocks’ of protein, but this may be in whole-body protein or in feathers. There are indications that feathering may be given preference, an issue which ideally should be clarified. Another challenging factor is the interactions involving amino acids that exist. The digestive dynamics of non-bound and protein-bound amino acids are diverse, which is an increasingly complicating factor in reduced-CP diets. Also, amino acids interact amongst themselves as they compete for intestinal co-absorption with sodium via Na⁺-dependent transport systems, plus there are recognised antagonisms between certain amino acids. Amino acids also compete with glucose for intestinal uptake via their respective Na⁺-dependent transport systems, which is probably of lesser importance as amino acids are predominantly absorbed as oligopeptides. The catabolism of amino acids is a distinct problem as it elevates plasma NH₃ concentrations, and NH₃ is highly toxic and readily compromises growth performance. The transition of amino acids across the gut mucosa diminishes their entry into the portal circulation because they are captured by either catabolic pathways to become energy substrates and/or anabolic pathways for the synthesis of proteins, including mucin and endogenous digestive enzymes. In addition, amino acid imbalances contribute to catabolism as surplus amino acids derived from either the diet or generated by protein turnover are catabolised. Another issue which ideally should be clarified is the metabolic fates of amino acids in small intestinal enterocytes, as limited research has been completed in this area.

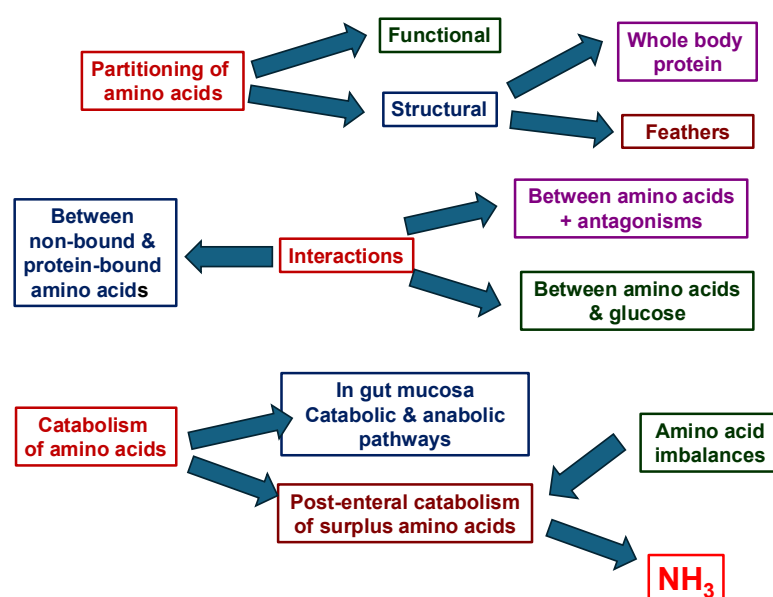


Figure 1. Schematic diagram illustrating some of the factors impacting the accuracy of ideal amino acid ratios in broiler chickens.

The feed grain basis has an enormous impact on growth performance in reduced-CP diets, where maize is usually superior to wheat for numerous possible reasons [175,176]. In addition, the feed grain basis of a broiler diet will impact the ideal amino acid profile. As shown in Table 10, the Chi-squared distance for maize (12.5) is less than that of wheat (21.2). The Chi-squared (X^2) distance was calculated by the Guilloteau et al. [177] methodology to compare amino acid composition of proteins from the Rostagno et al. [147] data for amino acid concentrations in feedstuffs. The equation used to calculate X^2 is presented as follows:

$$X^2 distance = 17 \sum_{k=1}^{k=17} \frac{(AA_{ik} - AA_{jk})^2}{\frac{(AA_{ik} + AA_{jk})}{2}}$$

In the equation, i represents the concentration of the k th amino acid in the IAAR, whereas j represents the concentration of the k th amino acid in a specific feedstuff. The X^2 distance value indicates the degree of similarity between the amino acid profiles of the two protein sources, with lower X^2 distance values representing greater similarity between amino acid profiles. Similarly, the lower X^2 distance value obtained for SBM compared with other feedstuffs indicates that the amino acid profile of SBM is more closely aligned with the IAAR recommended for broiler chickens in [30]. Thus, the maize amino acid profile is more closely aligned to the IAAR recommended for broilers than the amino acid profile of wheat. This means that ideal amino acid profiles for maize-based diets are likely to be more accurate than diets based on barley, sorghum and wheat (Table 10).

