

Influence of age and sex on the content of bioactive peptides and the fatty acid profile of venison (*Cervus elaphus*)

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Due to its intense flavour and high nutritional value, game meat has long been highly sought after. In Poland the main source of game meat is deer. The aim of this study was to examine the functional properties of venison, i.e. contents of anserine and carnosine and the fatty acid profile, and to determine if these properties varied depending on the age and sex of the animal from which the meat came. The meat from 42 deer (14 males, 14 females and 14 young deer (calves) shot during four battues between December 2015 and January 2016) was used in this study. Data was statistically analysed using one-way ANOVA and Tukey's post-hoc test.

In the analyses significant differences were found in the contents of collagen, fat, water and protein in stag, hind and calf meat. The highest protein content was found in calf meat (23.06%) and the lowest in stag meat (18.96%). Analysis of fatty acids showed that unsaturated fatty acids had the highest percentage share (on average around 44%). There was a significantly higher ($P \leq 0.01$) percentage of monounsaturated fatty acids in the fat from hind meat (32.08%) compared to stag (24.73%) and calf meat (25.44%). Fat in calf meat calves had a higher share of polyunsaturated fatty acids (27.17%) than fat of stags or hinds. A significantly higher ($P \leq 0.01$) content of carnosine and anserine was found in meat from hinds at 3.54 and 1.94 mg / g, respectively, compared to stag or calf meat. Results of this study suggest that further research should be conducted to confirm health benefits of venison.

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The red deer (*Cervus elaphus*) is found virtually all over Europe (except for northern Scandinavia, Ireland, southern Italy, Greece and Albania), in North America (mainly in the western United States), and in Asia and Africa. In Poland deer populations can mostly be found in the Carpathians, Masuria and the north-western part of the country.

The body mass of deer is affected by geographical distribution. Bergmann's rule seems to apply to deer, as the body mass of individuals living in the mountains and in the north-east of Europe is greater compared to those from the lowlands and the south-west. In Poland the body mass of adult males (stags) is 120-290 kg and females (hinds) 60-150 kg. Height at the withers can reach up to 150 cm, while the total body length is 160-270 cm. The main sources of food for deer include grass, herbs, young plant shoots, root crops, fruits, acorns and chestnut fruits. Deer browse on shoots mainly of young trees and strip the bark of conifers, which is very often a source of conflict who will pay for the damage caused by animals. [Milner *et al.* 2006, Okarma and Tomek, 2008].

Meat has been the food of humans since the earliest times. Meat and meat products are sources of protein, they contain low levels of cholesterol and fat, while being rich in unsaturated fatty acids [Wangang *et al.* 2010]. The quality and texture of meat have a key impact on its market success. Meat quality is determined by its sensory and physical properties, chemical composition, etc.

Nowadays the human diet is characterized by excessive food consumption, especially highly processed food. Consumption is not the resultant of the needs of the body and consumed food is often high in saturated fat, cholesterol, sugar and salt [Babicz-Zielińska and Zabrocki 2007]. Excess contents of cholesterol and fatty acids in the diet can lead to cardiovascular diseases, which are the leading causes of death and one of the main causes of illness and disability in developed countries [Wang *et al.* 2006]. The most common causes of death related to cardiovascular diseases include atherosclerosis and its clinical consequences (coronary heart disease, myocardial infarction, stroke, arterial hypertension or heart failure). For this reason consumers are increasingly looking for food products that are nutritious and healthy, but still flavoursome [Hoffman and Wiklund 2006].

The aim of this study was to analyse the health-related quality of venison by examining anserine and carnosine contents and the fatty acid profile as well as determine if they vary depending on the age and sex of the animal.

Material and methods

Animals

The deer included in this study were shot according to the national regulations on game and hunting (Official Journal of the Republic of Poland, 2005). Deer from

two hunting reserves were used – OHZ Szubin and OHZ Krośniewice, both located in central Poland. Both hunting reserves comprise an area of 23 441 ha and have a similar habitat and feeding options, as well as identical hunting management.

