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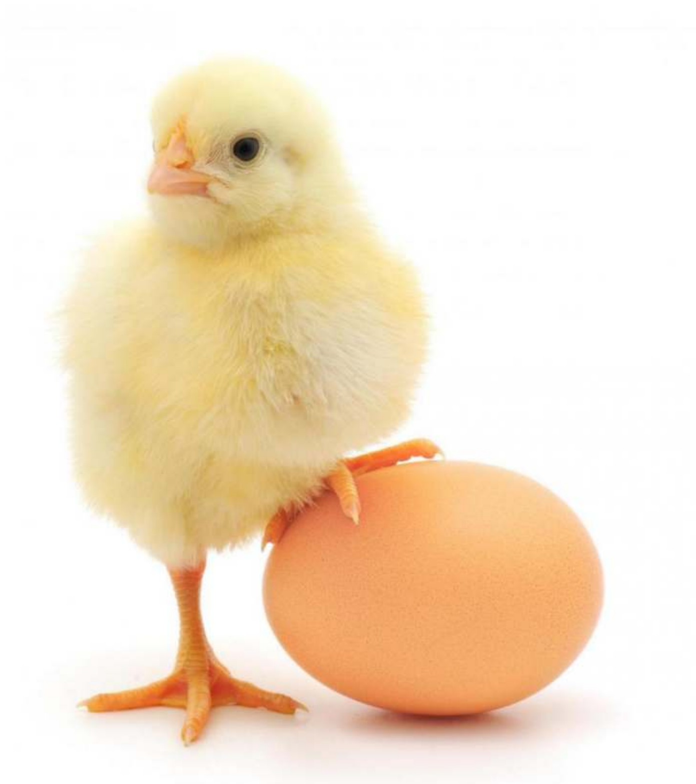

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Comparison of Plasma Amino Acid Levels of Two Breeds of Japanese Native Chicken and a Commercial Layer Line

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Abstract: The objective of the present study was to conduct amino acid profiling of two Japanese indigenous hens (Tosa-jidori; TJI and Ukokkei; UKO) and compared with a commercial hen (JL). Asparagine, leucine and proline levels in commercial layers were higher than those in both native Japanese chickens. Lysine and glutamate in UKO were higher than those in others and taurine was also higher than in JL. Serine in UKO was lower than those in others and methionine and cysteine were also lower than in JL. Arginine in TJI was lower than those in JL and UKO. No significant differences between breed/line were observed in histidine, threonine, glutamine, glycine, alanine, valine, isoleucine, tyrosine, phenylalanine and tryptophan. These results suggest that levels of dietary amino acid requirements might be different between native Japanese chickens.

Key words: Amino acid profiling, tosa-jidori, ukokkei, Japanese native chicken

INTRODUCTION

Animal scientists became particularly concerned about the potential loss of indigenous breeds during the early 1990's and as a consequence programme was launched by the FAO for the genetic conservation of poultry resources (FAO, 2007). There is a great variety of indigenous chicken bioresources (approximately 50 breeds) in Japan and almost all of them were developed for special plumage, crowing and fighting traits (Tsudzuki, 2003). The study of Japanese native breeds as a genetic resource has received very little scientific attention, while recent research efforts have been directed primarily towards enhancing commercial production systems. In fact, new meat-type chickens have been produced in some prefectural livestock research institutes using native Japanese chickens (Kubo *et al.*, 2009; Rikimaru *et al.*, 2009). However, there are fewer studies on the various advantages of native Japanese chickens for breeding.

Chickens can synthesize all of the amino acids necessary to build proteins except for the essential amino acids and therefore, the latter must be included in the diet or supplemented in adequate amounts. Failure to provide sufficient amounts of even one of these essential amino acids has serious health implications and can result in the degradation of the body's proteins. Muscle and other protein structures may be dismantled to obtain the needed amino acid. One method to estimate the amino acid requirements in poultry is by analyzing the amino acid composition of tissues (e.g., Hurwitz and Bornstein, 1973). Plasma amino acid levels appear to reflect skeletal muscle amino acid levels which constitute the majority of the amino acid pool (Gan and Jeffay, 1967). Since there were studies showing differences between native and commercial chickens in their amino acid or protein requirements (e.g., Kingori *et al.*, 2003; Li *et al.*, 2003), it is important to determine the optimum dietary amino acid pattern in indigenous chickens using various parameters such as plasma amino acid levels.

