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Research Article

Comparative Study on Muscle Fiber Types of Longissimus Dorsi of Xinjiang Brown Cattle and Angus Cattle of Different Months

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The longissimus dorsi muscle of Xinjiang brown cattle and Angus cattle at the age of 3, 7, 12, and 24 months under the same feeding and management conditions were selected to explore the differences of muscle 4 fiber types in this study. The muscle histological and molecular biological reasons for the quality difference between Xinjiang brown cattle and Angus beef were discussed. The morphology of the muscle was compared by ATP enzyme staining and SDH enzyme staining, and its gene expression was detected by qRT-PCR. The mRNA expression levels of Myhc-I in 3-month-old Xinjiang brown cattle were significantly higher than those in Angus cattle of the same age ($P < 0.01$). The 4 fiber types of 7-month-old Xinjiang brown cattle were significantly lower than those of Angus cattle of the same age ($P < 0.01$). The expression level of type I and IIb in 12-month-old Xinjiang brown cattle was significantly higher than that in 12-month-old Angus cattle ($P < 0.05$). Type I and IIa of 24-month-old Xinjiang brown cattle were significantly lower than those of Angus cattle of the same age ($P < 0.05$). However, in our study, the basic characteristics of longissimus dorsi of Xinjiang brown cattle and Angus cattle, such as color, pH, shearing force, and other characteristics were not detected, which is lacking in this aspect. Overall, with the increase of age, the growth trend of muscle fiber morphology of Xinjiang brown cattle and Angus cattle is roughly the same, but from the point of view of muscle fiber types, the Xinjiang brown cattle are more suitable for the production of early fat calves and to make some reference for improving the quality of beef cattle in China.

1. Introduction

Tenderness is not only the decisive factor and sensory characteristic of meat quality, but also an important index to evaluate muscle juiciness [1]. There are many factors affecting tenderness, and the characteristics of muscle fiber is one of them. Regardless of carcass weight or age, the total number of muscle fibers, muscle fiber diameter, cross-sectional area, and fiber type composition are of great significance to the growth performance and muscle quality of varieties [2]. By measuring the skeletal muscle tissue of adult animals, it was found that there were four muscle types of myosin heavy chain (Myhc), namely, type I, type IIa, type IIx, and type IIb [3], which were slow oxidation type (type I), rapid oxidation type (type IIa), intermediate type (type IIx), and rapid glycolysis type (type IIb) [4], respectively. It is also reported that the smaller diameter and the number of muscle fibers, that the smaller shear force required to cut the cross section of the muscle, and the tenderness of meat will be better [5]. The muscle with high content of muscle fiber has a higher ability to resist shear force, and the tenderness of meat is relatively low [6].

Most studies on meat quality are obtained from pig muscle, and it seems that the quantity and quality characteristics of meat products will change with the degree of fiber type profile change [7]. Thus far, the research on the effect of muscle fiber type expression on meat quality is mainly focused on the fiber type. The muscle fiber characteristics are different, and the meat quality traits are also significantly different. In muscle, the meat with the highest muscle fiber density is the most tender. The difference in meat quality between muscles can be explained by differences in muscle

fiber characteristics, especially high-quality muscle [8]. There are many studies on other cattle breed muscle fiber types [9–14], but there is a lack of research on gene expression and morphology of Xinjiang brown cattle. This paper studies the morphological comparison and gene expression difference of muscle fiber types between Xinjiang brown cattle and Angus cattle to compare the differences of muscle between the two breeds and explore the causes of quality traits of Xinjiang brown beef and provide guidance for beef breed breeding, improvement, and production in China.

2. Materials and Methods

2.1. Animals. We randomly selected 12 Xinjiang Brown cattle and 12 Angus cattle from Xinjiang Bole Tianlai Animal Husbandry Co., Ltd. Under the same feeding and management conditions, the high-quality forage planting base was located at 42 north latitude, 826 meters above sea level, golden latitude, and moist soil vein with full natural essence of nature and standardized scientific planting management. The longissimus dorsi muscles were collected from the Xinjiang Brown cattle and Angus cattle at the age of 3, 7, 12, and 24 months. 3 samples were collected in each period and stored in liquid nitrogen at −80°C.

