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

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8 **Abstract**

9 Poultry meat has been shown to be a rich source of carnosine and anserine (CRC) but
10 little is known of the effects of bird species and the system under which it is reared have
11 on the concentrations of CRC. Retail samples of breast meat from conventional chicken,
12 free range chicken and pheasant, and breast meat from wild caught pheasant were
13 procured and subjected to five different cooking methods: frying, grilling, boiling,
14 microwaving and roasting. CRC were greater in uncooked pheasant than chicken ($P <$
15 0.05) and greater in free range than conventionally reared chicken ($P < 0.05$). There were
16 no differences in CRC between retail and wild caught pheasant. Cooking method affected
17 CRC content; boiling and microwaving resulted in lower CRC contents than grilling,
18 roasting or frying ($P < 0.05$). Pheasant is a richer source of CRC than conventionally
19 reared chicken, although free range chicken produces meat of similar CRC content to
20 pheasant.

21 **Key words**

22 Chicken, Pheasant, Carnosine, Anserine, Cooking

23

24 **1. Introduction**

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

33 Both carnosine and anserine can be synthesised in the human body from β -alanine and
34 histidine, but an alternative source is the exogenous supply of these dipeptides from the
35 diet (Peiretti et al., 2011). These dipeptides are absorbed intact from the gut and are then
36 hydrolysed by serum and tissue carnosinases to their constituent amino acids (Kubomara
37 et al., 2009). Plasma concentrations of carnosine increased 15 min after the ingestion of
38 carnosine from beef, reached a maximum 2.5 h after meal ingestion and were undetectable
39 after 5.5 h (Park et al., 2005). Both carnosine and anserine appear to be transported across
40 the human intestinal epithelium by the H^+ /peptide co-transporters 1 and 2 (Geissler et al.,
41 2010). The rate of clearance of anserine when incorporated into a food (meat) appears to
42 be slower than when the anserine is consumed as a supplement (Kubomara et al., 2009),
43 and this may have some advantages with regard to the utilisation of this compound, and
44 its physiological efficacy.

45 Poultry meat has been shown to be a richer source of anserine when compared with other
46 meat sources (Peiretti et al., 2011; Rymer, 2012) and abundance varies with muscle type.

47 Exercise has been reported to influence muscle fibre type, as prolonged, vigorous training
48 has been shown to result in an increase in the abundance of type II muscle fibres (Harris
49 et al., 2012). Type II muscle fibres can be prone to fatigue and as such contain greater
50 concentrations of carnosine related compounds (CRC) (Sewell et al., 1992; Dunnett et al.,
51 1997). Therefore it is hypothesised that meat producing animals which are more active
52 may contain higher concentrations of these dipeptides within their muscle tissues than
53 those which are reared more intensively. Free range chickens may therefore have a higher
54 concentration of CRC in their flight (breast) muscles than conventionally reared chickens.
55 Game birds are likely to have even more active flight muscles, and it is hypothesised that
56 the breast meat of pheasants would be a richer source of CRC than either conventionally
57 reared or free range chicken.

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

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

69

70 **2. Materials and Methods**

71 2.1 **Sample procurement and preparation**

72 Retail chicken (free range [n=3] and conventional [n=3]) and pheasant breast fillets (n=3)
73 were purchased fresh from a single local supermarket at weekly intervals for three weeks.
74 During the same period of time wild pheasant breast tissue (n=3) was obtained from a
75 local shoot at similar weekly intervals. Samples were obtained on the morning before
76 preparation and cooking with the exception of wild pheasant which was hung for four
77 days prior to collection and preparation.

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

92 2.2 **Analysis of carnosine and anserine**

93 The method used for the analysis of carnosine and anserine was based on that developed
94 by Peiretti et al. (2011). A sample of freeze-dried, ground tissue (100 mg) was transferred

95 to a plastic tube and trichloroacetic acid (0.6 M, 6 ml) was added. The tube and its
96 contents were thoroughly mixed, and incubated at room temperature for 5 min to denature
97 the proteins. An aliquot (approx. 1 ml) was then transferred to an Eppendorf tube, which
98 was then centrifuged (9000 x g, 15 min, MSE Micro Centaur, London, UK). An aliquot
99 of the supernatant was then transferred to a vial and diluted 10-fold with distilled water.
100 The vial was sealed and stored refrigerated (4°C) pending analysis. Analysis of the
101 anserine and carnosine contents of the samples was done by LC-MS (Bruker, Germany)
102 using the method described by Peiretti et al. (2011).

