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A Novel Beef Production System Based on Grass Resources by Applying Metabolic Imprinting

Takafumi GOTOH[1], Tomiko FUMITA[1], Tetsuji ETOH[1], Yuji SHIOTSUKA[1], Keisuke HAYASHI[1], Yoshinori NAKAMURA[1], and Jochen WEGNER[2]

[1] Kuju Agricultural Research Center, Faculty of Agriculture, Kyushu University 8780201, Kuju, Japan,

[2] Research Institute for Biology of Farm Animals, 18196 Dummerstorf, Germany

INTRODUCTION

Grain is food that human being can eat. In recent time, global shift to produce bio-energy, i.e., ethanol from grain happens because of shortage of oil. The price of grain has become higher. In some of developed countries, a considerable grain feeds are fed to cattle. In Japan, Wagyu (Japanese Black cattle) are known for their excellent marbled beef which is achieved by feeding them with a considerable amount of concentrate (4000 to 5000 kg altogether, until slaughter at 28-30 mo of age). Cattle are ruminant, which means they have an important ecological niche that capitalizes on the symbiotic relationship between fiber fermenting ruminal microbes and mammalian demand for usable nutrients. We aim to produce a high-quality safe beef product while maximizing the used of domestic grass resources. This thing would contribute and could apply the beef production in lowland and upland of South East Asian countries. It has been shown, however, that alterations in fetal and early postnatal nutrition and endocrine status may result in developmental adaptations that permanently change the structure, physiology, and metabolism in adult life in rats, domestic species and mouse and human (Levin *et al*, 2000). This phenomenon is referred to as "metabolic imprinting" based on the medical research regarding "developmental origins of health and disease (DOHaD)". However, there are very few reports of metabolic imprinting in cattle.

In this study, in order to study the effects of metabolic imprinting on beef production, a fattening experiment was conducted. In this experiment, we feed mainly roughage and investigated how metabolic imprinting affects the expressions of meat quality or quantity and related genes in Japanese Black steers.

MATERIALS AND METHODS

In this study, Japanese Black steers were nursed artificially until 2 mo of age when they were divided into two groups: groups R (n=7) and C (n=7). In group R, the calves were fed only

roughage. In group C, they were fed a considerable amount of concentrate (over 2.5% of their body weight) and given *ad libitum* access to Italian ryegrass hay (roughage). After 10 and until 26 months of age both groups were fattened with only Italian ryegrass hay. Muscle samples were taken by biopsy from longissimus muscles (LM) at 2, 10, 17 and 22 months of age. Total RNA was isolated from these tissues with ISOGEN. Semi-quantitative analysis of realtime reverse transcription-polymerase chain reaction (RT-PCR) was used to measure the mRNA expression of meat quality-related (intra-muscular adipogenesis) (PPARγ2, C/EBPα, β, and δ, Leptin, G6P, SCD, FASN, ADRP) and quantity-related genes (myostatin, IGF-I, IGF-I receptor, MyoD, myogenin, MRF4, Myf5, and PGC-1α). G3PDH was used as a standard for each PCR reaction. Volume percentages of intramuscular fat were measured by the Soxhlet method. Adipose cell size diameter was observed using Oil-red O staining methods. ANOVA was used for statistical analyses

RESULTS AND DISCUSSION

The average live weight was 2.2-fold higher in group C (265 ±18 kg) than in group R (117 ± 12 kg) just after 10 mo of age ($p<0.001$). At slaughter, group C was just 1.3-fold heavier (472 ± 42 kg) than group R (357 ± 25 kg) ($p<0.001$) (Fig.1).

The expression of PPARγ2 mRNA in LM of group C was significantly higher than in group R only at 22 mo of age ($p<0.05$). The expressions of C/EBPα, β and δ, Leptin, G6P, ADRP, SCD and FASN in LM were significantly higher in group C than in group R at 5 or10, and 22 months of age. The diameter of intramuscular adipose cells in LM was significantly larger in group C than in group R at 10 and 22 months of age. The percentage of intramuscular fat content in LM at 26 months of age was significantly higher in group C (10.3 %) than in group R (6.2 %) ($p<0.05$). In both groups, subcutaneous fat thickness was quite thin (group C: 0.8mm, group R: 0.2mm) at 26 months of age. These results indicate that the effect of metabolic imprinting, or fattening regimen during the early growth period affected the expression of adipocyte differentiation factors and the accumulation of intramuscular adipose tissue. On the other hand, with this feeding system, waste fat such as subcutaneous did not greatly accumulate in group C.

The mRNA expressions of MyoD, IGF-I in LM were significantly higher in group C than in group R at 5 months of age (respectively, $p<0.05$, $p<0.01$). The mRNA expressions of myogenin and MRF4 and myostatin in LM were significantly higher in Group C than in group R at 10 months of age (respectively, $p<0.01$, $p<0.001$). For, myostatin, the mRNA expression in LM was significantly higher in group R than in group C at 22 months of age ($p<0.05$). On the other hand, the mRNA expressions of IGF-I receptor in LM were significantly higher in group R

than in group C at 5, 10 and 22 months of age ($p<0.05$). In Myf5, there were no significant differences in mRNA expressions in LM between two groups from 2 to 22 months of age. The MRFs family plays important roles as transcription factors during myogenesis (Berkes and Tapscott, 2005).

MyoD and Myf5 are expressed mainly in "determination" at an early stage during myogenesis. On the other hand, after determination, myogenin and MRF4 are expressed mainly in differentiation from myoblast to myotube, and maintain and mature myotube. In vivo, satellite cells may be on an equality level with precursor or stem cells in vitro. In this study, the high expressions of MyoD at 5 months of age and myogenin and MRF4 at 10 months in Group C might suggest that "determination" and "differentiation" occurred and that myoblasts differentiated from satellite cells, which may influence the hypertrophy and hyperplasia of muscle fiber.

In conclusion, the feeding level during the early growth stage influenced mRNA expressions in skeletal muscle. The growth size, meat quantity and quality were markedly different between groups. This may be caused by the effect of metabolic imprinting induced by a high feeding level during the early growth stage.

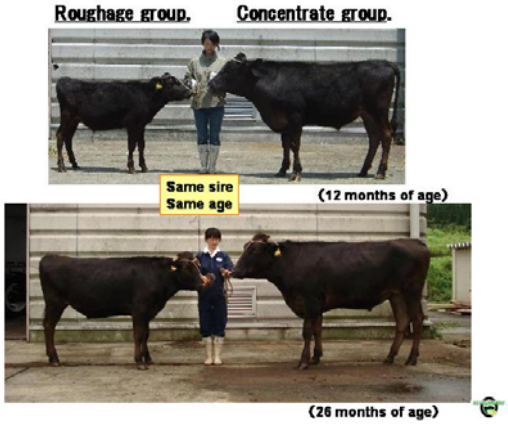

Fig.1. Comparison of steers at 12 and 26 months of age between group R and C.

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