



Proximate Composition, and L-Carnitine and Betaine Contents in Meat from Korean Indigenous Chicken

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ABSTRACT: This study investigated the proximate composition and L-carnitine and betaine content of meats from 5 lines of Korean indigenous chicken (KIC) for developing highly nutritious meat breeds with health benefits from the bioactive compounds such as L-carnitine and betaine in meat. In addition, the relevance of gender (male and female) and meat type (breast and thigh meat) was examined. A total of 595 F₁ progeny (black [B], grey-brown [G], red-brown [R], white [W], and yellow-brown [Y]) from 70 full-sib families were used. The moisture, protein, fat, and ash contents of the meats were significantly affected by line, gender, and meat type ($p < 0.05$). The males in line G and females in line B showed the highest protein and the lowest fat content of the meats. L-carnitine and betaine content showed effects of meat type, line, and gender ($p < 0.05$). The highest L-carnitine content was found in breast and thigh meats from line Y in both genders. The breast meat from line G and the thigh meat from line R had the highest betaine content in males. The female breast and thigh meats showed the highest betaine content in line R. These data could be valuable for establishing selection strategies for developing highly nutritious chicken meat breeds in Korea. (**Key Words:** Korean Indigenous Chicken Lines, Meat, L-Carnitine, Betaine)

INTRODUCTION

Meat is a highly nutritious food because it can provide not only all the essential amino acids but also micronutrients such as minerals and vitamins (Biesalski, 2005). Among meat sources, chicken meat contains higher protein as well as lower fat and cholesterol contents than red meat, and consequently is considered superior for human health (Choe et al., 2010). In addition, it is cheaper than pork and beef and has fewer religious restrictions. For these reasons, the consumption of chicken meat has increased and is predicted to increase as much as 34% by 2018 (OECD-FAO, 2009). In Korea, chicken meat has mainly been produced from broiler breeds, which have the benefits of fast growth, low production cost, and high meat

yield, by selection with an intensive fattening system (Jung et al., 2014). Recently, increasing consumer interest in eating healthier meat has resulted in an increasing interest in indigenous chicken breeds because the meat of indigenous chicken breeds has higher protein and lower fat contents as well as unique flavors compared with broiler breeds (Choe et al., 2010; Jung et al., 2011; Jayasena et al., 2013). Therefore, indigenous chicken breeds are regarded as good sources for the production of meat that has high nutritional value (Jung et al., 2013).

Chicken and other meats contain various endogenous compounds known to be beneficial to humans (Schmid, 2009; Lee et al., 2015). In addition to the basic nutrients, these compounds have received much attention in terms of their bioactivities and their contribution to the nutritional value of meat (Jung et al., 2013). Among the bioactive compounds in meat, L-carnitine (γ -trimethylamino- β -hydroxybutyric acid) is a small, water-soluble, nitrogen-containing compound that is biosynthesized from lysine and methionine (Hoppel, 2003; Schmid, 2009). Its key role in the body is fat metabolism. The inner membrane of

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Submitted Mar. 22, 2015; Revised May 8, 2015; Accepted May 22, 2015

mitochondria is impermeable to fatty acyl-coenzyme A (CoA). However, as an ester with L-carnitine fatty acyl-CoA is transported through the inner membrane of mitochondria and subsequently undergoes β -oxidation (Arslan et al., 2003; Schmid, 2009). In addition, L-carnitine has other roles such as buffering the ratio of acyl-CoA to CoA, branched chain amino acid metabolism, removal of excess acyl groups, and peroxisomal fatty acid oxidation (Hoppel, 2003; Steiber et al., 2004). Betaine (*N-N-N*-trimethylglycine) is a zwitterionic compound and a methyl derivative of glycine. Betaine is used as an organic osmolyte and as a source of methyl groups (Craig, 2004). Consequently, it has been suggested to be an important nutrient in humans (Craig, 2004).

