Effect of grazing in the latter fattening period on the nutrient content and gene expression in steer muscle

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Abstract— **This study investigated the nutrient content in muscle and gene expression for muscle development between indoor concentrate-fed and outdoor grazed steers. Ten Japanese Black steers were randomly selected and divided into two groups: grazing (GR) and concentrate (CT) groups. Crude protein and extractable lipid in muscle tissue were analyzed. The gene expression of myosin heavy chain (MyHC), myostatin, peroxisome proliferator-activated receptor γ2 (PPARγ2), and CCAAT/enhancer binding protein α (C/EBPα) in skeletal muscles was evaluated by real-time PCR. In the *semitendinosus* (ST) and *longissimus lumborum* (LL) muscles, extractable lipid content was lower in the GR group than in the CT group. Crude protein content in the LL muscle in the GR group was higher than that in the CT group. MyHC expression in LL muscle in the GR group was greater than that in the CT group. A decrease in the expression of myostatin (a specific negative regulator of skeletal muscle growth) was detected in the GR group compared with the CT group in both muscles. In the ST and LL muscles, PPARγ2 gene expression was lower in the GR group than in the CT group. These results suggest that the increase in protein content and decrease in fat accumulation in LL and/or ST muscles of the grazing group were affected by the expression of myostatin, MyHC, PPARγ2, and C/EBPα genes.**

Keywords— **grazing, gene expression, steer.**

I. INTRODUCTION

Our previous gene expression analysis revealed that increases in protein content and decreases in fat accumulation in skeletal muscle are affected by the regulation of myostatin, myosin heavy chain (MyHC), and CCAAT/enhancer binding protein α (C/EBPα) gene expression when steers are fed a large amount of grass hay indoors [1]. Skeletal muscle proteome analysis of grazed cattle revealed that muscle fiber type conversion from fast- to slow-twitch tissue occurs with changes in the expression of energy metabolic enzymes [2]. The objective of this study was to investigate the differences in gene expression involved in muscle development and the nutrient content in skeletal muscle between indoor concentrate-fed and outdoor grazed steers.

II. MATERIALS and METHODS

Ten Japanese Black steers of 10 months of age that had been bred at WeNARC were randomly selected and divided into two groups: the grazing (GR) group and concentrate (CT) group. They were housed individually in a stall barn and fed concentrate *ad libitum* and Italian ryegrass hay at 1.5 kg/d from 10 to 21 months of age. After this control period, the four steers of the GR group were placed on an outdoor pasture, while the other six steers of the CT group were continued on the concentrate and grass hay diet in the stall barn. Skeletal muscle tissues from the *semitendinosus* (ST) and *longissimus lumborum* (LL) muscles were obtained by biopsy at 27 months of age. These samples were rapidly frozen in liquid nitrogen and stored at -80°C until RNA extraction. The steers were slaughtered at 27 months of age at the WeNARC abattoir, and skeletal muscle tissues from the ST and LL muscles were obtained for nutrient content analysis.

Muscle tissues were minced to determine the crude protein content and extractable lipid content. Crude protein was calculated by quantitative analysis of nitrogen using the Kjeldahl method with copper sulfate and potassium sulfate as catalysts [3]. Lipid

was extracted with diethyl ether for 16 h using a Soxhlet extractor [3].

Total RNA was extracted from muscle tissues using 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^- reverse transcriptase (Invitrogen) with oligo(dT) primer (Amersham Pharmacia Biotech, Piscataway, NJ). After reverse transcription, gene expression of MyHC isoforms (MyHC-2x, -2a, and -slow), myostatin, peroxisome proliferator-activated receptor γ2 (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 MyHC gene was represented as the sum of MyHC-2x, -2a, and -slow gene expression. The first-strand cDNA was diluted with deionized water and amplified using SYBR Green PCR Master Mix or TaqMan Universal PCR Master Mix (Applied Biosystems) with the gene-specific TaqMan probe and primers. The real-time PCR reaction was carried out initially for 2 min at 50°C, then for 10 min at 95°C, then 50 cycles for 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).

Gene expression data were represented as means. The relationships between the relative mRNA levels of the target genes and treatment groups were analyzed using one-way ANOVA and a post-hoc Fisher test. A p value of <0.05 was considered statistically significant. An asterisk in the figures and table indicates that the means differed between two groups in the same muscle ($p < 0.05$).

III. RESULTS

Crude protein in the LL muscle of the GR group was significantly greater than that of the CT group (Table 1). In contrast, there was no significant difference in protein content in the ST muscle between the two groups. Extractable lipid in the ST and LL muscles of the CT group was significantly higher than that of the GR group (Table 1).