For improving poultry productivity as well as the conservation of indigenous chickens, it is useful to investigate various biochemical characteristics in chickens. The aim of this study was to survey the amino acid profiles of two Japanese indigenous chicken breeds and compared them with those in commercial chickens.

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MATERIALS AND METHODS

Animals: The data were collected from two Japanese chicken breeds, the indigenous Tosa-jidori (TJI) and Ukokkei (UKO: Japanese Silkie) breeds, together with a commercial layer-type chicken (Julia Light: JL). All hens (30-wk old) were housed in individual cages under ambient temperature (20-24°C). They were provided ad libitum with commercial layer feed (17% CP, 2, 800 kcal/kg of AME_n) and had free access to water under all conditions. The henhouse were maintained on a 14L: 10D light schedule with light turned on at 0800h and off at 2200h.

Analytical procedures: Blood samples were taken from the right brachial vein from 1300h to 1400h. Blood was immediately centrifuged at 4, 500 rpm and kept in room temperature for 15 min to obtain plasma. Protein was precipitated from diluted plasma samples with 3.0% sulfosalicylic acid. Supernatants were filtered through a 0.2 µm filter. Then, 50 µL of the filtrate was applied to an Amino Acid Analyzer (JLC-555/V: JEOL Ltd., Tokyo, Japan). Norleucine (50 nmol/mL) was used as an internal standard. The handling of birds was performed in accordance with the regulations of the Animal Experiment Committee of Hiroshima University.

Statistical analysis: ANOVA was used to determine overall statistical significance due to treatment. When a treatment effect was significant ($p<0.05$), the Tukey-ramer test was used to compare the significance among means. Results are presented as means±SEM.

RESULTS

Levels of plasma amino acid in the native Japanese chickens (TJI and UKO) and the commercial line chicken (JL) are given in Table 1. Twenty amino acids in the plasma could be detected by our method but significant differences between breed/line were observed in ten amino acids (lysine, arginine, glutamate, serine, asparagine, leucine, methionine, cysteine, taurine and proline). Asparagine, leucine and proline levels in JL were higher than those in both native Japanese chickens (TJI and UKO). Concentrations of plasma lysine and glutamate in UKO were higher than those in others and taurine was also higher than in JL but not in TJI. Conversely, serine level in UKO was lower than those in others and methionine and cysteine were also lower than in JL but not in TJI. As for arginine, there was no difference between JL and UKO but the level in TJI was lower than those in JL and UKO. No significant differences between breed/line were observed in histidine, threonine, glutamine, glycine, alanine, valine, isoleucine, tyrosine, phenylalanine and tryptophan.

DISCUSSION

It is known that the Japanese Silkie fowl (UKO) possesses numerous unique characteristics (Tsudzuki, 2003) and its genetic relationship with commercial

Table 1: Levels of amino acid in blood of native Japanese chicken breeds

Amino acids (nmol/mL)	TJI (n = 6)	UKO (n = 6)	JL (n = 8)	P-value
EAA				
Arginine	223.3±11.5 a	278.4±21.7 b	308.8±6.9 b	0.001
Lysine	190.4±21.8 a	329.1±39.2 b	180.5±22.4 a	0.003
Methionine	62.2±6.1 ab	53.7±1.8 a	75.1±4.5 b	0.012
Isoleucine	106.3±8.7	86.2±3.6	105.5±4.1	0.043
Leucine	175.8±12.6 a	185.1±8.7 a	284.3±9.0 b	<0.001
Valine	227.2±23.5	174.6±8.7	223.2±10.3	0.046
Phenylalanine	86.6±5.0	86.4±4.0	97.0±3.0	0.105
Threonine	393.7±41.6	430.5±41.0	336.5±29.7	0.201
Tryptophan	62.0±3.6	67.1±3.7	68.3±3.6	0.465
NEAA				
Histidine	99.0±8.9	111.3±10.6	137.8±12.8	0.068
Glycine	505.3±27.1	453.8±14.7	488.9±21.8	0.300
Glutamate	84.7±10.3 a	124.7±5.8 b	88.2±4.3 a	0.001
Glutamine	682.8±116.4	830.1±39.5	816.0±45.2	0.317
Asparagine	92.6±14.4 a	111.7±7.6 a	194.3±9.5 b	<0.001
Serine	655.6±43.3 a	395.8±37.3 b	715.0±25.7 a	<0.001
Alanine	465.0±66.8	366.4±44.0	415.5±48.3	0.479
Cysteine	50.1±7.7 ab	52.0±3.9 a	33.4±2.4 b	0.019
Tyrosine	96.3±6.0	114.5±9.0	119.2±9.2	0.173
Proline	160.7±9.2 a	222.7±16.2 a	509.3±24.1 b	<0.001
Taurine	209.9±40.8 ab	340.2±82.5 a	76.9±9.6 b	0.004