103 **2.3 Statistical analysis**

104 The concentration of carnosine, anserine and carnosine related compounds (determined
105 as the sum of carnosine and anserine) in dried samples were determined. This was then
106 converted to carnosine, anserine and CRC contents of fresh meat (either cooked or
107 uncooked) by allowing for the determined dry matter content of the meat. The effect of
108 sample type (conventional chicken, free range chicken, retail pheasant or wild caught
109 pheasant) on the carnosine, anserine and CRC content of uncooked breast tissue was
110 determined by analysis of variance (ANOVA) using a General Linear Model (GLM).
111 Means were separated by the Fisher multiple comparisons test

112 The effect of cooking method and sample type on the carnosine, anserine and CRC
113 content of cooked breast tissue was determined by ANOVA using a GLM. Means were
114 separated by the Fisher multiple comparisons test.

115 The effect of cooking method on the percentage loss of carnosine, anserine and CRC in
116 cooked breast tissue was determined by ANOVA using a GLM, treatment means were
117 separated by the Fisher multiple comparisons test. Statistical analysis was done using the
118 Minitab version 17 software package (Minitab Inc, Pennsylvania, USA).

119

120 **3. Results and Discussion**

121 **3.1 Effect of species on breast CRC content**

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

144 current study it is unlikely that either the retail pheasants or their wild caught counterparts
145 received additional β -alanine supplements during their rearing period when compared
146 with chickens. The similarity in anserine and total CRC content observed between the
147 two pheasant sources and between free range chickens and retail pheasants, with
148 conventionally reared chickens being lower than pheasants, may instead reflect levels of
149 activity within each group of birds, and explain the differences observed in anserine and
150 CRC content. Pheasants, whether sourced through a retail outlet or through a local shoot,
151 are reared in more extensive, free range conditions and as a consequence the flight
152 muscles (breast tissue) of pheasants are more likely to be active than those of commercial
153 chickens. Furthermore, the lack of difference between retail and wild caught pheasant
154 may reflect the similarity in which both are managed and sourced. There is very little
155 published material available concerning the effects that physical activity has on muscle
156 anserine and CRC content, although exercise in rats has been reported to increase muscle
157 anserine content (Stvolinski et al., 1992) and the results from this study would tend to
158 support this finding.

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

167 **3.2 Effect of cooking on breast CRC content**

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

182 However, if the difference in CRC content between uncooked and cooked samples is
183 calculated then estimated CRC losses (Table 3) are highest in boiled and microwaved
184 samples, accounting for approximately 20% of the CRC content of the uncooked sample.
185 This greater loss of CRC content associated with boiling has been reported in other studies
186 (Peiretti et al., 2012). Both carnosine and anserine have been shown to be highly soluble
187 in water (Quinn et al., 1992; Nielsen et al., 2002) and it is likely that the larger losses
188 associated with boiling may in part be a consequence of CRC being leached into cooking
189 water. Microwave cooking in the current study was also shown to result in similar CRC
190 losses as boiling, whereas other research has recommended microwaving as a better
191 cooking method if trying to minimise CRC losses (Peiretti et al., 2012). This disparity in
192 findings may be a consequence of differences in microwave cooking technique, which

193 may have had an impact on the type and quantity of cooking losses. However, if losses
194 in both boiled and microwaved samples are a consequence of leaching then it is likely
195 that the resultant broth is likely to contain these leached CRC compounds and that
196 consumption of this broth would compensate for any reduction in the CRC content of the
197 cooked meat. However, the concentration of CRC in cooking losses were not determined
198 in the current study and as such it is not possible to confirm this hypothesis.

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

206 **Conclusions**

207 Breast meat from birds that have had access to range (and therefore more exercise) is a
208 richer source of anserine and CRC than that of conventionally reared chicken, but there
209 is no significant difference between free range chicken and retail pheasant, or retail
210 pheasant and its wild caught counterpart in this regard. The method of cooking also
211 affects the anserine and CRC content of cooked breast meat; boiling and microwaving
212 resulted in greater losses of CRC than other, dryer methods of cooking. These greater
213 losses may be a consequence of leaching and could reflect the water soluble nature of
214 both carnosine and anserine.

215

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219

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321

322

323

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

	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

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

	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

	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