Research Article

Received: 25 November 2013 Revised: 7 July 2014 Accepted article published: 23 August 2014 Published online in Wiley Online Library: 15 September 2014

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6882

Endogenous functional compounds in Korean native chicken meat are dependent on sex, thermal processing and meat cut

Dinesh D Jayasena,[a,b] Samooel Jung,[a] Sun Hyo Kim,[a] Hyun Joo Kim,[c] Amali U Alahakoon,[a] Jun Heon Lee[a] and Cheorun Jo[c*]

Abstract

BACKGROUND: In this study the effects of sex, meat cut and thermal processing on the carnosine, anserine, creatine, betaine and carnitine contents of Korean native chicken (KNC) meat were determined. Forty 1-day-old chicks (20 chicks of each sex) from a commercial KNC strain (Woorimatdag™) were reared under similar standard commercial conditions with similar diets, and ten birds of each sex were randomly selected and slaughtered at 14 weeks of age. Raw and cooked meat samples were prepared from both breast and leg meats and analyzed for the aforementioned functional compounds.

RESULTS: Female KNCs had significantly higher betaine and creatine contents. The breast meat showed significantly higher carnosine and anserine contents, whereas the leg meat had a higher betaine and carnitine content. The content of all functional compounds was significantly depleted by thermal processing.

CONCLUSION: This study confirms that KNC meat is a good source of the above-mentioned functional compounds, which can be considered attractive nutritional quality factors. However, their concentrations were significantly affected by thermal processing conditions, meat cut and sex. Further experiments are needed to select the best thermal processing method to preserve these functional compounds.

Keywords: Korean native chicken; sex; meat cut; thermal processing; betaine; dipeptides

INTRODUCTION

Meat from indigenous chicken breeds has captured a unique place in the meat industry of many countries in recent years.[1] Many consumers are willing to pay a higher price for indigenous chicken meats owing to their distinct meat characteristics.[1,2] For example, Korean consumers prefer Korean native chicken (KNC) meat to commercial broiler meat.[2,3] Several researchers have conducted experiments on the quality characteristics of KNC meat during the past few years and revealed that KNC has nutritional qualities, including its unique flavor and texture.[2,3] Concurrently, there has been an increase in research interest in the endogenous functional compounds found in meat, such as carnosine, anserine, creatine, taurine, ubiquinone, betaine and carnitine.[4–7] However, the literature regarding the presence of these endogenous functional compounds in native chicken breeds is sparse, particularly for KNCs. Recently, Jung *et al.*[8] compared the carnosine, anserine and creatine contents of raw meat from five different pure-line KNCs.

Recent research findings have conveyed the potential health benefits and functional characteristics of these endogenous compounds present in meat. Histidine-derived dipeptides such as carnosine and anserine have strong buffering and antioxidant properties.[7] Furthermore, carnosine has a strong defense mechanism against glycation and oxidation[9] in addition to anti-aging properties.[4] Anserine is the most abundant histidine dipeptide found in most types of poultry meat, including KNC.[8] Creatine and creatine phosphate are vital for metabolism in the skeletal muscle, which provides the energy necessary for vigorous muscle contraction.[10] There is evidence that creatine can provide certain sensory properties to food, contributing to the overall flavor of meat extracts.[11]

Carnitine plays a vital role in fatty acid metabolism in animals, transporting long-chain fatty acids across the inner mitochondrial membranes for β-oxidation.[12] In addition, it has a strong buffering potential against excess acetyl group formation during exercise.[13] In most animal species, considerably higher carnitine concentrations have been found in the skeletal muscles than in other tissues.[12] Betaine acts as an osmolyte that protect cells, proteins

* *Correspondence to: Cheorun Jo, Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute for Agriculture and Life Science, Seoul National University, Seoul 151-921, Republic of Korea. E-mail: cheorun@snu.ac.kr*

a *Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 305-764, Republic of Korea*

b *Department of Animal Science, Uva Wellassa University, Badulla 90000, Sri Lanka*

c *Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute for Agriculture and Life Science, Seoul National University, Seoul 151-921, Republic of Korea*

and enzymes from environmental stress. Being a methyl donor, betaine is also involved in the methionine cycle,[14] and Alirezaei *et al.*[15] reported that it has the potential to improve the growth performance and fat distribution of broilers.

