HISTOLOGICAL CHARACTERIZATION OF THE FIBER TYPES IN THE *M. PECTORALIS* OF EGYPTIAN GEESE: A SOUTHERN AFRICAN WILDFOWL SPECIES

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Abstract – The Egyptian goose is a Southern African wildfowl species which is hunted on a regular basis. The utilisation of the meat is imperative, but with the current absence of scientific literature it is vital to investigate the meat quality as well as the factors of influence thereupon. One of the key aspects of meat quality is the composition of the muscle fibers. The *pectoralis* muscle of Egyptian geese is mainly comprised of fast, oxidative-glycolytic fibres (84%) with a cross-sectional area of 1283.9 μm^2. A small percentage of fast, glycolytic fibers (16%) are also present. This is attributable to the strenuous requirements of long distance flight. The large proportion of small, fast, oxidative-glycolytic fibers may be responsible for the perceived toughness of Egyptian goose breast meat.

I.   INTRODUCTION

In southern Africa, the gamebird industry and the sport of wingshooting is becoming increasingly popular. The Egyptian goose (*Alopochen aegyptiacus*) is one of the leading gamebird species hunted [1]. This wildfowl species is also considered to be a very serious agricultural pest by crop farmers in the Western Cape, South Africa [2]. In an effort to manage the population numbers and thereby reduce the damage they cause, wingshooting of the geese is recommended. The utilisation of the meat is therefore imperative, but with the current absence of scientific literature on the meat quality and composition of this species, it is vital to investigate the meat quality as well as the factors of influence.

The composition of muscle fibers is a critical determinant of meat quality [3]. Muscle fiber type can influence the colour, tenderness, juiciness and even the flavour of meat, which in turn may affect the consumer acceptability of the meat. The attribute which is most affected by muscle fiber type is the tenderness of meat. Factors such as the total number of fibers, the cross sectional area and diameter of the fibers as well as the composition of the muscle fiber types are significant in terms of meat tenderness [3].

It is thus necessary to determine the muscle fiber composition of the breast portion (*M. pectoralis*) of Egyptian geese. This will provide valuable insight regarding the perceived toughness of the meat. Investigating the fiber types present in this muscle will also provide vital information in terms of the type of activity (locomotive/flight and postural) this muscle is used for. This will assist in explaining other characteristics such as the colour, pH and intramuscular fat content of the breast portion as reported by Geldenhuys *et al*. [4].

II.   MATERIALS AND METHODS

Harvesting

Egyptian geese were harvested on the University of Stellenbosch's experimental farm, Mariendahl (ethical clearance: 10NP_HOF01), by the use of double barreled shotguns. A total of 3 mature geese were harvested and collected in the field.

Sample preparation

Superficial muscle samples were removed (1 hour post mortem) from the sternobrachialis region of the right *pectoralis pars thoracicus* muscle within the cranial third of the muscle. Each block of muscle (0.7 x 0.5 x 0.5 cm) was mounted on a cardboard square with the muscle fiber orientation perpendicular to the cardboard surface. Muscle specimens were coated with Jung tissue freezing medium (Leica Biosystems, Midrand, South Africa) and frozen in iso-pentane cooled to -160°C by liquid nitrogen [5] and stored at -80°C. Serial cross-sections (12 μm) were cut from each muscle sample using a cryostat (Leica, Germany) maintained at -25°C and mounted onto microscope slides (Lasec, SA).

Immunohistochemistry and NADH histochemistry

Three sections from each series were stained for immunofluorescence by a modified version of

the method described by Rosser *et al.* [6]. Sections were first blocked in a solution of 5% natural goat serum (NGS), 1% bovine serum albumin (BSA) and phosphate buffered saline (PBS) for 20 minutes at room temperature. The sections were then incubated with the primary antibodies; F59 and S46 (Developmental Hybridoma Studies Bank, University of Iowa, USA) respectively at 4°C overnight. The primary antibodies were used at the dilution of 1:20 in blocking solution. The sections were then rinsed in PBS and incubated at room temperature for 1 hour in a 1:200 dilution of secondary antibody (Alexa Fluor 488 goat anti-mouse IgG, Life Technologies, Carlsbad, USA) in PBS. The sections were then rinsed in PBS and fixed in 4% paraformaldehyde (PFA) for 3 minutes, after which a cover slip was mounted on the sections with fluorescent mounting media (DAKO, Denmark). So as to ensure that the immunoflorescent stain was effective and the antibodies specific, a serial section of the *sartorius*, a thigh muscle containing both slow and fast fibers, was stained as a control.

