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Original Research Article

Comparison of the amounts of endogenous bioactive compounds in raw and cooked meats from commercial broilers and indigenous chickens

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ABSTRACT

A study was conducted to compare carnosine, anserine, betaine and carnitine contents of breast and leg (combined thigh and drumstick) meat from Korean native chickens (KNCs) and commercial broilers (CBs) at their market ages (100 and 32 d, respectively) and to determine the changes in these compounds during moist heat cooking. In general, KNCs showed significantly higher histidyl dipeptide and carnitine contents and a lower betaine content than CBs ($p < 0.05$). Significantly higher histidyl dipeptide contents were observed in breast meat, while leg meat had more betaine and carnitine contents ($p < 0.05$). Significant decreases in the content of all compounds analysed in this study occurred during cooking ($p < 0.05$). Meat from KNCs is a good source of carnosine, anserine, and carnitine compared to that from CBs, which has a higher content of betaine. In addition, the contents of these endogenous compounds are significantly affected by the meat portion and the cooking process ($p < 0.05$).

1. Introduction

With rapid economic growth and globalisation of the food industry in Asian countries, including South Korea, meat production and consumption have increased remarkably in recent years. Accordingly, an approximate five-fold increase in *per capita* chicken meat consumption was reported during the last four decades in Korea (Jayasena et al., 2013). This increasing demand for chicken meat is mainly fulfilled by a few fast-growing commercial broiler (CB) strains (Choe et al., 2010), with little contribution from the slow-growing indigenous chicken breed known as the Korean native chicken (KNC). Because of their unique flavour and texture, KNCs are highly preferred to CBs by Korean consumers (Jayasena et al., 2013). In addition to these unique characteristics, these indigenous chickens contain considerable amounts of certain endogenous bioactive compounds such as carnosine and anserine (Jung et al., 2013); these can be considered additional nutritional quality factors.

Potential health-promoting and bioactive characteristics of carnosine, anserine, betaine, and carnitine have been revealed in recent studies. Both carnosine and anserine are histidyl dipeptides with strong buffering roles and antioxidant properties (Peiretti et al., 2012). In addition, carnosine possesses good anti-ageing properties (Purchas et al., 2004) and promotes defence mechanisms against glycation and oxidation (Peiretti et al., 2011). In addition to its ability to improve growth performance

Abbreviations: KNC, Korean native chicken; CB, commercial broiler; HPLC, high performance liquid chromatography.

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and fat distribution, betaine has osmoregulatory properties and can act as a methyl donor in tissue (de Zwart et al., 2003). Carnitine is a lysine-derived molecule that plays a vital role in fatty acid metabolism (Arslan et al., 2003). Carnitine is biosynthesised in the kidneys, liver, and brain, and can also be found in different food sources (Rigault et al., 2008).

Recently, Jung et al. (2013) quantified the contents of carnosine and anserine in KNC meat. They showed that the contents of these compounds in raw meat were affected by the line and sex of KNCs. No scientific literature was found regarding the factors affecting the availability of these bioactive compounds in KNCs and CBs, except that of Jung et al. (2013). Although meat quality characteristics of KNCs and CBs were compared previously (Choe et al., 2010; Jayasena et al., 2013), comparisons of these bioactive compounds in these two breeds are still unavailable. Therefore, the present study was primarily designed to compare the carnosine, anserine, betaine and carnitine contents of breast and leg meat from KNCs and CBs at their respective market ages and to determine the changes in these compounds during the cooking process.

