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Variability of anserine and carnosine concentration in the wild boar (*Sus scrofa scrofa*) meat

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Wild game meat becomes an important part of the diet, and its participation in consumption in recent years has increased. Several studies have confirmed that European wild boar meat has some favorable characteristics that distinguish it from other red meats. Notwithstanding, the information regarding the antioxidant capacity of wild boar meat, particularly of the protein fraction – concentration of anserine and carnosine, is scare. Carnosine and anserine have been shown to prevent lipid peroxidation and to act as neurotransmitters, modulators of enzymatic activities and chelates of heavy metals.

The aim of the study was to evaluate the antioxidant capacity of the wild boar meat, through determining the anserine and carnosine content in relation to age, sex, and type of muscle. The samples of wild boar meat used in this study were sampled from 58 wild boars (14 males, 16 females and 28 shoats) shot during 4 battues in December 2015 and January 2016. The data were analyzed statistically by three-way ANOVA, and Tukey's post-hoc test.

The study reviled that, sex, age, and muscle type played a significant role in shaping the concentration of both bioactive compounds. The concentrations of carnosine and anserine in mg g-1 of meat ranged between 2.13-3.47 and 1.38-3.82, respectively. In females, the concentrations of the dipeptides increased visibly with age, reaching about 41% (8% higher than in the early postnatal period) in longissimus dorsi (MLD), and about 50% (11% higher) in Semimembranosus (MS). In males, no significant age caused changes in carnosine concentration have been observed, while anserine concentration decreased by about 11% in MLD, and increased some 14 % in MS.

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Based on the present results, it can be assumed that the synthesis of carnosine and anserine were dependent on animal age and sex – concentrations of both dipeptides were higher in females than in males and increased with age. Therefore, from the concentration of carnosine and anserine point of view, culling of sows should take place laterin their lives while culling of the boars can take place earlier, to control the wild boar population.

KEY WORDS: anserine / carnosine / meat quality / wild boar

Wild boar (Sus scrofa scrofa), often called wild swine or Eurasian wild pig, is a species of the pig genus Sus, part of the biological family Suidae. The wild boar population in Poland has been constantly growing since the 2000s [Bombik *et al*. 2007, Kniżewska and Rekiel 2013]. In 2015, it exceeded 264 thousand heads (GUS). The set of factors, such as a limited number of natural enemies or rural depopulation has promoted heightening of wild boar densities [Sales and Kotrba 2013, Kniżewska and Rekiel 2013]. In addition, when environmental conditions are favorable, a substantial complicity of the youngest group in the reproduction causes that the population may soon be doubled [Bieber and Ruf 2005].

Wild game meat becomes an important part of the diet, and its participation in consumption in recent years has increased [Popczyk 2012]. One of the most important traits of wild boar meat, which can be used in promotion strategies, is its low intramuscular fat content [Sales and Kotrba, 2013, Quaresma *et al*. 2011, Guzek *et al*. 2013]. Despite the fact that consumer's interest for wild boar meat is gradually increasing, the information concerning its nutritional quality, particularly of the protein fraction – concentration of anserine and carnosine is scarce. Carnosine (β-alanyl-L-histidine) and anserine (β-alanyl-1-methyl-histidine) are endogenous dipeptides present in a comparatively high concentration in muscle [Boldyrev *et al.* 2013]. Carnosine is synthesized from β-alanine and L-histidine by carnosine synthetase, while anserine is created from carnosine by the enzyme S-adenosylmethionine [Penafiel *et al*. 2004]. Carnosine may chelate metal ions (e.g. copper, zinc, iron, and cobalt), and in consequence regulate their amount. This is very desirable because their inordinate concentration in the nervous system may have a toxic effect, causing an increased risk of development of neurological diseases [Łukasiewicz *et al*. 2015]. Shengying and Decker [1999] reported, that carnosine decreased by 44% of headspace trans-2-hexenal. These results suggest, that carnosine could be essential for reducing the toxicity of lipid oxidation products. Consequently, due to its biological properties, carnosine should be regarded as an important bioactive ingredient of human food [Bauchart *et al.* 2007].

