http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.0180104

Dietary Supplementation with Lysine and Threonine Modulates the Performance and Plasma Metabolites of Broiler Chicken

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Here, we investigated whether the optimal threonine (Thr) to lysine (Lys) ratio in high Lys diet improves the growth performance of modern broiler chickens at finisher period and determined the possible mechanism underlying improvement in the growth performance of chickens fed with high Lys or Lys + Thr diet using metabolome analyses. Eighteen 21-day-old chickens housed in individual cages were randomly divided into three groups of six chickens fed with different diets as follows: control diet, high Lys diet (150% Lys content of National Research Council requirement), and high Lys + Thr diet (0.68 of Thr/Lys in high Lys diet). Body weight gain (BWG) increased in chickens receiving high Lys diet as compared with those fed with the control diet ($P<0.05$); no significant difference was observed in BWG of chickens from high Lys + Thr and high Lys groups. Feed conversion ratio (FCR) was lower in chickens fed with high Lys or high Lys + Thr diet than in those on the control diet. Serotonin concentration increased in the plasma of chickens fed with high Lys diet as compared to those fed with other diets. A negative correlation was observed between plasma serotonin concentration and FCR. These results provide the first evidence on the use of high Lys in broiler diets to reduce FCR during finisher period, which may be associated with change in plasma serotonin concentration. These findings suggest that high Lys content in finisher diet, but not high Thr + Lys diet, may affect the peripheral serotonergic metabolism and improve FCR. Thus, plasma serotonin may serve as a biomarker of FCR in broilers.

Key words: amino acid, broiler chicken, lysine, metabolome analysis, serotonin, threonine

J. Poult. Sci., 56: 204-211, 2019

Introduction

The National Research Council (NRC, 1994) determined the nutritional requirements of broiler chickens, including amino acid content of diet. Nutritional requirements of modern broiler chickens have undergone changes owing to improvements in genetic selection and breeding, resulting in further improvements in growth rate, feed conversion ratio (FCR), and breast meat yield (Razaei *et al*., 2004; Sterling *et al*., 2004). Some leading breeding companies such as Aviagen have revealed the nutritional composition of the feed used to induce maximum performance in broiler chickens. However, body weight gain (BWG) and FCR in broiler chickens may be improved with diets supplemented with amino acids (Kidd *et al*., 2004b; Dozier *et al*., 2007, 2008). The contents and compositions of dietary amino acids for modern broiler chickens may need further improvement. Therefore, expansion of the relevant knowledge of amino acid contents and compositions for broiler chickens is desirable (Dozier *et al*., 2008).

Lysine (Lys) is the second limiting amino acid in broiler chicken diets based on corn-soybean. An optimum Lys content of 1.09% higher than NRC levels was reported to improve FCR, breast meat yield, and BWG with low abdominal fat pad weight (Leclercq, 1998). The growth performance improved in broiler chickens fed with finisher diet supplemented with Lys (Leclercq, 1998; Razaei *et al*., 2004; Sterling *et al*., 2004). Hence, Lys may be considered as an important amino acid that increases meat production and efficiency of broiler chickens.

Threonine (Thr) is the third limiting amino acid in corn-soybean-based diet of chickens. BWG, FCR, and immune

Received: October 1, 2018, Accepted: November 19, 2018
Released Online Advance Publication: January 25, 2019
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function improved with an increase in Thr contents in the diet of broiler chickens (Leclercq, 1998; Kidd, 2000, 2001, 2004a; Ciftci and Ceylan, 2004; Corzo *et al*., 2009; Star *et al*., 2012; Mejia *et al*., 2012; Mohammad, 2013; Sigolo *et al*., 2017). In a former meta-analysis, 0.73% and 0.75% of Thr contents in the finisher diets, higher than the requirements of NRC (3–6 week 0.74% and 6–8 week 0.68%), demonstrated maximum BWG and FCR, respectively (Ahmadi *et al*., 2010). Thr requirements of broiler chickens are dependent on environmental conditions such as heat stress (Mohammad, 2013). Thus, Thr also serves as an important amino acid that may improve meat production in broilers, as observed with Lys.