The meat used in this study was collected from 42 deer: 14 stags, 14 hinds and 14 calves, shot during four battues between December 2015 and January 2016, one week apart from each other. The deer were shot during a night hunt and a veterinary inspector certified that the carcasses were sanitary [Official Journal of the Republic of Poland 2005]. Immediately after the hunt samples of meat were taken from the central part of the *longissimus dorsi* muscle and then transported in a cooler (4°C) to the Milk and Meat Testing Laboratory of the Warsaw University of Life Sciences for analysis.

Chemical analysis

Approximately 200 g of meat was ground in a grinder, mixed and placed onto an analytic plate. The gross composition of meat, i.e. fat and protein content, was determined by automated infrared analysis with FoodScan™ equipment (Foss Electric; Hillerød, Denmark).

Carnosine and anserine were extracted from the meat and analysed with an 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 a Jupiter column C18 300A (Phenomenex, Torrance, USA). The identification of carnosine and anserine peaks was confirmed by a comparison with standards: carnosine, Lot: BCBB7948; and anserine, Lot: BCBF45160 (Sigma-Aldrich, St Louis, MO, USA).

Lipid extraction of meat samples was performed according to the Folch procedure [AOAC, 1990]. Fatty acid methylation was carried out according to the *trans*-esterification method as specified in EN ISO 5509 [2000]. Fatty acid standards were identified and individual fatty acids in crude fat were quantitatively determined using the Agilent 7890A GC system (Agilent, Waldbronn, Germany) with the HP ChemStation software, a flame-ionisation detector and a Varian Select FAME column (Agilent Technologies, Waldbronn, Germany). The analysis involved a programmed run with temperature ramps under the conditions and temperatures described by Puppel [2011]. Each peak was identified and quantified using pure methyl ester standards: PUFA no. 1, Lot LB 75066; PUFA no. 2, Lot LB 83491; FAME Mix RM-6, Lot LB 68242; FAME Mix Mix 37, Lot LB 68887; CLA, Lot BCBV3726 (Supelco, Bellefonte, PA, USA).

Atherogenic (AI) and thrombogenic (TI) indices were calculated according to the formulas given by Ulbricht and Southgate [1991]:

$$AI = (C12:0 + (4 \times C14:0) + C16:0) / [MUFA + PUFA\ n-6 + PUFA\ n-3]$$

$$TI = (C14:0 + C16:0 + C18:0) / [(0.5 \times MUFA) + (0.5 \times PUFA\ n-6) + (3 \times PUFA\ n-3) + (PUFA\ n-3/PUFA\ n-6)]$$

Statistical analysis

The data obtained were analysed statistically with one-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 the standard error of the mean.

The statistical model was:

$$y_{ijkl} = \mu + A_i + e_{ijkl}$$

where:

y_{ijkl} – the dependent variable; μ is the overall mean;

A_i – the fixed effect of group ($i=1, 2, 3,$).

The computations were performed using the SPSS 23.0 programme [IBM Corp 2017].

Results and discussion

Venison is highly appreciated by consumers due to its flavour. It is also considered to be a healthier alternative to meat from farmed animals. Venison is dark red-brown in colour. In 2015 in Poland a total of 5 232 tonnes of meat of this species were acquired. The largest quantity of deer meat was acquired from the West Pomeranian province at 1 141 tons, which accounted for nearly 22% of the total amount, while the lowest in Świętokrzyskie with only 27 tons [CSO - Forestry 2016]. The average carcass weight of the stags and hinds was 110.0 ± 15.90 and 70.26 ± 11.40 kg, respectively, while carcass weights of the calves were 40.20 ± 6.88 kg.

Many authors emphasize the high sensory quality of venison [Hoffman and Wiklund 2006, Daszkiewicz *et al.* 2007, Postolache *et al.* 2011, Żochowska-Kujawska 2016]. Research conducted by Daszkiewicz *et al.* [2007] showed that such attributes as smell, juiciness and tenderness do not depend on the sex of the animal, from which the meat was obtained. On the other hand, Teubner [2010] stated that meat obtained from older animals during the mating season is characterised by an unpleasant smell and taste that disappears after 3-5 months of freezing. Daszkiewicz *et al.* [2007] found that meat from stags is of a significantly higher quality of flavor compared to hind meat. In turn, Postolache *et al.* [2011] also showed that the sex of deer influenced meat aroma and taste, both of which were found to be better in meat obtained from stags.

