EFFECTS OF GRASS-FED AND GRAIN-FED DIETS ON EXPRESSION OF GENES FOR MUSCLE DEVELOPMENT IN JAPANESE BLACK STEERS

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***Abstract*—this study aimed to investigate gene expression for muscle development in steers fed a large amount of roughage during the indoor fattening period. Ten, 10-month-old Japanese Black steers were randomly separated into grass-fed and grain-fed groups. All steers were fed a combination of concentrate *ad libitum* and grass hay until 16 months of age in a stall barn. After this control period, the grass-fed steers were fed a combination of grass hay *ad libitum* and concentrate at 2.0 kg/day, whereas the grain-fed steers continued the concentrate diet in the same environment. The steers were slaughtered at 28 months of age. Analysis of gene expression for C/EBPα, PPARγ2 and myosin heavy chain (MHC) was performed by real-time PCR. C/EBPα gene expression in skeletal muscle was higher in the grain-fed group than in the grass-fed group at the end of fattening. Gene expression of PPARγ2 showed a similar tendency, although the grain-fed and grass-fed groups were not significantly different. C/EBPα gene expression in the LL muscle of the grass-fed group was lower after feeding the experimental diet than before feeding it. Gene expression of MHC in the LL muscle was greater in the grass-fed group than in the grain-fed group. These results demonstrate that the expression of genes involved in muscle growth was altered by feeding a large amount of roughage.**

***Index Terms*— myosin heavy chain, adipogenic gene, beef cattle, roughage**

I. INTRODUCTION

Most beef cattle in Japan are individually reared in free stanchion barns and are generally fattened indoors on a concentrate-based diet until slaughter. Recently, beef cattle production has made use of grazing, with effective utilization of the roughage in the field. In Japanese Shorthorn steers, concentrations of *α*-tocopherol and *β*-carotene are higher in the muscle of pasture-fed steers than in concentrate-fed steers (Muramoto et al., 2005). Skeletal muscle proteome analysis of cattle that were grazed during the fattening period, revealed the conversion of muscle fiber type from fast- to slow-twitch tissues with the change in the energy metabolic enzymes (Shibata et al, 2009a). A previous study showed that the final body weight and daily weight gains of steers reared by restricted feeding of concentrate and roughage *ad libitum* (grass-fed group) were lower than in steers reared by feeding of concentrate *ad libitum* (grain-fed group) (Shibata et al, 2009b). We also showed that the crude protein content in the *M. semitendinosus* (ST) muscle was significantly higher in the grass-fed group than in the grain-fed group. In contrast, the skeletal muscle lipid content tended to be lower in the grass-fed group than in the grain-fed group. To further assess changes in lipid and protein content in the skeletal muscle of grass-fed steers, this study investigated gene expression for muscle development in steers fed a large amount of roughage during the indoor feeding period.

II. MATERIALS AND METHODS

A. Animal Management

Management of steers and all procedures were performed according to the Animal Experimental Guidelines of the National Agricultural Research Center for Western Region (WeNARC), Japan. Ten, 10 month old Japanese Black steers, which had been bred at WeNARC, were randomly divided into two groups: grass-fed and grain-fed. All steers were housed individually in a stall barn and fed a combination of concentrate *ad libitum* and grass hay at 1.5 kg/d until 16 months of age. After this control period, the five grass-fed steers were fed a combination of grass hay *ad libitum* and concentrate at 2.0 kg/d in the stall barn; the other five grain-fed steers continued the concentrate diet in the same environment. Skeletal muscles were obtained by slaughter or biopsy. Muscle tissues from the ST and *M. longissimus lumborum* (LL) muscle were obtained by biopsy from before and after (aged 13 and 28 months, respectively) feeding of the experimental diet. The biopsy procedure was carried out according to a previous study (Shibata et al, 2006). Steers were slaughtered at 28 months of age, and skeletal muscle tissue from the ST and LL muscle were obtained for analyses. All samples were rapidly frozen in liquid nitrogen and stored at -80°C until RNA extraction.

B. RNA Isolation and Quantitative Real Time PCR

Total RNA was extracted from muscle tissues using the TRIZOL reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The first strand complementary DNA (cDNA) was synthesized from 3 μg of total RNA using SuperScript II RNase H^{-} (Invitrogen) reverse transcriptase with oligo dT primer (Amersham Pharmacia Biotech, Piscataway, NJ). After reverse transcription, gene expression of myosin heavy chain (MHC) isoforms (MHC-2x, -2a, -slow), PPARγ2, and C/EBPα were performed by real time PCR using an ABI 7700 detection system (Applied Biosystems, Foster City, CA). The expression of the MHC gene was represented as the sum of MHC-2x, -2a and -slow gene expression. The first strand cDNA was diluted with deionized water and amplified using TaqMan Universal PCR Master Mix (Applied Biosystems) with the gene specific TaqMan probe and primers. Each pair of oligonucleotide primers was designed to amplify a region that included at least one intron. The real time PCR reaction was carried out initially for 2 min at 50°C, then for 10 min at 95°C, 50 cycles of 15 s at 95°C, and then for 1 min at 60°C. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a normalizing control. The TaqMan probe and primers were designed using Primer Express (Applied Biosystems).

