

PAPER

Digestible lysine requirement of broilers based on practical diet

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Abstract

The aim of this study was to estimate the requirement for digestible lysine for broilers from 35 to 49 days of age. Two hundred and forty chicks with a mean weight of 44 ± 1 g were used in a completely randomized design, made up of male and female chicks and 6 digestible lysine levels. Experimental diets were formulated to be isoenergetic and isonitrogenous. Fitted broken lines on different responses indicated break points at 0.93, 0.93 (for body weight), 0.98, and 0.92 for feed conversion ratio for male and female, respectively. The results showed that the digestible lysine requirement of male broilers for maximum breast yield percentage, plasma free lysine and antibody titer against Newcastle disease virus exceeded the range of lysine levels tested. Dietary lysine had a significant effect in increasing the plasma free lysine, albumin, total protein, immunoglobulin, antibody titer against sheep red blood cell, Newcastle disease virus and heterophil to lymphocyte ratio. In conclusion, lysine requirements of broilers for performance were lower than breast yield percentage and immune responses. Broken-line analysis showed that the concentrations of plasma free lysine were useful physiological indicators for determining the digestible lysine requirement of male and female broilers. Our results suggest that the estimated requirements based on exponential response curves were higher than estimated requirements obtained using a broken-line model.

Introduction

Genetic selection by primary breeding companies has resulted in greatly improved growth rate and breast meat yield of broiler chickens (Dozier *et al.*, 2008a,b). Therefore, today broilers require higher dietary amino acid concentrations to optimize performance and breast meat yield than in the past (Dozier *et al.*, 2008b).

Market weights of broiler chickens have increased to meet the demand for breast fillets and value-added products. Breast meat is relatively high in lysine (Lys) (7%) compared with other amino acids (AA). AAs/crude protein has a strong impact on the cost of broiler diet; diet costs represent approximately 65% of total live production costs. Formulating broiler diets on a digestible AA basis and utilizing the economically advantageous commercial AA supplements (methionine, Lys and threonine sources) result in diets with marginally less crude protein that support equal broiler growth compared to broilers fed diets containing higher crude protein with excess AAs. Digestible AA values are more beneficial than requirements expressed on a total basis. Lys is used as the reference AA for the ideal AA profile because more information is known about its concentration in feedstuffs. Lys is also the second-limiting AA in poultry diets (after methionine) its main purpose being protein synthesis (Baker, 1994). Ayasan and Okan (2011) randomly assigned broilers to five dietary treatment groups: 0.70 (control diet), 0.75, 0.80, 0.85 and 0.90% of threonine. The results obtained showed that the highest body weight gain occurred at 0.75% threonine; the tendency towards a better feed conversion ratio in the group was reflected with 0.80% threonine for female broilers. Their results suggest that the requirement calculated according to the feed conversion ratio is higher than that calculated according to body weight gain (Ayasan and Okan, 2011).

As all AAs are in relation to Lys in an ideal ratio, it is important to have an accurate Lys digestible requirement for the group of animals for which the diets are being formulated. If errors are made in the determination of the Lys requirement, all other AAs will also be estimated incorrectly. A review of the literature revealed that no research has been conducted to determine the digestible Lys requirement for broiler chicks based on immune response, breast meat yield and blood parameter. Unfortunately, few data are available on the

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digestible Lys requirements based on immune response, breast meat yield and blood parameters. This study aimed to determine the digestible Lys requirement for male and female broilers during the finisher period based on body weight, feed conversion ratio, immune responses, breast meat yield and blood parameters. It has become a common practice to include synthetic AAs such as Lys and methionine in rations for young broilers. The supply of optimal digestible Lys levels will reduce feed costs and decrease nitrogen excretion (Baker, 1994; Firman, 2001). Mehri *et al.* (2010) conducted an experiment to determine the digestible lysine requirement. They formulated basal diet for straight-run broilers from 15-28 days of age based on wheat, triticale and corn gluten meal, and provided 6 treatments: 0.60, 0.72, 0.84, 0.96, 1.08, and 1.20%. They estimated digestible lysine requirements by using a linear broken-line 0.95 and 1.08% for body weight gain and feed conversion ratio, respectively. The fundamental difference between our experiment and theirs was the type of response. We estimated digestible lysine requirement based on blood parameter, breast meat yield and immune response.

The primary objective of this research was to compare the determined digestible Lys requirement of male broilers for maximum performance (body weight gain and feed conversion ratio), immune response and breast meat yield in the finisher period using practical diets formulated on a digestible AA basis.

Materials and methods

Birds, experimental design and bird diets

For this experiment, 240 35-day-old broiler chicks were sorted according to gender and randomly assigned to the dietary treatments so that each pen had a similar initial weight (the initial body weight averaged 44 ± 1 g for both male and female chicks) and weight distribution. The birds received the experimental diets consisting of 6 graded levels of digestible Lys. Each dietary treatment had 4 replicates each for male and female chicks. There were 15 birds in each pen in the battery studies. Feed and water was available *ad libitum*, and a 24-h constant light schedule was maintained. For blood parameters and immune evaluation, 3 birds per pen were randomly selected to be wing-banded. Venous blood samples were col-

lected at the end of the experiment. A corn-soybean meal-corn gluten meal diet was formulated to meet or exceed the National Research Council (1994) recommendations for all nutrients except Lys (Table 1). Graded levels of L-Lys-HCl were added to the diet. Changes in the dietary ingredients were made to ensure that all diets were isoenergetic, isonitrogenous, and equal in electrolyte balance (DCAD).

