Effect of Sex and Muscle on the Fiber-Type Composition and Cross-Sectional Area of Springbok (Antidorcas Marsupialis) Muscle

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Abstract: This study aimed to compare the fiber-type composition and fiber cross-sectional area (CSA) of male and female springbok (Antidorcas marsupialis) Longissimus thoracis et lumborum (LTL) and Biceps femoris (BF) muscles. Frozen samples from 4 male and 3 female springbok were fiber-typed immunohistochemically using the primary antibodies A4.74, BA-D5 and BF-35. The CSA of the fibers was determined using the software Image J. Type IIX fibers accounted for 64 to 77% of the fibers in all samples, with type IIA (12.7 to 19.1%), type IIAX (6.4 to 9.3%) and type I (2.4 to 8.5%) making smaller (P < 0.001) contributions. Female springboks’ muscles contained more type IIX fibers than males’ (P = 0.004) and the BF contained more type I fibers and fewer type IIA and IIAX fibers than the LTL (P < 0.001). CSA values did not differ between sexes or muscles; however, they increased with apparent fiber glycolytic capacity (I < IIA < IIAX < IIX; P < 0.001). The glycolytic nature suggested by the fiber-type composition of springbok muscle found in this study is in contrast with previous reports on the physicochemical nature of springbok meat. This casts doubt on the application of standard associations between fiber-type and meat quality to this species. However, it may provide some explanation for the low shear force values found for springbok meat in previous studies.

Keywords: immunohistochemistry, myosin heavy chain isoform, venison

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Introduction

The game industry currently utilizes approximately 24.4% of the available grazing land in South

Africa and is one of the fastest growing industries in the agricultural sector (Hoffman et al., 2005; Hoffman and Wiklund, 2006; The National Agricultural Marketing Council, 2006). Springbok (Antidorcas marsupialis) are one of the most important species for game meat production, representing more than 80% of the carcasses exported in 2005 (Hoffman and Wiklund, 2006). However, in order for springbok meat production to reach its full potential, the nature of springbok muscle must be fully understood.

Skeletal muscle is an extremely complex and highly organized tissue consisting of 4 levels of organization, namely the myofibril, the muscle fiber, the fascicle and the whole muscle (Bailey, 1972; Kohn et al., 2011). Of these structures, the myofibril represents the functional unit of contraction while the muscle fiber can be considered the functional metabolic unit (Bailey,
Variation in the fiber-type composition and thus the contractile and metabolic nature of muscle exists to allow the wide variety of types of contraction required by a functional body (Klont et al., 1998; Lefaucheur, 2010). This variation also affects the contraction of the muscle during rigor; the rate of decline in the pH and the rate and degree of tenderness during ageing (Klont et al., 1998). Muscle fiber-type composition is one of the most important intrinsic factors influencing meat quality, including shear force, drip loss, cooking loss, pH, and sensory parameters (Lawrie and Ledward, 2006; Lefaucheur, 2010).

The different fiber-types can be identified and classified according to the pH or formaldehyde sensitivity of myosin ATPase, the activity of the metabolic enzymes or the reactivity of the myosin heavy chain (MHC) isoforms with specific monoclonal antibodies (Dingboom and Weijs, 2004). This results in the classification of fibers as types I, IIA, IIx (in some literature this is referred to as IID or IIB) and IIB, as well as various hybrid fibers (Dingboom and Weijs, 2004). Type I fibers are typically described as slow-twitch oxidative, type IIA as fast-twitch oxidative and type IIx as fast-twitch glycolytic (Kohn et al., 2011). Type IIB fibers have the highest speed of contraction and are relatively rare in the skeletal muscles of large mammals (Dingboom and Weijs, 2004; Lefaucheur, 2010).

While fiber-typing has been performed relatively extensively on species such as cattle, sheep and pigs (Henckel et al., 1997; Moreno-Sánchez et al., 2008; Sazili et al., 2005; Valin et al., 1982; Vestergaard et al., 2000), only 1 study thus far has identified the fiber-type composition of springbok muscle (Curry et al., 2012).

