

Genetic effects on fatty acid composition of carcass fat of Japanese Black Wagyu steers

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ABSTRACT: Two hundred ninety-three Japanese Black Wagyu steers derived from 34 sires were used to investigate genetic effects on the fatty acid composition of carcass fat. All steers were fed identical diets for 365 d and slaughtered at similar ages. If the percentage of genetic contribution of sire A, B, or C was not lower than 25%, steers were classified into groups A, B, and C, respectively. Fatty acid compositions differed depending on deposit sites. Mean percentage of monounsaturated fatty acids (MUFA) tended to be higher in the outer parts than in the inner parts of the body. Percentage of MUFA in carcass fat was negatively correlated with withers height and BW and positively correlated with meat quality score and marbling score. Fatty acid compositions of the 34 sire groups varied,

and mean percentages of MUFA in i.m. fat ranged from 47.71 to 54.77%. Steers in the C group grew larger than those in the A or B group. Mean percentages of MUFA for i.m. fat in the A, B, and C groups (52.83, 51.88, and 50.33%, respectively) differed ($P < 0.05$) from each other. Steers in the C group had higher ($P < 0.05$) percentages of saturated fatty acids than those in the A or B groups. Percentages of genetic contribution of sires B ($P < 0.05$) and C ($P < 0.001$) were negatively correlated with percentage of MUFA in i.m. fat. These results suggested that genetic factors affected fatty acid composition of carcass fat in Japanese Black Wagyu cattle and that some sires had potent genetic factors affecting this composition.

Key Words: Carcass Composition, Fatty Acids, Genetics, Steers

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Introduction

Beef quality depends not only on the degree of marbling but also on fatty acid composition. Beef with the most desirable flavor has lower percentages of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) and higher percentages of monounsaturated fatty acids (MUFA) in the carcass fat (Dryden and Marchello, 1970; Westerling and Hedrick, 1979; Melton et al., 1982). Fatty acid composition of bovine tissues is affected to some degree by factors such as sex (Waldman et al., 1968; Clemens et al., 1973), breed (Eichhorn et al., 1986; Huerta-Leidenz et al., 1996; Perry et al.,

1998), diet (Melton et al., 1982; Marmer et al., 1984; Mandell et al., 1998), and age (Leat, 1975; Huerta-Leidenz et al., 1996; Rule et al., 1997). Japanese Black Wagyu steers have higher percentages of MUFA than Holstein, Japanese Brown, Charolais, or Angus steers (Yoshimura and Namikawa, 1983; May et al., 1993; Zembayashi et al., 1995). The percentage of MUFA in beef from Japanese Black Wagyu steers was also reported to depend on the regions in Japan from which the samples were obtained (Sturdivant et al., 1992). It was not clear whether the differences of fatty acid composition among the regions depended on genetic or nutritional factors. Zembayashi and Nishimura (1996) suggested that differences in the rate of maturation among three Japanese Black sires might have been related to differences in the fatty acid composition of s.c. neutral lipids.

This study was conducted to evaluate genetic effects on fatty acid composition in Japanese Black Wagyu steers that were fed identical diets and slaughtered at similar ages.

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Materials and Methods

Animals

Two hundred ninety-three Japanese Black Wagyu steers, derived from 34 sires (6 to 10 per sire), were used to evaluate the effects of genetics on fatty acid composition. Fattening started at a mean age of 266.4 d (230 to 289 d). All steers had ad libitum access to the identical concentrate diet and timothy hay with a small amount of chopped rice straw. The concentrate diet consisted of 45% rolled barley, 30% flaked corn, 20% wheat bran, 5% soybean meal, and 1% mineral mixture. Body weight was measured at the beginning and end of fattening, and withers height or height of the top of the third thoracic vertebrae was measured at the end of fattening. All steers were slaughtered after a 365-d fattening period. The carcasses were chilled for 24 h, and their meat quality was evaluated between the sixth and seventh ribs by official Japanese graders in accordance with the Japan Meat Grading Standards (JMGA, 1988). Carcasses were graded on meat quality, marbling, beef color, longissimus muscle area, and s.c. fat thickness. Meat quality is assessed based on marbling and beef color and given a score from 1 (poor) to 5 (excellent). Marbling is scored from 1 (poor) to 12 (very abundant) according to the Beef Marbling Standard. Beef color was scored from 1 (light) to 7 (dark) according to the Beef Color Standard.