Venison is considered to be high quality meat [Florek and Drozd 2013]. The bioactive compounds such as anserine, carnosine, taurine, coenzyme Q10, iron, vitamin E and fatty acids present in venison have a positive effect on human health [Florek and Drozd 2013]. Physicochemical properties and the structure of proteins and peptides are expressed as their functional properties.

Carnosine and anserine are endogenic peptides present in skeletal muscle proteins. Their content depends on the chromoproteins found in the meat. The darker the meat, the greater their content [Iwaniak and Dziuba 2009]. Venison is of a darker colour

compared to livestock meat [Hoffman *et al.* 2005]. This is also confirmed by research by Hutchison *et al.* [2010].

The content of bioactive peptides is an important therapeutic factor for many diseases, in which oxidative and carbonyl stress plays a role in disease pathogenesis. These diseases include neurodegenerative, metabolic and cardiovascular diseases [Zięba 2007]. The results of analysis of bioactive peptides in the deer meat of this study are presented in Table 1. Carnosine and anserine contents in hinds were 3.54 and 1.94 mg / g, respectively, being statistically significantly higher ($P \leq 0.01$) than the contents in stags and calves.

Table 1. The effect of gender and age on MLD carnosine and anserine contents (mg g⁻¹ of meat) and chemical compositions (%)

Parameters	Group					
	stags		hinds		calves	
	LSM	SEM	LSM	SEM	LSM	SEM
Collagen (%)	1.56	0.071	2.12 ^a	0.320	1.35 ^a	0.101
Fat (%)	4.40 ^A	0.396	2.92 ^A	0.153	1.63 ^A	0.062
Water (%)	68.79 ^A	0.466	69.41 ^B	0.221	71.61 ^{AB}	0.266
Protein (%)	18.96 ^A	0.789	20.37 ^B	0.550	23.06 ^{AB}	0.081
Anserine (mg/g)	1.32 ^A	0.155	1.94 ^{Ab}	0.212	1.54 ^b	0.049
Carnosine (mg/g)	3.11 ^A	0.222	3.54 ^B	0.206	2.47 ^{AB}	0.048

^{aA...}Means within a column bearing the same superscripts differ significantly at: small letters – $p \leq 0.05$; capitals – $p \leq 0.01$.

SEM – standard error of mean. LSM – least-square of mean.

Carnosine is a natural compound found in the skeletal muscle, brain, skin and kidneys of higher vertebrates and humans [Hipkiss, 2005]. The content of carnosine is the highest in muscle (it constitutes as much as 0.5% of mass in some muscles) and ranges from 200 to 500 mg/100 g/muscle depending on the type of muscle and species [Schmidt 2009]. It is an endogenous peptide (β -alanyl-L-histidine) composed of two amino acids: beta-alanine and histidine. In humans carnosine is absorbed after oral administration, mainly from the small intestine.

Contents of carnosine and anserine in human muscle decrease with age. Between ten and seventy years of life the content of these peptides drops by 63%, which corresponds to the normal decrease in muscle mass that occurs in old age. It is therefore suggested that a diet rich in carnosine and anserine may be important for the elderly. One of the processes underlying the effects of aging is connected with oxidative stress and damage caused by free radicals [Harman 2001]. In various studies an increase in oxidative stress in various tissues has been noted, including the liver [Wolf *et al.* 2005, Wong *et al.* 2006, Parildar-Kapuzoglu *et al.* 2008], the heart [Wolf *et al.* 2005, Wong *et al.* 2006, Parildar *et al.* 2008] as well as the brain [Siqueira *et al.* 2005, Wolf *et al.* 2005].

