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Muscle fiber type and metabolic profiles of four muscles from the African black ostrich

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ABSTRACT

Muscle fiber type, fiber cross-sectional area (CSA), enzyme activities (citrate synthase (CS), 3-hydroxyacetyl Co A dehydrogenase (3HAD), lactate dehydrogenase (LDH) and phosphofructokinase (PFK)) and glycogen content were analyzed in the *M. iliotibialis cranialis* (ITC), *M. iliotibialis lateralis*, *M. gastrocnemius* (G) and *M. fibularis longus* (FL) muscles from 24 ostriches. Type I and II fiber proportions were similar across the 4 muscles, but the ITC had overall the smallest fibers. CS activity was the highest in the ITC, but similar between the remainder of the muscles. 3HAD activities were very low in all muscles, ranging between 1.9 and 2.7 μmol/min/g protein, indicating poor β-oxidation. The ITC also had the lowest PFK activity. Glycogen content averaged ~85 mmol/kg dry weight across the muscles with large intramuscular variations. The 4 ostrich muscles present with low fat oxidation capacity and low glycogen content, which could have significant implications on meat quality attributes.

1. Introduction

Ostrich (*Struthio camelus var domesticus*) farming for their meat is still globally a valuable source of lean meat cuts (Hoffman, Muller, Cloete, & Brand, 2008; Hoffman & Wiklund, 2006). World production of ostrich meat is 12,000 to 15,000 tons per annum and South Africa is responsible for approximately 60% of this ostrich meat (Hoffman et al., 2008). Although studies have investigated the physicochemical composition of some ostrich meat cuts, there is still very little known about the skeletal muscle properties of these birds that may assist in a better understanding of their muscle function and metabolism that could affect meat quality after slaughter.

Muscle fiber type and their respective cross-sectional areas (CSA) are important parameters that may influence overall meat quality as found in other species, such as beef and pork (Lefaucheur, 2010). In most domesticated farm animals (excluding the pig), three fiber types are found in their large muscle groups, each brought about by the specific myosin heavy chain (MHC) isoform it expresses. The three fiber types are type I (slow oxidative, SO), IIA (fast oxidative-glycolytic (FOG)) and IIX (fast glycolytic (FG)) fibers (Kohn, Hoffman, & Myburgh, 2007). The pig expresses a fourth isoform, giving rise to type IIB fibers, being highly glycolytic and low in mitochondrial numbers compared to type I, IIA and IIX fibers (Lefaucheur, 2010). All these isoforms have been confirmed using antibodies specific to the MHC isoform, their respective migration patterns on SDS-PAGE gels and contractile properties (Kohn, Hoffman, & Myburgh, 2007; Toniolo et al., 2004). Using the conventional ATPase stain in combination with a mitochondrial stain, Velotto and Crasto (2004) reported three fiber types for the ostrich using the nomenclature SO, FOG and FG. However, the authors could only identify all three fiber types in one of the muscles studied, with the remaining muscles only containing SO and FOG. This method does have limitations in that it is highly dependent on the pre-incubation pH and stability of the myosin ATPase enzyme. In addition, the mitochondrial stain (also employed in the present study) has its own limitations in that the assay is highly dependent on incubation temperature and time (Curry, Hohl, Noakes, & Kohn, 2012). Curry et al. (2012) and Kohn (2014) showed that fibers typed using antibodies showed significant overlap in fiber oxidative staining intensities within and between type I, IIA and IIX fibers, which

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could result in misclassifying IIX fibers as type IIA (FOG) or type I (SO).

Equally important and not yet studied in ostrich muscle, is the metabolic profiles of the muscle fibers, which include enzyme activities representing the various ATP generating metabolic pathways and glycogen content. The latter has been highlighted as playing an important role in the maturing process of muscle to meat (Lefaucheur, 2010).

Thus, the aim of this study was to determine the muscle fiber type composition using commercially available antibodies, CSA of the fibers, enzyme activities representing the four metabolic pathways and glycogen content in the *M. iliotibialis cranialis* (ITC), *M. iliotibialis lateralis* (ITL), *M. gastrocnemius* (G) and *M. fibularis longus* (FL) of the ostrich. The ITC is one of the muscles with the highest selling price per kilogram, whereas the remaining 3 muscle groups represent cuts that are frequently found on the South African market as high quality meat (Hoffman et al., 2008).

2. Materials and methods

2.1. Ethical approval

This study was approved by the Elsenburg Agricultural Research farm and the University of the Western Cape (AR20/3/1).

