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Full Length Research Paper

Reevaluation of lysine requirement based on performance responses in broiler breeder hens

J. Fakhraei[1]*, H. Loutfollahian[2], M. Shivazad[1,3], M. Chamani[1] and S. A. Hoseini[2]

[1]Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2]Research Institute of Animal Science, Karaj, Iran.
[3]Animal Science department, Tehran University, Tehran, Iran.

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This experiment was conducted to study lysine requirement in broiler breeder hens. The treatments consist of 6 levels of lysine (0.50, 0.57, 0.64, 0.71, 0.78 and 0.85%) in a completely randomized design with 4 replicates and 8 hens in each pen, from 52 to 62 weeks of rearing period. The strain of hens was Arian. The first 3 weeks were considered as a period of depletion. The hens selected were of nearly the same body weight (3600±15 g) for maximum uniformity in treatments groups. Each pen was equipped with a four-hole nest, a tube feeder, and an automatic waterier. Amino acid profile and composition of feed ingredients were analyzed and results of them were used for diet formulation. The results showed that different levels of lysine had significant effects on egg production (%), egg mass (g/h/d), egg content (g) and settable egg (%) ($P < 0.05$). There were no significant differences in double yolk eggs, small eggs, hatch of fertile eggs and chicks weight ($P > 0.05$). The broken-line model was used for determination of requirement. Broken-line regression analysis indicated that lysine requirement for egg production (%), egg mass (g/h/d), egg content (g) and settable egg (%) were 0.64 , 0.67 , 0.67 and 0.63%, respectively. Finally, the results indicated that lysine requirements for egg mass and content and also R2 were higher than determination of lysine requirements based on egg production and settable egg.

Key words: Broiler breeder hens, lysine, performance, requirement.

INTRODUCTION

Protein and amino acids were the major nutrients representing a high percentage of total cost of the diets (Gunawardana et al., 2008). It is an accepted practice to control body weight (BW) of broiler breeders in the laying house by limiting the amount of feed they are given. It is also recognized that hens must have a certain intake of essential nutrients if maximum production is to be obtained (Harms and Ivey, 1992). The dietary balance of amino acids affects the efficiency of protein utilization significantly. Supplementation of poultry diets with crystalline amino acids is an additional tool to optimize the dietary amino acid balance. Lysine is generally accepted as the second limiting amino acid following the methionine in corn-soybean meal diets. Additionally, lysine is utilized as the basis for evaluating the content of other essential amino acids for ideal amino acid balance (Samadi and Liebert, 2007). Baker (1997) revealed that the reasons why lysine is chosen as reference amino acid is, that almost all of lysine in the diet is used for protein accretion and lysine is considered as a limiting amino acid in diet based on corn-soybean meal.

The scientists suggested that NRC (1994) recommended requirement of 765 mg/day/hen for lysine is low. Liu et al. (2005) and Wu et al. (2007) reported that increasing lysine level significantly improved egg production (EP), egg weight (EW), egg mass (EM), feed consumption, feed conversion and body weight (BW) of hens. Fisher (1998) reports that the available lysine requirements for broiler breeders (BB) ranges from 1080 mg/day/hen at 28 - 29 weeks to a slightly lower intake requirement of 975 mg/day/hen at the end of the laying cycle. Harms and Ivey (1992) suggested the daily lysine requirements for EP, EW and egg output to be 824, 806, and 819 mg per day, respectively, when protein intake

*Corresponding author. E-mail: j_fakhraei86@yahoo.com.

was greater than 18.6 g/day. Soares et al. (1988) reported that 45 to 60 week old broiler breeders required 915 mg lysine/day when consuming 18.5 g protein/day, producing an average 48 g egg mass/day, with a body weight of 3.5 kg at 60 weeks. Bowmaker and Gous (1991) conducted a study on the effects of lysine and methionine in broiler breeder hens EP, reporting that the highest mean of EW resulted from the highest concentration of lysine and methionine (1272 and 524 mg/bird/day respectively). They also observed that when there were severe deficiencies of lysine and methionine, EP was reduced to a greater degree than was EW. Waldroup et al. (1976) developed growth and egg production curves for different ages of breeder and the average change in body weight was calculated.

