

## Estimation of Standardized Ileal Digestible Lysine Requirement of Starting Broiler Chicks Fed Soybean-and Cottonseed Meal-Based Diets

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**Abstract:** The research was conducted to determine the Standardized Ileal Digestible Lysine (SID Lys) requirement of male broiler chicks from 0-14 days of age and to study the carry-over effects of early Lys deficiency in growing chickens. In the starter, graded levels of SID Lys in the experimental diets were achieved by addition of Lysine-HCl at the expense of cornstarch obtaining dietary SID Lys ranging from 0.88-1.28% of diet. Linear regression analysis was significant ( $p < 0.05$ ) for Body Weight Gain (BWG), Feed Intake (FI), Breast Meat (BM) and Thigh Yield (THG) to dietary Lys. Both of Linear Broken-Line (LBL) and Quadratic Broken-Line (QBL) models were fitted to determine SID Lys requirement. Regarding response criteria and regression models, SID Lys requirements of starting broiler chicks were from 1.06-1.29% of diet. Assuming 83% lysine digestibility of basal diet, the requirements were 1.28-1.55% of diet on total amino acids basis which were much higher than NRC recommendations. From 14-28 days of age, chickens were fed common grower diet according to NRC. Linear regression analysis was significant for BWG, Feed:Gain (FG), BM and TGH yield ( $p < 0.05$ ). Early lysine deficiency significantly reduced BWG, feed efficiency, BM and TGH yield ( $p < 0.05$ ). This study clearly showed that the very lysine-deficient fed chickens could not mount a compensatory growth.

**Key words:** Lysine, broiler chicks, modeling, compensatory growth, lysine-deficient, India

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### INTRODUCTION

Although, all Amino Acids (AA) have regulating effects on growth and protein metabolism (Tesseraud *et al.*, 2008), it has been documented that Lys effects on protein accretion is critical especially on breast meat yield (Tesseraud *et al.*, 1996a, b, 2008) and Lys deficiency significantly increased protein degradation in pectoralis major muscle due to heightening the expression of m-calpain and cathepsin B in breast muscle (Tesseraud *et al.*, 2008). Leclercq (1998) showed that higher levels of Lys in the diet would affect body composition by increasing breast meat yield and decreasing abdominal fat percentage. In comparison, other AAs such as Threonine (Thr) and Valine (Val) had no such pronounced effects (Leclercq, 1998). Dietary Lys is the most critical amino acid to breast muscle formation early in development (Tesseraud *et al.*, 1996b, 2008). Labadan *et al.* (2001) clearly showed that Lys requirement for breast meat yield is higher than for weight gain and

feed efficiency. Breast muscle contains a high concentration of Lys so it would be expected that dietary Lys concentrations can have a large influence on breast meat development (Kerr *et al.*, 1999). In NRC (1994) starter period for broiler chickens is defined as a first 3 week of age but in practical situation, starter period for the modern broiler strains with high growth rate has been considered as first 2 week of age. The aims of this study are determination of SID Lys requirements of male broiler chicks from 0-14 days of age and evaluation of the carry-over effects of early Lys deficiency in the grower period.

### MATERIALS AND METHODS

**Animal and diets:** Regarding amino acid requirements of starting broiler chicks, most of the research addressing the requirements during Days 7-21 of age and there is little information on the first 2 week of age. A corn-soybean-cottonseed meal basal diet was formulated to meet or exceed the NRC (1994) requirements for all nutrients

except for Lys. Digestibility of amino acids in feedstuffs were obtained from AminoDat (Version 3.0, Degussa AG, Germany) and calculated metabolizable energy, crude protein, calcium and available phosphorus were taken from NRC (1994). Three hundred sixty 1 day old male Ross 308 chicks were used in a complete randomized design consisted of six treatments with four replications each having 15 birds. The experimental diets were isocaloric and isonitrogenous in which CSM substituted for SBM at 50% by weight (Table 1) and then graded levels of digestible Lys were obtained with addition of Lysine-HCl at expense of cornstarch (Table 2). The birds were offered feed and water

Table 1: Lysine content of experimental diets

Lysine content	Added lysine-HCl to the basal diet					
	0	0.1	0.2	0.3	0.4	0.5
SID lysine of the diet	0.88	0.96	1.04	1.12	1.20	1.28
Lysine:CP ratio	4.19	4.57	4.95	5.33	5.71	6.09
Increment: 0.08%						

