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MICROSTRUCTURAL CHARACTERISTICS OF THE *Longissimus dorsi* MUSCLE IN BRAHMAN CROSS CATTLE

Hamny Sofyan[1*], Muhammad Jalaluddin[1], Erdiansyah Rahmi[2], Sri Wahyuni[1], Taufan Hidayat[3], Mulyadi Adam[4], Fadli A Gani[1], Mustafa Sabri[1], and Mudhita Zikkrullah Ritonga[1]

[1]Anatomy Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, Indonesia
[2]Histology Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, Indonesia
[3]Agroclimatology Laboratory, Faculty of Agriculture, Syiah Kuala University, Banda Aceh, Indonesia
[4]Physiology Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, Indonesia
*Correspondence author: hamny@unsyiah.ac.id

ABSTRACT

This study aimed to analyze the muscle microstructure of longissimus dorsi Brahman cross (BX) cattle slaughtered at the slaughterhouse of North Aceh District. The meat samples for the study were taken from three male BX cattle aged 18-30 months with body weights ranging from 400 kg to 500 kg and the average body condition scores (BCS) of 3-4. The muscle used was the longissimus dorsi muscle. The mean (±SE) diameter and cross-sectional area (CSA) of muscle fibers and the percentage of fast fibers in the longissimus dorsi BX muscle were 71.69±1.23 µm, 4263.43±305.69 µm^2, and 48.17±8.81%, respectively. This study concluded that the longissimus dorsi muscle of the BX has relatively large muscle fibers, some of which in each fascicle are composed of fast muscle (fast fiber/type II).

Key words: BX cattle, CSA, loin muscle, muscle microstructure

ABSTRAK

Penelitian ini bertujuan menganalisis mikrostruktur otot longissimus dorsi sapi persilangan Brahman (BX) yang dipotong di Rumah Potong Hewan Kabupaten Aceh Utara. Sampel daging berasal dari tiga ekor sapi BX jantan umur 18-30 bulan dengan bobot badan antara 400–500 kg dengan rata-rata skor kondisi tubuh (BCS) 3-4. Otot yang digunakan adalah otot longissimus dorsi. Rerata (±SE) diameter dan luas serabut otot serta persentase serabut cepat pada otot longissimus dorsi sapi BX masing-masing adalah 71,69±1,23 µm; 4263,43±305,69 µm^2; dan 48,17±8,81%. Kesimpulan penelitian ini adalah otot longissimus dorsi sapi BX memiliki serabut otot yang relatif besar dan sebagian dari serabut otot pada setiap fasikulus tersusun atas otot cepat (fast fiber/tipe II).

Kata kunci: sapi BX, CSA, otot loin, mikrostruktur otot

INTRODUCTION

Brahman cross cattle (BX cattle) is a type of beef cattle most commonly slaughtered in slaughterhouses (RPH) in Indonesia, including in Aceh Province. Most of the BX cattle in Aceh Province are imported from North Sumatra and Lampung to meet the needs of the Acehnese for beef. The survey conducted by Sofyan (2020) found that beef cattle farmers and traders in Banda Aceh City and Aceh Besar District still carefully maintain and sell BX cattle because of their high productivity and carcass percentage, thus giving higher profit. Delfa *et al*. (2007) stated that 97% of carcass weight is determined by skeletal muscle mass.

Skeletal muscle is the main structure that forms meat or carcass composed of heterogeneous muscle fibers. This is due to differences in the ultrastructure, biochemistry, and physiology of the myofibrils that constitute skeletal muscle (Aberle *et al*. 2001; Zhang and Gould 2017). Muscles are composed of muscle fibers (myofibrils) with different characteristics. Schiaffino and Reggiani (1994) and Schiaffino and Reggiani (1996) stated that each type of muscle fiber has different biochemical and biophysical characteristics, such as glycolytic and oxidative capacity, speed of contraction, muscle fiber size, myoglobin, and glycogen content.