The objective of this study was to evaluate the antioxidant capacity of the wild boar meat, through determining the anserine and carnosine content in relation to the age, gender, and type of muscle.

Experimental Design

Animals

The wild boars sampled in this study were shot in compatibility with the regulations of national laws on game and hunting (Official Journal of the Republic, 2005). The experiment comprises wild boars from two hunting reserves – OHZ Szubin and OHZ Krośniewice, located in central Poland. Both hunting reserves take the area of 23 441 ha and share a common habitat, similar feeding options, and equal hunting management.

The wild boar meat used in this study was collected from 58 wild boars; 14 adult males, 16 adult females and 28 shoats (14 young males and 14young females) shot during 4 battues in December 2015 and January 2016, one week apart from each other. The wild boars were shot during the night shooting, and the sanitary verification was performed by the veterinary inspector [Official Journal of the Republic 2005]. The carcass weights of the adult males and adult females were 71.12±14.85 and 58.56 ±10.25 kg, respectively, while the carcass weights of the shoats were 28.26± 7.53. Immediately after shooting samples of meat were taken from the central part of longissimus dorsi (MLD) and semimembranosus (MS); subsequently, they were transported in refrigeration (4°C) to the Milk and Meat Testing Laboratory of the Warsaw University of Life Sciences for analyses.

Chemical analysis

Approximately 200 g of muscle sample was grinded in a machine with revolving blades, mixed and placed onto an analytic plate. The gross composition of the meat, i.e. fat and protein were determined by automated infrared analysis with a FoodScanTM equipment (Foss Electric; Hillerod, Denmark).

Carnosine and anserine were extracted from boar meat and analyzed by Agilent 1100 Series reverse phase HPLC system (Agilent Technologies, Waldbronn, Germany) using methods adapted by Łukasiewicz et al. [2015]. Separations were performed at ambient temperature on Jupiter column C18 300A (Phenomenex, Torrance, USA). The identification of peaks of carnosine and anserine was confirmed by comparison with standards: carnosine, Lot: BCBB7948; anserine, Lot: BCBF45160 (Sigma-Aldrich, St Louis, MO, USA).

Statistical analysis

The analyses were performed using SPSS 23.0 [IBM Corp]. The data obtained were analyzed statistically by three-way ANOVA, and Tukey's post-hoc test. Significant differences were present among the means at a 95.0% confidence level. Data were presented as least-squares means with standard error of the mean. Only significant interactions between factors ($P \leq 0.01$ or $P \leq 0.05$) were considered in this study.

The statistical model was:

$$y_{ijkl} = \mu + A_i + B_j + C_k + (A_i \times B_j) + (A_i \times C_k) + (B_j \times C_k) + e_{ijkl}$$

where:

y_{ijkl} – the dependent variable;
μ – the overall mean;
A_i – fixed effect of age (i=1, 2, 3,);
B_j – fixed effect of gender (j=1, 2);
C_k – fixed effect of muscle type (k=1,2);
$(A_i \times B_j)$ – the interaction between age and gender;
$(A_i \times C_k)$ – the interaction between age and type of muscle;
$(B_j \times C_k)$ – the interaction between gender and type of muscle;
e_{ijkl} – the residual error.

Results and discussion

Sexual dimorphism of the content of protein and fat was observed in both muscles (Fig. 1 and 2). Based on the literature review, it can be noted that fat concentration of the wild animal's meat is lower (1-4%), while the protein content is higher (21-25%) compared with domestic animals [Skobrák *et al.* 2011, Sales and Kotrba 2013]. The present results, demonstrated that fat content in the meat samples ranged from 1.55% to 3.52% (Fig. 1). Similar results have been reported by Dannenberger *et al.* [2013].

