THE MUSCLE FIBRE CHARACTERISTICS OF SPRINGBOK (*ANTIDORCAS MARSUPIALIS*) *LONGISSIMUS THORACIS ET LUMBORUM* AND *BICEPS FEMORIS* MUSCLE

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Abstract – This study aimed to determine the fibre-type composition and fibre cross-sectional area (CSA) of springbok (*Antidorcas marsupialis*) *Longissimus thoracis et lumborum* (LTL) and *Biceps femoris* (BF) muscles. Snap-frozen samples from four male and three female springbok were fibre-typed immunohistochemically using the primary antibodies A4.74, BA-D5 and BF-35. The CSA of the fibres was determined using the software Image J. Type IIX fibres accounted for 64% - 78% of the fibres in all samples, with type IIA (11.9 – 21.2%), type IIAX (5.8 – 10.9%) and type I (1.8 - 10.4%) making significantly smaller contributions. The glycolytic nature ~~this suggests~~ may contribute to springbok meat's tenderness. The BF contained more type I fibres and fewer type IIA fibres than the LTL in male springbok. CSA values were within the range reported for domestic livestock species. They did not differ significantly across muscles and fibre-types in females but increased with glycolytic capacity (I < IIA < IIAX < IIX) in males.

Key Words – Game meat, Immunohistochemistry, Myosin heavy chain isoform

I. INTRODUCTION

The South African game industry is one of the fastest growing in the agricultural sector and springbok (*Antidorcas marsupialis*) are one of the most important species for meat production [1-3]. In order for the quality of springbok meat products to be improved the nature of the meat from this species needs to be fully understood.

Skeletal muscle is a highly organized and complex tissue [4], and variation in the nature of the myofibrils and muscle fibres exists in order for the diverse range of motions required in the animal body to be achieved [5, 6]. This variation in the contractile and metabolic nature of the muscle also impacts the changes that take place in the muscle *post-mortem* (PM), influencing the degree of contraction during rigor, the rate of decline in the pH and the rate and degree of tenderization during ageing [5].

The aim of this study was to contribute to the existing information on springbok fibre-type composition and cross-sectional area as well as explore the effect that this may have on meat quality.

II. MATERIALS AND METHODS

Three male and four female springbok were harvested at night using spotlights and 30-06 or .270 calibre rifles (Ethical clearance number SU-ACUM13-0034). At approximately eight hours PM, 0.5 cm^3 blocks of muscle from the centre of each *Longissimus thoracis et lumborum* (LTL) and *Biceps femoris* (BF) muscle were excised and snap frozen in liquid nitrogen.

Immunohistochemical fibre-type identification as described by Kohn *et al*. [4] was performed on serial 10 µm cross-sections of the snap frozen blocks. The primary antibodies A4.74 (specific to myosin heavy chain [MHC] isoforms IIa and IIx), BA-D5 (specific to MHC I) and BF-35 (specific to MHC I and IIa) (Developmental Studies Hybridoma Bank, Iowa) were used in conjunction with the secondary antibody Cy3 donkey anti-mouse (Jackson ImmunoResearch Laboratories, Pennsylvania, USA). All slides were visualized and photographed using a fluorescent-capable Nikon Eclipse 80i and a Canon 650D camera. Fibres were identified as type I, IIA, IIAX or IIX by comparing the intensity of the fluorescent staining for each primary antibody in the sequential sections. Between 500 and 1400 fibres were counted for each sample.

The cross-sectional area of each of the muscle fibre-types was determined using the software program Image J (version 1.47, http://rsb.info.nih.gov/ij), with each fibre being outlined and the area enclosed by the outline determined by the program. Up to 100 fibres of each type were measured per sample.

Statistical analysis of the data was performed using Statistica (version 12, Statsoft inc. 2013), with muscle, fibre-type and their interaction being tested as main effects using repeated measures of analysis of variance (ANOVA's). The data was analysed separately for the genders in order to allow some comparison; however this difference could not be statistically tested due to the small sample size used.

III. RESULTS AND DISCUSSION

Fibre-type composition

The samples in this study consisted of predominantly type IIX fibres (Table 1). This is in agreement with the results of Curry *et al.* [7] and is in contrast with the more oxidative nature reported for muscles from Svalbard reindeer (*Rangifer tarandus platyrhynchus*), fallow deer (*Dama dama*) and cattle [7-9].

The large proportion of type IIX fibres is in agreement with the high activity of the glycolytic enzymes reported for springbok muscle by Curry *et al*. [7] as well as the considerable sprinting ability of the species [4,10]. However, it appears to contradict the previous finding that extensive rearing systems and low levels of nutrition tend to increase the proportion of oxidative fibres [9,11,12]. It is also unexpected considering the high iron levels and low L* values reported for springbok meat, as these are commonly associated with high proportions of type I and type IIA fibres [11,13-16]. This apparent contradiction may be due to variation in the metabolic characteristics of the muscle fibres existing within each myosin ATPase-based type [11,17,18], and suggests that the classification of muscle fibres from springbok purely according to the myosin heavy chain isoform present may not provide the full picture.

The fibre-type composition found for springbok muscle in this study may reduce shortening during rigor, as type I fibres have been found to shorten more than the other fibre types [5,11].