Muscle characteristics are determined by various factors, including species, breed, sex, age, birth weight, livestock activity, hormone use, slaughter weight, muscle type and location, and sampling location in muscle (Joo *et al*. 2013). The diameter, area, and type of muscle fibers are some of the muscle microstructural variables that act as the main determinants of muscle mass and are closely associated with meat quality because they affect the level of meat tenderness (Sofyan *et al*. 2021). Muscle fibers with a large diameter and area tend to produce meat with a low level of tenderness (Joo *et al*. 2017). Muscle fibers are also divided into several types. Muroya *et al*. (2009) divided the types of bovine muscle fibers into three types; type I, type IIA, and type IIB. Type I is a slow fiber muscle, whereas type II is a fast fiber muscle (Frandson *et al*. 2009). The *longissimus dorsi* muscle is one of the muscles that causes loin meat to be in great demand because of its high tenderness (Senaratne *et al*. 2010; Safitri *et al*. 2018; Sofyan *et al*. 2021). As a result, it has a higher economic value (Sofyan 2020). The tenderness of meat produced from the *longissimus dorsi* muscle is influenced by the anatomical location and function of this muscle. The *longissimus dorsi* muscle is located in the dorsal part of the body and functions as a retaining muscle; thus, it is not much involved in the movement process. Since it does not play an active role in the movement process, this muscle has a different muscle structure and composition (muscle fibers, connective tissue, and fat tissue) from the muscles that do (Listrat *et al*. 2016; Listrat *et al*. 2020a; Listrat *et al*. 2020b).

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Knowledge and understanding of the structure and composition of muscle fibers are important in studies focusing on muscle characteristics because they are ultimately related to meat quality. Studies examining muscle microstructure as a factor that affects beef quality in Indonesia have been reported (Safitri *et al*. 2018; Sofyan *et al*. 2021); however, not all of the reported variables were strongly related to the type of muscle fiber in BX cattle. Therefore, this study aimed to analyze the microstructure, especially the diameter, area, and type of *longissimus dorsi* muscle fibers in BX cattle. The results of the research were expected to be the basis for a beef cattle livestock development program to produce quality beef for consumers.

MATERIALS AND METHODS

Animals and Research Samples

This study used samples of *longissimus dorsi* muscle from three adult Brahman cross (BX) bull with body weights ranging from 400 to 500 kg and the average body condition scores (BCS) of 3-4. The BX cattle used in the study showed clinically healthy symptoms. Muscle samples were obtained shortly after the cattle were slaughtered at the slaughterhouse (RPH) of North Aceh District, Aceh Province.

Muscle Histology Preparation

The *longissimus dorsi* muscle used was the sinister one (left) with a size of 1 cm x 1 cm x 1 cm and was immediately put into a 10% neutral-buffered formalin (NBF) fixation solution until the tissue was processed to the next stage. The dehydration step was carried out using a graded alcohol solution (70%, 80%, 90% and absolute alcohol), followed by a clearing process using xylol 3 times and liquid paraffin infiltration and implantation in paraffin to form tissue blocks. The tissue blocks were cut using a microtome with a thickness of 5 µm. Then, the tissue was placed on a glass object.

Hematoxylin-Eosin Stain

Hematoxylin-eosin (HE) staining was used to measure the diameter and cross-sectional area (CSA) of muscle fibers. Each individual cow was made into 3 slides of histology preparations (a total of 9 slides of tissue). The HE staining procedure was started with the deparaffinization process using xylol 3 times, followed by the rehydration process using a graded alcohol solution (absolute alcohol, 90%, 80%, and 70%), cleaning with running water, and distillation with water. The next step was immersion of tissue slides into hematoxylin solution and eosin, followed by dehydration, clearing and mounting. The measurement of the diameter and area of muscle fibers was carried out using IMAGE J software. The diameter was measured by measuring the long and short diameters of each muscle fiber (Figure 1A) and averaged. The measurement of the muscle fiber area was done by first making a circle mark on the endomysium layer of one muscle fiber (Figure 1B), then the IMAGE J software measured the muscle fiber area based on the boundaries that had been made. Measurements of the diameter and area of each muscle fiber were carried out on 50 muscle fibers (by not taking into account the number of observed visual fields). Measurements of the diameter and CSA of muscle fibers were carried out at 40 times magnification.

