PE1.35 Effect of dietary protein level on meat quality and glutamate metabolism in breast muscle(ICoMST 2009) 239.00

Hiroyuki Kobayashi (1) f07l004g@mail.cc.niigata-u.ac.jp, Atsushi Eguchi(1), Wataru Takano (1), Masahiro Shibata (2), Motoni Kadowaki (1), Shinobu Fujimura (1)
(1)Niigata University, Japan
(2)National Agriculture Research Centre for Western Region, Japan

***Abstract*— Glutamate (Glu) is one of the major taste-active components in muscle. Previously, we reported that free Glu content in muscle was significantly increased by feeding high protein diet (HCP). Although Glu contents in muscle were controlled by nutritional factors, the regulation mechanism was not clear. Therefore, we examined how Glu content was regulated in muscle, especially when animals fed different CP level to provide more efficient feeding model. Chicks were fed HCP (CP30%) and control diet (CP20%) for 10 days. As results, Glu content in breast muscle was significantly increased ($P<0.05$) by 72% in chicks fed HCP diet. At this time, glutaminase (GA) activity and abundance of GAmRNA was significantly decreased by feedback inhibition ($P<0.05$) of Glu. Furthermore, mRNA expression of lysine alpha-keto glutarate reductase (LKR), which is rate limiting enzyme of Lys degradation pathway containing Glu generate reaction, were changed in HCP group ($P<0.001$). The mRNA expression of branched-chain amino acid transaminase (BCAT) which provides Glu as by-product, was not changed by HCP diet. Hence, our results suggested that muscle free Glu content was increased when the levels of dietary CP was higher than the NRC level, and it was especially regulated by GA and LKR reaction.**

H. KOBAYASHI is with the Graduate School of Science and Technology, Niigata University, Niigata, 950-2181, Japan (corresponding author to provide phone: +81-25-262-6664; fax: +81-25-262-6674; e-mail: f07l004g@ mail.cc.niigata-u.ac.jp).

M. SHIBATA, is with National Agriculture Research Centre for Western Region, Shimane, 694-0013, Japan (e-mail: shibatam.affrc.go.jp)

M. KADOWAKI is with the Graduate School of Science and Technology, Niigata University, Niigata, 950-2181, Japan (e-mail: kadowaki@ agr.niigata-u.ac.jp).

S. FUJIMURA is with the Graduate School of Science and Technology, Niigata University, Niigata, 950-2181, Japan (e-mail: fujimura@ agr.niigata-u.ac.jp).

***Index Terms*—dietary protein, glutamate, muscle, Lys metabolism, lysine alpha-ketoglutarate reductase.**

I. INTRODUCTION

GLUTAMATE (GLU) has one of the five basic taste, *umami*, which indicates delicious, umami and brothy tastes [10]. 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 [4]. Moreover, dietary Glu is not directly absorbed into circumstance [14], so it seemed to be very difficult to change the 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 [5-6,9] and administration of dietary leucine [8] 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 [6,8-9]. These increases of muscle free Glu content was considered to change the muscle Glu metabolism, since almost all dietary Glu was not absorbed into circumstance [14]. 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 [7]. GS synthesizes Gln from Glu and ammonia, and it is the only enzyme can synthesize Gln in the body. Ordinarily, internal Lys is degraded through saccharopine pathway which is the major Lys metabolic pathway of mammal and avian species. In this pathway, one mole Lys degradation produces two mole Glu as by-product. In general, it is believed that Lys metabolism is mainly conducted in liver, in contrast, muscle is not important because it does not indicate the activity of Lys metabolic enzymes. However, Manangi et al. [11] demonstrated that the rate limiting and primary enzyme of Lys degradation - lysine alpha-keto glutarate reductase (LKR), existed into the chicks muscle. In addition, they showed that muscle LKR activity was 1.6-hold higher than that of liver when the activity was expressed as activity per total tissue weight. Therefore, Lys metabolism in muscle is also important for production of muscle free Glu. Branched-chain amino acid (BCAA) is very unique amino acid in terms of the main metabolic tissue is muscle. Branched-chain amino acid transaminase

(BCAT) is the primary enzyme of BCAA catabolism. As results of BCAT reaction, corresponding branched- chain keto acid (BCKA) and Glu are produced.

Muscle free Glu is important for improvement the meat taste, and that content is changed by diet which includes the HCP.

To clarify how free Glu content in muscle can establish more efficient feeding model for improving meat taste. In this study, we determined the activity and expression of some enzymes which concern to Glu metabolism in 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) and high CP diet (HCP; CP30%, 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 -80degrees C until each experiment. Plasma and muscle extracts were prepared as described by Imanari *et al*. [8]. 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 show [8]. Lys metabolite enzyme, LKR and BCAA metabolic enzyme, BCAT, were determined mRNA expressions 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

Glu is the major taste active components of meat, therefore the increase of free Glu content in muscle may improve the meat taste. In previous study, we suggested that the HCP diet increased muscle free Glu content [6]. However, muscle Glu regulation mechanism of which the chick fed HCP diet was not clear. Therefore, it was objective to present study to elucidate how Glu content is regulated by HCP diet for establish more efficient feeding model. In our study, plasma free Glu concentration was not changed among the experimental diets. These results were according to previous observation [14]. Plasma free Val, Met, Ile, Leu, His and Lys concentration were significantly increased in HCP group ($P<0.01$). These elevation of six amino acids level in plasma were due to increase the absorption of those amino acid from diet, since HCP diet included ~2.2-fold each amino acids compared to control diet. Conversely plasma free Ser and Tyr concentration were significantly decreased in chicks fed HCP diet ($P<0.05$). Free Glu content in muscle was significantly increased by 72% in HCP group ($P<0.05$) without increasing of plasma Glu concentration. In this time, free Val, Ile and Lys contents were also significantly increased in HCP group ($P<0.05$). These results suggested that the increase in levels of these three amino acids in muscle is derived from the diet via plasma. No differences were found in other amino acids.

In this study, we determined some of Glu, Lys and BCAA metabolic enzymes to clarify the Glu regulation mechanism in chick fed HCP diet. Activity and expression of GS and GDH were not altered between the experimental diets. On the other hand, GA activity was significantly decreased in HCP diet ($P<0.05$). GAmRNA expression tended to decrease in chick fed HCP diet but no significantly difference ($P<0.10$). GA activity is strongly inhibited by Glu *in vitro* [3]. Therefore, our results suggested that decrease of GA activity and expression were due to feedback regulation by elevated muscle free Glu content in chick fed HCP diet. Most of internal Lys is degraded through saccharopine pathway. In this pathway, LKR is rate limiting and primary enzyme. Saccharopine pathway can produce two moles Glu as by-product when one mole Lys is degraded. In the central nerve system, one third of Glu is derived from saccharopine pathway[13]. In our study, muscle LKRmRNA expression was significantly increased by feeding HCP diet ($P<0.001$). Previous reports suggested that HCP diet increased liver LKR activity [1-2,12]. Therefore, elevation of muscle LKRmRNA translation rate might be contribute to increment of muscle free Glu content. On the other hand, BCATmRNA expression was not changed by feeding HCP diet. Therefore, the change of translation levels of BCATmRNA might not contribute to increase of muscle free Glu content in our experimental situation.

IV. CONCLUSION

In conclusion, high CP (HCP) diet increased free Glu contents in muscle which is a major taste-active component. Our results suggested that GA and LKR reaction contributed to the regulation of free Glu content in muscle.

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