

Effects of Dietary Methionine or Arginine Levels on the Urinary Creatinine Excretion in Broiler Chicks

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Two experiments were conducted to evaluate the usefulness of urinary creatinine levels as a criterion for the estimation of protein and amino acid requirements in poultry. Here we studied the effects of dietary precursor levels of creatinine, methionine and arginine, on urinary creatinine excretion in experiments. Both experiments used 15 Chunky broilers chicks that were 8 days old. The chicks were assigned to three dietary groups, with five chicks each, and were fed an experimental diet for 7 days. The experimental diets mainly consisted of corn and soybean meal, and contained deficient, adequate, or excessive methionine and arginine levels in experiments 1 and 2, respectively. Excreta were collected for the last 3 days of the feeding trial, and chicks were terminated by dislocation of the neck at the end of the feeding trial to collect their livers. Creatinine concentration in the excreta and hepatic L-arginine-glycine amidinotransferase (AGAT) activities were determined.

Urinary creatinine levels increased with increasing both dietary methionine and arginine levels from deficient to adequate recommended by Japanese feeding standard ($P < 0.05$), and then remained constant in experiments 1 and 2, respectively. The hepatic AGAT activity decreased when both dietary creatinine precursors levels were increased from deficient to adequate levels ($p < 0.05$), and then remained constant.

These results suggested that creatinine excretion was changed with both increasing dietary methionine and arginine, dose-dependently.

Key words: arginine, broiler chicks, creatinine excretion, methionine

J. Poult. Sci., 54: 167-172, 2017

Introduction

In laying hens, a switch in the dietary composition is important at every life stage. Furthermore, the environmental factors affect the physiological condition of poultry and influence their energy and protein requirements (Geraert *et al.*, 1996; Valdkamp *et al.*, 2000). Therefore, it is necessary to revise the dietary energy protein ratio according to environmental changes such as heat or cold stress.

In dairy cattle, the nutritional status is usually monitored using the lactating quantity and quality as a criterion because the genetic diversity of cows is relatively large and the lactating periods are not the same among individual cows (DePeters and Cant, 1992; Ikuta *et al.*, 2000).

However, for poultry it is difficult to judge how daily changes in the nutritional status are affected by environmental stress because the effects are observed the next day on egg

production and blood sampling is too stressful for poultry. The final amino acid product levels in the urine are non-invasive parameters which can be used to estimate the protein requirement. With this criterion, it is also possible to test the same animals repeatedly. Therefore, it is useful for monitoring the nutritional condition.

Creatinine is one of the final amino acid products in the urine (Fig. 1). Creatinine has a precursor, creatine which mainly exists in the skeletal and cardiac muscle tissue and supplies phosphate to ADP. Creatine biosynthesis needs two enzymes, L-arginine-glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase. The first of two steps in the creatine biosynthesis is rate-limiting step and catalyzed by AGAT. Creatine is synthesized from the following three amino acids: arginine, glycine, and methionine (Walker, 1979; Wyss and Kaddurah-daouk, 2000; Brosnan, 2011). These amino acids are essential amino acids for poultry. Methionine, in particular, is a primary source of methyl groups, and it is liable to be first limited amino acid. Arginine is used synthesizing ornithine, NO, and polyamine. Excess arginine in a diet decreases the growth rate of broiler chicks, and a growth depression can be alleviated by sup-

