Animal Science Journal (2009) **80,** 62–69 doi: 10.1111/j.1740-0929.2008.00594.x

ORIGINAL ARTICLE

Effects of GH gene polymorphism and sex on carcass traits and fatty acid compositions in Japanese Black cattle

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ABSTRACT

To investigate the effects of bovine growth hormone (bGH) gene polymorphism on carcass traits and fatty acid compositions in Japanese Black cattle caused by nucleotide substitution of CTG (allele A)/GTG (allele B) at codon 127 and of ACG (allele A and B)/ATG (allele C) at codon 172 of bGH, GH genotypes of 135 cattle were determined using allele specific-multiplex polymerase chain reaction (PCR). Allele A gave greater rib thickness and lower melting point of fat (MP) while allele B gave higher C18:1% ($P < 0.05$). Allele C gave higher C18:1, monounsaturated fatty acid (MUFA), unsaturated fatty acid (USFA) percentages ($P < 0.05$). It also gave lower saturated fatty acid (SFA) percentages, higher MUFA/SFA and USFA/SFA ratios, and lower MP ($P < 0.05$). Interactions of sex and GH alleles were analyzed. In heifers, allele A gave higher carcass weight, daily carcass gain, rib eye area, rib thickness, subcutaneous fat thickness, and BMS while allele B gave greater rib eye area and rib thickness ($P < 0.05$). Allele C gave higher C18:1 ($P < 0.01$), MUFA ($P < 0.01$), USFA percentages ($P < 0.05$) and MUFA/SFA and USFA/SFA ratios ($P < 0.01$), and lower C16:0 and SFA percentages ($P < 0.05$) and MP ($P < 0.01$). GH gene polymorphism affected carcass traits and fatty acid compositions although the effects were more pronounced in heifers.

Key words: *carcass traits, fatty acids, GH genotype, Japanese Black, polymorphism.*

INTRODUCTION

Japanese Black cattle are famously known for their highly marbled beef and higher concentrations of subcutaneous (s.c.) and intramuscular (i.m.) MUFA compared with Holstein, Japanese Brown, and Charolais cattle (Zembayashi *et al.* 1995). The high level of MUFA in beef contributes to the fat softness and palatability (Yang *et al.* 1999; Smith *et al.* 2006). A previous study on Japanese Black steers fed the same diet and slaughtered at same age suggested that the grade of beef marbling and fatty acid composition was affected by a genetic factor carried by some Japanese Black sires (Oka *et al.* 2002).

Nowadays, much study into the use of genetic markers to select superior animals has been carried out. The bovine growth hormone (bGH) gene has been intensively studied as a candidate genetic marker because it has important roles in regulating animal growth and production. Moreover, exogenous GH is believed to be effective in increasing average daily

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Received 11 September 2007; accepted for publication 23 January 2008.

gain, feed efficiency and edible lean, and reducing carcass fat in steers (Moseley *et al*. 1992; Schlegel *et al*. 2006). Previously, it has been reported that a single nucleotide polymorphism (SNP) in the bovine GH gene caused by a nucleotide substitution of CTG/GTG at codon 127 was detected in different rates in Holstein and other major dairy breeds (Chikuni *et al*. 1991; Lucy *et al*. 1993; Schlee *et al*. 1994; Lee *et al*. 1996; Yao *et al*. 1996; Sorensen *et al*. 2002). Also, in Japanese Black and Brown cattle, the polymorphic substitution of ACG/ATG was observed at codon 172 (Chikuni *et al*. 1994, 1997). Codon 127 that codes for either Leu or Val, is represented by allele A or B, respectively, and codon 172 that codes for Met is represented by allele C. Finally, animals are categorized into 6 GH genotypes, namely genotype AA, AB, BB, AC, BC, and CC.

Some studies of relationship between GH gene polymorphism on bGH codon 127 and 172 and carcass traits in Japanese Black cattle have been established in Japan (Kono 2005; Oka *et al*. 2007; Katoh *et al*. 2008). The average body weight of Japanese Black bull calves with GH genotype BB was found to be significantly lower than those with genotype AA or AB. Also, calves with genotype AA were found to have higher concentrations of GH and IGF-1 after GHRH treatment than calves with other genotypes (Katoh *et al*. 2008). Another result showed that Japanese Black steers with GH genotype AA has greater carcass weight and overall mean concentration of plasma GH than those with genotype BB (Oka *et al*. 2007).