It seems that there are some considerable view points about reviewing the lysine requirements for broiler breeders. There were different reports about lysine requirements for broiler breeders. Though most of the latest studies used purified or semi purified diets in the mash forms it seems that they are not suitable for recommendation to industries. Therefore according to mentioned points, the objective of the present study was to obtain further information on the lysine requirements of broiler breeder hens using practical diet and to determine daily intakes of lysine that will support satisfactory performance and egg production in second phase of production.

MATERIALS AND METHODS

Hens

After the birds were weighted at 52 weeks of age, the heavier and lighter birds were discarded to achieve 192 females and 24 males. These hens had the same body weight (3600±15 g) and so there was maximum uniformity in treatments groups. The selected birds were randomly allocated (8 females and 1 male) to each of the 24 floor pens (1.2 m wide×2.1 m length). These hens were palpated on 21 successive day and not laying hens were replaced. Each pen was equipped with a four-hole nest, a tube feeder, and an automatic waterier. The house was heated to maintain a minimum temperature of 24°C throughout the experiment period. They were maintained in a windowless house and given artificial light (16 h light: 8 h dark).

Diets

Six experimental diets were fed (Table 1) with different lysine levels of 0.5, 0.57, 0.64, 0.71, 0.78 and 0.85%. All of the diets had a similar protein (16%) and metabolizable energy (2700 Kcal/Kg). Formulation of diets was based on analyzed values of corn, soybean, and wheat bran for amino acids. The amino acids content of feed ingredients were determined by high performance liquid chromatography (HPLC) according to the procedures described by Ravindran et al. (1999). Chemical analyses of diets were determined according to the methods of AOAC (1990). UFFDA program was used for diets formulation. The levels of the other amino acids were equal or exceed in all diets to ensure that the lysine was the first-limiting amino acid in diets. For achieving this purpose the amino acid requirements of broiler breeders relative to lysine suggested by Fisher (1998) were used.

The diets were formulated to be comparable to those used in the commercial industry and were prepared in the pellet form with size of 4 - 5 mm. Four pens with each of them containing eight hens were fed each diet. Water was consumed *ad libitum* and each hen received an average daily allocation of feed (150 g per hen). The experiment was conducted for 11 weeks with 3 weeks considered as period of depletion, as suggested by Harms and Ivey (1992) and 8 weeks were used to evaluate diets. The hens were weighted at the beginning and end of the experiment and weight gain (WG) was calculated.

Performance responses

Egg production, feed intake and egg weight was recorded daily on a replicate basis. Egg mass (EM) was calculated by multiplying egg production (EP) and egg weight (EW). Each pen was allocated an equal amount of feed each day. In most of the pens all of the feed was consumed; therefore, these pens did not have any variation in feed intake. In the experimental period in addition to EP, EW and EM, double yolk egg, settable egg (SE) and small egg (<51 g) were measured. Settable eggs consisted of all egg production except small, double and abnormal eggs. Egg content (EC) was calculated by multiplying EP by (EW - shell weight).

During the experimental period, hatchability and weight of chicks were measured every 15 days. Since in this experiment there were 8 females and 1 male in each pen, hatchability was calculated based on fertile eggs.

Statistical analysis

Analysis of variance was performed on all data using General linear Models (GLM) procedure of the SAS (SAS Institute, 2002). Significant treatment effects were separated by Duncan's range test (1955). The requirements of lysine were determined using broken-line regression as described by Robbins et al. (2006). The 4 different broken-line models were used to estimate lysine requirements as follows:

1) The single-slop straight broken-line model: $Y = L + U (R - X)$, where $(R - X)$ was defined as zero when $X > R$.
2) The two-slop straight broken line model: $Y = L + U (R - X) + V (X - R)$, where $(R - X)$ was defined as zero when $X > R$, and $(X - R)$ was defined as zero when $X < R$.
3) One-slop, quadratic broken line model: $Y = L + U (R - X)^2$, where $(R - X)$ is zero at values of $X > R$.
4) Two-slop, quadratic broken-line model: $Y = L + U (R - X)^2 + V (X - R)$, where $(R - X)$ is zero at values of $X > R$ and $(X - R)$ is zero at levels of $X > R$.

