EFFECT OF LOW PROTEIN DIET AND AMINO ACIDS SUPPLYMENTED LOW PROTEIN DIET ON MEAT TENDERNESS OF BROILER CHICKENS

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Abstract –21 days old chickens were fed one of three experimental diets, control, low protein (LCP), and low protein supplemented with synthetic essential amino acid (ELCP) diet, for 10 days to elucidate the effect of the LCP or the ELCP diet on meat tenderness. We measured shear force value (SFV) of pectoralis major muscle. We also measured cross-sectional area (CSA), collagen content, ultimate pH and drip loss of the muscle to elucidate the mechanism relevant to control meat tenderness. The SFV of the ELCP group was lowered 47% (P<0.01) compared with that of the control group. However, the SFV of the LCP group was not affected. The CSA of the LCP and the ELCP group was lower (P<0.05) than that of control group. Collagen content of muscle was not affected. Ultimate pH of the LCP group, but not the ELCP group, was lower than that of the control group (P<0.05). Although drip loss in the LCP group was greater than that of the others, the differences were not significance. Our results suggested that the short-term feeding of the ELCP diet might be effective to produce tender meat. However, the mechanisms explaining the lower SFV in the ELCP group is still unclear.

Key Words – Low protein diet, Muscle fiber, Shear force value, Ultimate pH.

- INTRODUCTION

Feeding a low protein diet to livestock is effective to reduce feed cost and to reduce nitrogen excretion to the environment. A number of studies elucidated the effect of a low protein diet on growth performance. On the other hand, only a few study evaluated the effect of a low protein diet on meat qualities although meat qualities (color, texture, and taste etc.) are one of main interests of consumers. Tenderness of meat affects consumer's choice; tender beef is preferred by consumers [1]. Meat sensory tenderness had negative correlations with shear force values [2]. In addition, shear force value is determined by several factors such as size of myofiber [3], content of intramuscular collagen [4], content of intramuscular fat [2] and ultimate pH of muscle [5]. The diameter of chicken breast muscle fiber was reduced by feeding a low-protein and low-energy diet [6]. Thus, feeding of a low protein diet may produce tender meat by reducing size of myofiber.

As shown in previous studies, growth performance is hindered by a low protein diet. Supplementation of essential amino acids (EAA) to a low CP diet to satisfy the requirements restored growth performance. Thus, a low CP diet supplemented with synthetic EAA that meets the standard requirements may be useful for saving feeding costs and for reducing the environmental load while maintaining growth performance. However, the effects of a low CP diet supplemented with EAA on meat qualities have not been elucidated yet.

The aims of this study were to elucidate the effects of a low CP diet and an ELCP diet on tenderness of broiler breast muscle and to obtain the insights into the underlying mechanisms how dietary levels of protein and/or amino acids regulate meat tenderness. Therefore, in this study, we elucidated the effects of a low CP diet and an ELCP diet on tenderness of broiler breast muscle. Furthermore, we measured cross-sectional area of myofibers, collagen content, pH and drip loss of chicken breast muscle because they are determinants of meat tenderness.

- MATERIALS AND METHODS

In this study, male Chunky strain 21 days old chickens were assigned randomly to the following three diet regimes: Control diet (crude protein (CP) 20%, metabolizable energy (ME) 3.2 kcal/g), low protein diet (LCP; CP15%, ME3.2 kcal/g), or a low protein diet supplemented with essential amino acid to satisfy the NRC (1994) recommendations (ELCP; CP15%, ME3.2 kcal/g). In this study, 7 or 8 chickens per group were used. The chickens were fed one of three experimental diets for 10 days. At the end of the experiment, final body weights of chickens were measured, and chickens were killed. Pectoralis major muscle (breast muscle) was collected for analyzing shear force value, muscle fiber size, collagen content, pH of muscle and drip loss. Muscle sample for cross-sectional area (CSA) and collagen analysis were frozen in liquid nitrogen cooled isopentane or liquid nitrogen, respectively and stored at -80°C until analysis. The sample for measurement of shear force value (SFV) was kept at 4°C for 24 hours. Muscle sections measuring 10 m thick was cut using a cryostat and CSA of myofiber was measured as described as previously [7]. The CSA of more than 400 myofibers were measured in each muscle sample. Extraction and determination of total and soluble collagen of muscle were performed by the procedure described as previously [7]. The breast muscle samples were aging at 4°C for 24 hour and then heated at 70°C for 60 min. The SFV was determined according to the procedure described as previously [7]. Muscle pH was measured after aging for 24 hour at 4°C. Then the samples were homogenized in the buffer including sodium iodoacetate. Homogenate after centrifugation was measured pH using a pH meter. The breast muscle, 2 × 2 × 1 cm block was used for analyzing of drip loss. The 24-hour drip loss at 4°C was determined by the bag method according to previous paper [8]. One-way analysis of variance (ANOVA) was performed using the General Linear Models procedure in the Statistical Analysis System (SAS: version 9.2; SAS Institute Inc., Cary, NC, USA). Significant differences between means were determined using Tukey’s multiple comparison test. The results were expressed as the means ± standard errors. Differences were considered significant at the $P < 0.05$ level.

