EFFECT OF LOW-ENERGY AND LOW PROTEIN DIET ON MUSCLE FREE GLUTAMATE CONTENT

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Abstract—*The major taste active component, glutamate (Glu), improves the taste of meat. In this study, we investigated the effect of a short-term low-metabolizable energy (LME) and low-protein (LCP) diet feeding on the intramuscular free Glu content. Furthermore, we elucidated how the muscle free Glu content was controlled by the LME and the LCP diet. Chicks were fed the control diet, the LME diet or the LCP diet for 10 days. Plasma and muscle free amino acid concentrations, and activity and mRNA expression of muscle enzymes related to Glu metabolism were determined. Muscle free Glu content was decreased by 47% in the LME group ($P<0.05$), but not altered in the LCP group. Activity of glutamate dehydrogenase (GDH) were significantly decreased in the both the LME and the LCP groups ($P<0.05$). GDH mRNA expression was decreased by LME diet feeding ($P<0.05$). Glutaminase (GA) and glutamine synthetase (GS) activity were not changed among the experimental groups. These results suggested that dietary LME feeding diminish the muscle free Glu content.*

Index Terms—*amino acid, diet, glutamic acid, muscle, metabolizable energy*

I. INTRODUCTION

Free glutamate (Glu) has one of the basic taste, *umami*, which indicates delicious, umami and brothy tastes (Lawrie, 2006). Therefore the increase of muscle free Glu content enhances the above taste, and improves the meat taste. However, in the past, free Glu content in muscle was believed that it was not changed by feeding treatment (Farmer, 1999). Moreover, dietary Glu is not directly absorbed into circumstance (Reeds *et al*. 1996), so it seemed to be very difficult to change the free Glu content in muscle by feeding animals diets containing Glu-rich foods or administration of crystalline Glu itself to the livestock. However our previous studies showed that restricted feeding, feeding short term (10days) high protein diet (Fujimura *et al*. 2001, 2006, Kobayashi *et al*. 2007) and administration of dietary leucine (Imanari *et al*. 2007) could change the Glu contents in muscle. Especially, feeding short term high protein diet or low leucine diet significantly increased Glu contents in muscle, and then the meat taste were improved (Fujimura *et al*. 2006, Imanari *et al*. 2007, Kobayashi *et al*. 2007). These increases of muscle free Glu content were considered to change the muscle Glu metabolism, since almost all dietary Glu was not absorbed into circumstance (Reeds *et al*. 1996). In the muscle, glutaminase (GA), glutamate dehydrogenase (GDH) and glutamine synthetase (GS) are major Glu related enzymes. GA catalyzes the deamination of glutamine (Gln). As result, Glu and ammonia are produced. Although the GDH reaction is reversible, its reaction directs the formation of Glu from α-ketoglutarate and ammonia (Hudson and Daniel 1993). GS synthesizes Gln from Glu and ammonia, and it is the only enzyme can synthesize Gln in the body.

Muscle free Glu is important for improvement the meat taste, and that content is changed by diet which includes the HCP. Although, HCP diet can increase muscle free Glu content, it has the assignments in terms of feeding cost and environmental load. Therefore, to improve the meat taste using the diet in low cost and low environmental load, it is necessary to use the LCP diet or the LME diet. However, even the effect of these diets on muscle free Glu content much less the influence on meat taste is not clear. Therefore, in this paper, we examined whether the LME or the LCP diet affect on the muscle free Glu content and the Glu metabolism. For these purposes, we gave control (fulfil the nutrient demand), LME or LCP diet to chick and determined amino acid content in plasma and muscle. Moreover, to investigate what kind of metabolism participated in the muscle free Glu content, we determined activity and mRNA expression of several enzymes which concern to Glu metabolism in the muscle.

II. MATERIALS AND METHODS

In our studies, 14 days old female chunky strain chicks were used. Chicks were randomly divided into two groups. Chicks were given one of two experimental diet-control (CP20%, ME 3.2kcal/g), low-ME (LME; CP20%, ME 2.4kcal/g) and low-CP (LCP; CP15%, ME3.2kcal/g) for 10 days. All chicks were allowed free access to feed and water.

The end of experiment, chicks were weighted and blood were collected. Then the chicks were killed and the pectoral muscle was removed immediately. Muscle was frozen by liquid nitrogen and then it was stored at -80 degrees C until each experiment. Plasma and muscle extracts were prepared as described by Imanari *et al*. (2007). Plasma and muscle amino acid concentration were determined by amino acid analyzer (JLC-500/V, JEOL, Tokyo, Japan). Enzyme activities and mRNA expressions concern to muscle Glu metabolism such as GA, GDH and GS were determined as previous report (Imanari *et al*. 2007, Kobayashi *et al*. 2010). mRNA expressions were determined by real time PCR (LightCycler 1.5, Roche Diagnostics, Basel, Switzerland). Enzyme activity and abundance of mRNA were expressed as percent of control. The date were compared using one-way ANOVA, where appropriate difference in group were compared using LSD.

