

Effects of Tryptophan to Large Neutral Amino Acid Ratios and Overall Amino Acid Levels on Utilization of Diets Low in Crude Protein by Broilers¹

J. Si,* C. A. Fritts,* P. W. Waldroup,*² and D. J. Burnham†

Poultry Science Department, University of Arkansas, Fayetteville, Arkansas 72701; and †Ajinomoto Heartland LLC, Chicago, Illinois 60631

Primary Audience: Nutritionists, Feed Manufacturers, Researchers

SUMMARY

It has been suggested that inadequate ratios of Trp to large neutral amino acids (LNAA; sum of Ile, Leu, Val, Phe, and Tyr) may inhibit feed intake of animals fed low CP diets. This study was conducted to examine this relationship as a potential contributor to the reduced performance of broilers fed low concentrations of CP. Using corn and soybean meal of known composition, diets were formulated to provide a minimum of 100 and 110% of NRC [1] amino acid requirements with 22, 20, 18, or 16% CP. The dietary electrolyte balance was maintained at a minimum of 250 mEq/kg. The Trp:LNAA ratio (Trp as percentage of LNAA) ranged from 4.14 to 4.72% in these diets; additional diets were supplemented with Trp to attain Trp:LNAA ratios of 5.0 to 6.0%. Each treatment plus a positive control 23% CP diet were fed to 6 replicate pens of 6 male chicks from 1 to 21 d. Performance of chicks fed the 22% CP diets, with either 100 or 110% of NRC amino acid recommendation, did not differ significantly from that of chicks fed the 23% CP positive control diet. A further reduction of CP to 20% or less resulted in significant reduction in BW and increased feed conversion ratio. Increasing minimum amino acids from 100 to 110% of NRC did not help overcome this reduction in performance and, in fact, may have contributed to reduced performance of chicks fed diets containing 16% CP. The ratio of Trp:LNAA had no significant effect on any of the parameters measured; therefore, the reduced performance at lower CP concentrations does not appear to be a deficiency of Trp or due to an imbalance among these amino acids.

Key words: broiler, crude protein, tryptophan, large neutral amino acids, nitrogen

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²To whom correspondence should be addressed: Waldroup@uark.edu.

DESCRIPTION OF PROBLEM

Attempts to reduce overall CP content of broiler diets have been successful to a point, but most researchers agree that at some reduced level of CP the overall performance of the bird suffers even though theoretically one has met all requirements for those amino acids considered as essential. Failure to attain optimum performance may be attributed to a number of factors, including reduced levels of potassium or altered ionic balance, lack of sufficient nitrogen pool to provide for synthesis of dispensable or nonessential amino acids, and imbalances among certain amino acids, such as Arg:Lys or branched-chain amino acids.

Inadequate ratios of Trp to large neutral amino acids (LNAA) may be a factor inhibiting feed intake of animals fed diets low in CP [2, 3, 4, 5, 6, 7, 8]. Trp serves as a precursor to serotonin, a neurotransmitter highly involved in feed intake regulation. In pigs, LNAA have been shown to compete with Trp for its passage through the blood-brain barrier, prior to serotonin synthesis in the brain; the resulting decrease in hypothalamic serotonin concentration below a threshold level was associated with lower feed consumption and decreased growth rates [7]. Peisker et al. [9] suggested that low Trp:LNAA ratios did not support maximum performance in young piglets; however, this may have been due to a deficiency of Trp per se rather than a true effect of Trp:LNAA ratios.

Another factor that must be considered in attempting to reduce CP concentrations by amino acid supplementation is that little research has been done with practical type diets to assess the requirements for many of the essential amino acids, other than Met, Lys, and Thr. The objective of the present study was to evaluate the possible effects of concentrations of essential amino acids and Trp:LNAA ratios on performance of broilers fed diets formulated primarily of corn and soybean meal supplemented with crystalline amino acids.

