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A Comparative Morphometrical Analysis of the Amount and Distribution of Fat within Muscles of Japanese Black Cattle, Japanese Shorthorn, and their Crossbred (F_1) Steers

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Summary

The amount and distribution of fat within muscles of Japanese Black Cattle (JB), Japanese Shorthorn Cattle (JN) and their crossbred (F_1) steers were studied by microscopic morphometry. Fourteen muscles were removed from the transverse sections between the 5th and 6th ribs of third grade carcasses. The percentages of intramuscular and interfascicular fat in muscles were greater in the M. trapezius pars thoracica of the JB steers than of the JN steers. The percentages of intrafascicular fat were greater in the M. pectoralis profundus and RM. transversus thoracis of JB than of JN steers. The percentages of intrafascicular fat in the M. longissimus thoracis were greater in JB steers than in F_1 and JN steers. The M. trapezius pars thoracica, M. semispinalis capitis, M. pectoralis profundus, and M. transversus thoracis had higher percentages of intrafascicular fat in the JB than in the JN steers. The M. latissimus dorsi of the F_1 steers had higher percentages of intramuscular fat than in the JN steers. Generally, the JB and F_1 steers tended to have larger amounts of the intramuscular and the inter- and intrafascicular fat than the JN steers. The diameters of the arterioles running in the central region of muscle bundles (Ia-B-art) were generally largest in the JB steers and smallest in the JN steers, indicating that the degree of ability to store intrafascicular fat is associated with the diameters of the Ia-B-art. These results suggest that the JB steers have the ability to store more inter- and intrafascicular fat than the JN steers.

The amount and distribution of intramuscular fat (marbling) in the skeletal muscles of beef cattle is one of the major factors in estimating meat quality. The amount and distribution of fat in muscles or carcasses depends on sex and feeding conditions (1-4). The layer of the subcutaneous fat is thicker in Japanese Shorthorn (JN) than in Japanese Black (JB) Cattle (3, 4). Results obtained by

morphometry with an image analyzer in a previous report (2) have shown that JB steers have an ability to store more intramuscular fat than JN steers and that beef quality is closely associated with the amount of intramuscular fat. In beef production, it is desirable to produce beef cattle with a moderate amount of fat within muscles and carcasses for acceptable meat quality. However, it is difficult to determine a reasonable degree of fat storage within muscles. The results of a previous study (5) have indicated that the skeletal muscles of cattle consist of muscle bundles regarded as functional units in the muscular tissue and suggested that the intramuscular fat can be divided into two categories of fat storage. The present study was designed to clarify differences in storage and distribution of fine intramuscular fat among three different steers by microscopic morphometry using an image analyser. In addition, fat storage between and within the muscle bundles was examined for its relation to beef quality.

Materials and Methods

Three Japanese Black (JB) steers, six Japanese Shorthorn (JN) steers, and three of their crossbred steers (F_1 : JB X JN) were used in this study. They were fattened at the Tohoku University Farm, Kawatabi, Narugo. Details of the procedures for the feeding and management of the animals were described in the previous report (2). The average weights of carcasses were 313.7 kg for the JB steers, 355.7 kg for the JN steers, and 360.7 kg for the F_1 steers. The carcasses of all the steers were graded at third class according to the standards of the Japan Grading Association. The muscle samples were taken from 14 skeletal muscles in the transverse sections that were cut between the 5th and 6th ribs of cooled carcasses (Fig. 1), and were fixed in 15% formalin-calcium solution. The cross sections of the muscle samples were cut at a thickness of 5 μm with a microtome and stained with hematoxylin-eosin. Photomicrographs of these sections of muscular tissues were taken at the same magnification and used for the microscopical morphometry.

Intramuscular fat was distinguished as the fat between the muscle bundles (fascicle) and the fat within the muscle bundles in the photomicrographs. The fat lying between muscle bundles (interbundle fat) was designated as Ir-B-fat, and the fat located within muscle bundles (intrabundle fat) as Ia-B-fat. The morphometrical procedure used to determine the amount of fat contained in skeletal muscles with an image analyzer (TAS plus, Leitz Co.) has been described in the previous report (2). The amount of fat was measured within the defined area enclosed by the mask displayed on the television moniter screen (Fig. 2). The total area occupied by all intramuscular fat (Fig. 2A), the area occupied by interfascicular fat (Fig. 2B), the area occupied by muscle bundles (Fig. 2C), and the area occupied by intrafascicular fat (Fig. 2D) were measured, and their

percentages of fat in relation to the muscles were calculated.