2.2. Animals and sample collections

Ostriches were hatched and reared at the Kromme Rhee Experimental Farm in the Western Cape Province of South Africa. They walked freely within alfalfa camps and fed ad libitum. Twenty-four adult mixed sex ostriches between the ages of 10 and 12 months old (11.2 ± 0.2 months), with an average live weight at slaughter of 94 ± 2 kg (range 82–114 kg) were included in the study. Ostriches were transported to a commercial abattoir and humanely harvested (refer to Hoffman et al. (2008) for a detailed description of the process). The mean carcass weight amounted to 49 ± 1 kg (range 40–61 kg). Samples from the ITC, ITL, G and FL were collected within a 10 min time frame after slaughter

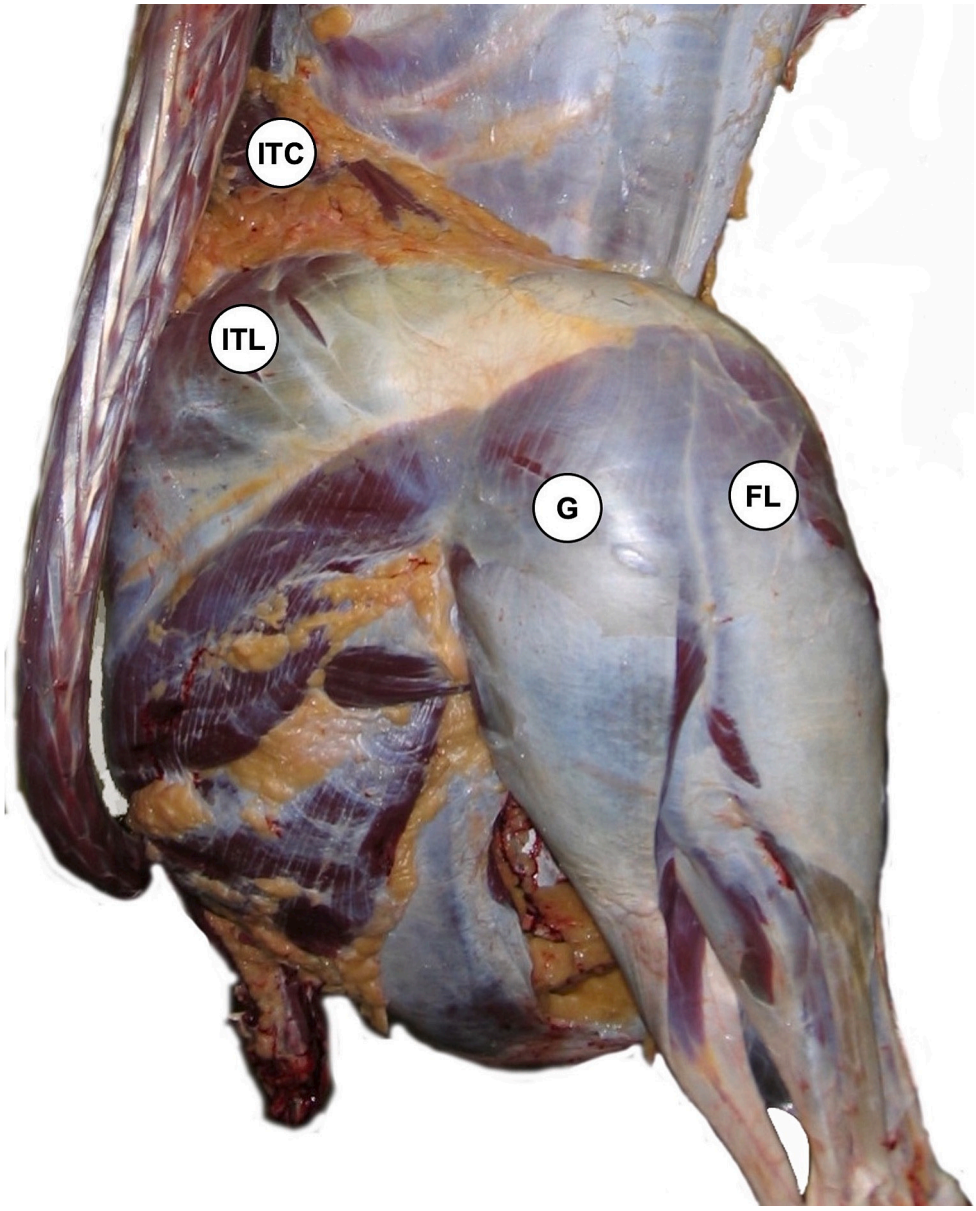

Fig. 1. Sites of sampling from the *m. Iliotibialis cranialis* (ITC), *m. Iliotibialis lateralis* (ITL), m. *Gastrocnemius* (G) and *m. Fibularis longus* (FL).

(Fig. 1), rapidly frozen in liquid nitrogen on site, and stored at −80 °C until analyses.

2.3. Histology

Samples were randomized into 8 batches of 12 and serial cross-sections (10 μm) were prepared using a cryostat (Leica, Germany) and stained for mitochondrial density using the NADH-nitro blue tetrazolium stain to visualize mitochondrial density (Kohn, Curry, & Noakes, 2011) (Fig. 3).

