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Validation of the Lysine Requirement as Reference Amino Acid for Ideal In-Feed Amino Acid Ratios in Modern Fast Growing Meat-Type Chickens

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Abstract

Nitrogen (N) balance studies were conducted with male growing broiler chickens to reevaluate the lysine (Lys) requirement of a modern broiler strain (Ross 308), making use of eight diets with graded crude protein (CP) supply (6% - 34% CP as-fed). Wheat, soy protein concentrate, wheat gluten, fishmeal and crystalline amino acids (AAs) were the protein sources in the experimental diets with Lys as limiting AA. Following an adaptation period of five days, two consecutive excreta collection periods (2 × 5 d) were conducted: 10 - 20 d of age (starter period) and 25 - 35 d of age (grower period). Statistical evaluation of N balance data utilized an exponential modelling approach. Based on different dietary Lys efficiency, Lys requirement data were derived by modelling depending on average body weight (BW) during starter and grower period and targeted body protein deposition (PD), respectively. In addition, the influence of graded feed intake was taken into account. For the starter period at 600 g BW and assumed 10 g daily body PD, Lys requirement data between 741 mg and 823 mg per day were observed. The corresponding Lys in-feed concentration was 1.06% and 1.18%, dependent on supposed Lys efficiency at 70 g daily feed intake. For the grower period (average BW 1800 g), 1272 mg to 1473 mg Lys per day was needed to yield 16.5 g daily PD. The corresponding required Lys in-feed concentration was between 0.85% and 0.94% Lys for 150 g daily feed intake.

Keywords

Growing Chicken, Lysine Requirement, N Utilization Model, Amino Acid Efficiency

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1. Introduction

Currently, fast growing chickens are supposed both to achieve high yields of protein deposition and minimized nitrogen (N) excretions per unit N deposition. One approach to realize these nutritional aims is the concept of dietary "Ideal Amino Acid Ratio" (IAAR) which was introduced by Cole *et al.* [1] and later on by the British Agricultural Research Council [2]. However, even before the 1980s nutritionists emphasized the importance of an "ideal protein" and its contribution to an effective feed conversion [3]-[5]. Currently, the IAAR concept is widely accepted in poultry nutrition as reviewed by Baker [6]. Thereby, the individual indispensable amino acids (AAs) are related to a reference AA, mostly lysine (Lys) which is almost exclusively utilized for body protein accretion [7]-[9].

Many reports evaluated the Lys requirement of modern meat-type chickens. Generally, the up to now suggested in-feed concentrations of Lys differ widely from 0.7% to 1.4% and 0.6% to 1.1% for starter and grower diets, respectively. As indicated below, such high variation of recommended dietary Lys content is observed both in terms of total and digestible Lys. Accordingly, a valid recommendation is still required. Based on earlier applications of a non-linear N utilization model [10]-[20] the present study aimed to reevaluate Lys requirement data of male broiler chickens dependent on age period, daily body protein deposition and real feed intake to enable further application of the modeling approach as a prediction device for promising concepts to improve traditional procedures for IAAR concepts [6]-[9] [21]-[26].

2. Materials and Methods

The experiments were conducted at the facilities of the Division Animal Nutrition Physiology, Department of Animal Sciences at Georg-August-University of Goettingen, Germany, and approved by the Lower Saxony Federal Office for Consumer Protection and Food Safety (LAVES).

2.1. Housing and Management

Day old male meat-type chicken (Ross 308) from a commercial hatchery were reared in a floor pen under standardized housing and feeding conditions up to the start of the N balance trials involving both starter and grower period. At day 5 and day 20 chicken (36 birds in each period) were randomly allotted to the experimental diets and individually housed in metabolic cages with wire floors (25 × 30 cm for starter and 80 × 80 cm for grower period), individual feeders and a self-drinking system. The room temperature was gradually reduced from 30˚C to 23˚C with increasing age of the birds. Humidity was maintained between 60% - 70% and light was provided for 23 hours per day. The study utilized only male chicken to eliminate gender effects on results of individual N balance measures.