Received: August 17, 2016, Accepted: November 29, 2016

Released Online Advance Publication: December 25, 2016

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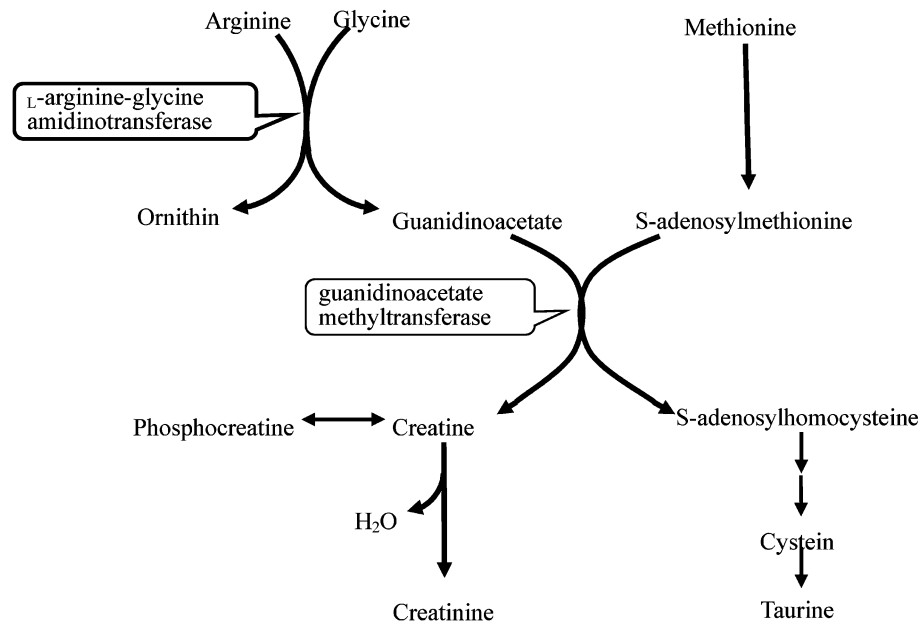


Fig. 1. Metabolic pathway of creatinine.

plementing methionine (Keshavarz and Fuller, 1971a, b).

Chamruspollert *et al.* (2002b) reported that dietary arginine and methionine levels influenced muscle creatine levels in broiler chicks. Furthermore, Chamruspollert *et al.* (2002a) suggested that muscle creatine levels can be used as a criterion to assess arginine requirement. Creatinine is non-enzymatically converted from creatine and excreted in the urine without any resorption. It indicated that creatinine excretion might respond to muscle creatine levels or creatine synthesis. Thus, creatinine excretion might reflect dietary its precursors levels.

In order to judge whether creatinine excretion will be criterion for estimation of amino acid and protein requirements or not, at first, the responses of creatinine excretion to dietary methionine and arginine were made sure in presented study.

Materials and Methods

Animals

For both experiments, 8-day-old Chunky broilers chicks were used. They were hatched in our laboratory, and grouped a stock diet that contained 3,200 kcal metabolizable energy (ME)/kg diet and 23% crude protein (CP), until 8 days of age.

When they were 8 days old, the chicks were divided into three experimental dietary groups, with five male chicks with an average body weights per group. The chicks were housed in different pens based on group for 7 days and fed three levels of methionine (0.25%, 0.50%, and 0.75%) or arginine (0.85%, 1.44%, and 2.04%), with water *ad libitum*. At 11 days of age, chicks were divided into metabolic cages (30 × 50 × 25) individually. Excreta were collected twice daily

and pooled for the last 3 days of feeding trial.

At the end of feeding trial, chicks were terminated by dislocating their necks to collect the livers for analyze the hepatic AGAT activities, and then terminal ileal contents for analyze the creatinine concentration. Three centimeter from a boundary with the colon was defined as a terminal ileum.

Housing, handling, feeding and killing procedures were in accordance with policies of Nippon veterinary and life science university committee on laboratory animal care.

Diets

Diets for both experiments mainly consisted of corn and soybean meal and contained 3,200 kcal ME/kg of diet and 16.2% CP in experiment 1 (Table 1 and 2) and 20.7% of CP in experiment 2 (Table 3 and 4), respectively. Diets experiment 1 was based on Ohta and Ishibashi (1994; 1997a; 1997b). Because materials of arginine limiting diet were different form materials of methionine limiting diets, in experiment 2, dietary composition was different from experiment 1. The experimental diets included three levels of methionine (0.25%, 0.50%, and 0.75%; experiment 1) or arginine (0.85%, 1.44%, and 2.04%; experiment 2), representing a range from deficient to excessive levels in both experiments (Table 2 and 4).

Crystalline amino acids were added, if the essential amino acid in the feed was deficient. Other nutrients were added to be not less than Japanese feeding standard for poultry (2011).

