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The Expression of Type III Collagen Connective Tissue in Gluteobiceps and Psoas Mayor Muscles of Aceh Cattle

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ABSTRACT

Collagen is one of the determinant factors of beef tenderness. This study analyzed the expression and content of type III collagen connective tissue in two muscle types of Aceh cattle through immunohistochemical staining. Meat samples were obtained from the gluteobiceps and psoas major muscles of three Aceh cattle bulls weighing 250-300 kg, with a body condition score of three. Histology preparations were created from samples and subjected to immunohistochemical staining utilizing specific antibodies targeting type III collagen. Qualitative data were analyzed descriptively, while quantitative data were analyzed using IMAGE J software. The results showed that the expression of type III collagen connective tissue was found in the endomysium and perimysium layers of both muscles. The expression of type III collagen connective tissue varied significantly among the muscles ($P<0.05$). The gluteobiceps muscle had an average percentage of the area containing type III collagen connective tissue of 4.47±2.41%, while the psoas major muscle was lower at 2.51±1.69%. This study concludes that the gluteobiceps muscle of Aceh cattle has a higher amount of type III collagen connective tissue than the psoas major muscle.

Keyword: Collagen expression, IHC, Intramuscular connective tissue, Loin beef, Meat quality

ABSTRAK

Kolagen merupakan salah satu faktor penentu keempukan daging sapi. Tujuan dari penelitian ini adalah untuk menganalisis ekspresi dan kandungan jaringan ikat kolagen tipe III pada dua jenis otot yang berbeda pada sapi aceh menggunakan pewarnaan imunohistokimia. Sampel daging berasal dari otot gluteobiceps dan psoas mayor dari 3 ekor sapi aceh jantan dengan kisaran berat badan 250-300 kg dan BCS 3. Sampel dibuat preparat histologi dan diwarnai dengan metode pewarnaan imunohistokimia menggunakan antibodi spesifik untuk kolagen tipe III. Data kualitatif dan kuantitatif dianalisis secara deskriptif dan IMAGE J. Hasil penelitian menunjukkan bahwa ekspresi jaringan ikat kolagen tipe III ditemukan pada lapisan endomisium dan perimisium kedua otot. Persentase area ekspresi jaringan ikat kolagen tipe III berbeda secara signifikan antara kedua otot ($P<0,05$). Otot gluteobiceps memiliki rata-rata persentase area yang mengandung jaringan ikat kolagen tipe III sebesar 4,47±2,41%, sedangkan otot psoas mayor lebih rendah yaitu 2,51±1,69%. Kesimpulan dari penelitian ini adalah kandungan jaringan ikat kolagen tipe III pada otot gluteobiceps sapi aceh lebih banyak dibandingkan dengan otot psoas mayor.

Kata kunci: Daging sapi loin, Ekspresi kolagen, IHK, Jaringan ikat intramuskular, Kualitas daging

INTRODUCTION

Background

Aceh cattle are a cross between *Bos sondaicus* and *Bos indicus*, recognized as one of Indonesia's local cattle breeds with a native geographical distribution in Aceh Province, and have been breed for generations (BSN, 2020). The potential and advantages of Aceh cattle and other local Indonesian cattle breeds have not been adequately studied and utilized to produce high-quality meat. Aceh cattle meat is considered inferior to other breeds, such as Bali cattle and Brahman cross (BX), particularly concerning meat tenderness (Sofyan et al., 2020, 2021; Safitri et al., 2018). Nonetheless, the quality of Aceh cattle meat requires further investigation to fully realize its potential in accordance with its genetic characteristics, thus establishing it as a viable source of animal protein for the community. Previous studies indicate that Aceh cattle meat has a lower fat content compared to general beef, making it a suitable option for consumers seeking lower-fat beef alternatives (Sofyan et al., 2021).