While each amino acid improves the performance of chickens, limited information is available on the effect of the combination of high Lys and Thr in finisher diet on BWG, feed intake (FI), and FCR in broiler chickens. Everett *et al*. (2010) evaluated the effect of Lys (2 levels) and Thr (4 levels) factorial responses in commercial male broilers (28 to 42 days of age) and suggested that high levels of Lys and Thr in diet may improve some live performance parameters. In starting broiler chickens, the ideal ratio of digestible Thr to digestible Lys was 70% for BWG and 66% for FCR (Mehri *et al*., 2012). However, the appropriate Thr content at higher Lys levels in finisher diet of broiler chickens is unknown. The growth performance during finisher period is an important characteristic for meat production.

In the present study, we evaluated the effect of an optimal Thr-to-Lys ratio (68%) in high Lys diet on growth performance indicators such as BWG and FCR in modern broiler chickens during finisher period. We used metabolome analysis to examine the possible mechanism underlying the improvement in growth performance of broiler chickens.

Materials and Methods

Birds

One-day-old male broiler chickens (Ross 308 strain) obtained from a local hatchery (Mori breeding farm Co., Ltd, Koriyama, Japan) were used in the present study. The birds were housed in electrically-heated battery brooders and fed with a corn-soybean meal-based diet (basal diet; 220 g of crude protein [CP]/kg and 3.0 Mcal metabolizable energy [ME]/kg from 0 to 7 days of age and 200 g/kg of CP and 3.1 Mcal/kg of ME from 8 to 21 days of age) *ad libitum* for 21 days. Selected 21-day-old chickens had similar body weights to ensure body-weight uniformity and were individually reared in stainless-steel wire cages (one bird in one cage) in a temperature- (25℃) and light (23 h/day)-controlled room.

Table 1. **Test diets for the 21 to 38 d period (% as is basis)[1]**

Ingredients	Control	High Lys	High Lys + Thr
Corn	65.70	66.09	66.13
Soybean meal	22.52	22.52	22.52
Canola meal	3.00	3.00	3.00
Yellow grease	4.35	4.35	4.35
CaCO3	0.67	0.67	0.67
Ca3(PO4)2	1.66	1.66	1.66
NaCl	0.16	0.16	0.16
Choline Cl	0.03	0.03	0.03
Vitamins and minerals[2]	0.10	0.10	0.10
L-Lysine-HCl	0.12	0.75	0.75
DL-Methionine	0.25	0.25	0.25
L-Threonine	0.04	0.04	0.38
L-Gultamic acid[3]	1.40	0.38	—
Total	100	100	100
Nutrient analysis			
ME, Mcal/kg	3.2	3.2	3.2
CP	18.00	18.00	18.00
Ca	0.89	0.89	0.89
Available P	0.40	0.40	0.40
Lysine	1.00	1.50	1.50
Threonine	0.68	0.69	1.02
Arginine	1.12	1.12	1.12

[1] High Lys is the amount of 1.5% lysine in test diet. High Lys + Thr is the amount of 1.5% Lys and 1.02% Threonine in test diet (Thr / Lys=0.68).
[2] Premix provided the following per kilogram of diet: vitamin A (vitamin A acetate) 9,200 IU; cholecalciferol 4,200 IU; vitamin E (source unspecified) 15 IU; menadione, 2.9 mg; B12, 13 μg; choline, 38 mg; riboflavin, 6.7 mg; niacin, 50 mg; D-biotin, 0.13 mg; pyridoxine, 3.4 mg; manganese, 130 mg; zinc, 100 mg; iron, 40 mg; copper, 16 mg; iodine, 1.3 mg.
[3] Corrected using glutamic acid to satisfy CP of caluculation in each test feed.

