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The effects of gender, age and region on macro- and micronutrient contents and fatty acid profiles in the muscles of roe deer and wild boar in Mecklenburg-Western Pomerania (Germany)

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ABSTRACT

Samples of M. *longissimus* were collected from a total of 203 feral roe deer (n = 118) and wild boar (n = 85) in two regions of Mecklenburg-Western Pomerania (Germany). The muscle lipid saturated fatty acid proportions of roe deer and wild boar ranged between 33 and 49 g/100 g total fatty acids and 31 and 35 g/100 g total fatty acids, respectively. The total $n-3$ PUFA proportions in roe deer muscle varied between 8.0 and 14 g/100 g fatty acids, and in wild boar muscle between 2.6 and 6.0 g/100 g fatty acids. The major vitamin E homologue, α-tocopherol, was determined to be between 5.8 and 13.1 mg/kg in roe deer muscles. Lower levels between 1.2 and 4.7 mg/kg were measured in wild boar muscles. The iron and zinc concentrations in roe deer and wild boar muscle ranged from 26.3 to 33.9 mg/kg and from 17.0 to 21.7 mg/kg, and from 13.6 to 39.3 mg/kg and 18.1 to 31.9 mg/kg, respectively.

1. Introduction

Total meat consumption in Germany ranged between 88.4 and 89.7 kg (including bones) per capita in the years 2007 to 2011 (http://www.bvdf.de). Pork is the primary type of meat consumed in Germany (54.4 kg per capita) followed by poultry meat (19.3 kg per capita) and beef and veal meat (12.5 kg per capita). The consumption of game meat in Germany has increased in recent years to approximately 1.5 kg per capita; however, it is negligible compared to pork, poultry or beef consumption. In Mecklenburg-Western Pomerania, 63155 head of roe deer and 75866 head of wild boar were culled in 2008/2009, and this frequency is increasing due to greater consumer interest in game meat in Germany. The risks associated with the consumption of red meat, including game meat, to human health (*e.g.*, cancer, diabetes and coronary heart disease) are currently a controversial topic (Corpet, 2011; Fretts et al., 2012, Wyness et al., 2011). To reduce the risk of cancer, the World Cancer Research Fund report recommends limiting the consumption of red meat to less than 500 g per week (World Cancer Research Fund, & American Institute for Cancer Research, 2009). The majority of evidence for the association of red meat with cancer shows an increase in cancer risk for consumers with the highest level of red meat consumption; however, the results of most studies have not reached statistical significance (Wyness et al., 2011). The German Nutrition Society (DGE) recommends the restriction of red meat consumption, including processed meat, to 600 g per week and a daily fat intake of up to 30% of the total daily energy intake. Less than 10% of this fat intake should be saturated fatty acids (SFAs), approximately 7–10% should be polyunsaturated fatty acids (PUFAs) and the remaining 10% should be monounsaturated fatty acids (MUFAs) (DGE, 2012).

Red meat is a source of high biological value protein and a significant source of important trace elements, *e.g.*, iron, zinc and selenium, and vitamins (A, B_6, B_{12}, D, and E). The fatty acids composition of adipose and muscle tissues can be affected by factors such as diet, species, fattiness, age/weight, depot site, gender, breed, and season and hormone levels. Levels of long-chain $n-3$ PUFAs in red meat were reported to be effectively elevated upon dietary supplementation with linseed/linseed oil, rapeseed cake/oil or algae or by pasture and grass silage feeding compared to maize silage feeding systems (Nuernberg, 2009). In addition, game meat, including wild boar and roe deer meat, has comparable muscle fat contents to domestic porcine- or beef muscle predominantly consisting of structural lipid molecules (phospholipids and cholesterol) and a high proportion of long-chain PUFAs. This content results in lipid profiles with desirable fatty acid compositions (*i.e.*, relatively low concentrations of SFAs and high concentrations of long chain $n-3$ and $n-6$ PUFAs) (Hoffman &

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Wiklund, 2006; Polak, Rajar, Gasperlin, & Zlender, 2008; Quaresma et al., 2011; Triumf et al., 2012).

Despite an increasing consumer interest in game meat, and wild boar and roe deer meat in particular, in Germany during recent years, information concerning the nutritional value of game meat is scarce (Nuernberg, Nuernberg, & Dannenberger, 2009). Limited data are available regarding the macro- and micronutrient compositions and fatty acid profiles of German wild boar and roe deer muscles at all. There are three different types of forest habitats in Mecklenburg-Western Pomerania (Germany): deciduous forest, mixed forest and coniferous forest (mainly pines). Between the two Forestry Commission offices in Schildfeld (north-western region of Mecklenburg-Western Pomerania) and Torgelow (north-eastern region of Mecklenburg-Western Pomerania), there were differences in the diet sources of feral animals. The north-western region of MW is predominantly mixed forest with a higher proportion of broadleaf forest. The north-eastern region of MW consists of greater than 90% coniferous forests (pines), and the soil is more nutrient poor than that in the north-western region. The forest area of the north-eastern region of Germany (Mecklenburg-Western Pomerania) covers 21%. There are different soil types with a high extent of agricultural use. Mecklenburg-Western Pomerania is one of the leading game meat suppliers for Germany and parts of Europe. The objective of this present study was to investigate the effects of gender, age and region on the macronutrients, micronutrients (vitamins and trace metals) and fatty acid profiles of wild boar and roe deer muscles in the Mecklenburg-Western Pomerania. The present study is the first more extensive survey of assessment of the nutritional values of game meat in Germany.