Moreover, in cattle, body growth rate and GH secretion are known to be regulated in a sex-specific manner. Male cattle were found to have greater rate of body growth since early postnatal life than female cattle (Gatford *et al*. 1998). Also, mean GH concentration, GH pulse amplitude, GH pulse frequency were greater in male than female cattle (Plouzek & Trenkle 1991). Gonadal steroids which have anabolic activity were also known to increase growth rate and to improve feed efficiency (Dikeman 2007). Intact male cattle had higher mean plasma GH concentration, area under the GH profile, and GH pulse amplitude than intact female and castrated male and female cattle. However, castration in females gave no impact on GH secretion pattern, which suggested that testosterone enhanced GH secretion (Plouzek & Trenkle 1991). Estrogen treatment has also been shown to increase circulating GH concentration in heifers (Preston 1975) and steers (Hayden *et al*. 1992). Castration in male cattle is known to reduce the carcass gain and increase meat tenderness (Laflamme & Burgess 1973; Unruh 1986). A previous study reported that steers had greater levels of circulating GH and IGF-1 than heifers (Brandt *et al*. 2007). Moreover, it was shown that steers had greater carcass weight, carcass weight gain per day, and rib eye area than heifers (Tanner *et al*. 1970; Brandt *et al*. 2007). These results suggested that castration in male cattle apparently did not change the fact that sex difference is involved in regulation of somatotropic axis.

However, the effects of variation of GH genotypes together with cattle sex on carcass quality in cattle have not yet been reported. Therefore, the objective of the present research was to determine the effects of interaction between GH gene polymorphism and sex on carcass traits and fatty acid compositions in Japanese Black cattle.

MATERIALS AND METHODS

Samples collection

Intramuscular (i.m.) adipose tissues at the cross-sectional location of the 6th to 7th of longissimus thoracis muscle and subcutaneous (s.c.) adipose tissues were collected as specimens of Japanese Black steers ($n = 91$) and heifers ($n = 44$) with 30.6 ± 2.7 months and 429.2 ± 62.6 kg of averaged slaughter age and carcass weight, respectively. Samples were stored at −20°C until genomic DNA extraction and carcass traits analysis were completed.

Bovine GH genotyping

Two hundreds milligrams of adipose tissue was collected from 135 Japanese Black cattle. Genomic DNA was then isolated using QuickGene-800 automatic nucleic acid isolation system (Fujifilm, Tokyo, Japan) and the eluted DNA concentration was measured using a GeneQuant DNA/RNA calculator (Amersham Pharmacia Biotech, Tokyo, Japan). The GH genotype of cattle was identified by allele specific-multiplex (ASM)-polymerase chain reaction (PCR) according to method of Chikuni *et al*. (1997) with a slight modification. ASM-PCR was carried out in 25 μL reaction mixtures containing 250 ng of template DNA, 10 mmol/L Tris HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 200 μmol/L each of dNTPs, 0.5 units of AmpliTaq Gold DNA polymerase (Applied Biosystem, Foster City, CA, USA), 10 pmol each of common primers GH4F and GH5R and 5 pmol and 3 pmol of specific primers GHAR and GHABR (Sigma Genosys, Sigma-Aldrich Japan, Ishikari, Japan), respectively. The sequences of primers are outlined in Table 1. One set of six standards of template DNA for GH genotype (AA, AB, BB, AC, BC, and CC) provided from livers of Japanese Black cattle was used for a test for PCR conditions. Template DNA was amplified using a PC808 thermal cycler (Astec, Fukuoka, Japan) as follows: first cycle of denaturation (94°C for 9 min), 38 cycles of denaturation (94°C for 30 s), annealing (55°C for 20 s),

and extension (72°C for 15 s), followed by one cycle of final extension (72°C for 7 min). PCR products were then analyzed by horizontal electrophoresis (100 V for 35 min) through 2% agarose gel (Invitrogen, Carlsbad, CA, USA), ethidium bromide staining (0.5 μg/mL) for 40 min, and visualized by UV fluorescence using Chemiimager digital imaging system (Alpha Innotech Corporation, San Diego, CA, USA).

