Inter- and Intra-breed Variation in the Fiber Type Composition of *M. longissimus lumborum* and *M. biceps femoris* in Japanese Black, Japanese Brown, Holstein and F_1 (Japanese Black × Holstein) Steers

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Absract Inter- and intra-breed variation in the fiber type composition of *M. longissimus lumborum* and *M. biceps femoris* in steers was examined on the biopsy materials. Fibers were divided into Type βR, αR and αW. Japanese Black (born in Ohita and Kagoshima prefecture) and Japanese Brown (Aso and Kikuchi, Kumamoto prefecture), Holstein and F_1 (Japanese Black bull × Holstein cow) steers were used. In both muscles, the marked inter- and intra-breed variation was observed on the percentages of Type βR and αW fibers with those reciprocal changes. Of all breeds, the Ohita-Black showed the largest percentage of Type βR fibers and the Kagoshima-Black the second in each muscle. On the other hand, the muscles of the Holstein were characterized by containing Type βR fibers at the lowest frequency. The Brown occupied middle position between the Blacks and Holstein in the percentage distribution of Type βR fibers and showed the largest percentage of Type αR fibers in the biceps muscle compared with the others. From these results it was suggested that the Japanese natives substantially differ in their fiber type composition in the muscles from those of the Holstein and also exhibit inter- and intra-breed variation.

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In beef cattle, *M. longissimus thoracis et lumborum* and *M. gluteobiceps* (*M. gluteus superficialis pars caudalis* + *M. biceps femoris*) are the largest and the second largest muscle of all, respectively[10]. Both Japanese native breeds, the Japanese Black and Japanese Brown, play an important role in beef production in Japan and show its different abilities both in the quality and quantity. Castrated Holstein male calf is used to produce beef at low cost. Holstein cow crossed with Japanese Black bull reproduces F_1 cattle for qualitative improvement of Holstein meat.

The percentage distribution of Type βR or red (Type βR + αR) fibers in the longissimus muscle has positive effects on the juiciness and tenderness of the meat in weathers and ewes[15] and in young bulls[14, 17]. However, Seidemann *et al.*[20] demonstrated the negative effects of red fibers in bulls. When 3 pair steers of Holstein twins were fattened at different planes of

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nutrition, the high plane was favored to increase the percentage of Type αW fibers[24].

On histochemical properties of the fibers, the breed-differences are reported in domestic animals with different athletic abilities, such as horse[6,21,22] and dog[6,7]. Different meat production abilities among breeds are also related to the different histochemical properties in pig[8,9,19] and cattle[10,12,25].

In the animals expected to be alive after histochemical examination, the properties of fibers can be examined on the biopsy materials[11,13,14]. Although breed-differences of the fiber type composition among the Black, the Brown and Holstein steers were clarified on the carcass materials[10], a number of the carcass was restricted because of its high cost. In the present study, biopsy materials of both muscles were gathered from a larger number of steers in the 3 breeds and F_1, and the fiber type composition was compared each other for demonstration of inter- and intra-breed differences.

Materials and Methods

M. longissimus lumborum materials

A total of 90 steers at 10–12 months of age were used. Japanese Black 10 steers (Tajiri strain sires, Ohita prefecture) were bred and raised in Kuju Agricultural Research Center, Kyushu University and the 16 steers (Tajiri strain sires, Kagoshima prefecture) were engaged in sire test in Kagoshima Prefectural Livestock Experiment Station. Japanese Brown 27 steers (Aso, Kumamoto prefecture) were going to be fattened in Green Hill farm, Aso-gun, Kumamoto prefecture. Holstein 16 steers and F_1 (Japanese Black bulls × Holstein cows) 21 steers were engaged in fattening test in Fukuoka Agricultural Research Center.

M. biceps femoris materials

A total of 143 steers at 10–12 months of age were used. Of those steers the 17 (Shiromatsu strain sires, Ohita prefecture) and 65 (Tajiri strain sires, Kagoshima prefecture) Japanese Black were engaged in sire or fattening test in Ohita and Kagoshima Prefectural Livestock Experiment Station respectively and the 25 Japanese Brown (Kikuchi, Kumamoto prefecture) also in Kumamoto Agricultural Research Center. The 18 Holstein and 18 F_1 steers were used in fattening test in the Fukuoka Center.

Ten steer groups (8 steers of each group) engaged in the test of 5 Tajiri strain sires and 5 Kedaka strain sires in the Kagoshima Station were also used.