With comparison of 4 models, it was clear that the second model has a higher R^2 and gives better fit line. So it has been discussed here. To assist in choosing an appropriate model, R^2 values were calculated as R^2 = (CTSS-SSE)/CTSS and for each trait, the model with maximum R^2 was selected (Robbins et al., 2006).

RESULTS AND DISCUSSION

The results showed that different levels of lysine had significant effects on EP, EM, EC and SE ($P < 0.05$). EP increased from 35.71% when the hens received the diet with 0.50% lysine to 45.26% when the diet contained

Table 1. Composition of experimental diets.

Ingredient and Analysis	Lysine level (% in diet)					
	0.50	**0.57**	**0.64**	**0.71**	**0.78**	**0.85**
Corn	58.71	58.81	58.96	59.16	59.36	59.55
Soybean meal (44% CP)	3.72	3.94	4.40	5.15	5.90	6.66
Wheat bran	16.83	16.66	16.34	15.85	15.35	14.86
Corn gluten	11.54	11.27	10.89	10.36	9.82	9.29
Oyster shell (38% Ca)	6.88	6.88	6.88	6.87	6.86	6.85
Dicalcium phosphate	1.20	1.20	1.20	1.20	1.20	1.20
Salt	0.21	0.19	0.17	0.15	0.14	0.13
Micro ingredients[1]	0.50	0.50	0.50	0.50	0.50	0.50
Choline HCl	0.14	0.14	0.14	0.14	0.14	0.14
Coccsidio stat	0.07	0.07	0.07	0.07	0.07	0.07
$NaHCo_3$	0.14	0.18	0.21	0.22	0.24	0.26
DL-Methionine	0.03	0.03	0.03	0.04	0.05	0.05
L-Lysine HCL	0.03	0.13	0.21	0.29	0.37	0.44
Calculated analysis[2]						
Protein	16.00	16.00	16.00	16.00	16.00	16.00
Cystine	0.33	0.33	0.33	0.33	0.33	0.33
Methionine	0.36	0.36	0.36	0.36	0.36	0.36
Tryptophan	0.17	0.17	0.17	0.17	0.17	0.17
Arginine	0.76	0.76	0.76	0.77	0.78	0.79
Threonine	0.56	0.56	0.56	0.56	0.56	0.56
Valine	0.76	0.76	0.75	0.75	0.75	0.75
Isoleucine	0.59	0.59	0.59	0.59	0.59	0.59
Calcium	3	3	3	3	3	3
Phosphore	0.36	0.36	0.36	0.36	0.36	0.36
Sodium	0.15	0.15	0.15	0.15	0.15	0.15

[1]Supplied per kilogram of diet: Vitamin A, 6600 IU; cholecalciferol, 2200 ICU; menadione dimethylpyrimidinol bisulfite, 2.2 mg; riboflavin, 4.14 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; vitamin B12, 0.022 mg; ethoxyquin, 125 mg; manganese, 60 mg; iron, 50 mg; copper, 6 mg; iodine, 1.1 mg; zinc, 35 mg; selenium, 0.1 mg. [2]Based on analysis of corn, soybean meal and wheat bran. All diets were calculated to be isocaloric (2700 kcal/kg).

Table 2. Effect of lysine levels on broiler breeder hens' performance.

Lysine level in diet (%)	Lysine intake (mg/d)	Egg production (%)	Egg weight (g)	Egg mass (g/h/d)	Egg content (g)	Body weight gain (g/d)
0.50	750	35.71^{b}	67.29	23.95^{b}	21.41^{c}	7.68
0.57	855	40.52^{ab}	65.07	26.36^{ab}	23.45^{bc}	9.19
0.64	960	45.26^{a}	66.65	29.80^{a}	26.87^{a}	7.68
0.71	1065	43.75^{a}	67.55	29.57^{a}	26.48^{a}	6.99
0.78	1170	44.44^{a}	66.27	29.59^{a}	26.27^{ab}	9.36
0.85	1275	43.49^{a}	66.29	29.00^{a}	25.70^{ab}	8.54
SEM	-	1.67	0.92	1.06	0.94	0.84

[a-c] Means within a column with no common superscript differ significantly (P < 0 .05).