The aim of this study was to add to the existing information on springbok muscle fiber-type composition and cross-sectional area as well as to examine the effect of sex and muscle on these parameters and how this relates to meat quality, as found in previous work on springbok.

**Materials and Methods**

Ethical clearance for this study was issued by the Stellenbosch University Animal Care and Use Committee (Ethical clearance number SU-ACUM13–0034).

**Harvesting and slaughter**

Seven mature wild springbok (4 male, 3 female) that had received no artificial diet nor had been exposed to any conventional farm management practices were harvested according to standard operating procedure number SU-ACUM13–00034 at Elandsberg nature reserve near Wellington in the Western Cape of South Africa (33°25’08.0”S 19°01’12.8”E) in January/February of 2014. Maturity was determined based on dentition and carcass weight, resulting in an estimated age range of 2 to 7.5 yr (Rautenbach, 1971). The springbok were either harvested in the early morning before dawn or at night after dark to allow the use of a spotlight to locate and temporarily immobilize them, thereby minimizing stress. They were killed with a shot to the head from a 30–06 or .270 caliber rifle. Each springbok was picked up immediately after being shot and the throat was cut to allow exsanguination. The bled carcasses were transported to the meat processing facility at the Department of Animal Sciences, Stellenbosch University, where they were skinned and eviscerated within 2 h post-mortem. Once dressed, the warm carcasses were placed in a cool room at 3 to 6°C to undergo rigor. All carcasses were suspended by both Achilles tendons to ensure equal contraction of the muscles in either side of the carcass.

**Sampling**

At approximately 8 h post-mortem, portions from the center of each Longissimus thoracis et lumborum (LTL) and Biceps femoris (BF) muscle were removed from the carcass. Several approximately 0.5 cm³ blocks of muscle were cut from each portion such that the muscle fibers aligned with the dimensions of the block. These blocks were frozen in liquid nitrogen and stored at –80°C until sectioning.

**Immunohistochemistry**

The fiber-type identification was performed using immunohistochemical methods as described by Kohn et al. (2011). Serial cross-sections of 10 μm each were cut perpendicularly to the direction of the fibers using a cryostat set to –25°C. The sections were fixed in acetone for 2 min and allowed to dry. They were subsequently rehydrated in phosphate buffered saline (PBS), pH 7.40, and blocked with 5% donkey serum (Sigma-Aldrich Pty. Ltd., St. Louis, MO) for 40 min at room temperature.

After blocking, the sections were incubated with primary antibody cocktails overnight at 4°C in a humidifying chamber. All primary antibody cocktails contained anti-dystrophin (MANDYS1 CLONE 3B7; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) and A4.74 (specific to myosin heavy chain Ila and IIx), BA-D5 (specific to MHC I) or BF-35 (specific to MHC I and Ila isoforms) primary antibodies (diluted 1:50 in PBS). These primary
antibodies were monoclonal antibodies raised in mice (Developmental Studies Hybridoma Bank).

The sections were washed in PBS for 2 min, prior to being incubated with Cy3 donkey anti-mouse secondary antibody (diluted 1:500 in PBS; Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 h at room temperature. In addition to the secondary antibody, the sections were incubated with diluted Hoechst (Sigma-Aldrich Pty. Ltd.) for 10 min prior to washing to visualize the nuclei. Sections were thoroughly rinsed in PBS and mounted using the fluorescent mounting medium MOWIOL, with anti-fade. MOWIOL was mixed using 30.0 g glycerol (Sigma-Aldrich Pty. Ltd.), 12.0 g Mowiol 4-88 (Merck Millipore, Darmstadt, Germany), 30.0 ml double distilled water and 60.0 ml 0.2 M Tris buffer at pH 8.5, with n-propylgallate (Sigma-Aldrich Pty. Ltd.) added as an anti-fading agent.