All basal diets were formulated to contain essential nutrients that met or exceeded recommended levels (NRC, 1994). All procedures were approved by the Animal Care and Use Committee of the Tokyo University of Agriculture and Technology.

Experimental Diets and Blood Sampling

Eighteen chickens (21 day of age) in individual cages were randomly divided into three groups of six chickens, and each group was provided with one of three experimental diets for 17 days *ad libitum*. The experimental diets are shown in Table 1. The control diet was a corn-soybean meal-based diet containing 180 g/kg of CP and 3.2 Mcal/kg of ME supplemented with 100% essential amino acid contents recommended by NRC (1994), including Lys and Thr requirements of 10.0 and 6.8 g/kg of diet, respectively. The high Lys diet contained 150% of Lys recommended by NRC (1994). This additional level of 150% Lys was determined from the findings of our previous study (Kobayashi *et al*., 2011). Based on the results of our previous study, we included high Lys + Thr diet (0.68 of Thr/Lys), which was reported to optimize broilers' performance at 21 to 42 days of age (Kidd *et al*., 2004a). The CP level of high Lys and high Lys + Thr diets was maintained by the dietary addition of l-glutamic acid. All other nutrients such as essential amino acids, vitamins, and minerals were adjusted in three experimental diets as per NRC requirements.

Blood samples were collected from the wing vein of six chickens from each dietary group after treatment and plasma was stored at −80℃ until further analysis.

Metabolome Analysis of Plasma

Metabolite levels in plasma were measured using capillary electrophoresis time-of-flight mass spectrometry (CE-TOF/MS) system (Agilent Technologies Inc., Santa Clara, CA, USA). A fused silica capillary (50 μm × 80 cm) was used. Detected peaks were analyzed by an automatic integration software (MasterHands ver. 2. 16. 0. 15; Keio University, Tokyo, Japan).

Measurement of Free Amino Acids

The characteristic peaks of plasma amino acids were measured with metabolome analysis. The free amino acid levels in blood were measured as described by Imanari *et al*. (2007) using amino acid analyzer (JLC-500/V; JEOL, Tokyo, Japan). A multi-segment tandem packed column (LC-500 AC4016, Li type, 4 mm diameter × 160 mm; JEOL, Tokyo, JAPAN) was used. The detection wavelengths were 440 and 570 nm.

Measurement of Serotonin Concentrations in Plasma

Detailed analysis was conducted on plasma serotonin (5-hydroxytryptamine, 5-HT), as its concentration in the Lys group was more than the double of that in Lys + Thr group in metabolome analysis. 5-HT levels in blood were measured using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's guidelines (BA E-8900; ImmuSmol SAS, Pessac, France).

Statistical Analysis

The SPSS application software package was used for statistical calculations (PASW Statistics 18.0, IBM, NY 10504). The group data for multiple comparisons were analyzed with analysis of variance (ANOVA) using a general linear model procedure followed by Tukey-Kramer test. Results are expressed as mean ± standard deviation (SD). Statistical significance was interpreted as values of $P < 0.05$. Correlation analysis was performed between the factors measured in the present study.

Results

Growth Performance

BWG increased in chickens that received high Lys diet as compared with those receiving the control diet, while no significant difference was observed in BWG of high Lys + Thr and other two groups (Table 2). Although FI was unchanged among different treatment groups, both high Lys and high Lys + Thr diets resulted in an improvement in FCR (Table 2).

Free Amino Acid Concentration in Plasma

The Lys content in the plasma of chickens fed with high Lys or high Lys + Thr diets was higher than that in the plasma of the control group, while arginine (Arg) content was lower in the plasma of chickens fed with high Lys diet than in plasma of those fed with the control diet ($P = 0.074$) (Table 3). The concentration of Thr increased in the plasma of chickens fed with high Lys + Thr diet (Table 3). No significant differences were observed in the levels of other free amino acids in the plasma of chickens from the three groups.