Levels of digestible Lys for the experiment were 0.77, 0.84, 0.91, 0.98, 1.05 and 1.12%. The true AA digestibility of the basal diets was determined using Rhone-Poulenc feed (1993). Diets were analyzed for nitrogen (the Kjeldahl method) (AOAC, 2006), crude protein (CP), dry matter (DM) (by drying in an oven at 103°C for 8 h) and acid insoluble ash (AOAC, 2006). All values were expressed according to DM. Samples of feed used for laboratory analysis were ground to pass through a 1 mm mesh in a micro-Wiley mill.

Measurement of performance parameters

Individual body weights, feed consumption and feed conversion ratio per pen were determined weekly and at 49 days of age. Body weight gain, feed intake, and feed conversion ratio measurements were taken during the 35-49 day period. No mortality was observed for any of the treatments; therefore, feed intake was not adjusted for mortality to calculate feed conversion ratio.

At the end of the assay, birds were individually weighed. The 3 birds within each pen that were closest to the mean body weight of that pen were killed with CO_2 , and breast meat (pectoralis major, without skin) was excised and weighed; abdominal fat, including fat surrounding the gizzard, was also removed and weighed (Chaiyapoom *et al.*, 2006; Dozier *et al.*, 2008a).

At the end of the experiment, on Day 49, all

Table 1. Composition of experimental diets.

	Treatments, digestible lysine %					
	0.77	0.84	0.91	0.98	1.05	1.12
Ingredients						
Corn, %	62.20	62.09	61.90	61.77	61.64	61.52
Soybean meal, %	23.64	23.65	23.66	23.67	23.68	23.68
Gluten meal, %	8.17	8.16	8.18	8.19	8.20	8.21
Corn oil, %	2.83	2.84	2.86	2.87	2.87	2.88
Dicalcium phosphate, %	0.89	0.88	0.98	0.98	0.98	0.98
Oyster shell, %	1.26	1.27	1.21	1.21	1.21	1.21
NaHCO_3 , %	0.17	0.20	0.24	0.29	0.33	0.38
Vitamin ^o and mineral ^f mix, %	0.50	0.50	0.50	0.50	0.50	0.50
Salt, %	0.14	0.12	0.09	0.06	0.03	0.001
DL-methionine, %	0.20	0.20	0.20	0.20	0.203	0.20
L-Lys HCL, %	-	0.09	0.18	0.26	0.35	0.44
L-arginine, %	-	-	-	-	0.003	-
Analysis results^s						
Metabolizable energy, Kcal/kg	3200	3200	3200	3200	3200	3200
Protein, %	18.25	18.40	18.40	18.50	18.50	18.50
Calcium, %	0.80	0.80	0.80	0.80	0.80	0.80
Available phosphorus, %	0.30	0.30	0.30	0.30	0.30	0.30
DCAB, meq/kg	192.80	192.50	192.50	192.50	192.50	192.50
Digestible methionine, %	0.53	0.53	0.53	0.53	0.53	0.53
Digestible methionine+cystine, %	0.83	0.83	0.83	0.83	0.83	0.83
Digestible threonine, %	0.68	0.68	0.68	0.68	0.68	0.68
Digestible isoleucine, %	0.80	0.80	0.80	0.80	0.80	0.80
Digestible arginine, %	1.19	1.19	1.19	1.19	1.19	1.19
Digestible tryptophan, %	0.19	0.19	0.19	0.19	0.19	0.19
Digestible leucine, %	2.07	2.06	2.06	2.06	2.06	2.06
Digestible valine, %	0.88	0.88	0.88	0.88	0.88	0.88
Crude fibre, %	3.13	3.13	3.12	3.12	3.12	3.12

^sFinisher period from 35 to 49 d of age. The diet contained (by calculation) 3200 kcal of metabolizable energy/kg and 18.50% crude protein; it also contained 0.77, 0.84, 0.91, 0.98, 1.05 and 1.12% digestible lysine; total lysine as determined by analysis of diet. ^oVitamin mix provided the following (per kg of diet): thiamin-mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B₁₂ (cobalamin), 12.0 mg; pyridoxine HCL, 4.7 mg; D-biotin, 0.11 mg; folic acid, 5.5 mg; menadione sodium bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 27.5 mg; transretinyl acetate, 1892 mg; all-rac α tocopheryl acetate, 11 mg; ethoxyquin, 125 mg. ^fTrace mineral mix provided the following (per kg of diet): manganese ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$), 60 mg; iron ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), 30 mg; zinc (ZnO), 50 mg; copper ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), 5 mg; iodine (ethylene diamine dihydroiodide), 0.15 mg; selenium (NaSeO_3), 0.3 mg. All of the amino acids were adjusted to broiler requirements in this period except lysine.

birds were starved for 3 h, and then 3 birds from each pen were killed by cervical dislocation for carcass chemical composition and breast meat yield measurements. Abdominal fat was also weighed. Carcass weight, abdominal fat weight and breast meat weight were expressed as absolute weights and relative to live body weight at processing.