Immunohistochemical Stain

Immunohistochemical staining (IHK) was performed to calculate the percentages of fast muscle fiber types (type II muscle fibers). Each individual cow was made into 3 slides of histology preparations (a total of 9 slides of tissue). The immunohistochemical staining used the IHK kit (cat no ab64264, Abcam, Cambridge, UK). The primary antibody used was an anti-fast skeletal myosin heavy chain antibody (cat no ab75370, Abcam, Cambridge, UK). The primary antibody on the negative control slide was replaced with phosphate-buffered saline (PBS). The calculation of the percentage of fiber types was based on Choe *et al*. (2008) that had been modified. The percentage of fast muscle fibers was obtained from the ratio of the types of fast muscle fibers to the total number of muscle fibers in one fascicle. The counting the number of fast muscle fibers (fast fiber) was carried out on 20 fasciculi with 4 times magnification.

Data Analysis

The measurement results of the diameter and area of muscle fibers, and the percentages of fast muscle fibers (fast fiber) were tabulated and expressed in terms of mean and standard error (SE), which were then analyzed descriptively.

RESULTS AND DISCUSSION

The average (±SE) muscle diameter of *longissimus dorsi* BX cattle was 71.69±6.95 µm with muscle fiber area reaching 4263.43±798.67 µm^2. The muscle fibers of the *longissimus dorsi* of this bovine mostly consist of fast muscle fibers with an average percentage of 48.17±7.85% (Table 1). The morphology of the *longissimus dorsi* bovine Brahman cross muscle fibers with HE staining is presented in Figure 1.

Table 1. Average (±SE) diameter, area, and type of fast muscle fibers in *longissimus dorsi* Brahman cross (BX) cattle

Individual	Muscle fiber diameter (µm) (n=136)	Muscle fiber CSA (µm^2) (n=136)	Percentage (%) of fast muscle fibers (fast fiber/type II)*
Bull 1	67.11±6.00	3688.22±542.99	53.14±2.69
Bull 2	75.28±7.42	4695.40±878.56	32.57±11.91
Bull 3	72.69±6.66	4406.43±805.60	58.81±3.58
Mean± SE	71.69±6.95	4263.43±798.67	48.17±7.85

SE= Standard errors; n= The number of muscle fibers measured/observed; *= Observed in 20 fasciculi

Compared to the size of the muscle fibers of Aceh cattle reported by Sofyan *et al*. (2021), the *longissimus dorsi* BX muscle in this study had a larger diameter and muscle fiber CSA. The *longissimus dorsi* muscle of Aceh cattle had an average diameter and area of 45.58 µm and 3409.66 µm^2, respectively (Sofyan *et al*. 2021). Many studies examined the relationship between muscle fiber morphometry and meat quality, especially with regard to the tenderness of meat produced from a specific type of muscle (Priyanto *et al*. 2015; Ebarb *et al*. 2016; Mendrofa *et al*. 2016; Baldi *et al*. al. 2017; Razmaite *et al*. 2017; Sofyan *et al*. 2021). Muscles of large size tend to produce meat with a low level of tenderness. However, several studies reported that large muscle fiber size does not always produce meat with low tenderness (Safitri *et al*. 2018; Sofyan *et al*. 2021).

