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Leucine Requirement of Female Cobb Broilers from 8 to 14 Days of Age

Research Article

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ABSTRACT

The objective of this study was to estimate the optimum requirement of the digestible leucine in ratio to lysine in female Cobb 500 broiler chicks from 8-14 days of age. In this study, 540 female broiler chickens (Cobb 500), were allocated to the experimental units in a completely randomized design with 6 treatments of 6 replicate pens of 15 birds in each. A basal diet (2890 kcal apparent metabolizable energy corrected for nitrogen (AMEn)/kg; 19% crude protein) was formulated to provide all of the nutrients with the exception of leucine. Lysine was sub-limiting and provided at 90% of recommended level of digestible lysine. Crystalline L-glutamic acid in basal diet was replaced by crystalline L-leucine to provide experimental diets containing 0.93, 0.98, 1.03, 1.08, 1.13, and 1.18 digestible leucine in ratio to lysine. Performance parameters were evaluated during a 7-day period (d 8-14) of *ad libitum* feeding. The body weight, weight gain and feed intake were significantly influenced by dietary treatments and showed quadratic effect with increasing digestible leucine in ratio to lysine. The groups fed 1.03 and 1.13 digestible leucine in ratio to lysine diet had statistically ($P<0.05$) greater feed intake than the group fed 0.93 digestible leucine in ratio to lysine diet. The birds fed 1.13 digestible leucine in ratio to lysine diet had higher body weight and weight gain ($P<0.05$) than the birds fed 0.93 digestible leucine in ratio to lysine diet. Using the curvilinear-plateau model and the quadratic regression analyses, the requirement of digestible leucine to lysine ratio for maximum feed intake were estimated as 1.05 and 1.04, respectively. The curvilinear-plateau model and the quadratic regression analyses estimated the requirement of digestible leucine to lysine ratio for maximum weight gain to be 1.07 and 1.06, respectively. However, the curvilinear-plateau model estimated the requirement of digestible leucine to lysine ratio for the minimum feed conversion ratio at 1.11. Based on the analysis of obtained data, female Cobb 500 broilers need lower ratio of leucine to optimize feed intake and weight gain than feed conversion ratio. In conclusion, the requirement of Cobb 500 female broilers from 8 to 14 d of age to support performance parameters was 1.08 digestible leucine in ratio to lysine.

KEY WORDS amino acid, dose-response, ideal ratio, leucine.

INTRODUCTION

Protein gain as a percentage of total body weight gain in young chicks is greater than older birds. So, the requirements of all amino acids, especially the amino acids that have stimulating effect on protein gain are interested. Leu as a functional amino acid has major effect on whole body growth. The amino acid stimulates muscle protein synthesis and acts as an inhibitor of protein degradation in skeletal muscle (Tischler *et al.* 1982; Nakashima *et al.* 2007). The probable stimulating mechanisms of Leu to promote translation initiation and inhibit autophagy (a key step in lysosomal proteolysis) in liver and muscle, may involve activation of the mammalian target of rapamicine (mTOR) signaling. Deng *et al.* (2014) found that the expression and phosphorylation of mTOR are more active in initial stage of broiler life, and the plasma Leu of broiler chicks on day 3 of age was significantly higher than days 7 and 14 of age. Furthermore, the requirement of valine (Val) and isoleucine (Ile), especially former one, are affected by the level of Leu due to antagonistic effect among branched chain amino acids (Ospina-Rojas *et al.* 2016). So, the amino acid in excess or in insufficient amount can negatively influence on the growth performance in broiler chicks. However, little is known about the dietary requirements of Leu in young chickens; although in diets based on corn-soy bean meal, the amino acid level is adequate to support maximum growth performance. Most of previous reports predicted amino acid need of broilers for at least two weeks. Baker (2003) and Rostagno *et al.* (2011) recommendations for digestible Leu in ratio to lysine (Leu:Lys) to maximize the performance of male broiler in starter phase (d 0-21 and 8-21, respectively) are 1.09 and 1.07, respectively. Since the duration of the breeding period for broilers has decreased, it is necessary to determine their nutrient requirements on a weekly basis. Also, the data on female broiler requirement is scarce, in addition the inherent capacity to store protein in females is different from male broilers, and may need different ratio of Leu. Therefore, the objective of the current study was to estimate the requirement of digestible Leu:Lys for female Cobb 500 broilers in second week of age (d 8 to 14).