Table 2: Composition of basal and grower diets

Ingredients	Basal diet (%)	Common grower (%)
Corn	56.37	58.20
Cotton seed meal	15.00	-
Soybean meal	15.00	28.71
Fish meal	5.66	5.20
Oil	4.54	5.00
DCP	-	0.94
Limestone	1.40	0.87
Corn starch	1.00	-
DL-Met	0.34	0.31
L-Thr	0.08	-
NaCl	0.10	0.10
Vit premix <sup>1</sup>	0.25	0.25
Min premix <sup>2</sup>	0.25	0.25
<b>Nutrient composition</b>		
ME (kcal kg <sup>-1</sup> )	3200.00	3200.00
CP (%)	21.00	21.00
Ca (%)	0.90	0.90
P <sub>avail</sub>	0.43	0.45
Lysine (%)	1.06	1.22
Dig Lysine (%)	0.88	1.10
Met (%)	0.69	0.66
Dig. Met (%)	0.64	0.63
Thr (%)	0.86	0.82
Dig. Thr (%)	0.70	0.69
Trp (%)	0.24	0.24
Dig. Trp (%)	0.20	0.21
Arg (%)	1.61	1.37

<sup>1</sup>Vitamin premix provides per kg of diet: vitamin A, 4,500 IU; vitamin D3, 2,250 ICU; vitamin E, 50 IU; thiamin HCl, 15 mg; riboflavin, 15 mg; nicotinic acid, 50 mg; folic acid, 6 mg; pyridoxine, 6 mg; biotin, 0.6 mg; vitamin B12, 0.02 mg; choline Cl, 2,500 mg; d-calcium pantothenate, 20 mg; menadione sodium bisulfite, 1.5 mg; butylated hydroxytoluene, 100 mg; glucose to make 12 g. <sup>2</sup>Mineral premix provides per kilogram of diet: CaCO<sub>3</sub>, 25.6 g; CaHPO<sub>4</sub>·2H<sub>2</sub>O, 5.6 g; KH<sub>2</sub>PO<sub>4</sub>, 14 g; NaCl, 5.1 g; MnSO<sub>4</sub>·H<sub>2</sub>O, 0.33 g; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.33 g; KI, 0.003 g; CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.05 g; ZnO, 0.1 g; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.0017 g; NaMoO<sub>4</sub>·2H<sub>2</sub>O, 0.0083 g; Na<sub>2</sub>SeO<sub>3</sub>, 0.0004 g

*ad libitum*. In the growing period, all birds were fed grower diet according to the NRC (1994) from 14-28 days of age and compensatory growth was measured for performance traits.

Feed intake was measured weekly and chicks were weighted at 0, 7, 14, 21 and 28 days of age. Feed:gain was determined as feed consumption divided by the live weight adjusted for mortality. Two birds of each pen were used for breast meat and thigh dissection. The breast muscle was carefully dissected and immediately weighted.

**Statistical analysis:** The calculated SID Lys values were used for statistical analysis with treatment levels ranging from 0.88-1.28% in increments of 0.08%. Five analyses were conducted: PROC REG (SAS, 2002) using linear response to evaluating potential effects of digestible lysine; PROC REG (SAS, 2002) using quadratic response to evaluating potential effects of digestible lysine (SAS, 2002); PROC NLIN (SAS, 2002) conducting linear broken-line model based on Robbins *et al.* (2006); PROC NLIN (SAS, 2002) conducting quadratic broken-line model based on Robbins *et al.* (2006) and PROC GLM (SAS, 2002) to separate differences among means using LSD option of SAS (Table 3). Digestible Lys requirements were estimated using both linear broken-line and quadratic broken-line models when a significant response occurred (p<0.05) (Fig. 1a-c). R<sup>2</sup> and sum of square residual of each model were used to compare nonlinear models (Table 4). Regression models for estimation SID Lys requirements were as follow:

$$\text{Linear Broken-Line model: } y = L+U \times (R-x) \text{ if } R < x, \text{ else } (R-x) = 0$$

$$\text{Quadratic Broken-Line model: } y = L+U \times (R-x) (R-x) \text{ if } R < x, \text{ else } (R-x) = 0$$

Table 3: Growth performance of broiler chicks fed varying levels of digestible lysine from days 0-14 of age