To identify muscle fiber type, various antibodies from the Developmental Studies Hybridoma Bank (DSHB, Ohio, USA) were tested for binding reactivity to the MHC isoforms. Some of these have also been validated to react and distinguish the various isoforms expressed in wild animals, including various felids (e.g. lions, cheetahs, tigers), antelope species (e.g. black wildebeest, springbok) and baboons (Dada, Henning, Feldmann, & Kohn, 2018; Hyatt, Roy, Rugg, & Talmadge, 2010; Kohn, 2014; Kohn, Burroughs, Hartman, & Noakes, 2011). These included monoclonal antibodies against mammalian isoforms namely N2.261 (reacting with MHC I and MHC IIA), A4.74 (reacting with MHC IIA and IIX), BF-35 (reacting with MHC I and IIA), 6H1 (reacting with MHC IIX), BA-D5 (reacting with MHC I), SC-71 (reacting with MHC IIA and IIX) and BF-F3 (reacting with MHC IIB). Additionally, antibodies specific to avian and mammalian MHC isoforms were also tested, namely F18 (reacting with multiple fast isoforms), F27 (reacting with multiple fast isoforms), F30 (reacting with multiple fast isoforms), F47 (reacting with multiple fast isoforms) and S22 (reacting with multiple slow isoforms). Only the antibody F18, specific to the fast avian MHC isoforms, was able to distinguish between type I (slow twitch) and type II (fast twitch) fibers (Fig. 3), whereas the remainder of the antibodies showed no binding. Fibers were therefor only characterized as type I or II. Weak binding of the antibody suggested a hybrid fiber but were negligible. Type I and II fibers were counted, and each type expressed as a percentage of the total number of fibers counted (average of 215 fibers per muscle, Table 1). The cross-sectional areas (CSA) of each fiber type were determined from a minimum of 30 fibers (Table 1).

2.4. Enzyme analyses

Each of the four enzyme assays was performed as one batch, with samples being randomized. Approximately 100 mg of the rapidly frozen tissue was freeze-dried overnight at −50 °C under vacuum (Virtis,

Table 1
Metabolic and muscle fiber characteristics in four ostrich muscle groups. Values are expressed as mean ± SEM.

Muscle group	*Iliotibialis cranialis*	*Iliotibialis lateralis*	*Gastrocnemius*	*Fibularis longus*
Fiber type distribution				
Type I fibers (%)	44 ± 2	42 ± 2	46 ± 2	51 ± 3
Iliotibialis lateralis	$P > 0.999$			
Gastrocnemius	$P > 0.999$	$P > 0.999$		
Fibularis longus	$P = 0.713$	$P = 0.120$	$P = 0.614$	
Type II fibers (%)	56 ± 2	58 ± 2	54 ± 2	49 ± 3
Iliotibialis lateralis	$P > 0.999$			
Gastrocnemius	$P > 0.999$	P > 0.999		
Fibularis longus	$P = 0.720$	$P = 0.135$	$P = 0.603$	
Fiber size				
CSA All (μm^2)	3433 ± 238	5682 ± 327	5360 ± 303	6598 ± 277
Iliotibialis lateralis	**$P < 0.001$**			
Gastrocnemius	**$P < 0.001$**	$P > 0.999$		
Fibularis longus	**$P < 0.001$**	$P = 0.349$	$P = 0.079$	
CSA Type I (μm^2)	2620 ± 203	4408 ± 327	4718 ± 276	5310 ± 102
Iliotibialis lateralis	$P = 0.086$			
Gastrocnemius	**$P = 0.009$**	$P > 0.999$		
Fibularis longus	**$P < 0.001$**	$P > 0.999$	$P > 0.999$	
CSA Type II (μm^2)	4223 ± 286	6962 ± 383	6041 ± 373	8039 ± 330
Iliotibialis lateralis	**$P < 0.001$**			
Gastrocnemius	$P = 0.069$	$P > 0.999$		
Fibularis longus	**$P < 0.001$**	$P > 0.999$	$P = 0.139$	
Metabolism				
CS (μmol/min/g prot)	47 ± 4	29 ± 1	34 ± 2	30 ± 2
Iliotibialis lateralis	**$P < 0.001$**			
Gastrocnemius	**$P = 0.034$**	$P > 0.999$		
Fibularis longus	**$P < 0.001$**	$P > 0.999$	$P > 0.999$	
3HAD (μmol/min/g prot)	2.7 ± 0.2	1.9 ± 0.1	2.5 ± 0.1	1.9 ± 0.1
Iliotibialis lateralis	**$P < 0.001$**			
Gastrocnemius	$P > 0.999$	**$P = 0.003$**		
Fibularis longus	**$P < 0.001$**	$P > 0.999$	**$P = 0.006$**	
PFK (μmol/min/g prot)	44 ± 11	124 ± 10	158 ± 12	157 ± 7
Iliotibialis lateralis	**$P = 0.007$**			
Gastrocnemius	**$P < 0.001$**	**$P = 0.046$**		
Fibularis longus	**$P < 0.001$**	$P > 0.245$	$P > 0.999$	
LDH (μmol/min/g prot)	1553 ± 107	1164 ± 44	1517 ± 74	1298 ± 40
Iliotibialis lateralis	**$P = 0.003$**			
Gastrocnemius	$P > 0.999$	**$P < 0.001$**		
Fibularis longus	$P = 0.591$	$P > 0.365$	$P = 0.1664$	
Glycogen (mmol/kg dw)	66 ± 9	86 ± 12	108 ± 15	81 ± 8
Iliotibialis lateralis	$P > 0.999$			
Gastrocnemius	$P = 0.347$	$P > 0.999$		
Fibularis longus	$P = 0.647$	$P > 0.999$	$P > 0.999$	

CS, citrate synthase; 3HAD, 3 hydroxyacetyl coenzyme A dehydrogenase; PFK, phosphofructokinase; LDH, lactate dehydrogenase; CSA, cross-sectional area; dw, dry weight.