2.2. Histochemical Reactions. Adenosine triphosphatase (ATPase) staining was performed. The samples frozen at −80°C were cut that using a cryostat (LEICA CM3050 S, Germany); then, the samples were cut into 10 μm thickness. All sections were incubated with 18 mM·$CaCl_2$ and 4 mM·ATP (Sigma) at 37°C for 30 min [15]. We used distilled water to wash the muscle suctions three times for 1 min per. Then, we used 2% cobalt chloride solution (Sigma) to wash three times for 1 min per and washed with distilled water. The last, we used 1% ammonium sulfide solution (Sigma) to immerse for 30 s and washed with tap water and dehydrated.

Succinic dehydrogenase (SDHase) staining was performed. The sections in serial with those stained for myosin ATPase were stained for SDH [16]. The SDHase staining methods were referred from those of the study conducted by Wang et al. [17]. We used the Nikon electron microscope to examine the muscle sections and used image analysis software (EZ-MET) to count the density and cross-sectional area (CSA) of muscle fibers according to the method of counting up and down, and left and right under the visual field of 10 to 20 times. The CAS of muscle fibers was calculated [18]:

$$d = \frac{\text{Long trail} + \text{short trail}}{2}. \quad (1)$$

2.3. Quantitative Real-Time PCR Protocol. The method used quantitative real-time fluorescence PCR (qRT-PCR) was referred to the article of Heid et al. [19]. The tissue samples were first processed and the total RNA by Trizol (abm (MasterMix-EL) Company) methods. Then, the DNA was extracted. The methods of extracted DNA were referred to the study by Zurmanová et al. [20]. The primers used for real-time PCR are presented in Table 1 and obtained from Shanghai Shenggong Company, which were the same as those of Zheng Yue et al. [11]. The four myosin Myhc-I, Myhc-IIa, Myhc-IIb, and Myhc-IIx genes in longissimus dorsi muscle fibers were transcribed and expressed at the transcriptional level. The optimum annealing temperatures were 53.4°C, 69.3°C, 53.4°C, 56.0°C, and 53.4°C, respectively, and the optimum cycles were 34, 32, 34, 34, and 31 cycles, respectively. The relative quantification was carried out by using EvaGreen Express 2 × qPCR MasterMix kit and ABI QuantStudioTM 6 Flex Real-Time PCR System instrument. The reaction system is described in Table 2.

2.4. Statistical Analysis. The data were analyzed by the SPSS 22.0 statistical software. The mismatched independent sample T test was used to compare the varieties and transform the gene expression data into 2-ΔΔCT. All the data were expressed by mean ± standard error. At the same time, univariate analysis of the data was carried out by using the statistical software GraphPad Prism 7.0. "*" means significant difference ($P < 0.05$), "**" means extremely significant difference ($P < 0.01$), and no mark means no significant difference ($P > 0.05$).

3. Results and Discussion

3.1. Comparison of Density Determination. To compare the determination of density of Xinjiang brown cattle and Angus cattle, the results are presented in Table 3 and Figure 1 and Figure 2. It showed that the densities of type IIb and type IIx muscle fibers of 3-month-old Xinjiang brown cattle were significantly lower than that of Angus cattle ($P < 0.05$), and the type IIa density of Xinjiang brown cattle was higher than that of Angus cattle. Type I, IIb, and IIx muscle fiber type density of 7-month-old Xinjiang brown cattle were lower than those of Angus cattle, type IIa density of Xinjiang brown cattle was higher than that of Angus cattle, and the changing trend of density ratio of 12-month-old Xinjiang brown cattle were similar to 7-month-old of Xinjiang brown cattle. Type I, IIa, IIb, and IIx densities of 24-month-old Xinjiang brown cattle were lower than those of Angus cattle ($P > 0.05$). The fiber density of type I of Xinjiang brown cattle was lower than that of Angus cattle at 3, 7, 12, and 24 months. The type IIa fiber density of Xinjiang brown cattle at the age of 3, 7, and 12 months was higher than that of Angus cattle, and the change trend of type IIx muscle fiber was the same as that of type IIa muscle fiber, and the type IIb density of Xinjiang brown cattle at the age of 3, 7, and 24 months was lower than that of Angus cattle. When Maltin et al. [21] and Choi et al. [22] studied found that there was a high positive correlation between the proportion of type I muscle fibers and meat tenderness. In our study, we found that the type I muscle fiber density of Xinjiang brown cattle were lower than that of Angus cattle at the age of 3, 7, 12, and 24 months, indicating that Xinjiang brown cattle were tender and juicy and had better meat quality. Stavaux et al. [23] studied showed that with the increasing time, the type I, II a, and II b

Table 1: Primer sequences used in quantitative polymerase chain reaction.