Blood chemical parameters

At 49 days, a blood sample was collected with a heparinized syringe from the wing vein of the chickens that had been wing banded. Blood samples were collected, placed on ice, centrifuged and stored as plasma at -18°C until the time of analysis. Parameters measured were total protein, creatinine, albumin, and globulin (Hiller *et al.*, 1927) uric acid (Fossati *et al.*, 1980; Donsbough *et al.*, 2010). Levels were measured spectrophotometrically (Shimadzu, UV 2100, Kyoto, Japan) using commercial kits.

Blood samples were collected in non-heparinized blood collection tubes to obtain the serum levels. The total number of leukocytes in the blood was assessed using a hemocytometer. Blood samples were put into heparinized tubes at the end of the experiment and from each sample, 4 smears were prepared and air-dried. The smears were fixed in glutaraldehyde-acetone solution, pH 4.8 for 3 min at -10°C . Two of the smears were stained with May Grunwald-Giemsa and peripheral blood leukocyte percentages of the samples were determined by counting 200 leukocytes on each specimen (Campbell, 1988; Post *et al.*, 2003). The results were expressed as percentages. Serum chemical parameters were analyzed using test-kits supplied by Merck (Darmstadt, Germany): total protein and hemoglobin (Dacie and Lewis, 1984). Hematocrit was determined by using heparinized capillaries for blood sampling and a micro-hematocrit centrifuge. Heterophils and total lymphocytes were counted microscopically. One hundred cells were counted to each ratio. Heterophil/lymphocyte (H/L) ratios were determined by dividing the number of heterophils by the number of lymphocytes (Gross and Siegel, 1983).

Humoral immune response

Antibody titers against sheep red blood cell

For humoral immune response, on Day 49, primary antibody response to sheep erythrocytes was measured in 3 randomly selected birds from each pen. Birds were intravenously injected with a 10% solution of sheep erythrocytes at Day 42. The blood samples from all the

sheep red blood cell (SRBC) injected birds were collected on Day 5 post inoculation for HA antibody titer estimation by the microtiter hemagglutination procedure (Siegel and Gross, 1980; Deng *et al.*, 2005).

Antibody titers against Newcastle disease

Birds were vaccinated with commercial NDV La Sota vaccine on Day 4 via eye drop and on Day 21 intramuscularly (inactive vaccine). The dose and vehicle of vaccine was used according to the manufacturer's instructions. Each bird received the same amount of NDV vaccine. At 49 days of age, blood samples were collected from 3 birds from each pen (Nanthakumar *et al.*, 2000). Serum antibodies to NDV were determined by a hemagglutination inhibition test (micromethod) (Isakov *et al.*, 2005). Antibody of serum samples was read as the highest dilution that can inhibit agglutination. Titer is expressed as Geometric Mean Titer (\log_2) to simplify numerical writing.

Statistical analysis

The statistical analysis was carried out with PROC GLM SAS (2007) with the fixed effect of sex and digestible Lys when significant interaction was also considered. Differences between treatments were tested for significance using the Duncan test. A P value <0.05 was considered significant.

To estimate the digestible AA requirements, multiple regression models (quadratic, exponential and one-slope broken-line; Robbins *et al.*, 2006) were developed:

Exponential:

$$Y = a + b(1 - e^{-c(x-d)})$$

One-slope broken line:

$$Y = L + U(R - X_{LR}) \text{ when } Lys < Lys_0, \text{ and } Y = \text{constant, when } Lys > Lys_0$$

where Y = BW, feed conversion ratio, plasma Lys, SRBC, H/L; all variables were analyzed (AT IG, etc.).

a = Y at the basal dietary Lys level;

b = maximum response to supplemental dietary Lys;

c = curvature steepness;

d = Lys content of basal diet (g/kg);

x = dietary Lys content (g/kg).

Values of R^2 were calculated to describe the goodness of fit.

Results and discussion

No mortality was observed between Days 35 and 49. There was a significant quadratic, bro-

ken-line and exponential response to increasing dietary levels of digestible Lys based on BWG and feed conversion ratio for females (Table 2). Subjecting the growth data to broken-line analysis indicated that the digestible Lys requirement for maximum body weight was 0.93% for both males and females (Table 2). The requirement for optimum feed conversion ratio was higher in males: 0.98% for males and 0.94% for female broilers (Table 2). When comparing males and females, there was some evidence that males and females differed in their digestible Lys requirement estimates. Ninety-five per cent of the asymptote in body weight was achieved at digestible Lys concentrations of 1.036 and 1.009% in males and females, respectively. Mehri *et al.* (2010) estimated digestible lysine requirements by using a linear broken-line 0.95 and 1.08% for body weight gain and feed conversion ratio, respectively (regardless of gender parameter). A higher digestible Lys requirement for FCR than for BWG has been reported (Mack *et al.*, 1999; Baker *et al.*, 2002). Body composition and growth differences in the birds used in those studies could explain the discrepancy (Kidd *et al.*, 2004). Lys limit protein accretion and increase fat accretion of broilers at low levels of supplementation (Moran and Bilgili, 1990) Another explanation for the effect of Lys on feed conversion ratio is the higher proportion of energy spent for maintenance. The poor growth rate (BW) and feed efficiency of chickens fed the diets including 0.77 and 0.84% digestible Lys at this period, indicated that the diets were, indeed, deficient in Lys. The decrease in performance and efficiency at the highest experimental digestible Lys level is due to less efficient use of AAs above the requirements for protein synthesis (imbalance). In contrast, the lower growth and high FRC at low experimental digestible Lys levels may be attributed to limiting supplies of the essential AAs. These results support the general principle that chick diets should be formulated to provide sufficient amounts of all AA corresponding subtle requirement for protein synthesis (Sklan and Plavnik, 2002). Lys and Arg are involved in the release of the growth hormone, insulin like growth factor I (IGF-I) and modulate bone growth by differentiation of osteoblastosis and collagen synthesis. New evidence has been provided by Sakomura and Coon (2003) and by Nonis and Gous (2008).