Metabolome Analysis in Plasma

To elucidate the effect of high Lys and high Lys + Thr diets on plasma metabolites, we determined metabolite levels using the CE-TOF/MS system. A total of 149 compounds

Table 2. **Live performance measurements of male broiler as affected by high level of Lys and Lys + Thr administrered from 21–38 d of age**

	Control	High Lys	High Lys + Thr
Initial BW (g)	1010.8 ± 7.2	1012.7 ± 10.6	1013.6 ± 6.2
BWG (g)	1507.1 ± 153.3^{b}	1673.9 ± 39.9^{a}	1534.1 ± 124.1^{ab}
Feed intake (g)	3180.6 ± 292.4	3294.8 ± 65.1	3080.5 ± 210.2
FCR	1.686 ± 0.056^{b}	1.584 ± 0.044^{a}	1.609 ± 0.040^{a}

Values are means ± SD for 6 birds.
Values with different superscripts are significantly different (a, b, c $P < 0.05$)
Lys, lysine; Thr, threonine; BWG, body weight gain; FCR, feed conversion ratio.

Table 3. **Effect of high Lys diet and high Lys + Thr diet on free amino acid concentration in plasma on broilers (nmol/mL plasma)**

	Control	High Lys	High Lys + Thr
Thr	401.3±72.9[A]	334.9±103.1[A]	2585.0±772.4[B]
Ser	486.3±50.3[a]	551.8±118.8[ab]	673.6±89.8[b]
Glu	174.1±18.8	185.2±46.3	134.8±14.5
Gln	1151.3±180.6	1348.1±152.9	1378.5±96.6
Gly	592.3±42.0	557.1±136.6	613.7±76.7
Ala	1074.1±205.4	1068.4±234.6	991.0±159.5
Val	135.4±23.1	135.4±26.3	135.7±16.5
Met	82.5±8.4	98.7±13.1	94.9±19.8
Cys	50.5±2.6	49.0±3.6	48.9±7.8
Ile	65.9±15.2	70.4±19.9	67.4±11.9
Leu	275.0±33.5	257.5±33.5	277.8±23.8
Tyr	225.6±51.4	269.7±40.6	267.1±41.6
Phe	133.0±16.7	119.0±17.4	121.5±8.0
His	86.9±46.9	46.2±12.2	52.6±3.6
Lys	113.8±73.9[A]	636.9±177.5[B]	547.6±64.9[B]
Arg	313.0±102.3	199.6±72.7[†]	208.2±48.2

Values are means±SD, and values in each row with different susperscripts are significantly different at A, B $P<0.01$, or a, b $P<0.05$.
† tend to be lower than in the control group ($P=0.074$).
Ala, alanine; Arg, arginine; Cys, cystine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.

Table 4. **Classification of metabolites by fold-change**

	Fold-change of high Lys vs. control			Total	Fold-change of high Lys + Thr vs. control			Total
	<0.8 (down regulated)	0.8–1.2 (unchanged)	1.2< (up regulated)		<0.8 (down regulated)	0.8–1.2 (unchanged)	1.2< (up regulated)	
Number of metabolites	21	93	35	149	32	92	22	146
% of total metabolites	14.1	62.4	23.5	100	21.9	63.0	15.1	100

Table 4. **(continued)**

	Fold-change of high Lys + Thr vs. high Lys			Total
	<0.8 (down regulated)	0.8–1.2 (unchanged)	1.2< (up regulated)	
Number of metabolites	36	99	13	148
% of total metabolites	24.3	66.9	8.8	100

Chicks were fed the control, high Lys or high Lys + Thr diet from 21 to 38 days old.
All samples in each group were pooled.
Metabolites are classified into three groups: up-regulated (>1.2 -fold), down-regurated (<0.8-fold) and unchanged (0.8–1.2).