All slides were visualized and photographed using a fluorescent-capable Nikon Eclipse 80i (Nikon Corporation, Tokyo, Japan) and a Canon 650D camera (Canon Inc., Tokyo, Japan).

**Image analysis**

The fibers in each sample were identified by comparing the intensity of the fluorescent staining for each primary antibody in the sequential sections (Fig. 1). They were classified as type I, I/IIA, IIA, IIX or IIX, and the number of each type in the image was counted. Between 500 and 1,400 fibers were counted for each sample.

Once the fibers had been identified and labeled, the average cross-sectional area (CSA) of each type was determined. This was done using the program Image J (version 1.47, http://rsb.info.nih.gov/ij), with each fiber being outlined and the area enclosed by the outline determined by the program. In the event that fewer than 100 fibers of a specific type were present in the image the CSA was determined for all the fibers, otherwise 100 fibers were measured.

**Statistical analysis**

The trial had a completely randomized design with the main effects (sex, muscle and fiber-type) and their second- and third-order interactions, being tested. The software program Statistica (version 12, Statsoft Inc., 2013) was used to analyze the data. As part of the analysis, normality was tested using normal probability plots. The Variance Estimation, Precision and Comparison module of Statistica was used to perform mixed model analyses of variance on the data to determine the significance of each main effect and interaction. A significance level of \( P \leq 0.05 \) was used.

**Results**

Very few type I/IIA fibers were identified and as they were not present in all the samples they will not be discussed in this paper. However, it may be noted that these fibers were only found in samples from male springbok, and a maximum of 3.9% was found in a single male BF sample.

The statistical analysis of the results showed no sex by muscle by fiber-type interaction (\( P = 0.51 \)), but differences in the proportions of the different fiber-types between the sexes (\( P_{sex \times type} = 0.004 \)) and the muscles (\( P_{muscle \times type} < 0.001 \)). The difference between the sexes was most distinct in the type IIX fibers, with muscles from female springbok containing a larger (\( P \leq 0.05 \)) proportion of
this type than those from male springbok (Table 1). The muscle differences showed the opposite pattern (Table 1), with no difference in the proportion of type IIX fibers, but the LTL having more type IIAX and IIA and less type I than the BF \( (P \leq 0.05) \). Despite the second-order interactions, a similar pattern of prevalence was found across the muscles and sexes, with all the samples consisting of predominantly type IIX fibers \( (P_{\text{type}} < 0.001) \).

The fiber CSA showed less variation than the fiber-type composition, with differences only being found between the fiber-types \( (P < 0.001) \). This also only reflected a higher CSA value for the type IIX fibers \( (P \leq 0.05) \), with the other fiber-types not differing in size (Fig. 2).

### Discussion

#### Fiber-type composition

The high prevalence of type IIX fibers found in this study (Table 1) is in agreement with the fiber-type composition reported by Curry et al. (2012) for springbok muscle. It also suggests greater similarity in this regard between springbok and kudu (Tragelaphus strepsiceros), than springbok and blesbuck (Damaliscus dorcas phillipsi), black wildebeest (Connochaetes gnu) or blue wildebeest (Connochaetes taurinus; Kohn et al., 2007). In addition, springbok muscle contained more type IIX fibers and fewer type I fibers than reported for Svalbard reindeer (Rangifer tarandus platyrhynchus) and a number of other species (Kiessling and Kiessling, 1984; Table 2). However, it appeared to contain fewer type IIX fibers and more type I and IIA fibers than domestic pig Longissimus dorsi; although the values reported by Ruusunen and Puolanne (2004) represent the percentage area rather than the percentage number of the different fiber-types.

The relatively large proportion of type IIX fibers found in springbok muscle relates well with the high activity of the glycolytic enzymes, as reported by Curry et al. (2012), as well as the considerable sprinting ability of the species (Kohn et al., 2011; Smithers, 1983). However, this apparently glycolytic nature appears to contradict the relatively high iron levels and low \( L^* \) values previously reported for springbok meat, as these are most commonly associated with high proportions of type I and IIA fibers (Choe et al., 2008; Dingboom and Weijs, 2004; Henckel et al., 1997; Hoffman et al., 2007a, 2007b).

