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Research Article

Study on Muscle Histological Determination of Three Cattle and It's Correlation with Meat Quality Traits

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Abstract

In order to understand the histological differences between different beef types and their correlation with meat quality traits, six cattle (Holstein cattle and Xilin buffalo) were selected for meat quality traits and histological morphological determination. The results showed that: in terms of muscle fiber diameter, Xilin buffalo >Longlin scalper >Holstein cattle, the density of muscle fibers is the opposite; Type Ⅰ muscle fiber proportion ,Xilin buffalo >Holstein cattle >Longlin scalpers showed significant differences among each other (P<0.05); II type A muscle fiber proportion Westwood Xilin buffalo >Longlin scalpers , no significant difference, but ii type A and B ii muscle fiber proportion is put in bigger difference, the alkaline type A muscle fiber ratio is relatively high acid dyeing, ii type B muscle fiber ratio was lower than those of acid dyeing. The diameter of muscle fiber was significantly correlated with marbling, tenderness, pH and intramuscular fat. Type i muscle fiber proportion and marble, intramuscular fat and tender degree of positive correlation, while ii B in proportion to the muscle fibers and pH2 has significant negative correlation.

Keywords: Scalper; Buffalo; Meat quality trait; Muscle fiber

Introduction

The characteristics is a key factor for the market of beef cattle and consumers usually chose beef based on its visual appearance of beef such as color, marbling, and package purge. Fresh beef color is the first visual cue evaluated by consumers and is probably the greatest quality trait hat drive consumer decision of meat purchase at the point of sale. Breed, sex, age, contractile and metabolic muscle properties, muscle composition and characteristics such as connective tissue content, intramuscular fat, pH, and others are important intrinsic factors affecting the meat color. The meat quality and palatability are greatly determined by the amount of intramuscular fat and back fat is responsible in determining cutability.

The quality of beef has a direct impact on the economic benefits of the cattle industry, especially the taste, flavor and tenderness are closely related to the quality of beef. To improve the quality of beef has been the direction of efforts of beef producers and researchers. Beef quality includes the appearance, flavor, nutrition, health, and other physical and chemical shapes related to processing and eating of fresh beef meat and processed meat. In recent years, with the development of analysis and detection technology, great progress has been made in the research of meat quality evaluation methods. For example, tenderness is usually measured by shearing force, penetration method and TPA method, meat color is measured by spectrophotometry and CIEL* A* B* method, flavor includes taste and fragrance, and nutritional value is mainly determined by moisture, ash, fat and protein types and content of meat. However, the determination of histologically beef and meat quality traits of meat quality differences between different association study are less, so the test of longlin Westwood water buffalo, cattle, holstein cows, and to study the muscle fiber diameter, density and type, discusses three cow muscle histologically differences and meat quality traits of correlation, will produce high quality beef to provide relevant theoretical basis.

Materials and Methods

Experimental animals

Six Longlin scalpers, Holstein cattle and Xilin buffalo with the same sex and similar age (2.5-3 old year) were selected as the experimental animal's freedom to gather the food, drinking water, every day according to concentrate feed accounted for 0.8% of body weight, green coarse feed accounted for about 3% of body weight feeding, each breed of cattle is fed the same concentrate feed and coarse feed, and the feeding and management conditions are the same.

Sample collection

In order to reduce the contents of digestive tract and prevent it from contaminating meat quality, cattle were fasted for 24h before slaughter and stopped drinking water 12h before slaughter.

During the period of fasting, ensure that their drinking water is clean, give them a quiet rest environment, to prevent the production of stress meat; Shower the cattle (water temperature about 20°C) to clean the dirt on the body surface, improve the slaughtering environment and ensure the quality of bloodletting.

Slaughtered after the left back muscle 12~13 rib, quickly along the direction of muscle fibers with a scalpel, scissors and tweezers article take less than 5 mm in diameter of meat, frozen storage method based on He Maozhang hanjie xuebao/transactions [1], such as step-by-step frozen tabernacle in combination with the actual situation to make adjustment as follows: first, to fetch the meat into the ice precooling of isopentane in 10 s, reoccupy tweezers clips from the top of the liquid nitrogen level place to stay for 10s, again into the liquid nitrogen precooling of isopentane in 10s, remove the add 10s completely frozen in liquid nitrogen, put in cryopreserved tubes to - 80°C.