were detected in the plasma of each group; these compounds were divided into three groups as follows: upregulated (>1.2-fold), downregulated (<0.8-fold), and unchanged (0.8–1.2) (Table 4). The extracted metabolites changed more than 1.5 times or less than 0.7 times among different groups (Table 5). General Lys metabolites such as 2-aminoadipic acid, β-alanine, and pipecolic acid increased in the plasma of broilers fed with high Lys or high Lys + Thr diets as compared with the plasma of broilers from the control group. The metabolites related to urea cycle, including Arg, ornithine, citrulline, urea, and uric acid, decreased in the high Lys group as compared with the control group. The metabolites of creatine pathway also showed changes in response to high Lys or high Lys + Thr diet. Characteristic changes in the plasma serotonin concentrations were detected (Table 5). The analysis of plasma serotonin level was confirmed by ELISA. Serotonin content significantly increased in the plasma of chickens fed with high Lys diet as compared with those from the control and high Lys + Thr groups (Fig. 1).

Correlation Analysis between Serotonin and Other Factors

Similar experiment was conducted to evaluate the correlation between plasma serotonin concentration and BWG, FI,

Table 5. **Effect of high Lys or high Lys + Thr diets on detected metabolites in blood of broilers at 38 days**

Compound name	Relative Area			Comparative analysis (fold-change)		
	Control	High Lys	High Lys + Thr	High Lys vs. control	High Lys + Thr vs. control	High Lys + Thr vs. high Lys
Lysine metabolites						
Lysine	3.49×10^{-2}	2.05×10^{-1}	1.74×10^{-1}	5.9	5.0	0.8
2-Aminoadipic acid	2.98×10^{-4}	9.04×10^{-4}	7.53×10^{-4}	3.0	2.5	0.8
Pipecolic acid	1.26×10^{-3}	2.98×10^{-3}	2.91×10^{-3}	2.4	2.3	1.0
N6-Acetyllysine	1.41×10^{-3}	3.50×10^{-3}	3.76×10^{-3}	2.5	2.7	1.1
N-Acetyllysine	2.77×10^{-4}	4.85×10^{-4}	5.25×10^{-4}	1.8	1.9	1.1
Carboxymethyllysine	3.36×10^{-4}	3.06×10^{-4}	3.83×10^{-4}	0.9	1.1	1.3
Metabolites increased more than ≧ 1.5 fold-change						
Specificallys in high Lys vs. control						
Serotonin	2.10×10^{-4}	8.89×10^{-4}	6.47×10^{-5}	4.2	0.3	0.1
Ethanolamine	1.05×10^{-3}	1.63×10^{-3}	1.18×10^{-3}	1.5	1.1	0.7
Specificallys in high Lys + Thr vs. control						
Threonine	9.63×10^{-2}	8.46×10^{-2}	5.66×10^{-1}	0.9	5.9	6.7
2-Hydroxy-4-methylvaleric acid	2.38×10^{-4}	1.78×10^{-4}	3.47×10^{-4}	0.7	1.5	2.0
Common high Lys and high Lys + Thr vs. control						
β-Alanine	7.13×10^{-3}	1.55×10^{-2}	1.11×10^{-2}	2.2	1.6	0.7
Homocitrulline	7.33×10^{-5}	1.08×10^{-4}	1.12×10^{-4}	1.5	1.5	1.0
Metabolites increased less than ≦ 0.7 fold-change						
