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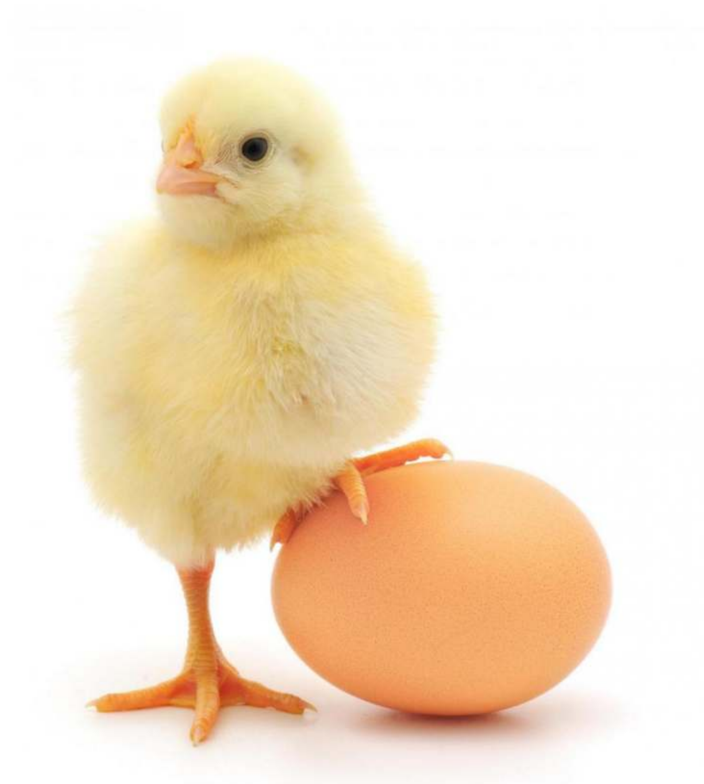

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Influence of Branched-Chain Amino Acid Balance in Broiler Diets[1]

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Abstract: Two experiments were conducted to evaluate the influence of balance among the branched-chain amino acids Leu, Ile, and Val in broiler diets when levels of these amino acids were deemed adequate. High levels of Leu were obtained by either increasing the quantity of corn gluten meal (disproportionately high in Leu relative to Ile and Val) or by supplementing a corn-soybean meal diet with crystalline Leu. Supplements of Ile and Val were added to aliquots of the high Leu diets to maintain Ile:Leu:Val ratios similar to those observed in diets at the lowest level of Leu. Live performance and organ weights of chicks grown to 21 d on these diets were evaluated. The results of the present studies suggest that an antagonism among or between Leu, Ile, and Val is not likely to result in depressed performance of broilers fed practical type diets when levels of these amino acids are above their minimum requirements. The primary effect noted in these studies was a reduction in feed intake as the level of corn gluten meal increased, attributed primarily to changes in texture of the diet. Further research is needed to evaluate the potential impact of an imbalance in diets with reduced levels of crude protein where one or more of the branched-chain amino acids may be at minimal dietary levels with high levels of Leu from corn protein.

Key words: Broilers, amino acids, branched chain amino acids, antagonism

Introduction

Although nutritionists have long been aware of adverse interactions among certain groups of amino acids (Elvehjem, 1956; Harper, 1956; Harper *et al*., 1964; D'Mello and Lewis, 1971) there has been little reason to consider these in formulating practical broiler or turkey diets. The most classic interaction, arising from excessive Lys in relation to Arg, is almost impossible to develop using practical ingredients, as casein is one of the few ingredients in which Lys is in great excess of Arg. An interaction that has received less attention is that among the branched-chain amino acids (BCAA) Leu, Ile, and Val. These amino acids share common enzymatic pathways (Featherston and Horn, 1973; Nonami *et al*., 1995; Denoya *et al*., 1995). Excesses of Leu are extremely disruptive to utilization of Ile and Val, especially when these two amino acids are marginal or limiting (D'Mello and Lewis, 1970; Boldizsar *et al*., 1973; Smith and Austic, 1978).