2.2. Diets and Feeding

A computer-aided feed optimizing program (Fumi for Windows 4, HYBRIMIN® Computer + Programme GmbH & Co. KG, Hessisch Oldendorf, Germany) was utilized for formulation of an AA balanced reference diet (**Table 1**) with AA pattern near to IAAR as derived from literature data [14] [15]. Based on a mixture of wheat, soy protein concentrate, wheat gluten, fishmeal and crystalline feed AAs, the postulated IAAR could be achieved for most of the indispensable AAs, except for histidine, leucine and phenylalanine.

According to principles of the applied approach, the AA under investigation is needed in a limiting position. Consequently, to ensure Lys as limiting AA (LAA) all experimental diets (**Table 1**) only contained native feed protein sources without L-lysine·HCl at a total Lys level of 85% (5.01 g/16 g N) as compared to the reference diet (5.89 g/16 g N). The formulated initial high protein summit diet with 38% crude protein (CP) in dry matter (DM) was subsequently stepwise diluted with wheat starch to yield eight experimental diets with graded CP contents between 38% and 6% of DM. According to these principles of diet dilution technique [29], a consistent protein quality (AA pattern) in all experimental diets was ensured (**Table 2**). Further details about diet composition and nutritive value were already reported by Pastor *et al.* [14].

During N balance experiments, birds were fed slightly restricted to minimize feed spillage and excreta contamination. Feed intake was under daily control and adapted to birds need according to Samadi and Liebert [11].

Table 1. Ideal amino acid ratios (IAAR) related to lysine (Lys = 100) in the experimental diets compared with literature data and targeted reference diet.

	NRC [27]	GRRS [28]	Pastor *et al.* [14]	Reference diet[1]	Experimental diets[2]
Lysine (Lys)	100	100	100	100	100 (5.01)
Methionine (Met)	42	37	38	49	57 (2.87)
Methionine + Cystine (Met + Cys)	72	71	74	74	87 (4.37)
Threonine (Thr)	74	67	65	65	77 (3.84)
Tryptophan (Trp)	18	16	17	17	20 (1.00)
Arginine (Arg)	110	108	106	106	124 (6.22)
Histidine (His)	32	32	34	41	48 (2.39)
Isoleucine (Ile)	73	69	68	69	81 (4.04)
Valine (Val)	82	-	79	79	93 (4.67)
Leucine (Leu)	109	112	110	122	143 (7.16)
Phenylalanine (Phe)	65	65	66	79	93 (4.67)
Phenylalanine + Tyrosine (Phe + Tyr)	122	118	118	139	163 (8.18)

NRC: National Research Council; GRRS: German Recommendations for Requirement Standards. [1]Amino acid balanced computer-aided reference diet (5.89 g lysine/16 g N) based on feed protein sources (see **Table 2**) including crystalline L-lysine·HCl. [2]In experimental diets N1-N8 no L-lysine·HCl was supplemented to set lysine in limiting position (in parentheses: amino acid concentrations in g per 16 g N).

Table 2. Composition of the experimental diets[1] based on a constant mixture of feed protein sources with adjusted graded dietary protein contents according to diet dilution technique.

Ingredients	Wheat	Soy protein concentrate	Wheat gluten	Fish meal	DL-Met[2]	L-Val[2]	L-Thr[2]	L-Trp[2]
Mixture (%)[3]	47.48	40.53	6.86	4.22	0.59	0.20	0.12	0.007

[1]Dietary crude protein contents in the experimental diets (g/kg DM): N1 = 65, N2 = 108, N3 = 152, N4 = 197, N5 = 241, N6 = 286, N7 = 332, N8 = 378 [14], [2]Crystalline feed amino acids: DL-methionine, L-valine, L-threonine, L-tryptophan, [3]Ratio of feed protein sources in all experimental diets (in percent as fed).