0.64% lysine (Table 2). Mean EP of the hens receiving diets with 0.71, 0.78 and 0.85% lysine was not different from the EP of hens receiving diet with 0.64% lysine. The estimated requirements of lysine for broiler breeder hens and the estimated parameters for broken-line with two slop model are shown in Table 4. Broken-line regression of EP on the lysine intake resulted in a requirement of 952.1 mg/h per day with R^2 = 0.548 (Figure 1). According

Table 4. The estimated requirement of lysine in broiler breeder hens and the estimated parameters for broken line with two slop model.

Trait	Requirement	Estimated parameters of model				R^2 (%)
		L	U	V	R	
EM	969.9	29.9339	-0.0280	-0.00271	969.9	57.38
EP	952.1	44.9625	-0.0458	-0.00439	952.1	54.80
SE	950.1	43.0858	-0.0473	-0.00413	950.1	52.83
EC	969.9	26.8972	-0.0260	-0.00373	969.9	58.06

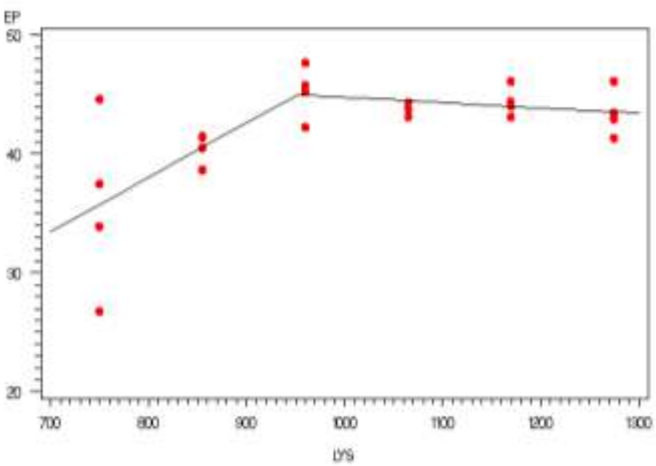

Figure 1. The broken-line with two slop model fitted for egg production response to various doses of lysine in broiler breeder hens. Model: EP= 44.96 -0.046 (952.1-x) -0.0044 (x-952.1), where (952.1-x) is zero at values of x > 952.1 and (x-952.1) is zero at values of x < 952.1.

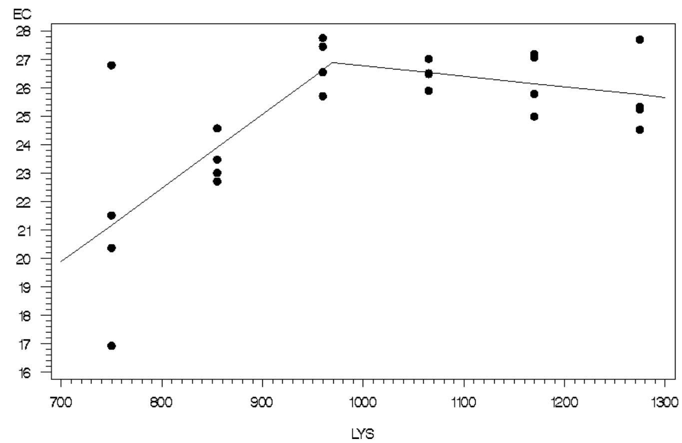

Figure 3. The broken-line with two slop model fitted for egg content response to various doses of lysine in broiler breeder hens. Model: EC= 26.89 -0.026 (969.9-x) -0.00373 (x-969.9), where (969.9-x) is zero at values of x > 969.9 and (x-969.9) is zero at values of x < 969.9.