Dig. lysine (diet %)	BWG (g)	F:G (g/g)	FI (g)	BM yield (g)	THG yield (g)	Carcass yield (%)
0.88	231.000 <sup>b</sup>	1.4300 <sup>a</sup>	332.000 <sup>b</sup>	34.2000 <sup>c</sup>	19.4000 <sup>c</sup>	67.5000
0.96	248.000 <sup>b</sup>	1.3500 <sup>ab</sup>	335.000 <sup>b</sup>	38.7000 <sup>bc</sup>	21.5000 <sup>bc</sup>	69.0000
1.04	273.000 <sup>a</sup>	1.3600 <sup>ab</sup>	371.000 <sup>a</sup>	45.0000 <sup>ab</sup>	23.4000 <sup>ab</sup>	69.4000
1.12	277.000 <sup>a</sup>	1.3500 <sup>ab</sup>	374.000 <sup>a</sup>	44.7000 <sup>ab</sup>	23.8000 <sup>a</sup>	70.9000
1.20	271.000 <sup>a</sup>	1.3600 <sup>ab</sup>	367.000 <sup>a</sup>	47.3000 <sup>a</sup>	24.0000 <sup>a</sup>	70.0000
1.28	282.000 <sup>a</sup>	1.2600 <sup>b</sup>	357.000 <sup>a</sup>	48.9000 <sup>a</sup>	23.7000 <sup>ab</sup>	69.0000
SEM	12.8000	0.0700	13.5000	4.9000	1.5300	3.2500
<b>p&gt;F</b>						
Lysine	0.0001	0.0922	0.0007	0.0040	0.0023	0.7537
Linear	<0.0001	0.1818	<0.0001	0.0006	0.0001	0.1675
Quadratic	0.0106	0.2417	0.0516	0.2697	0.0932	0.5901

<sup>a-c</sup>Values within rows without a common superscript are significantly different (p≤0.05)

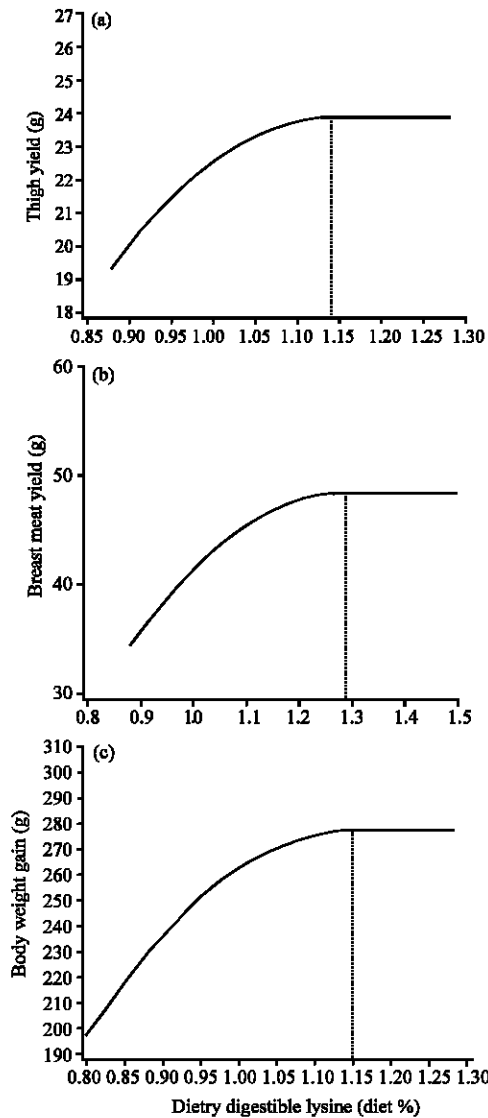


Fig. 1 (a-c): Estimation lysine requirement for breast meat yield using Quadratic broken-line model

Table 4: Lysine requirements (% of diet) of starting broiler chicks using different models and response criteria

Analysis	BWG		BM yield		THG yield	
	LBL <sup>1</sup>	QBL <sup>1</sup>	LBL	QBL	LBL	QBL
Lysine req.	1.06	1.15	1.15	1.29	1.06	1.14
R <sup>2</sup>	69.8	68.5	55.2	57.0	61.4	61.1
SE <sup>2</sup>	0.028	0.073	0.062	0.160	0.034	0.083
SSR <sup>3</sup>	3257.272	3402.807	474.050	455.030	42.480	42.842
95% CI <sup>4</sup>	1.00-1.11	1.00-1.29	1.04-1.28	0.98-1.60	0.99-1.23	0.98-1.3
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