2.3. Sample Collection

Following a 5 d adaptation period, two consecutive quantitative collection periods (5 d each) were conducted from d 10 - 20 in starter and from d 25 - 35 in grower period to obtain two individual measures per bird and age period, respectively. Excreta were collected every 12 hours and stored at −20˚C until further analysis. Feathers and spilled feed pellets were carefully removed from excreta. Additionally, spilled feed was quantified for precise measures of daily feed intake.

2.4. Laboratory Analysis

Diets were ground to 1 mm and analyzed for N, DM, starch, sugar and fat, according to German Standards [30]. Individual AA content of the protein sources was analyzed by ion-exchange chromatography (Biochrom 30, ONKEN Ltd., Gruendau, Germany) following acid hydrolysis both with and without an oxidation step for quantitative determination of the sulphur-containing AAs.

Excreta were carefully defrosted and homogenized. Subsequently, representative samples were taken and analyzed for N according to the DUMAS method (TruMac©, LECO, Moenchengladbach, Germany) and for DM, according to German Standards [30].

2.5. Model Application and Statistics

Data analysis utilized current applications of an exponential N utilization model [10]-[15] [19] [20] [31]-[34] based on the fundamental work of Gebhardt [35]:

$$\mathrm{NR} = \mathrm{NR}_{\max}\mathrm{T}\left(1-\mathrm{e}^{-b\cdot \mathrm{NI}}\right) \tag{1}$$

$$\mathrm{ND} = \mathrm{NR}_{\max}\mathrm{T}\left(1 - e^{-b \cdot \mathrm{NI}}\right) - \mathrm{NMR} \quad (2)$$

$$b = \left[\ln \mathrm{NR}_{\max}\mathrm{T} - \ln\left(\mathrm{NR}_{\max}\mathrm{T} - \mathrm{NR}\right)\right] / \mathrm{NI} \quad (3)$$

where: NR (mg/$BW_{kg}^{0.67}$) is the daily N retention; $NR_{max}T$ (mg/$BW_{kg}^{0.67}$) is the theoretical maximum for daily N retention; e is the basic number of natural logarithm (ln); *b* is the slope of the N retention curve (indicating the feed protein quality independent on N intake); NI (mg/$BW_{kg}^{0.67}$) is the daily N intake; ND (mg/$BW_{kg}^{0.67}$) is the daily N deposition; and NMR (mg/$BW_{kg}^{0.67}$) is the daily N maintenance requirement.

Transformation of Equation (3), were N intake (NI) is replaced by the intake of LAA (LAAI), yields Equation (4) and allows deduction of AA requirement data for a targeted NR resp. ND according to earlier reports [10]-[12] [20] [31] [33]:

$$\mathrm{LAAI} = \left[\ln \mathrm{NR}_{\max}\mathrm{T} - \ln\left(\mathrm{NR}_{\max}\mathrm{T} - \mathrm{NR}\right)\right] / 16 \cdot bc^{-1} \quad (4)$$

where: LAAI (mg/$BW_{kg}^{0.67}$) is the daily intake of the LAA; ln is the natural logarithm; $NR_{max}T$ (mg/$BW_{kg}^{0.67}$) is the theoretical maximum for daily NR; NR (mg/$BW_{kg}^{0.67}$) is the daily N retention (ND + NMR); *b* is the slope of the N retention curve (indicating the dietary protein quality independent on N intake); *c* is the concentration of the LAA in the dietary protein (g/16 gN); bc^{-1} is the slope between *c* and *b* (model parameter, indicating the dietary AA efficiency). The multiplier 16 results from LAA concentration in the dietary protein (g/16 gN).