Because corn protein is disproportionately high in Leu relative to Ile and Val (NRC, 1994), this imbalance is more likely to occur under practical conditions than the Arg-Lys interaction. Use of corn gluten meal or blood meal as a protein source markedly increases Leu levels relative to Ile and Val. In addition, increased usage of supplemental amino acids in broiler or turkey diets generally results in a reduction in the overall proportion of protein derived from soybean meal and an increase in the proportion derived from corn, thus exacerbating the adverse balance of Leu to Ile and Val.

It has been commonly observed that low-protein amino acid-fortified diets often do not support performance of broilers similar to that observed on diets of higher crude protein content from intact protein sources (Mendonca and Jensen, 1989; Holsheimer and Janssen, 1991; Jensen, 1991; Surisdiarto and Farrell, 1991). This reduction may be due in part to possible suppression of feed intake brought about by an adverse balance among the BCAA even though minimum requirements are met. The objective of the present study was to evaluate the relationship of BCAA balance in practical diets formulated to contain adequate levels of all amino acids for broiler chicks.

Materials and Methods

Diet Formulation: Two experiments were conducted. In the first experiment, diets were formulated to provide a minimum of 105% of NRC (1994) amino acid recommendations. All diets contained 5% of a blended animal protein[2], as most practical broiler diets in the United States contain some animal protein source. The blended animal protein, corn, soybean meal, and corn gluten meal were subjected to crude protein and amino acid analysis prior to formulation[3]. Cornstarch was included for eventual replacement with amino acids to

[1]Published with approval of the Director, Arkansas Agricultural Experiment Station
[2]Pro-Pak, H. J. Baker and Bro., Stamford CT
[3]Protein and amino acid assays kindly conducted by Degussa Corporation, Allendale NJ 07401

maintain diets relatively isocaloric. One diet (Diet 1, Table 1) was considered as the positive control and calculated to contain 23% CP. A second diet (Diet 2, Table 1) was similar in composition to Diet 1 but had no minimum CP imposed; this diet was calculated to contain 21.95% crude protein. Diets 3 through 7 (Table 1) had no minimum CP level and contained increasing quantities of corn gluten meal, ranging from 5 to 25% in increments of 5%. This increase in amount of corn gluten meal was done to exacerbate levels of Leu relative to Ile and Val in the diets. Crude protein content of these diets ranged from 22.65 to 29.47% as the level of corn gluten meal increased.

In the second experiment, the diets did not contain any animal protein but were formulated in a manner similar to that described above (Table 2). This resulted in an increase in the amount of supplemental poultry oil and slight changes in levels of Leu, Ile, and Val. The positive control diet with a minimum of 23% CP was eliminated from this experiment because performance of the chicks fed Diet 2 in the first experiment (no corn gluten meal; no minimum crude protein) did not differ from that of chicks fed the positive control with 23% CP.

Experimental Treatments

Experiment 1: In one series of experimental treatments, diets with increasing amounts of corn gluten meal (Diets 2 through 6, Table 1) were fed "as is" to provide diets with increasing ratios of Leu to Ile and Val from intact protein (intact protein [IP] series). In a second series of experimental treatments, Ile and Val were added to diets 3 through 7 to maintain Ile:Leu:Val ratios of 100:215:121, the same ratios observed in Diet 2 (free amino acid [FAA] series). The Ile and Val were added at the expense of cornstarch to maintain approximately the same metabolizable energy content of the diets, using energy values reported by NRC (1994). In a third series of experimental treatments, Leu was added to Diet 2 at the expense of cornstarch to provide the same percentage of Leu as calculated in diets 3 through 6 but without the inclusion of corn gluten meal (intact protein plus Leu [IP+Leu] series). This resulted in a 3 x 6 factorial arrangement of treatments with three types of basal diets in combination with six levels of Leu, with the diet containing 1.90% Leu considered as the first level of Leu for all three basal diets. All diets were pelleted with steam (3 mm die) using a laboratory model pellet mill[4]. Each of the diets was assayed for crude protein and amino acid content as previously described.