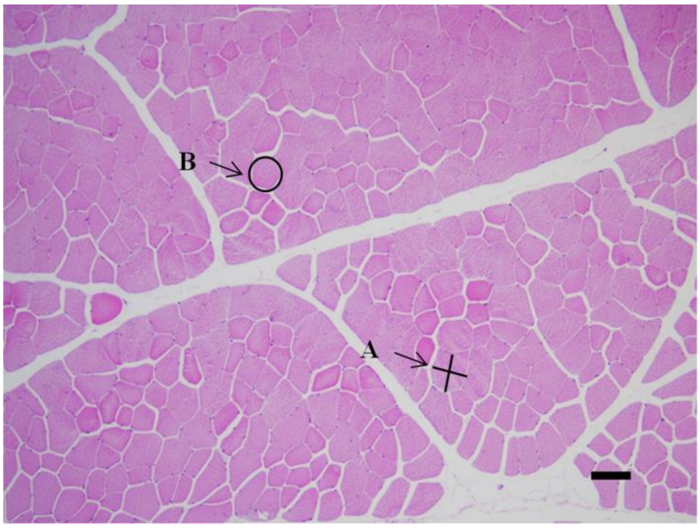

Figure 1. Histological structure of *longissimus dorsi* muscle fibers in BX cattle. Measurement of diameter (A) and area (B) of muscle fibers. HE staining. Bars: 100 µm

