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Cholesterol concentration and fatty acid profile of red deer (*Cervus elaphus*) meat

T. Polak, A. Rajar, L. Gašperlin, B. Žlender *

Department of Food and Technology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1111 Ljubljana, Slovenia

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ABSTRACT

The effects of gender and age on intramuscular fat (IMF) levels, cholesterol concentration, and fatty acid composition were investigated in the *semitendinosus* (ST) and *triceps brachii* (TB) muscles of feral red deer (*Cervus elaphus*). Six stags of >2 years of age, four hinds of 1 year, and six calves of 6 months were shot in Slovenia. Generally, all parameters measured were influenced by interaction of muscle and treatment group (hinds, stags and calves) at the 5% level or less. In ST muscle, the IMF levels were highest for hinds. In the TB muscle, cholesterol was lower for stags than for hinds and calves. The saturated fatty acids were the highest for stags and the mono-unsaturated fatty acids for hinds. The polyunsaturated fatty acids (PUFAs) were the highest for calves and lowest for hinds. The *n*−3 PUFAs were the lowest for hinds. In both muscles, the calves had higher *n*−6 PUFAs than stags and hinds. Only the ST muscle of the hinds contained >1% (1.44%) of the conjugated linoleic acid isomer 18:2*cis*-9,*trans*-11, while in the TB of hinds and calves this fatty acid was higher than with stags. We conclude that gender and age of feral red deer influence the IMF content, the cholesterol concentration, and the fatty acid composition of the meat.

1. Introduction

In the human diet, meat is seen as a major source of fat, and especially of saturated fatty acids (SFAs), which have been implicated in diseases associated with modern life (various cancers and coronary heart disease), mostly in developed countries. The World Health Organization (WHO, 1990) recommends that the daily fat intake be reduced to 30% of the total energy intake, and that saturated fats should be limited to 10% of this caloric intake. It is also advised that cholesterol intake should not exceed 300 mg per day. At the same time, the recommended ratio of polyunsaturated fatty acids (PUFAs) to SFAs (P/S) should be above 0.4, with the normal P/S ratio for meat at around 0.1 (Wood et al., 2003). The ratio of *n*−6/*n*−3 PUFAs is considered to be a risk factor in cancers and coronary heart disease, and it is recommended that this ratio be less than 4.0 (Enser et al., 2001).

Ulbricht and Southgate (1991) suggested that the index of atherogenicity (IA) is a more suitable measure of the atherogenicity of foods than the P/S ratio; the IA is highest for the most atherogenic dietary components, which might start or accelerate the process of atherogenesis (the formation of lipid deposits in the arteries).

Volpelli, Valusso, Morgante, Pittia, and Piasentier (2003) indicated that in comparison with domestic ruminant meat, fallow deer (*Dama dama*) venison contains less intramuscular fatty acids, with their composition being richer in PUFAs and poorer in monounsaturated fatty acids (MUFAs) and SFAs. Crawford, Gale, Woodford, and Casped (1970) indicated that the meat of wild, free-living ruminants is low in total fat, but rich in both linoleic and linolenic acids, as well as their elongation products.

There is little data available on meat composition of wild, free-living red deer (*Cervus elaphus*) and its nutritional value in the human diet. Likewise, the influence of age and gender of wild red deer on meat composition has rarely been investigated. Thus, the purpose of the present study was to determine the content of intramuscular fat, the fatty acid composition and the cholesterol content in two muscles (the *triceps brachii* and *semitendinosus*) of feral red deer stags, hinds and calves from the forest area in Slovenia.

2. Materials and methods

2.1. Care and use of animals

All animals included in the study were treated in accordance with the provisions of the laws on game and hunting enacted by the Government of the Republic of Slovenia (Official Journal of the Republic of Slovenia, 2004).