Experiment 2: Treatments in this experiment were similar to those in Experiment 1. One series of treatments utilized diets with increasing amounts of corn gluten meal to provide diets with increasing ratios of Leu to Ile and Val from intact protein (IP series). In a second series of diets L-Leu was added to the low protein corn-soybean meal control (Diet 1, Table 2) at the expense of corn starch to provide the same amount of Leu as calculated in diets 2 through 6 but without the inclusion of corn gluten meal (FAA series). In addition, both the intact protein series and the free amino acid series of diets were supplemented with L-Ile and L-Val at the expense of corn starch to maintain Ile:Leu:Val ratios of 100:195:109, the same ratios observed in Diet 2. This resulted in a 2 x 2 x 6 factorial arrangement of treatments with two types of basal diets, with and without additional L-Ile and L-Val supplementation, and six levels of Leu, with the diet containing 1.84% Leu considered as the first level of Leu for both basal diets. Diets were pelleted and assayed for crude protein and amino acid content as previously described.

Birds and Housing: Day-old male chicks of a commercial broiler strain[5] were obtained from a local hatchery. In the first experiment, six chicks were randomly assigned to each of 108 pens in electrically heated battery brooders with raised wire floors. In the second experiment, six chicks were randomly assigned to each of 126 battery pens. Fluorescent lights in each pen provided continuous 24 h illumination. Six pens were assigned to each of the dietary treatments (twelve pens for the positive control group in Experiment 1) in a randomized block design with block being a tier of the battery. Test diets and tap water were provided for ad libitum consumption.

Measurements: Each pen of birds was weighed at day-old and at 21 d. Feed consumption during the period was determined. Mortality was checked twice daily; birds that died were weighed for adjustment of feed conversion. Feed conversion ratio (FCR) was calculated as total feed consumed divided by weight of live birds plus mortality weight. At the conclusion of the first experiment, two randomly chosen birds per pen were killed by CO_2 inhalation. They were dissected and the weights of the heart, liver, and abdominal fat pad determined and expressed as percentage of total live body weight.

Statistical analysis: Pen means were the experimental unit. Data were subjected to the analysis of variance as a factorial arrangement of treatments using the General Linear Models procedure of SAS (SAS Institute, 1991). The model considered the main effects of diet type and Leu level in Experiment 1 and basal diet type, L-Ile and L-Val supplementation, and Leu level in Experiment 2. All possible two-way (Experiment 1 and 2) and three-way

[4]California Pellet Mill Company, Crawfordsville IN 47933

[5]Cobb 500, Cobb-Vantress, Inc., Siloam Springs, AR 72761

Table 1: Composition (%) and nutrient content of diets with different levels of branched chain amino acids (Experiment 1)

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Yellow corn	53.510	54.300	56.178	56.352	54.872	53.360	51.830
Soybean meal	32.035	29.729	24.286	20.286	17.730	15.177	12.625
Poultry oil	6.194	5.653	4.178	2.956	1.991	1.024	0.056
Pro-Pak[1]	5.000	5.000	5.000	5.000	5.000	5.000	5.000
Corn gluten meal	0.000	0.000	5.000	10.000	15.00	20.000	25.000
Limestone	1.095	1.098	1.123	1.143	1.160	1.178	1.195
Dicalcium phosphate	1.013	1.037	1.055	1.062	1.060	1.058	1.056
Iodized salt	0.400	0.400	0.400	0.400	0.400	0.400	0.400
Vitamin premix[2]	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Trace mineral mix[3]	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Corn starch	0.000	2.000	2.000	2.000	2.00	2.000	2.000
DL-Methionine (98%)	0.153	0.183	0.132	0.067	0.019	0.000	0.000
L-Lysine HCl (98%)	0.000	0.000	0.048	0.134	0.168	0.203	0.238
Total							
Nutrient analysis[4]							
ME, kcal/kg (C)	3200.00	3200.00	3200.00	3200.00	3200.00	3200.00	3200.00
CP, % (C)	23.00	21.95	22.65	23.95	25.77	27.62	29.47
CP, % (A)	23.17	22.06	22.48	24.02	25.63	27.32	29.7
Ile, % (A)	0.94	0.89	0.91	0.96	1.03	1.11	1.18
Val, % (A)	1.12	1.07	1.10	1.15	1.23	1.31	1.39
Leu, % (A)	1.99	1.90	2.23	2.60	3.01	3.42	3.83
Met, % (A)	0.56	0.57	0.55	0.53	0.54	0.57	0.60
Lys, % (A)	1.29	1.22	1.18	1.16	1.17	1.19	1.16
TSAA, % (A)	0.95	0.98	0.96	0.95	0.98	1.04	1.12