2. Materials and methods

2.1. Animals and processing

Eighty one-day old male chicks each from a commercial KNC strain (Woorimatdag™) and a CB strain (Ross) were allotted to 10 floor pens (16 chicks of same strain per separate pen) within a single house with similar standard commercial conditions to a chicken farm (Gimcheon, Korea). Chicks were fed commercial starter (3100 kcal ME/kg, 23% CP during first 7 days), grower (3200 kcal ME/kg, 20% CP from 8th to 21st day) and finisher (3200 kcal ME/kg, 18% CP from 22nd day to respective age) diets *ad libitum*, and they had free access to water. Two birds each from CBs and KNCs were randomly selected from each replication pen at 32 and 100 d of age and subjected to a 10-h feed withdrawal period. Subsequently, birds were exsanguinated by a conventional neck cut and were bled for 2 min. The carcasses were then defeathered and eviscerated manually. After chilling (4 °C) for 24 h, each carcass was split into two halves.

2.2. Preparation of raw and cooked meat samples

Raw meat samples were obtained by dissecting both breast and a combination of thigh and drumstick (hereinafter referred to as "leg") meat from the left half of each carcass. After trimming the visible skin, fat, and connective tissues from each of the dissected raw meat samples, they were minced (CH180; Kenwood, Shenzhen, China) separately and used for subsequent analysis.

The right half of each carcass was separately boiled in stainless steel containers with water (1:1.5, w/v). When a core temperature of 72 °C was reached in breast and leg meat as checked using a digital thermometer (YF-160A Type-K; YFE, Hsinchu City, Taiwan), carcasses were removed from boiling water and vacuum-packed separately. After cooling the vacuum-packed carcasses under running water, cooked breast and leg meat samples from each half of the carcasses were dissected and deboned separately. Finally, deboned samples were manually chopped into small pieces and used for analysis.

2.3. Determination of carnosine and anserine contents

Amounts of carnosine and anserine were determined according to the modified method described by Jung et al. (2013). Each meat sample (2.5 g) was homogenised with 0.01 N HCl (7.5 mL) at 13,500 rpm for 1 min [T25b; Ika Works (Asia), Sdn. Bhd, Rawang, Malaysia] and centrifuged at 17,030 × *g* for 15 min at 4 °C (HM-150IV, Hanil Co., Ltd., Incheon, Korea). The supernatant (250 μL) was mixed with 750 μL of acetonitrile, and after holding at 4 °C for 20 min, it was centrifuged at 10,000 × *g* for 10 min (4 °C; Hanil). The resulting supernatant was injected into a high-performance liquid chromatography (HPLC) column with a Waters 1525 pump and a Waters 717 plus auto sampler (Millipore Corporation, Milford, MA, USA). An Atlantis HILIC silica column (4.6 × 150 mm, 3 μm, Millipore) was used. To determine carnosine and anserine contents, a Waters 2487 diode array detector (Millipore) was used at 214 nm. A standard curve of each compound was used to calculate the content of the particular compound in the samples. Carnosine (≥99.0%) and anserine (≥99.0%) standards were obtained from Sigma Co. (St. Louis, MO, USA).

2.4. Determination of betaine and carnitine contents

Betaine and carnitine contents in raw and cooked meat samples were determined by the method of Li et al. (2007) with some modifications. Each meat sample (3 g) was homogenised at 13,500 rpm for 30 s (Ika Works) with 10 mL of acetonitrile–methanol solution (9:1, v/v) and centrifuged at 2090 × *g* for 5 min (4 °C; Hanil). 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 (Hanil) 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 were mixed with 810 mg of Na_2HPO_4 and 90 mg of Ag_2O (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 shaker for 30 min and centrifuged again (Hanil) at 2090 × *g* for 5 min at 4 °C. A 0.5-mL aliquot of each supernatant sample was then mixed with 0.5 mL of derivatising 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 analysed by HPLC to determine betaine and carnitine contents. The HPLC system used was the same as that used to determine the dipeptide contents (Millipore), except that the partitioned fractions were detected at 254 nm. Mobile phase A was 25 mM ammonium acetate in which pH was adjusted to 3.0 using formic acid, and mobile phase B was acetonitrile. The mobile phase was supplied at 1.4 mL/min for 20 min with isocratic elution (90% A:10% B). Betaine and carnitine contents were calculated using the standard curve of each compound. Betaine (≥99.0%) and L-carnitine hydrochloride (≥98.0%) standards were obtained from Sigma Co. (St. Louis, MO, USA).