Biopsy methods

The steers were lightly anesthetized by Xylazine hydrochloride (Rompun, Bayer, Germany) and locally by Lidocaine hydrochloride (Xylocaine, Fujisawa, Japan) in the surgical region after shaving. Biopsy needle (14 G, Baxter, USA) was inserted into 3 serial small holes of skin about 1cm apart incised with scalpel and 5 biopsy materials with parallel fiber striation were taken in each muscle. In *M. longissimus lumborum,* the needle was inserted cranioventrally about 2 to 4 cm depth at 45° through the sagittal lined 3 holes cut open at the lateral side of two thirds of its width on the level of the 1st and 2nd lumber vertebrae (Fig. 1). In the cranial portion of *M. biceps femoris,* the proximal-distal lined 3 holes were located on the surface region under *Trochanter major* of *Os femoris.* The biceps muscle materials were taken from about 2 cm depth of the proximal-superficial region with the needle inserted to *Tuber ischiadicum.* The surgical region was applied antibiotic agent (Terramycin, Pfizer, UK).

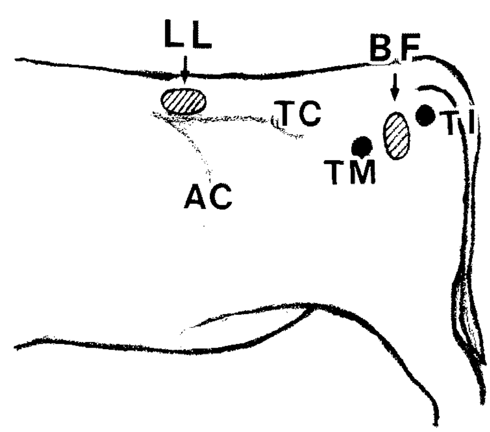

Fig. 1. Illustration of biopsy region in *M. longissimus lumborum* (LL) and *M. biceps femoris* (BF). AC ; *Arcus costalis,* TC ; *Tuber coxae,* TI ; *Tuber ischiadicum,* TM ; *Trochanter major* of *Os femoris.*

Histochemical observation

The 5 materials were gathered in a block by embedding protective agent (Tissue-tek, Miles, USA) and placed into dry ice-isopenthane mixture. Serial frozen sections (8μm thick) were obtained from the block and stained by histochemical reactions for reduced nicotinamide adenine dinucleotide dehydrogenase activity[17] and myosin adenosine triphosphatase activities[18] after acid (pH 4.3) or alkaline (pH 10.5) preincubation[2,23].

Microscopic photographs were taken at the same tissue location on different stained serial sections and printed off as pictures magnified 250 times. Fiber type classification was carried out on the pictures. Nomenclature of fiber types using the method of Ashmore *et al.*[1] was employed. The percentage distribution of fiber types was estimated from the number of each type in a total of 400 to 700 fibers. Means and standard deviations were calculated and used for the examination of significant inter- and intra-breed differences in *t*-test.

Results

Fiber type composition in M. longissimus lumborum

The Ohita (Tajiri) Black steers was found to have the largest percentage of Type βR fibers and conversely Type αW fibers had the smallest of all breeds (Table 1). Another (Tajiri) Black of Kagoshima and the Aso-Brown showed almost the same percentage in every fiber type. In the Holstein steers, the percentage of Type βR fibers was significantly smaller than in the other breeds and conversely the Type αW fibers were observed at higher frequency than the Ohita-Black and the Aso-Brown ($P<0.05$). As the F_1 steer's longissimus contained Type βR fibers at a significantly smaller percentage as compared with the Holstein's ($P<0.05$), no effects of Japanese Black bull crossing were recognized in these F_1 steers.

Fiber type composition in M. biceps femoris

In the Ohita (Shiromatu) Black steers, about half of the fibers were categorized into Type βR, showing the largest percentage of all breeds (Table 2). On the other hand, the percentage of Type αW fibers in this Ohita-Black was the smallest. The Holstein steers were recognized to have conversely the smallest percentage of Type βR fibers and the largest of Type αW. Although the Kagoshima (Tajiri) Black and the Kikuchi-Brown did not show breed-difference in the percentage of Type αW fibers, the biceps muscle contained Type βR fibers at a significantly higher percentage and the Type αR was lower in the former ($P<0.05$). The Kikuchi-Brown exhibited the largest percentage of Type αR fibers. In the F_1 steers, the percentage of Type βR fibers was larger and conversely Type αW was smaller than the Holstein steers ($P<0.05$). From these results, it was indicated that the F_1 muscle had obtained another fiber type composition different from the Holstein's by crossing with Japanese Black bull.