MATERIALS AND METHODS

To estimate the requirement of digestible Leu:Lys of female broilers from 8 to 14 days of age, a dose-response trial was conducted in university of Tabriz, using 540 females broiler chickens.

General procedures

One-old day female Cobb 500 broiler chickens were feather-sexed at the hatchery, vaccinated against Newcastle disease and infectious bronchitis, and received a common corn-soy bean meal diet (23% crude protein (CP), and 3200 kcal apparent metabolizable energy corrected for nitrogen (AMEn)/kg) from days 1 to 7 of age. On d 8, the birds were individually weighted and distributed into 36 floor pens (1.4×1.4 m; 15 birds/pen; 0.13 m^2 per bird) in a manner that all the pens had similar average body weight (181±0.33 g). The chicks were fed the experimental diets from 8 to 14 day of age. The experimental diets were in mashed form and formulated on ideal protein basis, using digestible amino acid values to meet the nutrient recommendations suggested by Rostagno *et al.* (2011), with the exception of Leu and Lys levels. To obtain a good response to increasing test amino acid levels, Lys was sub-limiting and supplied at 90% of digestible Lys recommendation (1.05%).

During the experiment, the poultry unit was equipped with thermostatically controlled heating system, one feeder (10-kg capacity), and one bell drinker per pen. The bird's house temperature was set at 32 ˚C at the start (from days 0 to 3) and was gradually reduced to 28 ˚C on d 14. Feed and water were provided *ad libitum* and lighting was continuous.

Body weight (BW) and feed intake (FI) were recorded at the beginning and the end of the experimental period (8 to 14 d) after a 6-h fasting to remove any residual feed from the gastrointestinal tract. Mortality was recorded daily and corrected to its effect on FI. Feed conversion ratio (FCR) was calculated as the unit of eaten feed per unit of weight gain (WG). Protein efficiency ratio (PER), and European poultry efficiency factor (EPEF) was calculated according to the formulas:

PER (g/g)= WG (g) / protein intake (g)

EPEF= (BW (kg)×livability (%)/breeding period (day)×FCR) × 100

Treatment

To estimate the requirement of digestible Leu:Lys, 540 female broiler chickens (Cobb 500), were distributed in a completely randomized design with 6 treatments of 6 replicate pens of 15 birds in each. To create Leu deficient, a wheat-soy bean meal basal diet formulated to provide all of the nutrients with the exception of Leu and Lys. Wheat was used because of its inherent deficiency on Leu. To obtain the different digestible Leu:Lys in experimental diets, crystalline L-glutamic acid in basal diet was replaced by crystalline L-Leu, and 6 different ratios of digestible Leu (% of Lys) of 0.93, 0.98, 1.03, 1.08, 1.13, and 1.18 were formulated.

In the experiment the pens were provided with wood-shaving litter (7 cm height). Wheat and soybean meal were analyzed for their total amino acid contents (by Ajinomoto Eurolysine S. A. S. using HPLC method) and the values converted to digestible amino acid (by Rostagno's digestibility coefficient) to use as the basis of formulation. The ingredient and nutrient composition of the basal diet is presented in Table 1.

Statistical analysis

This experiment was conducted in a completely randomized design with 6 treatments and 6 replications per treatment. Pen was used as the experimental unit for analysis. The obtained data were analyzed using the generalized linear model (GLM) procedure of SAS (SAS, 2006). Significance was accepted at the P ≤ 0.05 level, and mean differences were separated using Duncan's test.