MATERIALS AND METHODS

Diet Formulation

Corn and soybean meal were sampled prior to diet formulation to determine moisture and CP content. Based on the results of these assays,

the amino acid concentrations suggested for these ingredients by NRC [1] were adjusted proportionally. In all diets, several stipulations were established. The dietary electrolyte balance ((Na + K) - Cl) was set at a minimum of 250 mEq/kg as suggested by Mongin [10]. Sodium and chloride were maintained at a ratio of 1:1 as suggested by Hurwitz et al. [11] with a minimum sodium level of 0.25% as suggested by Murakami et al. [12]. A positive control diet was formulated that provided 23% CP and a minimum of 110% of recommended [1] amino acid concentrations (diet 1, Table 1). Diets were then formulated in which the CP was fixed at 22, 20, 18, and 16%. Within each of the CP series, diets were formulated in which the minimum concentrations of essential amino acids were either 100 or 110% of NRC recommendations. The CP and ME contributions of the amino acids [1] were considered in formulation. The composition of the diets is shown in Table 1.

Calculations were made of the balance between Trp and LNAA for the basal diets; these ranged from 4.14 to 4.72% Trp as percentage of LNAA. Additional diets were obtained by supplementing aliquots of these diets with sufficient Trp to produce Trp:LNAA ratios of 5.0 and 6.0%. This resulted in a $4 \times 2 \times 3$ factorial arrangement of treatments with 4 concentrations of CP, 2 concentrations of amino acid fortification, and 3 Trp:LNAA ratios. Along with the positive control diet, this resulted in a total of 25 diets. Each of the test diets was fed to 6 replicate pens of 6 male broilers in battery brooder pens; the control diet was fed to 18 replicate pens of 6 male broilers in each pen.

Birds and Housing

Day-old male chicks of a commercial broiler strain [13] were obtained from a local hatchery where they had been vaccinated in ovo for Marek's disease and had received vaccinations for Newcastle disease and infectious bronchitis posthatch via a coarse spray. They were randomly assigned to compartments in electrically heated battery brooders with raised wire floors. Six chicks were placed in each of 162 compartments. The experimental diets, offered in mash form, and tap water were available for ad libitum consumption. Fluorescent lamps provided 24-h lighting.

TABLE 1. Composition (g/kg) of diets with different levels of CP and amino acids

Ingredient	Positive control	22% CP		20% CP		18% CP		16% CP	
		100	110	100	110	100	110	100	110
Yellow corn	509.83	540.38	540.96	598.75	601.82	660.00	674.09	737.07	777.45
Dehulled soybean meal	378.96	353.78	352.56	301.67	295.54	243.37	223.72	165.61	109.92
Poultry oil	68.91	64.02	63.72	55.04	54.09	45.34	41.65	31.45	19.38
Dicalcium phosphate	20.32	20.48	20.50	20.85	20.91	21.28	21.45	21.87	22.36
Ground limestone	10.73	10.76	10.76	10.81	10.80	10.85	10.86	10.90	10.92
Sodium chloride	3.45	3.45	3.45	3.41	2.89	2.67	1.96	1.66	0.49
Sodium bicarbonate	2.07	2.02	2.02	1.97	2.73	2.96	3.97	4.30	5.96
Potassium sulfate	0.00	0.00	0.00	1.78	2.05	4.04	4.84	7.08	9.36
Trace mineral mix ^A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Broiler vitamins ^B	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
DL-Met (98%)	2.73	2.11	3.03	2.72	3.71	3.41	4.58	4.35	5.98
L-Thr	0.00	0.00	0.00	0.00	0.83	0.88	2.04	2.19	3.99
L-Lys HCl	0.00	0.00	0.00	0.00	1.63	2.20	4.37	5.17	8.75
L-Arg	0.00	0.00	0.00	0.00	0.00	0.00	1.74	2.40	5.63
L-Val	0.00	0.00	0.00	0.00	0.00	0.00	1.01	1.43	3.69
L-Ile	0.00	0.00	0.00	0.00	0.00	0.00	0.47	0.97	3.12
L-Trp	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.34	0.85
L-Phe	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21	3.89
L-Leu	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.24
Gly	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.07
L-His	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.95
Total	1,000.00	1,000.00	1,000.00	1,000.00	1,000.00	1,000.00	1,000.00	1,000.00	1,000.00

^AProvided per kilogram of diet: Mn (from MnSO₄·H₂O) 100 mg; Zn (from ZnSO₄·7 H₂O) 100 mg; Fe (from FeSO₄·7 H₂O) 50 mg; Cu (from CuSO₄·5 H₂O) 10 mg; I (from Ca (IO₃)₂·H₂O) 1 mg.