Gardiner, NY, USA). The dried samples were weighed and homogenized in chilled 100 mM potassium phosphate buffer, pH 7.30, in a ratio of 1 mg tissue: 400 µl homogenizing buffer, followed by sonication on ice (Virtis Sonicators, Gardiner, USA). Protein content of each sample was determined using the method developed by Bradford (1976). Enzyme activities were measured fluorometrically (Biotek FL800) as previously described (Kohn, Essen-Gustavsson, & Myburgh, 2007; Webster et al., 2020), and served as markers for the respective metabolic pathways: phosphofructokinase-1 (EC2.7.1.11) for flux capacity through glycolysis; lactate dehydrogenase (EC1.1.1.27) for lactate production from pyruvate; citrate synthase (EC2.3.3.1) for Krebs cycle oxidative capacity; 3-hydroxyacetyl-CoA dehydrogenase (3HAD; EC1.1.1.35) for fat oxidation capacity. The sample volume for CS and 3HAD were 5 µl and for LDH and PFK 2.5 µl. The assay was started with the addition of 250 µl working reagent. The appearance or disappearance of NADH was measured at an excitation wavelength of 340 nm and emission wavelength of 460 nm at 25 °C. An NADH standard curve was constructed with known concentrations and used to express activities as µmol/min/g protein (prot).

2.5. Glycogen content

The glycogen content was determined by the digestion of muscle followed by the degradation of the glycogen to glucose, as previously described (Passonneau & Lauderdale, 1974; Webster et al., 2016). The extraction of glycogen was performed in batches of 4, each batch comprising 24 randomized samples. For each sample, approximately 7 mg freeze-dried muscle tissue was digested in 200 µl 40% KOH at 95 °C for 30 min. The glycogen was precipitated with 800 µl absolute alcohol at 4 °C overnight. After centrifugation and washing the glycogen pellet with absolute ethanol, the glycogen was hydrolyzed to glucose by the addition of 200 µl 2 M HCl, and heated to 95 °C for 3 h. This suspension was neutralized with NaOH and the glucose concentration determined fluorometrically as one batch of randomized samples (Passonneau & Lowry, 1993). Glycogen was expressed as mmol glucose equivalents/kg dw.

2.6. Statistical analyses

Data are expressed as mean ± standard error of the mean (SEM). Statistical analyses of the various variables were compared between muscle groups using a Kruskal-Wallis one-way analyses of variance for non-parametric data (GraphPad Prism ver. 9, GraphPad Software, LLC). Significance was set at $P < 0.05$.

3. Results

3.1. Muscle fiber type

The color of the muscle fibers was distinguishable without any staining under a stereo microscope when illuminating the muscle specimen from the bottom (Fig. 2). Distinct light-red and clear (white) fibers were observed that is assumed to correspond to type I and II fibers, respectively.

Only one antibody that is specific to fast type II MHC isoforms in birds and mammals was able to bind to fibers with the typical low mitochondrial content that characterize these glycolytic type fibers (Fig. 3). Some of these type II fibers, labelled II*, appeared to have stained darker in intensity, representing a greater number of mitochondria, which subjectively appeared closer to the staining intensity of the type I fibers. For a few fibers, the antibody did show intermediate binding, which can be denoted as hybrid fibers (type I/II - Fig. 3), which express both MHC isoforms.

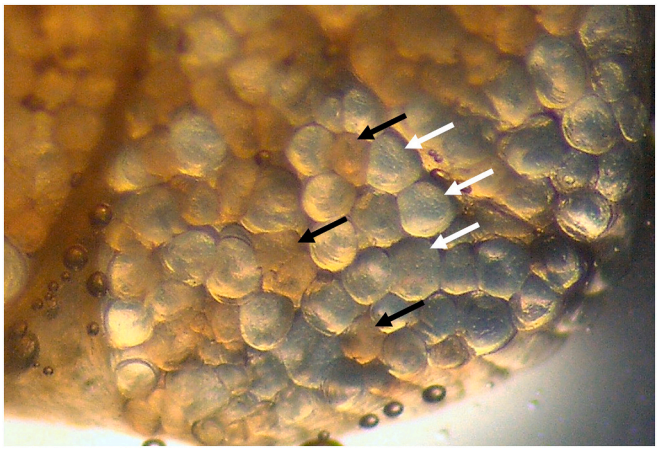

Fig. 2. Stereo image of a muscle sample from the *m. Fibularis longus* indicating large white fibers (white arrows) and smaller red fibers (black arrows). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Muscle fiber type of the various muscle groups

Overall, all four muscle groups presented with similar percentages of type I and II fibers and in relatively similar proportions that ranged, on average, between 42 and 51% and 49–58% for type I and type II fibers, respectively (Table 1). No differences in fiber proportions between the four muscle groups were found.