Specificallys in high Lys vs. control						
2-Hydroxy-4-methylvaleric acid	2.38×10^{-4}	1.78×10^{-4}	3.47×10^{-4}	0.7	1.5	2.0
Ornithine	5.29×10^{-3}	3.90×10^{-3}	4.30×10^{-3}	0.7	0.8	1.1
Citrulline	1.59×10^{-3}	1.15×10^{-3}	1.44×10^{-3}	0.7	0.9	1.3
Uric acid	1.93×10^{-2}	1.39×10^{-2}	1.52×10^{-2}	0.7	0.8	1.1
2-Hydroxyisobutyric acid	3.78×10^{-4}	2.56×10^{-4}	3.68×10^{-4}	0.7	1.0	1.4
Taurocholic acid	4.37×10^{-4}	2.41×10^{-4}	4.28×10^{-4}	0.6	1.0	1.8
3-Methylhistidine	1.60×10^{-2}	7.55×10^{-3}	6.52×10^{-3}	0.5	0.4	0.9
Specificallys in high Lys + Thr vs. control						
N-Acetylmuramic acid	2.69×10^{-4}	2.29×10^{-4}	1.84×10^{-4}	0.8	0.7	0.8
Sulfotyrosine	1.61×10^{-4}	1.37×10^{-4}	1.08×10^{-4}	0.9	0.7	0.8
Ectoine	4.88×10^{-4}	4.46×10^{-4}	3.21×10^{-4}	0.9	0.7	0.7
γ-Butyrobetaine	8.05×10^{-4}	7.43×10^{-4}	5.19×10^{-4}	0.9	0.6	0.7
Diethanoiamine	3.64×10^{-4}	3.50×10^{-4}	2.30×10^{-4}	1.0	0.6	0.7
Nicotinamide	9.74×10^{-4}	9.71×10^{-4}	5.95×10^{-4}	1.0	0.6	0.6
Hypoxanthine	1.32×10^{-3}	1.50×10^{-3}	7.77×10^{-4}	1.1	0.6	0.5
Taurine	1.30×10^{-2}	1.50×10^{-2}	6.25×10^{-3}	1.2	0.5	0.4
3-Methylhistidine	1.60×10^{-2}	7.55×10^{-3}	6.52×10^{-3}	0.5	0.4	0.9
Serotonin	2.10×10^{-4}	8.89×10^{-4}	6.47×10^{-5}	4.2	0.3	0.1
Common to high Lys and high Lys + Thr vs. control						
2-Hydroxyglutaric acid	3.20×10^{-4}	2.28×10^{-4}	2.11×10^{-4}	0.7	0.7	0.9
Arginine	1.19×10^{-1}	8.04×10^{-2}	8.18×10^{-2}	0.7	0.7	1.0
Dyphylline	3.56×10^{-3}	2.64×10^{-3}	1.69×10^{-3}	0.7	0.5	0.6
Glyceric acid	2.00×10^{-3}	1.46×10^{-3}	1.18×10^{-3}	0.7	0.6	0.8
Glycerol	1.20×10^{-2}	8.64×10^{-3}	4.70×10^{-3}	0.7	0.4	0.5
Creatine	1.35×10^{-2}	9.69×10^{-3}	5.39×10^{-3}	0.7	0.4	0.6
Carnitine	2.30×10^{-3}	1.61×10^{-3}	1.20×10^{-3}	0.7	0.5	0.7
Xanthine	3.64×10^{-4}	2.42×10^{-4}	2.09×10^{-4}	0.7	0.6	0.9
Lauric acid	1.73×10^{-3}	1.08×10^{-3}	1.28×10^{-3}	0.6	0.7	1.2
Urea	8.06×10^{-3}	4.83×10^{-3}	4.31×10^{-3}	0.6	0.5	0.9
Histidine	2.73×10^{-2}	1.30×10^{-2}	1.45×10^{-2}	0.5	0.5	1.1
Metabolites increased more than twice in high Lys + Thr vs. high Lys						
Threonine	9.63×10^{-2}	8.46×10^{-2}	5.66×10^{-1}	0.9	5.9	6.7
Metabolites decreased to less than half in high Lys + Thr vs. high Lys						
Taurine	1.30×10^{-2}	1.50×10^{-2}	6.25×10^{-3}	1.2	0.5	0.4
Serotonin	2.10×10^{-4}	8.89×10^{-4}	6.47×10^{-5}	4.2	0.3	0.1

Chicks were fed the high Lys or high Lys + Thr diet from 21 to 38 days.
All samples in each group were pooled (pooled by $n=6$).
Fold-change is the ratio of the reactive areas (between 2 groups in each group)
The metabolite of up-regulated or down-regulated was shown separately except for general lysine metabolites.