2.5. Statistical analysis

Data of the birds from the same pen were averaged and five replications from each breed were used for the statistical analysis of each parameter. The effects of cooking, meat portion, and the breed of chicken were estimated using three-way ANOVA and using the GLM procedure. After grouping the data according to each state of meat (raw or cooked) with each meat portion, the data were analysed by one-way ANOVA using the GLM procedure to confirm the associations and effects of the breed, meat portion, and state of meat. Mean separation was conducted using Tukey's multiple range test ($p < 0.05$). All tables indicate the mean values and SEM. The SAS software system was used for all statistical analyses (version 9.3, SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

The effects of the breed of chicken, meat portion, and cooking on the contents of different endogenous bioactive compounds in chicken meat were studied during the present experiment. No scientific publications that compare these bioactive compounds in different breeds of chickens are available. Only a few studies have reported the presence of these endogenous compounds in meat from chicken and other species.

3.1. Carnosine and anserine contents

Pre- and post-cooking contents of carnosine and anserine in breast and leg meat from different breeds of chicken are given in Tables 1 and 2. According to the pooled data, carnosine content in chicken meat was significantly affected ($p < 0.05$) by meat portion, cooking and breed, in order of significance. Furthermore, meat portion showed the greatest influence on anserine content, followed by breed and cooking (Table 2). Regarding the interaction effects of main factors, the interaction between the meat portion and cooking influenced the abundance of carnosine and anserine ($p < 0.05$).

Carnosine and anserine contents of breast meat were approximately 2–3-fold greater (average of 144 and 495 mg/100 g, respectively) than those of leg meat (average of 66.6 and 208 mg/100 g, respectively), irrespective of the breed of chicken and both in raw and cooked meat ($p < 0.05$). Previous studies have shown similar differences between breast and leg meat of poultry (Davies et al., 1978; Jung et al., 2013; Maikhunthod and Intarapichet, 2005; Plowman and Close, 1988; Tian et al., 2007) and this effect has been attributed to the different muscle fibre compositions of the two muscles. According to Verdiglione and Cassandro (2013), breast meat mainly contains fast-twitch glycolytic white fibres (type IIB muscle fibres), which rely on anaerobic metabolism for ATP generation (Jung et al., 2013). In contrast, leg meat primarily comprises slow-twitch oxidative red fibres known as type I muscle fibres (Lengerken et al., 2002). Therefore, lactic acid accumulation is often higher in breast muscle with more white fibres, compared to leg muscle (Maikhunthod and Intarapichet, 2005). Hence, breast muscle requires large amounts of endogenous compounds with high buffering potential, such as carnosine and anserine, which are well known for their potent buffering role (Dunnett and Harris, 1995; Jung et al., 2013; Purchas et al., 2004).

Cooking had a significant effect on the histidyl dipeptide content of chicken meat ($p < 0.05$; Tables 1 and 2). Carnosine and anserine contents significantly decreased after cooking, with average values of 94.7 and 320 mg/100 g in cooked meat, respectively, compared to average values of 116 and 384 mg/100 g in raw meat, respectively ($p < 0.05$). However, individual comparisons between raw and cooked meat in each meat portion of each breed showed that the cooking effect on the carnosine and anserine contents was only significant in the breast meat of KNCs ($p < 0.05$; data not shown). Hence, it might be proposed that the effect of cooking on the content of carnosine and anserine is influenced by the breed of chicken and the meat portion. The observed loss of carnosine and anserine contents during cooking was mainly due to the higher water solubility of these compounds (Peiretti et al., 2012; Purchas et al., 2004), which caused their losses in cooking juices. Similar depletions in the carnosine and anserine content of beef and turkey meat after cooking, compared to respective raw meats, were previously revealed (Peiretti et al., 2012; Purchas et al., 2004). In contrast, microwave-based cooking causes small losses in histidyl dipeptide content and is, therefore, suggested as a better cooking method to preserve histidyl dipeptides in meat (Peiretti et al., 2012).

