Influence of Age and Sex on the CLA and Other Fatty Acids Content in Roe Deer Meat (*Capreolus capreolus* L.)*

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Meat is an important source of animal protein but, at the same time, it includes saturated fatty acids, which makes it a potential cause of different cardiovascular diseases and still little is known about influence of age and sex on these parameters in roe deer muscles. The aim of this study was to determine the effect of age and sex on the CLA and other fatty acids content in the *musculus longissimus lumborum* in 67 roe deer (*Capreolus capreolus* L.). In the meat from the oldest individuals a higher content of CLA was noted (89.76 [mg/kg]) when compared to the fawns (42 [mg/kg]). In this research meat from roe deer does showed in general a higher percentage proportion of SFAs and MUFAs, but lower of PUFAs, than the meat from bucks. These results may provide an important source of information for consumers of roe deer meat because of differences between CLA and other fatty acids content depending on age and sex of the animals. Meat from roebucks is the most advantageous for dietary purposes.

Key words: CLA, fatty acids, meat, roe deer, sex, age.

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Animal fat is important for human nutrition for its high energy value which is more than twice that of carbohydrates (MAHGOUB et al. 2002). While assessing health benefits of meat, a pivotal role of conjugated linoleic acid and other essential unsaturated and saturated fatty acids cannot be disregarded. Concentration of fatty acids determines not only nutritional value of the meat, but also its quality, including its shelf life and taste (WOOD et al. 2003; WARREN et al. 2008). Health benefits of meat are usually described by the ratio of saturated fatty acids (SFA) to polyunsaturated fatty acids (PUFA) as well as by n-6 to n-3 fatty acids ratio (WARREN et al. 2008). The latter is considered one of the most important indices of the fat quality (MONTEIRO et al. 2006), mainly due to positive impact of n-3 PUFA on circulatory system (BRUCKNER 2000).

The discovery of beneficial effects of the natural component of ruminants' fat, i.e. conjugated fatty acid (CLA), created new opportunities for obtaining and developing meat food products from those animals. Ruminants' meat and dairy products are

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an important source of CLA (CHIN et al. 1992; after DE LA TORRE et al. 2006). Noteworthy, meat from small ruminants contains a higher amount of conjugated linoleic acid comparing to monogastric animals (CHIN et al. 1992) due to the fact that it is synthesized naturally in the processes of incomplete dehydrogenation taking place in the rumen (JAHREIS et al. 1998; SHANTHA 1997; HUR et al. 2007). Depending on the animal species, tissue and diet, CLA content in ruminant meat products varies from 2.7 to 5.6 mg/g lipid (EVANS et al. 2002). Many research support the thesis that CLA exhibits anti-carcinogenic, anti-oxide (HUR et al. 2004), anti-atherogenic (SCHMID et al. 2006) and anti-inflammatory properties; it prevents adipogenesis, arteriosclerosis and strengthens the immune system as well as bones (HUR et al. 2007). The content of fatty acids in meat depends mainly on the species of the animal, level of its adiposity and diet (SCOLLAN et al. 2006). Recently, the impact of various diets on improving the CLA and PUFA concentration in animal food products has been widely investigated. Numerous studies reported an advantageous influence of feed on the actual level of CLA and PUFA in meat and dairy products from ruminants (SCOLLAN *et al.* 2001; SCOLLAN *et al.* 2006; POPAVA 2007; WARREN *et al.* 2008).

The aim of this experiment was to determine the effect of sex and age on CLA and other fatty acids content in roe deer meat.

Material and Methods

The investigations involved 67 roe deer (*Capreolus capreolus* L.), coming from Polish region dominated by food, chemical, electromechanical, timber and paper industries. Agricultural lands constitute 65% of the area. The research animals were hunted in their natural environment. The material was obtained during two hunting seasons, 2005/2006 and 2006/2007. The bucks were shot between 11 May and 30 September, while the does and fawns – since the beginning of October to mid-January.

The animals were shot by individual hunters and divided afterwards into age and sex groups: I group – from 2 to 3-year-old males (n=12), II – from 4 to 5-year-old males (n=10), III – from 6 to 7-year-old males (n=10), IV – from 2 to 3-year-old females (n=14), V- from 4 to 5 -year-old females (n=12) and VI – from 6 to 7-month-old females (n=9). The age of the animals was determined on the basis of their dentition, i.e. on the basis of the extent to which some parts of teeth were developed or worn. Roe deer are herbivores, feeding mostly on green parts of diverse plant species, especially grasses, herbs as well as tree leaves and buds. Due to the fact of obtaining the animals from their natural environment, no control over the quantity and quality of the food intake was possible, thus no chemical analysis of it was performed.