Although L-carnitine and betaine can be synthesized in humans, the intake of these compounds from food could be advantageous for maintaining or improving health. A previous study found that the supplementation of L-carnitine reduced body weight, serum triglycerides, and total cholesterol in mice (Jang et al., 2014). Flanagan et al. (2010) suggested that L-carnitine supplementation could prevent cardiovascular disease and control obesity. Betaine has been suggested as a nutrient that can protect cells and proteins from environmental stress and prevent chronic diseases in humans (Craig, 2004; Patrick, 2002). In addition, Cholewa et al. (2013) reported that betaine supplementation improved the performance and body composition of strength-trained males. Therefore, it seems likely that increasing the concentrations of L-carnitine and betaine would improve the nutritional value of meat.

Recently, a governmental organization in Korea has been trying to develop a new chicken breed that can produce high quality meat based on Korean indigenous chickens (KICs) to satisfy consumers' demands. For this project, five lines of KIC (i.e., black [B], grey-brown [G], red-brown [R], white [W], and yellow-brown [Y]) were proposed as candidates for selection. The present study was conducted to investigate the proximate composition and the L-carnitine and betaine contents of meats from five lines of KIC, which are proposed as candidates for selection, to obtain potentially useful information for establishing selection strategies to develop a new chicken breed that can produce highly nutritious meat with health benefits from the bioactive compounds such as L-carnitine and betaine in meat.

MATERIALS AND METHODS

Animals

A two-generation resource pedigree using 5 lines of KIC was established and managed in this study. Within each line, three sires were mated with 14 to 15 dams to produce F₁ chicks. In total, 595 F₁ progeny (B: 90 [male 45, female

45], G: 110 [male 52, female 58], R: 136 [male 68, female 68], W: 126 [male 63, female 63], Y: 133 [male 62, female 71]) from 70 full-sib families were used in this study. Chickens were raised at the National Institute of Animal Science (NIAS) of Korea and fed *ad libitum* commercial-formula feed containing 18.2% concentrated protein and 2,859 kcal/kg metabolizable energy. Chicken care facilities and the procedures were performed met or exceeded the standards established by the Committee for Accreditation of Laboratory Animal Care at NIAS in Korea. This study was also conducted in accordance with "The Guide for the Care and Use of Laboratory Animals" published by the institutional Animal Care and Use Committee of NIAS, Korea. Chickens were weighed individually and slaughtered after 4 h of feed withdrawal at 20 weeks of age using conventional neck cuts and bleeding for 2 min; the feathers were then removed and the chickens were eviscerated. The carcasses were vacuum-packed after chilling in ice-cold water and stored in refrigerator at 4°C for 24 h. And then vacuum-packed carcasses were frozen in a freezer at -20°C until analysis.

Sample preparation

Before analysis, the frozen carcasses were thawed in a refrigerator at 4°C for 24 h. Breast and thigh muscles were dissected from each thawed carcass. Then, the right and left breast and thigh muscles from each chicken were minced separately using a food mixer (CH180A, Kenwood Ltd, Hampshire, UK) for 30 s. Minced meat samples were used for analysis.

Determination of proximate composition

The proximate composition of meats was determined by a slightly modified method of the Association of Official Agricultural Chemists (1995). Moisture content was determined by drying 3 g of samples placed in aluminum dishes for 15 h at 104°C. Crude protein content was measured by the Kjeldahl method (VAPO45, Gerhardt Ltd., Idar-Oberstein, Germany). The amount of nitrogen obtained was multiplied by 6.25 to calculate the crude protein content. Crude fat content was measured by the Soxhlet extraction system (TT 12/A, Gerhardt Ltd., Germany). Crude ash content was measured by burning 2 g of sample overnight in a furnace at 600°C.