^BProvides per kilogram of diet: vitamin A (from vitamin A acetate) 7,714 IU; cholecalciferol 2,204 IU; vitamin E (from DL- α -tocopheryl acetate) 16.53 IU; vitamin B₁₂ 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; choline 465 mg; menadione (from menadione dimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; thiamin (from thiamine mononitrate) 1.54 mg; pyridoxine (from pyridoxine hydrochloride) 2.76 mg; DL-biotin 0.066 mg; ethoxyquin 125 mg; Se 0.1 mg.

Measurements

Birds were group weighed by pen at 1, 7, 14, and 21 d. Feed consumption during each period was determined. Mortality was checked twice daily; all birds that died were weighed, and the weight used to adjust feed conversion [gram feed consumed/(weight of live birds + weight of dead birds)]. All diets were analyzed in duplicate for CP and total amino acid concentrations by a commercial laboratory specializing in amino acid analysis [14]. At d 18, excreta were collected for a 24-h period, freeze dried, and analyzed in quadruplicate for total nitrogen content.

Statistical Analysis

Pen means served as the experimental unit. Data were first analyzed using single-degree comparisons of performance of chicks fed the test diets to that of the positive control. Following that, the positive control group was removed

from analysis, and the data analyzed as a complete factorial arrangement with main effects of CP, amino acid level, Trp:LNAAs ratios, and all possible 2-way and 3-way interactions. Mortality data were transformed to $\sqrt{n+1}$ prior to analysis; data are presented as natural numbers in tables. The data of excreta nitrogen content were subjected to regression analysis using the PROC REG function of SAS software [15]. Statements of probability are based on $P \leq 0.05$.

RESULTS AND DISCUSSION

Analyzed CP and amino acid values are presented in Table 2. Though analyzed CP values were higher than calculated, analyzed value of individual amino acids were in good agreement with calculated ones. Therefore, discussion of concentrations of amino acids or ratios of amino acids in the results is based upon calculated values, since these were derived from a database

TABLE 2. Nutrient analysis of diets with different levels of CP and essential amino acids

