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Standard Body Weight and Serum Estradiol and Progesterone Concentrations in Response to Total Lysine Content in Female Broiler Breeders from 14 to 42 Days after Hatch

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ABSTRACT

This study was conducted to determine the total lysine requirement for female broiler breeders from days 14 to 42. Two-hundred and ten female broiler breeders were used in a completely randomized design with 6 replicates per treatment and 5 chicks per pen under restricted feeding. The contents of total lysine used in this experiment were 0.68, 0.72, 0.76, 0.80, 0.84, 0.88 and 0.92% in the diet. A basal diet was formulated to meet or to exceed the Ross 308 female broiler breeders' nutrient specifications except for the lysine. Body weight and feed intake were measured to calculate feed efficiency, and body weight uniformity was defined on a weekly basis. One chick per pen was randomly selected to collect blood samples, organ and an abundance of sexual maturity associated miRNAs (miR-21, mi-26a and mi-375) in the plasma was measured on day 42. Increasing total lysine contents in the diet improved body weight gain and feed conversion ratio from days 14 to 42. The combined values from the two models for BW, average daily gain and feed efficiency were estimated at 1.04, 1.00 and 1.21% total lysine, respectively. Total lysine contents did not affect the ovary weight, serum estradiol-17β, serum progesterone and plasma urea nitrogen or the expression levels of the three miRNAs on day 42. Therefore, the results from the current study indicated that female broiler breeders fed on 0.68% total lysine could achieve the recommended BW, suggested by field practice when data were fitted into the overlapped point of linear-, and quadratic-plateau models.

INTRODUCTION

Lysine is an indispensable nutrient for farm animals, although it is the second-limiting amino acid when diets are formulated mainly with corn and soybean meal for poultry ration. In addition, the diets with either deficient or excessive lysine caused poor performance and (or) increased the feed cost in broilers (Dozier *et al.*, 2010; Mehri *et al.*, 2010).

Owing to genetically higher growth potential in meat-type breeder hens, they are generally subjected to feed restriction to control their obesity and (or) to meet their target body weight (BW). Over or under BW is strongly related to poor reproductive performance of female broiler breeders including ovarian dysfunction and diabetes, were associated with metabolic imbalance (Robinson *et al.*, 1993; Chen *et al.*, 2006; Mohiti-Asli *et al.*, 2012a). Chen *et al.* (2006) described that 50 to 60% of daily ad libitum feed reduced metabolic disease, and enhanced egg quality in female broiler breeders. In this regard, commercial broiler breeder feeds are formulated with low energy and crude protein levels (Ekmay *et al.*, 2014). For instance, when the diets contained excess lysine, it could result in a higher muscle deposition

but in a lower egg production and fertility rate during laying periods (Coon, 2004). Although restricted feeding in the growing phase of female broiler breeder is necessary to prevent obesity, their daily ration must contain appropriate nutrients to accomplish target BW, puberty and onset of laying in sequence. Lysine requirement for offspring was well described, only few data, however, are available in total lysine requirement for the growth of broiler breeder, (Bowmaker & Gous, 1991; Fisher, 1998; Ekmay *et al.*, 2013; Sakomura *et al.*, 2015). Nonetheless, a gap existed for the lysine requirement between commercial breeding companies (i.e. Cobb-Vantress and Aviagen) due to perhaps its different genetic potential, and hence it is necessary to evaluate the nutritional needs (i.e. total lysine) of broiler breeders during rearing. The objective of the present study was to investigate the total lysine requirement of female broiler breeders from 14 to 42 days of age.

MATERIALS AND METHODS

All practices and procedures for this experiment were reviewed and approved by the Animal Ethics Committee of the Chungnam National University (CNU-00744).

Experimental design: This experiment was conducted in a completely randomized design with 7 total lysine levels. The 7 total lysine levels used in this experiment were 0.68, 0.72, 0.76, 0.80, 0.84, 0.88 and 0.92%. The chicks were randomly allotted in 42 pens with 5 birds per pen under standard management conditions. Birds had a 2-week adaptation period to prevent the adverse effects on early starter phase before the beginning of the study. Birds were fed with the respective experimental diets from 14 to 42 days. All experimental diets were fed as crumble form, and the study lasted for 28 days.