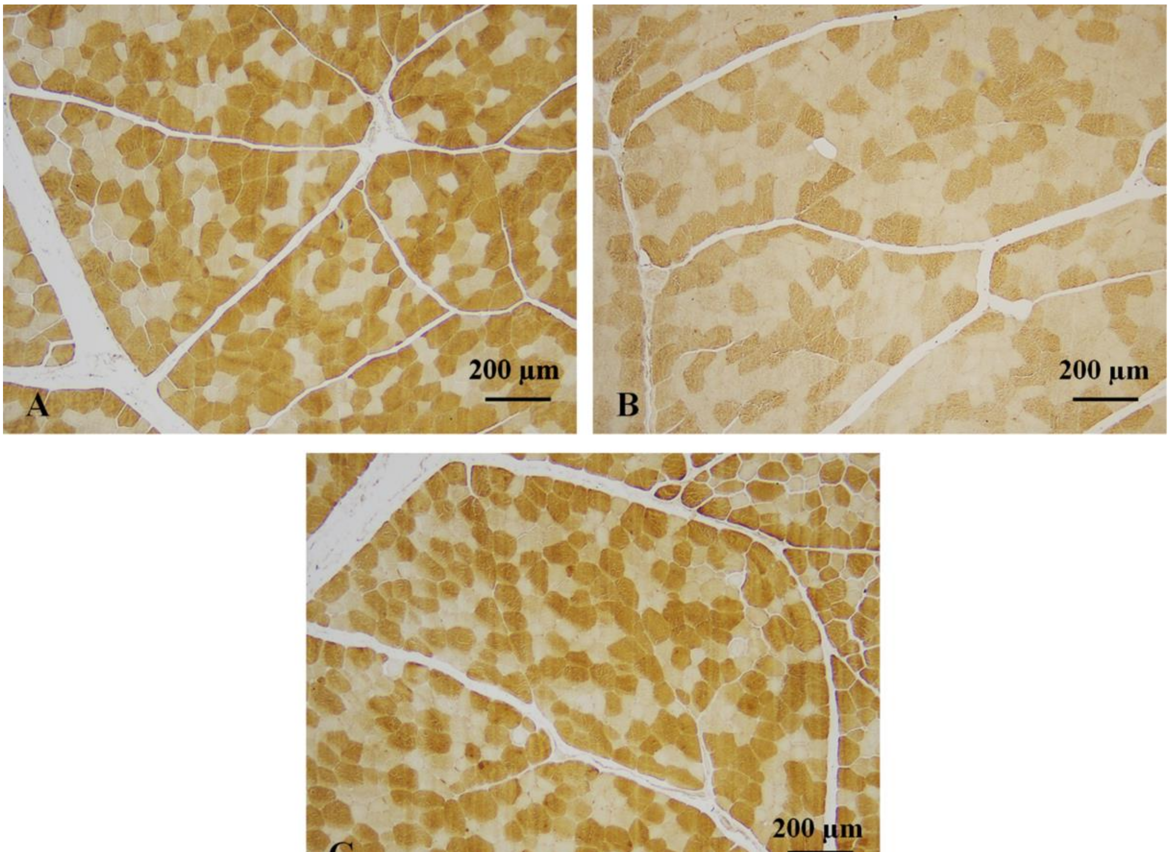

Figure 2. *Longissimus dorsi* muscle fiber type in Brahman cross (BX) cattle. The dark brown color indicates type II muscle fibers (fast muscle fibers). Figures A, B, and C represent each BX cow used in the study. The *longissimus dorsi* muscle from cattle 1 (A) and 3 (C) is dominated by type II muscle fibers, while the *longissimus dorsi* muscle from cattle 2 (B) has low type II muscle fibers. Immunohistochemical stain

This is due to the composition factor of the type of muscle fibers between fast muscle fibers and slow muscle fibers.

The composition of muscle fiber types varies from one muscle type to another (Schiaffino and Reggiani 2011). The composition of fiber type is influenced by muscle type (Listrat *et al*. 2020a; Sofyan *et al*. 2021), sex, age, breed of livestock, and type of livestock (Listrat *et al*. 2020a). In this study, BX cattle had an average percentage of fast fiber (type II) with 48.17±7.85% in one fasciculus (Table 1 and Figure 2). However, the slow fiber type (slow fiber/type I) was not observed in this study. The *longissimus dorsi* muscle from two BX cattle was dominated by fast muscle fiber type (type II) and while the other BX cow showed a low number of type II muscle fiber type. The differences in the composition of muscle fiber types are assumed to be influenced by the BX cattle used in this study, which were not raised on the same farm, so it was assumed that the management of cattle development was not the same. Listrat *et al*. (2016) stated that the composition of muscle fibers is determined by nation, gender, age, physical activity, environmental temperature, and feeding practice.

Type II muscle fibers are known as fast muscle fibers, whereas type I fibers are known as slow muscle fibers (Aberle *et al*. 2001). Type II fibers contract rapidly (fast-twitch) and last for a short period. Type I muscle fibers contract slowly (slow twitch) and last for a long period. Type II muscle fibers tire more quickly than type I muscle fibers (Frandson *et al*. 2009). In general, meat dominated by fast-type muscle fibers tend to have a low level of tenderness (Listrat *et al*. 2020b). However, Listrat *et al*. (2020b) discovered different trends related to the relationship between meat tenderness and the composition of muscle types in several muscle types. Fast muscle fibers also contain higher levels of glycogen and lactate than slow muscle fibers, resulting in a paler color of meat produced from fast muscle fibers, high drip loss, and a faster postmortem glycolysis process (Choe *et al*. 2008).

The type of muscle also affects the size of the muscle fibers. Safitri *et al*. (2008) reported that the semitendinosus muscle of Brahman cross cattle has an area of 2564.7±345.7 μm^2. The result of his study revealed that the *longissimus dorsi* muscle of Brahman cross cattle had an area of 4263.43±798.67 μm^2. Each type of muscle is assumed to have a different size. This is related to the function of these muscles. Active muscles tend to be smaller and denser, and vice versa.

Klont *et al*. (1998) reported that the variation in meat quality between livestock and muscle types was closely related to the distribution of muscle fiber types. Muscles with different types of muscle fibers will result in differences in the changes that occur postmortem during the conversion process of muscle into meat, and will subsequently affect the quality of the meat (Ryu and Kim, 2005). Variations in metabolic content, particularly glycogen, lactate, and muscle fiber type composition, may explain variations in meat quality characteristics (Ryu and Kim, 2006).

CONCLUSION

The *longissimus dorsi* muscle of the Brahman cross has a relatively large fiber size, and some of the muscle fibers in each fasciculus are composed of fast muscle (type II). There are other factors that affect the composition of muscle fiber types in the *longissimus dorsi* muscle of the Brahman cross cattle that must be studied further.

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