Nutrient (%)	Positive control	22 CP 100%	22 CP 110%	20 CP 100%	20 CP 110%	18 CP 100%	18 CP 110%	16 CP 100%	16 CP 110%
CP (C) ^A	23.00	22.00	22.00	20.00	20.00	18.00	18.00	16.00	16.00
CP (A) ^B	25.20	24.50	23.10	20.50	22.20	20.00	19.10	16.70	16.20
Met (C)	0.64	0.56	0.66	0.60	0.69	0.63	0.74	0.68	0.81
Met (A)	0.63	0.55	0.61	0.59	0.67	0.63	0.69	0.64	0.75
Lys (C)	1.33	1.26	1.25	1.10	1.21	1.10	1.21	1.10	1.21
Lys (A)	1.23	1.16	1.16	1.14	1.23	1.11	1.21	1.10	1.22
Trp (C)	0.27	0.26	0.26	0.24	0.23	0.21	0.22	0.20	0.22
Trp (A)	0.26	0.24	0.24	0.21	0.22	0.21	0.22	0.20	0.20
Thr (C)	0.93	0.89	0.89	0.80	0.88	0.80	0.88	0.80	0.88
Thr (A)	0.92	0.86	0.85	0.85	0.91	0.77	0.84	0.78	0.84
Ile (C)	1.18	1.12	1.12	1.00	0.99	0.88	0.88	0.80	0.88
Ile (A)	1.17	1.01	1.06	0.93	0.97	0.83	0.84	0.78	0.73
His (C)	0.59	0.56	0.56	0.51	0.50	0.44	0.42	0.36	0.39
His (A)	0.61	0.60	0.60	0.59	0.58	0.49	0.47	0.42	0.44
Val (C)	1.24	1.18	1.18	1.07	1.05	0.94	0.99	0.90	0.99
Val (A)	1.15	1.03	1.05	1.01	0.99	0.84	0.96	0.99	0.77
Leu (C)	1.95	1.89	1.89	1.75	1.73	1.59	1.53	1.37	1.32
Leu (A)	1.90	1.79	1.82	1.81	1.75	1.51	1.51	1.33	1.27
Arg (C)	1.71	1.63	1.62	1.46	1.44	1.27	1.38	1.25	1.38
Arg (A)	1.56	1.42	1.44	1.41	1.38	1.13	1.22	1.05	1.20
Phe (C)	1.25	1.20	1.19	1.08	1.07	0.95	0.90	0.79	1.03
Phe (A)	1.14	1.06	1.07	1.04	1.02	0.84	0.82	0.73	0.91
Met + Cys (C)	0.99	0.90	0.99	0.90	0.99	0.90	0.99	0.90	0.99
Met + Cys (A)	0.98	0.89	0.95	0.93	1.00	0.93	0.98	0.91	0.99
Phe + Tyr (C)	2.14	2.05	2.05	1.85	1.83	1.63	1.54	1.34	1.47
Phe + Tyr (A)	1.94	1.76	1.79	1.72	1.66	1.42	1.38	1.16	1.28
Gly + Ser (C)	2.17	2.07	2.06	1.86	1.84	1.62	1.54	1.31	1.38
Gly + Ser (A)	2.10	1.95	1.95	1.94	1.91	1.58	1.55	1.31	1.40
Total EAA ^C	14.53	13.83	13.89	12.56	12.69	11.38	11.58	10.32	11.10
EAA as % of CP ^D	53.60	53.63	53.74	53.73	53.93	53.91	54.31	54.45	56.04
Total LNAA ^E	6.52	6.25	6.24	5.68	5.60	5.03	4.94	4.41	4.66
Trp as % of LNAA ^F	4.20	4.19	4.18	4.15	4.14	4.10	4.45	4.53	4.72
Na + K - Cl, mEq/kg	261.98	252.15	251.10	250.00	250.00	250.00	250.00	250.00	250.00

^AC = calculated from NRC [1] adjusted to moisture and CP content of intact ingredients.

^BA = analyzed value from Ajinomoto Heartland LLC, Chicago, IL.

^CEAA = essential amino acids. Sum of Met, Lys, Trp, Thr, Ile, His, Val, Leu, Arg, Phe, Cys, and Tyr. Result is based on calculated values for appropriate amino acids.

^DBased on calculated values for appropriate amino acids and CP.

^ELNAA = large neutral amino acids. Sum of Leu, Val, Ile, Phe, and Tyr. Result is based on calculated values for appropriate amino acids.

^FResult is based on calculated values for appropriate amino acids.

consisting of hundreds of samples rather than individual assays.

Performance of birds fed the diets with 22% CP with either 100 or 110% of NRC [1] recommended amino acid concentrations did not differ significantly from that of chicks fed the 23% CP positive control diet for BW, feed conversion, or mortality at any age (data not shown). Therefore, the performance of birds fed the diets with 22% CP was considered valid as a standard of comparison for the various dietary factors evaluated in the trial.

The effects of dietary CP, amino acid level, and TRP:LNAA ratio on BW are shown in Table 3. The Trp:LNAA ratio had no significant effect on 21-d BW, and there was no interaction between Trp:LNAA ratio and the CP or amino acid content of the diet. The CP content, but not the amino acid status, of the diet significantly influenced BW; however, there was a significant interaction between these 2 factors. Overall, birds fed diets with less than 22% CP and a minimum of 100% of NRC-recommended amino acid concentrations weighed significantly

TABLE 3. Effects of dietary CP, amino acid status, and ratio of Trp to large neutral amino acids (LNNA) on 21-d BW of male broilers