Tenderness serves as a key criterion influencing consumer preferences in meat purchases. Meat tenderness can be linked to the location and function of muscles and their structural components, namely muscle proteins, muscle fiber size and type, and connective tissue (Sofyan et al., 2020, 2021). Collagen is one of the most abundant proteins in the extracellular matrix, including the components of intramuscular connective tissue (Anggorowati et al., 2017; McKee et al., 2019). Collagen maintains tissue integrity (Anggorowati et al., 2017). Collagen consists of 28 types identified in skeletal muscle (Csapo et al., 2020). However, types I and III are the primary collagens found in muscle tissue with varying ratios, particularly in the three layers of muscle sheaths: the epimysium, perimysium, and endomysium (McKee et al., 2019). The concentration of type III collagen in connective tissue differs based on the muscle type. It is estimated to constitute approximately 14–30% of the epimysium layer, 25–43% of the perimysium layer, and 53–58% of the endomysium layer (Purslow, 2002).

Collagen is recognized for its influence on the texture of meat produced (Listrat et al., 2000, 2019; Vierck et al., 2018). Collagen contributes to meat quality (He et al., 2023). The expression and content of collagen differ across various muscle types based on their anatomical locations, as each type possesses unique functions and adaptive capabilities (Listrat et al., 2000; Sofyan et al., 2021). Animal age is also known to influence the content of type I and III collagen in the rectus abdominis muscle (Calvi et al., 2011). An analysis of collagen content in the longissimus lumborum and semitendinosus muscles of Aceh cattle was conducted (Sofyan et al., 2021). The results indicated a difference in collagen content between the two muscle types. The longissimus lumborum muscle contains less collagen than the semitendinosus muscle. In this study, the distribution and content of type III collagen connective tissue were analyzed using immunohistochemistry on the gluteobiceps and psoas major muscles of Aceh cattle. The results of this study are expected to serve as a foundation for improving the quality of local beef, particularly Aceh beef, positioning it as a preferred option for consumers.

MATERIALS AND METHOD

Research Ethics

This study obtained ethical approval from the Animal Experimentation Ethics Committee of the Faculty of Veterinary Medicine, with Number 281/ KEPH/XII2023.

Research Design and Sample

This observational study used samples of Aceh beef from the gluteobiceps and psoas major muscles of two adult male Aceh cattle with a body weight range of 250-300 kg and a body condition score (BCS) of 3. The meat samples were obtained from the Keudah slaughterhouse in Banda Aceh. Sampling was conducted using the purposive sampling method. Meat samples from both muscle types were prepared histologically and stained using immunohistochemical (IHC) techniques with the avidin-biotin complex peroxidase (ABC method).

Research Procedure

Sampling

Meat samples measuring 2 x 2 x 2 cm were collected immediately after the slaughter of Aceh cattle at the slaughterhouse. The muscle samples were washed in physiological NaCl solution and fixed using the immersion fixation method in 10% neutral buffered formalin (NBF) for 24 hours. Following fixation, the samples were transferred and immersed in 70% alcohol to halt the process.

Preparation of Histology Specimens

The fixed samples were cut into 0.5 x 0.5 x 0.5 cm pieces and placed in a tissue cassette. The next step involved immersing the samples in alcohol with increasing concentrations (dehydration process), specifically 70%, 80%, 96%, and 95% alcohol, each for 2 hours, followed by absolute alcohol for 2 hours.

After dehydration, the samples were placed twice in a xylene solution (clearing process), each for 2 hours. The next step was tissue infiltration using paraffin infiltration (repeated twice), each for 2 hours. The tissue was then embedded in liquid paraffin and cast into tissue blocks. The tissue blocks were cut using a microtome at a thickness of 5 µm and mounted on glass slides coated with poly-L-lysine.

Immunohistochemical Staining (IHK)

The ABC method of IHC staining followed the manual kit instructions for the *mouse and rabbit-specific* HRP/DAB (ABC) *detection* IHC kit (ab64264, Abcam®, UK) with modifications. The first step involved deparaffinizing the tissue with xylene solution thrice for 5 minutes each. This was followed by rehydrating the tissue in alcohol at decreasing concentrations for 3 minutes each, rinsing with running water for 10 minutes, and distilled water for 5 minutes. The tissue was subsequently treated with a 3% H_2O_2 solution for 10 minutes in a moisture chamber and then washed twice with PBS for 5 minutes each. The tissue was then treated with a protein block solution and incubated for 10 minutes at room temperature, followed by washing twice with PBS for 5 minutes each. Subsequently, BSA was applied to the tissue, followed by the primary antibody (ab7778, Abcam®, UK) diluted 1:200 with Triton-X and incubated overnight at 4°C for 24 hours.