Analysis of Creatinine Concentration in the Excreta

In both experiments, the collected excreta were pooled for 3 days into 3% sulfosalicylic acid solution and homogenized. Five ml of homogenate was separated into tube and centrifuged at 3,000 rpm for 10 min. The supernatant was analyzed for creatinine concentration using a commercially avail-

able assay kit (Cayman Chemical Company, Michigan, USA) that is based on the Jaffe' reaction.

Analysis of Hepatic AGAT Activity

The hepatic AGAT activity was measured as described by Van Pilsum *et al.* (1970). In this method, one unit of hepatic AGAT activity is defined as the amount of enzyme that catalyzes the formation of 1 μ mol of l-ornithine /h at 37C.

Statistical Analysis

Statistically significant differences among the treatments were determined by one-way ANOVA using General Linear Model procedure of SAS software (SAS Institute, 2001).

Table 1. Composition of experimental diets in experiment 1 (%)

Ingredient	
Corn	74.20
Soybean meal (44% CP)	17.15
Soybean oil	2.80
Vitamin mineral premix ¹	0.25
Calcium carbonate	0.50
Calcium phosphate tribasic	2.20
Choline chloride	0.20
Sodium chloride	0.40
Amino acids ²	2.30
Total	100.00
Metabolizable energy (Mcal/kg)	3.20
CP (%)	16.18
Methionine (%)	0.26-0.75

¹Supplies per kilogram of diet: vitamin A, 6,500,000 I.U.; vitamin D₃, 2,500,000 I.U.; dl- α -tocopherol acetate 40,000 mg; menadion sodium bisulfite, 3,836 mg; thiamine-HNO₃, 2,000 mg; riboflavin, 4,500 mg; pyridoxine-HCl, 2,000 mg; cyanocobalamin, 10 mg; Ca-pantothenate, 7,500 mg; nicotinic acid, 30,000 mg; folic acid, 1,000 mg; d-biotin, 75 mg; FeSO₄, 54,400 mg; MnSO₄, 137,450 mg; ZnSO₄, 123,450 mg; CuSO₄, 18,840 mg; Ca(I₃)₂, 768 mg.

²See Table 2.

When differences among means were found, means were separated using the Turkey's multiple range test. The level of significance was based on $p < 0.05$, unless stated otherwise.

Results

In experiment 1, body weight gains increased with increasing dietary methionine levels from 0.25% to 0.50% ($p < 0.05$), and then remained constant (Table 5). There were no significant differences in feed intakes among three dietary groups. The feed efficiency was higher in chicks fed a 0.50% methionine diet than in those fed 0.25% ($p < 0.05$; Table 5). But chicks fed a 0.75% methionine diet was no significant differences with these fed a 0.25% and 0.50% diets.

Table 3. Composition of experimental diets in experiment 2 (%)

Ingredient	
Corn	62.40
Corn starch	5.03-5.50
Corn gluten	14.00
Soybean meal (44% CP)	8.00
Soybean oil	1.00
Vitamin mineral premix ¹	0.25
Calcium carbonate	1.20
Calcium phosphate tribasic	2.40
Choline chloride	0.25
Sodium chloride	0.30
Amino acids ²	3.35-3.38
Total	100.00
Metabolizable energy (Mcal/kg)	3.19
CP (%)	20.70
Arginine (%)	0.85-2.04

¹ See Table 1.

² See Table 4.

Table 2. Amino acid composition of mixtures and diets in experiment 1 (%)

Amino acid	Amino acid mixture	Experimental diet
Arginine	0.43	1.37
Histidine	0.00	0.35
Lysine	0.57	1.14
Isoleucine	0.11	0.76
Leucine	0.00	1.42
Valine	0.00	0.79
Tryptophan	0.05	0.22
Phenylalanine + Tyrosine	0.00	1.29
Threonine	0.16	0.76
Glycine + Serine	0.04	1.43
Cystine	0.14	0.40
Methionine	0.00-0.49	0.26-0.75
Glutamic acid	0.80-0.31	
Total	2.30	

Table 4. Amino acid composition of mixtures and basal diet in experiment 2 (%)