Genes	Primer sequence	Accession no.	Product size
Myhc-I	F: CTTCGGGAAATTCATTC R: GTCAAAGGCGTTATCAG	NM-174117	283 bp
Myhc-IIa	F: CACTTGCTAACAAGGACCTCTGAGTTCA R: ATCCAGGCTGCGTAACGCTCTTTGAGGTTGTA	XM-026521007	375 bp
Myhc-b	F: GATGTTCCTGTGGATGGTCA R: CTCGTTGGTGAAGTTGATGC	NM-001123141	148 bp
Myhc-x	F: GAAACCGTCAAGGGTCTACG R: CGCTTCCTCAGCTTGTCTCT	NM-001104951	153 bp
Beta-actin	F: GAGCGGGAAATCGTCCGTGAC R: GTGTTGGCGTAGAGGTCCTTGC	MF133532	278 bp

F: sense primer; R: antisense primer.

Table 2: Fluorescence quantitative PCR system.

Reagent	Volume (μL)
Eva green express 2 × qPCR master mix	5
Forward primer	0.3
Reverse primer	0.3
cDNA	1
RNase-free water	Up to 10

Table 3: Comparison of different types of muscle fiber density between the same month of Xinjiang brown cattle and Angus cattle of longissimus dorsi muscle. Bc means Xinjiang Brown cattle and An means Angus cattle. Type I is dark, type IIa and type IIb are light, and type IIx is gray.

Age (m)	Breed	Type I (ATP)	Type IIa (SDH)	Type IIb (SDH)	Type IIx (ATP)
3	Bc	178.64 ± 8.77	163.87 ± 7.13	94.05 ± 7.92^{a}	98.39 ± 7.48^{a}
	An	188.01 ± 7.36	152.85 ± 5.99	169.76 ± 6.65^{b}	160.30 ± 6.28^{b}
7	Bc	113.28 ± 9.52	74.34 ± 6.25	66.91 ± 5.62	99.47 ± 8.36
	An	136.19 ± 10.39	72.97 ± 5.57	71.18 ± 5.43	109.67 ± 8.37
12	Bc	107.79 ± 17.60	59.06 ± 9.65	62.06 ± 10.13	86.09 ± 14.06
	An	120.48 ± 21.40	49.78 ± 8.848	54.45 ± 9.67	84.30 ± 14.97
24	Bc	52.53 ± 10.09	50.32 ± 9.67	75.80 ± 14.56	57.32 ± 11.01
	An	83.58 ± 7.08	52.06 ± 4.41	81.33 ± 6.89	74.03 ± 6.27

In the same age, different groups marked different low case letters indicating statistical differences at $P < 0.05$. Different uppercase letters indicate statistical differences at ($P < 0.01$), and unmarked indicate statistical differences at ($P > 0.05$).