The apparent contradiction between the fiber-type composition and the physicochemical nature of springbok meat may be as a result of the variation in metabolic characteristics that exists within each myosin ATPase-based type (Choi and Kim, 2009; Dingboom and Weijs, 2004; Henckel et al., 1997; Hoffman et al., 2007a, 2007b).

### Table 1. The fiber-type composition of male and female springbok Longissimus thoracis et lumborum (LTL) and Biceps femoris (BF) muscles (LSMean ± SEM)\(^{1}\)

<table>
<thead>
<tr>
<th>Fiber-types(^2)</th>
<th>Sex</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female ( (N = 6) )</td>
<td>Male ( (N = 8) )</td>
</tr>
<tr>
<td>I</td>
<td>4.2 ± 1.17(^e)</td>
<td>6.8 ± 1.51(^d)</td>
</tr>
<tr>
<td>IIA</td>
<td>12.7 ± 0.83(^cd)</td>
<td>19.1 ± 1.44(^c)</td>
</tr>
<tr>
<td>IIAX</td>
<td>6.4 ± 1.51(^d)</td>
<td>9.3 ± 1.55(^d)</td>
</tr>
<tr>
<td>IIX</td>
<td>76.7 ± 1.56(^a)</td>
<td>64.3 ± 3.00(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Sex by fiber-type least square means reported with different superscripts differ significantly from each other \( (P \leq 0.05) \).

\(^a\) Muscle by fiber-type least square means reported with different superscripts differ significantly from each other \( (P \leq 0.05) \).

\(^1\) LSMean = least squares mean; SEM = standard error of the mean.

\(^2\) I, IIA, IIAX, IIX: Muscle fiber-types classified according to myosin heavy chain isoform as detected immunohistochemically.
without there being a change in the fiber-type (Choi and Kim, 2009; Vestergaard et al., 2000). In addition, the finding in horses that the oxidative capacity of type IIA fibers may be higher than that of type I fibers questions the traditional classification of fast-twitch glycolytic or slow-twitch oxidative fibers (Dingboom and Weijs, 2004). This suggests that the classification of muscle fibers from springbok purely according to the myosin heavy chain isoform present may not be sufficient to provide the full picture. The relatively high activity of the enzymes involved in oxidative metabolism found for springbok muscle by Curry et al. (2012), despite the low proportion of type I fibers, further supports this.

The large proportion of type IIX fibers found for springbok muscle may have a considerable influence on the nature of the meat. Fiber shortening during rigor could be reduced as a result of the small proportion of type I fibers, as these have been found to shorten more than other fiber-types during the rigor period (Dingboom and Weijs, 2004; Klont et al., 1998). In addition, muscles with a large proportion of fast glycolytic fibers have been reported to tenderize more rapidly than more oxidative muscles (Sazili et al., 2005), and the greater susceptibility of the z-line proteins in fast-twitch fibers to proteolysis (Choi and Kim, 2009).

The higher proportion of type IIX fibers found in samples from female springbok is similar to the results reported for sheep by Greenwood et al. (2007). This physiological difference may provide some explanation for the apparently higher susceptibility of male animals to DFD (Dransfield, 1994), as the low glycogen content of oxidative fibers makes ante-mortem glycogen depletion more of a risk (Dingboom and Weijs, 2004). However, it must be noted that the proportion of oxidative fibers in both sexes was very low and that more research specifically linking these 2 factors is needed.