Table 1. Muscle composition of the GR and CT groups in Japanese Black steers

	GR	CT
LL muscle		
Crude protein, %	19.65*	17.03
Extractable lipid, %	11.28*	23.95
ST muscle		
Crude protein, %	19.85	19.65
Extractable lipid, %	4.38*	9.45

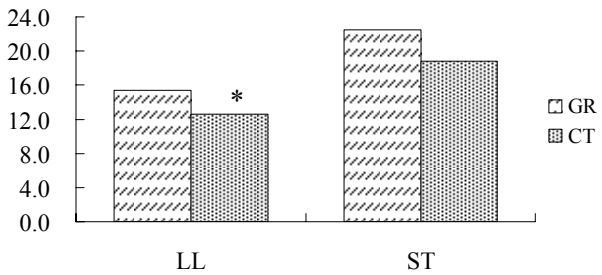

Fig. 1 Relative MyHC mRNA abundance in the LL and ST muscles of the GR and CT group

MyHC gene expression in the LL muscle of the GR group was significantly greater than that of the CT group (Fig. 1). The expression of MyHC gene in the ST muscle of the GR group tend to be also greater than that of the CT group ($p = 0.069$), but there was no significant difference between the two group. Change in the distribution ratio of the three MyHC isoforms (MyHC-2a, -2x, and –slow) was identified in the LL muscle (Fig. 2). The expression ratio of the MyHC-2a and -2x genes in the LL muscle was significantly lower in the GR group than in the CT group. In contrast, the expression ratio of the MyHC slow gene

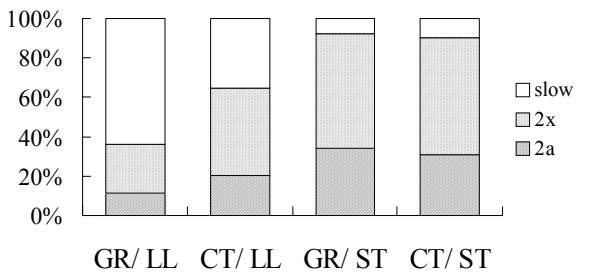

Fig. 2 mRNA composition of MyHC isoforms in the LL and ST muscles of the GR and CT group

in the LL muscle of the GR group was significantly greater than that of the CT group. No change in the distribution ratio of MyHC isoforms was identified in the ST muscle.

Expression of the myostatin gene in the ST and LL muscles of the GR group was significantly lower than that of the CT group (Fig. 3).

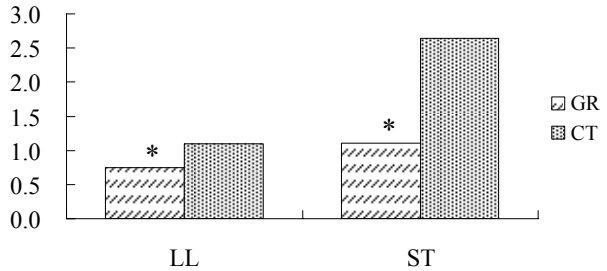

Fig. 3 Relative myostatin mRNA abundance in the LL and ST muscles of the GR and CT group

PPARγ2 gene expression in the ST and LL muscles of the GR group was significantly lower than that of the CT group (Fig. 4). In contrast, C/EBPα gene expression in the ST and LL muscles of the GR group tended to be lower than that of the CT group (p = 0.051 and 0.249, respectively), but there was no significant difference between the two groups (Fig. 5).

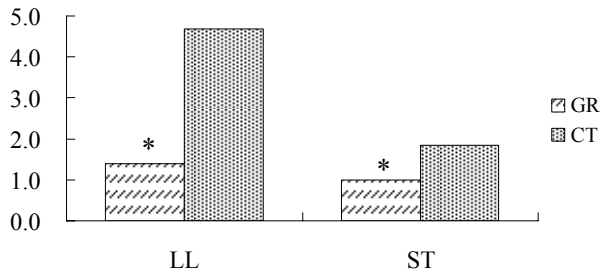

Fig. 4 Relative PPARγ2 mRNA abundance in the LL and ST muscles of the GR and CT group

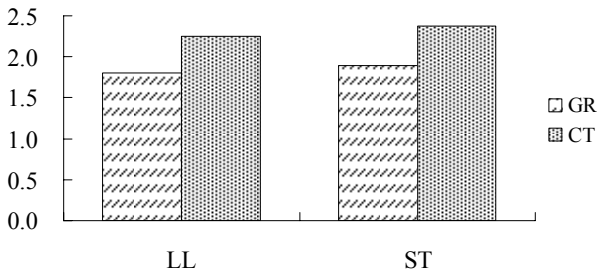

Fig. 5 Relative C/EBPα mRNA abundance in the LL and ST muscles of the GR and CT group