Table 1 The fibre-type composition and cross-sectional area (CSA) of muscle fibres in springbok *Longissimus thoracis et lumborum* (LTL) and *Biceps femoris* (BF) muscle (LSMean ± SEM)

		Female		**Male**	
		LTL	**BF**	**LTL**	**BF**
Fibre-type composition (%)	Type I	1.8^{c} ± 0.76	6.6^{c} ± 0.71	3.1$^{\mathbf{d}}$ ± 0.61	10.4$^{\mathbf{c}}$ ± 1.21
	Type IIA	13.5^{b} ± 0.57	11.9^{b} ± 1.57	21.2$^{\mathbf{b}}$ ± 2.53	16.9$^{\mathbf{c}}$ ± 0.56
	Type IIAX	7.0^{c} ± 1.84	5.8^{c} ± 1.15	10.9$^{\mathbf{bcd}}$ ± 2.26	7.7$^{\mathbf{cd}}$ ± 2.09
	Type IIX	77.7^{a} ± 1.84	75.7^{a} ± 2.79	64.7$^{\mathbf{a}}$ ± 5.09	63.9$^{\mathbf{a}}$ ± 4.00
Fibre CSA (μm^2)*	Type I	2112 ± 324.0		1367$^{\mathbf{c}}$ ± 91.0	
	Type IIA	1904 ± 219.1		1636$^{\mathbf{bc}}$ ± 153.3	
	Type IIAX	2101 ± 213.3		1904$^{\mathbf{b}}$ ± 147.9	
	Type IIX	2777 ± 401.2		2521$^{\mathbf{a}}$ ± 183.9	

LSMean: least squares mean; SEM: standard error of the mean.

$^{a, b, c}$; $^{\mathbf{a, b, c, d}}$ Within each gender, least square means reported for muscle, fibre-type and fibre CSA with different superscripts differ significantly from one another ($P \leq 0.05$).

*The values for the individual muscles have not been given for the fibre CSA due to no significant difference between the muscles being found.

In addition, muscles with a large proportion of fast glycolytic fibres have been reported to tenderize more rapidly than more oxidative muscles [19], which could provide some explanation for the rapid tenderization found by North *et al.* [20,21]. This more rapid tenderization has been attributed to the higher calpain to calpastatin ratio found in glycolytic muscles [19]. The z-line proteins in fast-twitch fibres have also been reported to be more susceptible to proteolysis [17].

Although the small sample size limited the value of comparing the muscles or genders, female springbok did appear to contain more type IIX fibres and fewer of the other fibre types than males. This may provide some explanation for the apparently higher susceptibility of male animals to DFD [22]; however, it must be noted that the proportion of oxidative fibres in both genders was very low.

The fibre-type composition of the muscles did not differ in females (P = 0.125), whereas in males the BF contained a higher proportion of type I fibres and a lower proportion of type IIA fibres than the LTL (P = 0.007). While this could influence the function of the muscle the effect on its PM behaviour may be negligible, as both type I and type IIA fibres are classified as oxidative according to their succinodehydrogenase activity [6,7].

Fibre cross-sectional area

While fibre size is thought to influence meat quality, and particularly texture, the results of studies attempting to correlate fibre cross-sectional areas (CSA) and tenderness are contradictory [11,17]. As the overall values found for springbok in this study were relatively similar to those reported for bovine LTL muscle [9], it seems unlikely that this aspect of springbok meat will affect consumer liking of the meat to any great extent.

Some variation in CSA was observed between the genders, with female springbok having considerably larger fibres than males for all the fibre-types and both muscles (Table 1). This is in contrast to the finding in most species that male muscles have larger fibres; however, similar results have been found for pigs [11].

In male springbok the average CSA tended to increase with glycolytic capacity for both muscles (P = 0.008). This progressive increase in size from type I to IIA, IIAX and IIX is consistent with literature, and has been reported in a number of other species [4,11,17].

IV. CONCLUSION

The high proportion of type IIX fibres found in springbok muscle suggests that the muscle is primarily glycolytic; however the physical and chemical characteristics of the meat do not support this. This discrepancy between the metabolic nature of the fibres and the MHC isoform they contain casts doubt on the application of standard associations between fibre-type and meat quality to springbok meat. However, the high proportion of fast-twitch fibres may explain the low shear force and rapid tenderization reported in literature.

The average cross-sectional area of the fibres was found to be low but within the range reported for other meat-producing species. The effect on consumer acceptance is thus likely to be relatively negligible.

ACKNOWLEDGEMENTS

The authors would like to thank the staff and students of the Animal and Food Science departments of Stellenbosch University for their assistance with sampling and Prof Martin Kidd from the Centre for Statistical Consultation at Stellenbosch University for performing the statistical analysis of the data. They would also like to thank Dr Tertius Kohn from the University of Cape Town's Research Unit for Exercise Science and Sports Medicine for his assistance with the fibre-typing. This work is based on research supported by the South African Research Chairs Initiative of the Department of Science and Technology and the National Research Foundation of South Africa. Any opinion, finding and conclusion or recommendation expressed in this material is that of the author(s) and the National Research Foundation does not accept any liability in this regard.

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