After shooting the animals, samples of *musculus longissimus lumborum* (from the area of the first three lumbar vertebrae) were collected and placed in sterile, tightly closed ziploc bags. These were cold-stored and taken as quickly as possible (up to 5 hours) to the laboratory. The samples were properly preserved, using various methods, such as freezing in a temperature of -18 °C (before marking CLA) or freeze-drying (other fatty acids).

According to the procedure of CLA determination in meat, the samples, after freeze-drying in Lyovac GT2 lyophilizing cabinet, were hydrolyzed in 2M NaOH solution at the temperature of 85° C for 30 min in closed test tubes. The hydrolyzates were cooled and then acidified to pH = 2 in 4M HCl. The extraction of the acids was performed using dichloromethane (four times, using 3.5 ml of dichloromethane). The following stage covered drying the organic layer in presence of Na₂SO₄ and evaporating the solvent in argon stream after adding an organic solvent. The sample residue was solved in acetonitrile and subjected to analytical procedures (CZAUDERNA *et al.* 2007).

The total CLA content was determined with the use of Merck HPLC (CZAUDERNA *et al.* 2007) equipped with UV/VIS detector, at the wavelength of 234 nm. Separation was performed on two 250×4.6 mm Nova-Pak C18 columns accompanied by a 10×6 mm guard column containing RP-C18 column (40 μ m), incubated in the temperature of 32° C in acetonitrile:water solvent system, with gradient elution and the flow of 1.5 [ml/min]. CLA identification in the samples was performed using Sigma standard 0-5507 (Octadecadienoic acid, conjugated) of molecular mass of 280.4 g/mol and the quantitative evaluation of CLA was calculated with use of calibration curve.

In order to determine the fatty acids profile in meat, the samples were first freeze-dried in Lyovac GT2 lyophilizing cabinet and then homogenized in a MPW-309 tissue grinder, using chloroform-ethanol (2:1) extraction mixture. Next, the fatty acid methyl esters (FAMEs) were prepared. For that purpose the fat was converted to FAMEs using methylation with 0.5M sodium methylate solution. The reaction took 22 hours and it was carried out in an incubator holding the temperature of 37°C. Afterwards isooctane was added in order to extract FAMEs. The detailed methodology of the samples preparation is included in A.O.A.C. (2000) and in EN ISO 5509:2000.

The analytical procedure was performed on Varian 3800 GC gas chromatograph equipped with FID detector. The fatty acid methyl esters were separated on 30 m × 0.25 mm × 0.25 μ m Supelcowax 10 column. Injection port temperature was 230°C and the detector temperature was 250°C. Flow rate of the carrier gas (helium) was 1.5 [ml/min] and the volume of the injected sample was 1 μ l. The detailed methodology of analytical procedure is included in Bulletin 855B Supelco (Sigma-Aldrich Co., 1998).

After separation, particular FAMEs in samples were identified by comparing the results to the following standards: Supelco PUFA-2 Animal Source and Supelco 37 Component FAME Mix. The results were divided into several groups in order to facilitate the fatty acids profiling: Saturated Fatty Acids (SFA); MonoUnsaturated Fatty Acids

The use and handling of animals for this experiment was approved by the Local Ethical Committee (no. 4/2006).

(MUFA); PoliUnsaturated Fatty Acids (PUFA) and PUFA n-3 and PUFA n-6.

The results obtained were statistically analyzed using Statistica 6.0 software, with application of non-parametrical one-factor variance analysis (Kruskal-Wallis test). The analysis was performed on an incomplete and unbalanced data (planning the quantity of samples was impossible for each group, for it was dependent on the number of the animals shot during the hunting season), so the principles in force had to be observed with scrutiny. Therefore the data analysis was performed using non-parametric tests.

Results and Discussion

Influence of age and sex on the CLA content in roe deer meat

In general, the amount of CLA in m. longissimus lumborum of the analyzed roe deer meat varied between animals from different age groups. The range of the results was between 42 [mg/kg] in fawns and 89.76 [mg/kg] in 6÷7 years old males (Table 1) and the last number is similar to the ones reported by BORYS et al. (1999) for lamb meat. More specifically, in the meat from milk lambs the amount of CLA was dependent on feeding, ranging from 75 [mg/kg] in animals fed with milk containing low CLA amount to 87 [mg/kg] in lambs fed with milk with high CLA content. Moreover, CLA amount in lamb meat varied among breeds, with the lowest value, 63 [mg CLA/kg] obtained in Suffolk and the highest, 112 [mg CLA/kg] – in Ile de France (BORYS et al. 1999). Furthermore, a