Carcass traits

Data of carcass traits of Japanese Black cattle were collected from documentation of the slaughterhouse in Sendai, Japan. Meat quality was evaluated by official graders according to Japan Meat Grading Association on the 6th to 7th rib section (JMGA 1988). The analyzed data included yield estimate (YE,%), carcass weight (CW, kg), daily carcass gain (DCG, kg/day), cross-sectional area of longissimus thoracis muscle which is also known as rib eye area (REA, cm^2), rib thickness (RT, cm), subcutaneous fat thickness (SFT, cm), and beef marbling score (BMS). DCG is calculated by dividing carcass weight (kg) with slaughter age (days).

Fatty acid analysis

Neutral lipid was extracted from i.m. adipose tissue using the following method. Twenty milligrams of i.m. adipose tissues was placed in a reaction tube with screw-cap to which 1 ml of n-hexane (Wako Pure Chemical Industries, Osaka, Japan) was added and vigorously mixed for 1 min. Then, 200 μL of 2N NaOH methanol was added and vortex-mixed for 1 min. The tube was incubated in a water bath at 50°C for 20 s and was vortex-mixed for 1 min afterwards. After cooling down the mixture to room temperature, 600 μL of hydrogen chloride methanol reagent (Tokyo Chemical Industry Co., Tokyo, Japan) was added into the tube and the tube was vigorously vortex-mixed again for 1 min. After the mixture separated into two layers, the upper layer was carefully transferred into another reaction tube using a capillary glass pipette. The upper layer, which contained fatty acid methyl ester, was then concentrated by placing it in a stream of nitrogen on a heating block at 55°C.

The methylated neutral lipid was analyzed using a flame ionization detector on a gas chromatograph (Hewlett-Packard 5890 Series II; Hewlett-Packard, Wilmington, DE, USA) equipped with a 30-m × 0.25-mm glass column (DB-23, J&W Scientific; Agilent Technologies, Folsom, CA, USA). The carrier gas was nitrogen and the column oven condition was programmed from an initial temperature of 100°C to a final temperature of 230°C at the rate of 4°C/min. The peak areas of fatty acid methyl ester were quantified with an electric integrator (C-R6A, Chromatopac; Shimazu, Kyoto, Japan). Identification of fatty acids (myristic acid, C14:0; palmitic acid, C16:0; palmitoleic acid, C16:1; stearic acid, C18:0; oleic acid, C18:1; linoleic acid, C18:2) was achieved by comparing the relative retention time of fatty acid methyl-ester peaks from samples with those of standards. The rising melting points were done according to the capillary tube method (AOAC 1975).

The data comprised percentages of C14:0 (myristic acid), C16:0 (palmitic acid), C16:1 (palmitoleic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), unsaturated fatty acid (USFA), and melting point of fat (MP,°C)

Statistical analysis

To determine the effects of GH alleles on carcass traits and i.m. fatty acid compositions, after GH genotyping, Japanese Black cattle were divided into 6 allele groups according to presence of related alleles, that is A or non-A, B or non-B, and C or non-C, as described in Table 2. Also, to analyze the effects of interaction between cattle sex and GH gene polymorphisms, animals were grouped based on their alleles and sexes. Effects of GH alleles and their interactions with sex on carcass traits and i.m. fatty acid compositions in Japanese Black cattle were analyzed by analysis of variance (ANOVA) using GLM procedure of SAS (SAS Institute, Cary, NC, USA). Slaughter age (month) was included in the model used for analyzing. Least squares means were computed and statistically separated with the PDIFF option. All data are presented as least squares means. Differences were considered highly significant at $P < 0.01$, significant at $P < 0.05$, and differences at $P < 0.10$ were considered to have tendency.

Table 1 Primers sequences for identification of bGH genotype polymorphism at GH codon 127 and 172 using ASM-PCR (Chikuni *et al*. 1997)

Name	Primer	Sequence
GH4F	Forward	5′-TCTATGAGAAGCTGAAGGACC TGGAGGAA-3′
GHAR	Reverse	5′-CGGGGGGTGCCATCTTCCAG-3′
GHABR	Reverse	5′-ATGACCCTCAGGTACGTCTCCG-3′
GH5R	Reverse	5′-CCAGAATAGAATGACACCTACT CAGACAAT-3′

Table 2 Animal grouping after GH genotype identification using ASM-PCR

Animal	Number	GH genotype						GH allele					
		AA	AB	BB	AC	BC	CC	A[1]	Non-A[2]	B[3]	Non-B[4]	C[5]	Non-C[6]
Steer	91	38	18	17	4	13	1	60	31	48	43	18	73
Heifer	44	20	4	13	3	3	1	27	17	20	24	7	37
Total	135	58	22	30	7	16	2	87	48	68	67	25	110

[1]GH genotype AA, AB, and AC; [2]GH genotype BB, BC, and CC; [3]GH genotype AB, BB, and BC; [4]GH genotype AA, AC, and CC; [5]GH genotype AC, BC, and CC; [6]GH genotype AA, AB, and BB.