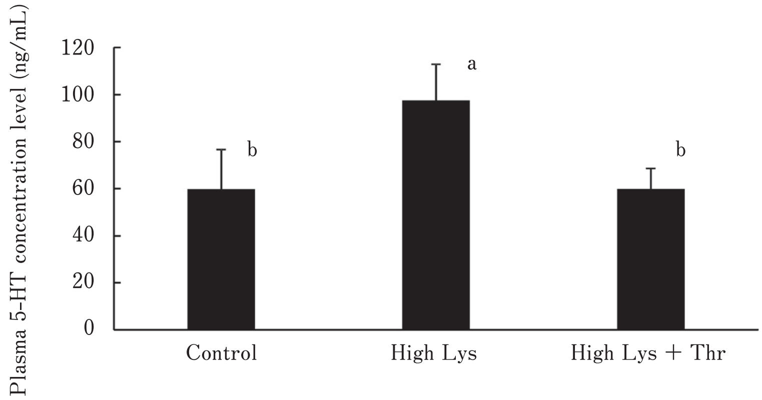

Fig. 1. **Effect of high Lys and high Lys + Thr diets on plasma serotonin (5-hydroxytryptamine, 5–HT) concentrations in broilers at 38 days.** Bars indicate the SD of the mean ($n=6$). Different superscripts indicate significant differences, $P<0.01$.

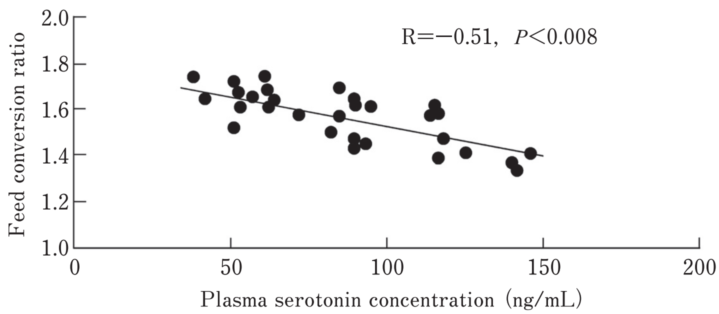

Fig. 2. **Correlation analysis between plasma serotonin concentration and feed conversion ratio in broilers at 38 days.** The treatment protocol is described in Materials and Methods ($n=30$).

and FCR ($n=30$). A negative correlation was detected between serotonin level and FCR ($P<0.008$, $r=-0.51$) (Fig. 2). No significant correlation was observed between other factors and plasma serotonin concentration.

Discussion

Lys is well known as the second limiting amino acid in corn-soybean-based diets. Here, we demonstrate that the supplementation of chicken diets with Lys at 15 g/kg diet concentration, which is 150% of NRC requirement (high Lys diet in the present study) resulted in an improvement in BWG and FCR (Table 2). These results suggest that dietary supplementation with Lys increases the meat production in birds. The supplementation of Lys in diet as per the NRC requirement may improve BWG, FI, and FCR in broiler chickens (Leclercq, 1998; Razaei *et al.*, 2004; Sterling *et al.*, 2004). It was reported that 128% Lys content in finisher diet of broilers significantly increased BWG as compared with supplementation with 100% Lys in 28 to 42-day-old chicken (Everett *et al.*, 2010). However, no report has evaluated the effect of 150% Lys content (high Lys content) in finisher diet on the performance of broiler chickens, while no significant improvement was observed in the broiler chickens fed with a diet containing 150% Lys at 14–24 days of age.