Following incubation with the primary antibody, the tissue was washed with PBS twice for 5 minutes each. The tissue was subsequently treated with biotinylated goat antipolyvalent (secondary antibody), incubated for 10 minutes at room temperature, and washed twice with PBS for 5 minutes each. Subsequently, streptavidin peroxidase was introduced, and the mixture was incubated for 10 minutes at room temperature, followed by two washes with PBS, each lasting 5 minutes. The next step was adding DAB chromogen to the tissue and incubating for 10 minutes, monitoring color changes under a light microscope.

Immunoreactivity was indicated by the formation of a brown color on the tissue, demonstrating a bond between the antigen and antibody. The tissue *slides* were then rinsed with PBS and washed with distilled water. The tissue *counterstain* process used Mayer's hematoxylin by dipping for 3 seconds, then rinsing with running water for several seconds and distilled water for 10 minutes. Dehydration was conducted with a series of alcohol solutions of increasing concentrations. Clarification was achieved using a xylene solution, and the tissue slides were mounted with a cover slip using slide adhesive (Entellan®).

Observation of Staining Results

The IHK staining results were assessed qualitatively. Qualitative observations were conducted to examine type III collagen connective tissue distribution in both muscle types using a light microscope with a photography device at 10x magnification. Images were captured with ToupView software, focusing on five fields of view for each tissue slide.

Data Anaylsis

Qualitative data on type III collagen expression in connective tissue were analyzed descriptively using ToupView software and presented as histological images. The average percentage of area expressing type III collagen was analyzed using IMAGE J software and the nonparametric Kruskal-Wallis test.

RESULTS

Immunohistochemical staining results indicated the presence of type III collagen connective tissue in the endomysium and perimysium structures of both the gluteobiceps muscle (Figure 1) and the psoas major muscle (Figure 2). This was derived from the expression of type III collagen in the connective tissue observed in the sample. Type III collagen connective tissue was more abundantly expressed in the perimysium of both types of muscle (asterisks in Figures 1 and 2) compared to the endomysium (arrows in Figures 1 and 2).

Figure 1 illustrates that the type III collagen connective tissue in the endomysium and perimysium exhibits varying thickness levels. In the perimysium, both types of muscle were composed of thicker type III collagen connective tissue compared to the endomysium. However, in this study, no thickness measurements of type III collagen connective tissue were performed. The thickness of type III collagen connective tissue in the endomysium is generally consistent between the gluteobiceps and psoas major muscles.

Macroscopic observations were supported by data from the analysis of the percentage of muscle tissue area expressing type III collagen connective tissue in the gluteobiceps and psoas major muscles of Aceh cattle. The content of type III collagen in connective tissue was quantified using Image J software. The average percentage of muscle tissue area expressing type III collagen connective tissue is presented in Table 1. Statistical analysis indicated that the average percentage of type III collagen connective tissue in the gluteobiceps muscle was significantly greater than in the psoas major muscle ($P<0.05$). This finding suggests