ASM-PCR, Allele Specific Multiplex PCR; GH, growth hormone.

RESULTS

Carcass traits

To evaluate the effect of every GH allele on carcass traits, cattle were grouped based on the presence of every allele into group A or non-A, group B or non-B, and group C or non-C after GH genotyping, regardless of the cattle sex. RT in group A was significantly greater than that in group non-A (8.36 vs. 7.59 cm). There were also tendencies for group A to have higher CW and DCG compared with group non-A (439.35 vs. 405.29 kg and 0.49 vs. 0.45 kg/day, respectively). Group B were found to have greater RT than group-non-B (8.26 vs. 7.69 cm), although the difference was only a tendency. Allele C had no effect on carcass traits.

Next, the significance levels of effects of cattle sex, GH alleles, and their interactions on carcass traits were analyzed as shown in Table 3. It is already known that cattle growth rate is higher in males than in females (Gatford *et al.* 1998), therefore we did not do any further analysis for this. However, there were some significant effects of interactions between cattle sex and GH allele A and B on carcass traits, although the significance levels varied among alleles and carcass traits. These results suggest that cattle sex might have an important role in determining the GH gene polymorphism effects on carcass traits.

Therefore, further analysis was carried out by grouping cattle based on sex and GH alleles as shown in Table 4. There was no significant differences between steers in group A and non-A. However, heifers in group A had significantly higher CW, DCG, REA, RT, SFT, and BMS values than those in group non-A. There was also a tendency for heifers in group A to have higher YE. Steers in group B had lower YE, REA, BMS, and fat grade than those in group non-B, although the differences were not significant. In contrast, heifers in group B have significantly higher REA and RT than those in group non-B. There were no significant differences between cattle in group C and non-C for both sexes.

Intramuscular fatty acid composition

To evaluate the effect of GH gene polymorphism on i.m. fatty acid compositions, cattle were grouped into group A or non-A, group B or non-B, and group C or non-C after GH genotyping, regardless of the cattle sex. Cattle in group A had significantly lower MP than those in group non-A (27.20 vs. 31.03°C). Cattle in group B had significantly higher percentage of C18:1

Table 3 Significance levels of effects of sex, GH alleles, and interaction between sex and GH alleles on carcass traits in Japanese Black cattle ($n = 135$)

Source	YE	CW	DCG	REA	RT	SFT	BMS
Sex	NS	**	**	†	NS	NS	NS
A	NS	†	†	NS	*	NS	NS
B	NS	NS	NS	NS	†	NS	NS
C	NS	NS	NS	NS	NS	NS	NS
Sex*A	*	*	*	**	**	NS	**
Sex*B	*	†	†	*	*	NS	*
Sex*C	NS	NS	NS	NS	NS	NS	NS

†$P < 0.10$, *$P < 0.05$, **$P < 0.01$.

CW, carcass weight; DCG, daily carcass gain; GH, growth hormone; NS, non-significant; REA, rib eye area; RT, rib thickness; SFT, subcutaneous fat thickness; BMS, beef marbling score; YE, yield estimate.

Table 4 Effects of interaction between cattle GH gene polymorphism and sex on carcass traits in Japanese Black cattle[a]

Traits	Steer		Heifer		Steer		Heifer		Steer		Heifer	
	A[1]	Non-A[2]	A[1]	Non-A[2]	B[3]	Non-B[4]	B[3]	Non-B[4]	C[5]	Non-C[6]	C[5]	Non-C[6]
YE (%)	73.89	74.69	75.20†	73.08	73.91	74.67†	75.18	73.10	74.18	74.41	74.06	74.22
CW (kg)	462.10	466.87	416.60*	343.71	458.60	470.37	405.45	354.86	474.96	454.01	401.03	359.27
DCG (kg/day)	0.52	0.52	0.46*	0.38	0.51	0.52	0.45	0.40	0.53	0.50	0.45	0.40
REA (cm^2)	56.65	60.84	60.84*	44.19	56.03	61.46†	58.68*	46.35	60.51	56.97	53.74	51.29
RT (cm)	8.11	8.25	8.60*	6.92	8.11	8.25	8.41*	7.11	8.10	8.26	8.15	7.38
SFT (cm)	2.77	2.57	2.98*	2.69	2.72	2.63	2.70	2.98	2.83	2.52	3.11	2.56
BMS	6.19	7.71	7.43*	4.20	6.14	7.76†	7.39†	4.24	6.59	7.31	5.57	6.05