Significant differences in carnosine and anserine contents were found between the two chicken breeds ($p < 0.05$; Tables 1 and 2). In this regard, KNCs had a significantly higher carnosine content in the raw breast meat than CBs ($p < 0.05$; Table 1). In addition, they had a greater ($p < 0.05$) anserine content compared with CBs except in cooked breast meat. The carnosine and anserine contents of meat are governed by muscle type, species, breed, gender, age, and breeding (Abe and Okuma, 1995; Chan and Decker, 1994). In contrast, Jayasena et al. (2014) recently showed that the age of KNC had no significant effect on the carnosine and anserine content of their meat. Hence, the higher histidyl dipeptide content of KNC meat compared to that of CB meat may be attributed to breed. Similarly, in a comparison between a native chicken breed (Black-Bone silky fowl) and a commercial breed (White Plymouth Rock),

Table 1
Effect of meat portion and cooking on carnosine content (mg/100 g) of chicken meat from two different breeds ($n = 5$).

Breed	Raw meat		SEM	Cooked meat		SEM	Meat portion	Cooking	Breed
	Breast	Leg		Breast	Leg				
Korean native chicken	182^{ax}	76.8^{b}	17.8	128^{a}	75.4^{b}	12.0			
Commercial broiler	138^{ay}	66.2^{b}	8.29	128^{a}	47.9^{b}	9.46			
SEM	16.5	9.86		11.6	9.61				
p-value							<0.0001	0.0211	0.0242
F-value							82.1	6.03	5.73

[a,b]Mean values in the same raw with different superscripts within same state of meat differ significantly ($p < 0.05$).
[x,y]Mean values in the same column with different superscripts differ significantly ($p < 0.05$).

Table 2
Effect of meat portion and cooking on anserine content (mg/100 g) of chicken meat from two different breeds ($n = 5$).

Breed	Raw meat		SEM	Cooked meat		SEM	Meat portion	Cooking	Breed
	Breast	Leg		Breast	Leg				
Korean native chicken	614^{ax}	273^{bx}	47.1	439^{a}	246^{bx}	26.6			
Commercial broiler	482^{ay}	167^{by}	22.2	447^{a}	148^{by}	19.4			
SEM	42.9	10.2		28.0	15.2				
p-Value							<0.0001	0.0032	0.0003
F-Value							205	10.2	16.82

[a,b]Mean values in the same raw with different superscripts within same state of meat differ significantly ($p < 0.05$).
[x,y]Mean values in the same column with different superscripts differ significantly ($p < 0.05$).

the native breed had significantly higher ($p < 0.05$) carnosine content in its meat than the commercial breed (Tian et al., 2007). When comparing the contents of the two histidyl dipeptides analysed in the present study, we found that anserine content was higher than carnosine content in chicken meat, irrespective of the breed, meat portion, and cooking status. This result is in agreement with previous findings of Abe and Okuma (1995) and Peiretti et al. (2011), who showed that anserine was the predominant histidyl dipeptide in poultry meat.