Earlier reports [36]-[38] also validated the linear relationship between LAA concentration (*c*) in the feed protein and the response on dietary protein quality (model parameter *b*). In consequence, the quotient bc^{-1} defines the slope of the linear relationship and indicates the efficiency of the LAA in the diet [10]-[12] [14] [15] [19] [20] [33]. The term "efficiency" summarizes the AA utilization for NR in the body taking into account both digestion, absorption and post-absorptive AA utilization.

Model parameters NMR and $ND_{max}T$ were basically predicted in a previous study [14]. However, according to earlier reports [11] [33] the modelling of Lys requirement data applied averaged "working values" for both NMR and $ND_{max}T$ (**Table 3**). Accordingly, N balance data from Pastor *et al.* [14] were adapted to actual NMR and $ND_{max}T$, respectively. As expected, within age periods no significant difference of dietary protein quality (*b*) between the experimental diets was observed.

Modelling of Lys requirements considered, approximated zoo-technical data of male Ross 308 chicken (Aviagen, 2014) for mean body weight (BW: 600 g and 1800 g), body weight gain (BWG: 60 g/d and/100 g/d) and daily feed intake (FI: 80 g/d and 170 g/d) during starter and grower period as reference criteria, respectively (**Table 4**).

3. Results

The results of modelling Lys requirement data depending on BW, age period, daily body protein deposition (PD), dietary Lys efficiency and level of feed intake (FI) are summarized in **Table 4**. Current objectives for zoo technical parameters of male Ross 308 broilers [39] for mean BW, BWG and FI within both of the age periods are utilized as reference criteria. Based on our database and taking into account results of Fatufe *et al.* [40], a CP content of 16.5% in the BWG of chicks was assumed. Accordingly, an average PD of 10 g and 16.5 g per day was assumed for starter and grower period, respectively. Furthermore, according to earlier reports [10]-[12] [20] [31] [33] the modelling of Lys requirement data applied graded dietary Lys efficiency estimates based on mean

Table 3. Summarized model parameters for daily nitrogen maintenance requirement (NMR)[1] and age-depending theoretical potential for daily nitrogen deposition ($ND_{max}T$)[1] of male meat-type Ross 308 chickens, and calculated protein quality (*b*) and lysine efficiency (bc_{Lys}^{-1}) parameters of the utilized experimental diets (Mean ± standard deviation).

Age Period	NMR	$ND_{max}T$	b	bc_{Lys}^{-1}
	[mg N/$BW_{kg}^{0.67}$ per day]		[$b \cdot 10^6$]	[$bc_{Lys}^{-1} \cdot 10^6$]
Starter (d 10 - 20)	240	4000	266 ± 29	53.1 ± 5.9
Grower (d 25 - 35)	240	3200	323 ± 41	64.5 ± 8.3

[1]Applied as averaged "working values" from previous experiments [11] [14] [34].

Table 4. Model calculation of lysine requirement for male meat-type chickens (Ross 308) during starter and grower period depending on assumed performance data, supposed different dietary Lys efficiency and predicted daily feed intake.

Starter period (d 10 - 20, mean body weight 600 g)									
PD (g/d)		9			10			11	
BWG (g/d)		55			61			67	
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
bc_{Lys}^{-1}	53.1	50.4	47.8	53.1	50.4	47.8	53.1	50.4	47.8
Lys required									
(mg/$BW_{kg}^{0.67}$)	901	948	1001	1044	1099	1160	1206	1270	1340
(mg/d)	640	673	711	741	780	823	857	902	952
Lys concentration needed in the starter diet (%)									
FI (g/d)									
70	0.91	0.96	1.02	1.06	1.12	1.18	1.22	1.29	1.36
80	0.80	0.84	0.89	0.93	0.98	1.03	1.07	1.13	1.19
90	0.71	0.75	0.79	0.82	0.87	0.92	0.95	1.00	1.06
Grower period (d 25 - 35, mean body weight 1800 g)									
PD (g/d)		15			16.5			18	
BWG (g/d)		91			100			109	
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
bc_{Lys}^{-1}	64.5	61.3	58.1	64.5	61.3	58.1	64.5	61.3	58.1
Lys required									
(mg/$BW_{kg}^{0.67}$)	753	793	837	858	903	953	975	1026	1083
(mg/d)	1117	1175	1241	1272	1339	1413	1446	1522	1606
Lys concentration needed in the grower diet (%)									
FI (g/d)									
150	0.74	0.78	0.83	0.85	0.89	0.94	0.96	1.02	1.07
170	0.66	0.69	0.73	0.75	0.79	0.83	0.85	0.90	0.95
190	0.59	0.62	0.65	0.67	0.70	0.74	0.76	0.80	0.84