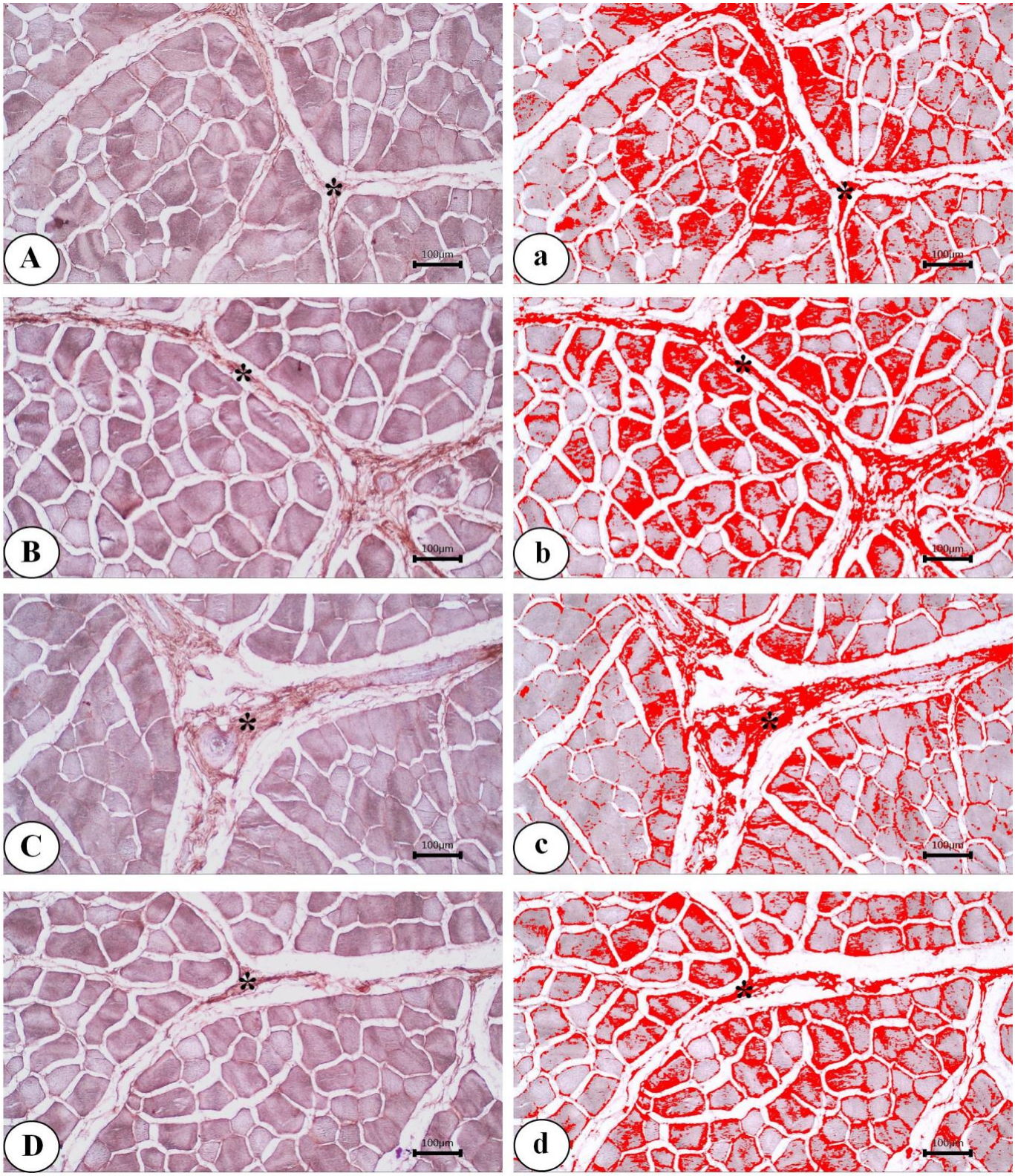

Figure 1. Expression of type III collagen connective tissue in the gluteobiceps muscle of Aceh cattle. Immunohistochemical staining (A-D) and Image J analysis results (a-d). Type III collagen connective tissue expression was found in the endomysium (arrow) and perimysium (*), marked by brown color in the tissue. Type III collagen connective tissue is more extensively expressed in the perimysium compared to the endomysium. Scale bar: A-D and a-d: 100 µm.