Trp:LNAA ratio (%)	Amino acid (% of NRC) ^A	Dietary CP content				
		21-d BW (g)				Mean
		16%	18%	20%	22%	
< 5 ^B	100	608	653	666	751	669
	110	546	648	730	747	667
	Mean	577	650	698	749	668
5	100	606	634	656	724	655
	110	533	668	701	751	663
	Mean	570	651	678	738	659
6	100	598	648	669	737	663
	110	548	673	716	703	660
	Mean	573	661	692	720	661
Mean	100	604 ^c	645 ^b	663 ^b	737 ^a	662
	110	542 ^d	663 ^b	715 ^a	734 ^a	664
	Mean	573 ^z	654 ^y	689 ^x	735 ^w	
Factor	<i>P</i> ≥ <i>F</i>					
CP	0.0001					
Amino acid (AA)	0.88					
Trp:LNAA (Trp)	0.56					
CP × AA	0.0001					
CP × Trp	0.77					
AA × Trp	0.79					
CP × AA × Trp	0.63					
CV	6.84					

^{a-d}Within comparisons, means with common superscripts do not differ significantly ($P < 0.05$).

^{w-z}Within row, means with common superscripts do not differ significantly ($P < 0.05$).

^AMinimum level of essential amino acids as percentage of NRC [1].

^BThis diet not supplemented with additional Trp. See Table 2 for exact ratios within each level of CP. Supplemental Trp added to attain ratios of 5 and 6.

less than those fed the diets with 22% CP at all ages (data for 7 and 14 d not shown for brevity). However, when the minimum amino acid content of the diet was increased to 110%, performance of birds fed 20% CP diet did not differ significantly from that of the birds fed the 22% CP diet. Increasing the minimum amino acid concentrations in diets containing 18% CP was of no benefit. When diets contained 16% CP, increasing the minimum amino acid concentrations actually resulted in a reduction in BW.

The effects of dietary CP, amino acid status, and TRP:LNAA ratio on feed conversion by broilers is shown in Table 4. The TRP:LNAA ratio had no significant effect on feed conversion, with no significant interactions between the TRP:LNAA ratio and the CP or amino acid content of the diet. Both the CP content and the amino acid status of the diet significantly

influenced feed conversion, but there was no significant interaction between CP content and amino acid status of the diet. Birds fed diets with 110% of NRC [1] recommended amino acid concentrations had significantly improved feed conversion compared with those fed 100% of the minimum recommendations. Birds fed diets with 22% CP had better feed conversion than those with 18 or 20% CP, which in turn had better conversion than those with 16% CP.

Reducing the CP content of the diet to 16% resulted in increased mortality at 21 d of age compared with those fed 18 or 22% but not those fed 20% (Table 5). There was a significant interaction between Trp:LNAA ratio and amino acid concentrations on mortality at 21 d; however, this followed no consistent trend. Mortality is subject to considerable variation, and caution should be taken in interpreting these data due

TABLE 4. Effects of dietary protein, amino acid status, and ratio of Trp to large neutral amino acids (LNAA) on 0 to 21 d feed conversion by male broilers

Trp:LNAA ratio (%)	Amino acid (% of NRC) ^A	Dietary CP content				Mean
		0–21 d Feed:gain ratio (kg:kg)				
		16%	18%	20%	22%	
< 5 ^B	100	1.572	1.469	1.473	1.369	1.471
	110	1.586	1.473	1.407	1.376	1.461
	Mean	1.579	1.471	1.440	1.372	1.466
5	100	1.597	1.547	1.511	1.394	1.512
	110	1.608	1.432	1.443	1.346	1.457
	Mean	1.603	1.490	1.477	1.370	1.485
6	100	1.561	1.553	1.473	1.365	1.488
	110	1.557	1.474	1.439	1.352	1.455
	Mean	1.559	1.514	1.456	1.359	1.472
Mean	100	1.577	1.523	1.485	1.376	1.490 ^a
	110	1.584	1.460	1.430	1.358	1.458 ^b
	Mean	1.580 ^x	1.491 ^y	1.458 ^y	1.367 ^z	
Factor	<i>P > F</i>					
CP	0.0001					
Amino acid (AA)	0.009					
Trp:LNAA (Trp)	0.42					
CP × AA	0.14					
CP × Trp	0.63					
AA × Trp	0.32					
CP × AA × Trp	0.78					
CV	4.68					

^{a,b}Within columns, means with common superscripts do not differ significantly ($P \leq 0.05$).