According to the latest scientific research, dipeptides are responsible for the strength, endurance and resistance of the body to fatigue. Dipeptides are the result of two amino acids bonding in a condensation reaction. The dipeptides carnosine

and anserine play an extremely important role in the body, particularly in athletes, as they increase the muscle ability to buffer hydrogen ions. This prevents excessive acidification of muscles, allows for longer and more strenuous training and accelerates the process of muscle regeneration and renewal.

The culinary and processing value of meat is primarily determined by its chemical composition. Venison, similarly as meat of other game, is characterized by a high protein content, with an advantageous amino acid composition. Typical muscle tissue consists of about 75% water, 20% protein, 3% fat and 2% soluble non-protein substances.

The chemical composition of deer meat from this study is presented in Table 1. Similarly to fallow deer and roe deer meat, red deer meat is characterized by high protein and low fat contents. Such favourable proportions may be related to the life style of this species as well as the specific biological diversity of its natural feeding grounds. In this study the contents of collagen, fat, water and protein in the meat were found to be significantly different in stags, hinds, and calves. Collagen is the main component of connective muscle tissue affecting the quality of meat. The high content of this protein in muscle tissue contributes to the reduction of digestibility, and thus results in lower tenderness and lower nutritional value of meat. In addition, collagen is a defective protein due to the lack of tryptophan and low contents of sulfuric and aromatic amino acids. The highest amount of this protein is usually found in beef meat (1.0-15.0) and in the muscles most active during animal life [Domaradzki *et al.* 2010].

The highest protein content was observed in calves (23.06%) and the lowest in stags (18.96%). According to Boisteanu *et al.* [2009], deer meat contains 21.6-22.1% protein. A slightly lower value is given by Postolache *et al.* [2011] – 21.57-21.7% for stags and 21.42-21.82% for hinds, while Daszkiewicz *et al.* [2007] gave higher values of 22.01 and 22.41% for stags and hinds, respectively. The highest protein content found in the literature was for the meat of stags from Hungary (22.03%) [Daszkiewicz *et al.* 2013]. The lowest protein content was reported by Bleier *et al.* [2013] at 20.56%.

Numerous studies confirm the low fat content in venison. It must be remembered that it is fat that gives meat its flavour and aroma. This study found significant ($P \leq 0.01$) differences in the percentage share of fat between stags, hinds and calves. The lowest share of fat was found in calves (1.63%) and the highest in stags (4.40%). According to Postolache *et al.* [2011], the meat from hinds has a slightly higher percentage of fat (3.0-3.7%) compared to stags (2.4-3.0%), but this was not confirmed in our research. Boisteanu *et al.* [2009] gave generally a value between 2.1 and 3.5%. According to Daszkiewicz *et al.* [2013], the percentage share of fat in stag meat is only 1.07% and a similarly low value was given by Bleier *et al.* [2013] (1.31%), Strazdina *et al.* [2011] (1.60%) and Strazdina *et al.* [2013] (1.90%). Fat content of fawn meat is 1.25-1.44% according to Polak *et al.* [2008]. It should be noted that differences in the percentage share of fat may not only be due to the influence of sex, but also depend on the time of year that the animal was killed and the area, from which the animal came. Our results suggest that older stags were used in our study, as fat content increases with age.

According to the literature, 73.5-76.5% of deer meat is water and 23.5-26.5% is dry matter [Dzierżyńska-Cybulko and Fruziński 1997, Postolache *et al.* 2011, Bleier *et al.* 2013, Daszkiewicz *et al.* 2013]. In this study slightly lower values in adults were observed. It was 68.70% for stags and 69.41% for hinds, resulting in good quality animal product. Lower water content leads to extended durable life of meat thanks limited microbial growth, in contrast to material with a greater water content. Ash content was almost 1%, which is similar to other game species [Postolache *et al.* 2011].

Detailed studies on the chemical composition of individual carcasses of stags indicate that the highest content of dry matter can be found in the loin (24.59%), while it is lowest in the shoulders (23.34%) and neck (23.43%). In addition, significantly lower protein contents were found in shoulder meat (21.57%) compared to meat from the loin (22.25%) or leg (22.09%). The lowest fat content was recorded in the neck (0.74%) and the highest in the shoulder (1.04%) [Daszkiewicz *et al.* 2013].