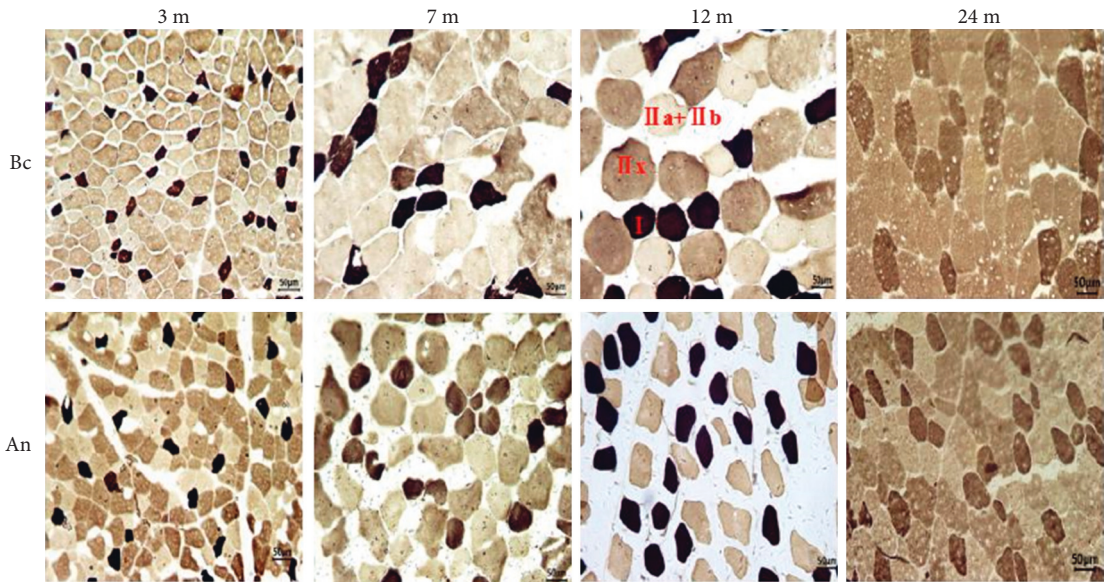

Figure 1: The longissimus dorsi muscle ATPase staining of Xinjiang brown cattle and Angus cattle (×200).

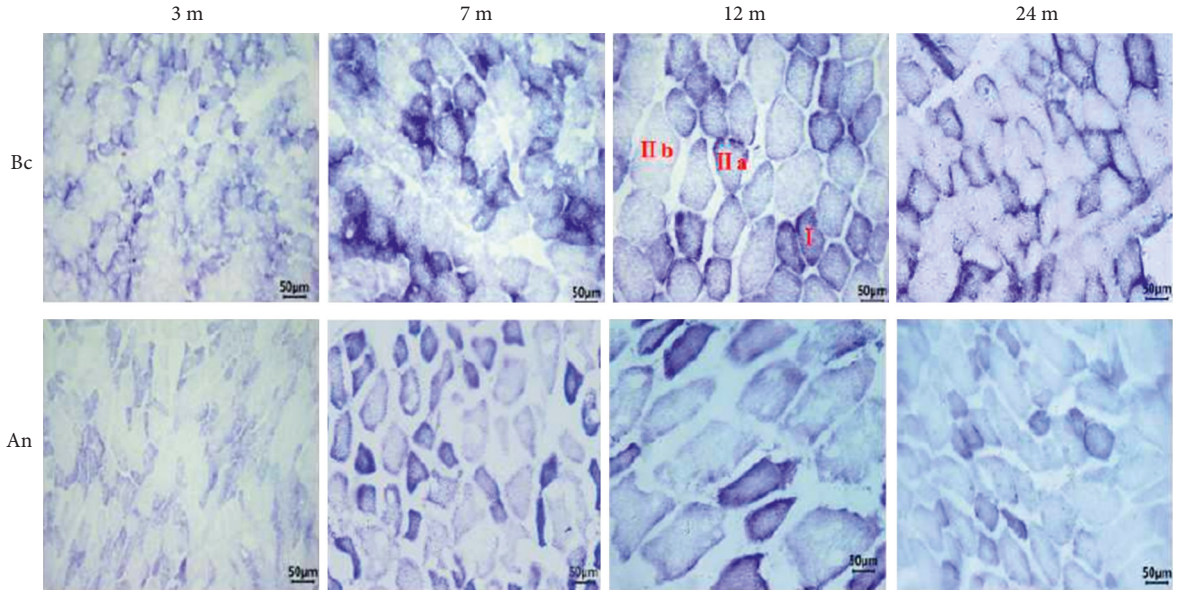

Figure 2: The longissimus dorsi muscle SDHase staining of Xinjiang brown cattle and Angus cattle (×200).

at age of 2 months were lower than three muscle fiber types at age of 7 months. However, in our study, we found that Xinjiang Brown cattle's and Angus cattle's type I, IIa, IIb, and IIx of density gradually decreased with the increasing age.

3.2. Comparison of CSA Determination. Table 4 shows that the CSAs of type I and IIa muscle fibers of Xinjiang brown cattle were significantly smaller than that of Angus cattle at the age of 3 months ($P < 0.01$), and the CSA of type IIb muscle fibers was significantly larger than that of Angus cattle ($P < 0.01$). There was no significant difference in the CSA of type IIx muscle fiber between Xinjiang brown cattle and Angus cattle ($P > 0.05$). The change trend of CSA of Xinjiang brown cattle muscle fiber was IIa < I < IIx < IIb, and Angus cattle muscle fiber was IIa < IIx < I < IIb.