^{x-z}Within rows, means with common superscripts do not differ significantly ($P \leq 0.05$).

^AMinimum level of essential amino acids as percentage of NRC [1].

^BThis diet not supplemented with additional Trp. See Table 2 for exact ratios within each level of CP. Supplemental Trp added to attain ratios of 5 and 6.

to the minimal number of birds involved in the study.

Excreta nitrogen content as percentage of dry matter significantly decreased in a linear manner with the reduction of dietary CP, with the dietary CP ranging from 16 to 23% (Figure 1). No higher order than linear regression was demonstrated to fit the data. The regression model ($R^2 = 0.82$) is as follows:

$$Y = 0.263X - 0.883$$

where Y is excreta nitrogen content (percentage of dry matter); X is the analyzed dietary CP (%). For every 1% decrease of dietary CP, there was about 0.3% less nitrogen excreted in the excreta. This is in agreement with Schutte [16] and Si et al. [17]. Schutte concluded that in broiler chick diets based on corn-soybean meal with adequate Lys and Met, the protein level could be reduced

1.5 to 2%, and the nitrogen excretion would be reduced by 15 to 20%.

It is interesting to speculate as to the reasons for the reduced performance on the diets with 16% CP. The reduction does not appear to be due to a deficiency of Trp per se or to an imbalance between Trp and the LNAA. The percentage of LNAA in the diet decreased with the ratio of Trp to LNAA increasing slightly as the diet was reduced in CP (Table 2). The ratio of essential amino acids as a percentage of total protein remained a rather constant proportion of the dietary CP. Bedford and Summers [18] suggested an optimal essential: nonessential amino acid balance of 55:45; a ratio close to that observed overall CP concentrations in this study. Dietary electrolyte balance was maintained at a minimum of 250 mEq/kg over all diets as suggested by Mongin [10], although a previous study in our laboratory indicated that maintaining a constant

TABLE 5. Effects of dietary protein, amino acid status, and ratio of Trp to large neutral amino acids (LNAA) on 0 to 21 d mortality by male broilers

Trp:LNAA ratio (%)	Amino acid (% of NRC) ^A	Dietary CP content				Mean
		16%	18%	20%	22%	
< 5 ^B	100	10.00	0.00	0.00	3.33	3.33 ^b
	110	16.66	0.00	3.33	0.00	5.00 ^{ab}
	Mean	13.33	0.00	1.66	1.66	4.16
5	100	3.33	0.00	10.00	3.33	4.16 ^{ab}
	110	3.33	4.00	4.00	0.00	2.83 ^b
	Mean	3.33	2.00	7.00	1.66	3.50
6	100	3.33	0.00	0.00	0.00	0.83 ^b
	110	13.33	4.00	13.33	4.00	8.66 ^a
	Mean	8.33	2.00	6.66	2.00	4.75
Mean	100	5.55	0.00	3.33	2.22	2.77
	110	11.11	2.66	6.88	1.33	5.50
	Mean	8.33 ^x	1.33 ^y	5.11 ^{xy}	1.77 ^y	
Factor	<i>P</i> > <i>F</i>					
CP	0.001					
Amino acid (AA)	0.09					
Trp:LNAA (Trp)	0.76					
CP × AA	0.40					
CP × Trp	0.07					
AA × Trp	0.02					
CP × AA × Trp	0.59					
CV ^C	3.96					

^{ab}Within columns, means with common superscripts do not differ significantly ($P \leq 0.05$).

^{xy}Within rows, means with common superscripts do not differ significantly ($P \leq 0.05$).

^AMinimum level of essential amino acids as percentage of NRC [1].

^BThis diet not supplemented with additional Trp. See Table 2 for exact ratios within each level of CP. Supplemental Trp added to attain ratios of 5 and 6.

^CThe CV of transformed means.