Birds, housing and diets: Two hundreds and ten 1-day-old Ross308 female broiler breeders were obtained from a commercial hatchery (Eum-seong chicken farm, Eumseong-gun, Chungcheongbuk-do, Korea). At the beginning, birds were randomly divided into 7 treatments with 6 replicates having 5 birds in each pen. This experiment was conducted from April to May 2016. Seven treatments were formulated to meet and exceed Ross 308 parent stock nutrition specification except for lysine (Aviagen, 2013). Experimental diets contained total lysine content from 0.68% to 0.92% in 0.04% increment (Table 1). From day 1 to 14, birds were fed with common commercial starter diet (2,800 kcal/

Table 1 – Composition of experimental diets (% as fed)

Item	Total lysine, %						
	0.68	0.72	0.76	0.80	0.84	0.88	0.92
Corn	48.84	47.84	46.24	57.77	56.27	54.57	53.27
Wheat	15.00	14.60	14.60	-	-	-	-
Wheat bran	17.00	17.00	17.00	17.00	17.00	17.00	17.00
Soybean meal	14.00	15.40	17.00	19.50	21.00	22.70	24.00
Vegetable oil	1.00	1.00	1.00	1.30	1.30	1.30	1.30
Limestone	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Monocalcium Phosphate	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin-Mineral premix[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Arginine	0.04	0.04	0.04	-	-	-	-
DL-Methionine	0.11	0.11	0.11	0.26	0.26	0.26	0.26
L-Threonine	0.05	0.05	0.05	0.12	0.12	0.12	0.12
L-Isoleucine	0.02	0.02	0.02	0.06	0.06	0.06	0.06
L-Valine	0.04	0.04	0.04	0.09	0.09	0.09	0.09
Calculated composition[2]							
ME, kcal/kg[3]	2,800	2,800	2,800	2,800	2,800	2,800	2,800
Crude protein, %	18.0	18.1	18.1	18.0	18.0	17.9	18.0
Calcium, %	1.0	1.1	1.1	1.1	1.1	1.1	1.1
Available Phosphate,%	0.5	0.5	0.5	0.4	0.5	0.5	0.5
Total lysine, %	0.68	0.72	0.76	0.80	0.84	0.88	0.92

[1]Provided the following nutrients (per kg of air-dry diet): Vitamins: A 12,000 IU, D 33,000 IU, E 15 mg, K 2 mg, thiamine 2 mg, riboflavin 6 mg, pyridoxine 2 mg, calcium pantothenate 0.03 mg, folic acid 0.2 mg, niacin 45 mg, biotin 0.15 µg. Minerals: calcium 0.5%, Co 0.5 mg (as cobalt sulphate), Cu10 mg (as copper sulphate), iodine 0.9 mg (as potassium iodine), iron 80 mg (as ferrous sulphate), Mn 80 mg (as manganous oxide), Se 0.2 mg (as sodium selenite), Zn 80 mg(as zinc oxide).

[2]The values are calculated according to the values of feedstuffs in NRC (1994).

[3] ME; Metabolizable energy.

kg, 15% CP, and 0.74 % total Lysine/kg) ad libitum and thereafter birds were fed with the experimental diets from day 14 to 42. During the experimental periods, birds were subjected to restrict feeding in accordance with the management guide of the Ross 308 Parent Stock (Aviagen, 2011). Fresh water was available at all times. Birds were reared following a photoperiod of 24 h of light (20 lux) for the first 3 days post hatch which was gradually reduced to a photoperiod of 13L:11D (10 lux). Brooding temperature was maintained 34°C from day 1 to 3 and then reduced 1°C every three days until it reached 25°C.

Post-mortem procedures and collection of ovaries: At the end of the experiment, the birds were subjected to 6 h of feed deprivation before slaughtering. One bird from each pen was selected randomly and weighed. The selected bird was euthanized by cervical dislocation, then bleeding via neck cutting. After bleeding, ovaries were isolated and weighed.

Data collection: To monitor BW and average daily gain (ADG), all birds were weighed weekly from the 14th to the 42nd day of age. The mortality was recorded daily and the feed conversion ratio (FCR) and BW uniformity were calculated every week.