The difference in fiber-type composition between the muscles likely reflects differences in the functions of the LTL and BF (Lawrie and Ledward, 2006). The lower proportion of type I fibers in the LTL is also in agreement with the results of previous studies and the general classification of the LTL as a white muscle (Kirchofer et al., 2002; Ruusunen and Puolanne, 2004; Żochowska-Kujawska et al., 2007). However, the effect on the muscle’s post-mortem behavior may be negligible, as both type I and type IIA fibers are classified as oxidative and the LTL contained a higher proportion of the latter (Curry et al., 2012; Lefaucheur, 2015). This more rapid tenderization has been attributed to the higher calpain to calpastatin ratio found in glycolytic muscles (Sazili et al., 2005), and the greater susceptibility of the z-line proteins in fast-twitch fibers to proteolysis (Choi and Kim, 2009).

### Table 2. Muscle fiber-type composition (percentage of total number) and cross-sectional areas (µm²) for a variety of species, as reported in literature

<table>
<thead>
<tr>
<th>Species</th>
<th>Muscle</th>
<th>Composition (%)²</th>
<th>CSA³ µm²</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capreolus capreolus</td>
<td>BF</td>
<td>25.3 29.3 45.3</td>
<td>– – 2036</td>
<td>Żochowska-Kujawska et al., 2007</td>
</tr>
<tr>
<td>Cervus elaphus</td>
<td>BF</td>
<td>45.9 21.6 32.6</td>
<td>– – 1287</td>
<td>Żochowska-Kujawska, 2016</td>
</tr>
<tr>
<td>Sus scrofa</td>
<td>BF</td>
<td>39.5 21.7 38.8</td>
<td>– – 1605</td>
<td>Żochowska et al., 2005</td>
</tr>
<tr>
<td>Bos taurus</td>
<td>LTL</td>
<td>30.4 25.4 41.8</td>
<td>1672 2228</td>
<td>Vestergaard et al., 2000</td>
</tr>
<tr>
<td>Bos taurus</td>
<td>LTL</td>
<td>35.0 21.8 43.2</td>
<td>1430 2432</td>
<td>Kirchofer et al., 2002</td>
</tr>
<tr>
<td>Bos taurus</td>
<td>LTL</td>
<td>33.1 14.9 52.6</td>
<td>– – 1605</td>
<td>Hwang et al., 2010</td>
</tr>
<tr>
<td>Capreolus capreolus</td>
<td>LTL</td>
<td>15.9 39 45.1</td>
<td>– – 1605</td>
<td>Żochowska-Kujawska et al., 2007</td>
</tr>
<tr>
<td>Cervus elaphus</td>
<td>LTL</td>
<td>35.5 26.1 38.4</td>
<td>– – 875</td>
<td>Żochowska-Kujawska, 2016</td>
</tr>
<tr>
<td>Dama dama</td>
<td>LTL</td>
<td>7 42 51</td>
<td>– – 2212</td>
<td>Curry et al., 2012*</td>
</tr>
<tr>
<td>Ovis aries</td>
<td>LTL</td>
<td>8.3 25.9 65.8</td>
<td>– – 2173</td>
<td>Hemnings et al., 2009</td>
</tr>
<tr>
<td>Sus scrofa</td>
<td>LTL</td>
<td>14.4 35.8 49.8</td>
<td>4718 2588</td>
<td>Solomon and West, 1985</td>
</tr>
<tr>
<td>Sus scrofa</td>
<td>LTL</td>
<td>– – –</td>
<td>3400 3490</td>
<td>Ruusunen and Puolanne, 2004</td>
</tr>
<tr>
<td>Sus scrofa</td>
<td>LTL</td>
<td>35.5 19.3 45.2</td>
<td>– – 1187</td>
<td>Żochowska-Kujawska et al., 2012</td>
</tr>
<tr>
<td>Sus scrofa domesticus</td>
<td>LTL</td>
<td>13.0 4.9 83.2</td>
<td>2173 3885</td>
<td>Henckel et al., 1997</td>
</tr>
<tr>
<td>Sus scrofa domesticus</td>
<td>LTL</td>
<td>– – –</td>
<td>2950 5790</td>
<td>Ruusunen and Puolanne, 2004</td>
</tr>
</tbody>
</table>

* MHC isoforms detected using SDS-PAGE.
1 BF: Biceps femoris muscle; LTL: Longissimus thoracis et lumborum or Longissimus dorsi or Longissimus thoracis muscle.
2 I, IIA, IIX(B): Muscle fiber-types as determined immunohistochemically or using the myosin ATPase method.
3 CSA: Muscle fiber cross-sectional area.

