Lysine Need of Heavy Broiler Males Applying the Ideal Protein Concept

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ABSTRACT An experiment was conducted to measure the response of broiler males to dietary lysine progressing from 0.75 to 1.15% between 42 and 56 d of age. Chicks (Ross × Ross 308) were placed in floor pens (30 pens having 35 chicks each) of an open-sided house and provided common feeds to 42 d of age. From 42 to 56 d, a corn-soybean meal diet (18% CP and 3,250 kcal/kg ME) having total lysine at 0.75% was supplemented with additions of 0.10% until 1.15%. All other essential amino acids were "ideally" balanced to one another within the limits of practicality assuming 0.85% total lysine. Birds had continuous access to feed, water, and light. Live performance during experimentation was particularly favorable. Weight gain between 42 and 56 d of age was similar among birds receiving all levels of lysine, while feed conversion was optimized at 0.85%. Depot fat removed from the abdominal cavity, yield of the resultant chilled carcass, and the amount of fillet (pectoralis major) cone deboned from the breast were unaltered by dietary lysine level. However, yield of tenders (pectoralis minor) decreased as supplemental lysine increased, whereas the incidence of myopathy (green muscle disease) increased. The lysine requirement of 0.85% as advocated by NRC (1994) for broilers between 42 and 56 d of age is in agreement with present results and may have been predisposed by its favorability of balance with all other essential amino acids.

(*Key words*: amino acid balance, broiler requirement, carcass quality, ideal protein, lysine)

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INTRODUCTION

The protein and essential amino acid requirements advocated for broilers by NRC (1994) are largely based on experimentation conducted several decades ago. The rate of broiler growth and its terms of production have changed markedly. In an attempt to accommodate expected needs associated with this additional growth, levels of the limiting amino acids commercially employed have generally increased (Agri Stats, 2001). Most of these amino acids are now being supplemented in free form, enabling dietary crude protein to decrease below NRC (1994) recommendation, while improving overall balance. The balance of amino acids, one to the other, that would be ideal for broilers has been estimated using digestibilities (Baker, 1994) and by combining amounts in body protein gain with the estimates for maintenance (Mack et al., 1999).

Lysine usually represents the basis to which all other amino acids are related when generating an ideal balance. The existing requirement of 0.85% for broilers between 42 and 56 d of age originated from research in the early 1970s using common formulations without ready benefit of most limiting amino acids in free form (NRC, 1994). Lysine associated with most formulations at these ages is usually second limiting and particularly important to assuring yield of skinless boneless breast meat with today's broiler. Lysine values used in practice have escalated to approximate 0.95% (Agri Stats, 2001) which has been supported by research on high breast meat yielding strains given feeds formulated to satisfy NRC (1994) specifications (Acar et al., 1991; Bilgili et al., 1992).

Present experimentation used feed having protein supplemented with amino acids to approach an ideal balance, then lysine was added for the total to progress from inadequate to a level considered excessive for broiler males between 42 and 56 d of age. The NRC (1994) requirement of 0.85% lysine served as the basis to which all other essential amino acids would be "ideally" related (Mack et al., 1999). Measurements involved live performance and quality of resultant carcasses as well as nitrogen balance conducted at midpoint in a separate study.

MATERIALS AND METHODS

Commercial source Ross × Ross 308 male day-old chicks were randomized into floor pens of an open-sided house having thermostatically controlled heating, curtains, and cross-ventilation (30 pens; 35 birds/pen; 0.118 m^2/bird). Each pen had fresh pine shavings and was equipped

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TABLE 1. Composition of experimental feeds and days duration fed (% as is)

Ingredient	0–21 d	21–42 d	42–56 d[1]
Corn	54.66	59.02	69.99
Soybean meal (48% CP)	35.39	31.51	18.58
Poultry oil	5.17	5.12	3.30
Corn gluten meal	—	—	3.16
DL-Methionine	0.23	0.19	0.15
Biolys[2]	0.16	0.12	—
Dicalcium phosphate	1.91	1.77	1.57
Limestone	1.50	1.40	1.25
Sodium chloride	0.45	0.32	0.32
L-Threonine	—	—	0.13
L-Arginine	—	—	0.14
L-Glutamic acid	—	—	0.85
Other[3]	to 100%	to 100%	to 100%
Calculated analyses			
CP (%)	22.50	20.50	18.0
ME (kcal/g)	3.15	3.20	3.25
Lysine (%)	1.24	1.12	0.75
TSAA (%)	0.90	0.75	0.75
Calcium (%)	1.08	1.00	0.88
Available phosphorus (%)	0.48	0.38	0.35

[1]Basal feed from 42 to 56 d of age.