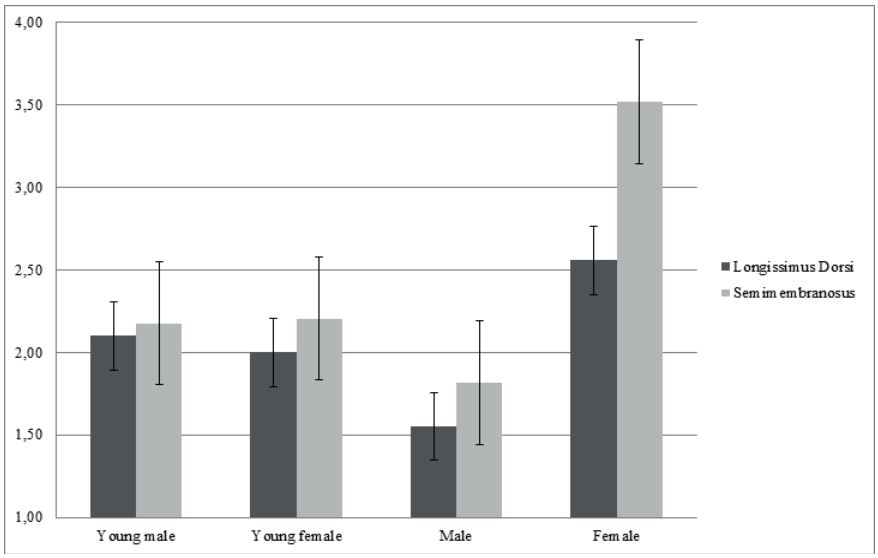

Fig. 1. Influence of gender and age on MLD and MS fat content (%)

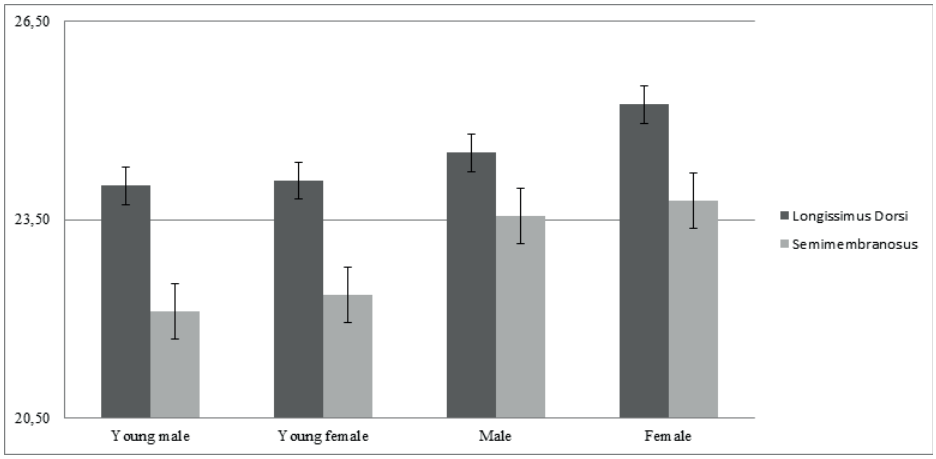

Fig. 2. Influence of gender and age on MLD and MS protein content (%)

Studies have shown, that wild boar meat is a good source of protein. In the analyzed muscles, the protein contents spread from 22.11% to 23.781% and showed significantly higher values in females (Fig. 2). A similarly high protein content in MLD obtained from wild boar of both sexes was determined by Dannenberger et al. [2013]. In turn, Batorska *et al*. [2016] reported, that wild boar meat, due to its health-promoting benefits, may provide an alternative to the meat of farm animals.

Carnosine exhibits a wide spectrum of properties, including anti-oxidation, anti-glycation, anti-aging and chelation of metal ions [Liu 2011], so it is important to consume products that are a natural source of this nutrient. The concentration of carnosine present in mg g-1 of meat in our study amounted to 2.13-3.47 (Tab. 1). In raw beef meat, demonstrated carnosine concentrations range from 2.28 to 5.72 mg g-1 [Purchas *et al*. 2005, Liu 2011] in turn for lamb meat from 2.51 to 4.91 mg g-1 [Purchas *et al.* 2005]. In the case of swine, carnosine concentration range from 2.40 to 4.66 mg g-1; 2.40 mg g-1 in longissimus dorsi, 2.76 in shoulder and legs [Easter and Baker 1977], and 4.66 mg g-1 in loin [Carnegie *et al*. 1983]. Maikhunthod and Intarapichet [2005] indicated carnosine levels about 7 times higher in chicken breast (2900.1 μg g-1) than in thigh muscle (419.9 μg g-1). Nagai *et al.* [2003] reported that muscle contraction or exercise influenced all aspects of carnosine metabolism, including synthesis, degradation, and transportation. Additionally, Mora *et al.* [2008] reported, that carnosine contents are associated both with the type of muscle metabolism and also with production system [Purchas *et al.* 2005] and slaughter age.