†$P < 0.10$, *$P < 0.05$, **$P < 0.01$.

[a]Values are expressed as least squares means ($n = 135$).

[1]GH genotype AA, AB, and AC; [2]GH genotype BB, BC, and CC; [3]GH genotype AB, BB, and BC; [4]GH genotype AA, AC, and CC; [5]GH genotype AC, BC, and CC; [6]GH genotype AA, AB, and BB.

BMS, beef marbling score; CW, carcass weight; DCG, daily carcass gain; GH, growth hormone; REA, rib eye area; RT, rib thickness; SFT, subcutaneous fat thickness; YE, yield estimate.

Table 5 Significance levels of effects of sex, GH alleles, and interaction between sex and GH alleles on carcass traits in Japanese Black cattle ($n = 79$)

Source	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	SFA	MUFA	USFA	MUFA/SFA	USFA/SFA	MP
Sex	NS	*	NS	NS	*	NS	*	*	*	*	*	NS
A	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
B	*	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	†
C	NS	†	NS	†	*	NS	*	*	*	*	*	*
Sex*A	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sex*B	*	†	NS	NS	†	NS	NS	NS	NS	NS	NS	NS
Sex*C	NS	*	NS	NS	†	NS	†	*	†	*	*	†

†$P < 0.10$, *$P < 0.05$, **$P < 0.01$.

GH, growth hormone; MP, melting point; MUFA, monounsaturated fatty acids; NS, non-significant; SFA, saturated fatty acids; USFA, unsaturated fatty acids.

Table 6 Effects of interaction between GH gene polymorphism and sex on i.m. fatty acid compositions in Japanese Black cattle[a]

Traits	Steer		Heifer		Steer		Heifer		Steer		Heifer	
	A[1]	Non-A[2]	A[1]	Non-A[2]	B[3]	Non-B[4]	B[3]	Non-B[4]	C[5]	Non-C[6]	C[5]	Non-C[6]
C14:0	2.19	2.02	1.97	2.30	2.12	2.10	1.71	2.56**	2.08	2.14	2.05	2.22
C16:0	23.89	23.35	21.45	23.28	23.73	23.51	20.99	23.74*	23.73	23.50	21.10	23.63*
C18:0	12.63	13.98	12.36	12.41	12.95	13.66	12.66	12.12	12.84	13.77	11.42	13.36
C16:1	3.72	3.43	3.64	4.02	3.61	3.55	3.41	4.26	3.67	3.48	3.95	3.71
C18:1	55.11	54.87	58.24	55.64	55.19	54.81	59.01*	54.88	55.25	54.74	59.14**	54.74
C18:2	2.45	2.34	2.33	2.35	2.42	2.38	2.23	2.45	2.42	2.37	2.35	2.33
SFA	38.71	39.35	35.78	37.99	38.80	39.27	35.36	38.42	38.65	39.41	34.56	39.21*
MUFA	58.84	58.31	61.89	59.66	58.79	58.36	62.41	59.14	58.93	58.22	63.09**	58.46
USFA	61.29	60.65	64.22	62.01	61.20	60.73	64.64	61.58	61.35	60.59	65.44*	60.79
MUFA/SFA	1.53	1.51	1.76	1.59	1.53	1.51	1.79†	1.56	1.54	1.50	1.84**	1.51
USFA/SFA	1.59	1.57	1.82	1.65	1.59	1.57	1.85	1.63	1.60	1.56	1.90**	1.57
MP (°C)	27.88	31.65	26.52	30.42	29.15	30.38	25.70	31.24†	29.21	30.32	25.08	31.86**

†$P < 0.10$, *$P < 0.05$, **$P < 0.01$.

[a]Values are expressed as least squares means ($n = 79$).

