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Research Article

The effect of rearing system and cooking method on the carnosine and anserine content of poultry and game meat

Darren Juniper*, Caroline Rymer
Division of Animal, Dairy and Food Chain Science, School of Agriculture Policy and Development, University of Reading, UK

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*Corresponding author (E-mail: d.t.juniper@reading.ac.uk)

Abstract

Poultry meat has been shown to be a rich source of carnosine and anserine (CRC) but little is known of the effects of bird species and the system under which it is reared have on the concentrations of CRC. Retail samples of breast meat from conventional chicken, free range chicken and pheasant, and breast meat from wild caught pheasant were procured and subjected to five different cooking methods: frying, grilling, boiling, microwaving and roasting. CRC were greater in uncooked pheasant than chicken ($P< 0.05$) and greater in free range than conventionally reared chicken ($P<0.05$). There were no differences in CRC between retail and wild caught pheasant. Cooking method affected CRC content; boiling and microwaving resulted in lower CRC contents than grilling, roasting or frying ($P < 0.05$). Pheasant is a richer source of CRC than conventionally reared chicken, although free range chicken produces meat of similar CRC content to pheasant.

Keywords: Chicken, Pheasant, Carnosine, Anserine, Cooking

Introduction

Carnosine and anserine are dipeptides of the amino acids β-alanine and histidine and are found in the muscles of a number of different vertebrates (Kohen *et al.,* 1988). Carnosine is more abundant in mammalian tissues, whereas anserine, the methylated form of carnosine, is found in greater concentrations in the muscles of avian species (Wolf and Wilson, 1935; Amend *et al.,* 1979). Both carnosine and anserine have been shown to serve a number of important physiological functions; these include inhibition of glycation (Hipkiss *et al.,* 1998), pH buffering in muscles (Davey, 1960; Harris *et al.,* 1998) as well as a role in antioxidant protection (Boldyrev *et al.,* 1993; Chan *et al.,* 1994).

Both carnosine and anserine can be synthesised in the human body from β-alanine and histidine, but an alternative source is the exogenous supply of these dipeptides from the diet (Peiretti *et al.,* 2011). These dipeptides are absorbed intact from the gut and are then hydrolysed by serum and tissue carnosinases to their constituent amino acids (Kubomara *et al.,* 2009). Plasma concentrations of carnosine increased 15 min after the ingestion of carnosine from beef, reached a maximum 2.5 h after meal ingestion and were undetectable after 5.5 h (Park *et al.,* 2005). Both carnosine and anserine appear to be transported across the human intestinal epithelium by the H^+/peptide co-transporters 1 and 2 (Geissler *et al.,* 2010). The rate of clearance of anserine when incorporated into a food (meat) appears to be slower than when the anserine is consumed as a supplement (Kubomara *et al.,* 2009), and this may have some advantages with regard to the utilisation of this compound, and its physiological efficacy.

Poultry meat has been shown to be a richer source of anserine when compared with other meat sources (Peiretti *et al.,* 2011; Rymer, 2012) and abundance varies with muscle type. Exercise has been reported to influence muscle fibre type, asprolonged, vigorous training has been shown to result in an increase in the abundance of type II muscle fibres (Harris *et al.,* 2012). Type II muscle fibres can be prone to fatigue and as such contain greater concentrations of carnosine related compounds (CRC) (Sewell *et al.,* 1992; Dunnett *et al.,* 1997). Therefore it is hypothesised that meat producing animals which are more active may contain higher concentrations of these dipeptides within their muscle tissues than those which are reared more intensively. Free range chickens may therefore have a higher concentration of CRC in their flight (breast) muscles than conventionally reared chickens. Game birds are likely to have even more active flight muscles, and it is hypothesised that the breast meat of pheasants would be a richer source of CRC than either conventionally reared or free range chicken.

Meat, particularly poultry meat, should be cooked through thoroughly prior to eating. Cooking has been shown to reduce the CRC content of meat (Purchas *et al.,* 2004; Bauchart *et al.,* 2006; Peiretti *et al.,* 2011) with reductions being lower in grilled samples when compared with those which had been boiled (Peiretti *et al.,* 2011). If poultry meat were to be marketed as a source of CRC, this effect of cooking would have repercussions on the value of the meat as a CRC source, and so does need to be confirmed.