In the Kagoshima station, the biceps muscle in the 5

Table 1. Fiber type composition of *M. longissimus lumborum* in the Japanese Black, Japanese Brown, Holstein and F_1 steers

Breeds	No. of animals	Percentage distribution		
		Type βR	Type αR	Type αW
Japanese Black (A)	10	30.5 ± 5.5^{a}	18.3 ± 4.5^{ab}	51.3 ± 4.2^{c}
Japanese Black (B)	16	23.8 ± 3.2^{b}	14.8 ± 4.5^{b}	61.5 ± 4.3^{ab}
Japanese Brown	27	25.1 ± 3.2^{b}	15.8 ± 3.0^{b}	59.2 ± 3.4^{b}
Holstein	16	21.4 ± 2.5^{c}	16.7 ± 3.7^{ab}	61.9 ± 4.7^{a}
F_1	21	18.8 ± 3.7^{d}	18.4 ± 2.8^{a}	62.8 ± 3.6^{a}

Mean ± the standard deviation. Japanese Black (A) ; born in Ohita prefecture. Japanese Black (B) ; in Kagoshima prefecture. Japanese Brown ; in Aso, Kumamoto prefecture.
[a,b,c,d] Means with different superscripts in the same colum differ significantly at 5% level.

Table 2. Fiber type composition of *M. biceps femoris pars cranialis* in the Japanese Black, Japanese Brown, Holstein and F_1 steers

Breeds	No. of animals	Percentage distribution		
		Type βR	Type αR	Type αW
Japanese Black (A)	17	47.0 ± 10.6^{a}	21.2 ± 8.6^{b}	31.8 ± 7.2^{d}
Japanese Black (B)	65	35.1 ± 4.5^{b}	24.3 ± 6.0^{b}	40.6 ± 5.2^{bc}
Japanese Brown	25	32.8 ± 5.5^{c}	28.2 ± 4.5^{a}	39.1 ± 5.2^{c}
Holstein	18	28.3 ± 4.2^{d}	25.6 ± 3.5^{b}	46.0 ± 4.0^{a}
F_1	18	32.1 ± 5.5^{c}	25.3 ± 3.7^{b}	42.6 ± 4.7^{b}

Mean$\pm$the standard deviation. Japanese Black (A) ; born in Ohita prefecture. Japanese Black (B) ; in Kagoshima prefecture. Japanese Brown ; in Kikuchi, Kumamoto prefecture.

^{a,b,c,d} Means with different superscripts in the same colum differ significantly at 5% level.

steer groups reproduced with the Tajiri strain sires was composed of Type βR fibers 34.4$\pm$2.6%, Type αR 24.6$\pm$5.2% and Type αW 40.8$\pm$3.2%. The muscle in the 5 Kedaka sire groups contained Type βR fibers at 34.8$\pm$2.0%, Type αR at 25.8$\pm$2.7% and Type αW at 39.4$\pm$2.5%. No significant difference was found between the two groups.

Discussion

In the biceps muscle of the Kagoshima-Black steers, no strain-difference of the fiber type composition was observed between the Tajiri and Kedaka strain sires. When the mother's strains were evaluated from the grandfather's strains, the Kagoshima steers reproduced by the intra- and inter-strain crossing were used in the present study. In spite of the different sire strains, the Kagoshima steers are the mixture of Tajiri and Kedaka strains, indicating the genotype characteristic to the Kagoshima strain. For the same reason, the Ohita steers are the mixture of Tajiri and Shiromatsu strains. As the Tajiri, Kedaka and Shiromatsu strains had been introduced recently from Hyogo, Tottori and Shimane prefecture, it was considered that the strains native to Kagoshima and Ohita prefecture had also some effect on genotypes of the present steers. For these reasons, the strains of the steers used in the present study were designated by the names of the prefecture where they were born. The Brown breed also has three genotypes characteristic of the local strains.

In the Ohita-Black steers (23–25 months of age) of the previous study[10], the longissimus muscle contained 30.5% of the fibers as Type βR, 18.3% as Type αR and 51.3% as Type αW respectively at the 11th thoracic vertebra. In the longissimus muscle, however, Goto *et al.*[4] obtained a little larger percentage of Type βR fibers (32.3–35.3%) and conversely a little smaller of Type αW (46.0–48.0%) at the 11th thoracic vertebra and the 5th lumber in the Ohita-Black steers at 28–30 months. On the biopsy materials in the present study, the Ohita-Black steers (10–12 months) showed the same fiber type composition in the longissimus muscle as the Ohita-Black (23–25 months) on the carcass materials[10] (Table 1).