<sup>1</sup>Linear Broken-Line (LBL) and Quadratic Broken-Line (QBL) are  $y = L + U(R - x)$  and  $y = L + U(R - x)^2$ , respectively if  $R < x$ . L = Ordinate value; U = Random component of the slope; R = Abscissa of the break point; <sup>2</sup>SE = Standard Error; <sup>3</sup>SSR = Sum of Squared Residual; <sup>4</sup>CI = Confidence Interval

## RESULTS AND DISCUSSION

In a dose-response experiment, SID Lys requirements were obtained at 1.29 and 1.15% for breast meat yield using QBL and LBL models respectively (Table 4). Sum of Squared Residuals (SSR) and R<sup>2</sup> were used to compare two models (Hernandez-Llamas, 2009; Pesti *et al.*, 2009). Sum of squared residuals of linear and quadratic broken-line models for BM yield were 455.030 and 474.050, respectively. It represents clearly that QBL model fitted data better than LBL model. Also, R<sup>2</sup> of the QBL model for BM yield is higher than for LBL model regression. Quadratic broken-line model resulted in a wide confidence interval and in comparison with LBL model, QBL model covered higher range of the SID Lys requirements (Table 4). Depending on response criterion, R<sup>2</sup> and SSR for each model is variable. For example, regarding BWG, the value of SSR of LBL model is lower than value for QBL model, showing better fitting data with LBL model. Pesti *et al.* (2009) stated that model efficacy would be affected by response criterion. Using QBL model, SID Lys requirements for BM, BWG and THG yield were 1.29, 1.15 and 1.14%, respectively. Choosing different responses resulted in a variety of requirement values.

The extent of deficiency significantly affected the performance of broiler chicks during 14-28 days of age (Table 5). Significant effects of linear regression model revealed that the low lysine-basal diet was deficient from 0-14 days of age. Lysine deficiency significantly reduced the performance in starter and grower periods ( $p < 0.05$ ). The compensatory response was different among lowest and highest Lys-diets. Chickens fed 0.88% digestible lysine could not compensate growth in the following growth period at all. This effect was pronounced on BWG, FG and BM yield (Table 4). Linear regression analysis revealed that increasing dietary SID Lys in starter period resulted in better response in terms of BWG, FG, TGH and BM yield ( $p < 0.05$ ) during the growth period.

Table 5: Growth performance of broiler chicks fed varying levels of digestible lysine from days 14-28 of age

Dig. lysine (diet %)	BWG (g)	F:G (g/g)	FI (g)	BM yield (g)	THG yield (g)	Carcass yield (%)
0.88	521.000 <sup>c</sup>	1.4300 <sup>a</sup>	1134.000 <sup>ab</sup>	180.000 <sup>b</sup>	78.0000 <sup>b</sup>	73.2000
0.96	535.000 <sup>bc</sup>	1.3400 <sup>b</sup>	1105.000 <sup>b</sup>	189.000 <sup>b</sup>	83.000 <sup>b</sup>	75.0000
1.04	564.000 <sup>ab</sup>	1.3400 <sup>b</sup>	1182.000 <sup>a</sup>	209.000 <sup>a</sup>	84.0000 <sup>a</sup>	75.0000
1.12	585.000 <sup>a</sup>	1.3100 <sup>b</sup>	1179.000 <sup>ab</sup>	197.000 <sup>ab</sup>	86.5000 <sup>a</sup>	74.6000
1.20	557.000 <sup>ac</sup>	1.3000 <sup>b</sup>	1128.000 <sup>b</sup>	199.000 <sup>ab</sup>	85.0000 <sup>a</sup>	73.9000
1.28	572.000 <sup>ab</sup>	1.2600 <sup>b</sup>	1137.000 <sup>ab</sup>	215.000 <sup>a</sup>	85.0000 <sup>a</sup>	74.8000
SEM	25.0000	0.0500	51.3000	13.6000	3.7200	1.3500
<b>p&gt;F</b>						
Lysine	0.0187	0.0101	0.2672	0.0207	0.0540	0.4111
Linear	0.0058	0.0030	0.4492	0.0452	0.0073	0.6497
Quadratic	0.0677	0.3098	0.2073	0.0894	0.0910	0.0486