Frozen section making

Will move in specimens of -80°C to -20°C refrigerator temperature for 3-5 h, Leica CM3050S frozen section machine set temperature adjustment for -20°C, after being temperature stability, transferred to the frozen sections chassis, using tweezers to take out the meat column and the blade is perpendicular to the direction of muscle fiber cut out of a flat cross section, column the uneven surface of the meat paste on OCT with glue of frozen, after waiting for the fixed completely on the sample. First set the thickness of the thin slice using manual cutting mode, adjust good meat column transverse direction and the blade after cutting in the same direction, adjust the slice thickness of 10 microns, using automatic cutting patterns at a moderate speed cutting many times in a row, make adjustments after the uneven edge again in the process of smooth down proof rolled plate in formally slices with semi-automatic cutting patterns.

He dyeing

The freshly adsorbed frozen sections were moved from the frozen slicing machine to the ventilator for air drying for more than 10min, fixed with 10% neutral formalin for 6s, washed with distilled water for 5s, and soaked with hematoxylin for 10min.Then they were washed with tap water for 10min, stained with eosin for 30-35s, washed with distilled water for 5s, dehydrated with 95% and 100% ethanol gradient for 2min each, and finally transparent with xylene for 5s, sealed with neutral gum and dried.

Photography and measurement

Nikon microscopes take pictures at 4x10 times. The image is saved in JP2 format with size information, the open-source image analysis software ImageJ was used to calculate the diameter and density of muscle fiber bundles as a unit. The number of myofiber bundles measured was more than 6.

Calculation method of muscle fiber density: Divide the number of muscle fibers in the muscle fiber bundle by the area occupied by the muscle fiber bundle.

Calculation method of muscle fiber diameter: Because the space between muscle fibers in this section was still very small even at 10x10 times, and the muscle cells basically did not shrink, the average cross-section area of a single muscle fiber was obtained by dividing the area of muscle fiber bundle by the number of muscle fiber roots contained in the muscle fiber bundle, and then its diameter was obtained by fitting it into a circle.

ATP staining

After the frozen sections are cut and dried, ATPase staining is performed immediately on the same day (if not enough time can be transferred to -80°C for storage). For staining procedures, refer to the instructions of GENMED ATPase Frozen Section Staining Kit for Animal Muscle Tissue Typing (GMS80063.2V.A), some of the steps were appropriately adjusted according to the actual usage: after alkaline solution was added to alkaline slices, the incubation time at room temperature was increased from 2min to 5-10min, and the incubation time of reaction working solution at 37°C was adjusted to 1.5-2h (if the time was too long, it should be noted that distilled water should be added midway to prevent dry slices).Incubation time of 37°C reaction working solution for acid slices was adjusted to 1h. After the section was colorized, photos were taken directly, ten visual fields were randomly selected at 10x10 times to count the number of muscle fibers, and the number of muscle fibers at the edge of all visual fields was counted as half.

Data analysis

The data were input and sorted by Office Excel 2016, and the independent sample T test and Duncan multiple comparison were performed by IBM SPSS Statistics 24 for the comparison of two breeds of cattle. The results were expressed as mean±standard

deviation. One-way ANOVA was performed for three cattle comparisons, and Duncan multiple comparisons were performed. The results were expressed as mean±standard error. Finally, IBM SPSS Statistics 24 was used for correlation analysis using bivariate Pearson.

Results

Determination results of muscle fiber density and diameter of Longlin scalpers, Holstein cattle and Xilin buffalo

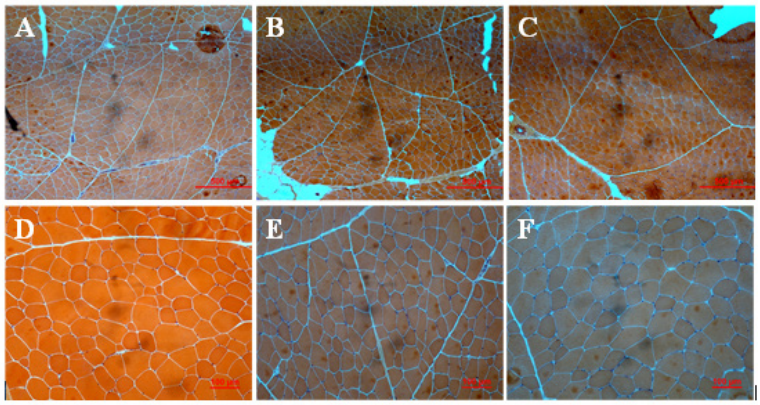

Figure 1: Frozen sections of muscle fibers prepared from Longlin scalpers, Holstein cows, and Xilin buffalo.