Among the ancestors of 293 steers, three sires, A, B, and C, whose semen was commonly used for artificial insemination, were selected for analysis. Percentages of genetic contribution of sires A, B, and C were calculated to the fourth generation from these sires by using pedigree information (i.e., sons of a sire: 50.0%, grandsons of a sire: 25.0%, great-grandsons of a sire: 12.5%, great-great-grandsons of a sire: 6.25%) and expressed as the sum of the values of paternal and maternal lines. If the percentage of genetic contribution of sire A, B, or C was not lower than 25%, steers were classified into the A, B, and C groups, respectively.

Sample Collection

After approximately 24 h of chilling, samples were scraped off longissimus muscle, intermuscular adipose tissue, and s.c. adipose tissue at the sixth-seventh rib and perinephric adipose tissue with a slide glass. Samples were stored in sample tubes at -40°C until they were used for fatty acid analysis.

Fatty Acid Analysis

Total lipids were extracted from approximately 100-mg samples using 2 mL of chloroform:methanol (2:1, vol/vol) according to the method of Folch et al. (1957). The lipids were methylated by the method of O'Keefe et al. (1968). Methylated lipid samples were analyzed using a flame ionization detector on a gas chromatograph (Shimadzu GC14A, Kyoto, Japan) equipped with

a 30-m \times 0.32-mm capillary column coated with HR-SS-10. The column was programmed to warm from 150° to 220°C at $3^{\circ}\text{C}/\text{min}$ followed by 3 min at 220°C . The injector and detector temperatures were 250° . The pressures of the gases were $0.6\text{ kg}/\text{cm}^2$ for the carrier gas (helium), $0.6\text{ kg}/\text{cm}^2$ for the hydrogen, $0.6\text{ kg}/\text{cm}^2$ for make-up gas (helium), and $0.5\text{ kg}/\text{cm}^2$ for the combustion air. Chromatograms were recorded with a computing integrator (Shimadzu Chromatopac C-R6A). Identification of sample fatty acids was made by comparing the relative retention times of standard fatty acid methyl-esters (Funakoshi, Tokyo, Japan), and the relative proportions were determined as percentages of summed peak areas.

Statistical Analysis of Data

Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Models included fat deposit site, sire and sire group separately as fixed effects, and slaughter age as a covariate. LSMEANS was used to perform multiple comparison tests. Pearson correlation coefficients were calculated between fatty acid composition and withers height, BW, carcass weight, longissimus muscle area, or subcutaneous fat thickness. Spearman correlation coefficients were calculated between fatty acid composition and percentage of genetic contribution, meat quality score, marbling score, or beef color score. Differences were considered significant at $P < 0.05$.

Results and Discussion

Withers height, BW, carcass weight, and carcass characteristics of steers varied greatly (Table 1). All of the steers were clinically normal, and we considered none of them pathologically small.

Fatty acid composition differed depending on deposit sites (Table 2). Subcutaneous adipose tissue lipid had higher percentages of myristoleic (14:1), palmitoleic (16:1), and oleic (18:1) acids and MUFA, a higher 18:1/stearic acid (18:0) ratio, and lower percentages of 18:0 and SFA than lipid from other sites ($P < 0.05$). In contrast, perinephric adipose tissue had lower percentages of 14:1, 16:1, 18:1, linoleic acid (18:2), MUFA, and PUFA, a lower 18:1/18:0 ratio, and higher percentages of myristic acid (14:0), palmitic acid (16:0), 18:0 and SFA than lipid from other sites ($P < 0.05$). These results were consistent with findings of previous researchers who reported that bovine tissues near the body surface have higher percentages of MUFA than internal tissues (Waldman et al., 1968; Ozutsumi et al., 1983; Sturdivant et al., 1992).