2.2. Animals

A total of 16 red deer (6 stags, 4 hinds and 6 calves) were included in this study. All of the deer were shot in the forest area in the southern part of Slovenia, near Kočevje, from September to November, 2006. At the time of shooting, the stags were >2 years of age, the hinds were 1 year, and the calves were 6 months.

* Corresponding author. Tel.: +386 01 4231161; fax: +386 01 2566296.
E-mail address: bozidar.zlender@bf.uni-lj.si (B. Žlender).

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The deer were shot during the rutting season. The carcasses were eviscerated immediately upon shooting and then transported to a receiving plant, where they were chilled to a temperature of +1 °C until 48 h post-mortem. The carcasses were then frozen and stored in the skin at a temperature of −21 ± 1 °C for 14 days. The average weights of the eviscerated carcasses with the skin were 128 kg for stags, 52 kg for hinds and 32 kg for calves.

2.3. Tissue sampling

The carcasses were defrosted at +4 °C for 36–72 h. After defrosting and skinning, samples from the left side of each carcass, across the whole width of the middle sections of the trimmed muscles, were taken from the *triceps brachii* (TB) and *semitendinosus* (ST) muscles. The samples were homogenized in a blender, packed into polyethylene bags, frozen and stored at a temperature of −21 ± 1 °C until analysis (for a maximum of 7 days). After thawing, the drip from samples was blended into the homogenate.

2.4. Intramuscular fat content

The IMF content was determined by the method described in the AOAC Official Method 991.36. Fat (Crude) in Meat and Meat Products (AOAC 991.36, 1997). The samples were analysed in duplicate.

2.5. Cholesterol content

The cholesterol content in the red deer meat was determined according to a modified method from Naeemi, Ahmad, Al-Sharrah, and Behbahani (1995), and followed by HPLC analysis; the data are expressed as mg/100 g fresh meat. Ten milliliter saturated methanolic KOH was added to accurately weighed well-ground meat samples (2.00 ± 0.01 g) spiked with an internal standard (5α-cholestane), in a 50 mL screw-capped vial. The vial was capped and then heated for 30 min at 60 °C. Ten milliliter hexane was added to the cooled vials (at 20 °C), which were then closed and shaken vigorously for 2 min. These samples were then centrifuged at 1000*g* for 2 min. An aliquot of the hexane extract (5 mL) was dried under vacuum and freed of solvent using a nitrogen flush, before being dissolved in 2 mL mobile phase; 20 μL was injected into the HPLC system for analysis. This latter was an Agilent Technology system 1100, consisting of a G1379A Micro Vacuum Degasser, a G1312A binary pump, a thermostated G1367A Autosampler, a G1316A thermostated column compartment, a diode array and a G1315B multiple wavelength detector. The analytical column was a Hypersil ODS 5 μm, 150 mm × 4.6 mm. The mobile phase consisted of isopropanol/acetonitrile (45:55) at a flow rate of 1.0 mL/min. The absorption was measured at 210 nm. Determination of reliability and accuracy of the analytical method for the quantitative determination of cholesterol was ensured by the use of the certified reference matrix – CRM 163 (Blend beef–pork fat – BCR) and they were in good agreement with the certified values.

2.6. Fatty acid composition

The fatty acid composition of the samples was determined by gas–liquid chromatography (GLC). The method chosen was in situ transesterification (ISTE) (Park & Goins, 1994). The content of fatty acid methyl esters (FAMEs) was determined by GLC, on an Agilent Technologies 6890 gas chromatograph with a flame ionisation detector and a capillary column Agilent Technologies HP-88 (Cat. No. 112-88A7) (100 m × 0.25 mm × 0.2 μm). Separation and detection were performed under the following conditions: temperature programme, 150 °C (hold 10 min), 2 °C/min to 180 °C (hold 40 min), 3 °C/min to 240 °C (85 min); injector temperature, 250 °C; detector temperature 280 °C; injector: split:splitless, 1:30; volume, 1 μL; carrier gas, He 2.3 mL/min; make-up gas: N_2 45 mL/min; detector gases: H_2 40 mL/min; synthetic air (21% O_2) 450 mL/min.