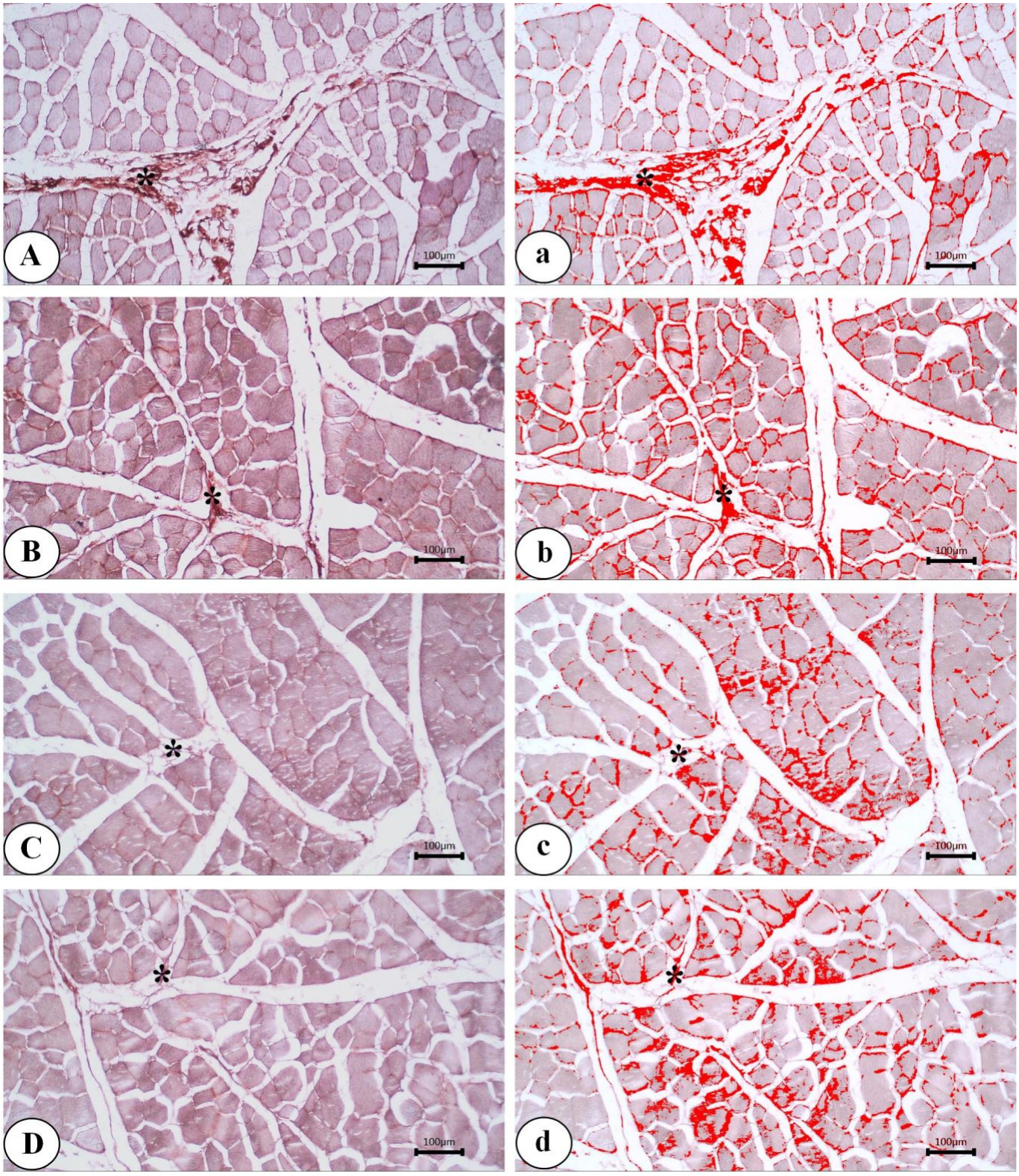

Figure 2. Expression of type III collagen connective tissue in the psoas major muscle of Aceh cattle. Immunohistochemical staining (A-D) and Image J analysis results (a-d). Type III collagen connective tissue expression was found in the endomysium (arrow) and perimysium (*), marked by brown color in the tissue. Type III collagen connective tissue is more extensively expressed in the perimysium compared to the endomysium. Scale bar: A-D and a-d: 100 µm.

Table 1. Average percentage (±SD) of collagen type III connective tissue expression area in the gluteobiceps and psoas major muscles of Aceh cattle.