- RESULTS AND DISCUSSION

The SFV of the LCP group was not different from that of the control group (Table 1). Interestingly, the SFV of the ELCP group was 46.8% lower than that of the control group ($P < 0.01$). Silva *et al*. [9] showed that SFV had a strong negative correlation with sensorial tenderness. Therefore, feeding an ELCP diet may be an effective method

Table 1 Effect of the LCP and ELCP diet on shear force value (SFV) of breast muscle

	control	LCP	ELCP
SFV (N)	20.1 ± 2.9^{a}	17.8 ± 2.9^{ab}	12.1 ± 1.3^{b}

The value represent the mean ± SE for 8 chickens in each group. $^{a\text{-}b}$Values with different superscripts are significantly different ($P < 0.01$). LCP, low protein diet;
ELCP, LCP diet supplemented with synthetic essential amino acids to meet the NRC recommendations.

for producing tender meat in short-term. In order to have insights into underlying mechanisms of the lower SFV of the ELCP group, the further measurements were made. Chen et al. [3] demonstrated that CSA of myofiber had a positive correlation to SFV. Therefore, we measured the CSA of myofibers (Figure 1). The means of CSA of the LCP and ELCP groups were lower than that of the control group ($P<0.05$). Although the CSA of the LCP and ELCP group was also reduced, the reduction of CSA of the LCP group did not affect to SFV. Therefore, CSA of myofiber was not a determinant of the reduction of SFV in the ELCP group.

The results of total and soluble collagen content were shown in Figure 2. Both total and soluble collagen contents of breast muscle did not differ among the experimental groups. These results suggested that the collagen contents were not a determinant of the lower SFV in the ELCP group. The ultimate pH of pig muscle indicated negative correlation between SFV and drip loss [5]. In addition, there is a negative correlation between drip loss and tenderness of pork [10].Therefore, we expected that ultimate pH and/or drip loss might contribute to reduce the SFV in the ELCP group. The results of muscle pH and drip loss were shown in Figure 3 and 4, respectively. The means of initial pH (0 hour) and 1 hour after aging of muscle were the same level among three experimental groups (data were not shown). The ultimate pH of the LCP group was lower than those of the control and the ELCP groups ($P<0.05$). The result of the LCP group agreed with a previous report where lower ultimate pH of muscle was observed in chickens fed a low protein diet [11]. Although the level of CP of the ELCP diet was also low, ultimate pH of the ELCP group was not different from that of the control group.

Figure 1. Effect of the LCP and ELCP diet on cross-sectional area (CSA) of myofiber of breast muscle.
The value represent the mean ± SE for 8 chickens in each group. $^{a\text{-}b}$Values with different superscripts are significantly different ($P < 0.05$). LCP, low protein diet;
ELCP, LCP diet supplemented with synthetic essential amino acids to meet the NRC recommendations.

Berri et al. [12] indicated that increase in dietary lysine level enhanced ultimate pH of broiler breast meat. In this study, level of lysine in the ELCP diet was higher than that of the LCP diet (1.10% vs. 0.83%, respectively). Therefore, this higher level of lysine might be a reason why the ELCP diet did not affect the ultimate pH. Drip loss in the LCP group was greater than those of the other two groups. However, significant difference was not observed due to the large variations. The drip loss of the ELCP group was not different from that of the control group. Thus, contrary to our first expectation, ultimate pH and drip loss might not

Figure 2. Effect of the LCP and ELCP diet on collagen content of breast muscle.
The value represent the mean ± SE for 8 chickens in each group. No significant difference was detect ($P > 0.05$). LCP, low protein diet;
ELCP, LCP diet supplemented with synthetic essential amino acids to meet the NRC recommendations.

Figure 3. Effect of the LCP and ELCP diet on ultimate pH of breast muscle.
The value represent the mean ± SE for 7 chickens in each group. $^{a\text{-}b}$Values with different superscripts are significantly different ($P < 0.05$). LCP, low protein diet; ELCP, LCP diet supplemented with synthetic essential amino acids to meet the NRC recommendations.

play a role in regulating SFV of broiler breast muscle. A positive correlation between SFV and cooking loss was reported [13]. Moreover, cooking loss had a negative correlation with ultimate pH [5]. Therefore, we need to investigate the effect of the ELCP diet on cooking loss of broiler breast muscle.

Figure 4. Effect of the LCP and ELCP diet on drip loss of breast muscle.
The value represent the mean ± SE for 7 chickens in each group. No significant difference was detect ($P > 0.05$). LCP, low protein diet; ELCP, LCP diet supplemented with synthetic essential amino acids to meet the NRC recommendations.

- CONCLUSION

In conclusion, results of this study suggested that short-term feeding of a low protein diet supplemented with EAA is an effective method for producing tender meat. We measured cross-sectional area of myofiber, content of collagen in the muscle, ultimate pH, and drip loss of breast muscle of broiler to clarify how the ELCP diet reduced the SFV of the muscle. However, the underlying mechanisms are still unclear. Therefore, further studies are necessary to elucidate the underlying mechanisms controlling the SFV by dietary CP levels, particularly by feeding a low CP diet supplemented with EAA.

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