[2]Biolys 60 (L-lysine sulfate a fermentation product, with a minimum content of 47.3% L-lysine, Degussa Corporation, Kennesaw, GA).

[3]Vitamin premix, 0.25% (supplied per kilogram of diet: vitamin A, 7,356 IU; vitamin D_3, 2,205 IU; vitamin E, 8 IU; cyanocobalamin, 0.02 mg; riboflavin, 5.5 mg; niacin 36 mg; d-pantothenic acid, 13 mg; choline, 501 mg; menadione, 2.2 mg; folic acid, 0.5 mg; pyridoxine, 2 mg; thiamine, 1 mg; biotin 0.1 mg; ethoxiquin, 125 mg). Mineral premix 0.25% (supplied mg/kg of diet): manganese, 65; zinc, 55; iron, 6; iodine, 1; copper, 6; selenium, 0.3); coccidiostat, 0.05% (60% salinomycin sodium premix, Roche Vitamins Inc., Parsippany, NJ).

with one hanging feeder (22.5-kg capacity) and one bell drinker. Chicks had been vaccinated for Marek's disease, Newcastle disease, and infectious bronchitis at the hatchery and at 12 d of age against infectious bursal disease. All birds received common corn-soybean meal feeds in crumbed form from placement to 21 d of age and as whole pellet from 21 to 42 d each of which generally exceeded NRC (1994) nutrient recommendations (Table 1).

At 42 d of age, bird number was equalized among pens (30/pen), then treatments were assigned to pens in order to provide a similar distribution of average bird weight at the start of experimentation. Treatments consisted of five total dietary lysine levels that progressed from 0.75% to 1.15%. Feed ingredients used from 42 to 56 d of age were corn, soybean meal, and corn gluten meal and were analyzed for protein bound and supplemented amino acids according to Llames and Fontaine (1994).

Formulation of the basal diet (Table 1) minimized lysine content (0.75%), while assuring the minimum levels of all other essential amino acids in a manner that would approach ideality as estimated by Mack et al. (1999), within the limits of practicality. The minimum amount of all essential amino acids was established using 0.85% lysine as the basis of balance. Progressive increments of 0.10% lysine were added to the basal diet at the expense of L-glutamic acid to generate the five treatment levels, while maintaining isonitrogenous conditions. Feed and water were offered ad libitum, and lighting was continuous. Feed conversions were corrected for mortality.

At 41 d of age, 90 birds were removed from the floor pens immediately before experimentation such that their individual body weights were within the upper and lower 5% of the total average. Birds were divided into groups that were heavier and lighter than the average, then these groups were placed in raised-wire floor batteries. Batteries had cages containing one trough waterer and one trough feeder (2 birds/cage). Room lighting was continuous, while environmental conditions were similar to those concurrently experienced by broilers that were maintained on the floor. Birds in cages received each of the experimental feeds until Day 48. Excreta were collected through 48 to 49 d and held frozen at –20 C for later lyopholization. Nitrogen retention was calculated from semi-Kjeldahl measurements on feed and excreta.

At 56 d of age, all birds in floor pens were placed in transportation coops and held about 14 h prior to slaughter. On-line processing was performed in a scaled down version of a commercial plant that involved a 9-min kill line followed with a 7-min evisceration line. Resulting warm carcasses were static chilled in slush ice for 4 h, then depot fat was removed from the abdominal cavity, and defects central to grade were itemized by type and location. The front half was removed from each carcass and held on flaked-ice until the following day. Removal of the fillets (pectoralis major) and tenders (pectoralis minor) was accomplished using stationary cones and experienced personnel. Incidence of blood contaminating the fillets and tenders exhibiting myopathy was noted during measurement of their weights. Fillets originating from the right side of the carcass were measured for light reflectance (CIE Scale) 24 h after removal from the carcass using a hand-held spectrophotometer (CM-2002),[2] which was placed at the center of the muscle surface that had been adjacent to the skin.

[2]Minolta Corp., Ramsay, NJ.

