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ORIGINAL ARTICLE

Effects of the fattening period on the fatty acid composition of fat deposits and free amino acid and inosinic acid contents of the longissimus muscle in carcasses of Japanese Black steers

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ABSTRACT

The effects of the fattening period on carcass characteristics, fatty acid composition of fat deposits, and muscle free amino acid (FAA) and inosinic acid (IMP) contents were evaluated in Japanese Black steers. Ten castrated, 10-month-old calves derived from the same sire were divided into five to be slaughtered at the age of 30 months after a 20-month fattening period (20-month group) and five to be slaughtered at the age of 34 months after a 24-month fattening period (24-month group). Concerning the fatty acid composition of subcutaneous fat, the percentage of palmitoleic acid was higher ($P < 0.05$) in the 24- than in the 20-month group, but no difference was noted in any other fatty acids. For intermuscular fat, no difference was observed in any fatty acids. The percentages of oleic acid and total monounsaturated fatty acid of intramuscular and perinephric fat were higher ($P < 0.05$) in the 24- than in the 20-month group. Of the FAAs in the longissimus thoracis muscle, the threonine and tyrosine contents were lower ($P < 0.05$) in the 24- than in the 20-month group. The IMP content was higher ($P < 0.05$) in the 24- than in the 20-month group, suggesting an effect of prolongation of the fattening period.

Key words: *fattening period, fatty acids, free amino acids, inosinic acid, steers.*

INTRODUCTION

The fatty acid composition of beef fat largely affects the sensory characteristics and flavor of beef, which are reported to be better as the percentage of oleic acid (C18:1) is higher, and the percentages of palmitic acid (C16:0) and stearic acid (C18:0) are lower (Dryden & Marchello 1970; Westerling & Hedrick 1979; Melton *et al.* 1982). The fatty acid composition is affected by many factors, such as the breed (May *et al.* 1993; Huerta-Leidenz *et al.* 1996; Perry *et al.* 1998), sex (Waldman *et al.* 1968; Yoshimura & Namikawa 1983; Zembayashi *et al.* 1995), and feed (Marmer *et al.* 1984; Mandell *et al.* 1998). In Japanese Black, fatty acid composition of carcass fat has been reported to vary with the sire and strain (Oka *et al.* 2002). Also, there have been reports that the fattening period or age at slaughter affects the fatty acid composition (Clemens *et al.* 1973; Leat 1975; Rule *et al.* 1997), but most studies were about fattening up to 24 months of age, and few studies were about fattening to more than 30 months of age.

Meanwhile, taste-producing factors such as free amino acids (FAAs) and inosinic acid (IMP) in muscle tissue are considered to contribute to the good taste of meat (Nishimura *et al.* 1988). The FAA levels have

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been reported to vary with the breed, postmortem period (Watanabe *et al.* 2004), and site in the muscle (Cornet & Bousset 1999). However, in cattle, there have been few studies on the effects of the fattening period on the FAA levels other than the one by Watanabe *et al.* (2004), and there has not been a report concerning IMP.

The average slaughter age of Japanese Black steer is 29.0 months (Wagyu Registry Association, 2008, unpublished data), and that in Hyogo prefecture is 31.7 months (our institute, 2008, unpubl. data). Recently, some farmers especially in the Hyogo prefecture empirically prolong fattening periods to attempt to produce more palatable beef. However, so far the effect of prolonged fattening period has not been scientifically determined.

In the present study, the effects of a prolonged fattening period on carcass characteristics, fatty acid composition of fat deposits, and muscle FAA and IMP contents were evaluated in Japanese Black steers by standardizing the sire and feed.

MATERIALS AND METHODS

Animals and dietary treatments

Ten 10-month-old Japanese Black steers derived from the same sire (mean body weight: 260.2 ± 6.9 kg) were divided into two groups: five animals to be slaughtered at the age of 30 months after a 20-month fattening period (20-month group) and five to be slaughtered at the age of 34 months after a 24-month fattening period (24-month group). The fattening of the animals and the experiment were performed according to the animal experiment guidelines of the Hyogo Prefectural Technology Center of Agriculture, Forestry, and Fisheries. The feed shown in Table 1 was given to both groups twice a day. Concentrated feed was restricted until the age of 17 months and given *ad libitum* thereafter. The animals were housed in pens and individually accessed feed *ad libitum* via electronic gates (American Calan, Northwood, NH, USA). Water and trace-mineralized salt were freely available.

Carcass measurement

Carcass characteristics were evaluated 48 h after slaughter between the 6th–7th ribs by certified graders according to the New Beef Carcass Grading Standards of the Japan Meat Grading Association (JMGA 1988).