The percentages of breast and weight of abdominal fat were significantly affected by digestible Lys levels and breast meat increased with increasing digestible Lys concentration (Table 3). The responses suggested that the digestible Lys requirement for maximal breast

Table 2. Results of estimation for the digestible lysine requirement base on different parameters.

Method	Trait	Sex	Equation	R ²	Estimation of digestible lysine requirement	
					% of feed intake	mg per day
Broken-line	Body weight	Male	$Y=3083.3-3558.9(0.93-X_{LR})$	0.996	0.93	2664.82
	Feed conversion rate		$Y=1.79+1.51(0.98-X_{LR})$	0.995	0.98	2808.09
	Free lysine		$Y=239.3-318.4(1.15-X_{LR})$	0.980	1.12<	-
	Sheep red blood cell		$Y=5.65-3.52(1.05-X_{LR})$	0.993	1.05	3008.67
	Heterophil/lymphocyte		$Y=0.61-0.42(1.11-X_{LR})$	0.996	1.11	3180.59
	A.T ^o		$Y=11.94-5.28(1.15-X_{LR})$	0.995	1.12<	-
	Ig ^f		$Y=3.70-4.72(1.08-X_{LR})$	0.996	1.08	3094.63
	Abdominal fat		$Y=1.79+3.66(0.98-X_{LR})$	0.997	0.98	2808.09
	Breast meat		$Y=14.93+31.34(1.34-X_{LR})$	0.991	1.12<	-
Exponential	Body weight	Male	$Y=2441.4+672.8[1-e^{-11.24(X-0.77)}]$	0.722	1.036	2968.55
	Feed conversion rate		Does not fit	-	-	-
	Free lysine		Does not fit	-	-	-
	Sheep red blood cell		Does not fit	-	-	-
	Heterophil/lymphocyte		$Y=-0.35+0.27[1-e^{-9.24(X-0.77)}]$	0.690	1.09	3123.29
	A.T ^o		$Y=4.30+4.72[1-e^{-1.72(X-0.77)}]$	0.681	1.12<	-
	Ig ^f		$Y=2.16+1.62[1-e^{-5.66(X-0.77)}]$	0.584	1.12<	-
	Abdominal fat		$Y=3.01-1.30[1-e^{-6.50(X-0.77)}]$	0.730	1.12<	-
	Breast meat		$Y=35.87-11.36[1-e^{-4.47(X-0.77)}]$	0.747	1.12<	-
Quadratic	Body weight	Male	$Y=-8405.4+22642.5X-11111.5X^2$	0.960	1.02	2922.71
	Feed conversion rate		Does not fit	-	-	-
	Free lysine		Does not fit	-	-	-
	Sheep red blood cell		$Y=0.95+3.81X-0.46X^2$	0.962	1.12<	-
	Heterophil/lymphocyte		$Y=-2.78+6.34X-2.99X^2$	0.955	1.06	3037.32
	A.T ^o		$Y=-5.37+17X-5.78X^2$	0.922	1.12<	-
	Ig ^f		$Y=-12.25+28.90X-13.26X^2$	0.945	1.09	3123.29
	Abdominal fat		$Y=15.27-24.70X+11.39X^2$	0.961	1.08	3094.63
	Breast meat		$Y=254-459.9X+229.2X^2$	0.970	1	2865.54
Broken-line	Body weight	Female	$Y=2583.4-3892.3(0.93-X_{LR})$	0.997	0.93	2169.97
	Feed conversion rate		$Y=1.85+3.21(0.94-X_{LR})$	0.993	0.94	2193.30
	Free lysine		$Y=220-342.9(1.06-X_{LR})$	0.990	1.06	2473.30
	Sheep red blood cell		$Y=5.35-4.66(1.05-X_{LR})$	0.996	1.05	2449.96
	Heterophil/lymphocyte		$Y=0.72-0.68(1.03-X_{LR})$	0.994	1.03	2403.30
	A.T ^o		$Y=6.40-6.3(1.04-X_{LR})$	0.997	1.04	2426.63
	Ig ^f		$Y=3.7-2.45(0.96-X_{LR})$	0.996	0.96	2239.97
	Abdominal fat		$Y=2.09+3.73(0.96-X_{LR})$	0.996	0.96	2239.97
	Breast meat		$Y=24.34+55.04(1.10-X_{LR})$	0.994	1.10	2566.63
Exponential	Body weight	Female	$Y=1894.1+700.6[1-e^{-12.5(X-0.77)}]$	0.586	1.009	2354.30
	Feed conversion rate		$Y=2.34-0.45[1-e^{-12(X-0.77)}]$	0.645	1.02	2379.97
	Free lysine		Does not fit	-	-	-
	Sheep red blood cell		$Y=3.85+5.02[1-e^{-1.39(X-0.77)}]$	0.656	1.12<	-
	Heterophil/lymphocyte		Does not fit	-	-	-
	A.T ^o		$Y=4.42+2.66[1-e^{-5.34(X-0.77)}]$	0.70	1.12<	-
	Ig ^f		Does not fit	-	-	-
	Abdominal fat		Does not fit	-	-	-
	Breast meat		Does not fit	-	-	-
Quadratic	Body weight	Female	$Y=-10203+25369.7X-12537.9X^2$	0.961	1.01	2356.63
	Feed conversion rate		$Y=10.97-18.23X+9.11X^2$	0.965	1	2333.30
	Free lysine		Does not fit	-	-	-
	Sheep red blood cell		$Y=-4.31+14.07X-4.54X^2$	0.940	1.12<	-
	Heterophil/lymphocyte		$Y=-3.82+9.02X-4.43X^2$	0.894	1.02	2379.97
	A.T ^o		$Y=-14.95+38.21X-16.91X^2$	0.931	1.129	2636.63
	Ig ^f		$Y=0.95+2.49X-0.10X^2$	0.940	1.12<	1134.98
	Abdominal fat		$Y=6.87-9.77X+5.03X^2$	0.970	0.97	2263.30
	Breast meat		$Y=350.2-663.5X+335.9X^2$	0.964	0.99	2309.97

