

Low lysine feeding enhances chicken meat taste by increasing of free glutamate

G. Watanabe¹, H. Kobayashi¹, M. Shibata², M. Kubota³, M. Kadowaki^{1,3} and S. Fujimura^{1,3}

¹Graduate School of Science and Technology, Niigata University, Japan

²National Agriculture Research Center for Western Region, Shimane, Japan

³Center for Transdisciplinary Research, Niigata University

Abstract – Taste is a crucial factor of meat quality, and amino acids are important taste-active components in meat. Here, the effects of dietary lysine (Lys) content on taste-active components in meat, especially free glutamate (Glu), were investigated. 28-day-old broilers were fed for 10 days with diets with graded Lys content of 90 or 100% of the recommended Lys requirement (according to the National Research Council, 1994). Free amino acid content in meat and sensory scores of meat soup were determined. Free Glu content, the main taste-active component of meat, was significantly increased by a reduction of dietary Lys. Compared with the Lys 100% group (control), free Glu was increased by 35.7% in the Lys 90% group ($P < 0.05$). Sensory evaluation of meat soup made from the Lys 100 and 90% groups indicated different meat tastes. Sensory scores for taste intensity, umami and kokumi tastes were significantly higher in the Lys 90% group. These results suggest that a reduction of dietary lysine increased free glutamate content in meat and improved its taste.

Key Words – amino acid, dietary lysine, glutamic acid

I. INTRODUCTION

The essential amino acid lysine (Lys) is typically the first or second limiting amino acid in corn and soybean meal-based diets of broiler chickens. [1]. It has been well documented that increasing dietary Lys generally results in improved feed intake, feed conversion, and body weight gain in broilers [2][3]. However, there have been few reports investigating the relationship between dietary Lys and meat quality.

Glutamate (Glu) contributes to meat taste, and is the main component of the ‘umami’ or ‘brothy’ taste. It is one of the most important amino acids for improving the taste of meat [4][5]. Fujimura *et al.*, (1996) reported that the umami taste,

sweetness and saltiness were significantly enhanced by the addition of Glu to chicken soup [5]. Therefore, increasing the concentration of Glu in meat might improve its taste. However, dietary Glu is not absorbed into the portal vein, because most is consumed by enterocytes as a respiratory fuel [6]. Therefore, it is difficult to improve meat taste by feeding animals diets containing Glu-rich feeds to increase the content of free Glu in muscle. However, dietary nutrient treatments, such as administration of high branched-chain amino acids and high crude protein (CP) diets do affect the Glu content of chicken muscle [7][8]. In addition, our recent study showed that short-term feeding of a high Lys diet also increased the level of free Glu in muscle [9]. Conversely, Katsumata (2008) reported that high plasma concentrations of free amino acids were observed in pigs on a low lysine diet [10]. Therefore, free amino acid content in muscle may be increased by a low Lys diet. Lys is the first or second limiting amino acid. Therefore, a low lysine diet is easy and cheap to produce. In the present study, the effects of a low Lys diet on free Glu levels in muscle were examined using broiler chickens. Moreover, a sensory evaluation of meat was performed to determine changes to meat taste.

II. MATERIALS AND METHODS

Animals

Ross strain female chickens at day 0 were purchased from a commercial hatchery. All chickens were allowed free access to feed and water. Chickens were raised on a commercial diet containing 200-g/kg CP and 3.2-kcal/g metabolizable energy (ME). On day 28, chickens were allocated to two groups, ensuring that the

average weight of animals was the same across groups (n = 16 per group). The study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Science Council of Japan. All experimental protocols were approved by Niigata University Animal Care Committee.

Experimental diets

Two experimental diets contained 100 or 90% of Lys requirement (100% Lys requirement, 10.0 g/kg of diet); (NRC, 1994). Both diets contained 3.2-kcal/g ME and 170-g/kg CP. Amino acids, ME, vitamins and minerals fulfilled the NRC requirements. Chickens in each group were fed their allocated diet for 10 days.