Previous studies have shown that the content of the above-mentioned functional compounds is generally determined by several factors, including species, breed, age, sex, muscle type and thermal processing.[4,6,11,14,16–18] Although the effects of sex and meat cut on the abundance of some of these functional compounds in raw KNC meat was studied,[8] their availability after thermal processing has not yet been well established. Therefore the aim of this study was to evaluate the effects of sex, thermal processing and meat cut on the carnosine, anserine, creatine, betaine and carnitine contents of KNC meat.

EXPERIMENTAL

All experimental procedures performed during this study followed the recommendations described in 'The Guide for the Care and Use of Laboratory Animals' published by the Institutional Animal Care and Use Committee (IACUC) of the NIAS (2012-C-037) in Korea.

Livestock and farming conditions

The KNCs used in this study were of a certified meat-type commercial strain (Woorimatdag™). During the study, the birds were raised under similar standard commercial conditions of temperature, humidity, ventilation and feeding at a commercial chicken farm (Gimcheon, Korea). In addition, similar chicken care facilities and procedures were carried out to meet or exceed the standards established by the Committee for Accreditation of Laboratory Animal Care at the National Institute of Animal Science in Korea.

Forty 1-day-old chicks (20 chicks of each sex) were allotted to five floor pens (four chicks of each sex per pen) within a single house. Chicks were fed *ad libitum* with commercial starter (3100 kcal metabolizable energy (ME) kg^{-1}, 230 g kg^{-1} crude protein (CP) during week 1), grower (3200 kcal ME kg^{-1}, 200 g kg^{-1} CP during weeks 2 and 3) and finisher (3200 kcal ME kg^{-1}, 180 g kg^{-1} CP from week 4 onwards) diets. In addition, the birds had free access to drinking water during the study period. The litter was regularly checked and maintained. Additional litter material was added if the birds were visibly dirty. The birds had no access to the outdoor environment.

Sample preparation

At 14 weeks of age, two birds of each sex were randomly selected from each of the five replicate pens (i.e. a total of ten birds of each sex). After withdrawing their feed for a period of 10 h, the birds were exsanguinated using a conventional neck-cut method and bled for 2 min. The carcasses were then manually defeathered and eviscerated, during which time the sex of each bird was confirmed. Following an air-chilling period of 24 h at 4 °C, the carcasses were vacuum packed and stored in a freezer at −20 °C until further analysis.

Each frozen carcass was thawed in a refrigerator (4 °C) for 24 h and split into two halves. Samples of raw breast and leg meat were separately prepared by dissecting each muscle from the left half of each carcass. After trimming the visible skin, fat and connective tissues, the raw meat samples from each carcass were minced separately with a CH180 mini-chopper (Kenwood, Shenzhen, China) and used for the analysis.

The cooked meat samples were prepared using the remaining halves of the 20 birds. They were cooked separately in water (1:1.5 w/v) for 40 min until a core temperature of >72 °C was reached, which represented the domestic boiling conditions for KNC meat. The temperature of the meat was measured using a YF-160A Type-K digital thermometer (YFE, Hsin-Chu City, Taiwan). The carcasses were then vacuum packed and cooled under running water. Finally, the breast and leg meats were separately dissected and deboned from each of the cooked half-carcasses. The cooked meat samples were manually chopped into small pieces and then used for the analysis.