3.2. Betaine content

Pooled data from this study revealed that the meat portion, breed, and cooking process significantly influenced the betaine content of chicken meat, in order of significance ($p < 0.05$; Table 3). In addition, the interaction between the meat portion and cooking had a significant effect on the betaine content. As shown in Table 3, the betaine content of chicken meat was significantly greater ($p < 0.05$) in its raw state (average value of 13.6 mg/100 g) than in its cooked state (average value of 10.3 mg/100 g). However, individual comparisons revealed that the cooking effect on the betaine content was only significant in the leg meat of both breeds ($p < 0.05$), but not for breast meat ($p > 0.05$; data not shown). Therefore, it might be suggested that the effect of cooking on the abundance of betaine is dependent on the meat portion. The betaine content of raw leg meat was reduced significantly during cooking due to its high water solubility (de Zwart et al., 2003). Similar to our data, lower content of betaine in cooked broiler meat and cooked livers and hearts of turkeys compared with their raw states was previously reported (Patterson et al., 2008). Moreover, processing or cooking methods can affect the amount of betaine (de Zwart et al., 2003). For instance, boiling results in higher depletion, whereas baking, microwaving, or frying cause no significant loss.

Regarding the portion of meat, leg meat had significantly higher betaine content both in raw and cooked meat compared to breast meat, irrespective of the breed of chicken ($p < 0.05$; Table 3). The average betaine contents of breast and leg meat were 8.87 and 15.0 mg/100 g, respectively (data not shown). A previous study showed that broiler drumsticks and thigh meat contained higher betaine content than breast meat (Patterson et al., 2008). Table 3 further shows the significant effect of the breed of chicken on the betaine content of meat, with the exception of raw leg meat. Between the two breeds, CBs expressed higher betaine content in raw breast meat, and cooked breast and leg meat ($p < 0.05$). It has been shown that the betaine content of meat decreases with the age of chicken (Jayasena et al., 2014). Hence, the lower betaine content of KNC meat compared to that of CB meat may be attributable to breed and age effects because KNCs are slaughtered at older ages than CBs.

3.3. Carnitine content

Table 4 shows the carnitine content of raw and cooked meat from KNCs and CBs. The main effect controlling the carnitine content of chicken meat was the breed, followed by meat portion and cooking process (Table 4). In addition, all possible interactions showed significant effects on the carnitine content of chicken meat ($p < 0.05$). Leg meat showed significantly higher ($p < 0.05$) carnitine content than breast meat, with average values of 7.29 and 5.50 mg/100 g, respectively. In the case of raw meat, leg meat from KNCs had significantly higher carnitine content than breast meat ($p < 0.05$). However, the effects of meat portion on carnitine content were not observed in raw broiler meat ($p > 0.05$). In addition, a significantly higher carnitine content was found in cooked leg meat of KNCs compared to breast meat of the same breed ($p < 0.05$). In contrast to raw meat, the carnitine content of cooked broiler meat differed significantly between the meat portions, where breast meat had higher values than leg meat ($p < 0.05$). Furthermore, carnitine has a potent buffering ability against excess acetyl group formation during exercise (Constantin-Teodosiu et al., 1996). In this regard, muscles rich in type I fibres, such as leg muscle (Jaturasitha et al., 2008), require more carnitine content to buffer the excess acetyl groups produced as a result of higher mitochondrial content compared to muscles with type II fibres (Constantin-Teodosiu et al., 1996). Significantly greater accumulation of acetylcarnitine in type I fibres during prolonged exercise further confirmed this phenomenon (Constantin-Teodosiu et al., 1996). Hence, greater fat and type I fibre contents may lead to increased carnitine content in leg meat.

Table 3
Effect of meat portion and cooking on betaine content (mg/100 g) of chicken meat from two different breeds ($n = 5$).

Breed	Raw meat		SEM	Cooked meat		SEM	Meat portion	Cooking	Breed
	Breast	Leg		Breast	Leg				
Korean native chicken	6.31^{by}	15.2^{a}	0.429	5.48^{by}	9.12^{ay}	0.359			
Commercial broiler	11.9^{bx}	20.8^{a}	1.69	11.8^{bx}	15.0^{ax}	0.890			
SEM	0.669	2.37		0.759	1.07				
p-Value							<0.0001	0.0032	<0.0001
F-Value							38.1	10.4	34.33

[a,b]Mean values in the same raw with different superscripts within same state of meat differ significantly ($p < 0.05$).
[x,y]Mean values in the same column with different superscripts differ significantly ($p < 0.05$).