Types of Muscles	Percentage of Type III Collagen Connective Tissue Expression Area (%)
Gluteobiceps	4.47±2.41[a]
Psoas mayor	2.51±1.69[b]

Note: Different superscripts in the same column indicate significant differences ($p<0.05$). SD: standard deviation

that the content of type III collagen connective tissue in the gluteobiceps muscle is higher than in the psoas major muscle of Aceh cattle. Aceh cattle's gluteobiceps muscle had an average area containing type III collagen connective tissue of 4.47±2.41%, while the psoas major muscle had a lower percentage of 2.51±1.69%.

DISCUSSION

Collagen is the primary protein component of the extracellular matrix in muscle tissue (Zhang et al., 2021; McKee et al., 2019). Collagen is found in the muscle sheath layers, namely the endomysium, perimysium, and epimysium. The endomysium, perimysium, and epimysium are layers of the extracellular matrix in skeletal muscle tissue. They maintain skeletal muscle morphology and are crucial for the physiological functions of muscle fibers, including mechanical force transmission, muscle fiber regeneration, and the formation of neuromuscular junctions (Zhang et al., 2021). Many studies indicate a correlation between collagen connective tissue and meat quality (Florek et al., 2022; Listrat et al., 2020; Safitri et al., 2018; Vierck et al., 2018). Research on collagen connective tissue content and its impact on meat tenderness in local Indonesian beef cattle remains scarce. Sofyan et al., (2021) Reported that collagen connective tissue in the longissimus dorsi muscle was less abundant and had higher tenderness than Aceh cattle's semitendinosus muscle, which had more collagen connective tissue and lower tenderness.

The immunohistochemical staining results indicate that type III collagen expression in the gluteobiceps and psoas major muscles of Aceh cattle shows similarities in its localization within the endomysium and perimysium. This is consistent with the findings reported by He et al., (2023). In this study, no analysis was conducted on the epimysium, the outermost layer of a muscle group. This study aimed to obtain information on the impact of collagen-type III connective tissue on meat tenderness, a key factor influencing consumer meat purchases. The epimysium is the outermost layer of a muscle group, which is often separable from the meat purchased by consumers. Consequently, it does not significantly impact the tenderness of meat processed into various dishes. Conversely, the endomysium and perimysium are essential components of muscle structure and affect the tenderness of meat derived from a particular muscle type.

The increased collagen type III content in the gluteobiceps muscle, relative to the psoas major muscle, is believed to be affected by anatomical location and functional variations. The gluteobiceps muscle is one of the large muscles in the lateral thigh region that plays a significant role in the movement of livestock. The psoas major muscle is a smaller muscle situated in the medial region of the pelvis, responsible for the rotation of the hind legs. Movement activities require greater strength and elasticity, necessitating the role of type III collagen connective tissue to ensure the muscles function properly. The increased collagen type III content is believed to contribute to reduced tenderness in meat from these muscles.

He et al., (2023) established a correlation between type III collagen in connective tissue and meat quality. The longissimus dorsi muscle appeared to contain less type III collagen than the semitendinosus muscle. The expression of Type III collagen is closely associated with genetic factors and is influenced by age (He et al., 2023). The quantity and type of collagen differ among various muscle types within the same livestock individuals, leading to variations in tenderness levels among meat from different muscle types (Velázquez dan Latorre, 2019). This study did not include examinations of meat tenderness by panelists or instruments; therefore, the results cannot be linked to the tenderness of Aceh beef from the gluteobiceps and psoas major muscles.

The gluteobiceps is a component of round cuts, while the psoas major belongs to sirloin cuts in commercial cuts. A study conducted by Sofyan et al., (2020) found that consumers frequently purchase both types of meat, and the selection process for both types of commercial cuts is based on the type of dish to be cooked. This study is expected to contribute to the advancement of meat quality in local Indonesian beef cattle in the future.

The research results indicate that type III collagen connective tissue is present in the endomysium and perimysium of the gluteobiceps and psoas major muscles of Aceh cattle, in varying amounts. The gluteobiceps muscle exhibited a higher abundance of type III collagen connective tissue than the psoas major muscle, as indicated by the average percentage

of muscle area expressing this type of collagen. In future studies, it is recommended to analyze the meat tenderness of both muscle types to determine the association between type III collagen content and reduced meat tenderness.

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