Determination of carnosine, anserine and creatine contents

The contents of carnosine, anserine and creatine were determined using the modified method of Jung *et al.*[8] Each meat sample (2.5 g) was homogenized separately with 7.5 mL of 0.01 mol L^{-1} HCl using a T25b disperser (IKA Works (Asia) Sdn. Bhd., Rawang, Malaysia) set at 1130 × *g* for 1 min and then centrifuged at 17 030 × *g* for 15 min (HM-150IV, Hanil Science Industrial Co. Ltd, Incheon, Korea). The supernatant (250 µL) was mixed with 750 µL of acetonitrile. Following a storage period of 20 min at 4 °C and centrifugation at 10 000 × *g* for 10 min, the supernatant was injected into an Atlantis HILIC silica high-pressure liquid chromatography (HPLC) column (4.6 mm × 150 mm, 3 µm; Waters Corp., Milford, MA, USA) using a 1525 pump and a 717 plus autosampler (Waters Corp.). The partitioned fractions were detected using a 2487 diode array detector (Waters Corp) at 214 nm to determine the creatine, carnosine and anserine contents. A gradient elution was performed where mobile phase B was supplied at 1.2 mL min^{-1} for 16 min with a linear gradient (0–100%) in a background of mobile phase A. Mobile phase A was 0.65 mmol L^{-1} ammonium acetate in water/acetonitrile (25:75 v/v, pH 5.5) and mobile phase B was 4.55 mmol L^{-1} ammonium acetate in water/acetonitrile (70:30 v/v, pH 5.5). Standards (carnosine, anserine and creatine) were purchased from Sigma (St Louis, MO, USA). The content of each compound in a sample was determined using the standard curve derived from the respective standard.

Determination of betaine and carnitine contents

The betaine and carnitine contents of each meat sample were determined using the method described by Li *et al.*[19] with some modifications. First, the meat samples (3 g) were homogenized separately in 10 mL of precipitating reagent (acetonitrile/methanol solution, 9:1 v/v) at 1130 × *g* for 30 s (IKA Works (Asia) Sdn. Bhd.) and centrifuged at 2090 × *g* for 5 min (HM-150IV, Hanil Science Industrial Co. Ltd). The supernatant was filtered into a 20 mL volumetric flask through a funnel plugged with glass wool. This procedure was repeated after adding 10 mL of precipitating reagent, and the supernatant was collected into the same volumetric flask. This filtrate was made up to 20 mL with the precipitating reagent, and 2 mL of the resulting solution was thoroughly mixed with 0.810 g of Na_2HPO_4 and 0.090 g of Ag_2O (9:1 w/w) in a 15 mL tube. The sample tubes were air dried in a shaking machine without their caps for 30 min and then centrifuged at 2090 × *g* for 5 min. The supernatant (0.5 mL) was added to 0.5 mL of derivatizing reagent (1.39 g of 2,4′-dibromoacetophenone and 0.066 g of 18-crown-6 in 100 mL of acetonitrile) in a 15 mL tube and vortex mixed. The mixture was heated at 80 °C for 60 min in a water bath and then cooled for 5 min under running water. This mixture was filtered through a 0.2 µm membrane filter and analyzed in an HPLC system to determine the betaine and carnitine contents. The HPLC system used was the same as that for

determination of the carnosine, anserine and creatine contents, except that the partitioned fractions were detected at 254 nm. An isocratic elution was performed where the mobile phase was a 9:1 (v/v) mixture of 25 mmol L^{-1} ammonium acetate (adjusted to pH 3 using formic acid) and acetonitrile. The mobile phase was supplied at 1.4 mL min^{-1} for 20 min. Standards (betaine and L-carnitine hydrochloride) were obtained from Sigma. The betaine and carnitine contents were calculated using a standard curve for each compound.

Statistical analysis

The effects of sex, cooking and meat cut were estimated using a three-way analysis of variance (ANOVA) model and the general linear model (GLM) procedure in SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Mean separation was performed using Tukey's multiple range test ($P < 0.05$). All tables report the mean values and standard errors of the mean (SEMs).

RESULTS AND DISCUSSION

In the present study we examined the effects of sex, type of meat cut and thermal processing on the content of endogenous functional compounds in KNC meat (Table 1). The sex of the bird had a significant effect on the creatine and betaine contents but not on the histidyl dipeptide and carnitine contents. The type of meat cut significantly affected the abundance of all functional compounds tested in this study, except for creatine. In addition, thermal processing significantly reduced the final concentration of these endogenous compounds.