2. Materials and methods

2.1. Animals and muscle samples

M. *longissimus* samples were collected from a total of 203 wild animals (wild boar and roe deer) in the region of Mecklenburg-Western Pomerania (Germany) between June 2006 and January 2009. The wild animals were shot in accordance with the Federal Game Law of Germany in the Forestry Commission offices of Schildfeld (north-western part of Mecklenburg-West Pomerania) and Torgelow (north-eastern part of Mecklenburg-West Pomerania) in the period between May and November (roe deer) and throughout the entire year (wild boar). Muscle samples of all animals were collected in the slaughterhouses of both Forestry Commission offices after the carcasses had been cooled for two days and were stored at −20 °C until required for further analysis. *Longissimus* muscles used for the analysis of macronutrients, micronutrients (vitamins and trace metals) and fatty acid profiles were taken from the 12th/14th rib (wild boar) and from the 11th/13th rib (roe deer), and stored at −20 °C until analysis. A total of 65 muscle samples of roe deer and 40 muscle samples of wild boar were taken at the Forestry Commission office in Schildfeld (Table 1), and 52 muscle samples of roe deer and 55 muscle samples of wild boar (Table 1) were collected at the Forestry Commission office of Torgelow. All wild boar and roe deer samples were grouped according to their corresponding region, *i.e.*, Schildfeld (north-western part of Mecklenburg-West Pomerania) and Torgelow (north-eastern part of Mecklenburg-West Pomerania).

The samples from each region were divided into male and female animal groups and further subdivided into two age classes: class 0 (animals younger than 1 year of age) and class ≥1 (animals ≥1 year of age).

Table 1
Number of *longissimus* muscle samples of roe deer and wild boar taken at forestry commission offices in Schildfeld (north-western region of Mecklenburg-Western Pomerania) and Torgelow (north-eastern region of Mecklenburg-Western Pomerania), Germany.

Region	North-West MW (Schildfeld)				North-East MW (Torgelow)			
Age class	0	≥1	0	≥1	0	≥1	0	≥1
Gender	Male	Male	Female	Female	Male	Male	Female	Female
M. *longissimus*								
Roe deer (n)	12	31	10	12	10	18	10	15
Wild boar (n)	10	11	10	9	7	11	17	10

Age class 0: animals < 1 year of age; age class ≥1: animals > 1 year of age.

2.1.1. Macronutrient analysis

The macronutrients (crude fat, crude protein and water) of M. *longissimus* samples were analysed as previously described by Seenger et al. (2008). Approximately 200 g of muscle sample was minced, mixed well and placed onto an analyte plate. The determination of the macronutrient levels was performed using a FoodScan™ Meat Analyser (FOSS Analytic, Hillerod, Denmark). The measurements were based on the near infrared (NIR) transmission and covered 16 measurement points. The results are the average of 16 measurements. The macronutrient contents were expressed as g/100 g of fresh muscle.

2.2. Fatty acid analysis

Samples of M. *longissimus* were thawed at 4 °C. After homogenisation (Ultra Turrax, IKA, Staufen, Germany; T25, 3 × 15 s, 12 000 rpm) and the addition of the fatty acid C19:0 as an internal standard, the total lipids were extracted in duplicate using chloroform/methanol (2:1, v/v) at room temperature. The detailed sample preparation procedure has been previously described (Nuernberg, Nuernberg, Dannenberger, Hagemann, and Paulke, 2011). Briefly, all of the solvents contained 0.005% (w/v) of t-butylhydroxytoluene (BHT) to prevent the oxidation of PUFAs. The extraction mixture was stored at 5 °C for 18 h in the dark and subsequently washed with 0.02% aqueous $CaCl_2$. The organic phase was dried with Na_2SO_4 and K_2CO_3 (10:1, wt/wt), and the solvent was subsequently removed under nitrogen at room temperature. The lipid extracts were redissolved in 300 µl of toluene, and a 25 mg aliquot was used for methyl ester preparation. Next, 2 ml of 0.5 M sodium methoxide in methanol was added to the samples, which were shaken in a 60 °C water bath for 10 min. Subsequently, 1 ml of 14% boron trifluoride (BF_3) in methanol was added to the mixture, which was then shaken for an additional 10 min at 60 °C. Saturated $NaHCO_3$ (2 ml) was added, and the fatty acid methyl esters (FAMEs) were extracted three times in 2 ml of *n*-hexane. The solvent containing the FAMEs was reduced to dryness under an oxygen-free nitrogen stream, and the FAMEs were resuspended in 100 µl of *n*-hexane and stored at −18 °C until used for gas chromatography (GC) analysis. The FAMEs were evaporated under an oxygen-free nitrogen stream and dissolved in *n*-heptane for GC analysis. The fatty acid analysis of the muscle lipids was performed using capillary GC with a CP-Sil 88 CB column (100 m × 0.25 mm, Chrompack-Varian, Lake Forest, CA, United States) that was installed in a PerkinElmer gas chromatograph Autosys XL with a flame ionisation detector and split injection (PerkinElmer Instruments, Shelton, United States). The detailed GC conditions were recently described (Shen, Dannenberger, Nuernberg, Nuernberg, and Zhao, 2011). Briefly, the initial oven temperature was 150 °C, which was held for 5 min; subsequently, the temperature was increased to 175 °C and then to 200 °C at a rate of 2 °C min^{-1} and held for 10 min. Finally, the temperature was increased to 225 °C at a rate of 1.5 °C min^{-1} and held for 25 min. Hydrogen was used as the carrier gas at a flow rate of 1 ml min^{-1}. The split ratio was 1:20, and the injector and detector were set at 260 °C and 280 °C, respectively.

2.3. Analysis of fat-soluble vitamins

Retinol (vitamin A) and tocopherol isomers were extracted according to the methodology recently described in detail by Mahecha et al. (2009). Briefly, three subsamples were prepared by homogenising