Chemical analyses: At the conclusion of the experiment, a selected bird from each replicate pen was bled for plasma metabolites and serum hormones analysis, and3mL of blood samples were collected from jugular vein into vacutainer and stored at 4°C until the transfer to the laboratory. The serum and plasma separated by centrifugation at 3,000rpm for 15 min (Micro 12, Hanil Science Co., Ltd., Korea) and then stored at -20°C for the assay. Quantitative determinations of estradiol-17β and progesterone in the serum was conducted using electro chemiluminescent immune assay kits (Roche, Mannheim, Germany) and using a Cobas®6000 (e601 module) analyzer (Roche, Mannheim, Germany). Plasma urea nitrogen levels

Table 2 – Effect of total lysine content on body weight, average daily gain and feed conversion ratio of female broiler breeders from 14 to 42 days of age[1]

Item	Total lysine, %							SEM[2]	P value[4]
	0.68	0.72	0.76	0.80	0.84	0.88	0.92		
Body weight (g)									
Initial	43.47	43.57	44.10	43.53	43.47	43.50	43.63	3.630	1.000
Day 14	257.57	256.30	258.30	257.33	257.77	256.30	253.30	11.052	0.993
Day 21	351.77^{a}	353.63^{a}	355.53^{a}	360.90^{ab}	368.13^{ab}	373.70^{b}	376.90^{b}	15.432	0.008
Day 28	442.97^{a}	448.87^{ab}	451.57^{abc}	459.00^{bc}	464.80^{cd}	478.00^{de}	480.57^{e}	17.083	0.001
Day 35	546.20^{a}	559.33^{ab}	566.30^{b}	569.30^{b}	589.00^{c}	598.93^{c}	602.07^{c}	23.713	0.001
Day 42	656.33^{a}	676.03^{ab}	687.43^{b}	692.37^{bc}	716.06^{cd}	728.00^{d}	725.93^{d}	31.859	0.001
Day 14 – 42	499.80^{a}	511.50^{ab}	514.73^{ab}	526.78^{bc}	531.56^{c}	546.81^{d}	547.98^{d}	20.509	0.001
Daily lysine intake (mg)									
Day 14	194	204	215	228	237	248	262		
Day 21	217	229	242	255	268	280	293		
Day 28	218	231	243	256	269	282	294		
Day 35	249	263	278	293	308	322	336		
Day 42	266	282	297	313	329	344	360		
Day 14 – 42	237	251	265	279	293	307	321		
Average daily gain (g/bird/day)									
Day 14 – 21	18.84^{a}	19.47^{a}	19.45^{a}	20.71^{ab}	22.07^{bc}	23.48^{cd}	24.71^{d}	2.823	0.001
Day 22 – 28	18.24^{a}	19.05^{ab}	19.121^{abc}	19.62^{abc}	19.33^{abc}	20.86^{a}	20.73^{bc}	1.511	0.019
Day 29 – 35	20.65^{a}	22.09^{ab}	22.95^{bc}	22.06^{ab}	24.84^{d}	24.19^{cd}	24.30^{cd}	1.922	0.001
Day 36 – 42	22.03	23.34	24.23	24.61	25.41	25.81	24.77	3.086	0.410
Day 14 – 42	19.94^{a}	20.99^{ab}	21.46^{b}	21.75^{bc}	22.91^{cd}	23.59^{d}	23.63^{d}	1.615	0.001
Feed conversion ratio (g/g)									
Day 14-21	1.69^{c}	1.67^{c}	1.64^{c}	1.54^{bc}	1.46^{ab}	1.36^{a}	1.29^{a}	0.193	0.001
Day 22-28	1.76^{c}	1.70^{bc}	1.67^{abc}	1.63^{abc}	1.67^{abc}	1.54^{a}	1.55^{ab}	0.131	0.019
Day 29-35	1.78^{c}	1.66^{bc}	1.59^{ab}	1.67^{bc}	1.48^{a}	1.52^{a}	1.51^{a}	0.137	0.001
Day 36-42	1.79	1.68	1.62	1.59	1.66	1.52	1.59	0.205	0.475
Day 14-42	1.75^{d}	1.68^{cd}	1.63^{bc}	1.61^{bc}	1.57^{ab}	1.48^{a}	1.48^{a}	0.112	0.001

[1]Results are mean with 6 replicates per treatment.

[2]Pooled standard error of mean.

[a-f]values in the same row with different superscripts differ significantly (p<0.05).

were analyzed by Enzymatic assay method using Automatic Analyzer 7180 (Hitachi, Japan).