At the age of 7 months, the CSA of type I of Xinjiang brown cattle was significantly smaller than that of Angus cattle ($P < 0.05$). The CSAs of muscle fibers of I, type IIa, type IIb, and type IIx of Xinjiang brown cattle were larger than that of Angus cattle, and the type I and type IIa were extremely significant between Xinjiang brown cattle and Angus cattle ($P < 0.01$), and the difference of type IIx was significant at the age of 12 months ($P < 0.05$). The change trend of muscle fiber CSA of two cattle was IIa < I < IIx < IIb.

At the age of 24 months, the CSAs of muscle fibers of type IIa, type IIb, and type IIx of Xinjiang brown cattle were larger than Angus cattle ($P < 0.01$), and type I of muscle fiber CSA was no significant difference ($P > 0.05$). The change trend of CSA of muscle fiber types in Xinjiang brown cattle was IIa < I < IIx < IIb, and Angus cattle was IIa < IIx < I < IIb.

The muscle have many histological characteristics. In particular, muscle fiber type and CSA histological characteristics determined meat quality tenderness, flavor, meat color, and other important factors [24]. Our results are the same with those of Lefaucheur et al. [25], in which the CSA of type I muscle fiber with the slowest contraction speed was smaller, the tenderness would be better, the proportion of type I muscle fiber was higher, the meat flavor would be rich, the muscle with higher type I fiber was generally tender and juicy, and the meat color would be bright and had better meat quality. Essén-Gustavsson et al. [26] found that the content of myoglobin and lipids in type I muscle fibers was higher than that of muscle fibers type IIa in the study of porcine longissimus dorsi muscle. Different fiber types and components affect the transformation of metabolic muscle and meat quality. Lepetit [27] found that the CSA of beef muscle fiber increased with the age, the finer muscle fiber will have the greater muscle fiber density, and the velvet-shaped CSA texture of muscle indicated good meat quality. There are significant differences in muscle fiber composition among different livestock breeds and different muscle tissues [28]. Chen and Opara [29] found that the CSA of beef muscle fibers in all eight parts were type I < type IIa < type IIb. Different from the results of this study, the CSA of Xinjiang brown cattle and Angus cattle at the age of 3, 7, and 12 months were type IIa < type I < type IIb. The reason may be that different breeds and different parts of the same animal lead to different activities [30]. Ozawa et al. [31] and Kirchofer et al. [32] found that the proportion of type IIb fibers in longissimus dorsi muscle was higher, which was the same as that in our study. Hwang et al. [33] also showed that the CSA of type IIb muscle fiber was larger than type I and type IIa muscle fiber when studying the relationship between muscle fiber type and meat quality of Korean cattle, but he concluded that the CSA of the three types of psoas major muscle fibers was not the smallest. Studies have shown that, compared with muscles with smaller muscle fiber CSA, muscles with larger muscle fiber CSA, especially type IIb fiber, the meat quality was not good [34]. In our study, we found that the number of type

Table 4: Comparison of CSA of different types of muscle fibers between the same month of Xinjiang brown cattle and Angus cattle in the longissimus dorsi muscle (μm^2).

Age (m)	Breed	Type I (ATP)	Type IIa (SDH)	Type IIb (SDH)	Type IIx (ATP)
3	Bc	361.03 ± 13.51 A	292.66 ± 12.19 A	981.99 ± 55.71 A	460.10 ± 16.54
	An	534.28 ± 28.99 B	407.87 ± 22.6 5 B	623.76 ± 31.40 B	492.24 ± 20.07
7	Bc	1031.45 ± 43.15a	1012.08 ± 63.10	2664.59 ± 231.43	1560.64 ± 105.23
	An	1174.43 ± 45.66 b	918.06 ± 55.51	2554.35 ± 252.66	1394.44 ± 94.00
12	Bc	1628.82 ± 97.05 A	1270.22 ± 68.88 A	2813.87 ± 210.34	1922.69 ± 98.83a
	An	1033.78 ± 39.08 B	990.01 ± 44.45 B	2641.40 ± 205.23	1669.91 ± 69.03 b
24	Bc	2287.74 ± 231.14	1699.44 ± 44.68 A	3157.79 ± 132.20 A	2355.23 ± 134.63 A
	An	2135.74 ± 305.02	1469.53 ± 35.89 B	2316.63 ± 92.25 B	1646.06 ± 58.74 B