Determination of L-carnitine and betaine contents

L-Carnitine and betaine contents in meat samples were determined by the method of Li et al. (2007) with some modifications. Each meat sample (3 g) was homogenized at 13,500 rpm for 30 s (T25b, IKA-Works Sdn Bhd, Selangor, Malaysia) with 10 mL of acetonitrile-methanol solution (9:1 v/v) and centrifuged at 2,090×g for 5 min at 4°C (Union 32R, Hanil Co., Ltd., Incheon, Korea). The supernatant was

filtered into a 20-mL volumetric flask through a funnel plugged with glass wool. The remaining filtrate was again mixed with 10 mL of acetonitrile-methanol solution and centrifuged under the same conditions. The resulting supernatant was collected in the same volumetric flask, which was then filled with acetonitrile-methanol solution. Subsequently, 2 mL of this sample was mixed with 810 mg of Na₂HPO₄ and 90 mg of Ag₂O (9:1 w/w) in a 15-mL tube by vigorous shaking and vortexing. Sample tubes were then dried by shaking without their caps in a shaking machine for 30 min and centrifuged again (Union 32R) at 2,090×g for 5 min at 4°C. A 0.5-mL aliquot of each supernatant was then mixed with 0.5 mL of derivatizing reagent (1.39 g of bromoacetophenone and 0.066 g of 18-crown-6 in 100 mL of acetonitrile) in a 15-mL tube, vortexed, and heated (80°C) for 60 min in a water bath. After cooling under running water, this mixture was filtered through a 0.2-µm membrane filter and injected into an Atlantis HILIC high-pressure liquid chromatography silica column (4.6 mm×150 mm, 3 µm, Waters) equipped with a Waters 1525 Pump and a Waters 717 Plus Autosampler (Millipore Co-Operative, Milford, MA, USA). A Waters 2487 Diode-Array Detector (Millipore Co-Operative, USA) was used at 254 nm to measure L-carnitine and betaine contents. The mobile phase A was 25 mM ammonium acetate in which pH was adjusted to 3.0 using formic acid, and the mobile phase B was acetonitrile. The mobile phase was supplied at 1.4 mL/min for 20 min with isocratic elution (90% of A and 10% of B). L-carnitine and betaine contents were calculated using standard curves of each compound. L-carnitine hydrochloride (≥98.0%) and betaine (≥99.0%) standards were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Statistical analysis

In this study, all meats of 595 chickens were analyzed, and all data (pooled data) were analyzed by multifactorial analysis of variance using the general linear model to investigate the effect of meat type (breast and thigh), gender (male and female), and line (five lines of KIC). After grouping the data by each meat type with each gender, the data were analyzed by the general linear model to confirm

the line effect in each meat type with each gender, and the gender effect in each meat type with line. Tukey's multiple range test was used to compare significant differences between least square mean values ($p < 0.05$). Least square mean values and standard error of the least square means are reported. Additionally, Pearson's correlation coefficients were calculated ($p < 0.05$). SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

RESULTS AND DISCUSSIONS

Proximate composition

The proximate composition of breast meat from lines B, G, R, W, and Y of KIC are presented in Table 1. The moisture content of breast meat was not significantly different among the 5 lines in both males and females. The protein content of breast meat was significantly higher in line G than in line Y for males, and higher in line B than in lines R, W, and Y for females ($p < 0.05$). Fat content was not significantly different in male breast meat among the 5 lines. However, the fat content in line B was significantly lower than that in line G females ($p < 0.05$). The males of lines W and Y and the females of lines R and Y had significantly higher ash content than that of the same genders in the other lines ($p < 0.05$). In the comparison of proximate composition between genders, the male breast meat in lines G and W had significantly higher moisture and ash contents than did the female breast meat ($p < 0.05$). On the other hand, the female breast meat in lines B and G had significantly higher protein and fat contents than did the male breast meat ($p < 0.05$).

In thigh meat, lines W and R had significantly higher moisture content in males than did lines B, G, and R ($p < 0.05$). However, there was no significant difference in moisture content in females (Table 2). Protein content of thigh meat was significantly higher in males in lines B, G, and R compared with that in males in lines W and R ($p < 0.05$); however, there was no significant difference in females. The thigh meats from males in lines B and G and females in line B showed significantly lower fat content

Table 1. Proximate composition (%) of the breast meat from Korean native chicken breeds