Table	1

Influence of age and sex on CLA content in the *longissimus lumborum* muscle of roe deer (means ± standard deviations)

Group	Age and sex of animals	CLA [mg/kg of fresh tissue]
Ι	2÷3 – year-old males	$53.43^{ac} \pm 30.39$
П	4÷5 – year-old males	66.58 ± 28.44
III	6÷7 – year-old males	$89.76^{\ b}\pm 27.76$
IV	2÷3 – year-old females	64.18 ± 21.48
V	4÷5 – year-old females	61.30 ± 43.87
VI	6÷7 – month-old females (kids)	$42.00\ ^{c}\pm 10.87$

Explanations – means with different superscript letters (a, b, c) in the same column differ significantly at $P \le 0.05$.

similar level of CLA in meat to the one reported in this paper, was obtained by WARREN *et al.* (2008) in one-year-old cattle, measured in the *longissimus dorsi* muscle. However, the CLA content in meat increased along with the animals' age, from 71 to 273 [mg/kg] in Aberdeen Angus cross (AA) and from 66 to 210 [mg/kg] in Holstein-Friesian (HF), which slightly surpassed the CLA amount in the roe deer meat. This phenomenon was also proved true in the analyses presented herein, as we obtained statistically significant differences of CLA content depending on the roe deer age. As presented in Table 1, the amount of CLA was higher in the meat from the oldest individuals, in comparison to the fawns.

The effect of animal sex on CLA content in meat was also analyzed, but only one statistically significant difference (P \leq 0.05) between the group of the oldest males and the fawns (kids) was found. However, it was assumed that the large age difference between the animals, rather than sex, can be a possible explanation of such result. But, due to the lack of literature data available concerning the effect of sex on CLA content in game, it was not possible to confirm this hypothesis. Nevertheless, roe deer meat can be considered one of the richest CLA sources, which is naturally produced in the rumen of the ruminants. Consumption of 300g of roe deer meat per day provides the organism with approximately 30 mg of CLA. According to KNEKT et al. (1996), daily consumption of 50 mg of CLA can potentially decrease occurrence of breast cancer in women.

Influence of age and sex on other fatty acids content in roe deer meat

Apart from CLA, other fatty acids and especially their dietary value, which is mainly influenced by the saturated: unsaturated fatty acids ratio, is also of vital importance for human health. As presented in Table 2, m. longissimus lumborum of roe deer contained from 37.59% to 47.14% of the saturated fatty acids (SFA), from 12.09% to 23.29% of monounsaturated fatty acids (MUFA) and from 29.57% to 47.06% of polyunsaturated fatty acids (PUFA). Similar results concerning the percentage proportion of SFA, i.e. 47%, were reported in the study of FINSTAD et al. (2007) on grazing free ranging reindeer. Moreover, the comparison of the results with the ones presented herein reveals that the meat of reindeer contains slightly more MUFA, i.e. 34.8%, and significantly less PUFA, i.e. 17.8%.

Within MUFA a prevalent compound was oleic acid C18:1; within PUFA – linoleic acid C18:2n6 and within SFA – stearic acid C18:0 and palmitic

