# HISTIDINE DIPEPTIDES CONTENTS IN BREAST AND THIGH MEATS FROM 5 LINES OF KOREAN NATIVE CHICKEN

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Abstract – This study examined histidine dipeptides, carnosine and anserine contents in breast and thigh meats from 5 lines of Korean native chicken (KNC). A total of 595 F<sub>1</sub> progeny [Black (B): 90 (male: 45, female: 45), Grey-Brown (G): 110 (male: 52, female: 58), Red-Brown (R): 136 (male: 68, female: 68), White (W): 126 (male: 63, female: 63), and Yellow-Brown (Y): 133 (male: 62, female: 71)] from 70 full-sib families were used for this study. The significant differences of the carnosine and anserine contents in breast and thigh meats from 5 lines of KNC were found. The influence of gender on carnosine and anserine contents was also confirmed. This data could provide useful information for selective breeding to develop a high quality meat-type chicken breed.

# Key Words – Chicken, Carnosine, Anserine

# I. INTRODUCTION

Selective breeding strategy for poultry has been mainly focused on quantitative traits related with growth rate and meat yield in the past decades. Consequently, the birds with the improved genetic potential by selection could achieve a market weight in 6 weeks raising term with high meat yield [1]. Recently, however, qualitative traits affecting nutritional and sensory quality of meat are valued rather than quantitative traits due to the increase of consumer's wellbeing trend.

Among the endogenous compounds in skeletal muscle of vertebrate animal, histidine dipeptides, carnosine and anserine, are known as bioactive substances [2]. Carnosine ( $\beta$ -alanyl-L-histidine) is a histidine dipeptide synthesized from  $\beta$ -alanine and L-histidine, and abundant in mammalian skeletal muscles [3]. Its biological activity is to act as pH buffer in muscle, anti-

anti-glycation, anti-oxidation, aging, and neurotransmitter function [2, 4]. Because of the biological activities of carnosine, that could militate as therapeutical substance in favor of suppression of various diseases such as diabetes, cataract, ischemia, ischemia, and Alzheimer's disease [5]. In addition, carnosine in human body attenuates acidosis and a loss of force during exercise (Baquet et al., 2010). Anserine  $(\beta$ -alanyl-*N*-methyl-L-histidine) is an Nmethylated derivative of carnosine, and mainly abundant in non-mammalian species such as poultry meat [6]. It has similar biological activity to carnosine [2].

Although dipeptides endogenously are synthesized in human body, the supplementation of them is recommended to keep or improve health, especially for elderly people because the age progress results in decrease of carnosine synthesis [7]. The supplementation of carnosine and anserine can be possible by taking meat whereas plant originated food cannot supply them sufficiently [2]. Nagai et al. [8] found that an improvement of recovery from mental fatigue when chicken essence including carnosine and anserine was ingested for subjects with heavy workload. Therefore. the increase of concentration in carnosine and anserine in meat could signify an improvement of nutritional and functional value.

Recently, the governmental organization in Korea (i.e., National Institute of Animal Scienc, RDA, Korea) has been responsible for progressing the project to develop a new chicken breed. This project mainly focused on developing the high quality meat-type breed by using Korean native chicken to meet demand of consumer and to conserve genetic resources [9]. The five lines of Korean native chicken (i.e., Black, Grey-Brown, Red-Brown, White, and Yellow-Brown) were proposed as candidates for selection. Therefore, the aim of this study was to investigate the concentration of carnosine and anserine in breast and thigh meats from the 5 lines of Korean native chicken.

# II. MATERIALS AND METHODS

# Animals

A two-generation resource pedigree using 5 lines of Korean native chicken (KNC) was established and managed for this study. Within each line, three sires were mated to 14~15 dams to produce  $F_1$  chicks. A total of 595  $F_1$  progeny [Black (B): 90 (male: 45, female: 45), Grey-Brown (G): 110 (male: 52, female: 58), Red-Brown (R): 136 (male: 68, female: 68), White (W): 126 (male: 63, female: 63), Yellow-Brown (Y): 133 (male: 62, female: 71)] from 70 full-sib families were used for this study. Chickens were raised in National Institute of Animal Science, Korea. Chickens were fed ad libitum with commercial formulated feed with 18.2% protein and 2859 concentrated kcal/kg metabolizable energy for 20 wks. Chickens were killed by conventional neck cut, bled for 2 min, removed feathers, and eviscerated. Breast and thigh muscle was dissected from the carcasses after chilling at 4°C for 24 h. Muscle samples were vacuum-packed and stored in a freezer at -20°C until analysis.