III. RESULTS AND DISCUSSION

In our study, plasma free Glu concentration was not changed among the experimental diets. These results were according to previous observation (Reeds *et al*. 1996). All muscle free amino acid determined in this study except Met, His and Lys, was significantly decreased ($P<0.05$) in the LME group compared to control. Muscle free Glu content in chick fed the LME diet was significantly decreased ($P<0.05$) by 47% without a corresponding decrease in the plasma Glu concentration. Fujimura *et al*. (2001) demonstrated that 50% restricted feeding significantly decreased the muscle free Glu content by 29.6% compared with *ad libitum* feeding, and they reported that the taste of meat from animals given restricted feeding was less favorable than that of *ad libitum* feeding in a sensory evaluation study. Similarly, Imanari *et al*. (2007) reported that a reduction of muscle free Glu content by 25% was identified as the degradation of the taste of meat in sensory evaluation. These results indicate that changing the muscle free Glu level by 25–30% affects the taste of meat. In our study, the muscle free Glu level was decreased by 47%; therefore, it is expected that the LME diet diminish the meat taste. The LCP diet significantly increased ($P<0.05$) muscle free Ser, Gln, Gly, Leu and Phe content. Differ from the LME diet feeding, the LCP diet feeding did not change the muscle free Glu content. In our previous study, increase of dietary CP level over the requirement significantly increased muscle free Glu content (Kobayashi *et al*. 2010). Conversely, in our present study, decrease of dietary CP level below the requirement did not change. These results suggested that the level of ME was one of the regulatory factor of muscle free Glu content, but not level of CP in the condition to be less than the quantity of NRC (1994) requirement.

In this study, we determined some of Glu metabolic enzymes to clarify the Glu regulation mechanism in chick fed the LME and the LCP diet. GA is one of the major enzyme that catalyzed the deamination of Gln. Therefore, it was expected that the change of GA activity concern to muscle free Glu content. In this study, activity and expression of GA was not altered in the LME group in spite of muscle free Glu content was decreased. In the LCP group, GA activity and expression were not changed. GS is the only enzyme, which synthesize Gln from Glu and ammonia. GS activity and expression was not changed by the LME and the LCP diet feeding. Although the GDH reaction is reversible, its reaction directs the formation of Glu from α-ketoglutarate and ammonia (Hudson and Daniel 1993). GDH activity was significantly decreased in the LME and the LCP diet ($P<0.05$). GDH mRNA expression was decrease in chick fed the LME diet ($P<0.05$). On the other hands, GDH mRNA expression was not changed by the LCP diet. Zhou and Thompson (1996) demonstrated that GDH activity in rat and chicken skeletal muscle was increased by leucine (Leu), but not isoleucine (Ile) or valine (Val) *in vitro*. Imanari *et al*. (2007, 2008) and Kobayashi *et al*. (2010) reported that low-Leu diet, high Ile + Val diet and HCP diet increased muscle free Glu content, although GDH activity in the muscle was not changed. Similarly in the present study, muscle free Glu content was only decreased in the LME group in spite of GDH activity was decreased by both LME and LCP diet feeding. So far, Leu is the only dietary amino acid which regulates GDH activity. Consider from result in this study, contribution of GDH for regulation of muscle free Glu content may not be big, although further studies are needed to better understand what kind of dietary factors regulate GDH activity and mRNA expression. In this present study, we could not clarify the regulation mechanism of muscle free Glu content by the LME and the LCP diet feeding. Saccharopine pathway is the major Lys degradation pathway in mammals and birds (Higashino *et al*. 1967, Hutzler and Dancis 1975, Grove and Roghair 1971, Wang and Nesheim 1972), and it concerns to free Glu production. Although in generally, it is believed that most of Lys metabolism occurs in the liver. However, recently Manangi *et al*. (2005) suggested that Lys metabolic enzyme (lysine α-ketoglutarate reductase; LKR) existed into the chicken muscle and they reported that the activity of LKR in chicken muscle was 1.6-fold higher than that of the liver if the activity was expressed per total tissue amount. Therefore, muscle appears to be another major tissue that catalyzed Lys degradation with secondary Glu generation. Kobayashi *et al*. (2010) suggested that muscle free Glu content was significantly increased along with the elevation of intramuscular LKR mRNA expression when chick fed the HCP diet. Therefore, similar to intramuscular free Glu regulation mechanism in HCP diet feeding, Lys metabolism may concern to regulation of muscle free Glu content when chick fed the LME and the LCP diet.

IV. CONCLUSION

In conclusion, we found that the muscle free Glu content, which is an active taste component of meat, was significantly decreased by short-term feeding of the LME diet. In contrast, we found that short-term LCP diet feeding didn't affect the muscle free Glu content

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