C. Statistical Analyses

Gene expression data were represented as means ± SEM. The relationships between the relative mRNA levels of the target genes and the growth stages and treatment groups were analyzed using one-way ANOVA and a post-hoc Fisher test. $P < 0.05$ was considered to be statistically significant.

III. RESULTS AND DISCUSSION

Table 1 shows adipogenic gene expression in the skeletal muscle of the grass-fed and grain-fed groups before and after feeding the experimental diet. Gene expression of C/EBPα in the ST and LL muscles at the end of fattening was significantly greater in the grain-fed group than in the grass-fed group. PPARγ2 gene expression in the two muscle types was also analyzed; it was (not significantly) higher in the grain-fed group than in the grass-fed group. This indicates that C/EBPα plays an important role in comparison with that of PPARγ2 in the adipogenesis of skeletal muscle.

In the LL muscle of the grass-fed group, C/EBPα gene expression was significantly lower after feeding the experimental diet than before feeding it (Table 1). Similarly, gene expression of PPARγ2 in the ST muscle was significantly lower after feeding the experimental diet than before feeding it. In contrast, in the ST muscle of the grain-fed group, gene expression of C/EBPα tended to be greater after the feeding experimental diet than before feeding it. When steers were reared by restricted feeding of concentrate and roughage *ad libitum*, the skeletal muscle lipid content tended to be greater in the concentrate-fed group than in the roughage-fed group (Shibata et al, 2009b). These results suggest that adipogenesis in the skeletal muscle is decreased by feeding a large amount of roughage during the indoor fattening.

Table 1 Changes in adipogenic gene expression in the skeletal muscles of steers during the experimental period

	C/EBPα						PPARγ2					
	ST			LL			ST			LL		
	Grass	Grain	*P*	Grass	Grain	*P*	Grass	Grain	*P*	Grass	Grain	*P*
Before	1.69	2.07	.730	2.83	3.02	.793	4.49	3.23	.373	5.85	5.10	.779
After	2.05	3.48	.047	0.79	2.41	.008	2.18	3.57	.093	3.17	5.25	.098
P value	.725	.069		.005	.337		.047	.744		.273	.922	

P: *P* value, Before: before feeding the experimental diet (aged 13 months), After: after feeding the experimental diet (aged 28 months)

Table 2 shows gene expression of myosin heavy chain (MHC) in the skeletal muscle of the grass-fed and grain-fed groups before and after feeding the experimental diet. A change in gene expression of MHC in the ST muscle of the grass-fed group was not identified between before and after feeding the experimental diet; however, it was significantly lower after feeding experimental diet than before feeding it in the three other muscles types. Gene expression of MHC in the LL muscle was significantly higher in the grass-fed group than in the grain-fed group at the end of fattening. The

ST muscle also exhibited a similar tendency of MHC gene expression, although there was no significant difference between the grass-fed and grain-fed groups. Shibata et al (2009b) showed that crude protein content in the ST muscle was higher in a grass-fed group than in a grain-fed group. These results suggest that muscle hypertrophy is activated during the later period of steers that are grass-fed during fattening.

Table 2 Changes in MHC gene expression in the skeletal muscle of steers during the experimental period

	ST			LL		
	Grass	Grain	*P*	Grass	Grain	*P*
Before	1633	1632	.998	2828	2797	.954
After	1379	881	.065	1770	1043	.005
P value	.534	.022		.006	.001	

P: *P* value, Before: before feeding of the experimental diet (aged 13 months), After: after feeding of the experimental diet (aged 28 months)

IV. CONCLUSION

Feeding a large amount of roughage to steers affects the expression of genes that are involved in muscle growth. C/EBPα gene expression decreased in the grass-fed group. Similarly, PPARγ2 gene expression was shown in the grass-fed group, but there was no significant difference between the grass-fed and grain-fed groups. These results suggest that adipogenesis in the skeletal muscle of grass-fed steers plays a more important role in the C/EBPα than it does in the PPARγ2. In terms of skeletal muscle synthesis, gene expression of MHC increased in the grass-fed group at the end of the fattening period. This suggests that skeletal muscle hypertrophy is induced in steers by feeding a large amount of roughage during the later period of fattening.

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