Table 10. Chi-squared distance values between amino acid profiles in feedstuffs and ideal amino acid requirements for broiler chickens.

Amino Acid (%)	Amino Acid Profile ¹	Standardised Ileal Digestible (SID) Amino Acid Concentrations ²				
		SBM ³	Maize	Barley	Sorghum	Wheat
Arginine	1.24	3.23	0.34	0.44	0.31	0.53
Histidine	0.49	1.13	0.22	0.22	0.19	0.26
Isoleucine	0.90	1.99	0.24	0.29	0.33	0.39
Leucine	1.59	3.36	0.91	0.60	1.12	0.75
Lysine	1.26	2.69	0.19	0.32	0.18	0.29
Methionine	0.58	1.21	0.15	0.15	0.13	0.18
Phenylalanine	0.95	2.33	0.33	0.45	0.45	0.52
Threonine	0.85	1.64	0.29	0.27	0.25	0.30
Tryptophan	0.21	0.61	0.06	0.10	0.09	0.12
Valine	0.99	2.00	0.31	0.38	0.41	0.46
Aspartic acid	0.93	1.74	0.49	0.31	0.76	0.33
Cysteine	1.89	4.90	0.45	0.53	0.50	0.53
Glutamic acid	0.32	0.62	0.14	0.21	0.14	0.25
Glycine	3.40	9.32	1.44	2.27	1.94	3.36
Proline	0.77	1.73	0.24	0.40	0.24	0.44
Serine	1.13	2.12	0.76	0.98	0.71	1.23
CP (%)		48.1	7.86	10.8	8.75	11.5
X^2 distance ⁴		1.79	12.5	13.8	18.2	21.2

¹ amino acid recommendations for broiler chickens. Adapted from Maharjan et al. [30]; ² SID amino acid concentrations for broilers from Brazilian tables. Adapted from Rostagno et al. [147]; ³ SBM: soybean meal; ⁴ X^2 = Chi-squared distance.

The digestive kinetics of feedstuffs in diets for broiler chickens were considered in depth by Liu et al. [178]. They are based on the simple premise that protein accretion, or growth, is dependent on the harmonious availability of an appropriate blend of amino acids and provision of energy, derived mainly from glucose, at sites of protein synthesis [179]. For example, in [178], the following multiple linear relationship ($r = 0.936$; $p < 0.001$) was detected for proximal ileal protein and starch disappearance rates:

$$y(\text{weight gain}) = 857.53 + 109.12 \times \text{protein}_{(\text{g/bird/day})} - 13.34 \times \text{starch}_{(\text{g/bird/day})}.$$

This equation shows that rapidly digestible protein will accelerate weight gains, whereas rapidly digestible starch will retard weight gains. Not surprisingly, the important caveat was issued that the digestive dynamics of protein/amino acids should not be considered in isolation from those of starch/glucose [11]. Moreover, one feed ingredient was shown to influence the disappearance rate of nutrients from other feed ingredients in a complex broiler diet [180].

As mentioned, the elucidation of the metabolic fates of amino acids and glucose in enterocytes would be beneficial as it may be possible to spare amino acids from catabolism in the gut mucosa by nutritional strategies. Albeit in pigs, Li et al. [181] concluded that sources of dietary starch profoundly influence the net appearance of amino acids and glucose in the portal circulation, and the starch source has important implications for the efficiency of nutrient utilisation. In broiler chickens, Yin et al. [182] compared three starch sources, maize, waxy rice and amylose, in diets with two CP levels. Waxy rice generated significantly higher plasma concentrations of 18 amino acids in blood taken from the anterior mesenteric vein than amylose by 22.4% (4490 versus 3668 nmol/L). Plasma concentrations supported by maize (3952 nmol/L) were intermediate. This outcome suggests that amino acid catabolism in the gut mucosa can be manipulated by nutritional strategies in broiler chickens.

Finally, the salient point made by Klasing [183] was that any unnecessary catabolism of amino acids has important economic implications because of the cost of the dietary protein component. Moreover, diminishing amino acid catabolism will decrease amino acid requirements, thereby impinging on dietary amino acid profiles. Thus, the likelihood is that optimal amino acid profiles in diets for broiler chickens will remain elusive targets given the complex impacts of amino acid catabolism. Importantly, acceptably accurate amino acid profiles can only be advantageous to sustainable chicken-meat production in the future. Nevertheless, they will remain elusive targets because amino acid metabolism in broiler chickens is extraordinarily complex, coupled with the fact that ideal amino acid ratios have effectively been made a moving target by ongoing selective breeding programmes.

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