TABLE 2. Actual amino acid analysis of experimental diets fed to broilers from 42 to 56 d of age, 88% dry matter basis[1]

Amino acid	Intended lysine (%) 0.75	0.85	0.95	1.05	1.15
Total lysine	0.81	0.88	0.97	1.05	1.20
Free lysine[2]	0.01	0.09	0.21	0.29	0.41
Arginine	1.19	1.16	1.14	1.14	1.19
Total methionine	0.46	0.43	0.46	0.43	0.47
Free methionine[2]	0.17	0.14	0.17	0.16	0.16
Cystine	0.32	0.31	0.31	0.30	0.31
TSAA	0.78	0.74	0.77	0.74	0.78
Threonine	0.78	0.75	0.77	0.76	0.78
Isoleucine	0.72	0.71	0.72	0.70	0.69
Valine	0.82	0.81	0.80	0.80	0.80
Glutamic acid	3.91	3.64	3.59	3.30	3.13

[1]Representative samples were analyzed in duplicate by Degussa-Huls Applied Technology Chemical Group, Allendale, NJ.

[2]Values represent supplemental free lysine and methionine.

TABLE 3. Live performance of male broilers from placement until initiation of experimentation at 41 d of age[1]

Basis	Live weight (g) Final	Live weight (g) Gain	F/G[2]	Mortality (% total)
		0–21 d[3]		
Mean	793	751	1.50	2.5
SEM	12.8	12.2	0.005	0.33
		22–41 d[4]		
Mean	2,818	2,025	1.73	3.7
SEM	22.3	18.3	0.007	0.49
		0–41 d		
Mean	—	2,776	1.66	6.2
SEM	—	21.8	0.005	0.70

[1]Values are grand means involving 30 pens each with 35 chicks at placement.

[2]Feed/gain values were corrected for mortality.

[3]Average temperature of 25 ± 2 C and RH of 52 ± 9%.

[4]Average temperature of 22 ± 3 C and RH of 60 ± 14%.

TABLE 4. Live performance of broiler males from 42 to 56 d of age in response to progressive supplemented dietary lysine[1]

Lysine %	BW (g) Final	BW (g) Gain	Consumption (g) Feed	Consumption (g) Lysine	Feed conversion[2] 42 to 56 d	Feed conversion[2] 0 to 56 d
0.75	4,258	1,425	3,333	25	2.34	1.88
0.85	4,280	1,457	3,315	28	2.28	1.86
0.95	4,253	1,429	3,287	31	2.30	1.86
1.05	4,263	1,440	3,289	35	2.28	1.86
1.15	4,265	1,442	3,288	38	2.28	1.84
SEM (24 df)	18.8	17.3	29.8	0.29	0.019	0.009
			Orthogonal polynomials[3]			
Contrast						
Linear	NS	NS	NS	***	NS	*
Quadratic	NS	NS	NS	NS	NS	NS
R^2	0.66	0.13	0.33	0.98	0.29	0.23

[1]Values represent observed means of six pens each having ca. 30 birds with an average temperature of 18 ± 2 C and RH of 61 ± 12%. All cubic responses were not significant, $P > 0.05$.

[2]Feed/gain values corrected for mortality.

[3]NS $P > 0.05$; *$P < 0.05$; ***$P < 0.001$.

TABLE 5. Chilled carcass yield of broiler males that received progressive supplemental lysine from 42 to 56 d of age[1]

	Abdominal fat[2]		Carcass without abdominal fat[3]	
Lysine %	Weight (g)	Carcass (%)	Weight (g)	Carcass (%)
0.75	82	2.75	2,990	70.2
0.85	82	2.73	2,997	69.9
0.95	81	2.72	2,989	70.2
1.05	79	2.66	2,992	70.1
1.15	78	2.63	2,991	70.1
SEM (24 df)	2.1	0.065	17.4	0.23
	Orthogonal polynomials[4]			
Contrast				
Linear	NS	NS	NS	NS
Quadratic	NS	NS	NS	NS
R^2	0.15	0.10	0.44	0.11

[1]Values are the observed means of six pens each providing ca. 30 carcasses. All cubic responses were nonsignificant, $P > 0.05$.

[2]Depot fat removed from the abdominal cavity of carcasses without neck and giblets after 4 h of slush ice chilling expressed on an absolute basis and relative to its entire weight.

[3]Chilled carcass without abdominal fat expressed on an absolute basis and relative to the full-fed live weight.

[4]NS, $P > 0.05$.

Data were evaluated by analysis of variance in a completely randomized design. Computations employed the general linear model procedure of SAS (1988). Mean separation procedure was performed by orthogonal polynomial techniques. Only linear and quadratic effects are presented on the respective tables because significance ($P > 0.05$) of higher order polynomials was not observed. Regression analysis was used to estimate lysine optimization (95% of the upper asymptote) whenever a significant quadratic response ($P < 0.05$) was detected. All percentage data for carcass defects and grade were transformed to arcsine $\sqrt{\%}$ for analysis.

