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Impact of season on the fatty acid profiles of male and female blesbok (*Damaliscus pygargus phillipsi*) muscles

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ABSTRACT

This study quantified the impact of season on fatty acid profiles of male and female blesbok muscles (*longissimus thoracis et lumborum*, *biceps femoris*, *semimembranosus*, *semitendinosus*, *infraspinatus*, and *supraspinatus*). Eight mature blesbok were harvested per season (winter and spring). Gender and muscle type influenced ($p < 0.05$) the fatty acid profiles of blesbok muscles, while season only influenced the C18:3ω3 (α-linolenic acid, ALA) percentages and therefore the total omega-3 poly-unsaturated fatty acids (total ω3 PUFA). Female muscles had higher C16:0 (palmitic acid) (21.01% ± 0.256 vs. 19.05% ± 0.296) and total MUFA percentages, while male muscles had higher ($p < 0.05$) C18:2ω6c, C20:5ω3, total ω3 PUFA (11.08% ± 0.382 vs. 8.50% ± 0.367), and total PUFA (43.03% ± 0.904 vs. 29.59% ± 1.164) percentages, contributing to higher poly-unsaturated to saturated fatty acid ratios (PUFA:SFA ratios). Differences in fatty acid profiles were attributed more to gender and anatomical location of muscles, than seasonal differences in diets.

1. Introduction

Red meat consumers primarily use the visible fat (intra- and intermuscular) (Hoffman, Muller, Schutte, Calitz, & Crafford, 2005) and nutritional claims on packaging as an indication of the healthiness of meat products (Issanchou, 1996). When considering the nutritional value of meat containing fat, three factors are important: the total fat content; the PUFA:SFA ratio; and the omega-6 to omega-3 fatty acid ratio (ω6:ω3) (Enser et al., 1998). However, the healthiness and sensory properties of meat are also determined by the overall fatty acid profile (Hocquette et al., 2010).

The meat industry has been successful in reducing the fat content and modifying the fatty acid profile of red meats in accordance with the demands by health conscious consumers (Higgs, 2000; Van Schalkwyk & Hoffman, 2010; Warriss, 2000). Decreases in the fat content of game meat is, however, not necessary since the fat content is known to be very low (2–3%) (Aidoo & Haworth, 1995; Van Schalkwyk & Hoffman, 2010). Difficulty also exists in modifying the fatty acid profile of meat from ruminant animals, since PUFA from forage are hydrogenated in the rumen to less unsaturated or saturated fatty acids (SFAs) (Warriss, 2000; Wood & Enser, 1997). Meat from ruminants will therefore have correspondingly lower PUFA:SFA ratios (Enser et al., 1998; Wood & Enser, 1997) as well as lower ω6:ω3 (especially in strictly grazing ruminants) (Enser et al., 1998). The fatty acid profiles of game meat has similarities with other red meat types, since the main fatty acids in the meat are usually C16:0 (palmitic acid), C18:0 (stearic acid) and C18:1ω9 (oleic acid) (Aidoo & Haworth, 1995). Fatty acid profiles can differ between genders, as the muscles from female animals often have higher quantities of intramuscular fat (Lawrie & Ledward, 2006).

Blesbok (*Damaliscus pygargus phillipsi*) is a popular game species hunted and consumed in South Africa. It is a free-ranging species that grazes selectively on short grass species (Bothma, Van Rooyen, & Du Toit, 2010; Du Plessis, 1972). Blesbok generally have regional and seasonal differences in the grass species available to them, as well as seasonal preferences towards specific grass species (Skinner & Chimimba, 2005). The composition of the forage consumed generally influences the quantity and quality of the fat present in the meat from ruminant animals (Warriss, 2000).