Analysis of miRNA expression: the miRNA was extracted from stored and pooled blood plasma samples using miRNeasy Serum/Plasma kit (Qiagen, Hilden, Germany) and reverse transcribed to cDNA using miScript II kit (Qiagen) with miScript HiFlex Buffer for quantification of mature miRNA and precursor miRNA. Quantitative real time polymerase chain reaction (qRT-PCR) was carried out utilizing a StepOnPlus real-time PCR system (SYBR Green Master Mix; Applied Biosystems, Foster City, CA, USA), and gene-specific primers (gga-miR-21 F; TAG CTT ATC AGA CTG ATG TTG, gga-miR-26a;TTC AAG TAA TCC AGG ATA GGC, gga-miR-375, F; TTT GTT CGT TCG GCT CGC GTT) and miScript Universal Primer (Qiagen). The relative quantification of gene expression was determined by the $2^{-\Delta\Delta Ct}$ method using 5S rRNA as an internal control (F; 5-TCT CGT CTG ATC TCG GAA GC-3; R; 5-AGG AGG TCT CCC ATC CAA GT-3).

Statistical analyses: Data were subjected to ANOVA using the GLM procedure of IBM SPSS statistics 22 (SPSS Inc., Chicago, IL) as a completely randomized design. Replicate pen was used as the experimental unit for growth performance index, and individual birds were used as the experimental unit for ovary weight, blood parameters measured, and miRNA expression. Statistical significance was accepted at $p<0.05$. Linear-plateau (LP) and quadratic-plateau (QP) models were fitted to estimate the optimal lysine requirement using a Nutritional Response Model (Version 1.1; Vedenov & Pesti, 2008).

RESULTS

All the female broiler breeders used in the study were healthy and well-performed, and achieved or exceeded the Ross 308 female broiler breeder BW target profiles throughout the entire experimental period.

Growth: Increased total lysine contents had higher BW, ADG and improved FCR from days 14 to 42 in the female broiler breeders ($p<0.05$; Table 2). In order to achieve maximum BW, ADG and minimum FCR, total lysine requirement was to be 0.94%, 0.89% and 0.94%, respectively, when data was fitted into LP regression model from 14 to 42 days of age in female broiler breeders (Figure 1, 2 and 3). However, QP model showed total lysine requirement for maximum BW and ADG, and for minimum FCR from 14 to 42 days of age were estimated to be 1.13, 1.27 and 1.48% of the

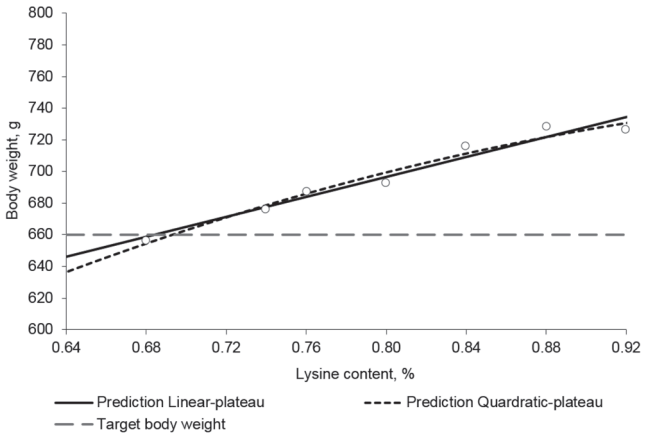

Figure 1 – Determination of total lysine requirement for maximum body weight based on linear- and quadratic-plateau model. Total lysine requirement for broiler breeders from 14 to 42 days of age for body weight determined by a quadratic-plateau model was 1.13 [Y = 752.93-477.47 (1.13-x)^2, R^2=0.97] (open line), and by a linear-plateau was 0.94 [Y = 741.35-315.34 (0.94-x), R^2=0.96] (closed line).Data points (o) represent least squares means of dietary treatment.