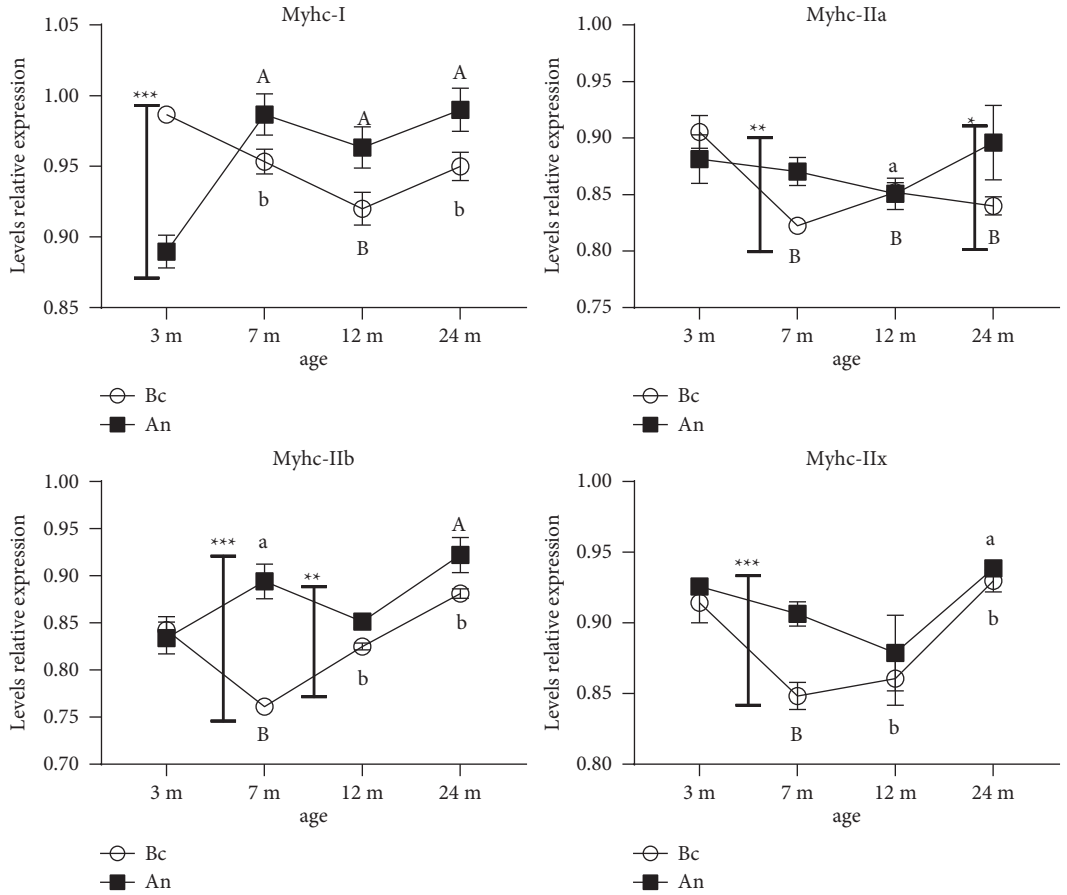

Figure 3: Cattle longissimus dorsi muscle Myhc-I, Myhc-IIa, Myhc-IIb, and Myhc-IIx relative amounts of mRNA in qRT-PCR comparing the expression of two breeds 3, 7, 12, and 24 months. The x-axis represents the age of the cow, and the y-axis represents the expression level of the four muscle types.

I muscle fibers of Xinjiang brown cattle decreased with the increase of age, and the proportion of type IIb muscle fibers increased gradually with the age of 7, 12, and 24 months old. The number of type I muscle fibers in Angus cattle was similar to that of Xinjiang brown cattle, but the proportion of type IIb muscle fibers changed irregularly with age. The result may be that the number of samples is too small. The CSA of type IIb muscle fiber is relatively large, the activities of glycogen, ATP enzyme, and glycolytic enzyme were very high, the activity of oxygen metabolic enzyme was low, and the contraction speed was fast, so the muscle with more type IIb fiber had rough texture, which will increase the shearing force of muscle, lighten the color of meat, and reduce flavor and tenderness [35].