Differences in the plane of nutrition and activity level are known to influence the fibre type composition of skeletal muscles (Lawrie & Ledward, 2006) subsequently causing variations in fatty acid profiles (Wood et al., 2003). Research on the factors influencing the chemical composition of the meat from various game species is usually limited to the *longissimus thoracis et lumborum* (LTL) (Hoffman, Kroucamp, & Manley, 2007; Hoffman, Mostert, Kidd, & Laubscher, 2009; Hoffman, Smit, & Muller, 2008; Hoffman, Van Schalkwyk, & Muller, 2008;

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Hoffman, Van Schalkwyk, & Muller, 2009; Purchas, Triumf, & Egelandsdal, 2010), since the commercial red meat industry considers the LTL to be the most representative muscle in domestic livestock carcasses (Warriss, 2000).

This study was therefore aimed at quantifying the impact of season on the fatty acid profiles of six commercially important blesbok muscles from male and female animals.

2. Materials and methods

2.1. Harvesting of blesbok

Blesbok were harvested on Brakkekuil farm (34°18′24.0″S and 20°49′3.9″E; 93 m.a.s.l.), near Witsand, Western Cape Province, South Africa. The study area is classified as the Coastal Renosterveld and receives 300–500 mm of non-seasonal rainfall (Chase & Meadows, 2007; Kruger, 2007; Rebelo, Boucher, Helme, Mucina, & Rutherford, 2006; Rutherford, Mucina, & Powrie, 2006).

Eight mature blesbok were harvested per season in June (winter) and October (spring) of 2010. The harvesting periods formed part of the general management strategies of the farm and therefore no preference was given to the selection of male or female blesbok (winter, three males and five females; spring, four males and four females); in addition both genders have horns and it is difficult to distinguish between the two sexes at a distance. The blesbok were harvested during the day and shot in the head or the high neck area with a .308 calibre rifle, so as to cause immediate death. No unnecessary ante mortem stress was experienced by the animals (ethical clearance number: 10NP_HOF02, issued by *Stellenbosch University Animal Care and Use Committee*). Exsanguination occurred within 2 min while in the field. Partially dressed carcasses were transported to slaughtering facilities where the head, legs and skin were removed and evisceration occurred according to the *Draft Meat Safety Act, 2000* (Act No. 40 of 2000).

2.2. Sample preparation

The dressed carcasses were cooled (0°–5 °C) shortly after dressing (≈45 min post mortem). After 24 h the *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF), *semimembranosus* (SM), *semitendinosus* (ST), *infraspinatus* (IS) and *supraspinatus* (SS) muscles were removed completely from the left side of each carcass. Muscles were weighed, homogenised, vacuum-packed and stored at −20 °C. Approximately four weeks after harvesting, the homogenised muscle samples were removed and thawed for 12 h at ≈4 °C, prior to fatty acid analyses.

2.3. Intramuscular fatty acids

Two grammes of each sample was extracted according to a method by Folch, Lees, and Sloane Stanley (1957). Extractions were performed with a chloroform:methanol (2:1; v/v) solution containing 0.01% butylated hydroxytoluene (BHT) as antioxidant. Samples were homogenised for 30 s in the extraction solvent, by use of a polytron mixer (WiggenHauser, D-500 Homogenizer). To enable quantification of the individual fatty acids in the original muscle sample, heptadecanoic acid (C17:0) was used as an internal standard. A sub-sample was taken from the extracted fats and transmethylated for 2 h at 70 °C with a methanol:sulphuric acid (19:1; v/v) solution. The sub-sample was cooled to room temperature after which the resulting fatty acid methyl esters (FAME) were extracted with the use of water and hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen. Fifty microlitres of hexane was added to the dried sample of which 1 μl was injected.