Amino acid	Amino acid mixture	Experimental diet
Histidine	0.00	0.35
Lysine	0.85	1.20
Isoleucine	0.06	0.80
Leucine	0.00	1.35
Valine	0.00	0.82
Tryptophan	0.09	0.23
Phenylalanine + Tyrosine	0.00	1.34
Threonine	0.14	0.80
Methionine + Cystine	0.13	0.93
Arginine	0.00-1.20	0.85-2.04
Glutamic acid	2.11-0.88	
Total	3.38-3.35	

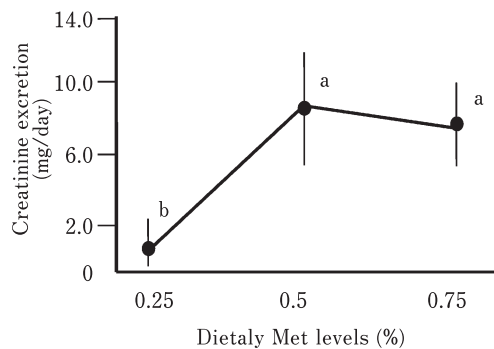


Fig. 2. Effects of dietary methionine (Met) levels on the creatinine excretion of 12 to 14-day-old broilers (experiment 1). Values are the means \pm SD for 5 chicks. ^{a,b}Means with no common letters differ significantly ($p < 0.05$).

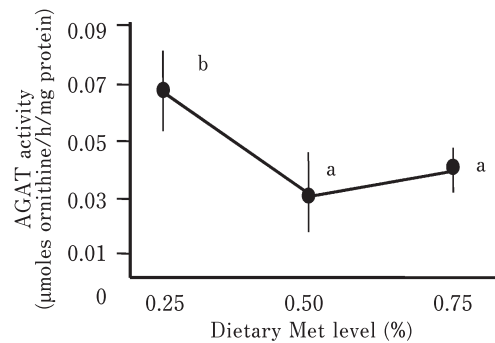


Fig. 3. Effects of dietary methionine (Met) levels on the hepatic L-arginine-glycine amidinotransferase (AGAT) activity of 14 day-old broilers (experiment 1). Values are means \pm SD of 5 chicks. ^{a,b}Means with no common letters differ significantly ($p < 0.05$).

Urinary creatinine excretion increased with increasing dietary methionine levels from 0.25% to 0.50% ($p < 0.05$), and subsequently remained constant (Fig. 2). On the other hand, the hepatic AGAT activity decreased with increasing dietary methionine levels from 0.25% to 0.50% ($p < 0.05$), and then remained constant (Fig. 3).

In experiment 2, body weight gains increased with increasing dietary arginine levels from 0.85% to 1.44% ($p < 0.05$), and then remained constant (Table 6). There were no significant differences in feed intakes among three the dietary groups. The feed efficiency was higher in chicks fed a 1.44% arginine diet than in those fed 0.85% and ($p < 0.05$; Table 6). But chicks fed a 2.04% arginine diet was no significant differences with those fed a 0.85% and 1.44% diets. Urinary creatinine excretion increased with increasing dietary arginine levels from 0.85% to 1.44% ($p < 0.05$), and subsequently remained constant (Fig. 4). The hepatic AGAT activity decreased with increasing dietary arginine levels from 0.85% to 1.44% ($p < 0.05$), and then remained constant (Fig. 5).

In both experiments, creatinine concentration of terminal ileal contents was not detected.

Discussion

The present study was conducted to evaluate the usefulness of urinary creatinine excretion as a criterion for the protein requirement in poultry. The effects of dietary precursor levels of creatine (i.e., methionine and arginine) on urinary creatinine excretion were studied in two experiments.

In both experiments, urinary creatinine excretion increased with increasing dietary creatine precursor levels from deficient to adequate ($p < 0.05$) and then remained constant. In contrast, hepatic AGAT activities decreased with increasing dietary creatine precursor levels from deficient to adequate ($p < 0.05$) and then remained constant.