3.3. Fiber CSA of the muscle groups

The mean fiber CSA for all fibers between the four muscle groups ranged between 3433 and 6598 μm^2 (Table 1). The ITC had the smallest fibers ($P < 0.001$) compared to the G, ITL and FL. The small fibers of the ITC were also confirmed by the smaller type I and II fibers ($P < 0.01$), whereas the ITL type I fibers ($P = 0.086$) and G type II fibers ($P = 0.069$) showed a trend to be larger than their equivalent fiber type of the ITC (Table 1). There was also a trend of $P = 0.079$ for the overall fiber CSA of the FL to be larger than the G. No statistical differences were found between the remaining muscle groups and fiber type sizes.

3.4. Metabolic profiles

CS was the highest in the ITC ($P < 0.05$), whereas no difference was found between the ITL, G and FL. The marker of β-oxidation (3HAD) was overall very low, with the ITC and G having the highest activity, and the ITL and FL the lowest. PFK activity was similar between the ITL, G and the FL, but was approximately 3.5× higher than the ITC ($P < 0.05$). Finally, LDH activity was the highest in the ITC and G, and lower in ITL ($P < 0.05$).

3.5. Glycogen content

The glycogen content varied significantly within the muscles and between the ostriches, ranging from low (20–30 mmol/kg dw) to high (160–170 mmol/kg dw), which resulted in large SEM, leading to no statistically significant difference between the muscle groups. Nevertheless, the average glycogen content ranged between 66 and 108 mmol/kg dw.

4. Discussion

This is the first study to investigate a combination of muscle fiber type, fiber size, metabolic enzyme activities and glycogen content in four muscle groups of the ostrich to better understand meat quality traits