Table 1 The ingredient and nutrient composition (%, as is) of the basal diet

Ingredients	Amounts (%)	Digestible amino acid:Lys
Wheat grain	76.51	
Soy bean meal (46%)	14.3	
Dical.phos	1.56	
Calcium-carbonate	1.01	
Soy bean oil	1.0	
Common salt	0.2	
$NaHCO_3$	0.3	
K_2SO_4	0.5	
Vitamin premix[1]	0.2	
Mineral premix[2]	0.2	
Coccidiostat[3]	0.05	
L-glutamic acid (98%)[4]	1.73	
L-lysine HCL (99%)	0.65	
DL-methionine (99%)	0.34	
L-threonine (98.5%)	0.35	
L-arginine (98%)	0.44	
L-isoleucine (92%)	0.24	
L-tryptophan (98%)	0.01	
L-valine (96.5%)	0.33	
L-histidine (98%)	0.06	
Enzyme (polysaccharidase)[5]	0.02	
Calculated nutrient composition		
AMEn (kcal/kg)	2890	
Crude protein (%)	19	
Calcium (%)	0.8	
Available phosphorus (%)	0.4	
Sodium (%)	0.17	
Chlorine (%)	0.19	
Calculated total amino acid content (%)[6]		
Arginine	1.35 (1.25)[7]	1.18
Glycine + serine	1.51 (1.37)	1.30
Histidine	0.47 (0.43)	0.41
Isoleucine	0.86 (0.78)	0.74
Leucine	1.06 (0.98)	0.93
Lysine	1.16 (1.05)	1
Methionine	0.56 (0.53)	0.50
Methionine + cysteine	0.86 (0.78)	0.76
Threonine	0.88 (0.76)	0.72
Phenylalanine	0.83 (0.78)	0.74
Phenylalanine + tyrosine	1.61 (1.39)	1.32
Tyrosine	0.78 (0.61)	0.58
Tryptophan	0.22 (0.20)	0.19
Valine	1.02 (0.90)	0.85

[1] The vitamin contained per kg of diet: vitamin A (retinyl acetate): 43200 IU; vitamin K (menadione): 3.2 mg; vitamin D_3 (cholecalciferol): 12800 IU; vitamin E (DL-α-tocopheryl acetate): 28 IU; Thiamin: 1.44 mg; Riboflavin: 6.6 mg; Niacin: 8 mg; D-pantothenic acid: 24 mg; Pyridoxine: 2.4 mg; Biotin: 4 mg and Choline chloride: 800 mg.
[2] The mineral contained per kg of diet: Mn (manganese oxide): 98 mg; Zn (zinc oxide): 156 mg; Fe (ferric sulfate): 28 mg; Cu (copper sulfate): 8 mg; I (calcium iodate): 0.83 mg and Se (selenium premix): 7 mg.
[3] Salinomycine.
[4] Supplemental L-Leu was added to the test diets at the expense of L-glutamic acid to derive dietary treatments.
[5] Grindazyme contain 18000 active unit of beta-1-4 glucanase and 43000 active unit of beta-1-4 arabinogesilanase per g.
[6] The amino acid values are shown as percentage of the diets. These values were converted to digestible amino acid by Rostagno's digestibility coefficient.
[7] The values in parentheses indicate the calculated digestible amino acid content.

Digestible Leu:Lys requirements for optimum performance were estimated by curvilinear-plateau model (Robbins *et al.* 2006) and quadratic equation. In fitting the quadratic equation to the data, when a significant quadratic response was observed, 95% confidence level was applied to avoid overestimation. The equations of the regression models are as follows:

For curvilinear-plateau model:

$$Y= Y_{max} \text{ for } X \geq R;\ Y= Y_{max} + U \times (R-X)^2 \text{ for } X < R$$

For quadratic response curve:

$$Y= aX^2 + bX + c$$

Where:
X: independent variable.
Y: variable response (FI, WG or FCR).
Y_{max}: maximum response.
R: requirement.
U: slope of the function.
a, b and c: estimated parameters.

RESULTS AND DISCUSSION

As described by Soumeh *et al.* (2015a), to estimate the requirement of individually amino acid based on ideal protein (relative to Lys), Lys must be marginally sub-limiting in the experimental diets. So, to obtain good responses at the present study, Lys was supplied at 90% of recommendation, and the other amino acids except for Leu were provided to meet broiler nutritional requirements suggested by Rostagno *et al.* (2011).

Amino acid analysis of formulated diets is presented in Table 2. The analyzed data confirmed calculated values. It means that, except for test amino acid (Leu), all of the experimental diets had same amino acid content and the obtained results of the present study was influenced by dietary Leu levels.