2004; Maltin et al., 2003). Studies have found that a change in the oxidative capacity of a fiber may take place without there being a change in the fiber-type (Choi and Kim, 2009; Vestergaard et al., 2000). In addition, the finding in horses that the oxidative capacity of type IIA fibers may be higher than that of type I fibers questions the traditional classification of fast-twitch glycolytic or slow-twitch oxidative fibers (Dingboom and Weijs, 2004). This suggests that the classification of muscle fibers from springbok purely according to the myosin heavy chain isoform present may not be sufficient to provide the full picture. The relatively high activity of the enzymes involved in oxidative metabolism found for springbok muscle by Curry et al. (2012), despite the low proportion of type I fibers, further supports this.
2010). It is however possible that the slightly higher proportion of type I fibers in the BF may contribute to its higher shear force as reported by North et al. (2015), by increasing shortening during rigor.

**Fiber cross-sectional area (CSA)**

The mean muscle fiber CSA found for springbok muscle was slightly lower than that reported for bovine LTL muscle and similar to results from previous studies on roe deer (Żochowska-Kujawska et al., 2007). It was much lower than values reported for domestic and wild pigs by Ruusunen and Puolanne (2004); however, there is a large amount of variation in the CSA values reported for Sus scrofa in literature (Table 2). Red deer CSA values were smaller than those found for springbok in this study (Żochowska-Kujawska, 2016).

The lack of difference in CSA values between the sexes is in agreement with the findings of Curry et al. (2012) for springbok and fallow deer. There was a similar lack of significant effect of muscle on the CSA values, which is in contrast with the results of Żochowska-Kujawska (2016), who found the BF to contain larger muscle fibers than the Longissimus lumborum in deer. However, in both the case of the sex comparison and the muscle comparison it must be noted that the lack of difference found could reflect the small sample size used. Further research using a larger number of animals may therefore be valuable.

The average CSA increased with the apparent glycolytic capacity of the fiber-type, with type IIX fibers being significantly larger than the other fiber-types (Fig. 2). This progressive increase in size from type I to IIA, IIAX and IIX is consistent with literature, and has been reported for a number of other species (Choi and Kim, 2009; Dingboom and Weijis, 2004; Kohn et al., 2011; Ruusunen and Puolanne, 2004). This is hypothesized to be due to the more rapid hypertrophy of the type IIX fibers than the type I and IIA fibers during postnatal growth (Ruusunen and Puolanne, 2004).

The fiber cross-sectional area is most important in terms of its effect on muscle texture and tenderness; however, the precise relationship between these factors is still to be completely elucidated (Choi and Kim, 2009; Dingboom and Weijis, 2004). The CSA found for springbok appears to be within the range of that reported for other domesticated and wild species, and it is therefore unlikely to affect consumer liking of the meat to any great extent.

**Conclusions**

Springbok muscle contains higher proportions of type IIX fibers than is found in domestic livestock and other wild species utilized for the production of red meat. This suggests that the muscle is primarily glycolytic in nature; however, the physical and chemical characteristics of springbok meat, as previously reported, do not support this. This discrepancy between the apparent metabolic nature of the fibers and the MHC isoform they contain casts doubt on the application of standard associations between fiber-type and meat quality to springbok meat. However, assuming that these associations still exist to some extent, it appears that the high proportion of fast-twitch fibers found in springbok meat may explain the low shear force and rapid tenderization reported in literature and support the short ageing periods recommended.

The average cross-sectional area of the fibers was low but within the range reported for other meat-producing species and increased with the apparent glycolytic capacity of the fiber-types, as previously reported. There was no significant effect of sex or muscle on the cross-sectional area.

**Literature Cited**


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