Line	Moisture			Protein			Fat			Ash		
	Male	Female	SEM	Male	Female	SEM	Male	Female	SEM	Male	Female	SEM
B	73.10	72.80	0.110	24.37 ^{aby}	24.67 ^{ax}	0.060	0.68	0.65 ^b	0.024	1.31 ^b	1.31 ^{bc}	0.030
G	73.10 ^x	72.78 ^y	0.097	24.51 ^a	24.55 ^{ab}	0.073	0.67 ^y	0.76 ^{ax}	0.025	1.26 ^b	1.25 ^c	0.024
R	73.12	73.01	0.084	24.40 ^{ab}	24.32 ^b	0.053	0.68	0.72 ^{ab}	0.023	1.35 ^b	1.43 ^{ab}	0.034
W	73.07	72.89	0.094	24.33 ^{ab}	24.42 ^b	0.052	0.68	0.74 ^{ab}	0.025	1.56 ^{ax}	1.38 ^{bcy}	0.034
Y	73.27	72.99	0.091	24.26 ^b	24.40 ^b	0.060	0.67	0.71 ^{ab}	0.024	1.60 ^a	1.54 ^a	0.047
SEM	0.098	0.090		0.058	0.061		0.025	0.024		0.038	0.039	

SEM, standard error of mean; B, black; G, grey-brown; R, red-brown; W, white; Y, yellow-brown.

^{a-c} Different letters among breeds differ significantly ($p < 0.05$).

^{x-y} Different letters between genders differ significantly ($p < 0.05$).

Table 2. Proximate composition (%) of the thigh meat from Korean native chicken breeds

Line	Moisture			Protein			Fat			Ash		
	Male	Female	SEM	Male	Female	SEM	Male	Female	SEM	Male	Female	SEM
B	75.02 ^b	75.04	0.150	22.22 ^a	22.17	0.189	1.07 ^b	1.07 ^b	0.071	1.20 ^b	1.18 ^b	0.019
G	74.88 ^b	74.58	0.154	22.32 ^a	22.17	0.189	0.99 ^{by}	1.42 ^{abx}	0.085	1.17 ^b	1.16 ^b	0.021
R	74.82 ^b	74.79	0.116	22.19 ^a	21.93	0.137	1.24 ^{ab}	1.41 ^{ab}	0.073	1.21 ^b	1.24 ^{ab}	0.017
W	75.59 ^{ax}	74.68 ^y	0.127	21.29 ^{by}	21.89 ^x	0.145	1.45 ^a	1.55 ^a	0.076	1.31 ^{ax}	1.22 ^{aby}	0.021
Y	75.92 ^{ax}	75.06 ^y	0.129	21.00 ^{by}	21.72 ^x	0.152	1.42 ^a	1.58 ^a	0.084	1.35 ^a	1.27 ^a	0.025
SEM	0.137	0.135		0.157	0.169		0.070	0.090		0.024	0.020	
	Line	Gender	Meat type	Line	Gender	Meat type	Line	Gender	Meat type	Line	Gender	Meat type
p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0317	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0069	<0.0001
F-value	7.29	31.85	950.38	9.69	4.62	1300.85	8.90	16.98	518.04	34.45	7.33	154.27

SEM, standard error of mean; B, black; G, grey-brown; R, red-brown; W, white; Y, yellow-brown.

^{ab} Different letters among breeds differ significantly ($p < 0.05$).

^{xy} Different letters between genders differ significantly ($p < 0.05$).

compared with lines W and Y ($p < 0.05$). Ash content was significantly higher in the thigh meat from males in lines W and Y and females in line Y compared with males in lines B, G, and R and females in line B and G ($p < 0.05$). In comparing proximate composition of thigh meat between genders, the males in lines W and Y had significantly higher moisture content and lower protein content than did females ($p < 0.05$). However, there were no significant differences in moisture and fat contents between genders in lines B, G, and R. The significant difference in fat content between males and females was found only in line G, in which females had higher values than did males ($p < 0.05$). Ash content of thigh meat from males was significantly higher than that of females only in line W ($p < 0.05$).