The sizes of muscle bundles were calculated from their areas with a TAS plus. The diameters of the arterioles within the muscle bundles (intrabundle arterioles : Ia-B-art) were measured to show the relationship of size to amount of Ia-B-fat. Diameters of 40 arterioles in five muscles were obtained by measuring the smallest dimension with an ocular micrometer.

Results

Table 1 shows the average sizes of muscle bundles from JB, JN, and F_1 steers. Muscle fibers were grouped into muscle bundles in the muscles except in the Mm. multifidi, Mm. intercostales externi, and Mm. intercostales interni, in which no muscle bundles were recognized. The mean values of the bundle sizes varied from 3.50 to 12.95 mm. The largest bundle was observed in the M. latissimus dorsi, but no significant differences were noted in the sizes of the bundles among the three steers.

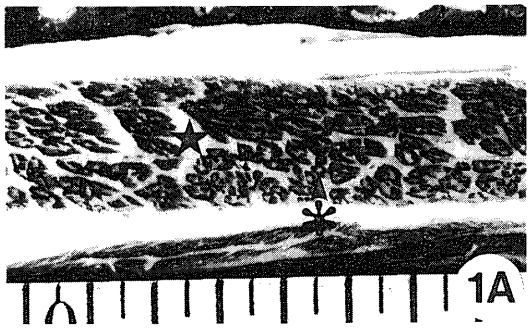
The visible fat in muscles was distinguished as being of two types : retiform type or dotty type (Fig. 1A). A histological observation showed that the retiform type corresponded to Ir-B-fat and the dotty type to Ia-B-fat (Fig. 1B). These

TABLE 1. *Average sizes of fascicles (muscle bundles)*

Name of muscle	Size of muscular bundles (mm^2)		
	JB	JN	F_1
M. trapezius, pars thoracica	6.96±0.40	6.84±0.85	6.44±1.94
M. rhomboideus thoracis	3.50±0.23	5.65±3.00	7.01±1.20
M. semispinalis thoracis	5.59±0.32	6.10±0.95	8.18±2.23
M. longissimus thoracis	5.77±0.65	6.64±2.98	5.75±1.00
M. semispinalis capitis	6.16±0.76	7.09±0.99	8.03±1.21
Mm. multifidi	—	—	—
M. iliocostalis thoracis	3.86±0.40	3.35±1.13	5.26±2.35
M. serratus dorsalis cranialis	5.72±2.53	4.89±1.06	5.36±1.69
M. latissimus dorsi	12.95±3.17	11.54±4.19	10.83±0.88
M. serratus ventralis thoracis	3.97±0.30	5.14±1.14	4.25±1.73
Mm. intercostales externi	—	—	—
Mm. intercostales interni	—	—	—
M. pectoralis profundus	6.19±0.24	7.91±2.64	6.58±4.77
M. transversus thoracis	7.53±0.14	6.73±2.70	7.44±1.25

JB : Japanese Black steers, JN : Japanese Shorthorn steers, F_1 : crossbred steers. Means±SD.