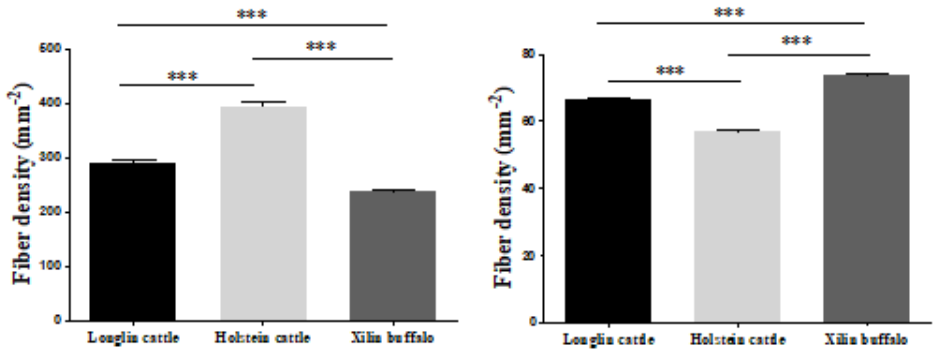

Figure 2: Density and diameter of muscle fiber in Longlin cattle, Holstein cattle and Xilin buffalo.

The results of ordinary frozen sections of Longlin scalper, Holstein cattle and Xilin buffalo were shown in Figure 1, and the results of fiber density and diameter were shown in Figure 2. In terms of muscle fiber density, Holstein cattle> Longlin scalper >Xilin buffalo, the mean values were 393.81, 296.10 and 232.23 cattle /mm^2, respectively, and there were significant differences among the three breeds($P<0.05$). In terms of muscle fiber diameter, Xilin buffalo >Longlin scalper >Holstein cattle, the mean values were 73.36μm, 66.40μm and 56.94μm, respectively, and there were significant differences among the three breeds($P<0.05$). (Figure 2)

Testing results of muscle fiber types of Longlin scalper and xilin buffalo

The results of ATPase staining section of longissimus dorsi muscle in Longlin scalper, Holstein cattle and Xilin buffalo in three groups were shown in Figure 3, in one of the basic section Figure 3a & 3c : light gray for I muscle fibers, moderate gray for II type A muscle fibers, dark grey and black for II B fibers; Acid slices (Figure 3b & 3d) black for I type fibers, white II A, gray for II B (holstein cattle II A and II B as the deal with the problem of sample with white).The statistics of the proportions of all muscle fiber types in different sections of Longlin scalper and Xilin buffalo are shown in Figure 4.

The composition of muscle fiber types between Longlin scalper and Xilin buffalo was consistent with that of alkaline section and acid section are as follows: in terms of type muscle fibers, Longlin scalper <Xilin buffalo; And the difference between them was significant ($P<0.05$), the mean values of the two were 21.36% and 25.28% in the alkaline section, and 20.15% and 24.03% in the acid section, respectively. In terms of II type A muscle fibers, Longlin scalper <Xilin buffalo but there was no significant difference between them

(P>0.05), the mean values of the two were 33.67% and 35.40% in the alkaline section, and 28.22% and 30.38% in the acid section, respectively. In terms of II B muscle fibers, longlin cattle & gt; And the difference between them was significant (P<0.05), the mean values of the two were 44.97% and 39.32% in alkaline section, and 51.63 and 45.59% in acid section, respectively.

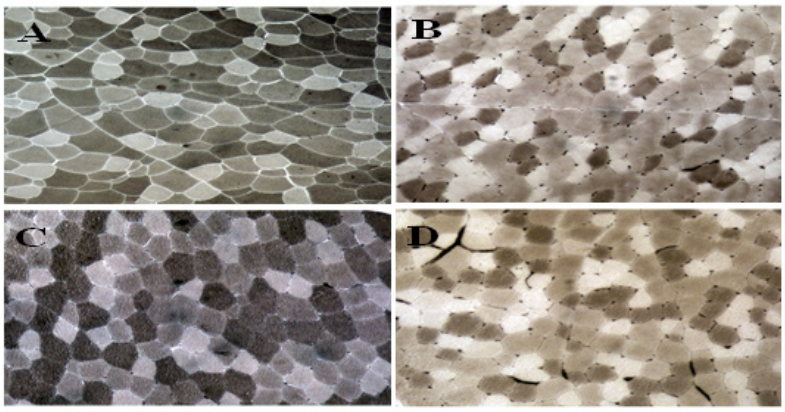

Figure 3: Three groups of ATP enzyme staining results of longissimus dorsi.