From the pooled data (presented as p and F values) of proximate composition in the present study, moisture, protein, fat, and ash contents were significantly affected by line but the differences were small, $p < 0.05$ (Table 2). Previous study reported that the protein, fat, and ash composition of breast meat and moisture, protein, fat, and ash composition of thigh meat between native breeds in Thailand and imported breeds were different (Wattanachant et al., 2004). In the present study, gender and meat type were also influential factors in proximate composition with significance ($p < 0.05$). Thomas et al. (1984) reported that gender was one of the factors affecting proximate composition of chicken meat; however, López et al. (2011) did not observe any such gender effect. Therefore, the effect of gender on the proximate composition of chicken meat remains an open question. Regarding the effect of meat type on proximate composition, Suchý et al. (2002) found that breast meat from Ross 308 and Cobb broilers had higher moisture, protein, and ash contents and lower fat content than that of the thigh meat. These results are at least partially consistent with those of the present study in which the protein and ash contents were high in breast meat, and fat content was higher in thigh meat than in breast meat.

However, the moisture content of breast meat was lower than that of thigh meat in the present study (data not shown).

L-Carnitine content

The L-carnitine content of breast and thigh meat from KICs is presented in Table 3. The male breast meat from lines B and Y had significantly higher L-carnitine content than that from lines G, R, and W ($p < 0.05$). Lines W and Y showed significantly higher L-carnitine content in female breast meat than did line R ($p < 0.05$). In thigh meat, L-carnitine content was significantly higher in line Y than that in males in lines R and W ($p < 0.05$). Line Y also exhibited the highest L-carnitine content in female thigh meat compared with other lines with significance ($p < 0.05$). Based on these results, the pooled data revealed that there was a highly significant effect of line on the L-carnitine content of breast and thigh meat from KICs ($p < 0.0001$). These data are consistent with a recent publication by Jayasena et al. (2015), which reported the existence of

Table 3. L-carnitine content (mg/100 g) of breast and thigh meat from Korean native chicken breeds

Line	Breast			Thigh		
	Male	Female	SEM	Male	Female	SEM
B	7.99 ^{ab}	7.09 ^{ab}	0.394	13.84 ^{ab}	13.00 ^b	0.358
G	7.91 ^b	6.97 ^{ab}	0.307	12.86 ^{abc}	12.77 ^{bc}	0.399
R	7.34 ^{bx}	6.24 ^{by}	0.231	12.15 ^c	11.48 ^c	0.304
W	7.42 ^b	7.25 ^a	0.249	12.72 ^{bc}	13.13 ^b	0.284
Y	8.66 ^{ax}	7.27 ^{ay}	0.259	14.00 ^a	14.59 ^a	0.338
SEM	0.299	0.256		0.328	0.354	
	Line	Gender	Meat type	Line	Gender	Meat type
p-value	<0.0001	0.0016	<0.0001	<0.0001	<0.0001	<0.0001
F-value	21.03	10.02	1,707.48	21.03	10.02	1,707.48

SEM, standard error of mean B, black; G, grey-brown; R, red-brown; W, white; Y, yellow-brown.

^{a-c} Different letters among breeds differ significantly ($p < 0.05$).

^{xy} Different letters between genders differ significantly ($p < 0.05$).

genetic effect on L-carnitine content in chicken meats.

The male breast meat in lines R and Y showed significantly higher L-carnitine content than did female breast meat, while there was no significant difference in L-carnitine content between genders in thigh meat. From the pooled data, it was found that gender had an effect on the L-carnitine content of meat from KICs ($p < 0.01$). However, the difference in L-carnitine content between males and females (10.43 vs 9.99 mg/100 g meat) was insignificant (data not shown). Previous studies reported that gender is an influential factor for L-carnitine content in animal tissues. The skeletal muscles of male rats had high L-carnitine content compared with those of female rats (Borum, 1978). Abuzaid (2010) found a higher L-carnitine content in beef from male Angus than in that from female Angus.