^oAntibody titration against of Newcastle; ^fimmunoglobulin.

yield differed greatly from that predicted from the feed efficiency data (Figure 1). Broken-line analysis suggests that the digestible Lys requirement for optimum body abdominal fat is 0.98 and 0.96% for male and female, respectively (Table 2). There was a great difference according to sex in the digestible Lys requirement estimates based on breast meat yield (Figure 1). In order to achieve 95% of the asymptote in breast meat weight in male chicks, digestible Lys need was higher than experimental digestible Lys levels. For optimized BW gain, feed conversion ratio and breast meat yield, male broilers had approximately 2.66, 2.81 and 3.84 g of digestible Lys intake per day, respectively. The amounts of digestible Lys intake necessary to optimize growth performance and breast meat yield of female broilers were 2.19 and 2.57 g per bird per day, respectively. Urdaneta-Rincon *et al.* (2005) reported that both protein synthesis and breakdown increased at levels of dietary Lys and CP above those required for maximum growth.

The results of Nasr and Kheiri (2011) suggested that additional lysine at a level of 120% of NRC in starter and grower diets optimized body weight gain, carcass and breast percentage in Arian broiler, whereas reductions in lysine level reduced growth and live weight (Nasr and Kheiri, 2011; Kerd *et al.*, 1998). Breast muscle (BM) constitutes the greatest portion of edible meat in broilers and, depending upon market conditions, it is generally the most valuable part of the carcass. The way in which the yield of BM changes as a bird grows is of considerable importance in deciding the optimal weight to slaughter, estimating accurate nutrient requirements, and evaluating nutritional effects (Gous *et al.*, 1999). In recent years, the importance of quality in meat production has increased. In this, some believe that feed is an important factor (Acar *et al.*, 1993; Gous *et al.*, 1999; Scheuermann *et al.*, 2003) and diet composition (Moran, 1999), especially protein and Lys levels, influences BM yield. It was believed that breast meat was

affected by digestible Lys level in diet (Farmer, 1999), but there have not been any reports on the determined requirement of digestible Lys based on breast meat yield. Immune response, including antibody against Newcastle and SRBC, H/L and immunoglobulin (Ig) of both males and females, followed a significant quadratic and broken-line response to increasing dietary levels of digestible Lys (Table 2).

Males had a higher digestible Lys requirement estimate for maximum antibody titration against Newcastle than experimental digestible Lys levels. However, based on antibody against SRBC, the estimates were similar for both sexes. In females, the estimated digestible Lys requirement by quadratic model based on immune response was higher than that based on breast meat yield (Table 4).

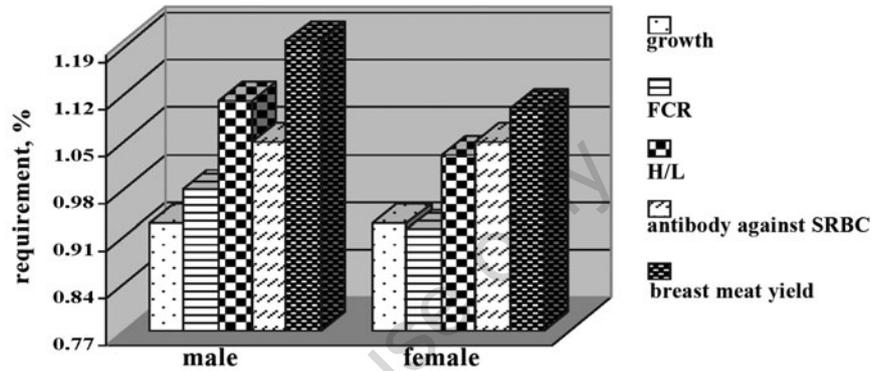


Figure 1. Comparison of digestible lysine requirements based on performance, breast meat yield and immune response.

Table 3. Effects (interaction sex × lysine) of digestible lysine levels on carcass parameters of male and female broilers.