The aim of this study was to determine whether the CRC content of a more active avian species (pheasant) was greater than that of chicken, and whether the way in which the bird was reared affected its CRC content. The study also aimed to determine whether different cooking methods affected the CRC content of the meat from these different sources.

Materials and Methods

Sample procurement and preparation

Retail chicken (free range [n=3] and conventional [n=3]) and pheasant breast fillets (n=3) were purchased fresh

Table 1: Effect of species and system of production on the carnosine, anserine and total carnosine related compounds (CRC) content of uncooked poultry breast tissue

Compound	Conventional Chicken (n=3)	Free Range Chicken (n=3)	Retail Pheasant (n=3)	Wild Caught Pheasant (n=3)	SEM	P-Value
Carnosine (mg g^{-1} FW)	4.89^{a}	6.81^{ab}	7.49^{ab}	8.16^{b}	0.605	0.025
Anserine (mg g^{-1} FW)	10.39^{a}	13.54^{b}	16.36^{bc}	16.82^{c}	0.939	0.004
CRC (mg g^{-1} FW)	15.27^{a}	20.34^{b}	23.86^{bc}	24.97^{c}	1.420	0.005
Carnosine:Anserine ratio	0.470	0.508	0.457	0.484	0.033	0.730

Different superscripts differ significantly (P<0.05) within row; FW = Fresh weight

from a single local supermarket at weekly intervals for three weeks. During the same period of time wild pheasant breast tissue (n=3) was obtained from a local shoot at similar weekly intervals. Samples were obtained on the morning before preparation and cooking with the exception of wild pheasant which was hung for four days prior to collection and preparation.

All skin, membranes and visible fat were removed prior to each breast being divided into six cubes of approximately 25g in weight. All cubes were kept to the same approximate dimensions to ensure even cooking. Five of the cubes were subjected to one of five methods of cooking (boiling, grilling, frying, roasting or microwaving). The sixth sample remained uncooked to determine CRC losses during cooking. The boiled sample was submerged in a beaker of boiling water on top of a hot plate, the grilled sample was grilled on both sides in a compact grill (George Forman model 17894, Spectrum Brands, China), the fried sample was dry fried in a frying pan on top of a hot plate, the roasted sample was roasted in a metal tin in an oven at 180ºC and the microwaved sample was cooked on a plate within a microwave oven (Kenwood model K20MSS10, Kenwood Ltd, China). All samples were cooked to an internal temperature of 80ºC. Following cooking all samples were freeze dried and the dry matter content of each sample was determined. Lyophilisates were subsequently ground to a fine powder using a hand blender (Bosch model MSM6150GB, China) and then stored frozen (–20ºC) pending analysis.

Analysis of carnosine and anserine

The method used for the analysis of carnosine and anserine was based on that developed by Peiretti *et al.* (2011). A sample of freeze-dried, ground tissue (100 mg) was transferred to a plastic tube and trichloroacetic acid (0.6 M, 6 ml) was added. The tube and its contents were thoroughly mixed, and incubated at room temperature for 5 min to denature the proteins. An aliquot (approx. 1 ml) was then transferred to an Eppendorf tube, which was then centrifuged (9000 x *g*, 15 min, MSE Micro Centaur, London, UK). An aliquot of the supernatant was then transferred to a vial and diluted 10-fold with distilled water. The vial was sealed and stored refrigerated (4ºC) pending analysis. Analysis of the anserine and carnosine contents of the samples was done by LC-MS (Bruker, Germany) using the method described by Peiretti *et al.* (2011).