Regarding the meat type, thigh meat exhibited high L-carnitine content compared with breast meat regardless of KIC line and gender. In addition, the effect of meat type on L-carnitine content of meat from KICs was the highest among the effects of line, gender, and meat type. This result is consistent with a previous study in which the L-carnitine content was higher in thigh meat than breast meat from a KIC (Jayasena et al., 2014). This result may be due to the differences in metabolic requirements for energy production between breast and thigh muscles in chickens. Breast and thigh muscles in chickens are composed of different muscle fiber types: I (slow-twitch oxidative red fiber), IIA (fast-twitch oxidative-glycolytic white fiber), and IIB (fast-twitch glycolytic white fiber). Thigh muscle, which is a red muscle, predominantly consists of type I muscle fibers with relatively large quantities of mitochondria and myoglobin, while breast muscle, which is white muscle, has a high ratio of type IIB muscle fibers and comparatively smaller quantities of mitochondria and myoglobin (Jayasena et al., 2015). Therefore, the energy production in thigh muscle relies on aerobic metabolism in mitochondria that requires L-carnitine as a carrier of fatty acids, which may result in the accumulation of L-carnitine in thigh muscle (Arslan et al., 2003; Ehrenborg and Krook, 2009). Shimada et al. (2004) also reported that the concentration of L-carnitine in red muscle was higher than in white muscle and suggested that oxygen metabolism and myofiber types were related to L-carnitine concentration in muscle. In addition, Rigault et al. (2008) reported that fat content in beef showed a positive correlation with L-carnitine content, while there was no correlation between L-carnitine and moisture or protein content. Jayasena et al. (2014) suggested that the differences in L-carnitine content between breast and leg meat in chickens might be due to the differences in fat content. In the present study, the moisture and fat contents showed a positive correlation with L-carnitine content, while protein and L-carnitine contents were negatively

Table 4. Correlation coefficient for L-carnitine and betaine content (mg/100 g) of breast and thigh meat with proximate composition (%)

Variable	Pooled data	Breast		Thigh	
		Male	Female	Male	Female
L-Carnitine					
Moisture	0.45***	0.06	0.06	-0.06	-0.08
Protein	-0.50***	-0.28***	-0.08	0.06	0.11
Fat	0.38***	0.03	0.09	-0.09	-0.01
Betaine					
Moisture	0.51***	-0.15*	-0.04	-0.01	0.11
Protein	-0.57***	0.23***	0.10	0.01	-0.12*
Fat	0.43***	-0.02	-0.10	-0.01	-0.03
L-Carnitine	0.58***	0.21**	0.10	-0.13*	-0.08

* $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

correlated (Table 4). However, moisture and fat content did not show any correlation with L-carnitine content in breast or thigh meats from both genders. Protein content exhibited a negative correlation with L-carnitine content in the male breast meat; however, this correlation was inconsistent in the female breast meat and the male and female thigh meat. Therefore, it is likely that the correlations of moisture, protein, and fat content with L-carnitine content of meat in the pooled data may be caused by the large differences in moisture, protein, and fat content, with a difference in L-carnitine content between breast and thigh meat. Based on this analysis, it seems that the fat content as well as the moisture and protein content of chicken meat are not influential factors for the L-carnitine content of chicken meat.

Betaine content

The betaine content of meat from KICs is shown in Table 5. The male breast meat from lines B and G contained

Table 5. Betaine content (mg/100 g) of breast and thigh meat from Korean native chicken breeds

Line	Breast			Thigh		
	Male	Female	SEM	Male	Female	SEM
B	9.01 ^a	8.50 ^{ab}	0.546	23.15 ^a	21.97 ^b	0.892
G	9.05 ^a	8.63 ^{ab}	0.352	21.50 ^{ab}	22.96 ^b	0.813
R	8.50 ^{ab}	9.48 ^a	0.364	23.84 ^{ay}	26.83 ^{ax}	0.878
W	7.32 ^b	7.66 ^b	0.271	18.77 ^b	20.17 ^b	0.662
Y	7.94 ^{ab}	7.78 ^b	0.303	21.09 ^{ab}	20.71 ^b	0.699
SEM	0.380	0.332		0.823	0.778	
	Line		Gender	Meat type		
p-value	<0.0001		0.0467	<0.0001		
F-value	22.44		3.96	2,446.43		

SEM, standard error of mean; B, black; G, grey-brown; R, red-brown; W, white; Y, yellow-brown.