Effect of meat cut

The carnosine and anserine contents of the leg meat were significantly lower than those of the breast meat (Table 1), irrespective of the sex and the cooking state. The breast meat had more than twice the carnosine content of the leg meat (Table 1). Similar results suggesting that the histidyl dipeptide content differs significantly between these two meat cuts of KNCs have been reported previously.[8] Moreover, white muscles have been shown to express higher levels of carnosine[20,21] and anserine[22,23] than dark muscles. The higher carnosine and anserine content of the KNC breast meat could be caused by the clear-cut difference between the breast and leg muscles with respect to the muscle fiber composition.[8] The breast muscle is mainly made up of fast-twitch glycolytic muscle fibers (type IIB), whereas the leg muscle contains more slow-twitch oxidative muscle fibers (type I).[24] Therefore anaerobic energy delivery is more important in the breast muscle[18] and hence the breast meat needs higher levels of carnosine and anserine,[4,25] which are well-known pH buffers in muscle systems,[18,25] to neutralize the pH changes caused by the accumulation of lactic acid.

No difference was found in the creatine content between the KNC breast and leg meats in this study ($P > 0.05$) (Table 1). Similarly, KNCs were recently demonstrated to have comparable levels of creatine in their breast and thigh meats.[8] In another study the creatine content did not differ significantly between the type I and II muscle fibers of rodents.[26] In contrast, glycolytic muscles of pigs had a higher creatine content than their oxidative muscles.[5] The demand for higher levels of phosphocreatine for immediate regeneration of ATP in type II muscle fibers results in the higher creatine content of glycolytic muscles.[10,11] Hence these results suggest that the effect of meat cut on the creatine content may vary depending on the animal species.

Table 1. Carnosine, anserine, creatine, betaine and carnitine contents (g kg^{-1}) of Korean native chicken meat as affected by sex, meat cut and degree of thermal processing

	Sex		Meat cut		Thermal processing		
Compound	Male	Female	Breast	Leg	Raw	Cooked	SEM
Carnosine	1.02	1.15	1.48a	0.69b	1.27a	0.91b	0.103
Anserine	3.59	3.93	5.24a	2.27b	4.28a	3.23b	0.254
Creatine	2.85b	3.20a	2.99	3.06	3.73a	2.32b	0.078
Betaine	0.07b	0.09a	0.05b	0.11a	0.09a	0.06b	0.001
Carnitine	0.08	0.08	0.06b	0.10a	0.09a	0.07b	0.002

Mean values in the same row with different letters within each effect differ significantly ($P < 0.05$). SEM, standard error of mean ($n = 20$).

The KNC leg meat had significantly higher betaine and carnitine contents than the breast meat (Table 1). Meat from the drumstick and thigh of broilers had a higher choline content than breast meat.[27] Hence this may be the cause of the greater betaine content of the KNC leg meat, since choline is endogenously converted to betaine.[28] Similarly to current data, the red muscle from laying hens had a significantly higher carnitine content than the white muscle.[12] The higher carnitine content in the KNC leg meat can be explained by the greater fat and type I fiber content of the leg muscles. Type I fibers contain greater numbers of mitochondria than type II fibers, enabling them to produce more acetyl groups.[13] As leg muscles are rich in type I fibers,[24] they require more carnitine to buffer the excess acetyl groups that are produced.[13] This has been confirmed by the greater accumulation of acetylcarnitine ($P < 0.05$) in type I fibers during prolonged exercise.[13] Rigault *et al.*[6] reported greater amounts of carnitine in high-fat meat cuts in comparison with lean cuts, confirming a correlation between the carnitine and fat contents of beef cuts. The KNC leg meat also had a higher fat content than the breast meat.[3]

Effect of sex

The effect of sex on the carnosine and anserine content of KNC meat (Table 1) is in agreement with several previous studies of the histidyl dipeptide content in the meat of several other species and strains; no significant effect of sex was found on the carnosine content of equine and bovine meat[29,30] and on the anserine content of meat from five pure-line KNCs.[8] In contrast, female birds from the same pure-line KNCs had a greater carnosine content in their meat than male birds,[8] whereas male animals contained higher carnosine contents in their muscles compared with females in rats[31] and humans.[32] Hence this suggests that the effect of sex on the carnosine content of skeletal muscle may vary depending on the animal species and the strain.