The FAMEs were determined through their retention times in comparison to a standard mixture (Supelco fatty acid methyl ester mix – 37 components (Cat. No. 18919-1AMP); Supelco PUFA No.1: Animal source (Cat. No. 47015-U); Supelco Linoleic Acid Methyl Ester cis/trans Isomer Mix (Cat. No. 47791); Supelco *cis*-7-octadecenoic methyl ester (Cat. No. 46900-U); *cis*-11-octadecenoic methyl ester (Cat. No. 46904); Fluka Methyl stearidonate (Cat. No. 43959); Natural ASA CLA 10t, 12c in CLA 9c, 11t; NuChek standards: GLC-68D, GLC-85, GLC-411 and GLC-546. The NuChek GLC-68D and GLC-85 standards mixtures were used to determine the response factor, Rf_i, for each fatty acid. The weight portion of each fatty acid in the sample was determined using the Rf_i and the factor of transformation of fatty acid content from FAME content. The determination of reliability and accuracy of the analytical method for the detection of fatty acids was ensured by the use of the certified reference matrix, CRM 163 (Blend beef–pork fat, BCR) and it was in good agreement with the certified values. The FAMEs were expressed as percentages of total fatty acid.

In our study, the *n*−3 fatty acids include 18:3*cis*-9,12,15, 18:4*cis*-6,9,12,15, 20:5*cis*-5,8,11,14,17, 22:5*cis*-7,10,13,16,19, and 22:6*cis*-4,7,10,13,16,19, and the *n*−6 fatty acids include 18:2*cis*-9,12, 18:3*cis*-6,9,12, 20:3*cis*-8,11,14, 20:4*cis*-5,8,11,14, 22:2*cis*-13,16, and 22:4*cis*-7,10,13,16. The 17:1*trans*-10, 20:2*cis*-11,14, 22:1*cis*-13, 22:4*cis*-7,10,13,16 and 24:1*cis*-15 fatty acids were determined too, but their concentrations were less than 0.01 g/100 g of total fatty acids. The index of atherogenicity was calculated as followed: IA = (C12:0 + 4 C14:0 + C16:0)/(Σ(*n*−6) + Σ(*n*−3) + C18:1*cis*-9 + other MUFA) (Ulbricht & Southgate, 1991).

2.7. Data analysis

The data for fat and cholesterol contents and fatty acid compositions were processed by the repeated measures analysis using the GLM procedure (SAS, 1999). The statistical model included the main effects of treatment group (stags, hinds and calves) and muscle (TB and ST), as well as the interaction between treatment group and muscle. The hypothesis was expressed as 'Does the pattern of differences between the mean values for treatment groups change for each muscle?', and it was examined by testing for a between-subjects by within-subjects interaction of treatment group by muscle. The least squares means of the experimental groups were obtained using the LSM procedure and were compared at the 5% probability level (SAS, 1999).

3. Results and discussion

3.1. Intramuscular fat and cholesterol contents

The intramuscular fat contents and concentrations of cholesterol are shown in Table 1 for the ST and TB muscles of the stags, hinds and calves, respectively.

In the ST muscle, the IMF content was the highest for hinds ($P < 0.05$), intermediate for calves ($P < 0.05$), and the lowest for stags ($P < 0.05$). In the TB muscle of stags, hinds and calves, the IMF content was similar.

Deer have very low lipid concentrations in their muscles, with those present primarily being structural lipids (phospholipids and cholesterol), with little contribution from triacylglycerols (Hoffman & Wiklund, 2006). The low IMF content in feral red deer meat (1.14–1.72%) in the present study is largely in agreement with the findings of Manley and Forss (1979), who reported 0.9–3.2% triacylglycerols in leg muscle of feral red deer. Similar results