# *Histidine dipeptides content*

The content of carnosine and anserine were determined by the method of Mora et al. [10]. Minced meat samples (2.5 g) were homogenized with 7.5 mL of 0.01 N HCl at 13,500 rpm/min for 1 min. The homogenate was centrifuged at  $17,000 \times g$  for 15 min, and the supernatant was mixed with 750 µL acetonitrile. After storing at 4°C for 20 min and centrifuging at 10,000 rpm for 10 min, the mixture was injected into a HPLC column with a Waters 1525 pump and a Waters 717 plus autosampler (Millipore Co-Operative, Milford, MA, USA). An Atlantis HILIC silica column (4.6  $\times$  150 mm, 3  $\mu$ m, Waters) was used. A diode array detector (Waters 2487, Millipore Co-Operative, Milford, MA, USA) was used at 214 nm for determining carnosine and anserine. The mobile phase A was

0.65 mM ammonium acetate in water/acetonitrile (25:75, v/v, pH 5.5) and B was 0.55 mM ammonium acetate in water/acetonitrile (70:30, v/v, pH 5.5). The mobile phases were supplied at 1.2 mL/min for 16 min with a linear gradient (0–100%). Standards were obtained from Sigma (USA).

# Statistical analysis

Analysis of variance was conducted by the procedure of General Linear Model using SAS program version 9.1 (2002-2003 by SAS Institute Inc.). Duncan's multiple range tests were used to compare the significant differences of the mean values of treatments (P < 0.05). Least squares mean values and its standard error (SEM) were reported.

# III. RESULTS AND DISCUSSION

Carnosine is a composite of  $\beta$ -alanine and Lhistidine by carnosine synthase [3]. Recently, ATP-grasp domain-containing protein 1 was reported as carnosine synthase, which is ligase [11]. Anserine is an N-methylated derivative of carnosine. It can be synthesized by three pathways: (1) combination of  $\beta$ -alanine and Lmethylhistidine, (2) direct N-methylation of carnosine, and (3)  $\beta$ -alanyl transfer from carnosine to L-methylhistidine [12]. The contents of carnosine and anserine in skeletal muscle is influenced by various factors [6].

The breast meat of male chickens from B, R, and W line had significantly higher carnosine content than that from G line (P < 0.05) (Table 1). The content of anserine in breast meat of male chicken was high in lines B, W, and Y compared to that in R (P < 0.05). However, there was no significant difference of carnosine and anserine contents in breast meat of female chickens among 5 lines. In thigh meat, the highest carnosine and anserine contents were found in male chicken from R and W line, respectively (Table 2) (P < 0.05). The female chicken from line R, also, had higher carnosine content than those from G, W, and Y (P < 0.05). The content of anserine in female chicken was high in W compared to that in R (P < 0.05). This results showed that the contents of carnosine and anserine in chicken meat were influenced by genetic differences in chicken. Intarapichet and

Maikhunthod [13] reported that breast and leg meats of chicken from Thai native 4-line cross breeds had different carnosine content. Also, contents of carnosine differed considerably between Black-Bone silky Fowl (native chicken in south China) and White Plymouth Rock (world's popular chicken breed) in both breast and thigh meats [14].

Except for line W, female chickens had significantly higher carnosine content than male chickens in both breast and thigh meats (P <0.05). A previous study found high carnosine content of breast and thigh meat from female chicken compared to those from male chicken in whole breeds with similar live weight. suggesting that the higher carnosine content in female chicken came from their old age [13]. However, the age of female and male chickens used in this study was the same. The effect of gender on the content of muscle carnosine was different depending on species. Liu [15] reported that the carnosine content of bovine longissimus muscle was higher in heifer than bull and steer. In rats and humans, muscle of female had lower carnosine content than that of male [16]. However, there was no significant effect on gender in the carnosine content of muscle from equines [17]. In the present study, the difference of the anserine contents between male and female chicken was inconsistent between breast and thigh meat.