In humans, males contain a higher proportion of fast-twitch type IIB muscle fibers.[18] Therefore it was expected that male KNCs would also have a higher type IIB muscle fiber content and thereby a higher creatine content in their meat than females. However, female KNCs showed a significantly higher creatine content in their meat than males (Table 1). Conversely, in five of the pure-line KNC strains studied, the sexes showed similar creatine contents of their meat ($P > 0.05$).[8] Therefore it might be proposed that the effect of sex on the creatine content of meat is influenced by the strain of KNCs. However, further investigations are needed to find out the reasons for the above-mentioned contradictions regarding the effect of meat cut and sex.

As shown in Table 1, female birds possessed higher levels of betaine in their meat than males, irrespective of the meat cut or the cooking state of the meat. This can be attributed to the superior ability of females to produce phosphatidylcholine endogenously,[28] which would result in higher levels of choline through the action of phospholipase D. Therefore higher choline levels in females may eventually yield a higher betaine content in their meat compared with that of males.

In contrast to our result showing a comparable carnitine content between male and female KNCs, several other researchers have demonstrated an effect of sex on the carnitine content of meat from several other species. Males of the Angus breed of cattle have been shown to have a higher carnitine content in their beef than female cattle.[33] Furthermore, the carnitine content of plasma and of the heart and skeletal muscles was higher in male than in female rats.[34] In addition, it was reported that the carnitine content increased with the age of the animal.[33] Hence the contradictory result found in this study compared with previous experiments regarding the effect of sex on carnitine content of meat is likely be due to the differences in age of the animals. However, future studies are required to confirm this phenomenon.

Effect of thermal processing

The significant depletions observed in the carnosine, anserine, betaine and carnitine contents of KNC meat during boiling (Table 1) can be explained in relation to the higher water solubility of these compounds,[4,7,16,35] which increases their rate of loss into the cooking liquor. Creatine is non-enzymatically converted into creatinine in muscle systems by the removal of water and the formation of a ring structure. This can occur easily at the temperatures generated by cooking.[4,11] The creatine content of KNC meat decreased significantly during thermal processing (Table 1), which may be primarily due to the formation of creatinine as described above. Several other researchers have also detected a similar depletion of muscle carnosine,[4,7] anserine,[7] creatine,[4,11,36] betaine[27] and carnitine[6,16] content during cooking.

In contrast, microwave cooking has been suggested as a better cooking method for reducing the loss of carnosine and anserine from meat during thermal processing.[7] Furthermore, boiling caused greater reduction in the betaine content than baking, microwaving or frying, none of which caused a significant loss. In addition, increasing the boiling time resulted in increased losses of carnitine.[16] Therefore the cooking method and conditions, including the temperature and duration, may affect the loss of these compounds from meat into the cooking liquor during thermal processing.

CONCLUSION

The sex of the bird only had a significant effect on the betaine and creatine contents of KNC meat. The type of meat cut influenced the abundance of the functional compounds in the KNC meat, with higher carnosine and anserine contents observed in the breast meat and higher betaine and carnitine contents in the leg meat. The levels of all functional compounds investigated were significantly reduced by thermal processing of the KNC meat, mainly owing to the higher water solubility of these compounds, leading to greater losses into the cooking liquor. Therefore further experiments should be conducted to find out the best thermal processing method and/or condition to preserve these functional compounds in KNC meat.

ACKNOWLEDGEMENT

This work was supported by a grant from the Next-Generation BioGreen 21 Program (No. 00813302), Rural Development Administration, Republic of Korea.