<sup>ab</sup>Values within rows without a common superscript are significantly different ( $p < 0.05$ )

To maximizing profit feed formulation, the importance of choosing the correct model which relates animal response to feed composition has been documented (Lerman and Bie, 1975). The exact model will be functions of feed quality, feed intake, genetics and environmental conditions (Pesti *et al.*, 2009). Among the nonlinear models, LBL has been used extensively for determination of amino acid requirements in the most species (Hernandez-Llamas, 2009). The most advantage of LBL model is the clearly definition of requirement for the performance variables (Pesti *et al.*, 2009). Since, it assumes an unrealistic, abrupt shift at the plateau when a smooth transition actually occurs. So, the requirement would be underestimated. Alternatively, Robbins *et al.* (2006) used curve-and-plateau approach to estimate amino acid requirement. They applied quadratic equation in combination with straight-line equation at the plateau. Quadratic broken-line regression resolve the problem of underestimation resulted from LBL regression to some extent and ascending quadratic segment models diminishing marginal productivity until the level of the requirement is reached (Pesti *et al.*, 2009). The results of this experiment are in agreement with Pesti *et al.* (2009) and Hernandez-Llamas (2009). To eliminating this underestimation, Booth modified LBL model so as to simulate a smooth curvilinear transition from ascending portion to the plateau and a breakpoint in the transition curve used for estimating requirement. They showed that diphasic model represents an improvement over the LBL model. As shown in Table 4, estimated SID Lys requirement using QBL model is 11% higher than value obtained from LBL model. Pesti *et al.* (2009) found 15% higher value using QBL model. Different dietary crude protein of basal diets and protein contributing ingredients of the experimental diets and also response criterion may be attributed to the difference between two experiments (Kerr *et al.*, 1999; Leclercq, 1998; Pesti *et al.*, 2009).

The SID Lys requirement for BM yield was higher than for BWG and THG yield (Table 4). Pesti and Miller (1997) suggested that the optimal levels of amino acids were not the same for the maximization of weight gain, feed conversion or breast muscle development. On other hand, the effect of dietary Lys on body composition has been demonstrated by several authors (Kidd *et al.*, 1998; Kerr *et al.*, 1999; Halevy *et al.*, 2000; Moran and Bilgili, 1990; Leclercq, 1998). This effect is pronounced on the BM especially. Kerr *et al.* (1999) suggested that feeding dietary lysine above required levels for BWG improves BM deposition. Additionally, Acar *et al.* (1993) demonstrated that *Pectoralis major* is more sensitive to Lys intake other than muscles. This response also has been documented in the current study. Regardless of

used model, lysine requirement for thigh yield is lower than for breast meat muscle synthesis (Table 4). It has been postulated that higher lysine requirement for BM yield and a large influence of dietary lysine on *Pectoralis major* may be related to the high lysine content of that muscle (Kerr *et al.*, 1999; Acar *et al.*, 1993). Labadan *et al.* (2001) estimated lysine requirement for BM yield at 1.32% of total amino acid in the diet which is lower than value estimated from this research. This difference may be related to the protein quality of the basal diet (Kidd and Fancher, 2001). Adverse effects of anti-nutritional factors in CSM and lower Lys digestibility of CSM would increase the needed Lys for normal growth and performance. Researchers used CSM in the basal diet and higher estimation of Lys requirement may be attributed to this event.

Halevy *et al.* (2000) found that reduction in muscle growth and low ability to mount a compensatory growth of chicken-fed lysine deficient diet may relate to the rapid maturation of fiber muscle in broilers and irreversible effect on fiber development. Kidd *et al.* (1998) also showed such effects of early malnutrition of lysine in broiler chicks and its detrimental influence on subsequent growth.

## CONCLUSION

Estimation the SID Lys needs of broiler chicks using QBL model released more accurate results than LBL model and underestimation resulted from LBL model would be eliminated, since quadratic ascending portion of QBL regression models diminishing marginal productivity. The results of this research clearly showed that inadequate Lys nutrition of starting broiler chicks lead to decrease performance in the subsequent growth period and chickens fed very low-Lys diets could not mount a compensatory response in terms of BWG, FG and BM yield.

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