Cianzio et al. (1985) listed adipocyte diameters of steers at 17 mo of age in descending order as perinephric, mesenteric, s.c., intermuscular, i.m. and brisket adipose tissue and found that numbers and diameters of adipocytes in six adipose tissue depots remained constant from 17 mo of age. Barber et al. (2000) reported

Table 1. Withers height, BW, and carcass characteristics of steers

Item	Mean	SD	Minimum	Maximum
Final withers height, cm	133.5	3.8	126	147
Final BW, kg	556.7	68.7	418	768
Hot carcass wt, kg	326.7	46.0	232	454
Meat quality score ^a	3.0	0.8	2	5
Marbling score ^b	4.0	1.4	2	9
Beef color score ^c	3.7	1.4	2	6
Longissimus muscle area, cm ^{2d}	42.1	5.4	30	60
Fat thickness, cm ^e	1.8	0.6	0.2	4.0

^{a,b,c,d,e}These carcass characteristics were evaluated according to the procedures of the Japan Meat Grading Association (JMGA, 1988).

^aA higher number indicates better quality (1 to 5).

^bA higher number indicates more marbling (1 to 12).

^cA higher number indicates a darker color (1 to 7).

that gene expression of stearoyl-CoA desaturase, which catalyzes conversion of SFA to Δ^9 MUFA, varied with adipose cell size for carcass depots except abdominal fat in sheep and was positively correlated with the amount of 18:1 per cell. Stearoyl-CoA desaturase is also present in bovine adipose tissues (St. John et al., 1991). Differences in fatty acid composition among depot sites might be partly due to variation in expression of stearoyl-CoA desaturase gene depending on cell size.

Percentages of MUFA in s.c. ($P < 0.001$), intermuscular ($P < 0.001$), and i.m. ($P < 0.05$) adipose tissue were negatively correlated with final BW (Table 3). Percentages of MUFA in s.c. ($P < 0.001$), intermuscular ($P < 0.001$), i.m. ($P < 0.001$), and perinephric adipose tissue ($P < 0.01$) were negatively correlated with withers height. Percentages of SFA in s.c. ($P < 0.001$), intermuscular ($P < 0.001$), i.m. ($P < 0.001$), and perinephric adipose tissue ($P < 0.05$) were positively correlated with withers height. These results suggested that larger steers had lower percentages of MUFA in their tissues. Percentages of MUFA in carcass fat were positively correlated with meat quality scores ($P < 0.001$ or $P < 0.01$) and marbling scores ($P < 0.01$ or $P < 0.05$). In

contrast, percentages of SFA and PUFA in carcass fat were negatively correlated with meat quality score ($P < 0.001$ or $P < 0.01$) and marbling score ($P < 0.001$, $P < 0.01$, or $P < 0.05$). Other workers previously found that the percentage of SFA was negatively related to marbling and quality grade and that the percentage of 18:1 was positively related to those factors and to triacylglycerol lipid content in longissimus muscle (Skelley et al., 1973; Kazala et al., 1999), whereas the percentage of 18:2 was negatively correlated with the U.S. marbling score (Dryden and Marchello, 1970) and the lipid content in longissimus muscle (Kazala et al., 1999). These findings were consistent with our results in Japanese Black Wagyu cattle.

In general, fatty acid composition in cattle was affected by age and time on feed (Waldman et al. 1968; Leat, 1975; Rule et al., 1997). Changes in fatty acid composition began early in life (Huerta-Leidenz et al., 1996), and a large change in major fatty acids occurred immediately after cattle entered the feedlot (Rule et al., 1997). In the present study, slaughter age did not affect fatty acid composition ($P > 0.05$); ages at slaughter were similar and relatively advanced.