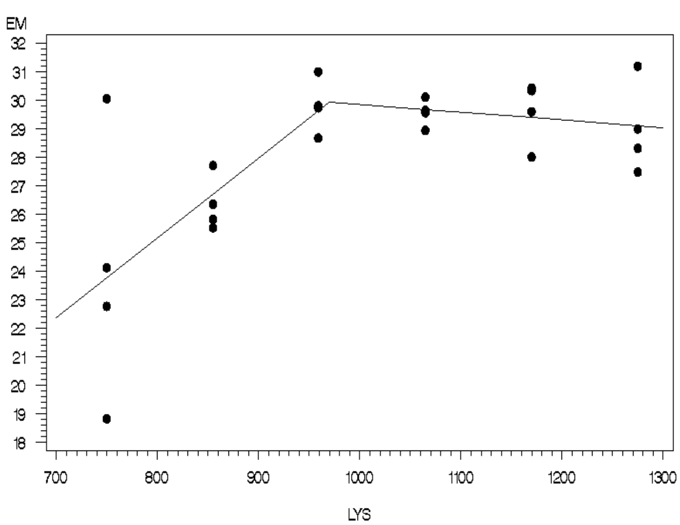

Figure 2. The broken-line with two slop model fitted for egg mass response to various doses of lysine in broiler breeder hens. Model: EM= 29.93 -0.028 (969.9-x) -0.0027 (x-969.9), where (969.9-x) is zero at values of x > 969.9 and (x-969.9) is zero at values of x < 969.9.

to the results different levels of lysine had no significant effects on body weight gain (BWG) and EW (Table 2). Egg mass followed the same trend as EP. The lowest and highest values for EM were observed when the diets that contained 0.5 and 0.64% lysine, respectively (Table 2). Broken-line regression of EM on lysine intake indicated a requirement of 969.9 mg/h per day with R^2 = 0.573 (Figure 2). Egg content followed the same trend as EP and EM. However, the EC of the hens receiving the diet with 0.5% lysine was significantly less than the EC of the hens receiving the diets with 0.64, 0.71, 0.78 or 0.85% lysine. Broken-line regression of EC on lysine intake indicated a requirement of 969.9 mg h per day with R^2 = 0.58 (Figure 3). Based on Harms' theory (1992), EC includes both EW and EP, hence it is necessary in evaluation of amino acid requirements.

There were no significant differences in EW and BWG as the levels of lysine in the diet ranged from 0.5 - 0.85% (Table 2). The highest BWG was observed when the hens received the diet with 0.78% lysine. Increase in BW by using different levels of amino acids has been observed previously (Harms and Ivey, 1992).

Table 3. Effect of lysine levels on broiler breeder hens' performance.

Lysine level in diet (%)	Lysine intake (mg/d)	Double yolk egg (%)	Small egg (%)	Settable egg (%)	Hatch of fertile egg (%)	Chicks weight (g)
0.50	750	0.97	1.11	33.63[b]	71.16	44.67
0.57	855	1.13	0.79	38.59[ab]	73.16	44.01
0.64	960	1.20	0.44	43.62[a]	64.99	43.45
0.71	1065	1.29	0.86	41.59[a]	68.95	46.60
0.78	1170	1.35	0.59	42.50[a]	58.80	46.10
0.85	1275	1.19	0.44	41.87[a]	67.60	45.55
SEM	-	0.18	0.22	1.78	6.98	0.90

[a-b] Means within a column with no common superscript differ significantly (P < 0 .05).