Sex	Digestible lysine, %	Breast weight, %	Abdominal fat, g/kg BW
Male	0.77	23.66±1.54 ^d	2.51±0.54 ^{bc}
	0.84	24.03±1.72 ^d	2.35±0.47 ^{abc}
	0.91	25.78±1.17 ^d	2.00±0.27 ^{ab}
	0.98	29.36±2.75 ^c	1.80±0.25 ^a
	1.05	36.37±3.49 ^a	1.76±0.26 ^a
	1.12	33.49±2.68 ^{ab}	1.81±0.26 ^a
Female	0.77	23.32±1.26 ^d	2.82±0.56 ^c
	0.84	24.10±2.63 ^d	2.54±0.49 ^{bc}
	0.91	24.89±1.15 ^d	2.30±0.26 ^{abc}
	0.98	25.33±3.38 ^d	2.01±0.21 ^{ab}
	1.05	30.50±2.28 ^{bc}	2.11±0.22 ^{ab}
	1.12	25.17±3.02 ^d	2.16±0.06 ^{ab}

BW, body weight; ^{a-d} means with different superscripts within same row are significantly different.

Table 4. Comparison of digestible lysine (in percentage) requirement estimate base on performance, blood parameters and immune response.

Sex	Performance		Immune response			Blood parameters	
	BW	FCR	H/L	Antibody against SRBC	Antibody against Newcastle	Total Ig	Free lysine of plasma
Male	0.93	0.98	1.11	1.05	1.15	1.08	1.15
Female	0.93	0.92	1.03	1.05	1.04	0.96	1.06

BW, body weight; FCR, feed conversion rate; SRBC, sheep red blood cell; H/L, heterophil/lymphocyte.

The plasma Lys concentration data could not be fitted either to a quadratic or exponential response curve. Therefore, an estimation of the digestible Lys requirement for plasma Lys concentration was obtained only by broken-line; although, based on this parameter, digestible Lys requirement estimates of males were slightly higher than that of experimental digestible Lys levels. Regardless of sex, the digestible Lys requirement based on plasma Lys concentration was consistently higher than that based on feed conversion ratio (FCR). Males had higher feed intake, BW, concentration plasma of creatinin and urea (Tables 5 and 6), and breast meat yield than females. From the results of this experiment, there appeared to be more effect of sex on the digestible Lys requirement during this period. Additions of Lys improved plasma Lys concentration and other blood parameters (Table 6). Likewise, total protein accretion is correlated with breast muscle yield. Optimizing the AA supply in diet formulation is one of the important tools to restrict wide range in N excretion of animals (Firman, 2001; Dozier *et al.*, 2008b;

Donsbough *et al.*, 2010). Other serum chemical parameters which are indicative of the protein synthesis capacity of the liver are total protein and the albumin. The low correlation between urea concentration and predicted protein depo-

sition implied that the former was not a useful physiological indicator of the latter (Cameron *et al.*, 2003). They also improved immune response (antibody titration against Newcastle, SRBC, H/L and total Ig) and hemoglobin in both

Table 5. The effects (interaction sex × lysine) of digestible lysine levels on growth performance of male and female broilers.

Sex	Digestible lysine, %	BWG, g	FI, g	FCR
Male	0.77	2000.75±53.32 ^e	3721.40±669.75 ^{ab}	1.86±0.27 ^{abc}
	0.84	2250.03±44.06 ^c	3982.55±655.56 ^{ab}	1.77±0.23 ^{abc}
	0.91	2499.00±41.30 ^b	4148.34±636.15 ^a	1.66±0.22 ^{ab}
	0.98	2600.03±47.95 ^a	4004.00±801.64 ^{ab}	1.54±0.25 ^{ab}
	1.05	2650.00±45.83 ^a	3895.50±788.79 ^{ab}	1.47±0.27 ^a
	1.12	2499.93±17.81 ^b	4074.89±731.39 ^a	1.63±0.25 ^{ab}
Female	0.77	1450.10±48.66 ^g	2972.71±512.05 ^b	2.05±0.26 ^c
	0.84	1700.02±71.47 ^f	3213.04±427.95 ^{ab}	1.89±0.19 ^{bc}
	0.91	1995.02±34.93 ^e	3192.03±566.94 ^{ab}	1.60±0.22 ^{ab}
	0.98	2150.00±40.01 ^d	3332.50±615.57 ^{ab}	1.55±0.24 ^{ab}
	1.05	2100.00±45.85 ^d	3399.00±696.27 ^{ab}	1.59±0.30 ^{ab}
	1.12	2000.05±18.97 ^e	3240.08±633.96 ^{ab}	1.62±0.26 ^{ab}

BW, body weight; FI, feed intake; FCR, feed conversion rate; ^{a-d}means with different superscripts within same row are significantly different.

Table 6. Effects (interaction sex × lysine) of digestible lysine levels on blood parameters of male and female broilers.