[1]H. J. Baker & Bro., 595 Summer Street, Stamford, CT 06901-1407.
[2]Provides per kg of diet: vitamin A (from vitamin A acetate) 7714 IU; cholecalciferol 2204 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadione dimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg; Se 0.1 mg.
[3]Provides per kg of diet: Mn (from $MnSO_4{\bullet}H_20$) 100 mg; Zn (from $ZnSO_4{\bullet}7H_2O$) 100 mg; Fe (from $FeSO_4{\bullet}7H_2O$) 50 mg; Cu (from $CuSO_4{\bullet}5H_20$) 10 mg; I from $Ca(IO_3)_2{\bullet}H_20$), 1 mg. [4]C = calculated value; A = analyzed value.

Table 2: Composition (%) and nutrient content of diets with different levels of branched chain amino acids (Experiment 2)

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Yellow corn	47.790	49.548	51.168	49.977	48.472	46.955
Soybean meal	37.491	32.165	26.855	24.059	21.516	18.974
Poultry oil	6.677	5.233	3.784	2.782	1.822	0.862
Corn gluten meal	0.000	5.000	10.000	15.000	20.000	25.000
Limestone	1.237	1.274	1.311	1.335	1.359	1.382
Dicalcium phosphate	1.784	1.800	1.818	1.817	1.815	1.813
Iodized salt	0.458	0.457	0.456	0.456	0.455	0.455
Vitamin premix[1]	0.200	0.200	0.200	0.200	0.200	0.200
Trace mineral mix[1]	0.263	0.213	0.163	0.085	0.037	0.000
Corn starch	4.000	4.000	4.000	4.000	4.000	4.000
DL-Methionine (98%)	0.263	0.213	0.163	0.085	0.037	0.000
L-Lysine HCl (98%)	0.000	0.010	0.145	0.189	0.224	0.259
Total						
Nutrient analysis[2]						
ME, kcal/kg (C)	3200.00	3200.00	3200.00	3200.00	3200.00	3200.00
CP, % (C)	21.93	22.58	23.35	25.08	26.91	28.75
CP, % (A)	22.04	22.67	23.41	24.98	27.02	28.67
Ile, % (A)	0.94	0.96	0.98	1.05	1.12	1.20
Val, % (A)	1.02	1.05	1.07	1.15	1.23	1.31
Leu, % (A)	1.84	2.17	2.50	2.90	3.31	3.72
Met, % (A)	0.59	0.57	0.56	0.53	0.53	0.54
Lys, % (A)	1.25	1.16	1.16	1.16	1.16	1.16
TSAA, % (A)	0.95	0.95	0.95	0.95	0.98	1.02

[1]See Table 1 for composition.
[2]C = calculated value; A = analyzed value.

Table 3: Branched chain amino acid relationships in experimental diets

Diet	% Corn Gluten Meal	Branched chain amino acid relationship[1]					
		Percent of diet			Relative proportion		
		ILE	LEU	VAL	ILE	LEU	VAL
Experiment 1							
1	0	0.94	1.99	1.12	100	212	120
2	0	0.89	1.90	1.07	100	215	121
3	5	0.91	2.23	1.10	100	246	121
4	10	0.96	2.60	1.15	100	272	120
5	15	1.03	3.01	1.23	100	292	119
6	20	1.11	3.42	1.31	100	309	119
7	25	1.18	3.83	1.39	100	325	118
Experiment 2							
1	0	0.94	1.84	1.02	100	196	109
2	5	0.96	2.17	1.05	100	226	109
3	10	0.98	2.50	1.07	100	255	109
4	15	1.05	2.90	1.15	100	276	110
5	20	1.12	3.31	1.23	100	296	110
6	25	1.20	3.72	1.31	100	310	109

[1]The NRC (1994) recommends minimum levels of 0.80% Ile, 1.20% Leu, and 0.90% Val for diets with 3200 ME kcal/kg to give an ILE:LEU:VAL ratio of 100:150:112.