Table 2

Fatty acids content [%]								
Group	Ι	II	III	IV	V	VI		
Age and sex of animals	2÷3 – year-old males	4÷5 – year-old males	6÷7 – year-old males	2÷3 – year-old females	4÷5 – year-old females	6÷7 – month-old females (kids)		
C14:0	$0.31^{\text{a}}\pm0.06$	$0.28^{\text{a}}\pm0.07$	$0.37^{a}\pm0.14$	0.68 ± 0.22	$0.75^{\text{b}}\pm0.36$	0.40 ± 0.10		
C16:0	$14.99^{ae}\pm1.15$	$14.46^{a}\pm1.32$	$17.78^{\text{ad}} \pm 4.06$	$20.60^{\text{cdef}}\pm2.23$	$21.35^{\texttt{bdf}}\pm3.76$	17.10 ± 0.90		
C16:1	0.80 ± 0.29	$0.58^{\text{A}} \pm 0.12$	$0.99^{\text{B}}\pm0.27$	$1.06^{\rm B}\pm0.18$	$1.03^{\rm B}\pm 0.26$	0.86 ± 0.18		
C17:0	0.75 ± 0.13	$0.70^{\rm a}\pm0.10$	0.83 ± 0.20	0.76 ± 0.18	$0.87^{\text{b}}\pm0.14$	$0.64^{\text{a}}\pm0.08$		
C18:0	20.38 ± 1.49	19.22 ± 0.62	21.16 ± 2.50	20.09 ± 2.09	20.75 ± 1.30	19.28 ± 1.18		
C18:1	12.59 ^{ac} ±1.96	$11.51^{a}\pm1.19$	$12.66^{\texttt{ac}} \pm 1.88$	$18.22^{\text{ce}}\pm4.52$	$22.26^{bde}\pm9.19$	$14.35^{\text{cde}}\pm2.41$		
C18:2n6	$28.55^{\text{a}}\pm4.10$	$29.55^{\text{ac}}\pm0.87$	27.34 ± 4.02	$21.27^{\text{b}}\pm4.49$	$15.81^{bc} \pm 8.41$	25.29 ± 1.22		
C18:3n3	$4.73^{a} \pm 1.21$	$5.17^{\text{b}}\pm0.86$	$5.22^{\text{b}} \pm 1.88$	$4.06^{\text{b}}\pm0.84$	$5.61^{\text{b}}\pm6.46$	$5.33^{\text{b}}\pm0.88$		
C20:4n6	$8.27^{\rm A}\pm1.05$	$8.94^{\rm A}\pm0.63$	$8.71^{\rm A}\pm0.72$	$5.43^{\rm B}\pm0.58$	5.34 ± 3.01	6.43 ± 0.91		
C24:0	2.58 ± 0.51	2.93 ± 0.78	$2.17^{\text{a}}\pm0.25$	$2.44^{a}\pm0.61$	$3.42^{\text{b}}\pm3.77$	3.01 ± 0.44		
C22:6 n3	4.19 ± 1.85	3.40 ± 1.38	2.76 ± 1.62	2.70 ± 0.69	2.81 ± 1.58	3.85 ± 2.13		
SFA	39.01 ± 2.25	37.59 ± 1.35	42.31 ± 6.37	44.57 ± 4.00	47.14 ± 7.05	40.43 ± 1.78		
MUFA	13.39 ± 2.10	12.09 ± 1.31	13.65 ± 2.01	19.28 ± 4.59	23.29 ± 9.25	15.21 ± 2.57		
PUFA	$45.74^{\mathtt{a}}\pm4.24$	47.06 ± 1.66	$44.03^{ad} \pm 5.25$	$33.46^{\text{b}}\pm6.28$	$29.57^{\text{b}}\pm10.36$	40.9 ± 2.79		
n-6	$36.82^{\mathtt{a}}\pm4.48$	$38.49^{a}\pm0.41$	$36.05^{\text{a}}\pm4.26$	$26.7^{\text{b}}\pm4.90$	$21.15^{b} \pm 11.21$	31.72 ± 1.45		
n-3	$8.92^{ACG} \pm 2.65$	8.57 ± 1.86	$7.98^{\text{BDF}}\pm\!2.23$	$6.76^{\text{CD}} \pm 1.47$	8.42 ± 6.37	$9.18^{\text{EFG}}\pm2.41$		
n-6/n-3	4.13 ± 1.33	4.49 ± 1.06	4.52 ± 0.66	3.95 ± 0.32	2.51 ± 1.56	3.45 ± 0.91		
P/S	1.17 ± 0.14	1.25 ± 0.08	1.04 ± 0.17	0.75 ± 0.20	0.63 ± 0.29	1.01 ± 0.1		

Influence of age and sex on fatty acids content in the *longissimus lumborum* muscle of roe deer (means ± standard deviations)

Explanations – means with different superscript letters (a, b, c, d...) in the same row differ significantly at $P \le 0.05$ and (A, B, C, D...) at $P \le 0.01$.

acid C16:0 (Table 2). Noteworthy, venison is abundant in trans unsaturated acids, such as trans 10 C18:1 and trans 11 C18:1, which are of high value to human health. However, in this paper all these isomers are considered collectively, as oleic acid C18:1. A similar percentage proportion of those acids was reported in the research of SAMPELS et al. (2006) on m. longissimus dorsi in free-ranging reindeer of different age and sex. Regardless of those two factors, prevalent compounds within SFA groups were stearic and palmitic acid, within MUFA - oleic acid and within PUFA - linoleic acid. Among the predominant acids listed above, a statistically significant difference between 10-monthold calves and 2-year-old adults of both sexes was noted only in the case of C16:0. In the calves this acid's content was lower, i.e. 10%, while in the older, regardless of sex, it amounted to 11%.