RESULTS AND DISCUSSION

Actual level of lysine calculated to be in each of the experimental diets as well as those supplemented to approach an ideal balance according to Mack et al., (1999) were in agreement with actual analyses (Table 2). Live

TABLE 6. Incidence of major defects to the carcass of broiler males that received progressive supplemental lysine from 42 to 56 d of age[1]

	Wings[2]			Drums[3]		Breast[4]		Back-thigh[5]			Grade[6]
Lysine %	Dislocated	Broken	Bruised	Broken	Bruised	Bruised	Broken	Bruised	Tear	Scratched	A
0.75	12.3	0.6	20.0	3.5	3.5	3.4	14.2	12.9	0.5	12.3	41.4
0.85	20.5	2.2	31.1	1.7	0.6	5.5	9.8	14.0	0.6	9.7	39.7
0.95	23.0	2.2	27.5	5.0	5.2	5.1	17.6	11.9	2.8	7.9	30.3
1.05	15.0	3.5	30.3	2.8	1.6	6.0	15.1	16.5	1.7	7.7	37.2
1.15	16.7	4.5	27.1	3.4	4.1	5.2	11.2	11.4	0.0	9.3	35.5
SEM (24 df)	2.78	1.29	3.20	1.55	1.24	1.92	2.66	3.11	0.65	3.09	3.82
	Orthogonal polynomials[7]										
Contrast											
Linear	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
Quadratic	*	NS	NS	NS	NS	NS	NS	NS	**	NS	NS
R^2	0.39	0.24	0.27	0.19	0.31	0.08	0.24	0.07	0.36	0.06	0.17

[1]Values of observed means of six pens each providing ca. 30 carcasses. Statistical analysis employed transformed values (arcsine $\sqrt{\%}$), whereas the respective SEM values were estimates derived from actual percentages. All cubic responses were nonsignificant, $P > 0.05$.

[2]Wings defects correspond to join dislocation, broken bones, and bruise, respectively.

[3]Drumstick defects correspond to broken and bruise, respectively.

[4]Breast defects correspond to bruise and broken clavicle, respectively.

[5]Defects on the back and thigh correspond to bruise, torn skin, and scratched skin, respectively.

[6]Proportion of carcasses without major defects.

[7]NS, $P > 0.05$; *$P < 0.05$; **$P < 0.01$.

TABLE 7. Yield and quality of breast fillets (pectoralis major) of broiler males that had received progressive supplemental lysine from 42 to 56 d of age[1]

	Yield			Light reflectance (CIE)[3]		
Lysine, %	Weight (g)	Carcass (g)	Blood[2] total (%)	L	a	b
0.75	731	24.4	1.7	57.8	1.40	8.19
0.85	727	24.3	1.9	57.8	1.36	8.04
0.95	730	24.4	3.4	57.4	1.38	8.43
1.05	732	24.4	3.5	57.2	1.34	8.27
1.15	729	24.4	3.2	56.8	1.25	7.87
SEM (24 df)	5.7	0.14	0.02	0.22	0.059	0.107
	Orthogonal polynomials[4]					
Contrast						
Linear	NS	NS	NS	*	NS	NS
Quadratic	NS	NS	NS	NS	NS	*
R^2	0.60	0.37	0.50	0.40	0.20	0.40

[1]Values represent observed means of six pens each providing ca. 30 carcasses. All cubic responses were nonsignificant, $P > 0.05$.

[2]Incidence of blood contaminating meat.

[3]Increasing values relate to increasing lightness for L, redness for a, and yellowness for b.

[4]NS, $P > 0.05$; *$P < 0.05$.

performance of broiler males from placement through the first 42 d and initiation of experimentation was favorable (Table 3). Live performance through the subsequent 42 to 56 d was also beyond expectation in practice; however, response to progressively increasing dietary lysine was not obvious (Table 4). Furthermore, dietary lysine did not result in altering the amount of depot fat removed from the abdominal cavity after processing and chilling or yield of resulting carcasses (Table 5). However, level of lysine in the final feed appeared to influence the incidence of several common defects associated with the whole carcass. Broken wings increased as dietary lysine was supplemented, whereas wings having bone dislocation at the joint and tearing of skin covering the back-thigh maximized when lysine approximated 0.95% then decreased (Table 6). Reason for these observations is elusive given similar live performance.