Sample collection

At the end of the trial, feed intake was recorded for each individual and chickens were weighed. Then six chickens were randomly selected for the free amino acid analysis and sensory evaluation. Blood samples were taken from the wing vein and chickens were killed by cutting the carotid arteries. The breast muscle from the left side was cut into small pieces. The breast muscle from the right side was using for the sensory evaluation.

Preparation of muscle extracts

The muscles were separately homogenized in perchloric acid with a high-speed homogenizer (Ultra-turrax T25 basic, Ikawerke, Staufen, Germany). The homogenate was then centrifuged and the supernatant was neutralized with 10% (w/v) potassium hydrate. After removal of potassium crystals by filtration, the filtrate volume was adjusted to 50 ml using double distilled water.

Measurement of free amino acids

The levels of free amino acid in blood and muscle were measured by an amino acid analyzer (JLC-500/V; JEOL, Tokyo, Japan). The detection wavelengths were 440 and 570 nm. Amino acids were detected using the ninhydrin method.

Sensory evaluation of meat

Chickens used for amino acid analysis were also used for sensory evaluation. Breast meat from the right side was used to prepare meat soup for sensory evaluation. Frozen breast muscles were thawed at 4°C for 24 h. Then, muscle samples

were cut into small pieces. Water was added to the pieces of meat (final volume was 1.5 times the original meat weight) and the mixture rapidly brought to the boil in a small pan and then simmered for 1 hour. Finally, the soup was filtered and the volume of soup was adjusted to 1.5 times the original meat weight, and sodium chloride was added to a final concentration of 0.3% of the soup. Fourteen trained panelists belonging to Niigata University, all in their 20s, performed the sensory evaluation. Tastes of both soups were compared using a closed panel method at room temperature (20 to 25°C). First, taste difference between soups was determined. Then umami, sweetness, bitterness, sourness, kokumi and taste intensity was determined by paired comparison test of 7-grade scale (-3 to +3). "Kokumi" is the food taste term meaning mouthfulness and increasing continuity [12].

Statistical analysis

Student's t-tests were carried out for statistical analysis of growth performance and concentration of free amino acid. Means and standard errors were calculated for both groups. Directional paired difference test was used for statistical analysis of the taste difference between soups of the Lys 100 and 90% groups [11]. Scheffé's paired comparison test was carried out for statistical analysis of scores of umami, sweetness, bitterness, sourness, kokumi and taste intensity [11]. Differences with probabilities of < 0.05 were considered significant.

III. RESULTS

Growth performance

Total Lys intake was significantly decreased in chickens fed the Lys 90% diet compared to the Lys 100% diet (P < 0.01). Feed intake was not different between groups. However, body weight gain was decreased by 6.0% in the Lys 90% group, compared with the Lys 100% group (P = 0.11). In addition, feed efficiency was significantly decreased (P < 0.05). Therefore, a 10% restriction of dietary Lys decreased growth performances.

Concentrations of free amino acids in plasma

The free Lys and Glu concentrations in plasma are shown in Fig 1. The free Lys concentration in plasma was significantly decreased in the Lys 90%

group compared with the Lys 100% group ($P < 0.01$). However, free Glu was unchanged in chickens fed the Lys 90% diet. In addition, there was no difference between groups for other amino acids (data is not shown).

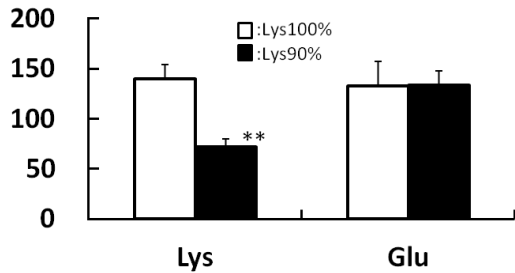


Fig 1. Effect of dietary Lys level on free Lys and Glu concentration in plasma of broilers (nmol/ml plasma)

Values in each row with different superscripts are significantly different at $**P < 0.01$.