— : No muscle bundles were observed.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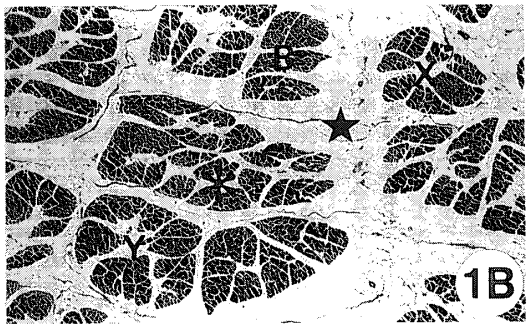
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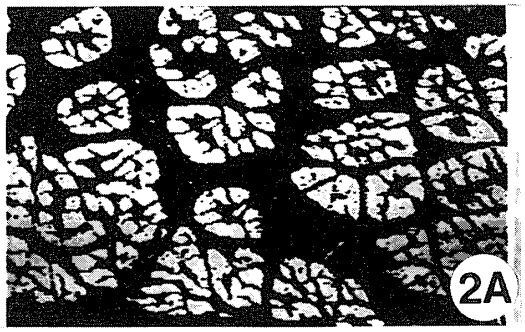
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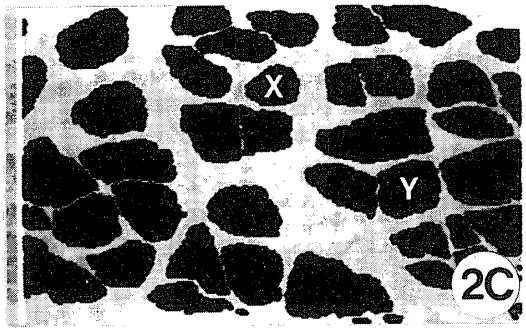
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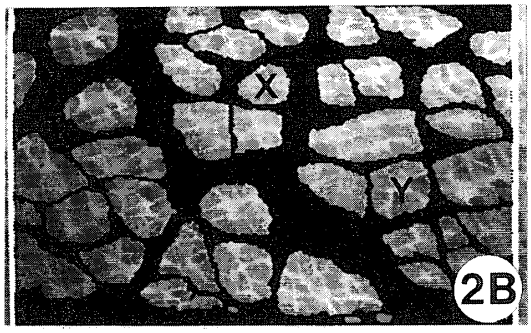
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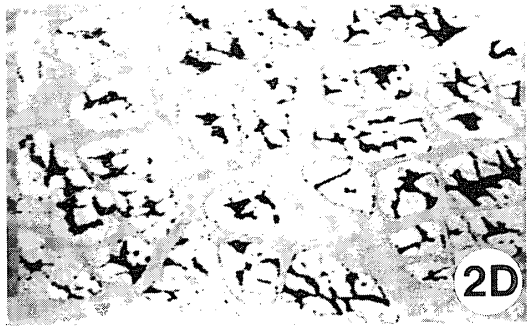
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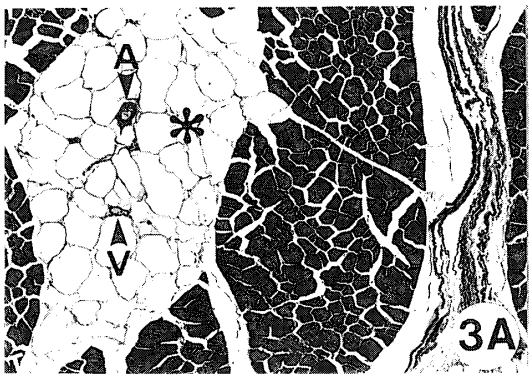
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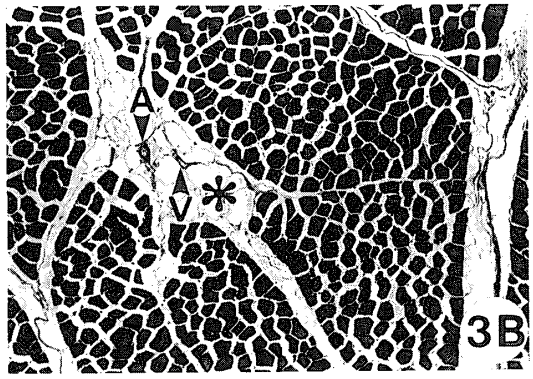
A
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3B

findings indicate that intramuscular fats can be classified into the two types from a morphological standpoint.

Table 2 shows the average percentages of areas occupied by the adipose tissue indicating intramuscular fat, Ir-B-fat, and Ia-B-fat, and differences in percentages among the steers. The percentages of intramuscular fat in each muscle varied from 10.84 to 37.6% in JB steers, from 8.35 to 32.07% in F_1 steers, and from 3.86 to 17.04% in JN steers. JB and F_1 steers tended to store larger percentages of intramuscular fat than did JN steers in all muscles except the M. semispnalis capitis, iliocostalis thoracis, and Mm. intercostales interni. In the M. trapezius pars thoracica, the percentages of intramuscular fat were greater in JB and F_1 than in JN steers. The percentages of intramuscular fat in JB and F_1 steers were similar in seven out of 14 muscles. The M. serratus ventralis thoracis had the greatest amount of intramuscular fat in all steers.

The percentages of interfasicular fat (Ir-B-fat) in the M. trapezius pars thoracis were greater in JB and F_1 than in JN steers (Table 2). The M. pectoralis profundus and M. transversus thoracis contained higher percentages of Ir-B-fat in JB than in JN steers. F_1 steers had higher percentages of Ir-B-fat in the M. latissiums dorsi than did JN steers. In the other seven muscles, JB and F_1 steers tended to have higher percentages of Ir-B-fat than did JN steers.