IV. DISCUSSION

The LL muscle crude protein content of the GR group was greater than that of the CT group. In terms of the expression of skeletal muscle formation genes, expressions of MyHC and myostatin genes differed between the GR and CT groups. MyHC has been shown to play an important role in skeletal muscle hypertrophy or growth. Myostatin is known as an impressive and specific negative regulator of muscle mass [4]; double-muscled cattle have a functional deficiency of this gene [5-7]. Furthermore, a previous study revealed that an increased muscle protein content of grass hay-fed steers was induced by regulation of myostatin and MyHC gene expressions [1]. In the present study, the ST and LL muscles of the GR group showed a decrease in the expression of myostatin compared with those of the CT group. This result indicates a release of inhibition of skeletal muscle growth by myostatin. On the other hand, an increase in the expression of MyHC was detected the GR group compared with the CT group in the LL muscle. At the end of fattening, skeletal muscle growth of the GR group may have been promoted by an increase in MyHC expression relative to the CT group. These results suggest that the increase in the LL muscle protein content of the GR group may have been induced by regulation of myostatin and MyHC gene expression.

The lipid content in the skeletal muscle was lower in the GR group than in the CT group. In terms of the adipogenic gene expression in skeletal muscle,

expression of PPARγ2 and C/EBPα genes underwent a quantitative analysis by real-time PCR. These two genes have been shown to play an important role in adipogenesis. PPARγ2 gene expression of the GR group was lower than that of the CT group in the ST and LL muscles. A decrease tendency in the C/EBPα gene expression of the GR group was identified in two muscles compared with that of the CT group. Furthermore, analysis of myostatin-null mice revealed that a decrease in body fat accumulation resulted from a downregulation of adipogenesis; therefore, leptin secretion was decreased [8, 9]. Moreover, a previous study suggested that a decrease in fat accumulation in the skeletal muscle of grass hay-fed steers was caused by declines in adipogenic gene and myostatin expressions [1]. In the present study, expression of myostatin in two muscles of the GR group was lower than that of the CT group. These results suggest that a decrease in fat accumulation in the skeletal muscles of the GR group may have been caused by the reductions in adipogenic gene and myostatin expressions.

In terms of the expression ratio of MyHC isoforms in skeletal muscle, MyHC of fast-twitch (-2a and -2x) and slow-twitch (-slow) gene expression was detected in all muscles of both groups. MyHC-slow in the ST muscle of grazed cattle increases as a result of muscle fiber type conversion [2]. Although it was only the LL muscle in the present study, the expression ratio of the MyHC-slow gene increased in the GR group. Moreover, a decrease in the expression ratio of the MyHC-2a and -2x genes was identified in the GR group. These results suggest that repartition of MyHC isoform expression is caused by grazing in the latter fattening period.

V. CONCLUSIONS

Grazing of steer in the latter fattening period may lead to an increase in protein content and a decrease in fat accumulation in LL and/or ST muscles by regulation of myostatin, MyHC, PPARγ2, and C/EBPα gene expression.

VI. ACKNOWLEDGMENTS

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VII. REFERENCES

1. Shibata M., Matsumoto K., Hikino Y. et al. (2011) Influence of different feeding systems on the growth performance and muscle development of Japanese Black steers. Meat Sci. (In press)
2. Shibata M., Matsumoto K., Oe M. et al. (2009) Differential expression of the skeletal muscle proteome in grazed cattle. J Anim. Sci., 87: 2700-2708.
3. AOAC. (1990) Official methods of analysis (15th ed). Arlington, VA: Association of Official Analytical Chemists.
4. McPherron A.C., Lawler A.M., and Lee S.J. (1997) Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member. Nature, 387: 83-90.
5. Grobet L., Martin L.J.R., Pirottin D. et al. (1997) A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. Nat. Genet., 17: 71-74.
6. Kambadur R., Sharma M., Smith T.P.L. et al. (1997) Mutations in myostatin (GDF8) in double-muscled Belgian Blue and Piedmontese cattle. Genome Res., 7: 910-915.
7. McPherron A.C., & Lee S.J. (1997) Double muscling in cattle due to mutations in the myostatin gene. Proc. Natl. Acad. Sci. USA, 94, 12457-12461.
8. Lin J., Arnold H.B., Della-Fera M.A. et al. (2002) Myostatin knockout in mice increases myogenesis and decreases adipogenesis. Biochem. Biophys. Res. Commun., 291: 701-706.
9. McPherron A.C. & Lee S.J. (2002) Suppression of body fat accumulation in myostatin-deficient mice. J. Clin. Invest., 109: 595-601.