3.3. Myhc Isoform Gene Expression. In Figure 3, the relative level of type I muscle fiber mRNA of 3-month-old Xinjiang brown cattle was significantly higher than that of Angus cattle, and the relative level of type I muscle fiber mRNA of 7-month-old, 12-month-old, and 24-month-old Xinjiang

brown cattle were significantly lower than that of Angus cattle ($P < 0.05$), and the relative level of type I muscle fiber mRNA of 7-month-old, 12-month-old, and 24-month-old Xinjiang brown cattle were lower than that of Angus cattle ($P > 0.05$). Kirchofer et al. [32] found that the higher proportion of type I muscle fiber, and lower proportion of type IIb muscle fiber could make the tenderness better. However, the beef had the larger muscle fiber CSA will result the worse tenderness. The relative level of type IIa muscle fiber mRNA of 7-month-old and 24-month-old Xinjiang brown cattle were significantly lower than that of Angus cattle. The relative level of mRNA of type IIb muscle fiber of 7-month-old Xinjiang brown cattle was significantly lower than that of Angus cattle, and that of 12-month-old Xinjiang brown cattle was significantly lower than that of Angus cattle. The relative level of type IIx muscle fiber mRNA of 7-month-old Xinjiang brown cattle was significantly lower than that of Angus cattle. Monoclonal antibodies were used to identify specific myosin isomers. For distinguishing myosin isomers in muscle fibers, muscle fibers were divided into type I, IIa, IIb, and IIx. Although the names of muscle fibers were different, they were highly consistent with each other. The red muscle fibers were equivalent to type I, and white muscle fibers were equivalent to type IIb [36]. Myhc gene could highly express mRNA levels in skeletal muscle and directly affects skeletal muscle type and meat quality, which was the one of the main factors that determine the composition of muscle fiber type. Therefore, the muscle fiber types were defined by Myhc subtypes [37]. Tanabe et al. [38] studied showed that the composition of Myhc subtypes affected the quality of meat. The results of our study were consistent with those of Myhc-IIx type was stronger than Myhc-IIa type in the longissimus dorsi muscle tissue of the two varieties. Our study results showed that the mRNA level of Myhc-type I muscle fiber of 3-month-old Xinjiang brown cattle was significantly higher than 7- and 24-month-old Xinjiang brown cattle, and the mRNA expression level of Myhc-IIa muscle fiber of 3-month-old Xinjiang brown cattle was significantly higher than that of Xinjiang brown cattle at 7-, 12-, and 24-month-old. The mRNA expression level of Myhc-IIb and Myhc-IIx in muscle fibers in 3-month-old Xinjiang brown cattle was significantly higher than 7- and 12-month-old Xinjiang brown cattle but significantly lower than 24-month-old Xinjiang brown cattle. The expression level of Myhc-I mRNA in 3-month-old Angus cattle was significantly lower than that of 7-, 12-, and 24-month-old Angus cattle, and the mRNA expression level of Myhc-IIb muscle fiber in 3-month-old Angus cattle was significantly lower than that of 12- and 24-month-old Angus cattle. The mRNA expression level of Myhc-IIb muscle fiber in 3-month-old Angus cattle was higher than that of 7- and 12-month-old Angus cattle but lower than that in 24-month-old Angus cattle. The proportion of IIb fiber was negatively correlated with meat quality. The higher the proportion will make the meat quality worse, and it was easy to produce PSE (pale, soft, and exudative) meat [39]. Nam et al. [40] showed that the higher the content of Myhc-type I muscle fiber would make the meat quality better. However, our study was different from that of this experiment, and the reason was the different varieties and sampling sites. Gagaoua et al. [41] found that the fiber types I, IIa, and IIx correlated with the beef tenderness. Our results showed that in 3 m, the mRNA expression level of types I, IIa, and IIx in 3-month-old Xinjiang brown cattle was higher than that in the Angus cattle.

4. Conclusion

From the perspective of muscle fiber histology, we found that the muscle fiber CSA of Xinjiang brown cattle was smaller than Angus cattle, indicating that the tenderness of Xinjiang brown cattle was better than Angus cattle. Except for the 3-month-old stage, the mRNA expression levels of Myhc-I, Myhc-IIa, Myhc-IIb, and Myhc-IIx genes in the longissimus dorsi of 7-month-old, 12-month-old, and 24-month-old Xinjiang brown cattle were significantly lower than those of Angus cattle, and the Myhc-I expression level of 3-month-old Xinjiang brown cattle was significantly higher than that of Angus cattle, indicating that the tenderness of 3-month-old Xinjiang brown cattle was better than that of Angus cattle. It is shown that Xinjiang brown cattle are more suitable for the production of early fat calves and to make some reference for improving the quality of beef cattle in China.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Ya-wei Sun and Miao Qu are contributed equally.

Acknowledgments

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