Table 4: Effects of increased dietary leucine from intact proteins or free amino acid and additional supplementation of isoleucine and valine on live performance of male broilers (Experiment 1)

% Dietary LEU	Source of increased LEU			
	Intact Protein Series[1]	Free Amino Series[2]	Intact plus ILE and VAL[3]	Mean
21 d body weight (g)[4]				
1.90	793 ab	793 ab	793 ab	793 pq
2.23	832 a	831 a	793 ab	819 p
2.60	774 ab	784 ab	797 ab	785 pqr
3.01	728 abc	775 ab	733 abc	745 s
3.42	781 ab	784 ab	702 bc	755 rs
3.83	632 cd	803 ab	579 d	671 t
Mean	757 y	795 x	733 z	
0 to 21 d feed per bird (g)[5]				
1.90	1046 ab	1046 ab	1046 ab	1046 p
2.23	1073 a	1064 a	1025 abc	1054 p
2.60	1010 abc	996 abc	1050 ab	1018 p
3.01	914 bcd	982 abc	914 bcd	936 q
3.42	998 abc	1003 abc	884 cde	962 q
3.83	828 de	1020 abc	745 e	864 r
Mean	978 y	1018 x	944 z	
0 to 21 d Feed:Gain ratio[6]				
1.90	1.321	1.321	1.321	1.321 a
2.23	1.289	1.281	1.293	1.288 bc
2.60	1.304	1.272	1.316	1.297 b
3.01	1.254	1.268	1.245	1.255 d
3.42	1.277	1.279	1.261	1.273 c
3.83	1.315	1.270	1.286	1.290 bc
Mean	1.293	1.282	1.287	

Source of variance	Body weight		Feed Intake		Feed:gain ratio	
	Prob > F	SEM	Prob > F	SEM	Prob > F	SEM
LEU level	0.0001	13	0.0001	17	0.0001	0.0009
LEU source	0.0001	9	0.0002	12	0.41	0.0006
Source x Level	0.0001	23	0.0001	29	0.45	0.015

[a-z]Within comparisons, means with common letters differ significantly ($P \leq 0.05$) [1]Obtained from incremental increases in corn gluten meal from 0 to 25%. [2]Obtained by supplementation of basal diet with L-LEU. [3] Obtained from incremental increases in corn gluten meal from 0 to 25% with L-ILE and L-VAL added to maintain 100:215:121 ILE:LEU:VAL ratio. [4]Positive control birds weighed 799 g. [5] Positive control birds consumed 1,100 g.[6] Positive control had a feed conversion ratio of 1.378.

Table 5: Effects of increased dietary leucine from intact proteins or free amino acid and additional supplementation of isoleucine and valine on carcass characteristics of 21 d male broilers (Experiment 1)

%Dietary LEU	Source of increased LEU			
	Intact Protein Series[1]	Free Amino Series[2]	Intact plus ILE and VAL[3]	Mean
Heart as % of BW[4]				
1.90	0.67	0.67	0.67	0.67
2.23	0.75	0.66	0.72	0.71
2.60	0.72	0.66	0.75	0.71
3.01	0.63	0.69	0.75	0.69
3.42	0.66	0.64	0.64	0.65
3.83	0.72	0.66	0.75	0.71
Mean	0.69 a	0.66 b	0.71 a	
Liver as % of BW[5]				
1.90	3.24	3.24	3.24	3.24
2.23	3.21	2.93	3.01	3.05
2.60	3.16	2.96	3.47	3.20
3.01	3.38	3.39	3.31	3.36
3.42	3.45	3.10	3.50	3.35
3.83	3.40	3.06	3.50	3.32
Mean	3.31 a	3.11 b	3.34 a	
Abdominal fat as % of BW[6]				
1.90	1.23	1.23	1.23	1.23 a
2.23	1.27	1.18	1.13	1.19 b
2.60	1.22	1.10	1.10	1.14 b
3.01	1.31	1.45	1.28	1.35 a
3.42	1.06	1.17	1.22	1.15 b
3.83	0.99	1.25	0.97	1.07 c
Mean	1.18	1.23	1.15	