Concentrations of free amino acids in muscle

The free amino acid concentrations in muscle are shown in Fig 2. The level of free Lys was unchanged. However, free Glu was significantly increased by 35.7% ($P < 0.05$) in the Lys 90% group. In addition, total free amino acid (AA) concentration was also significantly increased in Lys 90% group ($P < 0.05$).

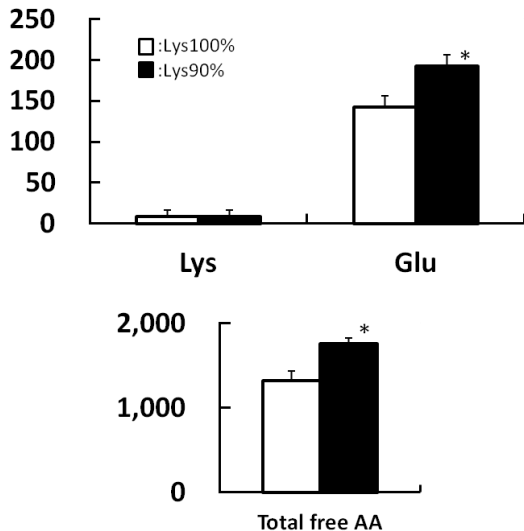


Fig 2. Effect of dietary Lys level on free Lys and Glu concentration in muscle of broilers ($\mu\text{g/g}$ muscle)

Values in each row with different superscripts are significantly different at $*P < 0.05$.

Sensory evaluation of meat soup

All 14 panelists scored a statistically significant difference between the two groups ($P < 0.001$). Scores of umami, kokumi and taste intensity in the Lys 90% group were significantly higher than those in the Lys 100% group ($P < 0.05$) (Fig. 3).

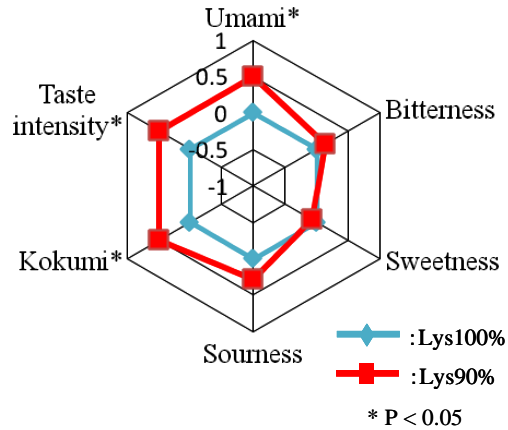


Fig 3. Scheffé's paired comparison test assessing meat soups between the Lys90 and 100% groups

IV. DISCUSSION AND CONCLUSION

In this study, effects of a low Lys diet on the level of free Glu in muscle were investigated with respect to improvement of meat taste. Compared with the Lys 100% group, free Glu content in the Lys 90% group was increased by 35.7% ($P < 0.05$). In addition, total free AA levels in muscle were also increased by the low Lys diet. However, the content of free amino acids in plasma, except Lys, were almost the same between Lys 100 and 90% groups. Our results suggest that the significant increase in the level of muscle free Glu and total free AA were not derived from plasma or other tissues. Higher contents of free amino acids in muscle by low Lys diet might be explained by a lower muscle protein synthesis rate in broilers fed the low Lys diet. In addition, in rats, high Lys conditions suppressed myofibrillar protein degradation through the autophagic-lysosomal pathway [13]. Therefore, protein degradation might be enhanced by low Lys conditions. These lower protein synthesis and higher degradation rates are likely to result in higher pooled levels of free amino acids (Watanabe *et al.*, in preparation). The sensory evaluation of meat soups made from the Lys 90% and Lys 100% groups, clearly

identified a difference in meat tastes. There were significant differences in taste intensity, umami and kokumi tastes between the Lys 100 and 90% groups. These results suggested that meat from broiler chickens of the Lys 90% group had a stronger taste intensity and greater umami and kokumi taste compared with that of the Lys 100% group. Umami is a desired taste and the main taste of Glu [14]. Therefore, meat taste might be improved by increasing free Glu content in muscle by decreasing dietary Lys level.