In order to distinguish between the two fast fiber isoforms (fast, oxidative-glycolytic and fast glycolytic) two sections from each series were stained for B-nicotinamide adenine dinucleotide (NADH) activity [5]. Slides were incubated in dinitroblue tetrazolium (NBT) (0.12g in 60 mL, 0.05 M Tris buffer) and NADH (0.1g in 60 mL, 0.05 M Tris buffer) (Sigma-Aldrich, St. Louis, USA) for 30 minutes at 37°C. Post incubation, the slides were rinsed with distilled water and washed in increasing concentrations of acetone (30%, 60% and 90%). This was followed by rinsing with distilled water and mounting of the cover slips with DPX mountant (Sigma-Aldrich, St. Louis, USA). All slides were kept at 4°C until ready to view.

Fiber identification, counting and cross-sectional area
All slides were visualised and photographed at 4x magnification using an Olympus Cell^R Microscope system (Olympus Biosystems GMBH) with an F-view-II cooled CCD camera (Soft Imaging systems). A Xenon-Arc burner (Olympus Biosystems GMBH) was used as the light source. Photos were taken at three locations on each of the sections. The three locations coincide on each of the serial sections. The Muscle fibers were identified [6] and counted on each of the three locations. The cross-sectional area (CSA) was determined on

100 muscle fibers within a designated rectangular area on each of the three locations by using Image J software (Version 1.41, 2009, http://rsbweb.nih.gov/ij/).

Statistical analysis
The data from each of the three areas obtained from the three *pectoralis* muscle samples (n=9) were subjected to an analysis of variance (ANOVA) and the t-Least Significant Differences (LSD) were calculated at a 5% significance level. Differences between the variables were accepted as being significant at P≤0.05. SAS[TM] statistical software (Version 9.2, USA) was used for the ANOVA.

III. RESULTS

The staining of the Egyptian goose *pectoralis* muscle for immunofluorescence revealed the presence of only fast twitch, oxidative-glycolytic (FOG/type IIa) and glycolytic (FG/type IIb) fibers. This is evident in Fig. 1. which indicates the positive labeling of the fast (F59) antibody (Fig. 1b) compared to the negative labeling of its slow (S46) counterpart (Fig. 1c) within the serial sections. For this reason, the staining of the sections for NADH activity (Fig. 1d) was necessary in order to differentiate between the two fast isoforms; FOG and FG present in the breast portion (*pectoralis* muscle).

The quantification of the two fast isoforms revealed that the *pectoralis* muscle predominantly consists (P≤0.05) of FOG fibers (84.4%) (Table 1). These FOG fibers were much smaller than the FG fibers. This is indicated by the lower (P≤0.05) CSA and diameter (Table 1) of the FG compared to the FOG fibers.

Table 1 The mean[1] (±SD) fiber composition (%), fiber CSA and fiber diameter of the *pectoralis* muscle of Egyptian geese

	FOG	FG
Total (%)	84.4[a] ± 9.3	15.6[b] ± 9.3
CSA (µm[2])	1283.9[b] ± 176.7	2793.3[a] ± 921.9
Diameter (µm)	40.3[b] ± 2.9	58.4[a] ± 12.9