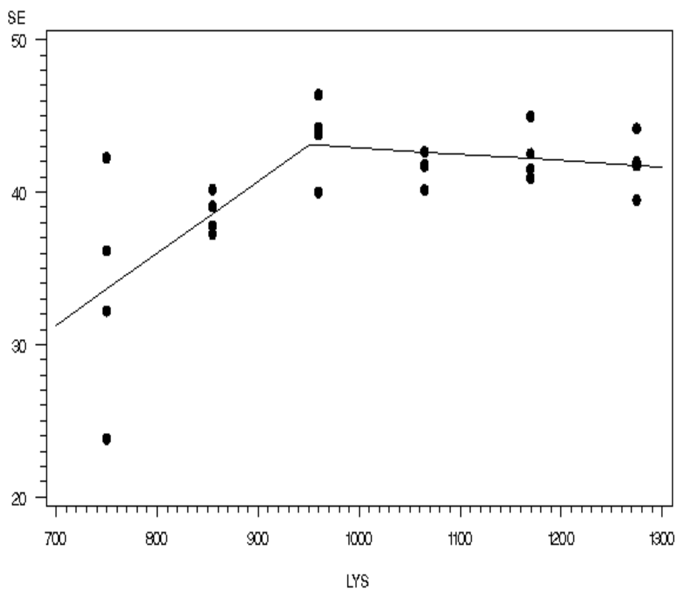

Figure 4. The broken-line with two slop model fitted for settable egg response to various doses of lysine in broiler breeder hens. Model: SE= 43.0858 -0.0473 (950.1-x) -0.00413 (x-950.1), where (950.1-x) is zero at values of x > 950.1 and (x-950.1) is zero at values of x < 950.1.

There were no significant differences in double yolk eggs, small eggs, hatch of fertile eggs and chicks weight (Table 3). The different levels of lysine had significant effect on settable egg (Table 3). Lower values were observed for SE when the diet contained less than 0.64% lysine. Broken-line regression of SE on the lysine intake showed a requirement of 950.1 mg/h per day with R^2 = 0.528 (Figure 4).

There is little information available on the needs of broiler breeder hens for amino acids at different phases of the laying period. The quality of dietary protein is determined by its amino acid content and their balances relative to overall requirement. Amino acid utilization for egg production is lower for broiler breeder hens as compared to egg laying hens (Bowmaker and Gous, 1991; Fisher, 1998). Egg production increased significantly as dietary levels of lysine increased from 0.50 to 0.64%. Bowmaker and Gous (1991) reported poor performance with low levels of lysine, and higher levels (918 - 1272 mg/bird/day) resulted in the best performance, especially in terms of egg production suggesting that the lysine needs could have initially been underestimated. A lysine requirement of 765 mg/bird/day is reported by the National Research Council. Wilson and Harms (1984) found that a daily intake of 808 mg of lysine/hen/day supported maximum EP and EW. Higher levels of lysine to support good performance have also been reported by other authors (Pearson and Herron, 1981; Soares et al., 1988; Harms, 1992; Spratt and Leeson, 1987). The most important dietary factors known to alter egg weight are dietary protein and certain amino acids.

It was not possible to fit a broken-line for egg weight; therefore it was not possible to determine a requirement. This information showed that small increments in intake of amino acids (lysine) that were closed to the optimum resulted in equal proportional response in rate of lay and egg weight, but that when there were severe deficiencies of lysine and methionine, egg production was reduced to a greater extent than was egg weight. Gous and Nonis (2010) realized that the number of eggs laid increased with lysine intake with little change in the mean egg weight over the 30 weeks of lay. It seems that when lysine intake is low, birds tend to adjust first their EP and secondly their EW. The highest EM was observed when the hens received the diet with 0.64% lysine and estimate of lysine requirement for EM response has the higher R^2 (R^2 = 0.573). This information suggested that to establish optimum lysine intake for broiler breeder hens, we must predict changes in EM rather than isolated responses to EP and EW. Fisher (1998) also shows the increase in amino acid requirements due to flock variability that were not used in some of the model calculations of Waldroup et al. (1976) in developing some of the NRC (1994) requirements.

As laying hens become older, the initial decrease in EP and increase in EW is due to a reduction in the rate of recruitment of yellow-yolky follicles, which grow to a layer size before ovulation (Williams and Sharp, 1978). Finally this study demonstrated that evaluation of lysine requirement is affected by some factors as kind of parameters of performance.

Conclusion

The results indicated that lysine requirement for producing egg mass and egg content were higher than determination of lysine requirement based on egg production and settable egg. Supplemental lysine can be of benefit in corn soybean meal diets for broiler breeder hens. The amino acid requirement is not one fixed figure. The requirement depends on certain physiological parameters, kind of keeping, immunological stress and definitely on the production status of the birds. The ability to model the amino acid requirement for breeders will require a better understanding of efficiency of utilization of amino acids for production.

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