Sample collection

The samples were collected 48 h after slaughter. Fat samples were collected from four sites, i.e. the subcutaneous tissue over the upper part of the latissimus dorsi muscle, between the iliocostalis and latissimus dorsi muscles, the longissimus thoracis muscle at the 7th rib, and around the kidney. Muscle samples of approximately 15 g were obtained from the longissimus thoracis muscle as a slice of about 2 mm thick. Each of the fat and muscle samples was placed in a sample tube, vacuum-packed, and stored at −30°C until analysis.

Fatty acid composition analysis

Total lipids were extracted from approximately 100 mg samples using 2 mL of chloroform: methanol (2:1, v/v) 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 (GC14A; Shimadzu, Kyoto, Japan) equipped with a 30-m × 0.32-mm capillary column coated with HR-SS-10 (Shinwa, Kyoto, Japan). The column was programmed to warm from 150°C to 220°C at 3°C/min followed by 3 min at 220°C. The injector and detector temperatures were 250°C. The pressures of the gases were 0.6 kg/cm^2 for the carrier gas (helium), 0.6 kg/cm^2 for the hydrogen, 0.6 kg/cm^2 for make-up gas (helium), and 0.5 kg/cm^2 for the combustion air. Chromatograms were recorded with a computing integrator (Chromatopac C-R6A;

Table 1 Composition of the experimental diets

Item	Months of age				
	10–12	13–15	16–20	21–26	27–34
Ingredients, % fed basis					
Barley	0.0	0.0	18.4	28.8	26.5
Steam-flaked maize	23.1	25.0	25.8	24.7	30.9
Wheat bran	20.8	22.5	18.4	24.7	26.5
Soybean meal	2.3	2.5	11.1	4.1	4.4
Rice straw dried	0.0	50.0	26.3	17.7	11.7
Timothy hay	53.8	0.0	0.0	0.0	0.0
Dry matter†(DM), %	86.1	86.7	87.4	87.1	87.1
Crude protein†, % of DM	12.7	10.5	15.4	13.6	14.0
Crude fiber†, % of DM	20.9	19.3	12.2	9.9	8.3
NDF†‡, % of DM	45.2	43.0	29.8	26.9	24.2
TDN†§, % of DM	72.1	62.8	73.8	76.7	79.4

†Calculated from the values in standard tables of feed composition in Japan (2001). ‡Neutral detergent fiber. §Total digestible nutrients.

Shimadzu). 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.

Free amino acids and other chemical analyses

The FAA, dipeptide, and IMP contents were determined by the method of Chikuni *et al.* (2002). About 5 g of raw meat sample was weighed, homogenized with 16 mL of distilled water, and adjusted to a total volume of 20 mL with distilled water. This homogenate was used for various analyses. For the determination of the FAA and dipeptide contents, 5 mL of the homogenate was mixed with 5 mL of 10% (w/v) trichloroacetic acid, stirred, and centrifuged at 1700 *g* for 15 min for deproteinization. The supernatant was set in an amino acid analyzer (L-8800; Hitachi, Tokyo, Japan) and assayed by the ninhidrin detection method. For the determination of the IMP content, perchloric acid was added to 5 mL of the homogenate to a final concentration of 6%, and the mixture was centrifuged at 1700 *g* for 15 min for deproteinization. The supernatant was adjusted to pH 6.5–6.8 using a potassium hydroxide solution, allowed to stand for 30 min in an ice bath, and filtered through a 0.45-μm microfilter to remove potassium perchlorate. The filtrate was adjusted to a total volume of 10 mL with distilled water, and was analyzed by a high-performance liquid chromatography using a ODS column (Shim-Pack CLC-ODS (6 mmID × 15 cm, 5 μm particle diameter), Shimadzu) and flowing mobile phases of 40 mmol/L potassium dihydrogen phosphate and 60 mmol/L dipotassium hydrogen phosphate at a flow rate of 1 mL/min. For the detection, a visual light-ultraviolet detector (L-4000; Hitachi) was used, and the absorbance at 254 nm was determined. The area of the peak of IMP observed at approximately 4.6 min was calculated, and the IMP content of the sample was estimated. The crude fat content was determined by the ether extraction method (AOAC 1990).

Statistical analysis

Statistical analysis was performed by analysis of variance using the general linear model (GLM) procedures of Statistical Analysis Systems (SAS Institute, Cary, NC, USA). Differences between means by treatment were tested using Student's *t*-test, and significance was evaluated at the 5% level.