REFERENCES

1 Wattanchant S, Benjakul S and Ledward DA, Composition, color, and texture of Thai indigenous and broiler chicken muscles. *Poult Sci* **83**:123–128 (2004).
2 Jeon H-J, Choe J-H, Jung Y, Kruk ZA, Lim D-G and Jo C, Comparison of the chemical composition, textural characteristics, and sensory properties of North and South Korean native chickens and commercial broilers. *Korean J Food Sci An* **30**:171–178 (2010).
3 Jayasena DD, Jung S, Kim HJ, Bae YS, Yong HI, Lee JH, *et al.*, Comparison of quality traits of meat from Korean native chickens and broilers used in two different traditional Korean cuisines. *Asian Australas J Anim Sci* **26**:1038–1046 (2013).
4 Purchas RW, Rutherfurd SM, Pearce PD, Vather R and Wilkinson BHP, Concentrations in beef and lamb of taurine, carnosine, coenzyme Q_{10}, and creatine. *Meat Sci* **66**:629–637 (2004).
5 Mora L, Sentandreu MA and Toldra F, Contents of creatine, creatinine and carnosine in porcine muscles of different metabolic types. *Meat Sci* **79**:709–715 (2008).
6 Rigault C, Mazue F, Bernard A, Demarquoy J and Le Borgne F, Changes in L-carnitine content of fish and meat during domestic cooking. *Meat Sci* **78**:331–335 (2008).
7 Peiretti PG, Medana C, Visentin S, Dal Bello F and Meineri G, Effect of cooking method on carnosine and its homologues, pentosidine and thiobarbituric acid-reactive substance contents in beef and turkey meat. *Food Chem* **132**:80–85 (2012).
8 Jung S, Bae YS, Kim HJ, Jayasena DD, Lee JH, Park HB, *et al.*, Carnosine, anserine, creatine, and inosine 5′-monophosphate contents in breast and thigh meats from 5 lines of Korean native chicken. *Poult Sci* **92**:3275–3282 (2013).
9 Peiretti PG, Medana C, Visentin S, Giancotti V, Zunino V and Meineri G, Determination of carnosine, anserine, homocarnosine, pentosidine and thiobarbituric acid reactive substances contents in meat from different animal species. *Food Chem* **126**:1939–1947 (2011).
10 Wyss M and Kaddurah-Daouk R, Creatine and creatinine metabolism. *Physiol Rev* **80**:1107–1213 (2000).
11 Mora L, Hernández-Cázares AS, Sentandreu MA and Toldrá F, Creatine and creatinine evolution during the processing of dry-cured ham. *Meat Sci* **84**:384–389 (2010).
12 Shimada K, Sakuma Y, Wakamatsu J, Fukushima M, Sekikawa M, Kuchida K, *et al.*, Species and muscle differences in L-carnitine levels in skeletal muscles based on a new simple assay. *Meat Sci* **68**:357–362 (2004).
13 Constantin-Teodosiu D, Howell S and Greenhaff PL, Carnitine metabolism in human muscle fiber types during submaximal dynamic exercise. *J Appl Physiol* **80**:1061–1064 (1996).
14 Craig SA, Betaine in human nutrition. *Am J Clin Nutr* **80**:539–549 (2004).
15 Alirezaei M, Gheisari HR, Ranjbar VR and Hajibemani A, Betaine: a promising antioxidant agent for enhancement of broiler meat quality. *Br Poult Sci* **53**:699–707 (2012).
16 Knüttel-Gustavsen S and Harmeyer J, The content of L-carnitine in meat after different methods of heat treatment. *Br Food J* **113**:1114–1126 (2011).
17 Chan KM and Decker EA, Endogenous skeletal muscle antioxidants. *Crit Rev Food Sci Nutr* **34**:403–426 (1994).
18 Derave W, Everaert I, Beeckman S and Baguet A, Muscle carnosine metabolism and β-alanine supplementation in relation to exercise and training. *Sports Med* **40**:247–263 (2010).
19 Li K, Li W and Huang Y, Determination of free L-carnitine in human seminal plasma by high performance liquid chromatography with pre-column ultraviolet derivatization and its clinical application in male infertility. *Clin Chim Acta* **378**:159–163 (2007).
20 Cornet M and Bousset J, Free amino acids and dipeptides in porcine muscles: differences between 'red' and 'white' muscles. *Meat Sci* **51**:215–219 (1999).
21 Tian Y, Xie M, Wang W, Wu H, Fu Z and Lin L, Determination of carnosine in Black-Bone Silky Fowl (*Gallus gallus domesticus* Brisson) and common chicken by HPLC. *Eur Food Res Technol* **226**:311–314 (2007).