Statistical analysis

The concentration of carnosine, anserine and carnosine related compounds (determined as the sum of carnosine and anserine) in dried samples were determined. This was then converted to carnosine, anserine and CRC contents of fresh meat (either cooked or uncooked) by allowing for the determined dry matter content of the meat. The effect of sample type (conventional chicken, free range chicken, retail pheasant or wild caught pheasant)on the carnosine, anserine and CRC content of uncooked breast tissue was determined by analysis of variance(ANOVA) using a General Linear Model (GLM). Means were separated by the Fisher multiple comparisons test. The effect of cooking method and sample type on the carnosine, anserine and CRC content of cooked breast tissue was determined by ANOVA using a GLM. Means were separated by the Fisher multiple comparisons test. The effect of cooking method on the percentage loss of carnosine, anserine and CRC in cooked breast tissue was determined by ANOVA using a GLM, treatment means were separated by the Fisher multiple comparisons test. Statistical analysis was done using the Minitab version 17 software package (Minitab Inc, Pennsylvania, USA).

Results and Discussion

Effect of species on breast CRC content

The concentration of anserine and CRC was significantly greater in uncooked meat from pheasant when compared with chicken (P<0.05), although free range chicken was not significantly different from retail pheasant, nor were there any differences between the retail pheasant and its wild caught counterpart (Table 1). There are a number of published works that show species differences in the carnosine, anserine and CRC content of breast tissue (Abe, 2000; Peiretti *et al.*, 2011; Rymer, 2012). These tend to focus on differences in CRC between chicken and turkey and there are marked differences between individual publications with regards absolute values of CRC and which of the two species are a richer source of CRC. There is very limited or no published data on the CRC content of pheasant tissue and available literature tends to focus on the differences in amino acid content and profiles between pheasants and chickens reared in identical conditions (Straková *et al.*, 2006). Although pheasants, which would have access to more extensive range than conventional chickens, had higher concentrations of anserine and CRC than the conventional chicken, it was interesting to note that there was no significant difference between the free range chicken and the retail pheasant. The largest variability in muscle CRC content is usually attributable to species/genotype (Harris *et al.*, 1990), but the similarity between free range chickens and pheasants in the current study might suggest that differences observed in CRC breast muscle content might not necessarily be a consequence of species differences but rather nutrition or environment. Nutrition, or more specifically the supply of β-alanine and histidine, have been shown to have a profound effect upon muscle CRC content in both humans (Peiretti *et al.*, 2011) and poultry (Park *et al.*, 2013; Lukasiewicz *et al.*, 2015). However, in the context of the current study it is unlikely that either the retail pheasants or their wild caught counterparts received additional β-alanine supplements during their rearing period when compared with chickens. The

similarity in anserine and total CRC content observed between the two pheasant sources and between free range chickens and retail pheasants, with conventionally reared chickens being lower than pheasants, may instead reflect levels of activity within each group of birds, and explain the differences observed in anserine and CRC content. Pheasants, whether sourced through a retail outlet or through a local shoot, are reared in more extensive, free range conditions and as a consequence the flight muscles (breast tissue) of pheasants are more likely to be active than those of commercial chickens. Furthermore, the lack of difference between retail and wild caught pheasant may reflect the similarity in which both are managed and sourced. There is very little published material available concerning the effects that physical activity has on muscle anserine and CRC content, although exercise in rats has been reported to increase muscle anserine content (Stvolinski *et al.*, 1992) and the results from this study would tend to support this finding.

It is also interesting to note that the ratio of carnosine to anserine was very similar between the two species. The carnosine : anserine ratio has been reported to be species specific and that it could be used to determine the species of origin of meat products (Plowman and Close, 1988; Abe and Okuma, 1995). The carnosine:anserine ratio in chicken has been reported to range between 0.28 and 0.81 whilst that of turkey has been shown to be lower at 0.18 to 0.27 (Plowman and Close, 1988). The carnosine : anserine ratio did not differentiate between the two species in this study, and might suggest that the physiological anatomy of these two species is similar.