In both experiments, creatinine concentration of terminal ileal contents was not detected. Therefore, creatinine in excreta was considered to be derived from urine. However, Karasawa and Maeda (1994) indicated the back-flow of urine from the croaca into the caecum. In the present results, it

Table 5. Effects of dietary methionine (Met) levels on performance of broilers from 8 to 14 days of age in Experiment 1

Met (%)	Body weight gain (g/day)	Feed intake	Feed efficiency (gain : feed)
0.25	18.0±5.4 ^b	42.5±1.6	0.34±0.07 ^b
0.50	28.3±4.8 ^a	50.3±5.1	0.46±0.05 ^a
0.75	26.9±5.0 ^a	50.9±2.7	0.44±0.08 ^{ab}

Values are means±SD for 5 chicks.

^{a,b} Means in the same column with no common superscripts differ significantly ($p<0.05$).

Table 6. Effects of dietary arginine (Arg) levels on performance of broilers from 8 to 14 days of age in Experiment 2

Met (%)	Body weight gain (g/day)	Feed intake	Feed efficiency (gain : feed)
0.85	16.1±6.2 ^b	48.7±7.9	0.32±0.10 ^b
1.44	29.0±3.3 ^a	58.1±4.3	0.53±0.11 ^a
2.04	22.4±5.6 ^a	55.5±4.6	0.41±0.13 ^{ab}

Values are means±SD for 5 chicks.

^{a,b} Means in the same column with no common superscripts differ significantly ($p<0.05$).

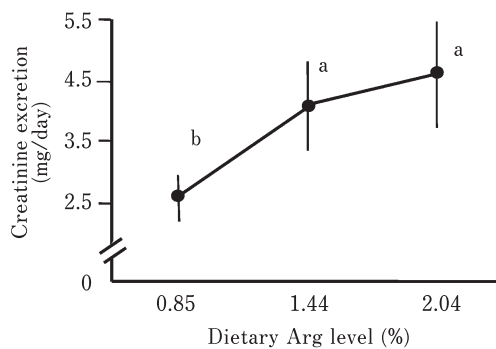


Fig. 4. Effects of dietary arginine (Arg) levels on the urinary creatinine excretion 12 to 14-day-old broilers (experiment 2). Values are means±SD of 5 chicks. ^{a,b} Means with no common letters differ significantly ($p<0.05$).

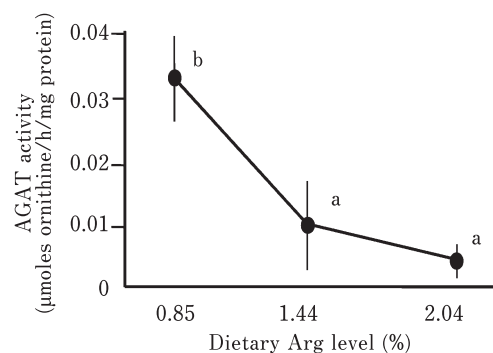


Fig. 5. Effects of dietary arginine (Arg) levels on chick hepatic L-arginine-glycine amidinotransferase (AGAT) activities of 14-day-old broilers (experiment 2). Values are means±SD of 5 chicks. ^{a,b} Means with no common letters differ significantly ($p<0.05$).

cannot be discussed whether intestinal bacteria in caecum influence the urinary creatinine or not.

Chamruspollert *et al.* (2002a) demonstrated that breast muscle creatine was affected by dietary arginine levels in broiler chicks. Creatine is irreversibly converted to creatinine, and creatinine is excreted in the urine without reabsorption (Walker, 1979; Wyss and Kaddurah-daouk, 2000; Brosnan, 2011). Accordingly, we hypothesized that urinary creatinine excretion would denote synthesized creatine levels.

When methionine or arginine was present in deficient in the diet, methionine or arginine might become limiting amino acid for creatine synthesis. Therefore, AGAT activities were high to meet the creatine demand. Because supply of crea-

tine precursor was low, creatine synthesis and urinary creatinine excretion were low.

In the 0.50% methionine or 1.44% arginine diet groups, the chicks' methionine or arginine requirements were satisfied; therefore, the AGAT activity was lower than that of the 0.25% methionine or 0.85% arginine diet groups. Because the supply of creatine precursor was satisfied, creatinine excretion increased.

When methionine or arginine was present in excess in the diet, other creatine precursors might become limiting amino acid for creatine biosynthesis. Therefore, no changes were observed in creatinine excretion and AGAT activities com-

pared with the sufficient methionine or arginine diet groups. Excess methyl groups of methionine or arginine might be metabolized by different pathways (Bertolo and McBreairty, 2013; Cynober *et al.*, 1995).