Table 2. Fatty acid compositions of s.c., intermuscular, and i.m. and perinephric fat of steers

Item	s.c. Fat	Intermuscular fat	i.m. Fat	Perinephric fat
Fatty acid, %				
14:0	3.16 ± 0.04 ^w	3.03 ± 0.03 ^x	3.19 ± 0.03 ^w	3.33 ± 0.03 ^y
14:1	1.96 ± 0.03 ^w	1.10 ± 0.02 ^x	0.89 ± 0.02 ^y	0.62 ± 0.01 ^z
16:0	26.10 ± 0.10 ^w	25.90 ± 0.13 ^w	28.95 ± 0.10 ^y	27.91 ± 0.15 ^z
16:1	6.01 ± 0.07 ^w	3.87 ± 0.04 ^x	3.40 ± 0.03 ^y	2.07 ± 0.03 ^z
18:0	8.76 ± 0.09 ^w	13.69 ± 0.11 ^x	13.93 ± 0.10 ^x	22.23 ± 0.16 ^y
18:1	51.69 ± 0.17 ^w	50.15 ± 0.18 ^x	47.43 ± 0.14 ^y	41.73 ± 0.21 ^z
18:2	2.20 ± 0.02 ^w	2.15 ± 0.03 ^w	2.16 ± 0.03 ^w	2.00 ± 0.02 ^x
18:3	0.12 ± 0.00 ^w	0.12 ± 0.00 ^w	0.04 ± 0.00 ^x	0.11 ± 0.00 ^w
SFA ^a	38.02 ± 0.17 ^w	42.62 ± 0.20 ^x	46.07 ± 0.15 ^y	53.47 ± 0.23 ^z
MUFA ^a	59.66 ± 0.16 ^w	55.12 ± 0.20 ^x	51.73 ± 0.15 ^y	44.42 ± 0.22 ^z
PUFA ^a	2.32 ± 0.03 ^w	2.27 ± 0.03 ^{wx}	2.20 ± 0.03 ^x	2.11 ± 0.03 ^y
Ratio of 18:1/18:0	6.09 ± 0.07 ^w	3.75 ± 0.04 ^x	3.46 ± 0.03 ^y	1.92 ± 0.02 ^z

^aSFA = saturated fatty acids (sum of 14:0, 16:0, and 18:0); MUFA = monounsaturated fatty acids (sum of 14:1, 16:1, and 18:1); PUFA = polyunsaturated fatty acids (sum of 18:2 and 18:3).

^{w,x,y,z}Means with different superscripts in the same row differ ($P < 0.05$).

Table 3. Simple correlation coefficients between withers height, BW, or carcass characteristics and SFA^a, MUFA^a, or PUFA^a percentages of carcass fat

Item	s.c. Fat			Intermuscular fat			i.m. Fat			Perinephric fat		
	SFA	MUFA	PUFA	SFA	MUFA	PUFA	SFA	MUFA	PUFA	SFA	MUFA	PUFA
Final withers height	0.22***	-0.25***	0.14*	0.28***	-0.30***	0.14*	0.22***	-0.25***	0.18**	0.15*	-0.16**	0.09
Final BW	0.21***	-0.23***	0.11	0.20***	-0.21***	0.07	0.13*	-0.14*	0.09	0.10	-0.09	-0.05
Hot carcass wt	0.17**	-0.18**	0.07	0.15**	-0.16**	0.03	0.08	-0.09	0.05	0.07	-0.06	-0.10
Meat quality score	-0.15*	0.19**	-0.20***	-0.20***	0.23***	-0.17**	-0.18**	0.24***	-0.28***	-0.17**	0.20***	-0.17**
Marbling score	-0.14*	0.18**	-0.25***	-0.12*	0.15**	-0.25***	-0.11	0.18*	-0.36***	-0.12*	0.15*	-0.18**
Beef color score	0.08	-0.09	0.10	0.00	-0.02	0.10	0.07	-0.08	0.12*	0.01	-0.01	0.01
Longissimus muscle area	0.02	0.00	-0.11	0.05	-0.04	-0.11	-0.03	0.05	-0.10	-0.03	0.05	-0.18**
Fat thickness	0.00	0.00	0.03	-0.11	0.12*	-0.08	-0.04	0.06	-0.11	-0.03	0.05	-0.14*

^aSFA = saturated fatty acids (sum of 14:0, 16:0, and 18:0); MUFA = monounsaturated fatty acids (sum of 14:1, 16:1, and 18:1); PUFA = polyunsaturated fatty acids (sum of 18:2 and 18:3).
 * $P < 0.05$.
 ** $P < 0.01$.
 *** $P < 0.001$.