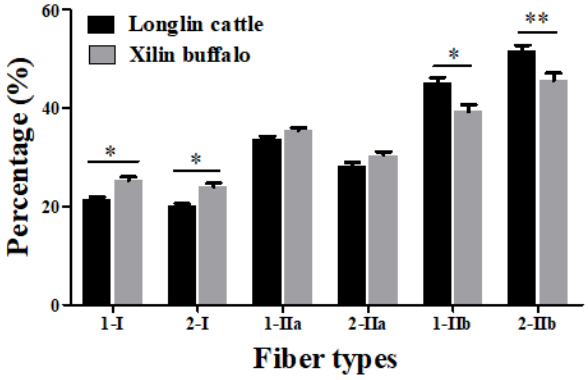

Figure 4: Comparison of all muscle fiber type percentage in different meats of Longlin cattle and Xilin buffalo.

Correlation analysis of muscle fiber diameter and meat quality traits in three cattle breeds

The correlation analysis results between muscle fiber diameter and meat quality traits of Longlin cattle, Holstein cattle and Xilin buffalo are shown in Table 1. There was a positive correlation between muscle fiber diameter and water loss rate, shear force and △pH in the three cattle breeds. Among them, there was a significant positive correlation between muscle fiber diameter and water loss rate in two cattle breeds (Holstein cattle and Xilin buffalo) (P<0.05), the diameter of muscle fiber and sheer force of Holstein cattle were significant (P<0.05), and no significant correlation was found in other items (P>0.05); The diameter of muscle fiber was negatively correlated with marbling, pH2 and intramuscular fat, among which, the diameter of muscle fiber was significantly correlated with marbling in all three types of bovine (P<0.05) or extremely significant (P<0.01), the diameter of muscle fiber was significantly correlated with pH2 and intramuscular fat (P>0.05), other items were not significant (P&T; 0.05); In addition, in terms of pH1, Longlin cattle and Holstein cattle had different correlations with Xilin buffalo, and none of them were significant (P>0.05).

Table 1: Correlation analysis between the diameter of muscle fiber and meat quality traits in Longlin cattle, Holstein cattle and Xilin buffalo.

	Diameter of Muscle Fiber of Longlin Scalper	Diameter of Muscle Fiber of Holstein Cattle	Diameter of Muscle Fiber of Xilin Buffalo
Marble	-0.962**	-0.912*	-0.969*
Filtration rate	0.533	0.887*	0.976*
Shear force	0.651	0.857*	0.595

pH1	-0.606	-0.051	0.827
pH2	-0.840*	-0.74	-0.899
△pH	0.541	0.763	0.904
Intramuscular fat	-0.906*	-0.733	-0.521

Table 2: Correlation analysis between all muscle fiber type percentage and meat quality traits in Longlin scalper, Holstein cattle and Xilin buffalo.

Item	Longlin Scalper			Xilin Buffalo			Holstein Cattle
	I	IIA	IIB	I	IIA	IIB	I
Marble	0.998*	0.771	-0.928	0.993	0.931	-0.996	0.998*
Filtration rate	-0.989	-0.713	0.892	-0.94	-0.669	0.85	-0.99
Shear force	-0.992	-0.728	0.902	-0.997	-0.917	0.992	-0.935
pH1	0.918	0.511	-0.748	-0.826	-0.464	0.692	-0.655
pH2	0.786	0.275	-0.554	0.961	0.978	-0.997*	0.929
△pH	-0.5	0.102	0.206	-0.921	-0.63	0.821	-0.894
Intramuscular fat	0.834	0.352	-0.62	0.655	0.933	-0.796	0.997*
Muscle fiber diameter	-0.973	-0.655	0.854	-0.991	-0.811	0.942	-0.984

The correlation analysis results of different types of muscle fiber ratio and meat quality traits among Longlin scalper, Holstein cattle and Xilin buffalo are shown in Table 2. the three type of cattle, Type I muscle fiber proportion and filtration rate, shear force, delta pH and muscle fiber diameter are negatively correlated, but were not significant;TypeImuscle fiber proportion and marble, there was a positive correlation pH2 and intramuscular fat, two of the cow (longlin cattle and holstein cows) type I muscle fiber proportion and marble was significantly positively related to was significantly positively related with intramuscular fat, other projects were not significant;TypeImuscle fiber proportion and pH1 correlation is different in the three cow, but were not significant.