No indication of the health problems or mortality attributed to dietary treatment was observed. Performance parameters of birds which fed with different digestible Leu:Lys were presented in Table 3. The FI, BW and WG showed a quadratic response (P<0.05) to increasing the digestible Leu:Lys; however, FCR was not affected. As expected, Leu intake was linearly increased with increasing dietary Leu ratio. The groups fed 1.03 and 1.13 digestible Leu: Lys diet had statistically (P<0.05) higher FI (319 and 320 g, respectively) than the group fed 0.93 digestible Leu:Lys diet (310 g), which were not different from the 0.98, 1.08 and 1.18 digestible Leu:Lys diets.

The WG increased in parallel with BW, and the birds fed 1.13 digestible Leu:Lys diet had higher WG (P<0.05) than the birds fed 0.93 digestible Leu:Lys diet, however, these values were not different from the other groups. The improvement of chicks WG with increasing digestible Leu:Lys, without significant FCR differences, probably raised from FI.

A diet lacking just one of the essential amino acids, restrict protein synthesis, inhibit growth and cause loss of body weight. Among the essential amino acids, the ability of Leu to stimulate protein synthesis and inhibit protein degradation is greater than the others, makes it special (Harris *et al.* 2004). So, deficiency of dietary Leu in the birds consumed 0.93 digestible Leu:Lys diet may be a cause of discrepancy between the WG of the birds with the groups fed 1.13 digestible Leu:Lys diet.

Because of stimulating effect on branched-chain α-ketoacid dehydrogenase, excess of Leu increase oxidation of other 2 branched-chain amino acids (especially Val) (Brosnan and Brosnan, 2006). However, it appears that a decline of the amino acids concentration on serum, leads to impaired growth performance (Soumeh *et al.* 2015b).

Working with pigs, Soumeh *et al.* (2015b) found that a moderate excess of Leu in growing pigs diet, has no or little effect on performance parameters; however, a greater excess of the amino acid could impair the FI and WG. May *et al.* (1991) showed the impaired performance in rats that consumed low-protein diets with excess Leu.

It seems that the high Leu level in the present study was not enough extra to offset the performance parameters. None of the PER and EPEF were affected by dietary treatment. The result of the performance data was in line with the finding of Franco *et al.* (2016), who reported that, WG and FI were quadratically (P<0.05) affected by dietary digestible Leu:Lys, but FCR was not affected. Franco *et al.* (2016) evaluated Leu ratio ranging from 0.93 to 1.21 in starter phase (d 8-17) of male broilers, and reported that the birds fed 1.14 digestible Leu:Lys had greatest FI (499.8 g), however, the group received 1.07 digestible Leu:Lys diet had greatest WG (389.3 g).

Curvilinear-plateau model and quadratic regression analyses estimate requirement of digestible Leu:Lys for maximum FI, at 1.05 ± 0.06 and 1.04, respectively (Figure 1). Digestible Leu:Lys requirements with curvilinear-plateau model and quadratic regression analyses for maximum WG were 1.07 and 1.06, respectively (Figure 2). The FCR did not show a quadratic response to increasing the dietary Leu ratio; so, only curvilinear-plateau model were used to estimate the digestible Leu:Lys for minimum FCR. The curvilinear-plateau model estimated the digestible Leu:Lys requirement for the lowest FCR at 1.11 ± 0.1 (Figure 3).

Both of WG and FI showed quadratic and curvilinear-plateau response (Table 4), and the estimated optimum digestible Leu:Lys was the average of the values estimated using each method.

The curvilinear-plateau model (1.07) and the quadratic regression (1.06) gave an estimated optimum average digestible Leu:Lys for WG of 1.07. For the FI, the estimated ratios were 1.05 and 1.04, using the mentioned models, respectively. The average digestible Leu:Lys was 1.05. According to the mentioned observations, we conclude the optimum digestible Leu:Lys for optimum WG and FI during the starter phase of Cobb 500 female broilers to be 1.06. However, the digestible Leu:Lys requirement for optimum FCR was estimated at higher ratio (1.1). So, it may be concluded that female Cobb 500 broilers need lower ratio of Leu to optimize FI and WG than FCR.