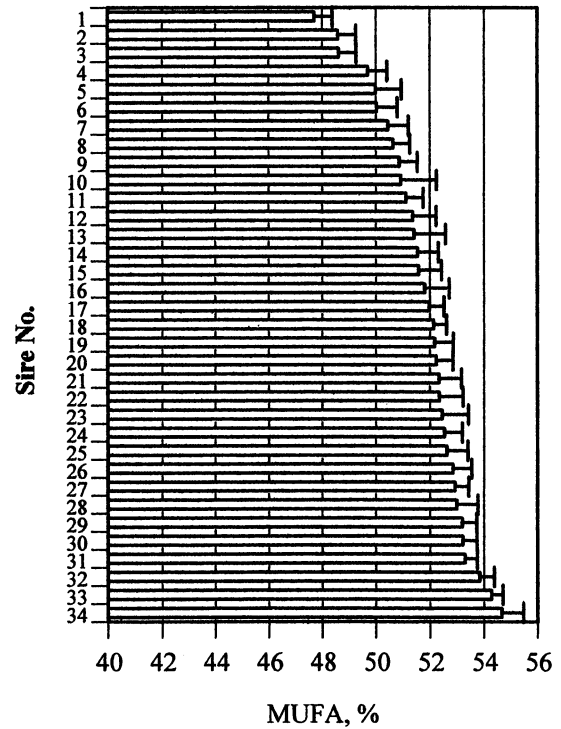


Figure 1. Monounsaturated fatty acid (MUFA) content in i.m. fat of steers derived from 34 sires. Values are means with their standard errors.

Japanese Black steers have higher percentages (approximately by 7%) of MUFA in s.c. and i.m. lipids than Holstein or Angus steers (Yoshimura and Namikawa 1983; May et al., 1993; Zembayashi et al., 1995). The higher the percentage of MUFA, the better the beef flavor. In the present study, the difference between maximum and minimum percentages of MUFA in i.m. fat of the 34 sire groups was approximately 7% (range: 47.71 to 54.77%; Figure 1). This difference was relatively large. Zembayashi and Nishimura (1996) also found that fatty acid composition of s.c. neutral lipids differed among progenies of three Japanese Black sires and suggested that differences in genetic factors including rates of maturation among sires might be related to differences in fatty acid composition. Thus, in Japanese Black Wagyu cattle, the genetic factors might be important for MUFA percentage or beef flavor.

In order to analyze further the genetic contribution to fatty acid composition, the steers of the 34 sire groups were classified into the A, B, and C groups. Mean values of withers height and BW in the C group were higher ($P < 0.05$) than those in the A and B groups (Table 4). This result suggested that steers in the C group had greater growth potential than those in the A or B group. Mean values of carcass weight, beef color score, longissimus muscle area, and s.c. fat thickness in the C group were higher ($P < 0.05$) than those in the other groups. However, there was no difference ($P > 0.05$) in meat quality scores or marbling scores among three groups. Mean percentages of MUFA in i.m. lipid in the A, B,

Table 4. Withers height, BW, and carcass characteristics of steers in A, B, and C sire groups

Item	Sire group ^a		
	A	B	C
Number of steers	68	65	49
Initial BW, kg	235.58 ± 3.26 ^x	227.21 ± 2.72 ^x	278.13 ± 3.78 ^y
Final BW, kg	513.66 ± 5.57 ^x	502.51 ± 4.65 ^x	608.33 ± 6.36 ^y
Final withers height, cm	132.06 ± 0.35 ^x	132.01 ± 0.33 ^x	134.63 ± 0.48 ^y
Hot carcass wt, kg	300.35 ± 3.93 ^x	290.43 ± 3.27 ^x	362.18 ± 4.37 ^y
Meat quality score ^b	3.01 ± 0.08	2.98 ± 0.07	2.96 ± 0.09
Marbling score ^c	4.19 ± 0.16	4.02 ± 0.14	3.96 ± 0.16
Beef color score ^d	3.49 ± 0.09 ^x	3.45 ± 0.10 ^x	4.04 ± 0.09 ^y
Longissimus muscle area, cm ² ^e	41.31 ± 0.58 ^x	40.97 ± 0.56 ^x	43.76 ± 0.75 ^y
Fat thickness, cm ^f	1.66 ± 0.06 ^x	1.54 ± 0.06 ^x	2.18 ± 0.10 ^y

^aThese groups comprised the steers whose percentages of genetic contribution of the A, B, or C sire were not lower than 25%.