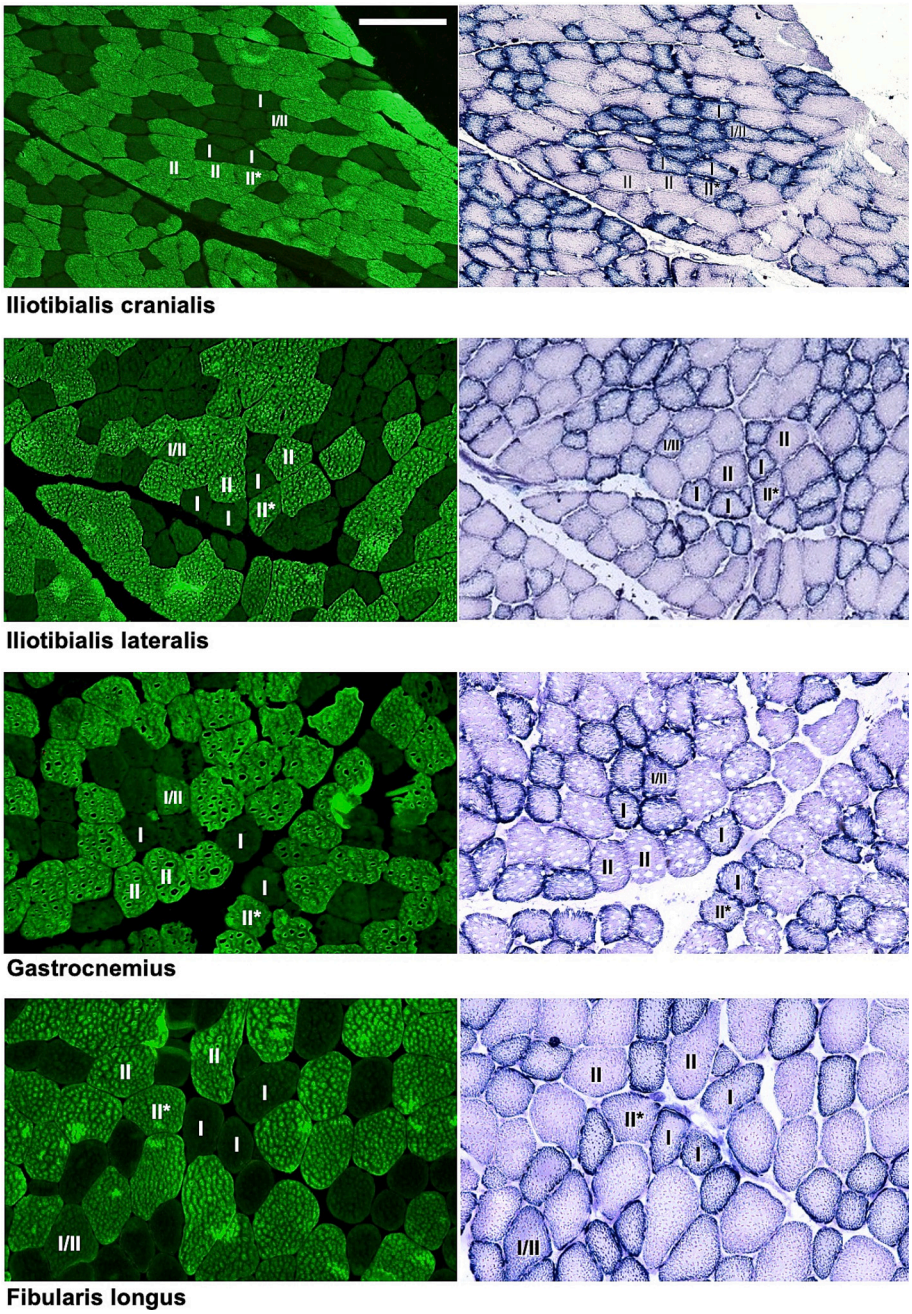

Fig. 3. Muscle fiber type and mitochondrial content (NADH stain) of the *m. Iliotibialis cranialis, m. Iliotibialis lateralis, m. Gastrocnemius* and *m. Fibularis longus*. Fibers were probed with anti-type II avian MHC isoforms (F18). Fibers were classified as type I, II or hybrid I/II fibers. Some type II fibers also had high mitochondrial content, identified as II* (refer to methods section). White scale bar = 200 μm.

in these animals. A previous study by Velotto and Crasto (2004) focused on fiber type and fiber CSA in similar muscle groups, namely the *m. gastrocnemius pars externa (current gastrocnemius)*, *m. tibialis cranialis caput tibiale*, *m. tibialis cranialis caput femorale* and *m. fibularis longus tendo caudalis*. These authors determined muscle fiber type using the conventional myosin ATPase enzyme stain at pH 4.6 or pH 10.0 that relies on the stability of the various myosin ATPases at predetermined acidic or alkaline pre-incubations, respectively. In addition, mitochondrial content was evaluated visually by co-staining for succinate dehydrogenase (SDH) activity using the same muscle sections and, based on the combined evaluations, fibers were classified as either SO, FOG or FG. The CSAs of these fibers were also determined.

To date, no study has evaluated fiber type using antibodies directed at the MHC isoforms and the metabolism (enzyme activities and glycogen content) in the muscles of the ostrich. As the ostrich is well-known for its lean meat cuts, these parameters can assist in predicting meat quality traits in this species. For all the muscle characteristics presented in this study, it needs to be acknowledged that sex and age do play a significant role in determining muscle composition but may be species specific. For example, the fiber type may be the same but the fiber CSAs are different between males and females; or no difference may be observed between the sexes (Chriki et al., 2012; Essén-Gustavsson & Rehbinder, 1985). Similarly, even though fast-growing chickens weighed more (2.0 kg vs. 1.65 kg) and were younger (37 days vs. 101 days), fiber type was not different, but the fiber CSAs significantly smaller compared to slow-growing chickens (Weng et al., 2022). In the present study, no distinction could be made between the sexes and were thus grouped before slaughter.

4.1. Muscle fiber type

There is no doubt that over the many years that it became apparent that skeletal muscles comprise of different fiber types, the way fibers are classified has evolved with the advent of technology and innovation (Frontera & Ochala, 2015). The initial fiber classification relied on color (i.e. red, intermediate and white), which reflected the myoglobin content of fibers. The discovery that certain fibers have stable myosin ATPase activities after pre-incubation at either acidic or alkaline pH, and in combination with metabolic stains (e.g. mitochondrial content using the NADH-nitro blue tetrazolium stain), produced a classification system of type I (SO), IIA (FOG) and IIB (FG) fibers in human, rodent and some domestic animals (e.g. horses and steers) (Karlström, Essén-Gustavsson, & Lindholm, 1994; Staron, 1997). Hybrids were also identified and added to the classification system as IC, IIC and IIAB (Staron, 1997).

It became clear that the metabolism of fibers is not the same between species and that metabolism can be up or down regulated in response to exercise or the lack thereof, respectively (Curry et al., 2012; Kohn, Curry, & Noakes, 2011; Schiaffino, Sandri, & Murgia, 2007). Additionally, micro-assays measuring single fiber oxidative enzyme activities (e.g. SDH, CS) have shown that there is great overlap in oxidative capacity within one fiber type and between fiber types from the same species (Pette, 1985). It was only in the past 30 years that the advent of antibodies against specific MHC isoforms became available and the successful separation of these isoform proteins by SDS-PAGE that fiber typing transitioned towards a classification system using the MHC protein as the primary determinant of fiber type (Bárány, Bárány, & Giometti, 1995; Frontera & Ochala, 2015; Kohn & Myburgh, 2006). In addition, it also led to the discovery that human fibers, previously classified as type IIB using the conventional ATPase stability method, in fact contained mRNA transcripts 94% homologous to that of the rat MHC IIX (Schiaffino & Reggiani, 1996). This was further confirmed with immunohistochemistry, single muscle fiber electrophoresis and Western blotting when the anti-MHC IIX antibody (6H1) strongly reacted with fibers from other species (including that from humans), but the anti-MHC IIB antibody (BF-F3) showed no reactivity (Dada et al., 2018; Kohn, 2014; Kohn, Burroughs, et al., 2011; Maccatrozzo, Patruno, Toniolo, Reggiani, & Mascarello, 2004). Thus, most mammals express the MHC IIX isoform in their large muscles and not the MHC IIB.