Table 2 Amino acids analysis of experimental diets[1]

Total amino acids (%)	Digestible Leu:Lys					
	0.93	0.98	1.03	1.08	1.13	1.18
Leucine	1.02	1.07	1.12	1.17	1.22	1.27
Lysine	1.17	1.18	1.17	1.18	1.16	1.17
Arginine	1.36	1.36	1.36	1.36	1.36	1.36
Histidine	0.46	0.46	0.46	0.46	0.46	0.46
Isoleucine	0.87	0.87	0.86	0.87	0.86	0.87
Valine	1.03	1.02	1.05	1.04	1.02	1.03
Methionine	0.56	0.56	0.55	0.55	0.55	0.55
Methionine + cysteine	0.85	0.85	0.85	0.85	0.85	0.85
Phenylalanine	0.79	0.79	0.79	0.78	0.78	0.78
Tryptophan	0.21	0.21	0.21	0.20	0.21	0.21
Threonine	0.89	0.87	0.89	0.89	0.86	0.87

[1] Representative samples were analyzed by Ajinomoto Eurolysine S. A. S. using HPLC method.

Table 3 The influence of the digestible Leu:Lys on growth performance of 8-14 d old female broilers[1]

Digestible Leu:Lys	Performance					EPEF	PER (g/g)
	Feed intake (g/bird)	Leu intake (mg/bird)	Weight gain (g/bird)	Body weight (g/bird)	FCR (g/g)		
0.93	310[b]	3165[f]	220[b]	401[b]	1.413	352.41	3.79
0.98	314[ab]	3362[e]	223[ab]	404[ab]	1.408	359.05	3.72
1.03	319[a]	3571[d]	227[ab]	408[ab]	1.401	357.59	3.78
1.08	317[ab]	3710[c]	228[ab]	409[ab]	1.389	365.40	3.79
1.13	320[a]	3905[b]	230[a]	411[a]	1.391	369.79	3.74
1.18	316[ab]	4017[a]	225[ab]	406[ab]	1.406	350.77	3.81
SEM	2.28	26.73	2.71	2.70	0.02	7.76	0.058
P-value							
ANOVA	0.063	0.001	0.118	0.119	0.959	0.49	0.88
Linear	0.028	0.001	0.031	0.031	0.468	0.15	0.63
Quadratic	0.034	0.05	0.036	0.036	0.479	0.14	0.62

[1] Each value represents the mean of 6 replicates with 15 birds per pen (90 birds in total).
FCR: feed conversion ratio; EPEF: European poultry efficiency factor calculated and PER: protein efficiency ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In addition to amino acid requirement, ideal protein ratio (amino acids relative to Lys) was also influenced by Lys level. NRC (1994) presented the essential amino acids requirement as percentage of diet, and when convert to ideal ratio (relative to lysine), the requirement of the amino acids may overestimate, probably because the reference amino acid, lysine, was too low relative to the other amino acids (Baker and Han, 1994). NRC (1994) and Baker (1997) recommended same value for Leu:Lys in the starter phase on 1.09. Rostagno *et al.* (2011) recommendation for Leu:Lys in the starter phase is 1.07. A review of the recent literatures shows that, there are few study reporting digestible Leu requirement on broiler chickens. Ospina-Rojas *et al.* (2016) found an interaction between the levels of Leu and Val on FI and WG; they reported that, the best WG and FCR of 21 to 42 day of age male broilers were achieved when the broilers received 1.15 and 0.86%, and 1.19 and 0.86% standard ileal digestible Leu and Val, respectively.

Franco *et al.* (2016) conducted three trials to estimate the optimal ratios of digestible phenylalanine + tyrosine, histidine, and Leu relative to digestible Lys in starter phase (d 8-17). In the study, male broilers were fed a semi-purified diet using corn starch, broken rice, fish meal and soybean meal; the growth performance and carcass characteristics data were analyzed using various requirement-estimation models, and the optimal digestible Leu:Lys estimated at 1.04.

Some experiments on amino acid need have reported lower amino acid needs for females (Chamruspollert *et al.* 2002; Dozier *et al.* 2008; Wang *et al.* 2016). Baker (2009) indicate that the Lys requirement of male broilers is more than females. Due to the mentioned reasons, the requirement of ideal Leu ratio in females may differ from male requirement.

However, the result of present study was in line with mentioned reports (NRC, 1994; Baker, 1997; Rostagno *et al.* 2011; Franco *et al.* 2016), and the exist difference between our results and previous reports may be due to the using of different gender (female *vs.* male) and statistical models to describe Leu requirement.