Percentages of intrafascicular fat (Ia-B-fat) in the M. longissimus thoracis were greater in JB than in F_1 and JN steers (Table 2). JB steers had larger percentages of Ia-B-fat than did JN steers for the M. trapezius pars thoracis, M. semispinalis capitis, M. pectoralis profundus, and M. transversus thoracis. The M. latissimus dorsi of F_1 steers stored more intrafascicular fat than that of the JN steers. The other five muscles of JB and F_1 tended to store more Ia-B-fat fat than did those of JN steers. The results suggest that JB steers have a greater ability to store Ir-B-fat and Ia-B-fat than do JN steers.

In the muscles, the arterioles branched out from the small arteries running

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FIG. 1. Distribution of fat in the transverse section of M. serratus ventralis thoracis of JB steers. Fig. 1A. A close-up photograph. Fig. 1B. Photomicrograph. Hematoxylin and eosin stain. ×7.5. @ Retiform type of interfascicular fat. * dotty type of intrafascicular fat. The retiform type correspond to interfascicular fat and the dotty type to intrafascicular fat (Fig. 2B). X and Y indicate the same muscle bundles as X and Y of Fig. 2.

FIG. 2. Microscopic measurements of Ir-B-fat and Ia-B-fat with an image analyzer (Leitz, TAS plus). Fig. 2A. Image of intramuscular fat indicated by area showing the dark portion. Fig. 2B. Dark portion indicates area between muscle bundles (fascicles). Fig. 2C. Dark portion indicates area of the fascicles. Fig. 2D. Dark portion indicates area of intrafascicular fat.

FIG. 3. Arterioles (Ia-B-art) located in the central region of the fascicle of M. trapezius pars thoracica. Ia-B-art of JB steers (Fig. 3A) is larger than that of JN steers (Fig. 3B). A : Ia-B-art ; V : venule ; * Ia-B-fat. Hematoxylin and eosin stain. ×50.

TABLE 2. *Percentages of area occupied by intramuscular, interfascicular, and intrafascicular fat in muscles*

Name of muscle	Portion	JB(a) %	JN(b) %	F_1(c) %	Significance		
					a-b	b-c	a-c
M. trapezius pars thoracica	1	27.60	9.50	23.97	*	*	NS
	2	19.31	8.12	19.94	*	*	NS
	3	8.29	1.38	2.79	*	NS	NS
M. rhomboideus thoracis	1	11.61	3.86	16.19	NS	NS	NS
	2	9.00	3.82	12.49	NS	NS	NS
	3	2.61	0.05	1.90	NS	NS	NS
M. semispinalis thoracis	1	22.68	15.02	20.23	NS	NS	NS
	2	15.60	13.71	18.59	NS	NS	NS
	3	7.13	1.44	1.64	NS	NS	NS
M. longissimus thoracis	1	18.80	12.07	13.88	NS	NS	NS
	2	13.30	10.99	12.22	NS	NS	NS
	3	5.51	1.08	1.66	*	NS	*
M. semispinalis capitis	1	15.07	11.89	11.40	NS	NS	NS
	2	10.90	11.24	9.36	NS	NS	NS
	3	4.17	0.65	2.06	*	NS	NS
Mm. multifidi	1	21.40	7.36	15.36	NS	NS	NS
M. iliocostalis thoracis	1	10.84	11.06	12.83	NS	NS	NS
	2	9.21	9.90	12.21	NS	NS	NS
	3	1.64	1.16	0.62	NS	NS	NS
M. serratus dorsalis cranialis	1	22.18	13.69	19.35	NS	NS	NS
	2	19.89	13.47	15.47	NS	NS	NS
	3	2.28	0.22	3.87	NS	NS	NS
M. latissimus dorsi	1	16.12	8.54	19.05	NS	*	NS
	2	13.50	7.92	15.02	NS	*	NS
	3	2.62	0.62	4.03	NS	*	NS
M. serratus ventralis thoracis	1	37.60	17.04	32.07	NS	NS	NS
	2	30.12	14.63	26.53	NS	NS	NS
	3	7.48	2.40	5.54	NS	NS	NS
Mm. intercostales externi	1	12.24	8.81	10.34	NS	NS	NS
Mm. intercostales interni	1	15.76	12.31	9.87	NS	NS	NS
M. pectoralis profundus	1	12.37	4.85	8.35	NS	NS	NS
	2	9.59	4.11	7.39	*	NS	NS
	3	2.79	0.73	0.97	*	NS	NS
M. transversus thoracis	1	15.24	6.17	14.21	NS	NS	NS
	2	14.11	5.94	13.33	*	NS	NS
	3	1.12	0.22	0.88	*	NS	NS

JB : Japanese Black steers, JN : Japanese Shorthorn steers, F_1 : crossbred steers.
1 : Intramuscular fat ; 2 : interfascicular fat ; 3 : intrafascicular fat. * $P<0.05$; NS : Not significant.