RESULTS AND DISCUSSION

No difference was observed in the body weight ($P = 0.21$) or height ($P = 0.24$) at the age of 30 months between the 20- and 24-month groups (Table 2). The body weight of the 24-month group did not increase from the age of 30 months to 34 months (Table 2). There was no difference in the TDN intake or feed efficiency during 10–30 months of age between the two groups (Table 2). There were no difference in the carcass weight, meat grade, marbling, meat color, longissimus muscle area, subcutaneous fat thickness, or crude fat content of the longissimus muscle between the two groups (Table 3). Okumura *et al.* (2007) reported the effects of a delay in the slaughter age from 24 to 30 months on the carcass characteristics and chemical composition of major muscles in 4 pairs of identical twins of Japanese Black steers. They indicated that the carcass weight was significantly heavier, and the crude fat content of the longissimus muscle was about 5.4% greater, in the 30- than in the 24-month group. The steers in the study of

Table 2 The effects of the fattening period on feed intake and growth performance

Item	Fattening period (months)	
	20 ($n = 5$)	24 ($n = 5$)
TDN intake, kg		
10–30 months of age	3242.1 ± 23.7	3172.0 ± 20.9
31–34 months of age	–	532.9 ± 34.1
TDN/gain, kg/kg		
10–30 months of age	8.5 ± 0.4	9.4 ± 0.5
Body weight, kg		
10 months of age	262.5 ± 9.1	259.2 ± 6.5
30 months of age	633.8 ± 20.6	598.4 ± 15.9
34 months of age	–	618.8 ± 19.7
Withers height, cm		
10 months of age	114.2 ± 0.9	113.0 ± 1.0
30 months of age	139.4 ± 1.4	136.6 ± 1.7
34 months of age	–	137.6 ± 1.8

Values are means ± standard error. TDN, Total digestible nutrients.

Table 3 The effects of the fattening period on carcass characteristics

Item	Fattening period (months)	
	20 ($n = 5$)	24 ($n = 5$)
Carcass weight, kg	390.8 ± 17.8	374.4 ± 12.3
Meat quality score†	4.2 ± 0.2	4.6 ± 0.2
Marbling score‡	6.0 ± 0.5	7.4 ± 0.8
Beef color score§	3.4 ± 0.2	3.8 ± 0.4
Longissimus muscle area¶, cm^2	49.4 ± 2.0	45.6 ± 3.9
Fat thickness††, cm	3.1 ± 0.3	3.2 ± 0.3
Intramuscular crude fat‡‡, %	33.7 ± 1.8	37.2 ± 2.6

†,‡,§,¶,††These carcass characteristics were evaluated according to the procedures of the Japan Meat Grading Association (JMGA 1988). †A higher number indicates a better quality (1 to 5). ‡A higher number indicates more marbling (1 to 12). §A higher number indicates a darker color (1 to 7). ‡‡Determined in longissimus muscle obtained from the 7th ribs. Values are means ± standard error.

Okumura *et al.* (2007) were fattened from 10 months of age similar to the present study. In the present study, there was no difference ($P = 0.29$) in the crude fat content of the muscle between the two groups. These results suggest that the impact of prolongation of the fattening period on carcass weight and the crude fat content of muscles is minimum after 30 months of age.

Table 4 shows the fatty acid composition of subcutaneous, intermuscular, intramuscular, and perinephric fat. While in subcutaneous fat, the percentage of palmitoleic acid (C16:1) was higher ($P < 0.05$) in the 24- than in the 20-month group, no difference was noted in the other fatty acids ($P > 0.05$). For intermuscular fat, no difference was noted in any fatty acid level ($P > 0.05$). For intramuscular fat, the percentages of C18:1 and total monounsaturated fatty acid (MUFA) were higher ($P < 0.05$) by 2.25% and 2.29%, respectively, and the percentage of total saturated fatty acid (SFA) was lower ($P < 0.05$) in the 24- compared with the 20-month group. In perinephric fat, the percentages of C18:1 and total MUFA were higher by 6.42% and 6.55% ($P < 0.05$), respectively, and the percentages of C16:0, C18:0, and total SFA were reduced ($P < 0.05$) in the 24- compared with the 20-month group. Clemens *et al.* (1973) reported that the percentage of C18:1 in intramuscular fat was markedly higher in the group slaughtered at the age of 18–24 months than in the group slaughtered at the age of 12–15 months, but no significant difference was observed in the fatty acid composition of subcutaneous fat regardless of the age at slaughter in Angus bulls and steers. These results suggest that the effects of the fattening period on the percentages of C18:1 and total MUFA of fat deposits are greater in intramuscular and perinephric fat in deep areas of the body than in subcutaneous and intermuscular fat located near the body surface.