TJI: Tosa-jidori; UKO: Ukokkei; JL: Julia Light; EAA: Essential amino acid; NEAA: Non-essential amino acid.
Values are means±SEM of the number of chickens in parentheses. Means with different letters are significantly different at P<0.05.

breeds is also more distant than that with other breeds (Okabayashi *et al.*, 1998; Ponsuksili *et al.*, 1999). Similarly, our results indicated that UKO was a unique chicken in the characteristics of its plasma amino acid levels as most differences were seen between UKO and JL and TJI seemed to show intermediate plasma amino levels.

One method to determine the optimum dietary amino acid pattern in poultry analyzes the amino acid composition of materials, such as carcass, feather and eggs (Hurwitz and Bornstein, 1973, 1978, 1980; Kim *et al.*, 1997). In general, it is known that levels of plasma free amino acids which are a part of the amino acid pool, are precisely controlled by various physiological mechanisms and therefore, the levels are maintained at a constant value. The plasma amino acid levels appear to reflect skeletal muscle amino acid levels which constitute the majority of the amino acid pool (Gan and Jeffay, 1967). In fact, we found the concentration of amino acid in plasma to show a positive relationship to that in skeletal muscles of layer-type chicken (unpublished data). The levels of the four essential amino acids showed significant differences between breed/line, suggesting that the levels of dietary amino acid requirements might be different among them since plasma amino acid levels appear to reflect skeletal muscle amino acid levels. Native chickens have their own characteristic requirements and utilization of nutrients because they show different growth rates from each other (Goto *et al.*, 2010). However, the diet formula for native birds is usually based on the recommendations for commercial line chickens. Hence, plasma amino acid profiling may be useful to determine the optimum dietary amino acid in Japanese native chickens.

Also, six non-essential amino acids in plasma showed significant differences between breed/line. There is the possibility that their amino acid metabolic patterns are different but no specific trends can be found with each amino acid in the metabolic pathway. For example, leucine in JL was higher than in both native chickens but we could not find any positive or negative correlation between leucine and its metabolism-related amino acids, alanine and glutamate (Block and Harper, 1984). Factors affecting amino acid metabolism exert different effects on each amino acid, while each amino acid has a different efficiency, turnover rate and oxidation rate in various tissues (Li *et al.*, 2003). Thus, it is difficult to consider the effect of these differences on nutritional physiology. Further research is needed to explain the difference of non-essential amino acids levels in plasma.

For breeding, it is necessary to investigate characteristics of meat quality and quantity. It is possible to indirectly evaluate increased meat quantity using body weight gain but it is difficult to assess meat quality changes with growth because large quantities of animals need to be measured. On the other hand, plasma levels represent a homeostatic balance between supply and utilization of amino acids making this approach ideal for repeated assessments to monitor progress of treatment. Following these viewpoints, plasma amino acid profiling conducted under the same rearing condition may be useful to evaluate muscle amino acid levels in an age-dependent manner without sacrificing rare Japanese chicken breeds.

Conclusion: Given the fact that there are breed differences in plasma amino acid levels, it can be assumed that the levels of dietary amino acid requirements might be different among native Japanese chickens. Further studies on the relationship between meat quality and plasma amino acid levels among Japanese native chickens are necessary to further evaluate the use of amino acid profiling as breeding parameters.

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