EFFECT OF DIETARY PROTEIN LEVELS ON MUSCLE FREE GLUTAMIC ACID CONTENTS AND THEIR REGULATION MECHANISM

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Introduction

Meat quality is important for consumers. Historically, targets for improving meat qualities by diet had been focused to mainly meat aroma and antioxidation. However, the study that paid its attention to meat taste and taste-active components was rare. It had been generally believed that diet doesn't change the meat taste by diet, although genotype and age can improve it.

Our recent studies showed that main active component, glutamic acid (Glu) contents, controlled by dietary restriction, crude protein (CP) and particular amino acids levels. Especially, dietary Leucine (Leu) and Lysine (Lys) level improved meat taste (Fujimura et al., Imanari et al., 2004, Watanabe et al., 2006). Our previous studies indicated that glutaminase (GA) and glutamate dehydrogenase (GDH) involve in Glu regulation of muscle. However, the Glu regulation mechanisms of muscle were not clarified. Watford (1995) reported GA activity from chicken muscle was inhibited by Glu *in vitro*.

The objective of this study was clarify the Glu regulation mechanism by dietary low to high CP levels.

Materials and Methods

The 14-day-old female Cobb strain broiler chickens were divided into four groups with six chicks in each group. The chicks were fed CP15.0 (as low CP model), 20.0 (as control), 30.0 (as high CP model) % diet in *ad libitum* for 10 days. All the chickens were kept in individual wire cages. On day 11th, all chicken were slaughtered, and breast muscles (*pectoralis superficialis*) were taken for subsequent analysis. Experiment 1: Contents of free amino acids in meat extract were measured by HPLC. Experiment 2: Glu-relate enzyme activities in muscle were measured. For the investigation of the mechanism of Glu regulation, the muscle glutamate dehydrogenase (GDH), glutaminase (GA) and glutamine synthetase (GS) activities were measured by enzymatic methods (Bergmeyer, 1974). Experiment 3: Effect of increased intramuscular Glu on GA activity by feedback regulation was studied. So we determined the GA activity in medium concluded several Glu concentration (0-5mM) *in vitro*. Experiment 4: Gene expressions of Glu-relate enzyme were measured. In order to clarify the involvement for muscle Glu regulation, we determined the relative quantity of GDHmRNA, GAmRNA, and GSmRNA by real time PCR. The data were expressed relative means (%) to expression of CP20% group.

The data were compared using one-way ANOVA, where appropriate differences in groups were compared using LSD.

Results and Discussion

In Experiment 1, free Glu content of muscle in CP30% group was significantly increased 72% ($P<0.05$) compared to CP20% (as control), but no change in CP15% compare to control (Figure 1). Plasma free Glu contents were constant among three groups. This result showed that only high CP diet increased Glu contents of muscle without increment of plasma Glu concentration. In Experiment 2, GS activity in muscle didn't show significant difference among three groups. On the other hand, GDH activity in muscle tended to increase in CP30% compared to control. Significant differences of GDH activity were not shown between CP15% and control (Figure 3). GA activity of CP15 and 30% groups were approximately 63% and 59% of control group (Figure 4). From these results, GDH and GA activities seem to affect the regulation of muscle Glu contents. In Experiment 3, we elucidated the inhibition of GA activity *in vitro.* As result, GA activity tended to decrease with increase in medium Glu concentration. Especially, when the Glu concentrations of medium were 2.5mM and 5.0mM, GA activity was significantly decreased ($P<0.05$). From this result, it seems to feedback regulation by increased intramuscular Glu affect degradation of GA activity in CP30% in Experiment 2. In Experiment 4, relative GSmRNA expression in CP15% showed about 239% increase compared to control, while relative GSmRNA expression of CP30% didn't show significant difference. Relative GAmRNA expression tended to

decrease both CP15 and 30% groups compared to control. It indicated that GAmRNA expression relate to the GA activity in muscle.

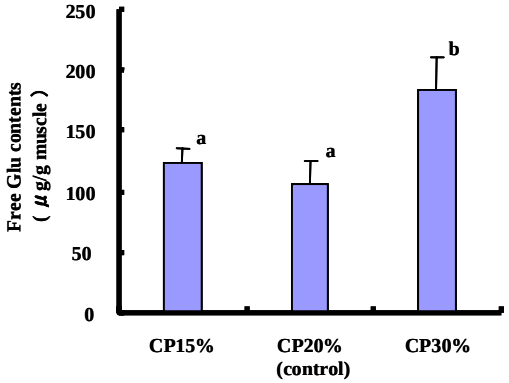

Figure 1. Effect of dietary CP levels on Glu contents in chicken muscle.

Values are means ± SEM (n=6). Bars with different superscripts[a-b] are significantly different, P<0.05.

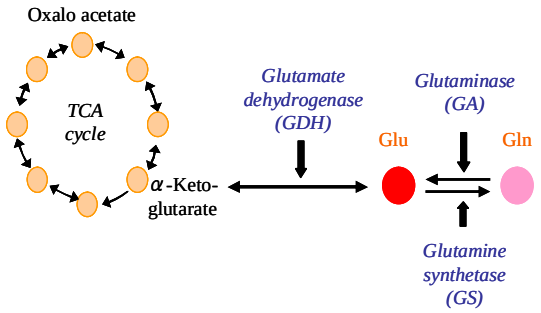

Figure 2. Glutamate metabolic pathway and Glu-related enzyme.

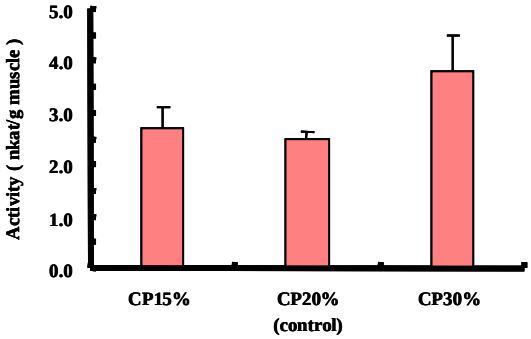

Figure 3. Effect of dietary CP levels on GDH activity in muscle.

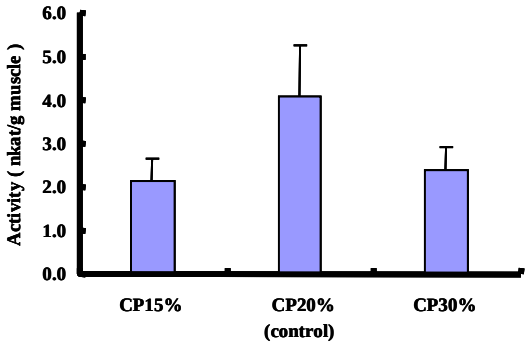

Figure 4. Effect of dietary CP levels on GA activity in muscle.

and GAmRNA expression. Therefore, it was suggested that lowering of GA activity in CP30% attribute to inhibition of GAmRNA.

Conclusion

In order to clarify the regulation mechanism of intramuscular Glu content by dietary CP levels, we measured free amino acids, Glu relate enzyme activity (GA, GDH and GS) and Glu relate gene expression in muscle. Our results suggested intramuscular Glu content increase in high CP diet, and GA and GDH activity controlled the Glu contents in muscle. Furthermore, reduction of GA activity in high CP diet showed attribute to inhibition of GAmRNA expression by increased intramuscular Glu.

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