These results suggested that creatinine excretion was changed with both increasing dietary methionine and arginine, dose-dependently. The fact indicated that it is valuable to study the dose response of creatinine excretion to dietary protein and other amino acid levels furthermore.

References

- Agriculture, forestry, and fisheries research council secretariat ministry of agriculture, forestry and fisheries. Japanese Feeding Standard for Poultry. pp. 16–17. Central Association of Livestock Industry, Tokyo, 2011.
- Bertolo RF and McBreairty LE. The nutritional burden of methylation reactions. *Current Opinion in Clinical Nutrition and Metabolic Care*, 16: 102–108. 2013.
- Brosnan JT. The metabolic burden of creatine synthesis. *Amino Acids*, 40: 1325–1331. 2011.
- Chamruspollert M, Pesti GM and Bakalli RI. The influence of labile dietary methyl donors on the arginine requirement of young broiler chick. *Poultry Science*, 81: 1142–1148. 2002a.
- Chamruspollert M, Pesti GM and Bakalli RI. Dietary interrelationships among arginine, methionine, and lysine in young broiler chicks. *British Journal of Nutrition*, 88: 655–660. 2002b.
- DePeters EJ and Cant JP. Nutritional factors influencing the nitrogen composition of bovine milk: A review. *Journal of Dairy Science*, 75: 2043–2070. 1992.
- Geraert PA, Padilha CF and Guillaumin S. Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: growth performance, body composition and energy retention. *British Journal of Nutrition*, 75: 195–204. 1996.
- Ikuta K, Kokamo M, Sasakura K and Hakogi E. Evaluation of nutritional condition on the basis of milk urea nitrogen and milk protein concentrations in dairy cows. *Journal of the Japan Veterinary Medical Association*, 53: 289–292. 2000.
- Karasawa Y and Maeda M. A role of caecum in nitrogen nutrition of the chicken fed moderate protein diet and low protein diet plus urea. *British Poultry Science*, 35: 383–391. 1994.
- Keshavarz K and Fuller HL. Relationship of arginine and methionine in the nutrition of the chick and the significance of creatine biosynthesis in their interaction. *Journal of Nutrition*, 101: 217–222. 1971a.
- Keshavarz K and Fuller HL. Relationship of arginine and methionine to creatine formation in chicks. *Journal of Nutrition*, 101: 855–862. 1971b.
- Morris SM Jr. Enzymes of arginine metabolism. *Journal of Nutrition*, 134: 2743–2747. 2004.
- Ohta Y and Ishibashi T. Dietary levels and ratio of methionine and cysteine for maximum performance of Broilers. *Japanese Poultry Science*, 31: 369–380. 1994.
- Ohta Y and Ishibashi T. Effects of days after changing diets, body weight and feed consumption on excretion of taurine in broilers. *Japanese Poultry Science*, 34: 282–291. 1997a.
- Ohta Y and Ishibashi T. Alleviatory effects of individual essential amino acids on reduced performance induced by dietary excess methionine in broilers. *Japanese poultry science*, 34: 414–418. 1997b.
- SAS Institute Inc., SAS[®] User's Guide Statistics. Version 8.2 Edition. SAS Institute Inc. Cary. NC., 2001.
- Van Pilsom JF, Taylor D, Zakis B and McCormick P. Simplified assay for transaminase activities of rat kidney homogenates. *Analytical Biochemistry*, 35: 277–286. 1970.
- Veldkamp T, Ferket PR, Kwakkel RP, Nixey C and Noordhuizen JPTM. Interaction between ambient temperature and supplementation of synthetic amino acids on performance and carcass parameters in commercial male turkeys. *Poultry Science*, 79: 1472–1477. 2000.
- Walker JB. Creatine: biosynthesis, regulation and function. *Advances in Enzymology and Related Areas of Molecular Biology*, 50: 177–242. 1979.
- Wyss M and Kaddurah-daouk R. Creatine and Creatinine metabolism. *Physiological Reviews*, 80: 1107–1213. 2000.