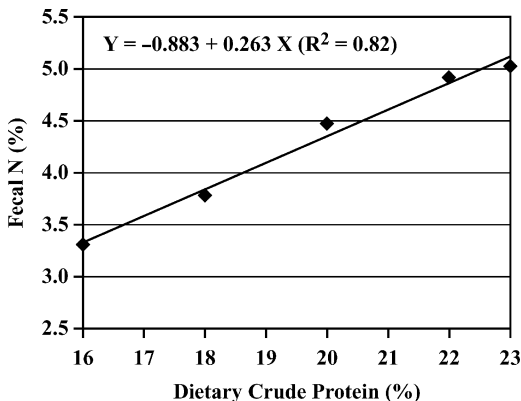


FIGURE 1. Observed (*) and estimated (—) N content (percentage of dry matter) from 24 h collection of excreta from 18-d-old broilers fed diets varying in analyzed CP content (means of quadruplicate analyses).

dietary electrolyte balance was ineffective in overcoming the adverse effects of reduced dietary protein [17].

Although most essential amino acid concentrations tend to decline toward their minimum requirements as dietary CP concentrations decrease, 1 notable exception exists. As dietary CP declines, the level of Met per se increased considerably above its minimum requirement, and especially when the minimum amino acid concentrations are increased to 110% of NRC [1] recommended concentrations. This increase in Met is the result of meeting minimum needs for TSAA by the addition of DL-Met, as supplemental cystine was not used in this study due to its extremely high cost. Adverse effects of excess concentrations of individual essential amino acids on broiler performance have been reported [19, 20, 21]. These reports considered

the addition of 3 to 4% excess of the essential amino acid needs; little or no information is available regarding the use of lower concentrations of amino acids. Methionine is known to be one of the more toxic of amino acids when fed in excess, and it is interesting to speculate as to whether this increased level may inhibit feed intake sufficiently on the low CP diets to account for the reduced BW gains.

Although all the essential amino acids concentrations are above NRC [1] recommended concentrations, most of them tend to decline toward their minimum requirements as dietary

CP concentrations decreases. It is possible that some essential amino acids whose requirements are based on limited studies, such as glycine and serine, may become limiting. In comparison to the 1.25% level of Gly + Ser suggested by NRC [1], Heger and Pack [22] reported that glycine and serine needs ranged from 1.5 to 1.6% at 17% CP up to 1.7 to 1.8% at 23% CP. Schutte et al. [23] recommended 1.9% of total Gly and Ser, when birds were fed low CP diets fortified with amino acids. More research needs to be done on requirements for individual amino acids other than Met, Lys, and Thr in diets low in CP.

CONCLUSIONS AND APPLICATIONS

1. Overall, birds fed diets with less than 22% CP and a minimum of 100% of NRC-recommended amino acid concentrations weighed significantly less than those fed the diets with 22% CP.
 2. When the minimum amino acid content of the diet was increased to 110%, performance of birds fed 20% CP diet did not differ significantly from that of the birds fed the 22% CP diet.
 3. Increasing the minimum amino acid concentrations in diets containing 18% CP was of no benefit and when diets contained 16% CP, increasing the minimum amino acid concentrations actually resulted in a reduction in BW.
 4. Within the range of concentrations evaluated in this study, the ratio of Trp to large neutral amino acids (LNAA; sum of Ile, Leu, Val, Phe, and Tyr) had no significant effect on overall performance and did not appear to contribute to reduced performance on amino acid supplemented diets low in CP.
 5. More research needs to be done on requirements for individual amino acids other than Met, Lys, and Thr in diets low in CP.
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REFERENCES AND NOTES