PD = average daily protein deposition (N deposition·6.25), BWG = average daily body weight gain (crude protein in BWG: 16.5%), bc_{Lys}^{-1} = Lys efficiency [(1) as observed, (2) 5% lower as observed, (3) 10% lower as observed], Lys required = lysine requirement for targeted PD, FI = average daily feed intake.

age-dependent dietary Lys efficiency data as shown in **Table 3**. Due to the current ingredient composition (**Table 2**), according to Evonik tables [41] a high level of standardized ileal digestible Lys (88%) was expected. Additionally, relating to the aimed level of dietary AA balance (**Table 1**), the observed dietary Lys efficiency (1) was stated in the upper level of practical diets for growing chicken. Consequently, in **Table 4** a graded decline of Lys efficiency by 5 percent (2) and 10 percent (3) was utilized to rank the yielded data more in line with expectations for practical feeds. Finally, graded expectations for daily feed intake according to Aviagen [39] were applied for predictions of needed in-feed concentration of Lys.

Generally, the metabolic BW-related Lys requirement (mg/$BW_{kg}^{0.67}$/d) declined with increasing BW and age, while the absolute daily Lys requirement (mg/d) increased. The needed in-feed Lys concentration also declined with advancing age. Accordingly, higher Lys supply is required both for birds with elevated daily PD and lower level of dietary Lys efficiency. However, lower FI levels reveal higher Lys concentrations to achieve optimal performance data.

Male starter chickens of 600 g BW require 750 to 950 mg Lys depending on the assumed Lys efficiency to yield 10 to 11 g daily PD (about 60 to 70 g/d BWG). At individual FI level between 70 and 80 g per day, 0.9% to 1.4% Lys in the starter feed is needed to achieve this PD with varying Lys efficiency. Male grower chicken of 1800 g BW and 100 to 110 g/d BWG (16.5 - 18 g/d PD) need 1.3 g to 1.6 g Lys per day corresponding to 0.7%

and 1.1% Lys in the diet when the variation of both FI and dietary Lys efficiency is taken into account.

4. Discussion

The observed variation of Lys requirement data yielded by applied modelling procedure is in line with variation of current recommendations. Growth response of growing meat type chickens depending on dietary Lys supply was in focus of numerous studies. Recommendations of NRC [27] based on a review of 20 references summarized 1.1% total Lys for birds between 0 and 3 weeks and 1.0% total Lys for birds between 3 and 6 weeks of age. However, the analyzed individual studies published between 1942 and 1981 ranged from 0.70% to 1.18% Lys and from 0.59% to 1.05% Lys in diets for the starter and grower period, respectively. Vazques and Pesti [42] evaluated 16 data sets from 7 reported studies and concluded 1.21% and 1.32% Lys in starter diets for the response criteria BWG and feed efficiency, respectively. The GRRS [28] reviewed more than 40 original papers and concluded optimal Lys contents in the DM of broiler diets between 1.3% and 1.1% during the first 5 weeks of age. Relandeau and Le Bellego [43] reported a total of 25 publications and results of 20 dose-response studies demonstrating that elevated Lys recommendations are needed for modern genotypes. Similar conclusions were reported by Aftab [44] following data analyses from 65 dose-response studies published from 1993 to 2010, involving Lys limiting diets with widely different ingredient composition. For maximum BWG and feed efficiency, an optimal digestible Lys content of 1.13% to 1.17% (1 to 21 d) and 1.04% to 1.08% (22 to 42 d) was recommended in starter and grower diets, respectively.