Sex	Digestible lysine, %	Total protein, mg/dL	Albumin, mg/dL	Free lysine of plasma, μ mol/mL	Urea nitrogen, mg/dL	Uric acid, mg/dL	Creatinin, mg/dL
Male	0.77	3.39±0.42 ^d	1.20±0.43 ^{ab}	130±12.00 ^a	4.50±0.49 ^a	8.90±0.77 ^f	0.54±0.07 ^{ab}
	0.84	3.90±0.46 ^{cd}	1.30±0.55 ^{ab}	150±9.76 ^{ab}	4.08±0.52 ^{ab}	9.70±0.62 ^{def}	0.50±0.03 ^{abc}
	0.91	4.30±0.54 ^c	1.31±0.52 ^{ab}	140±13.64 ^{ab}	3.60±0.67 ^{bc}	10.45±0.68 ^{bcd}	0.44±0.08 ^{bcd}
	0.98	4.50±0.52 ^{bc}	1.41±0.45 ^{ab}	160±19.11 ^b	2.43±0.57 ^d	11.52±0.91 ^{ab}	0.58±0.09 ^a
	1.05	5.40±0.51 ^a	1.80±0.52 ^b	220±8.79 ^{cd}	2.70±0.25 ^d	10.3±0.50 ^{cde}	0.40±0.06 ^{cd}
	1.12	5.50±0.50 ^a	1.80±0.39 ^b	240±18.26 ^d	3.40±0.26 ^c	9.50±0.55 ^{ef}	0.43±0.06 ^{cd}
Female	0.77	4.00±0.50 ^{cd}	1.00±0.44 ^a	130±14.72 ^a	2.49±0.41 ^d	10.00±0.63 ^{def}	0.47±0.06 ^{bc}
	0.84	4.30±0.49 ^c	1.10±0.36 ^{ab}	140±11.11 ^{ab}	2.19±0.44 ^d	11.40±0.66 ^{abc}	0.45±0.04 ^{bcd}
	0.91	4.20±0.43 ^c	1.30±0.49 ^{ab}	150±17.44 ^{ab}	1.40±0.28 ^{ef}	12.30±0.69 ^a	0.50±0.06 ^{abc}
	0.98	5.11±0.66 ^{ab}	1.60±0.49 ^{ab}	200±14.51 ^c	0.94±0.29 ^f	11.20±0.60 ^{abc}	0.35±0.06 ^d
	1.05	5.20±0.46 ^{ab}	1.50±0.42 ^{ab}	220±12.36 ^{cd}	0.90±0.10 ^f	10.70±0.44 ^{bcd}	0.35±0.06 ^d
	1.12	5.30±0.45 ^a	1.60±0.38 ^{ab}	220±11.55 ^{cd}	1.60±0.14 ^e	10.00±0.48 ^{def}	0.45±0.06 ^{bcd}

^{a-f}Means with different superscripts within same row are significantly different.

Table 7. Effects (interaction sex × lysine) of digestible lysine levels on blood parameters and immune response of male and female broilers.

Sex	Digestible lysine, %	SRBC, Log ₂	Total IG, mg/dL	H/L	Hematocrit, %	Hemoglobin, g/dL
Male	0.77	4.52±0.48 ^c	2.19±0.01 ^c	0.47±0.14 ^c	31.75±4.22 ^a	10.27±0.7 ^{3f}
	0.84	4.55±0.49 ^c	2.60±0.09 ^{bc}	0.49±0.10 ^c	31.83±2.79 ^a	10.73±0.69 ^{ef}
	0.91	4.59±0.44 ^c	2.99±0.51 ^{ab}	0.54±0.15 ^{bc}	32.29±3.05 ^a	11.47±0.74 ^{cde}
	0.98	4.68±0.47 ^{bc}	3.09±0.69 ^{ab}	0.52±0.11 ^{bc}	32.67±2.09 ^a	12.22±0.66 ^{bcd}
	1.05	5.58±0.35 ^a	3.60±0.60 ^a	0.60±0.12 ^{abc}	33.51±2.10 ^a	13.00±0.57 ^{ab}
	1.12	5.61±0.39 ^a	3.70±0.74 ^a	0.61±0.09 ^{abc}	33.60±2.67 ^a	13.43±0.53 ^a
Female	0.77	4.24±0.50 ^c	3.00±0.07 ^{ab}	0.55±0.10 ^{bc}	30.08±3.14 ^a	10.14±0.78 ^f
	0.84	4.27±0.37 ^c	3.20±0.13 ^{ab}	0.61±0.15 ^{abc}	30.27±2.60 ^a	10.50±0.63 ^{ef}
	0.91	4.32±0.60 ^c	2.89±0.21 ^{abc}	0.62±0.11 ^{abc}	30.54±3.22 ^a	11.34±0.58 ^{de}
	0.98	5.31±0.44 ^{ab}	3.51±0.18 ^a	0.71±0.14 ^{ab}	31.14±1.55 ^a	12.38±0.74 ^{bc}
	1.05	5.35±0.35 ^{ab}	3.70±0.47 ^a	0.75±0.10 ^a	31.56±2.00 ^a	12.45±0.46 ^{bc}
	1.12	5.35±0.35 ^{ab}	3.70±0.50 ^a	0.70±0.11 ^{ab}	31.22±1.66 ^a	12.25±0.41 ^{bcd}

SRBC, sheep red blood cell; IG, immunoglobulin; H/L, heterophil/lymphocyte; ^{a-f}means with different superscripts within same row are significantly different.

sexes (Table 7). Determination of serum titers to NDV after regular vaccination is a method which is often used to evaluate immunomodulating effects. There was no significant change in hemotocrit and feed intake according to differing levels of digestible Lys (Tables 5 and 7). The H:L ratio, as an indicator of stress, was found to be highly heritable and under the influence of few genes (AL-Murrani *et al.*, 2002). An inadequate supply of Lys would reduce antibody response and cell-mediated immunity in chickens (Geraert and Mercier, 2010).

Overall, these results indicate that digestible Lys levels required for males were higher for breast meat yield than growth performance and immune response variables (Figure 1).