Effect of cooking on breast CRC content

There was no significant interaction between sample type and cooking method on the concentration of carnosine (P=0.908), anserine (P=0.244) or CRC (P=0.551). The carnosine content of the meat was not affected by cooking method, but the anserine and total CRC content was (Table 2). Compared with the uncooked meat, the anserine (P=0.013) and total CRC (P=0.042) content was increased when the meat was grilled, roasted or fried; if the meat was microwaved this also increased total CRC content. Previous studies have reported reductions in the CRC content of meat following cooking (Purchas *et al.*, 2004; Bauchart *et al.*, 2006; Peiretti *et al.*, 2011) but the findings of the current study seem not to reflect this. When comparing uncooked breast meat with cooked meat, CRC concentrations were similar for uncooked, boiled and microwaved samples and greater in those that had been grilled, roasted or fried. This apparent increase in dipeptide content with these methods of cooking could be explained by the loss of other compounds during the cooking process which served to concentrate the dipeptides in the cooked meat.

However, if the difference in CRC content between uncooked and cooked samples is calculated then estimated CRC losses (Table 3) are highest in boiled and microwaved samples, accounting for approximately 20% of the CRC content of the uncooked sample. This greater loss of CRC content associated with boiling has been reported in other studies (Peiretti *et al.*, 2012). Both carnosine and anserine have been shown to be highly soluble in water (Quinn *et al.*, 1992; Nielsen *et al.*, 2002) and it is likely that the larger losses associated with boiling may in part be a consequence of CRC being leached into cooking water. Microwave cooking in the current study was also shown to result in similar CRC losses as boiling, whereas other research has recommended microwaving as a better cooking method if trying to minimise CRC losses (Peiretti *et al.*, 2012). This disparity in findings may be a consequence of differences in microwave cooking technique, which may have had an impact on the type and quantity of cooking losses. However, if losses in both boiled and microwaved samples are a consequence of leaching then it is likely that the resultant broth is likely to contain these leached CRC compounds and that consumption of this broth would compensate for any reduction in the CRC content of the cooked meat. However, the concentration of CRC in cooking losses were not determined in the current study and as such it is not possible to confirm this hypothesis.

Table 2: Effect of cooking method on the carnosine, anserine and total carnosine related compounds (CRC) content of poultry breast meat

Compound	Uncooked	Fried	Grilled	Boiled	Roasted	Microwave	SEM	P-Value
Carnosine (mg g^{-1} FW)	6.40	7.91	7.54	6.61	7.35	6.49	0.548	0.296
Anserine (mg g^{-1} FW)	13.4^{a}	16.1^{bc}	17.0^{c}	13.5^{ab}	16.8^{c}	14.0^{ab}	0.908	0.013
CRC (mg g^{-1} FW)	19.8^{a}	24.1^{bc}	24.5^{c}	20.1^{ab}	24.2^{c}	20.5^{abc}	1.39	0.042

Different superscripts differ significantly (P<0.05) within row;FW = Fresh weight

Table 3: Effect of cooking method on the estimated percentage loss of carnosine, anserine and total carnosine related compounds (CRC) in poultry breast tissue

Compound	Fried	Grilled	Boiled	Roasted	Microwave	SEM	P-Value
Carnosine	7.2	12.8	17.2	9.5	20.7	6.42	0.552
Anserine	7.4^{ab}	7.2^{ab}	19.9^{c}	2.9^{a}	18.7^{bc}	4.16	0.029
CRC	7.6^{ab}	9.3^{ab}	19.2^{b}	3.7^{a}	19.6^{b}	4.49	0.077

Different subscripts differ significantly (P<0.05) within row

CRC losses associated with grilling, frying or roasting in the current study were markedly lower than those seen with boiling or microwaving, accounting for approximately 5% of the CRC content of the uncooked sample (Table 3). CRC losses reported in other studies have shown that losses associated with grilling and broiling are lower than those seen in boiling, although the losses reported for grilling are notably greater than those seen in the current study (Peiretti *et al.*, 2012). This difference in findings between studies may also reflect differences in grilling technique.

Conclusions

Breast meat from birds that have had access to range (and therefore more exercise) is a richer source of anserine and CRC than that of conventionally reared chicken, but there is no significant difference between free range chicken and retail pheasant, or retail pheasant and its wild caught counterpart in this regard. The method of cooking also

affects the anserine and CRC content of cooked breast meat; boiling and microwaving resulted in greater losses of CRC than other, dryer methods of cooking. These greater losses may be a consequence of leaching and could reflect the water soluble nature of both carnosine and anserine.

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