Sexual dimorphism of carnosine and anserine contents was observed both in MLD and MS muscles. The influence of sexual differentiation on muscle dipeptide concentration is illustrated by the changes reported in carnosine and anserine values in meat of adult wild boars. Table 1 indicates that in the MS of wild boar shoats carnosine and anserine concentrations were similar in both male and female. Therefore, based

on the current results, it can be stated that the synthesis of carnosine and anserine were dependent on animal age. This may be justified in two ways – as the effect of developmental variation in S-adenosyl methionine: carnosine methyl transferase activity or as a result of changes in carnosine availability [Penafiel *et al*. 2004].

In females, both carnosine and anserine concentrations increased significantly with age, obtaining in the mature period values about 41% in MLD (8% higher than in young animals), and about 50% in MS (11% higher) respectively, than in the postnatal period (Tab. 1). In the boars, no significant changes in carnosine concentration were observed (Tab. 1) while anserine concentration decreased about 11% in MLD, and increased about 14% in MS. In all the samples, carnosine was more substantial than anserine; furthermore, concentrations of both dipeptides were higher in females than in males (P<0.01). A similar relationship has also been demonstrated in other species of animals. Liu [2011] reported, that gender of cattle significantly affected the concentrations of carnosine and anserine. Heifers had higher amounts of carnosine than did bulls, which indicated an opposite gender effect than in rodents and humans [Penafiel *et al.* 2004].

Table 1. Influence of gender and age on MLD and MS Carnosine and Anserine content (mg g^{-1} of meat)

Group		*Longissimus Dorsi*						*Semimembranosus*					
		anserine		carnosine		interaction		anserine		carnosine		interaction	
		LSM	SEM	LSM	SEM	anserine x group	carnosine x group	LSM	SEM	LSM	SEM	anserine x group	carnosine x group
Shoats	young male	1.65^{AB_c}	0.095	2.88^{AB}	0.188	p= 0.029	p=0.004	1.28^{aBC}	0.080	2.37^{AB}	0.153	p=0.095	p=0.000
	young female	1.45^{AD}	0.088	2.30^{ACD}	0.175			1.31^{aDE}	0.074	2.13^{ACD}	0.142		
Male		1.48^{B}	0.072	2.86^{CE}	0.143			1.46^{BD}	0.051	2.40^{CE}	0.098		
Female		1.57^{cD}	0.075	3.26^{BDE}	0.148			1.46^{CE}	0.055	3.47^{BDE}	0.097		

$^{aA...}$ Means within a column marked with the same letters differ significantly at: small letters – p≤0.05; capitals – p≤0.01.
SEM – Standard error of mean. LSM – Least square of mean.

It has been proved that oral intake of carnosine improves high-intensity exercise performance and endurance, facilitates wound healing, inhibits inflammation and has anti-ulcer effects due to membrane protection activity [Monhiani 2010]. Park *et al*. [2005] reported, that carnosine can be identified in plasma 15 minutes after beef consumption, and its maximum level has been recorded after 2.5 hours, which creates the possibility of carnosine supplementation in the case of deficiency of this ingredient in human diet.

Growing population of wild boar in Poland and Europe creates the possibility to utilize this species as a meat producer of elevated pro-health properties. The meat of wild boar is rich in nutritionally important substances e.g. carnosine and

anserine. Present study reviled that, gender, age, muscle fiber type composition play a significant role in moulding the concentration of those bioactive compounds that may be essential for human nutrition and health.

As a practical recommendation from the concentration of carnosine and anserine point of view, culling of sows should take place later in their lives while culling of the boars can take place earlier, to control the wild boar population.

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