It is not yet known how many MHC isoforms the muscles of the ostrich express as no information could be found about the gene sequence of the MHC isoforms. Previously, Velotto and Crasto (2004) and Rosser and George (1985) used conventional ATPase staining (preincubation at pH 4.6 or pH 10.4) in conjunction with SDH staining (the latter being semi-quantitative) that allow for fibers to be classified as SO, FOG and FG. According to this classification system, Velotto and Crasto (2004) only found FG fibers in the *m. gastrocnemius pars externa*, and none in the *m. tibialis cranialis caput tibiale, m. tibialis cranialis caput femorale* and FL. The present study was able to identify only two muscle fiber types using an avian and mammalian fast MHC isoform antibody, whereas approximately 10 other antibodies failed to bind (data not shown). Studies performing fiber type should therefore also include immunohistochemistry using antibodies specific to the various isoforms as metabolic profiles of fibers are not related to the MHC isoform (Hohl, Blackhurst, Donaldson, van Boom, & Kohn, 2020; Kohn, 2014). It is for this reason, even though the NADH mitochondrial stain was performed, that the present study did not sub-divide fibers into oxidative type II and glycolytic type II.

Nevertheless, the fiber type profiles of the ostrich pelvic limb muscles from the present study conforms with that found by a previous study (Velotto & Crasto, 2004), which on average amounts to a range of between 40 and 60% for either fiber type. In contrast, Weng et al. (2022) found that broilers (slow or fast growers) had predominantly type II muscle fibers, ranging between 73% for the soleus, 92% for the G and 100% for the breast muscle. The same authors were unable to determine the fiber type composition in the same muscles of the duck, but mRNA analyses of the MHC isoforms showed similar findings as for the chickens (Weng et al., 2021).

4.2. Fiber CSA of the muscle groups

The fibers from the present study were larger than previously reported (Velotto & Crasto, 2004). It is important to note that Velotto and Crasto (2004) obtained muscles from the Blue neck ostrich, which reaches maturity earlier and weighs approximately 10 kg heavier than the common Black African ostrich. The most likely explanation for the difference in fiber sizes between the two studies is related to the sampling time point, where the average weight from the study by Velotto and Crasto (2004) compared to the present study was 85 ± 10 kg and 94 ± 2 kg, respectively. The same observation was made in bulls slaughtered at two different weights, where the larger weight presented with larger fiber areas (Vestergaard, Oksbjerg, & Henckel, 2000). As was noted by these authors, CSA may be influenced by intense exercise and feeding regimes, but the ostriches of the present study roamed freely within their camps.

4.3. Metabolic profiles

Measuring the enzyme activities that represents the major metabolic pathways for ATP synthesis in muscle provides a clearer understanding of the muscle's fuel sources. Furthermore, CS activity has in the past been an excellent marker of mitochondrial content, but recent data proved otherwise (McLaughlin et al., 2020). In the present study, the ITC muscle appears to have the lowest type I fiber content, but highest CS and 3HAD activities and the lowest PFK activity of the four muscles analyzed (Table 1). In contrast, the FL appeared to have the highest type I fiber content, but lower CS and 3HAD activities compared to ITC.

Overall, CS, PFK and LDH activities in the ostrich muscles appear ~50% lower than that obtained in wild antelope (Curry et al., 2012; Kohn, 2014; Kohn, Curry, & Noakes, 2011). However, the 3HAD activities in the various ostrich muscles appear very low when compared to other species, such as reindeer, cattle, horses, wild antelope and humans (Essén-Gustavsson & Rehbinder, 1985; Hohl et al., 2020; Karlström et al., 1994; Kohn, Essen-Gustavsson, & Myburgh, 2007; Vestergaard et al., 2000). 3HAD is a key enzyme in β-oxidation for the oxidation of fats. Overall, the activities for beef and horse range between 24 and 28 μmol/min/g prot and 32–39 μmol/min/g prot, respectively, whereas antelope species range from 28 to 84 μmol/min/g prot. Furthermore, Azad, Kikusato, Sudo, Amo, and Toyomizu (2010) reported 3HAD activities in the breast muscle of broiler chickens averaging ~38 μmol/min/g prot. In strong contrast, the values obtained for the ostrich were low and ranged between 1.9 and 2.7 μmol/min/g prot. This would suggest that ostrich muscle have a poor capacity to derive energy from fat sources. Indeed, the chemical analyses of ostrich muscles comparing it to other species found that ostrich muscle contained 4.7-fold and 9.7-fold less intramuscular fat than chicken and beef, respectively (Sales & Hayes, 1996). However, having low intramuscular fat content does not equate to poor β-oxidation represented by low 3HAD activity. As mentioned above, wild antelopes have high 3HAD activities, but low intramuscular fat content and could purely be genetic in nature (Hoffman & Wiklund, 2006). Speculatively, ostriches are flightless birds and not considered migrators, which would mean that they would not require abundant energy production via β-oxidation for muscle contraction as is the case for certain antelope species, horses and endurance runners (Essén-Gustavsson, Karlström, & Lindholm, 1984; Kohn, 2014; Kohn, Essén-Gustavsson, & Myburgh, 2011).