[1]GH genotype AA, AB, and AC; [2]GH genotype BB, BC, and CC; [3]GH genotype AB, BB, and BC; [4]GH genotype AA, AC, and CC; [5]GH genotype AC, BC, and CC; [6]GH genotype AA, AB, and BB.

GH, growth hormone; MP, melting point; MUFA, monounsaturated fatty acids; NS, non-significant; SFA, saturated fatty acids; USFA, unsaturated fatty acids.

(57.10 vs. 54.84%) and tended to have lower melting point than those in group non-B (27.43 vs. 30.80°C).

Cattle in group C had significantly higher percentages of C18:1, MUFA, and USFA, and MUFA/USFA and USFA/SFA ratios than those in group non-C (57.20 vs. 54.74%, 61.01 vs. 58.34%, and 63.39 vs. 60.91% and 1.69 vs. 1.51 and 1.75 vs. 1.57, respectively). In contrast, animals in group C tended to have lower percentages of C16:0 and C18:0 (22.42 vs. 23.57% and 12.13 vs. 13.56%, respectively) and significantly lower percentage of SFA than those in group non-C (36.61 vs. 39.31%). Moreover, cattle in group C had significantly lower MP than those in group non-C (27.14 vs. 31.09°C).

As shown in Table 5, there were significant effects of interaction between sex and GH alleles on fatty acid composition, although the significance levels were different among factors. Therefore, further analysis using cattle grouped by sex and GH allele were carried out as described in Table 6. Unlike the result for carcass traits analysis, there were no difference of fatty acid compositions between steers and heifers in group A and non-A. There was no significant difference either between steers in group B and non-B. However, heifers in group B had significantly higher C18:1% and tended to have higher MUFA/SFA ratio ($P < 0.10$). Heifers in group B also had significantly lower percentages of C14:0 and C16:0 and tendency for lower MP than those in group non-B. There was no significant difference between steers in group C and non-C for fatty acid compositions. Nevertheless, some significant differences were found between heifers in group C and

non-C. Heifers in group C had significantly higher percentages of C18:1, MUFA, and USFA and MUFA/SFA and USFA/SFA ratios. They also had lower percentages of C16:0 and SFA and lower MP.

DISCUSSION

GH acts directly by binding to its receptors on precursor bone, muscle, and fat cells and triggers cell proliferation. Also, it acts indirectly by binding to receptors in liver and some other tissues to stimulate secretion of insulin-like growth factor-I (IGF-I) that has role in promoting cell growth (Kopchick & Cioffi 1991). GH induces lipolysis in adipose tissues, increases gluconeogenesis and proteosynthesis in liver, enhances protein uptake in muscle along with IGF-1, and promotes the growth of mammary gland (Renaville *et al*. 2002).

In the previous research, Japanese Black calves with GH genotype AA had higher concentration of basal GH than those with genotype BB. They also had the highest body weight at 10 months of age compared to those with GH genotype AB and BB (Katoh *et al*. 2008). It was also suggested that allele A had role in improving body weight in Japanese Black cattle whereas allele B is correlated with significant decreases in carcass weight (Oka *et al*. 2007). Moreover, GH genotype BB gave lower body weight and daily gain compared with genotype AA and AB (Kono 2005; Katoh *et al*. 2008) and cattle with GH genotype CC were found to have the lowest carcass weight among other genotypes (Oka *et al*. 2007). In the present research, CW, DCG, REA, and RT as the indicators for growth rate were significantly higher in heifers with allele A. Surprisingly, SFT and BMS as indicators for fat accumulation rate were also high in this group, in spite of previous results which showed that those two indicators were not significantly different between cattle with allele A and B (Oka *et al*. 2007).

It was expected that steers with allele A would have a higher growth rate than those without allele A. However, there were no significant differences found in the present research. These might be caused by interference of other effects, such as sires, age, and feed. It has been previously shown that effects of sires were significant for most of the carcass traits, such as hot carcass weight, rib eye area, carcass grade, etc. (Tanner *et al*. 1970; Xie *et al*. 1996). Also, carcass characteristics including muscle, fat, and bone weights increased with slaughter age (Jurie *et al*. 2005). A previous study showed that corn-fed steers had greater marbling scores than hay-fed steers (Chung *et al*. 2007). Also, a higher percentage of grain in the diet increased average daily gain (Woody *et al*. 1983), whereas feeding different sources of roughage in steers resulted in various levels of carcass weight (Vaz & Restle 2005). Indeed, the cattle used in the present research were collected from different farms, which use different sires and slaughtered at ages varying from 24.2 to 37.0 months. Moreover, there was no data on feed compositions available for this study. Therefore, other factors which affect the phenotypic measurement, such as genetic (sires) and environment (feed), might interfere with effect of allele A on body growth, especially in male cattle, despite of the obvious results previously shown in some other related studies.