Analysis of the fatty acid profile of the meat shows (Tabs 2-4) the highest content, on average around 44%, for saturated fatty acids (SFAs). A significantly higher ($P \leq 0.01$) percentage of monounsaturated fatty acids (MUFAs) was recorded in the intermuscular fat of hinds (32.08%) compared to stags (24.73%) and calves (25.44%). Calf meat contained a higher share of polyunsaturated fatty acids (PUFAs) (27.17%) compared to meat of hinds and stags (26.27% and 24.18%, respectively). In the literature palmitic and stearic acids are dominant in the fatty acid profile of venison [Polak *et al.* 2008, Strazdina *et al.* 2013]. The average SFA content has been found to be 41.13%, that of MUFAs to be 26.57% and PUFAs 23.47%, respectively [Strazdina *et al.* 2013]. In contrast, Valenciak *et al.* [2015] showed in their research lower contents of SFAs (30.8%) and MUFAs (8.4%) and a higher content of PUFAs (60.8%). In addition, those authors showed the effect of heat treatment on the fatty acid content of meat.

Table 2. Saturated fatty acid composition in MDL (g/100 g of fat)

Parameters	Group					
	stags		hinds		calves	
	LSM	SEM	LSM	SEM	LSM	SEM
C15:0	0.76 ^A	0.092	0.65 ^B	0.060	1.21 ^{AB}	0.037
C16:0	23.35	1.028	24.11 ^a	0.807	22.23 ^a	0.306
C17:0	0.60 ^A	0.028	0.73 ^{AB}	0.028	0.60 ^B	0.027
C18:0	15.00	0.859	14.76	0.415	15.25	0.475
C20:0	0.08 ^{ab}	0.004	0.10 ^{ac}	0.007	0.13 ^{bc}	0.006
C24:0	0.53	0.088	0.68 ^A	0.094	0.41 ^A	0.046
SFA*	44.02	1.882	44.05	1.203	44.68	0.649
AI	0.83 ^A	0.047	0.68 ^{AB}	0.027	0.84 ^B	0.025
TI	1.14 ^{Ab}	0.060	0.91 ^{Ac}	0.020	1.01 ^{bc}	0.028

^{aA...} Means within a column bearing the same superscripts differ significantly at: small letters – $p \leq 0.05$; capitals – $p \leq 0.01$.

SEM – standard error of mean. LSM – least-square of mean; *SFA – saturated fatty acids; AI – atherogenic index; TI – thrombogenic index.

Table 3. Monounsaturated fatty acids composition in MDL (g/100 g of fat)

Parameters	Group					
	stags		hinds		calves	
	LSM	SEM	LSM	SEM	LSM	SEM
C14:1	2.51 ^A	0.290	2.48 ^B	0.124	1.40 ^{AB}	0.077
C16:1	7.64 ^A	0.257	10.66 ^{AB}	0.477	6.85 ^B	0.372
C17:1	0.17 ^A	0.021	0.40 ^{AB}	0.041	0.20 ^B	0.008
C18:1 t9	0.13 ^A	0.011	0.17 ^b	0.015	0.32 ^{Ab}	0.060
C18:1 c9	14.24 ^{Ab}	0.726	18.32 ^{Ac}	0.743	16.58 ^{bc}	0.440
C20:1	0.04 ^A	0.001	0.04 ^B	0.002	0.09 ^{AB}	0.008
MUFA*	24.73 ^A	0.305	32.08 ^{AB}	0.792	25.44 ^B	0.478

^{aA...}Means within a column bearing the same superscripts differ significantly at: small letters – $p \leq 0.05$; capitals – $p \leq 0.01$.

SEM – standard error of mean. LSM – least-square of mean; *MUFA – monounsaturated fatty acids.