4.4. Glycogen content

No study has yet measured glycogen content in the muscles of ostriches. Glycogen content was not different between the 4 muscle groups, but, overall, was considered low in relation to other species

(Table 1). In particular, the muscles from beef contained glycogen in the amount of between 280 and 400 mmol/kg dw, and that of reindeer between 250 and 500 mmol/kg dw (Essén-Gustavsson & Rehbinder, 1985; McVeigh & Tarrant, 1982; Vestergaard et al., 2000). Higher glycogen content at the start of the maturing process of muscle to meat, where glycogen is converted to lactic acid via anaerobic glycolysis results in greater H^+ accumulation leading to lower post mortem muscle pH (Lefaucheur, 2010), but whether this leads to better or worse quality meat in the ostrich, has not yet been determined. Stress before slaughter can result in a significant decrease in muscle glycogen content, that would result in the pH post mortem not reaching an optimum acidity, resulting in dark colored meat (Lefaucheur, 2010). The ostriches in the present study were minimally stressed and were allowed an 18-h habituation period in enclosed camps at the abattoir before slaughter. Alternativelt, it may also be that ostriches naturally contain significantly less glycogen than their mammalian meat counterparts.

Historically, muscle fiber type and its respective metabolism were closely related, especially in rodent muscles (Delp & Duan, 1996; Kohn & Myburgh, 2007). However, since other species have been studied, especially wild animals, there appears little to no interaction between fiber type and its respective metabolism. Indeed, game species that include black wildebeest (*Connochaetes gnou*), springbok (*Antidorcas marsupialis*), blesbok (*Damaliscus pygargus phillipsi*), reindeer (*Rangifer tarandus*) and fallow deer (*Dama dama*), to name a few, have all shown that they harbor large numbers of so called FG (type IIX) fibers, but yet, these fibers have high mitochondrial numbers and high CS and 3HAD activities (Curry et al., 2012; Essén-Gustavsson & Rehbinder, 1985; Kohn, 2014; Kohn, Curry, & Noakes, 2011).

Both game and ostrich meat share similarities when it comes to their color. Both are dark red in color, indicative of muscle fibers that harbor significant quantities of myoglobin, which adds to the amount of iron present in these muscles. Indeed, previous analyses of ostrich, beef and chicken revealed that the former harbors significantly more iron (Sales & Hayes, 1996). Apart from myoglobin serving as a reservoir for oxygen storage, especially during vigorous muscle activity to facilitate mitochondrial respiration, it also contributes to the color of the meat.

4.5. Limitations

The primary limitation of this study is that meat quality parameters, such as cooking loss, drip loss, shear force and color were not measured in the same animals due to the study design and limited amount of tissue that was available during sampling. Measuring intramuscular fat content, ultimate pH in the different muscles as well as documenting the post mortem pH decline in each muscle would also contribute valuable information on the maturation of ostrich muscle to meat. Additionally, these animals were slaughtered when they reached a specific weight, and, although a small SEM in their age, some were not of the same age and should be considered in future studies. Finally, only one antibody that recognized supposedly all fast MHC isoforms in the ostrich, was able to distinguish between type I and type II fibers. However, it is still unknown how many MHC isoforms are expressed in the ostrich and is being investigated on single fiber MHC isoform protein, molecular and DNA level in our laboratory.

5. Conclusion

This is the first study to evaluate muscle structure (fiber type and CSA) and metabolism (enzyme activities and glycogen content) in four distinct muscle groups of the ostrich. These muscles contained relatively the same proportions of type I and II fibers, with the ITC showing significantly smaller fibers than the other muscle groups. Metabolically, it was found that ostrich muscles have low 3HAD activity, suggesting limited energy production from fat oxidation for muscle contraction. Finally, the glycogen content was low in all four muscle groups, which could affect meat quality.

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CRediT authorship contribution statement

Tertius A. Kohn: Supervision, Conceptualization, Validation, Formal analysis, Resources, Writing – original draft, Writing – review & editing. **Megan J. Anley:** Validation, Formal analysis, Writing – review & editing. **S'thandiwe N. Magwaza:** Validation, Formal analysis, Writing – review & editing. **Luqmaan Adamson:** Validation, Formal analysis, Writing – review & editing. **Louw C. Hoffman:** Resources, Investigation, Writing – review & editing. **Tertius S. Brand:** Resources, Investigation, Writing – review & editing.

Declaration of Competing Interest

The authors report no conflicts of interest.

Data availability

Data will be made available on request.

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