The present study shows that the increase in Thr content in high Lys diet negatively affected the performance of broilers, although Thr is the third limiting amino acid in corn-soybean-based diets in finisher period. The ratio of Thr and Lys in diet is an important factor that determines the performance of broilers, and the broilers between 21 and 42 days of age reguire 68% of digestible Thr to Lys ratio (Kidd *et al.*, 1997; Mejia, 2012). We, therefore, hypothesized that high Lys + Thr diet with 68% of Thr and Lys ratio may improve the performance of broilers as compared with control and high Lys diets. The present findings, however, show that BWG in high Lys + Thr group was not significantly different from BWG in the control group, while FCR was significantly lower in the chickens fed with high Thr + Lys diet than in those fed with control diet. This result suggests that the optimal Thr-to-Lys ratio (68%) in finisher diet of broiler chickens was not the appropriate ratio for maximal production, especially for BWG, in high Lys diet. Therefore, this is the first study to demonstrate that Thr supplementation at 68% of Thr/Lys in finisher diets may be insufficient to improve BWG of broilers. Further experiments evaluating the effects of dose-dependent supplementation of Thr in high Lys diet may allow elucidation of optimum Lys and Thr contents in finisher diet to support maximum performance of broilers.

We performed metabolome analysis of the plasma obtained from the chickens fed with high Lys and high Lys + Thr diets. Although high Lys and high Lys + Thr diets induced changes in several metabolites, a characteristic change was detected in the metabolite serotonin. In mammals, there are two independent serotonin systems: one in the central nervous system and the other in the periphery. Serotonin affects feeding behaviors and obesity in the central nervous system (Leibowitz and Alexander, 1998). In contrast, the role of peripheral serotonin has not been clarified; peripheral serotonin is thought to play an important role in the regulation of glucose and lipid metabolism (Kim *et al*., 2011; Watanabe *et al*., 2016). Serotonin injection in the hypothalamus of chickens increases FI values (Tachibana *et al*., 2001). Whether peripheral serotonin, particularly plasma serotonin, affects glucose and lipid metabolism as well as FCR in broiler chickens is unknown. A recent study showed that supplementation of broiler chicken diet with tryptophan may improve FCR under chronic and unpredictable stress, probably owing to serotonergic metabolism (Yue *et al*., 2017). Therefore, the metabolic role of peripheral serotonin has been identified in broilers in the present study. High plasma serotonin concentration detected in the chickens fed with high Lys diet (Fig. 1) correlated with FCR. These results suggest that plasma serotonin may be involved in FCR of broiler chickens during finisher period. This result indicates the possibility that plasma serotonin concentration may serve as a biomarker for the improvement in FCR of broiler chickens.

In the present study, Lys metabolites such as 2-aminoadipic acid, β-alanine, and pipecolic acid increased in the blood of chickens fed with high Lys diet. Similar changes in metabolites were observed in the muscle of chickens fed with high Lys diet (Watanabe *et al*., 2015). The fold changes in metabolites were higher in the muscles than in blood of chickens from control and high Lys diet groups. Hence, the changes in the metabolites of Lys were similar in the muscle and blood of chickens fed with high Lys diet.

Lys-Arg antagonism is induced by feeding chickens with high Lys diet (Austic and Scott, 1970; Austic and Nesheim, 1975). The optimum Lys content per Arg content in finisher diet of broilers is 1.18 (Labandan *et al*., 2001). Therefore, high Lys diet may likely induce negative effects on the performance of broilers, resulting in Lys-Arg antagonism. However, the contents of Lys, which was 150% of NRC requirement, in finisher diets improved the performance of broilers as compared with the Lys content in the control diet. In addition, the metabolites related to urea cycle, including Arg, ornithine, citrulline, urea, and uric acid, tended to decrease in high Lys group as compared with control group (Table 5). Here we suggest that high Lys diet, at least 150% of NRC requirement, failed to cause Lys-Arg antagonism or affect the live performance of modern broiler chickens.

In conclusion, Lys supplementation, particularly at 150% of NRC requirement, enhanced the performance in finisher period of broiler chickens, demonstrating that high Lys diet may be a useful component in poultry industry. In contrast, Thr may not be a valuable supplement to improve meat production with finisher diet supplemented with high Lys content. The present study reveals for the first time that plasma serotonin concentration regulates FCR of broiler chickens, suggesting that plasma serotonin may serve as a novel biomarker for the improvement of FCR in chickens.

Acknowledgments

We would like to thank the laboratory members for their help with the study.

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