The FAME were analysed by gas–liquid chromatography (Varian Model 3300 equipped with a flame ionisation detector) using a 60 m BPX70 capillary column of 0.25 mm internal diameter (SGE International Pty Ltd, 7 Argent Place, Ringwood, Victoria 3134, Australia). The hydrogen gas flow rate was 25 $ml \cdot min^{-1}$ and the hydrogen carrier gas flow rate was 2–4 $ml \cdot min^{-1}$. Temperature programming was linear at 3.4 $°C \cdot min^{-1}$ with the following temperature settings: initial temperature of 60 °C; final temperature of 160 °C; injector temperature of 220 °C; and detector temperature of 260 °C. The run time was ≈45 min with an injection volume of 1 μl. The FAME in the total lipids of each sample ($mg \cdot g^{-1}$ sample) were identified by comparing the retention times with those of a standard FAME mixture (Supelco™ 37 Component FAME Mix, 10 $mg \cdot ml^{-1}$ in CH_2Cl_2, Catalogue Number 47885-U. Supelco™, North Harrison Road, Bellefonte, PA 16823-0048, USA). The fatty acid profile was calculated and compared as a proportion of the total amount of fatty acids present in each sample.

2.4. Statistical analysis

Statistical analysis of data was performed using the Statistica 10 VEPAC module (STATISTICA, 2011). The mixed model repeated measures of analysis of variance (ANOVA) was conducted with animal as random factor nested in the fixed season and gender effects. Muscle type, also a fixed effect, was treated as a within subject effect. The total intramuscular fat percentage was also added as a covariate of the fatty acid percentage data. Fisher LSD was used for post hoc testing. Normal probability plots were continuously checked for deviations from normality and possible outliers. A 5% significance level was used as guideline for determining significant effects. Most of the values are reported as the Means and Standard Error of the Mean (SEM). Pearson correlations were used to test for relationships between measured variables.

3. Results

Table 1 depicts the differences in carcass weights (kg) and mean intramuscular fat percentages (means ± SD) of six blesbok muscles for both genders and seasons, as well as the respective p-values. It should be noted that this data refers to a previous publication by Neethling et al. (2014). Table 2 depicts the nature of the significant interactions (p-values) between the main effects (season, gender and muscle type) and the individual impact of each effect on the fatty acid profile of blesbok meat. The overall means and standard deviations of the fatty acid profile ($g \cdot 100\ g^{-1}$ total fatty acids) of blesbok meat (all muscles and seasons) are included in Table 2, so as to provide some insight into the importance of each fatty acid. Since the aim of the study was to quantify the impact of season, gender and muscle type on the fatty acid profile of blesbok meat, the results are discussed as percentage values (% fatty acid of all identified fatty acids within the intramuscular fat), rather than $mg \cdot g^{-1}$ of meat (fatty acid content), as the fatty acid content can vary with varying intramuscular fat content (as a result of significant differences or interactions between the main factors). The interactions depicted in Table 2 are only relevant to the $g \cdot 100\ g^{-1}$ total fatty acid values. The level of statistical significance (p-values) for the fatty acid calculated with intramuscular fat as a co-variant was also added to Table 2, however, due to the small changes in the p-values this will not be discussed further. The column with the $mg \cdot g^{-1}$ fatty acid values is for the LTL only (most representative muscle) and these values were added for nutritional tabulation purposes and will not be discussed further. The overall intramuscular fat percentages of blesbok meat from this study area were low (<3 $g \cdot 100\ g^{-1}$ meat; Neethling et al., 2014), as is generally the case for wild and free-living South African game species (Aidoo & Haworth, 1995; Ramanzin et al., 2010; Van Schalkwyk & Hoffman, 2010). The fatty acids present in very low percentages (≤1%) will therefore not be discussed further in detail.

The impact of the three-way interaction between the main effects (season, gender and muscle type) on the percentages of C20:3ω6 (homo-g-linolenic acid), C20:4ω6 (arachidonic acid), omega-3 polyunsaturated fatty acids (ω6 PUFAs) and ω6:ω3, is presented in Table 3. The homo-g-linolenic acid, arachidonic acid and ω6 PUFA percentages of male muscles from both harvesting seasons were higher ($p < 0.05$)