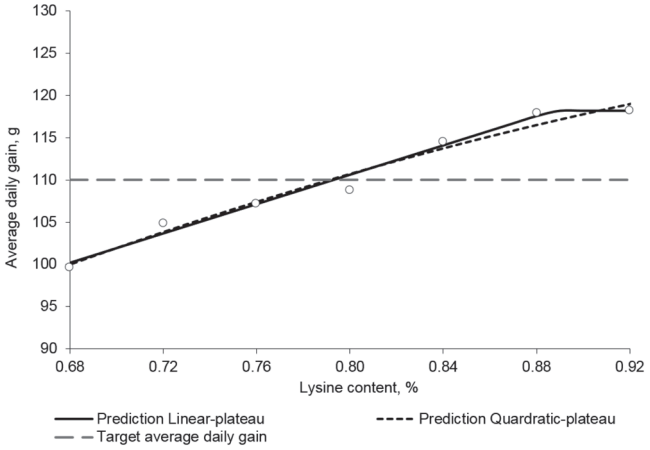

Figure 2 – Determination of total lysine requirement for maximum average daily gain based on linear- and quadratic-plateau model. Total lysine requirements of broiler breeders from 14 to 42 days of age for average daily gain determined by a quadratic-plateau model was 1.27 [Y = 129.22-84.7810 (1.27-x)2, R^2=0.97] (open line), and by a linear--plateau was 0.88 [Y = 118.16-86.86 (0.88-x), R^2=0.98] (closed line). Data points (o) represent least squares means of dietary treatment.

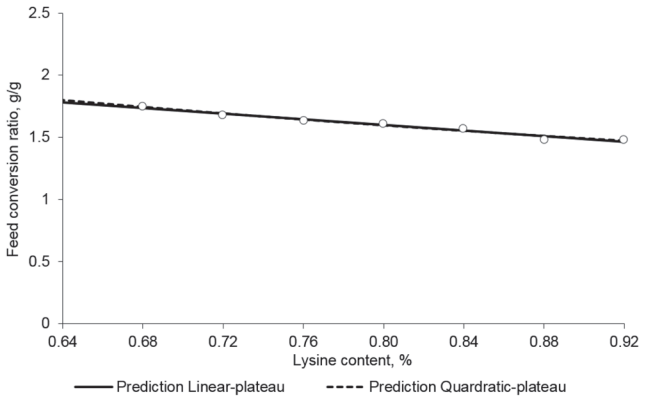

Figure 3 – Determination of total lysine requirement for minimum feed conversion ratio based on linear- and quadratic-plateau model. Total lysine requirements of broiler breeders from 14 to 42 days of age for feed conversion ratio determined by a quadratic--plateau model was 1.48 [Y = 1.21+0.83 (1.48-x)^2, R^2 = 0.97] (open line), and by a linear-plateau was 0.94 [Y = 1.44+1.13 (0.94-x), R^2 = 0.97] (closed line). Data points (o) represent least squares means of dietary treatment.

diet, respectively. Taking a mean value from the two models, recommended total lysine levels for female broiler breeders during 14 to 42 days of age were to be 1.04, 1.00 and 1.21% total lysine for maximum BW, ADG, and for minimum FCR, respectively (Table 3).

Table 3 – Estimated total lysine requirement and recommendations for female broiler breeders from 14 to 42 days of age based on linear-plateau and quadratic-plateau regression analysis[1]

Item	Requirement, %[2]	SE	R^2	Recommendation, %[3]
Mean body weight				
LP	0.94		0.96	1.04
QP	1.13	0.282	0.97	
Average daily gain				
LP	0.89	0.017	0.98	1.00
QP	1.27	0.550	0.97	
Feed conversion ratio				
LP	0.94		0.97	1.21
QP	1.48	1.199	0.97	

[1]LP; Linear-plateau regression analysis, QP; Quadratic-plateau regression analysis, SE; Standard error.

[2]Total lysine requirement based on regression analysis.

[3]Total lysine recommendation for each parameter based on both regression analyses.

Plasma metabolite, serum hormones and ovary weight: Female broiler breeders' visceral ovary weight and ovary weight correlated with BW by different total lysine levels are shown in Table 4.Total lysine levels did not affect (p>0.05) both ovary weight and ovary weight correlated with BW. Serum hormones and plasma metabolite values at 42 days of age are presented in Table 5. Total lysine levels did not affect (p>0.05) serum estradiol-17β, progesterone and plasma urea nitrogen concentrations.

Table 4 – Effect of total lysine content on ovary weight in female broiler breeders on day 42[1]

Total lysine (%)	Daily lysine intake (mg)	Ovary weight (g)	Relative ovary weight (%)
0.68	237	0.092	0.014
0.72	251	0.116	0.017
0.76	265	0.153	0.022
0.8	279	0.191	0.028
0.84	293	0.21	0.03
0.88	307	0.161	0.022
0.92	321	0.167	0.023
SEM[2]	-	0.098	0.014
p-value	-	0.421	0.525

[1]Results are mean with 6 replicates per treatment.