^{a,b} Different letters among breeds differ significantly ($p < 0.05$).

^{x,y} Different letters between genders differ significantly ($p < 0.05$).

significantly higher amounts of betaine than that from line W ($p < 0.05$). The betaine content of female breast meat was the highest in line R compared with lines W and Y ($p < 0.05$). In thigh meat, lines B and R showed significantly higher betaine content than line W ($p < 0.05$). The female thigh meat from line R had a significantly higher betaine content compared with those from the other lines ($p < 0.05$). From the pooled data, it was found that there was an apparent line effect on the betaine content of meat from KICs ($p < 0.0001$). A similar result was found in a previous study that showed significantly different betaine levels in meat from different chicken breeds (Jayasena et al., 2015). The gender effect on betaine content of meat from KICs was found in pooled data ($p = 0.0467$). However, an individual comparison of betaine content between genders showed that a significant difference was found only in thigh meat from line R, while other lines in thigh meat and all lines in breast meat did not show significant differences.

Based on the pooled data from KIC meat, it was found that betaine content was highly influenced by meat type ($p < 0.0001$). The mean betaine content in breast and thigh meats from KICs was 8.37 and 22.11 mg/100 g meat, respectively (data not shown). Previously, Jayasena et al. (2014) reported that chicken leg meat contained over a two-fold higher amount of betaine than chicken breast meat. Patterson et al. (2008) found that chicken drumstick and thigh meat had high betaine and choline contents compared with breast meat. Betaine is synthesized by a two-step oxidation of choline in which mitochondrial choline oxidase first catalyzes the production of betaine aldehyde, which is further oxidized by mitochondrial betaine aldehyde dehydrogenase to betaine (Dragolovich, 1994). Therefore, it is plausible that the higher choline content and number of mitochondria in thigh muscle compared with breast muscle of chickens result in the higher accumulation of betaine.

In the present study, the correlation coefficient of betaine content with moisture, protein, and fat contents in meat from KICs was analyzed (Table 4). From the pooled data, there was a positive correlation between moisture and fat contents, while protein and betaine contents were negatively correlated. However, those correlations may be caused by the large differences between betaine content and moisture, protein, and fat contents in breast versus thigh meat, because there was no consistent correlation within individual meat types and genders. Therefore, we conclude that there is no correlation between betaine content and moisture, protein, and fat contents in meat from KICs. Mahmoudnia and Madani (2012) reported that betaine acts as a methyl donor for the synthesis of L-carnitine. Indeed, we did find a positive correlation between betaine and L-carnitine contents in meat from KICs in the pooled data (Table 4). However, the correlations between betaine and L-carnitine content were positive in breast meat and negative

in thigh meat from males, while there were no correlations in females. Thus, we conclude that no correlation exists between the betaine and L-carnitine content of meat from KICs.

In the present study, the moisture, protein, fat, and ash content of meats from 5 lines of KIC were significantly different, but the differences are small. The L-carnitine and betaine contents, which can be considered positive nutritional factors with health benefit, differed significantly between the meats from the 5 KIC lines. To our knowledge, there have been no studies on the heritability of L-carnitine and betaine content in chicken meat. However, the heritability of both compounds would not be surprising because many endogenous compounds show heritability in animals (Mateescu et al., 2012). Therefore, we conclude that these data can be valuable in establishing selection strategies for developing a new chicken breed that can produce highly nutritious meat. However, an investigation of the heritability of L-carnitine and betaine content in chicken meat is warranted. In addition, a comparative analysis of the phenotype and genotype of each line is needed to clearly understand the characteristics of these five lines of KIC.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ACKNOWLEDGMENTS

This research was supported by Golden Seed Project, Ministry of Agriculture, Food and Rural Affairs (MAFRA), Ministry of Oceans and Fisheries (MOF), Rural Development Administration (RDA) and Korea Forest Services (KFS) and Institute of Green Bio Science and Technology, Seoul National University.

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