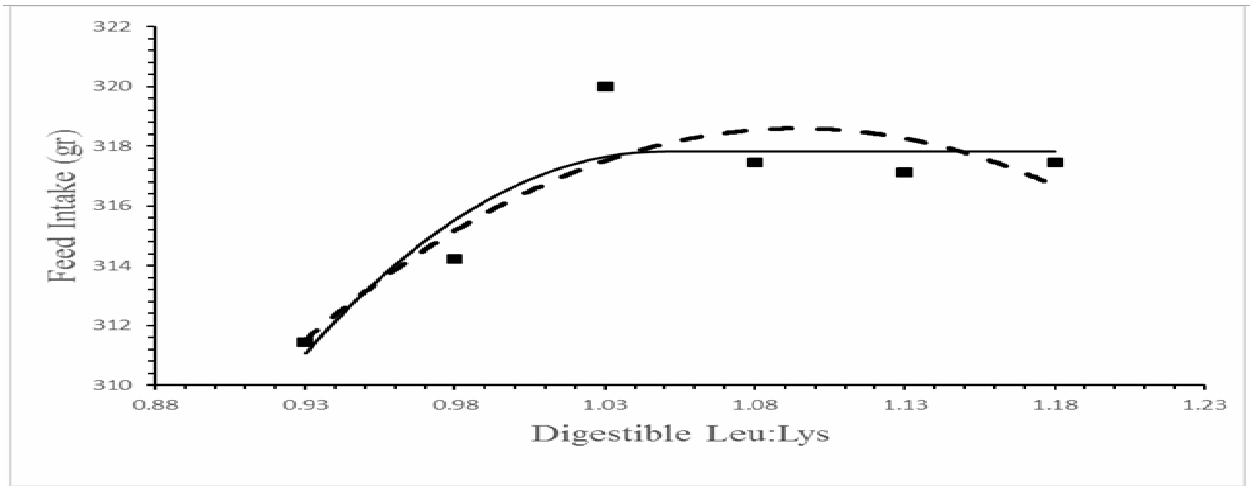

Figure 1 The requirement of digestible Leu:Lys to maximize feed intake determined by Curvilinear-plateau model was 1.05 ± 0.06 [(Y=317.8–474.7×(1.05–X)^2; r^2= 0.82] (—)
The data were also fitted to quadratic regression equation [(Y=4.9111+573.2539 (X)–261.9047 (X)^2; r^2= 0.77] (− −)
The level of digestible Leu:Lys that maximized feed intake was calculated to be 1.09, with 95% of this value being 1.04
Data points (■) represent mean for each dietary treatment (n=90 chicks per treatment)