TABLE 3. *Diameters of arterioles in fascicles (muscle buncle)*

Name of muscle	Diameters of arterioles (μm)		
	JB	JN	F_1
M. trapezius pars thoracica	35.78± 9.48[a]	18.38± 4.99[c]	27.56± 9.52[b]
M. semispinalis thoracis	34.19± 9.58[a]	19.41± 7.61[c]	26.01± 8.82[b]
M. longissimus thoracis	28.75± 8.47[a]	22.38± 7.59[b]	24.04± 9.09
M. serratus dorsalis cranialis	32.95± 8.70[a]	25.13± 8.53[b]	30.19± 9.79
M. pectoralis profundus	53.88±16.01[a]	38.94±12.13[c]	42.69±13.88[b]

JB : Japanese Black steers, JN : Japanese Shorthorn steers, F_1 : crossbred steers. Means±SD.
a, b, c : Means with superscripts within the same column differ significantly from the other column ($p<0.05$). A>B>C

between the muscle bundles to enter into the muscle bundles and lie longitudinally in the central region. The arterioles located in the central region were designated as Ia-B-art (Figs. 3A and 3B). As seen in Table 3, the average diameters of Ia-B-art were smallest in the JN steers and largest in JB steers in each muscle, and differences in the average diameters of the Ia-B-art among the steers were significant in almost all muscles. The Ia-B-fat was stored in the connective tissue surrounding the Ia-B-art (Figs. 3A and 3B), but such storage of Ia-B-fat was infrequently observed in some muscles of JN steers. In the areas containing a large amount of Ia-B-fat, the Ia-B-art with small amounts of Ia-B-fat was smaller in diameter than those with large amounts of Ia-B-fat.

Discussion

It is accepted that the amount of fat within muscles is closely associated with meat quality. Many researchers take an interest in precisely determining the amount and distribution of intramuscular fat in order to appraise beef quality. In many cases, intramuscualar fat has been quantified by a chemical analysis using an ether extract method. In the previous study (2), the amount of both intermuscular and intramuscular fat was measured by image analysis of the transverse sections of carcasses. The present study shows that the storage of intrafasicular fat in the rib eye muscle (M. longissimus thoracis) between 5th and 6th ribs was greater in JB steers than in JN and F_1 steers, and that the texture analyzing system (TAS) for quantitative measurement is a useful tool for determining the amount of the microscopic intramuscular fat, while in the previous study (2) it was impossible to analyze the distribution of the Ir-B-fat and Ia-B-fat in intramuscular fat. In the present study, the amount of the Ir-B-fat and the Ia-B-fat was measured separately by analyzing photomicrographs of the muscles.

The amount of intramuscualar fat measured in the analysis of the microscopic morphometry was 1.5 times more than that of the macroscopic morphometry in the previous report (2). This indicates that the microscopic morphometry makes it possible to measure the exact amount of the fine intramuscular fat: Ir-B-fat and Ia-B-fat. Thus, the image analysis of the microscopic structure proves to be an excellent tool for showing the differences in the amount and distribution of inter- and intrafasicular fat among muscles.

JB steers showed a tendency to store more Ir-B-fat and Ia-B-fat than did JN steers. F_1 steers were similar to JB steers in the amount and distribution of intramuscular fat. It seems that the meat quality of JB and F_1 steers is better than that of JN steers, because the storage of intramuscular fat, especially Ia-B-fat, is closely associated with meat quality.

A histological observation revealed that the diameters of Ia-B-art with large amounts of Ia-B-fat were larger than those with less Ia-B-fat. These findings indicate that the sizes of the diameters of Ia-B-art are associated with an ability to store Ia-B-fat. The diameters of the Ia-B-art were smallest in the JN steers and largest in the JB steers. It remains to be determined whether the differences in diameters of the Ia-B-art among the steers are due to functional properties or to genetic factors in cattle.

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