Serving as a precursor for protein synthesis, the most significant functions of Lys appear to be as a substrate for the synthesis of nitric oxide, polyamines and various hormones, all of which are capable of modulating immune responses (Wu and Morris, 1998). Kidd *et al.* (2001) reported that other than lymphoid organ weights, antibody responses were influenced by dietary Lys and Arginine varying around the NRC (1994) recommended level in commercial broilers. Dietary manipulations may also exert carry-over effects on immune responses in birds.

The higher levels of Lys may be required for the synthesis of Ig antibodies or perhaps for thymus derived T-cell helper function (Tsiagbe *et al.*, 1987). The antibody production against SRBC inoculation was increased with the level of digestive Lys in the present study. Similarly, Takahashi *et al.* (1997) reported significant improvement in the magnitude of antibody production to SRBC antigen. The dose-related antibody titers to SRBC inoculation in broilers, given different levels of Lys and methionine, were also reported by Dunnington *et al.* (1994).

In general the bursa (source of B lymphocytes that produce antibody) responds well to Lys deficiency by increasing its ability to take up Lys from the blood (relative to the abilities of other tissues, such as muscle) and maintains normal cell numbers. In contrast, the thymus (source of T lymphocytes that regulate immune function and kill virus-infected cells) responds poorly, does not augment its ability to compete for Lys, and the number of cells in the thymus decreases. This suggests that animals preserve the antibody-preserving arm of the immune system during malnutrition while the regulatory, T-lymphocyte arm of the immune response is diminished (Klasing, 2007). The requirements of the immune system have been met using a the direct approach by sum-

ming up the components of the immune system (cells and accessory proteins) and estimating the amounts of nutrients in them relative to the rest of the body (Klasing, 1998, 2003; Barnes *et al.*, 2002). Klasing (2007) estimated that rates of AA incorporation into leukocytes, Igs and accessory proteins indicate that the resting immune system utilizes only about 1.2% of the Lys intake in a healthy growing broiler chick. However, during a robust immune response, production of protective accessory proteins by the liver increases (hepatic acute phase response). These anabolic processes increase nutrient use by the immune system by almost 6-fold in the case of Lys (from 1.2 to 6.7% of Lys intake). For nutrients for which the immune system is most vulnerable due to a low priority for acquisition, requirements set based on maximal weight gain or FCR are likely to be inadequate for optimal disease resistance. Low immune response is possibly due to reduced protein availability for liver protein synthesis associated with immune response or antibody production (Murwani, 2008).

The results of previous experiments by other researchers indicated that the digestible Lys requirements of male broilers with purified diet only achieve maximum body weight gain and feed conversion ratio in the starter period (Zaghari *et al.*, 2002). Nevertheless accuracy of estimates is critical for applying data in practice to broiler diet formulation. Therefore, usual feed ingredient was used for practical evaluation of the determined digestible Lys requirements of male and female broilers by comparing the performance of chicks, immune response and breast meat yield.

NRC (1994) recommendations are based on experiments (total requirement) that were, in part, conducted more than 20 years ago, and it is questionable whether they are still applicable to modern breeds. Changes in the rate and composition of growth, namely fat and protein concentration in gained body weight, affect feed conversion ratio and, consequently, the required Lys concentration in the diet. Based on the breast meat yield for the finisher period, we recommend a dietary digestible Lys concentration of 1.10%, or 2.57 g/day and more than 1.12% for female and male, respectively. A comparison with the current NRC (1994) recommendations indicates that the recommendations for the other AAs should be urgently revised in order to adjust and change to digestible requirement for modern genotypes.

In general, the requirement of Lys is higher for increased immunity than for growth. The competition for limited resources may con-

tribute to a negative relationship between growth and immunity. Similarly, several authors (Liu *et al.*, 1995; Parmentier *et al.*, 1996) found that body weight and antibody titers are negatively correlated. Also, more immune competent birds have poor nutrient utilization ability. The results from the present study indicate that the Lys requirement for maximum antibody response was greater than for maximum growth for broilers (Klasing, 2007).

Results showing significant differences between sexes in all traits such as immune parameters may result from differences in sex steroid hormones. This hypothesis is supported by the findings of Daynes *et al.* (1990), who reported that dehydroepiandrosterone (a weak androgen) is an important enhancer of interleukin 2 (IL2) by helper T cells. IL2 is the most powerful growth factor as well as activating factor for lymphocytes. It promotes T-cell proliferation and activates B and T cells (Khajavi *et al.*, 2003).

Conclusions

Based on the results from this study, high-yield male broilers should be fed a minimum of digestible Lys 3.18 g/day (8.5 g/kg) from 35 to 49 days of age. In conclusion, supplementing L-Lys to meet the NRC (1994) recommended requirement for growth selectively decreased serum antibody and Ig levels. This effect probably increased mortality. However, BW, feed conversion ratio, plasma Lys concentration and other blood parameters, such as albumin, total protein, creatinin, uric acid, urea nitrogen, and antibody responses to SRBC were improved by adding digestible Lys level. The underlying mechanisms and the practical implication of these findings in disease resistance merit further studies, especially involving cytokines. Economically, the most important criterion is the breast muscle yield which clearly responded to Lys in our study. A 1% improvement in breast meat yield occurred among the treatments. This signifies an increase in revenue for a broiler company.

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