The difference of the carnosine and anserine content between breast and thigh meat regardless of lines was confirmed (Tables 1 and 2). Previous studies reported that breast meat included a large amount of carnosine and anserine when compared with thigh meat [6, 13]. This is due to the fact that different composition of muscle fiber types between breast and thigh meat. Breast muscle is predominantly composed by type II muscle fiber rather than thigh muscle [18]. During exercise, muscle fiber type IIA and IIB required sufficient amount of dipeptides, carnosine and anserine as a physic-chemical buffer against a proton produced by anaerobic glycolysis in muscle compared to muscle fiber type I. This resulted in an accumulation of carnosine [19].

# IV. CONCLUSION

The difference of the carnosine and anserine content of breast and thigh muscle was found among the 5 lines of KNC. This data could provide useful information for selection to develop a high quality meat-type chicken breed. However, the comparative analysis between dipeptide content and the genotype of each line is necessary to be more understanding of the difference among 5 lines of KNC.

Table 1. Carnosine and anserine content (mg/100 g) of breast meat from Korean native chicken breeds.

| -       | Carnosine          |                  | _   | Anse              | _                |      |
|---------|--------------------|------------------|-----|-------------------|------------------|------|
| -       | М                  | F                | SEM | М                 | F                | SEM  |
| В       | 155 <sup>ay</sup>  | 179 <sup>x</sup> | 8.1 | 880 <sup>a</sup>  | 823              | 21.2 |
| G       | 130 <sup>by</sup>  | 163 <sup>x</sup> | 7.3 | 851 <sup>ab</sup> | 829              | 23.0 |
| R       | 153 <sup>ay</sup>  | 186 <sup>x</sup> | 6.4 | 803 <sup>b</sup>  | 797              | 18.5 |
| W       | 159 <sup>a</sup>   | 172              | 7.4 | 918 <sup>ax</sup> | 830 <sup>y</sup> | 26.3 |
| Y       | 139 <sup>aby</sup> | 176 <sup>x</sup> | 7.4 | 914 <sup>ax</sup> | 847 <sup>y</sup> | 23.0 |
| $SEM^1$ | 6.9                | 7.8              |     | 23.1              | 22.7             |      |

B=Black, G=Grey-Brown, R=Red-Brown, W=White, and Y=Yellow-Brown; M=Male and F=Female. <sup>1</sup>Standard errors of mean.

<sup>a,b</sup>Different letters among breeds differ significantly (P < 0.05).

<sup>x,y</sup>Different letters between sex differ significantly (P < 0.05).

Table 2. Carnosine and anserine content (mg/100 g) of thigh meat from Korean native chicken breeds.

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|---|-------------------|-------------------|-----|-------------------|--------------------|------|--|--|--|
|   | Carn              | osine             |     | Ans               |                    |      |  |  |  |
|   | М                 | F                 | SEM | М                 | F                  | SEM  |  |  |  |
| В   | 85 <sup>aby</sup> | 98 <sup>abx</sup> | 3.6 | 321 <sup>by</sup> | 373 <sup>ax</sup>  | 8.7  |  |  |  |
| G   | 66 <sup>by</sup>  | 73 <sup>dx</sup>  | 2.1 | 330 <sup>by</sup> | 367 <sup>abx</sup> | 7.9  |  |  |  |
| R   | 92 <sup>ay</sup>  | 107 <sup>ax</sup> | 3.4 | 312 <sup>by</sup> | 343 <sup>bx</sup>  | 7.5  |  |  |  |
| W   | 81 <sup>b</sup>   | 85 <sup>c</sup>   | 3.1 | 360 <sup>a</sup>  | 374 <sup>a</sup>   | 10.3 |  |  |  |
| Y   | 76 <sup>by</sup>  | 93 <sup>bex</sup> | 3.5 | 327 <sup>by</sup> | 357 <sup>abx</sup> | 10.5 |  |  |  |
| $SEM^1$   | 2.9               | 3.6               |     | 9.5               | 9.1                |      |  |  |  |

B=Black, G=Grey-Brown, R=Red-Brown, W=White, and Y=Yellow-Brown; M=Male and F=Female.

Y=Yellow-Brown; M=Ma Standard errors of mean.

Standard errors of mean.

<sup>a-d</sup>Different letters among breeds differ significantly (P < 0.05). <sup>x,y</sup>Different letters between sex differ significantly (P < 0.05).

# **ACKNOWLEDGEMENTS**

This work was supported by a grant from the Next-Generation BioGreen 21 Program (No. PJ0081330), Rural Development Administration, Republic of Korea.

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