Regarding fatty acid compositions in i.m. adipose tissues, in heifers, allele C has significant effects on increasing contents of C18:1 (oleic acid), MUFA, and USFA. It also reduced contents of C16:0 (palmitic acid) and SFA, and consequently lowered MP. These results suggested that although effect of the GH allele C was invisible on carcass traits, it has stronger effects on fatty acid compositions. Also, effects of GH gene polymorphisms on fatty acid composition in Japanese Black cattle, as well as carcass traits, were found in a sex-specific manner.

It has been shown that increased GH concentration in postpartum Holstein cows contributed in severe reduction of stearoyl-CoA desaturase (SCD) mRNA level in adipose tissue (Beswick & Kennelly 2000). SCD, also known as delta-9 (Δ^9) desaturase, is involved in conversion of myristic (C14:0), palmitic (C16:0), and stearic acids (C18:0) into MUFA myristoloeic (C14:1), palmitoleic (C16:1), and oleic acids (C18:1), respectively (De Smet *et al*. 2004; Smith *et al*. 2006). Also, SCD mRNA expression was found to be significantly greater in both s.c. adipose tissues and longissimus dorsii muscle of Japanese Black steers than in those of Holstein steers (Taniguchi *et al*. 2004). Our previous results showed that there are only two GH alleles in Holstein breed, that is allele A and B, and allele B was found at very low frequency (data not shown). On the other hand, in Japanese Black cattle there are three GH alleles: A, B, and C. These reports suggested that it is necessary to further investigate the SCD gene expression and enzyme activity in animals with different GH genotype.

In the present research, although sex-mixed group cattle with allele A were shown to have lower MP, the effect was absent after the cattle were grouped by sex

and allele. It has been shown previously that MUFA increases with age (Huerta-Leidenz *et al*. 1996). Also, heifers had higher percentages of intramuscular MUFA than steers (Zembayashi *et al*. 1995). Therefore, it is important to eliminate the effects of other factors, such as sex difference, feed, slaughter age, and sires during the analysis GH gene polymorphism on carcass traits and fatty acid compositions.

We have shown that GH gene polymorphism at bGH codon 127 and 172 were not only useful as a tool for selecting cattle with superior ability, but also exerted its effects in a sex-specific manner. Nevertheless, it is still too early to draw a conclusion about how GH gene polymorphism significantly affects carcass traits and fatty acid compositions only in heifers, but not in steers, without gathering some more evidence on the relationship between GH gene polymorphism and GH biological activities in both sexes. GH biological activity itself may include the circulating GH level, GH molecular stability, GH binding affinity, GH receptor (GHR) activities, and/or to a large extent of stimulation of IGF-1 synthesis which is crucial to body growth. Besides, GH is synthesized and secreted in age- and sex-dependent manner (Trenkle 1971; Wehrenberg & Giustina 1992; Gatford *et al*. 1998). The regulation of GH synthesis was also shown to involve gonadal steroids. The androgenic hormones increased GHRH and SRIF release from bovine anterior pituitary cells; whereas estradiol increased GHRH but decreased SRIF release (Hassan *et al*. 2001).

In conclusion, SNP in bGH gene at bGH codon 127 and 172 were shown to be useful as genetic markers in the selection of Japanese Black cattle with superior traits, although the differences of carcass traits and fatty acid compositions were more pronounced in heifers. GH allele A is related with higher growth rate whereas GH allele C is related with higher contents of oleic acid, MUFA, and USFA, which subsequently contributes to softer beef in heifers. However, further studies are necessary to confirm the role of sex difference in determining the effect of GH gene polymorphism on carcass traits and fatty acid compositions, especially on endocrine levels and GHR activities.

ACKNOWLEDGMENT

This work was partly supported by Ministry of Education, Science, Sports and Culture of Japan (Grant #17380159 and #19380150 for K.K. and K.S.).

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