^{b,c,d,e,f}These carcass characteristics were evaluated according to the procedures of the Japan Meat Grading Association (JMGA 1988).

^bA higher number indicates better quality (1 to 5).

^cA higher number indicates more marbling (1 to 12).

^dA higher number indicates a darker color (1 to 7).

^{x,y}Means with different superscripts in the same row differ ($P < 0.05$).

and C groups differed ($P < 0.05$) from each other (Table 5). The C group had higher percentages of 14:0, 16:0, and SFA and lower percentages of 14:1 and 18:1 and a lower 18:1/18:0 ratio than the A or B group ($P < 0.05$). The A group had lower percentages of 18:0 and SFA and a higher 18:1/18:0 ratio than the B or C group ($P < 0.05$). Percentage of genetic contribution of sire A was not correlated ($P > 0.05$) with percentage of MUFA in i.m. lipid; however, those of sires B ($P < 0.05$) and C ($P < 0.001$) were negatively correlated with percentages of MUFA in i.m. lipid (Figure 2). These results suggested that genetic factors of some sires affected fatty acid composition of their progeny. Sturdivant et al.

(1992) reported that stearoyl-CoA desaturase might be responsible for the elevated MUFA observed in Wagyu adipose tissues. Activity of stearoyl-CoA desaturase might have contributed to the differences of fatty acid composition among the offspring of different sires in the present study.

Percentage of MUFA in carcass fat was negatively correlated with withers height and BW. Steers in the C group whose growth potential might be the greatest had a higher percentage of SFA and a lower percentage of MUFA than those in the A or B group. These findings suggested that larger Japanese Black Wagyu steers tended to have higher percentages of SFA and lower

Table 5. Fatty acid compositions of i.m. fat in steers in A, B, and C sire groups

Item	Sire group ^a		
	A	B	C
Number of steers	68	65	49
Fatty acid, %			
14:0	3.11 ± 0.05 ^x	3.22 ± 0.05 ^x	3.42 ± 0.08 ^y
14:1	1.00 ± 0.03 ^x	0.98 ± 0.02 ^x	0.82 ± 0.04 ^y
16:0	28.96 ± 0.18 ^x	28.90 ± 0.20 ^x	29.83 ± 0.26 ^y
16:1	3.57 ± 0.05 ^x	3.38 ± 0.06 ^y	3.53 ± 0.07 ^{xy}
18:0	13.11 ± 0.15 ^x	13.94 ± 0.17 ^y	14.33 ± 0.24 ^y
18:1	48.26 ± 0.26 ^x	47.52 ± 0.29 ^x	45.98 ± 0.43 ^y
18:2	1.95 ± 0.05	2.03 ± 0.05	2.05 ± 0.08
18:3	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
SFA ^b	45.18 ± 0.26 ^x	46.06 ± 0.03 ^y	47.58 ± 0.41 ^z
MUFA ^b	52.83 ± 0.27 ^x	51.88 ± 0.31 ^y	50.33 ± 0.43 ^z
PUFA ^b	1.99 ± 0.05	2.06 ± 0.05	2.09 ± 0.08
Ratio of 18:1/18:0	3.72 ± 0.06 ^x	3.45 ± 0.06 ^y	3.26 ± 0.07 ^z

^aThese groups comprised the steers whose percentages of genetic contribution of the A, B, or C sire were not lower than 25%.

^bSFA = saturated fatty acids (sum of 14:0, 16:0, and 18:0); MUFA = monounsaturated fatty acids (sum of 14:1, 16:1, and 18:1); PUFA = polyunsaturated fatty acids (sum of 18:2 and 18:3).