[1]Means in rows with different superscripts differ significantly at P≤0.05. Table abbreviations: Standard deviation (SD), fast, oxidative glycolytic (FOG), fast glycolytic (FG), cross-sectional area (CSA).
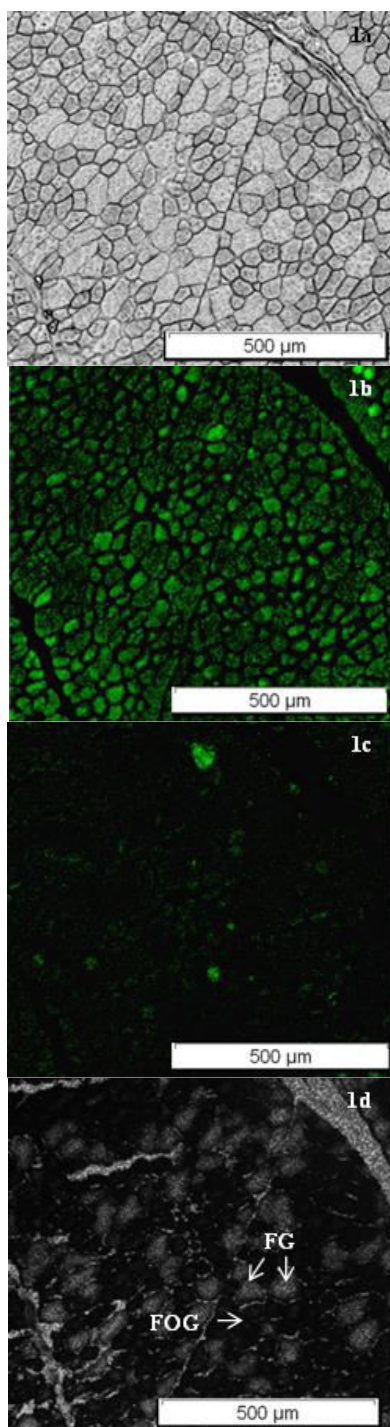
Figure 1. Serial sections of the Egyptian goose *pectoralis* muscle (a) Transverse section (b) immunocytochemical labelling by fast primary antibody (F59) (c) immunocytochemical labelling by slow primary antibody (S59) (d) histochemical demonstration of NADH activity and identification of FG vs. FOG fibers

IV.   DISCUSSION

The *pectoralis* muscle of avian species, which rely on this muscle for long distance flight is almost entirely composed of fast-twitch fibers [6,7,8]. A rigorous requirement for rapid, sustained muscle contraction is placed on this muscle for long term flight. This is responsible for the presence of mainly FOG fibers in the breast portion of volant species [7]. The small amount of FG fibers present is presumed to assist larger bodied birds when an increase in power is needed during take-off [7]. The results found in this study (Table 1) is in agreement with literature and shows that the *pectoralis* muscle of Egyptian geese is only comprised of fast-twitch fibers i.e. 84.4% FOG and 15.6% FG. Egyptian geese are volant gamebirds that fly long distances in order to forage on grain fields [2]. Their breast muscles therefore endure a much higher level of activity compared to terrestrial gamebird species such as guineafowl. Similar results have also been found in other Anseriforms (ducks and geese) such as Mule ducks [9], Canada geese [10] and several other species [6,7].

Muscle fiber composition is recognized as one of the major determinants of meat quality. With that said, the definite relationship between fiber type and meat tenderness is still somewhat unclear [11]. The general consensus seems to be that muscles containing slow, type I fibers are more tender because of the smaller fiber diameter which results in it being less resistant to chewing or mechanical shearing [12]. However, some studies [13,14] have found that FOG (type IIa) fibers are smaller than the slow fibers present. This suggests that fiber size may vary on account of species, muscle and exercise and that the generalization that slow fibers produce more tender meat may be partial. In light of all the controversy, Henckel *et al*. [13] and Maltin *et al*. [14] reported that the percentage of FOG fibers considerably affects tenderness. Another aspect to consider is the fact that with a decrease in fiber size there is a larger amount of fibers present, thus an increase in connective tissue (per unit area/volume) resulting in less tender meat [15]. In the Egyptian goose *pectoralis*, the FOG fiber area of 1283.9 μm^2 (Table 1) is small compared to the CSA of fibers measured in studies on pork and beef [13,14,16]. This suggests that the small FOG fibers may be a contributing factor to the overall perceived toughness of Egyptian goose breast meat.

The high proportion of FOG fibers also clarifies the higher (P≤0.05) intramuscular fat percentage [17] and dark, red colour [4] of Egyptian goose breast meat compared to other species such as guineafowl and broiler chicken. Other important factors relating to fiber type and meat quality is the variation in the post mortem muscle metabolism and the difference in the proteolytic enzyme/inhibitor concentrations. These factors may have an influence on the sensory tenderness of Egyptian goose meat and warrant further research.

V. CONCLUSION

The *pectoralis* muscle of Egyptian geese are mainly comprised of FOG fibres with a small percentage of FG fibers. This is attributable to the strenuous requirements of long distance flight. The large proportion of small, FOG fibers may be responsible for the perceived toughness of Egyptian goose breast meat.

ACKNOWLEDGEMENTS

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