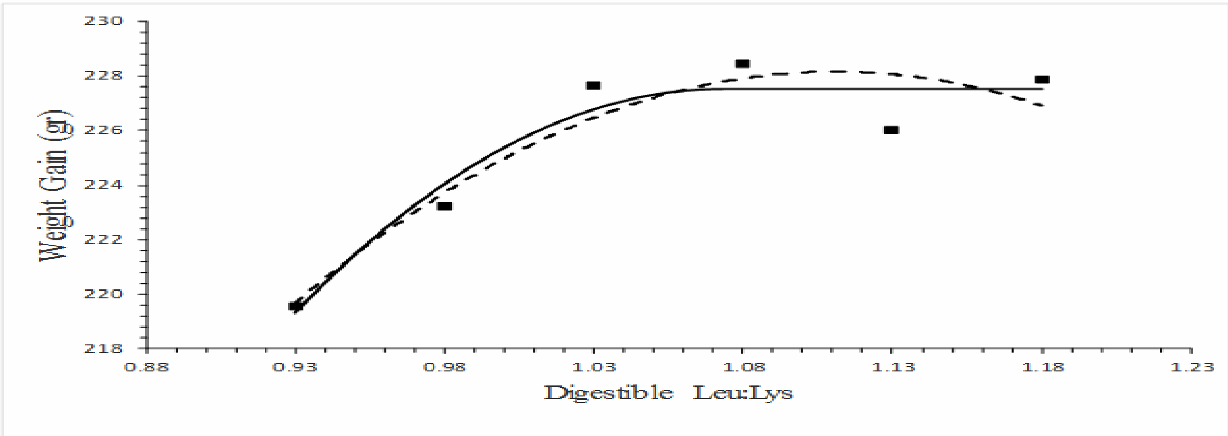

Figure 2 The requirement of digestible Leu:Lys to maximize weight gain determined by Curvilinear-plateau model was 1.07 ± 0.04 [(Y=227.6–400.4×(1.07–X)^2; r^2= 0.92] (—)
The data were also fitted to quadratic regression equation [(Y=–92.97+578.2857 (X)–260.317 (X)^2; r^2= 0.88] (− −)
The level of digestible Leu:Lys that maximized feed intake was calculated to be 1.11, with 95% of this value being 1.06
Data points (■) represent mean for each dietary treatment (n=90 chicks per treatment)

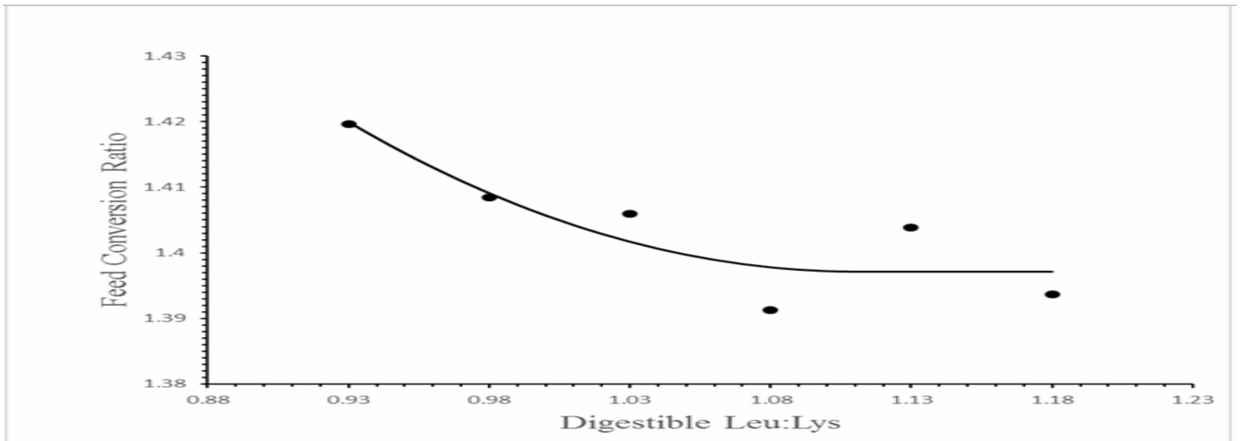

Figure 3 The requirement of digestible Leu:Lys to optimize feed conversion ratio determined by Curvilinear-plateau model was 1.11 ± 0.1 [(Y=1.3971 + 0.6973 × (1.11–X)^2; r^2= 0.78]
Data points (●) represent mean for each dietary treatment (n=90 chicks per treatment)

Table 4 Estimated parameters of the Curvilinear-plateau model and quadratic regression equation for feed intake (FI), weight gain (WG) and feed conversion rate (FCR) of female Cobb broiler (8 to 14 days of age) according to digestible Leu:Lys

Response criterion	Curvilinear-plateau model				R-squire	P-value
	Intercept (L)	Slope (U)	Requirement (R)			
FI (g)	317	- 474.7	1.05		0.92	0.01
WG (g)	227.6	-400.4	1.07		0.78	0.02
FCR (g/g)	1.397	0.697	1.11		0.82	0.10
	Quadratic regression equation					
	Intercept	X	X × X	Requirement		
FI (g)	4.911	573.25	-261.9	1.04	0.88	0.03
WG (g)	-92.99	578.28	-260.32	1.06	0.77	0.02

CONCLUSION

This study clearly showed that deficiency of Leu ratio affect performance parameters, and reducing dietary digestible Leu:Lys up to 0.98 in Cobb 500 female broilers from 8 to 14 d of age, reduces the FI and WG, however FCR not affected. Therefore, it can be concluded that increasing of FI have resulted in WG enhance. Female Cobb 500 broilers requirement of Leu to optimize FCR was higher than to FI and WG. Based on obtained data, the requirement of Cobb 500 female broilers from 8 to 14 d of age for optimizing performance parameters is concluded at 1.08 digestible Leu:Lys, which was in line with mentioned reports on male broiler.

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