22 Plowman JE and Close EA, An evaluation of a method to differentiate the species of origin of meats on the basis of the contents of anserine, balenine and carnosine in skeletal muscle. *J Sci Food Agric* **45**:69–78 (1988).
23 Davies AM, Wilkinson CC and Jones JM, Carnosine and anserine content of turkey breast and leg muscles. *Br Poult Sci* **19**:101–103 (1978).
24 Jaturasitha S, Srikanchai T, Kreuzer M and Wicke M, Differences in carcass and meat characteristics between chicken indigenous to northern Thailand (Black-boned and Thai native) and imported extensive breeds (Bresse and Rhode Island Red). *Poult Sci* **87**:160–169 (2008).
25 Dunnett M and Harris RC, Carnosine and taurine contents of type I, IIA, and IIB fibres in the middle gluteal muscle. *Equine Vet J* **27**:214–217 (1995).
26 Kushmerick MJ, Moerland TS and Wiseman RW, Mammalian skeletal-muscle fibers distinguished by contents of phosphocreatine ATP, and Pi. *Proc Natl Acad Sci USA* **89**:7521–7525 (1992).
27 Patterson KY, Bhagwat SA, Williams JR, Howe JC, Holden JM, Zeisel SH, *et al.*, *USDA Database for the Choline Content of Common Foods*. US Department of Agriculture, Beltsville, MD, pp. 12–36 (2008).
28 Olthof MR, Brink EJ, Katan MB and Verhoef P, Choline supplemented as phosphatidylcholine decreases fasting and postmethionine-loading plasma homocysteine concentrations in healthy men. *Am J Clin Nutr* **82**:111–117 (2005).
29 Marlin DJ, Harris RC, Gash SP and Snow DH, Carnosine content of the middle gluteal muscle in thoroughbred horses with relation to age, sex and training. *Comp Biochem Physiol A* **93**:629–632 (1989).
30 Mateescu RG, Garmyn AJ, O'Neil MA, Tait RG, Abuzaid A, Mayes MS, *et al.*, Genetic parameters for carnitine, creatine, creatinine, carnosine, and anserine concentration in longissimus muscle and their association with palatability traits in Angus cattle. *J Anim Sci* **90**:4248–4255 (2012).
31 Peñafiel R, Ruzafa C, Monserrat F and Cremades A, Gender-related differences in carnosine, anserine and lysine content of murine skeletal muscle. *Amino Acids* **26**:53–58 (2004).
32 Mannion AF, Jakeman PM and Willan PLT, Effects of isokinetic training of the knee extensors on isometric strength and peak power output during cycling. *Eur J Appl Physiol* **65**:370–375 (1992).
33 Abuzaid AA, Variation of carnitine concentrations in Angus beef. *MSc Thesis*, Graduate College, Iowa State University, Ames, IA (2010).
34 Borum PR, Variation in tissue carnitine concentrations with age and sex in the rat. *Biochem J* **176**:677–681 (1978).
35 De Zwart FJ, Slow S, Payne RJ, Lever M, George PM, Gerrard JA, *et al.*, Glycine betaine and glycine betaine analogues in common foods. *Food Chem* **83**:197–204 (2003).
36 Purchas RW, Busboom JR and Wilkinson BHP, Changes in the forms of iron and in concentrations of taurine, carnosine, coenzyme Q_{10}, and creatine in beef longissimus muscle with cooking and simulated stomach and duodenal digestion. *Meat Sci* **74**:443–449 (2006).