The fatty acid composition changes with the duration of fattening, age, carcass weight, and degree of fat deposition (Waldman *et al.* 1968; Leat 1975; Huerta-Leidenz *et al.* 1996; Zembayashi & Nishimura 1996). In the present study, the effects of genetic factors and feed were minimized using Japanese Black steers derived from the same sire and fattening them with the same feed. Therefore, the differences in the fatty acid composition in fat deposits between the two groups are considered to be due to the duration of fattening. The present study showed the percentage of C18:1 increased in intramuscular and perinephric fat with prolongation of the fattening period. C18:1 is

Table 4 The effects of the fattening period on the fatty acid composition of subcutaneous, intermuscular, intramuscular, and perinephric fat

Item	Subcutaneous fat		Intermuscular fat		Intramuscular fat		Perinephric fat	
	20 ($n = 5$)	24 ($n = 5$)	20 ($n = 5$)	24 ($n = 5$)	20 ($n = 5$)	24 ($n = 5$)	20 ($n = 5$)	24 ($n = 5$)
Fatty acid composition, %								
C14:0	2.38 ± 0.09	2.36 ± 0.12	2.52 ± 0.11	2.32 ± 0.09	3.04 ± 0.20	2.73 ± 0.12	2.78 ± 0.29	2.42 ± 0.17
C14:1	1.46 ± 0.11	2.25 ± 0.38	1.57 ± 0.12	1.65 ± 0.32	1.32 ± 0.21	1.22 ± 0.10	0.70 ± 0.19	0.68 ± 0.07
C16:0	22.50 ± 0.65	22.29 ± 0.50	23.65 ± 0.62	22.64 ± 0.58	28.27 ± 0.92	26.59 ± 0.15	26.36 ± 0.74	24.00 ± 0.68*
C16:1	5.14 ± 0.23	7.30 ± 0.76*	5.43 ± 0.25	5.72 ± 0.75	4.54 ± 0.46	4.69 ± 0.31	2.28 ± 0.45	2.50 ± 0.17
C18:0	8.95 ± 0.48	6.40 ± 0.94	8.60 ± 0.46	8.22 ± 0.94	9.86 ± 0.86	9.77 ± 0.43	18.97 ± 1.95	15.50 ± 0.99*
C18:1	56.61 ± 0.67	56.63 ± 0.90	55.32 ± 0.65	56.78 ± 1.11	50.55 ± 0.83	52.80 ± 0.36*	46.18 ± 1.83	52.60 ± 1.01*
C18:2	2.26 ± 0.17	2.05 ± 0.10	2.24 ± 0.18	2.04 ± 0.17	1.98 ± 0.34	1.81 ± 0.13	2.15 ± 0.48	1.78 ± 0.12
C18:3	0.11 ± 0.01	0.10 ± 0.00	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.11 ± 0.03	0.08 ± 0.01
C20:0	0.60 ± 0.04	0.58 ± 0.04	0.57 ± 0.04	0.53 ± 0.05	0.35 ± 0.05	0.31 ± 0.02	0.47 ± 0.06	0.44 ± 0.03
SFA	34.43 ± 0.98	31.63 ± 0.85	35.34 ± 0.89	33.71 ± 1.30	41.52 ± 0.57	39.30 ± 0.46*	48.58 ± 2.30	42.40 ± 0.94*
MUFA	63.20 ± 0.85	66.21 ± 0.94	62.31 ± 0.75	64.14 ± 1.30	56.41 ± 0.40	58.70 ± 0.45*	49.15 ± 2.32	55.70 ± 0.88*
PUFA	2.37 ± 0.18	2.16 ± 0.09	2.35 ± 0.19	2.14 ± 0.18	2.07 ± 0.36	1.89 ± 0.13	2.26 ± 0.51	1.86 ± 0.13

*Significant difference from value in the 20-month group ($P < 0.05$). Values are means ± standard error. MUFA, monounsaturated fatty acids (sum of 14:1, 16:1, and 18:1); PUFA, polyunsaturated fatty acids (sum of 18:2 and 18:3); SFA, saturated fatty acids (sum of 14:0, 16:0, 18:0, and 20:0).

synthesized from C18:0 by stearoyl-CoA desaturase (SCD). In cattle, high-level SCD expression is observed in adipose tissues (Cameron *et al.* 1994). The quantity of SCD mRNA expression in subcutaneous fat of Angus steers collected by biopsy reached a peak at the age of 12 months and decreased thereafter (Martin *et al.* 1999). In contrast, the SCD activity increased until the age of 28 months in subcutaneous fat of Wagyu on long-term fattening (Chung *et al.* 2007). In ovine adipose tissue, the quantity of SCD mRNA expression has been shown to vary with the sampling site (Barber *et al.* 2000). These reports suggest that expression of the SCD gene and SCD activity in fat deposits vary with the site and age. The differences in the percentage of C18:1 observed in the present study might have been due to the difference in the SCD activity of the fat deposit sites.