Source of variance	Heart % of BW		Liver % of BW		Fat % of BW	
	Prob > F	SEM	Prob > F	SEM	Prob > F	SEM
LEU level	0.06	0.018	0.13	0.089	0.005	0.050
LEU source	0.01	0.013	0.03	0.066	0.31	0.036
Source x Level	0.26	0.031	0.60	0.154	0.37	0.087

[abc]Within comparisons, means with no common superscript differ significantly ($P < 0.05$) [1]Obtained from incremental increases in corn gluten meal from 0 to 25%. [2]Obtained by supplementation of basal diet with L-LEU. [3]Obtained from incremental increases in corn gluten meal from 0 to 25% with L-ILE and L-VAL added to maintain 100:215:121 ILE:LEU:VAL ratio. [4] Positive control birds had heart weight of 0.62 of BW. [5]Positive control birds had liver weight of 2.64% of BW. [6]Positive control birds had abdominal fat weight of 1.20% of BW.

(Experiment 3) interactions were examined. Where significant differences existed ($P < 0.05$) means were separated by repeated t-tests using the lsmeans option of SAS (SAS Institute, 1991).

Results

Experiment 1: The diets used in these studies were formulated using supplements of lysine and methionine to contain nutrient levels similar to those used in the commercial broiler industry, and were not designed to be deficient in Leu, Ile, or Val. Consequently, levels of these three amino acids were in excess of their minimum requirements in both experiments (Table 3). The ratio of these amino acids at their minimum requirements (NRC, 1994) would be 100 ILE:150 LEU:112 VAL. The actual ratio of Val to Ile ranged from 118 to 120 parts Val to 100 parts Ile in the first experiment and 109 to 110 parts Val to 100 parts Ile in the second experiment, similar to the 112 to 100 NRC ratio. However, the ratio of Leu to Ile and Val was much greater than that at NRC minimums, ranging from 212 to 325 parts Leu to 100 parts Ile in the first experiment and 196 to 310 parts Leu to 100 parts Ile in the second experiment.

Both source and level of Leu had a significant effect on the 21-d body weight of broilers with a significant interaction of source and level (Table 4). Body weight of birds fed increasing levels of Leu from intact crude protein (primarily from increasing amounts of corn gluten meal) remained fairly constant until the level of Leu reached 3.83%, commensurate with a 25% inclusion rate of corn gluten meal. No such reduction in body weight was observed from the addition of L-Leu to the basal diet void of corn gluten meal, and was not alleviated by the addition of L-Ile and L-Val to the corn gluten meal diets to maintain the Ile:Leu:Val ratio constant with that observed at the lowest level of Leu. Therefore, it was considered that this reduction in body

Table 6: Effects of increased dietary leucine from intact proteins or free amino acid and additional supplementation of isoleucine and valine on live performance of male broilers (Experiment 2)

%LEU	Intact Protein			Free amino acid			Mean		
	Added ILE and VAL[1]			Added ILE and VAL			Added ILE and VAL		
	No	Yes	Mean	No	Yes	Mean	No	Yes	Mean
21 d BW (g)									
1.84	761	761	761 bc	761	761	761 bc	761	761	761 a
2.17	777	791	784 bc	783	760	772 abc	780	776	778 a
2.50	802	781	792 a	769	764	767 abc	786	773	779 a
2.90	805	775	790 a	764	769	767 abc	785	772	778 a
3.31	766	733	750 c	771	774	773 abc	769	754	761 a
3.72	740	700	720 d	744	752	748 c	742	726	733 b
Mean	775	757	766	765	763	764	770	760	
0 to 21 d feed intake (g/bird)									
1.84	1072	1072	1072	1072	1072	1072	1072	1072	1072 a
2.17	1078	1065	1072	1080	1047	1064	1080	1056	1068 a
2.50	1107	1061	1084	1080	1037	1058	1093	1049	1071 a
2.90	1084	1069	1077	1047	1053	1050	1065	1061	1063 a
3.31	1031	1019	1025	1077	1050	1063	1054	1034	1044 a
3.72	1025	965	995	1002	1016	1009	1013	990	1002 b
Mean	1066	1042	1054	1060	1046	1053	1063 x	1044 y	
0 to 21 d Feed:Gain ratio									
1.84	1.409	1.409	1.409	1.409	1.409	1.409	1.409	1.409	1.409 a
2.17	1.388	1.347	1.367	1.380	1.380	1.379	1.384	1.363	1.373 b
2.50	1.378	1.358	1.368	1.405	1.357	1.381	1.392	1.357	1.375 b
2.90	1.346	1.380	1.363	1.371	1.370	1.371	1.359	1.375	1.367 b
3.31	1.346	1.390	1.368	1.396	1.356	1.376	1.371	1.373	1.372 b
3.72	1.385	1.381	1.383	1.347	1.351	1.349	1.366	1.366	1.366 b
Mean	1.375	1.377	1.376	1.385	1.371	1.378	1.380	1.374	