The research results revealed high PUFA to SFA ratio, ranging from 0.63 to 1.25 (Table 2), while in

general PUFA:SFA ratio should exceed 0.45 and n-6: n-3 ratio should be lower than 4.0. Such proportion of fatty acids in diet is recommended in preventive treatment of cancer and cardiovascular system diseases (WILLIAMS 2000; SIMOPOULOS 2004; WARREN et al. 2008). The PUFA:SFA ratio in the study presented is consistent with the data of POLAK et al. (2008), according to which the proportion of PUFA to SFA in red deer ranges between 0.63-1.09, dependently on the experimental group (stags, hinds, calves) and the kind of muscle (triceps brachii and semitendinosus), as well as with the results of SAMPLES et al. (2004), obtained on fresh reindeer meat, in which the ratio was 1.02. A significantly lower value can be found in the research of PHILLIP et al. (2007) on the meat of red deer kept in a pen and slaughtered at the age of 15 months. Dependently on the diet of the animals, the values ranged from 0.09 to 0.12.

Similar results of the fatty acids composition were reported for the saddle of red deer, which contained 42.7% of saturated fatty acids and 57.3% of unsaturated fatty acids, including 38.4% of polyunsaturated fatty acids (DROZD & GRUSZECKI 1996). Similarly to the roe deer muscle, in the saddle of red deer oleic acid was prevalent and the PUFA:SFA ratio amounted to 1.41. To compare with other game – in the saddle of fallow deer within the SFA the palmitic (24.4%) and stearic (16.5%) acids were prevalent, while unsaturated fatty acids consisted mostly of oleic (16.7%) and linoleic (12%) acids. Generally, muscle fat of fallow deer contained more saturated acids (52.1%) at the cost of mono- and polyunsaturated fatty acids. PUFA: SFA ratio for saddle of red deer was as low as 0.94 (DROZD & GRUSZECKI 1996). Meat of red deer and fallow deer kept in the outdoor pen was for the most part rich in palmitic and stearic acids (AIDOO & HAWORTH 1995). Taking the above criteria into consideration, roe deer meat shows the most favorable proportion of unsaturated (MUFA + PUFA) to saturated (SFA) fatty acids, and therefore proves to be a potential valuable source of high dietary and health benefits. Rising PUFA content in the meat of all animals, including ruminants, is constantly becoming main breeding goal of animal breeders and scientists. Also in this case the feeding system plays a crucial role in fatty acids composition in meat (POPAVA 2007). Animal age and sex affect this parameter likewise, as it was proved in the former part of the discussion. The experiments presented herein established that meat of does shows in most cases a higher percentage proportion of SFA and MUFA, but lower of PUFA when compared to bucks (Table 2). This may be due to the fact that does and kids (fawns) are hunted during the autumnalwinter season, thus by that time, females – after several months of feeding on a natural, full-value feed – had deposited a thick layer of adipose tissue as the energy reserves for winter. In addition, older individuals showed a higher percentage proportion of all the groups of fatty acids considered in this experiment and the most beneficial unsaturated to saturated fatty acids ratio was noted for the group of 4÷5 years old males. RULE et al. (1997) showed that in the meat of white-tailed deer (Odo*coileus virginianus*) SFA increased and PUFA and MUFA decreased along with ageing of the animal. Similarly to the case of roe deer, the individuals obtained in the autumn-winter season (females) showed a higher share of saturated fatty acids. On the other hand, the research of LORENZO et al. (2010) showed no effect of foals' sex on the individual fatty acids content. The impact of age and sex on the content of fatty acids was also demonstrated by HOFFMAN et al. (2007) in their study on m. longissimus dorsi of antelope. Results of the

latter are interesting for one more reason: in longissimus dorsi of this ruminant - like in roe deer meat - linoleic acid was prevalent, the amount of which was similar to the results reported in this paper. The results of ORIANI et al. (2005) confirmed the impact of age on PUFA content in lamb meat the highest was in the oldest individuals. This factor, however, did not affect the amount of SFA and MUFA. CIFUNI et al. (1999) demonstrated that along with the lambs ageing the content of SFA in their meat rises and his results were similar, although slightly higher than the ones presented. Likewise, the research of MAHGOUB et al. (2002) showed the impact of body mass, which means also of age and sex, on fatty acid composition in goats' meat. The level of SFA lowered while the animal aged independently on the goats' sex and it was higher in females than in males. Moreover, the results obtained on these animals were slightly higher then the ones presented and they lied in the range of 52-55% for the bucks' meat and of 55-58% for the does' meat. The level of MUFA and PUFA rose along the animals' ageing and MUFA level was similar independently on the animals' sex, while PUFA level was lower in females (MAHGOUB et al. 2002).

Meat from wild roe deer, in comparison with slaughter animals, shows lower SFA concentration and higher CLA and PUFA content and the research confirms the influence of age and sex on the analyzed parameters in roe deer meat. Meat from roe deer bucks proves to be the most beneficial for dietary purposes.

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