FAAs and IMP are known to be typical taste-improving components of meat (Nishimura *et al.* 1988). FAAs have been reported to vary according to the breed, postmortem period (Watanabe *et al.* 2004), and site in the muscle (Cornet & Bousset 1999). In the present study, threonine and tyrosine content of the longissimus muscle were lower ($P < 0.05$) in the 24- compared with the 20-month group, but no difference was noted in any other FAAs, dipeptide, or total FAA level (Table 5). Watanabe *et al.* (2004) reported that slaughter age affected the FAA and dipeptide contents of the longissimus muscle, and that the contents of many FAAs and dipeptides decreased by a delay in the slaughter age from 25 to 35 months. The decreases in FAA and dipeptide contents are reportedly affected by reductions in the protein content associated with increases in intramuscular crude fat contents (Ueda *et al.* 2007). Non-significant differences in the FAA or dipeptide content of the present study might be partly due to no difference ($P = 0.29$) in the crude fat content between the two groups.

On the other hand, the IMP content was higher ($P < 0.05$) in the 24- compared with the 20-month group. IMP contents of beef were negatively correlated with intramuscular crude fat content (Tsuneishi 1999). In the present study, there was no difference ($P = 0.29$) in the crude fat content between the two groups. It is not clear why the IMP content was higher in the 24- compared with the 20-month group. In the heart of chicken, ATP content and AMP catabolism increased with age (Wegelin *et al.* 1995). Prolongation of the fattening period might influence ATP content and AMP catabolism, and consequently increase the IMP content of beef.

Table 5 The effects of the fattening period on free amino acid, dipeptide, and inosinic acid contents of the thoracic longissimus muscle

Item	Fattening period (months)	
	20 ($n = 5$)	24 ($n = 5$)
Free amino acid content, μmol/g		
Taurine	1.197 ± 0.109	1.112 ± 0.05
Aspartic acid	0.495 ± 0.042	0.589 ± 0.03
Threonine	0.237 ± 0.010	0.205 ± 0.01*
Serine	0.335 ± 0.018	0.319 ± 0.02
Glutamic acid	0.577 ± 0.054	0.450 ± 0.03
Proline	0.859 ± 0.073	0.739 ± 0.04
Glycine	0.872 ± 0.076	0.684 ± 0.07
Alanine	2.590 ± 0.208	2.714 ± 0.19
Valine	0.293 ± 0.017	0.336 ± 0.02
Methionine	0.079 ± 0.002	0.081 ± 0.00
Isoleucine	0.210 ± 0.009	0.198 ± 0.01
Leucine	0.337 ± 0.013	0.320 ± 0.01
Tyrosine	0.127 ± 0.003	0.116 ± 0.00*
Phenylalanine	0.136 ± 0.004	0.142 ± 0.00
β-Alanine	0.121 ± 0.017	0.109 ± 0.01
γ-Amino-n-butyric acid	0.016 ± 0.002	0.017 ± 0.00
Ornithine	0.131 ± 0.019	0.110 ± 0.02
Lysine	0.458 ± 0.050	0.384 ± 0.02
Histidine	0.173 ± 0.012	0.145 ± 0.01
Arginine	0.333 ± 0.029	0.309 ± 0.03
Total	9.723 ± 1.088	9.207 ± 0.87
Dipeptide content, μmol/g		
Anserine	2.669 ± 0.155	2.871 ± 0.20
Carnosine	9.965 ± 0.212	10.320 ± 0.59
Inosinic acid content, μmol/g	1.683 ± 0.076	2.166 ± 0.13*

*Significant difference from value in the 20-month group ($P < 0.05$). Values are means ± standard error.

These observations suggest that prolongation of the fattening period from 20 to 24 months in Japanese Black steers increases the percentage of C18:1 and total MUFA in intramuscular and perinephric fat and the IMP content of the longissimus muscle. Furthermore, the effect of the fattening period on the percentage of C18:1 and total MUFA was shown to be greater in fat in deep areas of the body than in fat near the body surface.

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