Source of variation	Body weight		Feed intake		Feed:gain ratio	
	Prob>F	SEM	Prob>F	SEM	Prob>F	SEM
LEU source (S)	0.75	4	0.88	6	0.87	0.006
LEU level (L)	0.0001	7	0.0001	11	0.03	0.009
S x L	0.02	10	0.25	15	0.55	0.014
Added BCAA	0.07	4	0.03	6	0.45	0.006
S x BCAA	0.15	6	0.56	9	0.33	0.008
L x BCAA	0.95	10	0.75	15	0.53	0.014
S x L x BCAA	0.28	14	0.67	22	0.34	0.02

[abc]Within comparisons, means with no common letters differ significantly ($P < 0.05$).
[1]Isoleucine and valine added to maintain an ILE:LEU:VAL ratio of 100:195:109.

weight was not the result of a toxic level of Leu *per se* or to an Ile:Leu:Val imbalance.

Feed intake was also significantly influenced by source and level of Leu with a significant interaction of source and level (Table 4) and is believed to be the primary cause of the reduced body weight observed in chicks fed the diets with the highest level of corn gluten meal. No reduction in feed intake was observed when L-Leu was added to the basal diet devoid of corn gluten meal, and the reduction in feed intake was not alleviated by the addition of L-Ile and L-Val to the corn gluten meal diets. Although corn gluten meal is a well-accepted feed ingredient, it is usually added at levels sufficient to provide xanthophyll pigments (5 to 10% of the diet) and is not normally added at these higher levels. Diets with the higher levels of corn gluten meal did not pellet well due to the minimal starch content in corn gluten meal. Although no attempt was made to quantitatively evaluate pellet quality, it deteriorated considerably with each increase in level of corn gluten meal. Thus, it was considered that the texture of the diets high in corn gluten meal may have inhibited feed intake, rather than the level of Leu *per se* or possible imbalance among Ile:Leu:Val. This conclusion was supported by the lack of an adverse effect of Leu source or level on feed conversion (Table 4). Feed conversion was significantly improved as the level of Leu increased.

Levels of leucine had little influence on heart and liver weight as percentage of body weight (Table 5). Chicks fed the intact protein diets with or without the additional L-Ile and L-Val had significantly higher heart and liver weights as a percentage of body weight than did chicks fed the free amino acid series of diets; this was due primarily to a reduction in body weight of these birds rather than an increase in heart or liver weight *per se*. Abdominal fat content was generally reduced as Leu levels increased, although this was not a consistent effect.

Experiment 2: Removal of the blended animal protein from the diets resulted in slightly higher usage levels of poultry oil to maintain a constant dietary energy level (Table 2). Although no quantitative measure of pellet quality was made, the pellet quality of the diets containing the higher levels of corn gluten meal was improved as compared to that observed in the first experiment.