1. National Research Council. 1994. Nutrient requirements of poultry. 9th rev. ed. National Academy Press, Washington, DC.
2. Uttecht, D. J., C. R. Hamilton, E. M. Weaver, and G. W. Libal. 1991. Interaction between dietary levels of neutral amino acids and tryptophan fed to 10-kg pigs. *J. Anim. Sci.* 69(Suppl. 1):364. (Abstr.)
3. Uttecht, D. J., C. R. Hamilton, E. M. Weaver, and G. W. Libal. 1992. Interactions between dietary levels of neutral amino acids and tryptophan for growing and finishing pigs. *J. Anim. Sci.* 70(Suppl. 1):65. (Abstr.)
4. Meunier-Salaun, M. C., M. Monnier, Y. Colleaux, B. Seve, and Y. Henry. 1991. Impact of dietary tryptophan and behavioural type on behavior, plasma cortisol and brain metabolites of young pigs. *J. Anim. Sci.* 69:3689-3698.
5. Adeola, O., and R. O. Ball. 1992. Hypothalamic neurotransmitter concentrations and meat quality in stressed pigs offered excess dietary tryptophan and tyrosine. *J. Anim. Sci.* 70:1888-1894.
6. Denbow, D. M., F. C. Hobbs, R. M. Hulet, P. P. Graham, and L. M. Potter. 1993. Supplemental dietary L-tryptophan effects on growth, meat quality, and brain catecholamine and indoleamine concentrations in turkeys. *Br. Poult. Sci.* 34:715-724.
7. Henry, Y., B. Seve, Y. Colleaux, P. Gainer, C. Saligault, and P. Jego. 1992. Interactive effects of dietary levels of tryptophan and protein on voluntary feed intake and growth performance in pigs, in relation to plasma amino acids and hypothalamic serotonin. *J. Anim. Sci.* 70:1873-1887.
8. Henry, Y., B. Seve, A. Mounier, and P. Ganier. 1996. Growth performance and brain neurotransmitter in pigs as affected by tryptophan, protein, and sex. *J. Anim. Sci.* 74:2700-2710.
9. Peisker, M., P. H. Simmins, H. Monge, and F. Liebert. 1998. Tryptophan as a feed intake stimulant. *Feed Mix* 6:8-12.
10. Mongin, P. 1981. Recent advances in dietary anion-cation balance in poultry. Pages 109-119 in *Recent Advances in Animal Nutrition*. W. Haresign, ed. Butterworths, London, UK.
11. Hurwitz, S., I. Cohen, A. Bar, and S. Bornstein. 1973. Sodium and chloride requirements of the chick: Relationship to acid-base balance. *Poult. Sci.* 52:903-915.
12. Murakami, A. E., S. E. Watkins, E. A. Saleh, J. A. England, and P. W. Waldroup. 1997. Estimation of the sodium and chloride requirements for the young broiler chick. *J. Appl. Poult. Res.* 6:155-162.
13. Cobb 500, Cobb-Vantress, Inc., Siloam Springs, AR.
14. Ajinomoto Heartland, LLC, Chicago.
15. SAS Institute. 1991. *SAS User's Guide*. SAS Institute, Inc., Cary, NC.

16. Schutte, B. 1994. Controlling nitrogen pollution—practical application of free amino acids in poultry diets. *Feed Mix*. 2:28–31.
17. Si, J., C. A. Fritts, P. W. Waldroup, and D. J. Burnham. 2004. Extent to which crude protein may be reduced in corn-soybean meal broiler diets through amino acid supplementation. *Int. J. Poultry Sci.* 3:46–50.
18. Bedford, M. R., and J. D. Summers. 1985. Influence of ratio of essential to nonessential amino acids on performance and carcass composition of the broiler chick. *Br. Poultry Sci.* 26:483–491.
19. Okumura, J., and K. Yamaguchi. 1980. Effects of excess of individual essential amino acids in diets of chicks. *Jpn. Poultry Sci.* 17:135–139.
20. Ueda, H., S. Yabuta, H. Yokota, and I. Tasaki. 1981. Involvement of feed intake and feed utilization in the growth retardation of chicks given excessive amounts of leucine, lysine, phenylalanine or methionine. *Nutr. Rep. Int.* 24:135–144.
21. Edmonds, M. S., and D. H. Baker. 1987. Comparative effects of individual amino acid excesses when added to a corn-soybean meal diet: Effects of growth and dietary choice in the chick. *J. Anim. Sci.* 65:699–705.
22. Heger, J., and M. Pack. 1996. Effects of glycine + serine on starting broiler chick performance as influenced by dietary crude protein levels. *Agric. Res.* 49:257–265.
23. Schutte, J. B., W. Smink, and M. Pack. 1997. Requirement of young broiler chicks for glycine + serine. *Arch. Geflügelkd.* 61:43–47.

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