^{x,y,z}Means with different superscripts in the same row differ ($P < 0.05$).

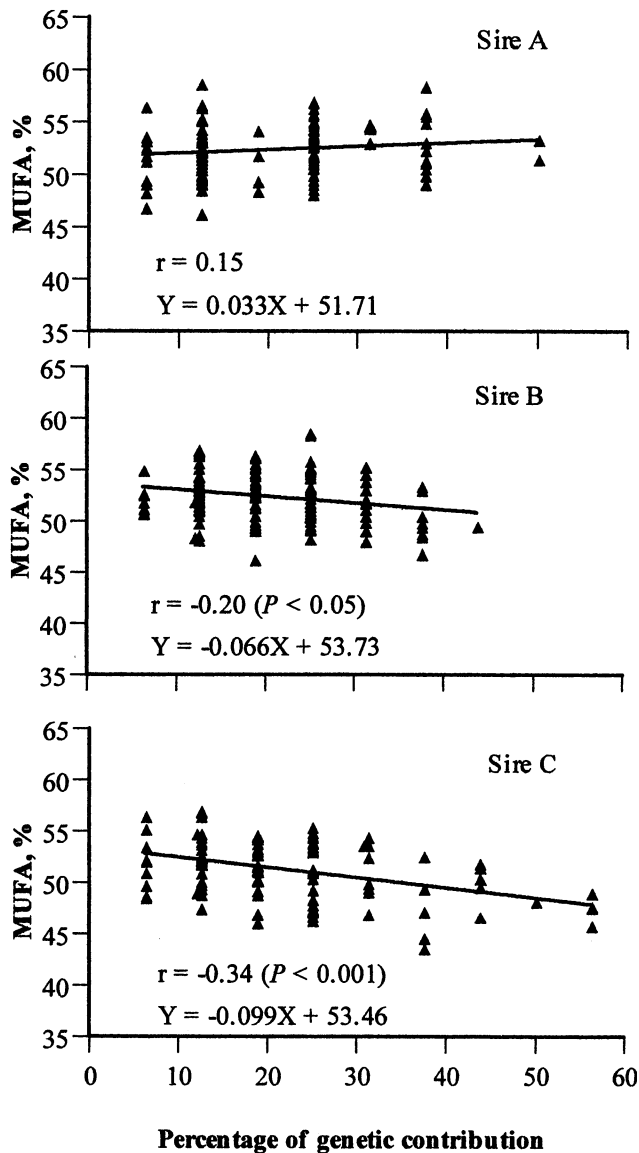


Figure 2. Relationship between monounsaturated fatty acid (MUFA) content in i.m. fat and percentage of genetic contribution of sire A, B, or C.

percentages of MUFA in their tissues. However, Rule et al. (1997) reported that progeny of high-growth-rate-potential sires tended to have less SFA and more MUFA in longissimus muscle than progeny of medium-growth-rate-potential sires. Perry et al. (1998) reported that fatty acid composition was affected by sire breed, independently of variation in carcass weight. It is not clear whether growth potential affects fatty acid composition of the carcass fat in Japanese Black Wagyu cattle.

Beef flavor has been shown to be positively correlated with 18:1 and MUFA (Dryden and Marchello, 1970; Mandell et al., 1998) and negatively correlated with 18:0 and 18:3 (Melton et al., 1982). Therefore, fatty acid composition is an important factor for producing high-quality beef. Because fatty acid composition is significantly affected by the genetic properties of the sires in

the same breed, we need to consider these properties for breeding purposes.

Implications

Percentage of monounsaturated fatty acids in carcass fat was negatively correlated with withers height and BW and positively correlated with meat quality score and marbling score. Fatty acid compositions of 34 sire groups varied. Steers in the C group whose growth potential might be the greatest had the lowest percentage of monounsaturated fatty acids in carcass fat. Percentages of the genetic contribution of sires B and C were negatively correlated with percentage of monounsaturated fatty acids in i.m. lipids. These results suggested that fatty acid composition of carcass fat was affected by genetic factors in Japanese Black Wagyu cattle.

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