Body weight of the broilers at 21 d was significantly influenced by level of dietary Leu (Table 6). No significant differences in body weight were observed among birds fed from 1.84 to 3.31% Leu; at the highest Leu level of 3.72%, body weight was significantly reduced. This was not influenced by the source of the additional Leu (increasing corn gluten meal or supplemental L-Leu); however, there was a significant interaction between source and level of Leu. A reduction in body weight was observed at the higher levels of Leu in birds fed both the intact protein diets with increasing levels of corn gluten meal or the free amino acid diets with supplemental L-Leu; however there was a sharper reduction in body weight for birds fed the diets with increasing levels of corn gluten meal, resulting in the interaction of source and level of Leu. As observed in the first experiment, the addition of L-Ile and L-Val to the diets in amounts sufficient to retain an Ile:Leu:Val ratio of 100:195:109 was not beneficial in overcoming the reduction in body weight seen at the higher levels of Leu; in fact the addition of these amino acids tended ($P = 0.07$) to reduce body weight.

Feed intake was reduced at the highest level of dietary Leu, in agreement with the first experiment (Table 6). This reduction was not related to the source of Leu (increasing levels of corn gluten meal or additional L-Leu as free amino acid). The addition of L-Ile and L-Val to maintain an Ile:Leu:Val ratio of 100:195:109 resulted in a significant depression in feed intake, regardless of whether they were added to the diet with increasing levels of corn gluten meal or the diet with L-Leu provided as the free amino acid.

The depressed feed intake at the higher levels of Leu appeared to be the primary cause of the reduced body weight, as the consumed feed was efficiently converted to gain (Table 6). Feed conversion was significantly higher on the lowest level of dietary Leu as compared to higher levels. There was no influence of source of the dietary Leu or to the addition of L-Ile and L-Val to the diets.

Discussion

The existence of an interaction among the BCAA, and especially the adverse effects of excess Leu, has been noted primarily in diets that are considered as marginal or deficient in Ile and Val for rats (Benton *et al.*, 1956; Harper *et al.*, 1964, 1970), chicks (D'Mello and Lewis, 1970; Allen and Baker, 1972; Barbour and Latshaw, 1992) and turkeys (D'Mello, 1975; Tuttle and Balloun, 1976; Jackson and Potter, 1984). However, in studies with diets based on practical ingredients meeting minimum requirements for Ile and Val, no adverse effects of high levels of Leu were apparent (Burnham *et al.*, 1992; Barbour and Latshaw, 1992).

The determination of minimum requirements for Ile, Leu, and Val has been the subject of several reports with a considerable range in estimated requirements (Grau and Peterson, 1946; Almquist, 1947; Klain *et al.*, 1960; Dobson *et al.*, 1964; Dean and Scott, 1965; D'Mello, 1974; Woodham and Deans, 1975; Baker *et al.*, 1979; Mendonca and Jensen, 1989; Farran and Thomas, 1990, 1992, 1996). Diets used in the present studies were not formulated to be at minimum levels of these amino acids but rather were formulated to be consistent with typical diets used in commercial broiler production utilizing corn and soybean meal with supplements of commercially available amino acids. As a result, all diets contained levels of Ile, Leu, and Val in excess of their minimum recommended levels (NRC, 1994), similar to the diets used by Burnham *et al.* (1992) and Barbour and Latshaw (1992). The lack of an apparent interaction among the BCAA in the present studies is in agreement with these cited works.

It has long been recognized that the feed intake of animals given a diet with an amino acid imbalance is depressed compared to that of control animals (Harper *et al.*, 1970). In the present studies, the primary effect observed was a reduction in feed intake that occurred in diets with high levels of Leu provided from corn gluten meal (Experiment 1), which may have been due in part to diet texture, and in diets fortified with additional L-Ile and L-Val regardless of the source or level of Leu (Experiment 2). Adverse effects of excess levels of individual essential amino acids on broiler performance have been reported (Okumura and Yamaguchi, 1980; Ueda *et al.*, 1981; Edmonds and Baker, 1987). 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 levels of amino acids or the effects of combinations of excess amino acids as might have occurred in the present studies.

The results of the present studies suggest that an antagonism among or between the branched-chain amino acids Leu, Ile, and Val is not likely to result in depressed performance of broilers fed practical type diets with levels of these amino acids above their minimum requirements. Further research is needed to evaluate the potential impact of a BCAA imbalance in diets with reduced levels of crude protein where one or more of the BCAA may be at minimal dietary levels with high levels of Leu from corn protein.

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