Generally, direct comparisons between results of modelling and earlier requirement estimates are questionable according to the direct dependencies on modulated PD, Lys efficiency and FI data. It is well documented that derived AA requirements of growing chickens depend on many animal and environmental factors [12] [44]-[51]. Genotype, gender, age period, BW and expected growth performance of birds have to be considered for valid recommendations. The fast breeding progress in meat-type chicken [52] [53] has an impact on derived Lys requirement data too. Modern broiler genotypes require significantly higher dietary Lys concentrations to optimize performance and body composition as compared to broilers of past years [54]-[59]. In addition, diet composition regarding the feed ingredients, dietary protein concentration, AA balance, AA availability and the presence of anti-nutritional factors may also act as factors of influence.

Furthermore, the applied experimental approach (dose-response studies, diet dilution technique, factorial methods, metabolic based procedures) and particularly the selected response criteria (BWG, feed efficiency, PD, carcass yields, physiological parameters) are additional factors of influence. Earlier reports showed that more Lys is necessary for maximum PD and carcass yields as compared to the supply needed for optimal feed efficiency and BWG [21] [48] [51] [55] [60]-[62]. Finally, the applied statistical model for assessing AA requirements (broken-line models, non-linear regression analyses, response surface methods, etc.) considerably impacts on validity of concluded Lys requirement data [9] [42] [48] [54]-[57] [63]-[66]. Our modelling approach involves both BW and graded performance data (PD, BWG). Additionally are considered, variation of dietary Lys efficiency and predicted FI data, respectively. Taking into account these important factors of influence, remarkable modulations of the derived optimal Lys concentration in the diet of modern meat-type chickens is not surprising (**Table 4**).

Even if requirement data are expressed in terms of total or digestible Lys, regardless of apparent, standardized or true ileal digestible Lys, the observed considerable variation of requirement data did not decline. Throughout the past 12 years, requirement estimates between 0.98% and 1.41% total Lys [67]-[71] vs. 0.93% and 1.38% digestible Lys [41] [51] [55] [58] [66] [69] [72]-[74] in starter diets and between 0.91% and 1.13% total Lys [57] [70] vs. 0.84% and 1.05% digestible Lys [41] [51] [55] [75]-[77] in grower diets of meat-type chickens were reported. Finally, a further explanation for the observed high variation is that the concluded requirements are only in part directly related to body PD. Assessing the ileal Lys digestibility meets only a part of losses during the total utilization process. Losses of Lys taking place during post-absorptive utilization processes [78] [79], as taken into account by the dietary AA efficiency approach [20], need more attention as an additional factor of influence on derived AA requirement data in the future.

5. Conclusions

The presented modelling of Lys requirements based on data evaluation by "Goettingen approach" demonstrates that an adequate dietary Lys supply to yield optimal performance data and feed efficiency in broiler chicken production is influenced by different animal factors, especially the age period, the assumed growth (PD) performance and the realized FI. In addition, dietary factors influencing the Lys efficiency have to be taken into account. The observed high variation of recommendations to meet the Lys needs in meat type chicken requires more investigations, but also more standardized experimental conditions for more validated conclusions.

Generally, a fine structured network of recommendations could be achieved by modelling of requirement data which are useful for integration into feeding systems by continuous adaptation to varying BW, PD and FI, respectively. However, more reliable data are needed due to the expected variation of AA efficiency in feed ingredients. By this way, application of physiologically based new procedures like "Goettingen approach" might contribute to more validated recommendations in the future and to reducing the observed variation in conclusion of Lys requirement data.

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