Table 4
Effect of meat portion and cooking on carnitine content (mg/100 g) of chicken meat from two different breeds ($n = 5$).

Breed	Raw meat		SEM	Cooked meat		SEM	Meat portion	Cooking	Breed
	Breast	Leg		Breast	Leg				
Korean native chicken	5.18^{b}	13.1^{ax}	0.626	5.16^{b}	7.60^{ax}	0.319			
Commercial broiler	5.76	4.72^{y}	0.336	5.89^{a}	3.69^{by}	0.298			
SEM	0.305	0.553		0.263	0.398				
p-Value							<0.0001	<0.0001	<0.0001
F-Value							39.9	32.5	94.12

[a,b]Mean values in the same raw with different superscripts within same state of meat differ significantly ($p < 0.05$).
[x,y]Mean values in the same column with different superscripts differ significantly ($p < 0.05$).

The cooking process had a significant effect on the carnitine content of chicken meat (Table 4). Raw meat had significantly higher average amounts of carnitine than cooked meat ($p < 0.05$; average values of 7.20 *vs.* 5.58 mg/100 g, respectively). By contrast, individual comparisons between raw and cooked meat in each meat portion of each breed showed that the cooking effect on the betaine content was only significant in the leg meat of KNCs ($p < 0.05$; data not shown). Therefore, it may be suggested that the effect of cooking on the betaine content is affected by the breed of chicken and the meat portion. The carnitine content of seven beef cuts and salmon was comparable ($p > 0.05$) between their raw and cooked states when frying, boiling, grilling, baking, microwave cooking, and steaming were used as cooking methods (Rigault et al., 2008). However, these authors used only short cooking times ranging from 2 to 10 min compared to the long cooking duration (40 min) used in the present study. Consequently, the loss of carnitine content in chicken meat during this study can clearly be attributed to the higher water solubility of carnitine (Arslan et al., 2003) and the longer boiling period. Similar to our findings, the carnitine content of salmon was shown to be significantly depleted during smoking, which involves several days of processing at various temperatures (Rigault et al., 2008).

Regarding the effects of breed on carnitine content, KNCs showed significantly higher carnitine content in leg meat both before and after cooking compared with CBs ($p < 0.05$; Table 4). No differences in the carnitine content of raw and cooked breast meat were found between the two chicken breeds tested in this study. The higher carnitine content in leg meat of KNCs compared to that of CBs can be explained by the differences in muscle fibre compositions between the two breeds. Jaturasitha et al. (2008) revealed that Thai native chickens contained significantly higher type I and IIA muscle fibres than imported fast-growing breeds such as the Rhode Island Red. Imported breeds are bred for higher muscle accretion, which generally involves a shift from oxidative to glycolytic muscle metabolism with more type IIB muscle fibres (Jaturasitha et al., 2008). In addition, Jayasena et al. (2014) revealed that the carnitine content of chicken meat was not affected ($p > 0.05$) by the age of chicken. Therefore, higher levels of type I and IIA fibres in KNCs compared to those in CBs may demand increased carnitine in order to buffer the excessive production of acetyl groups, as described earlier.

4. Conclusions

The breed of chicken, type of meat portion and cooking had significant effects on the levels of all bioactive compounds analysed in this experiment ($p < 0.05$). KNCs can be considered a better source of carnosine, anserine, and carnitine compared to CBs. Breast meat showed significantly higher histidyl dipeptide contents, whereas leg meat had higher betaine and carnitine contents ($p < 0.05$). Cooking of chicken meat caused significant losses in the contents of all endogenous compounds ($p < 0.05$). The results of this study are very important to breeders, producers, and consumers of poultry because no scientific literature comparing these endogenous bioactive compounds in meat from CBs and indigenous chickens, particularly KNCs, had been published prior to this study.

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