The fillets and tenders are adjacent breast muscles that were removed from the carcass in the same manner; however, each one responded differently to dietary lysine. The fillets were similar in yield and incidence of contaminating blood from ruptured vessels, regardless of lysine level, but their appearance based on light reflectance was modified to a measurable though minimal extent (Table 7). The extent of lightness decreased as lysine level progressively increased, while yellowness increased to maximize at 0.95% lysine and subsequently decreased.

Yield of tenders and myopathy incidence were influenced in a correlated manner ($r = 0.43$, $P < 0.05$) as dietary lysine increased (Table 8). This myopathy in broilers has long been known to be a necrosis that results in a progressive wasting and muscle loss, hence the alternative term "green muscle disease" (Wight et al., 1979; Richardson et al., 1980; Siller, 1985). Its occurrence has been attributed to extensive overall development of the breast muscles and induced by stimulated use such as wing flapping. The significant increase in the incidence of broken wings as lysine increased (Table 6) indirectly attests to a differen-

TABLE 8. Yield and quality of tenders (pectoralis minor) of broiler males that had received progressive supplemental lysine from 42 to 56 d of age[1]

	Yield		
Lysine %	Weight (g)	Carcass (%)	Myopathy[2] total (%)
0.75	155	5.2	2.1
0.85	153	5.1	6.5
0.95	153	5.1	8.2
1.05	151	5.1	9.8
1.15	150	5.0	11.8
SEM (24 df)	1.4	0.04	0.02
	Orthogonal polynomials[3]		
Contrast			
Linear	*	*	***
Quadratic	NS	NS	NS
R^2	0.47	0.27	0.38

[1]Values represent observed means of six pens each providing ca. 30 carcasses. All cubic responses were nonsignificant, $P > 0.05$.

[2]Incidence of myopathy (green muscle disease).

[3]NS, $P > 0.05$; *$P < 0.05$; ***$P < 0.001$.

TABLE 9. Nitrogen balance of broilers measured while receiving feeds having progressive lysine supplemented to the experimental basal from 44 to 48 d[1]

Contrasts	Intake (mg)	Retention (mg)	Retention (%)	Retention (mg/kg of BW)
Lysine %	NS[2]	NS	NS	NS
0.75	6,138	3,057	49.9	0.91
0.85	6,487	3,277	50.5	0.96
0.95	6,120	3,007	48.9	0.87
1.05	6,394	2,986	46.5	0.87
1.15	6,303	2,895	46.0	0.84
SEM	218.1	160.3	1.91	0.049
Linear	NS	NS	*	NS
Quadratic	NS	NS	NS	NS
R^2	0.14	0.35	0.47	0.49
BW[3]	NS	***	***	***
Heavy	6,264	2,775	44.3	0.78
Light	6,312	3,314	52.5	1.01
SEM	141.4	103.3	1.18	0.032

[1]Data represent a total of 45 cages each with two birds. Data are given as observed means of the main factor contrasts because their interactions were not significant ($P > 0.05$). All cubic responses were nonsignificant, $P > 0.05$.

[2]NS, $P > 0.05$; *$P < 0.05$; ***$P < 0.001$.

[3]Body weight corresponds to groups divided to be above and below the total sample average: heavy = 3,528 g, and light = 3,290 g, SEM = 27.4.

tial extent of stimulation and basis for incidence. Loss in tender yield suggests that these changes had occurred many days prior to preslaughter handling when dietary lysine might have been influential.

Nitrogen balance was conducted to measure use of dietary protein with lysine level (Table 9). The source of broilers was the same as for floor pens as were the diets received; however, their cage environment likely altered nutritional needs, particularly energy. Furthermore, results solely relate to the few days midway between 42 to 56 d rather than the accumulated effort that occurred in floor pens. The progressive supplementation of lysine did not provide a definitive level of advantage but led to a progressive decrease in retention. Broilers that were lighter in weight than the average retained more nitrogen than those heavier, and differential in this respect to lysine was not apparent.

A requirement for lysine could not be pinpointed between the 0.75 and 1.15% levels used in the present experiment. However, an overview of all measurements suggest that 0.85% dietary lysine approximates an optimal level. Given that the "ideal balance" used in experimentation was based on all other essential amino acids being related to 0.85% lysine, our aforementioned requirement might have been predisposed to the terms imposed. Similarly, the absence of definitive responses to alteration of lysine may also have arisen with the reasonably close interrelationship among the limiting amino acids and did not enable clear secondary or tertiary inadequacies to prevail.

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