[2]Pooled Standard error of mean.

[3]% = tissue weight/BW ×100.

Table 5 – Effect of total lysine content on serum estradiol (E2) concentration, serum progesterone(P4) concentration and blood nitrogen urea (BUN) of female broiler breeders on day 42[1]

Total lysine (%)	Daily lysine intake (mg)	Serum E2[3] (pg/mL)	Serum P4[4] (ng/mL)	PUN[5] (mg/dL)
0.68	237	5.18	0.05	0.358
0.72	251	5.75	0.043	0.345
0.76	265	5.73	0.04	0.395
0.80	279	5.45	0.048	0.288
0.84	293	5.08	0.065	0.293
0.88	307	7.00	0.063	0.303
0.92	321	5.75	0.048	0.240
SEM[2]		1.353	0.033	0.098
p-value		0.381	0.936	0.359

[1]Data represent means from 4 repetitions per treatment. [2]Pooled Standard error of mean. [3]Estradiol-17β. [4]Progesterone. [5]Plasma urea nitrogen.

Expression of miRNA associated with sexual maturity: To examine whether different lysine treatments affect sexual maturity of female broiler breeders, levels of miRNAs that are associated with sexual maturity and circuiting in the body fluid were measured in the broilers treated with different lysine contents from 0.68% to 0.92%. There were no differences (p>0.05) of expression levels of three miRNAs, gga-miR-21, gga-miR-26a, and gga-miR-375, although gga-miR-21 was relatively abundant at 0.88 and 0.92 % of lysine (Figure 4).

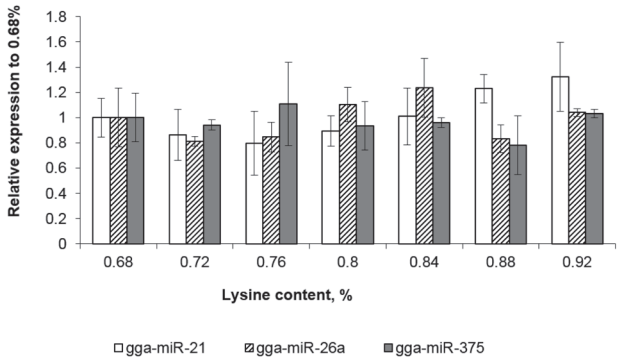

Figure 4 – Expression patterns of gga-miR-21a, gga-miR-26a, and gga-miR-375. The relative expression levels of miRNAs from blood plasma were determined by qRT-PCR. The expression levels were not significantly altered by Lysine contents on day 42. However, gga-miR-21 was slightly elevated at 0.88 and 0.92%, respectively (p=0.045).

DISCUSSION

In the present study, the levels of lysine requirement for optimum target BWs were investigated in female broiler breeders from 14 to 42 days of age. Also, reproductive organ weight, serum hormones, plasma urea nitrogen content, and circulating miRNAs were subsequently examined on day 42. For illustrative purposes, target performance objectives (i.e., BW, feed intake and etc.) for the Ross 308 Parent Stock female were used in this study (Aviagen, 2013).

Our results indicated that BW, ADG and FCR improved in a linear manner, as total lysine contents in the diet were increased. Harms & Ivey (1992) conducted a study with Arbor Acres broiler breeder hens fed 7 levels of protein ranging from 9.6% to 12.2% and 7 levels of total lysine ranging from 0.37 to 0.56% those increased BW as both levels of protein and total lysine intake increased during the laying period. Likewise, Brito *et al.* (2017) evaluated the performance in male Cobb 500 broilers fed 4 different dietary lysine levels and broilers showed higher weight gains and improved feed efficiency as levels of lysine were increased during the growing period. Since the diets were given the same amount due to restricted feeding, an increase in BW of female broiler breeders would be driven by lysine to protein accretion. This is because lysine is primarily used for body protein synthesis and muscle development, and subsequent both nitrogen and muscle deposition in chickens (Scott *et al.*, 1969; Sibbald *et al.*, 1986; Si *et al.*, 2001; Ball *et al.*, 2007). In the present study, birds fed over 0.72% total lysine diets showed higher BW (≥ 660 g) than target BW, in turn, the birds fed 0.68% total lysine diet no overweight was observed, but similar with recommended BW in female broiler breeders on day 42. According to NRC (1994), recommendations for total lysine of female broiler breeder during the growing period were not presented due to insufficient research conducted to determine the broiler breeders' nutrient requirements from hatch to maturity (Powell & Gehle, 1975; Harms & Wilson, 1984). The Ross 308 Parent Stock Nutrient Specification (2013) reported 0.74% of total lysine requirements for female breeders from 22 to 42 days of age. Our present study suggests that modern female broiler breeders may need less amount of lysine to achieve the target growth performance than that recommended by its company guideline. These gaps could be related to recent improvement in genetics and management practices over the decades that could yield significantly improved feed efficiency than the previous genotype (Rekaya *et al.*, 2013; Reyer *et al.*, 2015).