In two cattle (Longlin scalper and Xilin buffalo) II type A muscle fiber proportion and filtration rate, shear force and muscle fiber diameter were negatively correlated, but which were not significant; II type A muscle fiber proportion and marble, pH2 and intramuscular fat are positively correlated, but were not significant; II type A muscle fiber proportion and the correlation of pH1 and delta pH is different, but were not significant; II type B muscle fiber proportion and filtration rate, shear force, delta pH and muscle fiber diameter were positively correlated, but were not significant; II type B muscle fiber proportion and marble, pH2 and intramuscular fat are negatively correlated, one cattle (Xilin buffalo) and pH2 has significant negative correlation; II type B muscle fiber proportion and pH1 correlation correlation is different in the two bull but were not significant.

Discussion

Determination results of muscle fiber density and diameter of Longlin scalpers, Holstein cattle and Xilin buffalo

The diameter of muscle fiber has a certain relationship with the quality of meat to some extent. Generally, the thicker the diameter of muscle fiber, the lower the tenderness and the lower the department of hydraulic, but this rule is mainly reflected in the comparison of the meat quality of the same species, and the tenderness of meat is also affected by the content of connective tissue and intramuscular fat. In this experiment, the diameter and density of muscle fibers of Longlin scalper and Holstein cattle and Xilin buffalo were measured by making frozen sections.

The results showed that there were significant differences between cattle interspecific and between cattle species and buffalo species, the diameter of muscle fiber of Longlin cattle and Holstein cattle was significantly higher than that of Holstein cattle, but the density of muscle fiber was opposite. The results of comparison of muscle fiber diameter between Longlin scalper and Holstein buffalo and Xilin buffalo were that the scalpers was significantly lower than the buffalo, while the results of comparison of muscle fiber density were opposite. Otherwise[2] shows that guangxi cattle diameter was significantly higher than that of guangxi buffaloes, contrary to the test results, but the measurement method used is common paraffin section, speculation is the cause of two opposite results: the test measurement method is to make frozen section, as shown in figure 1 the muscle fiber gap even if is small, under 10 x10 times showed that muscle fibers in the process of production of frozen section shrinkage degree is small, basic to maintain the original size of muscle fibers; However, paraffin section will cause severe contraction of muscle fibers in the dehydration process. Due to the higher moisture value of Guangxi buffalo, the contraction degree may be much greater than that of Guangxi scalpers, so the opposite comparison results are formed.

Testing results of muscle fiber types of Longlin scalper and Xilin buffalo

Different types of muscle fiber have different colors, anti-fatigue ability and diameter sizes, etc. Therefore, different types of muscle fiber ratio of beef are different. To find out the histological

basis of the difference of meat quality between different beef, we compared the proportion of muscle fiber types between Longlin scalper, Holstein cattle and Xilin buffalo. This test at pase alkaline and acidic dyeing results as shown in figure 3, appropriate adjustments in the process of dyeing acid the processing time of each step can make AC biopsy and BD acid are the three kinds of color, including AC basic slices: light gray for I type muscle fiber , medium gray for II type A muscle fibers, dark grey or black for II B fibers; BD acid slices: black for I type fibers, white II A, gray for II B (holstein cattle II processing error and II B due to samples are white); At the same time, the coloring contrast of different muscle fibers in No.BD acid section was higher than that in No.1 alkaline section.