Table 4. Polyunsaturated fatty acids composition in MDL (g/100 g of fat)

Parameters	Group					
	stags		hinds		calves	
	LSM	SEM	LSM	SEM	LSM	SEM
C18:3 n3	3.15 ^{Ab}	0.165	4.22 ^{Ac}	0.208	3.72 ^{bc}	0.091
C18:2 n6	14.46	0.268	14.87	0.669	16.01	0.424
C18:3 n6	0.13	0.008	0.14	0.007	0.17	0.011
C18:4 n3	0.12	0.010	0.10	0.011	0.10	0.007
C20:4 n6	4.41 ^a	0.031	4.41 ^b	0.021	4.48 ^{ab}	0.025
C20:5 n3	1.01	0.071	1.01	0.070	0.88	0.050
C22:5 n3	0.66 ^A	0.063	1.20 ^A	0.078	1.52 ^A	0.080
C22:6 n3	0.20 ^{ab}	0.015	0.27 ^a	0.023	0.26 ^b	0.015
PUFA*	24.18 ^{ab}	0.448	26.27 ^a	0.716	27.17 ^B	0.608

^{aA...}Means within a column bearing the same superscripts differ significantly at: small letters – $p \leq 0.05$; capitals – $p \leq 0.01$.

SEM – standard error of mean. LSM – least-square of mean; *PUFA – polyunsaturated fatty acids.

When assessing the nutritional value of meat an important determinant is connected with the ratio of omega-6 to omega-3 fatty acids, which should be about 4:1. Analysis of the literature confirms that in the case of deer meat this value for hind meat does not exceed 4:1. In turn, Polak *et al.* [2008] reported that the lowest ratio found in meat from stags and the highest in calf meat. According to Strazdin *et al.* [2013] the ratio is 2.75:1; Hoffman and Wiklund [2006] found it to be 1.7:1; and Valencak *et al.* [2015] – 1.95:1, respectively. In our study two distinct indexes were investigated: the atherogenic index (AI) and the thrombogenic index (TI). These indexes take into account the different effects that individual fatty acids may have on human health, in particular on the probability of an increased incidence of pathogenic phenomena. Generally the atherogenic index (AI) indicates the relationship between the sum of the main saturated fatty acids and that of the main classes of unsaturated fatty acids, the

former being considered pro-atherogenic (favouring the adhesion of lipids to cells in the immune and circulatory systems), and the latter anti-atherogenic (i.e. diminishing the levels of esterified fatty acids, cholesterol and phospholipids, thus preventing the development of micro- and macrovascular diseases. The thrombogenic index (TI) shows the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic fatty acids (MUFAs, n6 and n3 PUFAs). Both AI and TI are highest for most atherogenic and thrombogenic dietary components. In the present study AI and TI values were advantageous (Tab. 2), as it is assumed that AI below 1 is beneficial to human health.

Allegations of acquiring game, which are often made by animal rights defenders, are often not confirmed by facts or result from ignorance of the aspects of populations. In order to meet today's challenges it is necessary to treat the acquisition of game in a very broad context, taking into account the tradition and culture of obtaining superior quality food (natural, free of foreign compounds and their residues) while considering animal welfare and sustainable management of natural resources.

Nowadays the quality of food and nutrition has a strong influence on consumer behaviour. Food quality may be determined by evaluating its sensory properties, durable life, reliability, authenticity, naturalness, safety (no antibiotics used) and health-related aspects, as well as purchase cost and availability. It should be noted that commercially available game meat is subject to the same quality and sanitary requirements as material of slaughter animals [Ziemińska and Krasnowska 2007]. In some families and cultures the traditional cuisine based on game meat is enthusiastically cultivated and results in a wealth of dishes and flavours.

Game dishes are highly valued by consumers. Consumers are willing to pay more for high quality products with health-promoting properties. It is therefore appropriate to conduct research to confirm high health benefits of venison. Considering the data on game consumption, including venison, it might be a viable alternative to meat of domestic species kept in an intensive system. The pro-health value as well as the taste of venison means that it is worth taking actions aimed at increasing the consumption of this meat.

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