Total lysine requirements (i.e., total lysine level) of the female broiler breeders were evaluated through both LP and QP models from Nørgaard *et al.* (2015) and Wickramasuriya *et al.* (2016) in the current study. The mean values from two models were higher than established lysine levels of female broiler breeders when growth performances were compared to practical target growth performances. This is because these two models for nutrient recommendations are usually appropriate for determining maximum growth performance indices (Vednov & Pesti, 2008). In this light, LP and QP models might not be suitable to determine the lysine requirement of female broiler breeders for optimal target growth performance, because BW of broiler breeders was already strictly controlled from an early age to reduce the reproductive problems in the future.

The coordinated activity of hypothalamic-pituitary-gonadal axis manages the production of steroid hormones such as estradiol-17β and progesterone to develop the reproductive organs and initiate ovulation (Ethches, 1998; Onagbesan *et al.*, 2006). Recently, intensive genetic selection for rapid growth rate has become a down trend due to a higher rate of lipolysis in broiler and relatively induced its obesity along with accumulation of adipose tissue in the ovary (Calabotta *et al.*, 1985; Siiteri, 1987; Mohiti-Asli *et al.*, 2012b). In the present study, all birds showed extremely lower concentrations (< 1 ng/mL) of sexual serum hormones (i.e., estradiol-17β and progesterone) on day 42. Tanabe *et al.* (1981) and Sharp (1975) reported that the sexual hormone levels were under 2 ng/mL from hatch to the 42nd day, then it surged between 140 and 147 days of age. No changes in sexual hormones could be explained by the fact that the birds did not reach maturity to produce these hormones on day 42. Thus, lower levels of estradiol-17β and progesterone may not have been enough to develop reproductive organ in this period. Consequently, further studies are warranted to accurately determine how sexual hormones and reproductive organs are altered by various lysine levels in female broiler breeders during growing- and (or) laying- period.

Furthermore, miRNAs associated with sexual maturity were examined. A previous study identified differentially expressed miRNAs (gga-miR1a, 21, 26a, 137 and 375) between days on 43 and 162 in chicken ovaries. For example, gga-miR-21 was elevated while gga-miR26a and gga-miR-375 were down-regulated in matured ovaries (Kang *et al.*, 2013). Interestingly, it was observed that the expression of target miRNAs was not altered by the different lysine treatment,

suggesting that expression levels of circulating miRNAs in the body fluid can be an indicator for sexual maturity in the chicken.

Birds have no mechanisms for storage of amino acids and therefore when they had excessive amino acids, the amino acids are conveyed to the liver where they are immediately deaminated and (or) converted to urea (Goldstein & Skadhauge, 2000; Weiner *et al.*, 2014). With this in mind, concentration of blood urea nitrogen is strongly associated with the amount of amino acid intake along with its efficiency as then it could be used as an indirect measurement to estimate nitrogen utilization efficiency (Kohn *et al.*, 2005; Donsbough *et al.*, 2010). In the present study, increasing lysine levels did not alter plasma urea nitrogen concentration, which agrees with results of Corzo *et al.* (2003) and Dozier *et al.* (2010) in broiler. Donsbough *et al.* (2010) suggested the determination of differences in plasma urea nitrogen needed for fasting and refeeding schedules due to the above mentioned notion. However, female broiler breeders in the present study had 6 h feed deprivation before slaughtering and did not conduct fasting and refeeding.

CONCLUSION

In conclusion, the results of the present study indicated that female broiler breeders fed 0.68% total lysine diet could be able to reach the target BW more precisely than those fed upper levels of total lysine diets from 14 to 42 days of age.

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