Because of Holstein cattle to dye the BD acid slice and can only be separated I type muscle fibers, so only in Longlin scalper and Xilin buffalo for AC alkaline dyeing and BD acid dyeing all types of muscle fiber ratio analysis; Another 3 cattle in BD, acidic slice I type muscle fiber proportion. Result shows: by type AC alkaline slice calculate Imuscle fiber ratio is slightly lower than BD acidic slice, but respectively calculated II A and II B muscle fiber ratio difference is larger, this situation may be II x muscle fibers (usually think of at pase staining acid II B there is heterogeneity in slices, some of these for II x type of muscle fiber, its characteristics between II A II and B) on I alkaline slices and the II A close to the color, in BD acid slices with II B similar results; On type I muscle fiber proportion, belong to cow kind of longlin scalper was significantly lower than holstein cattle, belong to Longlin scalper and Holstein cattle were significantly lower than Xilin buffalo; In the acid, alkali typeImuscle fiber proportion of two slices, longlin scalper were significantly lower than Xilin buffalo ;In the acid, alkali two slices II A muscle fiber proportion, Longlin scalper and Xilin buffalo of differences were not significant; In acid, alkali II B muscle fiber proportion of two slices, Longlin scalper were significantly higher than that of Xilin buffalo; In addition, through the analysis of other typeImuscle fiber proportion and its overall muscle fiber diameter (the type I muscle fiber proportion and overall muscle fiber diameter were significantly higher than the other two cattle) shows that its I muscle fiber diameter should be the most coarse, but according to Mr Transcribing guli, erquan [3] the test results show that the typeImuscle fiber diameter and moisture value was significantly negative correlation, Westwood buffalo meat moisture value, this may be the cause of the high.

Correlation analysis of muscle fiber diameter and meat quality traits in three cattle breeds

According to the results in Table 1, there is a significant or extremely significant correlation between muscle fiber diameter of at least one of the three types of beef and marbles, filtration rate, shear force, pH2 and intramuscular fat in each carcass and meat quality traits. Among them, three kinds of cattle were significantly or negatively correlated with marbling, which was basically consistent with the results of Zhu Shubin, et al. [4] and An Zhongzhu [5]. Two kinds of cattle (Holstein cattle and Xilin buffalo) were significantly positively correlated with the filtration rate. Although the remaining Longlin scalper were not significantly correlated with the filtration rate, they were also positively correlated. This result was basically consistent with the research results of Chen Jiebo, et al. [6] and An Zhongzhu. One type of cattle (Holstein cattle) was positively and significantly correlated with the shear force, while the remaining Longlin scalper and Xilin buffalo were not significantly correlated with the shear force. This result was basically consistent with the research results of Chen Qiliang, et al. [7]. One kind of cattle (Longlin scalper) was negatively and significantly correlated with pH2, while the remaining Holstein cattle and Xilin buffalo were negatively correlated with pH2, which was basically consistent with the research results of Zhao Yantong, et al. [8]. One type of cattle (Longlin scalper) showed a negative and significant correlation with intrascularis fat, while the remaining Holstein cattle and Xilin buffalo showed a negative correlation with intrascularis fat, which was basically consistent with the results of Zhu Shubin, et al., Zhang Donghong, et al. [9].

Correlation analysis of muscle fiber ratio and meat quality traits among three cattle breeds

According to the results of Table 2 shows that there are at least in three cattle of beef a I type muscle fiber proportion with the carcass, meat quality traits in marble and intramuscular fat a significant positive correlation, and two of the cattle (Longlin scalper and Holstein cattle) type I muscle fiber proportion and marble is significantly related to the remaining Xilin buffalo, although not significant, but also positively correlated with; One type of cattle (Holstein cattle) was positively correlated with intramuscular fat, while the rest Longlin scalper and Xilin buffalo were also positively correlated with intramuscular fat, although not significantly. In addition, three cattle type I muscle fiber proportion and filtration rate, shear force, pH2, delta pH, and the correlation of muscle fiber diameter is not significant but are consistent, and filtration rate, shear force, delta pH and muscle fiber diameter are negatively correlated, there was a positive correlation with pH2.

In two cattle (Longlin scalpers and Xilin buffalo) II type A muscle fiber proportion and meat quality traits do not exist significant correlation, but with the filtration rate, shear force, and muscle fiber diameter were negatively correlated, there was A positive correlation marble, pH2 and intramuscular fat; Xilin buffalo in two cattle II type B muscle fiber proportion and pH2 significant negative correlation, Longlin scalpers is not significant but negatively correlated.

The above analysis results are basically consistent with the research results of Nuziguli Tursun in Turpan Black Sheep and Altay Samples and Su Lin [10] in Bamei Mutton.

Above all I, II type A muscle fiber proportion of meat quality traits were no significant has significant and positive effect, but

the livestock and meat force related to the family of carcass traits has certain adverse effect, II B, on the other hand, this may, and II type B cross-sectional area of muscle fiber increased much faster than in the process of growth and development type I muscle fibers [11,12]. So, in time to improve the meat quality breeding meat animal should be appropriate to promote I, II type A muscle fiber proportion, but cannot to increase, otherwise it will affect the meat animal meat production ability.

Acknowledgement

None.

Conflict of Interest

No conflict of interest.

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