In conclusion, we found that levels of free amino acids in muscle were significantly increased by short-term feeding of a low Lys diet. A decrease in dietary Lys induced an increase in free amino acid content and increased tastes of umami, kokumi and taste intensity in meat. In particular, umami was enhanced by Glu content. These observations provide new insights into an effective method to improve meat taste. In addition, we used a 90% Lys feeding period of 10 d before testing; however, a shorter period may reduce the low growth performance, although this remains to be investigated (Watanabe *et al.*, in preparation). It is expected that the same effects of dietary Lys on meat quality would occur in other animals.

ACKNOWLEDGEMENTS

Part of this research was financially supported by a Grant-in-Aid for Scientific Research (C), JSPS KAKENHI Grant Number 26450375, from Japan Society for the Promotion of Science (JSPS) to S. Fujimura.

REFERENCES

- Baker, K., Firman, J. D., Blair, E., Brown, J. & Moore, D. (2003). Digestible lysine requirements of male turkeys during the 12 to 18 week period. *The Journal of World's Poultry Research* 2: 229-233.
- Holsheimer, J. P. & Veerkamp, C. (1992). Effect of dietary energy, protein, and lysine content on performance and yields of two strains of male broiler chicks. *Poultry Science* 70: 872-879.
- Sterling, K. G., Pesti, G. M. & Bakalli, R. I. (2006). Performance of different broiler genotypes fed diets with varying levels of dietary crude protein and lysine. *Poultry Science* 85: 1045-1054.
- Kato, H. & Nishimura, T. (1987). Taste components and conditioning of beef, pork, and chicken, in: Kawamura, Y. & Kare R. M., (Eds) *Umami: A Basic Taste*. New York: Marcel Dekker.
- Fujimura, S., Koga, H., Takeda, H., Tone, N., Kadowaki, M. & Ishibashi T. (1996). Role of taste-active components, glutamic acid, 5'-inosinic acid and potassium ion in taste of chicken meat extract. *Animal Science and Technology* 67: 423-429.
- Reeds, P. J., Burrin, D. G., Jahoor, F., Wykes, L., Henry, J. & Frazer, E. M. (1996). Enteral glutamate is almost completely metabolized in first pass by the gastrointestinal tract of infant pigs. *American Journal of Physiology* 270: 413-418.
- Kobayashi, H., Eguchi, A., Takano, W., Shibata, M., Kadowaki, M. & Fujimura, S. (2011). Regulation of muscular glutamate metabolism by high-protein diet in broiler chicks. *Animal Science Journal* 82: 86-92.
- Imanari, M., Kadowaki, M. & Fujimura, S. (2007). Regulation of taste-active components of meat by dietary leucine. *British Poultry Science* 48: 167-176.
- Watanabe, G., Kobayashi, H., Shibata, M., Kubota, M., Kadowaki, M. & Fujimura, S. (2015). Regulation of free glutamate content in meat by dietary lysine in broilers. *Animal Science Journal* 86: 435-442.
- Katsumata, M., Matsumoto, M., Kobayashi, S. & Kaji, Y. (2008). Reduced dietary lysine enhances proportion of oxidative fibers in porcine skeletal muscle. *Animal Science Journal* 79: 347-353.
- Stone, H. & Sidel, J. L. (2004). Discrimination testing, in: Taylor SL. (Ed.) *Sensory Evaluation Practices*, 3rd ed. San Diego CA: Academic Press.
- Ueda, Y., Tsubuku, T. & Miyajima, R. (1994). Composition of sulfur-containing components in onion and their flavor characters. *Bioscience, Biotechnology and Biochemistry* 58: 108-110.
- Sato, T., Ito, Y. & Nagasawa, T. (2013). Regulation of skeletal muscle protein degradation and synthesis by oral administration of lysine in rats. *Journal of Nutritional Science and Vitaminology* 59: 412-419.
- Okiyama, A. & Beauchamp, G. K. (1998). Taste dimensions of monosodium glutamate (MSG) in a food system: role of glutamate in young American subjects. *Physiology & Behavior* 65: 177-181.