On the other hand, in the present Ohita-Black steers the proximal-superficial part of the biceps muscle exhibited a fiber type composition different from that of the biceps muscle by Goto *et al.*[5] (Table 2). Goto *et al.*[5] observed Type βR, αR and αW fibers at 51.7%, 19.6% and 28.7% respectively in the Ohita-Black steers at 29 months. Although the fiber type composition changes markedly from the proximal region to the middle[5], the difference between these studies could be due mainly to the different maturation stages because of the biceps development with age[3,26].

The intra-breed difference of fiber type composition in the Black steers was clarified in both muscles. The Kagoshima-Black steers had the muscles containing Type βR fibers at smaller percentage and conversely Type αW fibres at larger than the Ohita (Tables 1 and

2). This intra-breed difference was larger than that compared with the inter-breed difference between the Kagoshima-Black and the Aso- or Kikuchi-Brown. The fiber type composition in the Kagoshima-Black differed only in the biceps muscle with that of the Kikuchi-Brown (Table 2).

In our previous study[10], the Brown steers were bred in the area of Kuma-gun, Kumamoto prefecture and were characterized by rapid growth. In the steers at 22–24 months, the longissimus muscle at the 11th thoracic vertebra was composed of 18.1% of Type βR, 18.4% of Type αR and 63.5% of Type αW fibers. Although the percentage of Type βR fibers increases and conversely that of Type αW decreases with age[4,10], the present younger Aso-Brown had already gotten the muscle with Type βR fibers at a higher percentage and Type αW at a lower compared with the previous older Kuma-Brown, indicating different types of longissimus (Table 1). In the Kuma-brown the middle-center of the biceps muscle contained 32.4% as Type βR fibers, 24.1% as Type αR and 43.6% as Type αW[10]. The percentages of Type αR and αW differed between the previous Kuma-Brown and the present Kikuchi-Brown, but they could not be compared under the term of age- and region-related differences because of those reverse changes[5].

The longissimus and biceps muscles in the Holstein steers were characterized by smaller percentage of Type βR fibers and larger of Type αW as compared with the both Japanese native breeds (Tables 1 and 2). However, the fiber type composition of the longissimus varies markedly from study to study. In our previous study[10], 16.0% of the fibers was recognized as Type βR, 14.8% as Type αR and 69.2% as Type αW in the Holstein steers at 20–22 months. Suzuki *et al.*[24] showed another fiber type composition in the Holstein's longissimus at 20 or 26 months, namely Type βR 25.0%, Type αR 24.9% and Type αW 50.1%. The younger steers (10–12 months) in the present study had the longissimus exhibiting an intermediate fiber type composition between those of the previous reports[10,24]. Because of the age-related change of fiber type composition, it is not clear that the present younger steers have another composition different from that of the older by Suzuki *et al.*[24]. However, the intra-breed difference of Holstein's fiber type composition is great between our previous study[10] and the other.

By crossing with Japanese Black bulls, Holstein cows are used to reproduce the F_1 calves with expectations to improve the meat quality in Japan. In the present study, although the F_1 steers showed a different fiber type composition of the biceps muscle from that in the Holstein steers, the other F_1 steers did not change the fiber type composition of the longissimus (Tables 1 and 2). It seemed that the Black bull father did not transmit its properties of the longissimus to the F_1 steers. As mentioned above, the Holstein steers showed great variation of the fiber type composition and so did the Black. It was supposed that Holstein mothers and Japanese Black fathers could transmit variable histochemical properties of their fibers to the F_1 steers.

In the longissimus muscle, interrelationship of fiber type composition to the meat quality has been examined in beef cattle[13,16,20]. When Type βR or red fibers occur at higher frequency, beef increases its juiciness and tenderness. However, the interrelationship of fiber type composition to marbling has not been clarified yet because of complex system of environmental factors. Although different nutritional planes of beef cattle cause change in the fiber type composition[24], all steers used in the present study were fattened under high plane.

In Japan, it is Japanese Black cattle that high quality beef with good marbling is obtained at the highest frequency, followed by Japanese Brown. Holstein steer is used to produce beef at low cost because of its rapid growth. The results in the present study confirm that Japanese Black steers have the largest percentage of Type βR fibers in the muscles and conversely Holstein gets the smallest percentage of Type βR fibers. In all the breeds, the intra-breed variation was also demonstrated in the results of the present and those of the previous studies[4,5,10].

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