Effects of feed withdrawal times on antemortem muscle protein degradation levels and postmortem muscle free amino acid concentrations in broiler chickens

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Objectives: Few studies have investigated the effects of feed withdrawal times on skeletal muscle protein degradation and free amino acids in chicken meat. Furthermore, plasma N$^{\tau}$-methylhistidine concentration has been used as an index of live muscle protein deg- radation, whereases no study has evaluated the relationship between antemortem muscle protein degradation levels and postmor- tem muscle protein degradation in chickens. Therefore, the objectives of this study were to determine the effects of different feed withdrawal (fasting) times before slaughter on antemortem plasma N$^{\tau}$-methylhistidine concentration and postmortem muscle free amino acid concentrations in broiler chickens.

Materials and Methods: Twenty-four broiler chicks (*Gallus gallus domestics*, Ross 308) at 0 days of age were provided water and a semi-purified diet with no animal protein *ad libitum* until 27 days of age. The chicks were weighed and collected blood samples from the wing vein at 27 days of age. The blood samples were used to evaluate plasma N$^{\tau}$-methylhistidine concentration before experiment. Then, they were assigned to four treatments: 0-hour of fasting (0H), 8-hours of fasting (8H), 16-hours of fasting (16H), or 24-hours of fasting (24H) to ensure no significant differences in body weights among the treatments. The chicks were fasted ac- cording to the fasting hours of each treatment. At 28 days of age, after body weight measurement and blood sampling were con- ducted, they were slaughtered by cervical dislocation following carbon dioxide anesthesia. The left pectoralis major and minor muscles were collected at the slaughter and weighed. A portion of the left pectoralis major muscle was immediately stored in liquid nitrogen. The right pectoralis major muscles were stored at 4°C for 48 hours. Plasma N$^{\tau}$-methylhistidine concentrations before experiment and just prior to slaughter were analyzed by the UHPLC system (NexeraX2, Shimadzu Co., Ltd., Japan) with a Kinetex
2.6 μm column (EVO C18; 100×3.0 mm) and fluorescence detector (RF-20Axs). Antemortem change in individual plasma N$^{\tau}$- methylhistidine concentrations before and after experiment was calculated as "concentration value of before experiment" minus "concentration value of just prior to slaughter". Free amino acid contents of pectoralis major muscles at slaughter and at 48 hours after postmortem storage (4°C) were analyzed by the UHPLC system. Data were analyzed with one-way ANOVA and post-hoc Tukey's multiple comparison using the PROC GLM procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC).

Results and Discussion: The body weights of the fasted chickens (16H and 24H) were decreased in a fasting time dependent man- ner, while that of the freely fed chickens (0H) was increased by 48.8 g during 24 hours before slaughter. The weight of pectoralis major muscle tended to be lighter in 24H than in 0H ($P < 0.1$), and that of pectoralis minor muscle was significantly lighter in 24H than in 0H ($P < 0.05$). The plasma N$^{\tau}$-methylhistidine concentrations just prior to slaughter were increased with the length of fast- ing time, and it was significantly higher in 24H than in 0H ($P < 0.05$). In addition, antemortem change in individual plasma N$^{\tau}$- methylhistidine concentrations was significantly increased in 8, 16, and 24H compared to that in 0H, respectively. These results suggested that antemortem muscle protein degradation levels at slaughter were enhanced by feed withdrawal treatment. After 48 hours of postmortem storage, glutamic acid contents, which is related to the umami taste in meat, were increased with the length of fasting time ($P < 0.05$), i.e., its contents of pectoral major muscle of 8, 16, 24H were significantly higher than that of 0H, respec- tively, and that of 24H was the highest among these 4 groups ($P < 0.05$). Interestingly, we found that there was a positive correla- tion between the antemortem changes in individual plasma N$^{\tau}$-methylhistidine concentrations and glutamic acid contents of pectoralis major muscle after 48 hours of postmortem storage ($P < 0.05$). On the other hand, total free amino acid contents of pectoralis major muscle in 24H was higher than 0H and 16H after 48 hours of postmortem storage, whereas no correlation was observed be- tween the changes in individual plasma N$^{\tau}$-methylhistidine concentrations and total free amino acid contents of pectoralis major muscle. These results suggest that postmortem changes in glutamic acid contents of chicken meat during storage reflect antemor- tem muscle protein degradation levels exerted by feed withdrawal treatment.

Conclusions: The data obtained from this study indicated that pre-slaughter feed withdrawal increased antemortem muscle protein degradation levels of broiler chickens, and consequently affected their postmortem glutamic acid contents in chicken meat after 48 hours of postmortem storage.

Key words: Pre-slaughter feed withdrawal, Skeletal muscle protein degradation, N$^{\tau}$-methylhistidine, Free amino acids, Chicken meat