EFFECT OF A RICE WHOLE CROP SILAGE DIET ON CARCASS CHARACTERISTICS AND EXPRESSION OF GENE INVOLVED IN MUSCLE GROWTH IN JAPANESE BLACK STEERS

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Abstract – This study aimed to investigate the differences in growth performance, carcass characteristics, and expression of genes involved in muscle growth between Japanese Black steers fed rice whole-crop silage (rWCS) and those fed concentrate. Steers were randomly separated into rWCS-fed (rWCS *ad libitum* and restricted feeding of concentrate) and concentrate-fed groups. Total digestible nutrient intake and daily gain (DG) decreased in rWCS-fed steers in comparison with concentrate-fed steers, whereas dressed carcass weight and final body weight did not significantly differ between the groups. Decreases in drip loss in the muscle of rWCS-fed steers may be caused by α-tocopherol and β-carotene in muscle. Feeding large amounts of rWCS to steers may maintain quantitative productivity of beef steers equally to a concentrate-based diet, and improve the qualitative productivity. Results of gene expression suggest that activation of skeletal muscle growth in rWCS-fed steers may occur at the late fattening period owing to a decrease in myostatin and increase in myosin heavy chain genes expression. This rearing system would allow the beef production to switch to rWCS-based diets from concentrate-based diets.

Key Words – beef steer, meat quality, rice whole-crop silage

I. INTRODUCTION

Beef cattle in Japan are reared with restriction of movement and are generally finished indoors on a concentrate-based diet throughout the fattening period until slaughter. However, several studies of beef cattle production have been performed investigating the effective use of roughage. Our previous study revealed that feeding a large amount of grass hay to steers during the indoor finishing period led to an increase in protein content and a decrease in fat accumulation in the muscle compared with feeding a concentrate-based diet, but grass hay-fed steers had a lower final body weight (BW) because of the lower total digestible nutrient (TDN) intake [1]. Recently, 'Tachisuzuka,' a new rice cultivar for whole crop silage (WCS) has been developed as a roughage source at the Western Region Agricultural Research Center, NARO (WARC/NARO), Japan. Tachisuzuka has a high straw yield, high sugar content, high digestibility of fiber, and low grain yield [2]. The present study investigated the influence of a diet largely comprising rice WCS (rWCS) on the growth performance and carcass characteristics of Japanese Black steers. Furthermore, this study also examined the expression of genes involved in muscle growth of the steers when they were fed a large amount of rWCS.

II. MATERIALS AND METHODS

Animal Management and Diets

Management of steers and all procedures were performed according to the Animal Experimental Guidelines of the WARC/NARO. Ten 10-month-old Japanese Black steers, bred at WARC/NARO, were randomly divided into two groups: a combined feeding with rWCS and concentrate diet (RS) group, and concentrate-fed (CT) group. Steers were housed individually in a tie stall barn and fed concentrate diet *ad libitum* and grass hay from 10 until 16 months of age. After this period, the four steers of the RS group were fed an experimental diet that consisted of rWCS *ad libitum* and restricted feeding of the concentrate diet until 28 months of age. The RS group was fed the concentrate diet at 4.5 kg/day and 2.5 kg/day during 16 to 22 and 22 to 28 months of age, respectively. The six steers of the CT group were continued on the concentrate diet and grass hay until 28 months of age. All steers were slaughtered at 28 months of age.

Sample Preparation and Carcass Evaluation
Skeletal muscle tissues from the *longissimus lumborum* (LL) muscle were obtained by biopsy. All samples were rapidly frozen in liquid nitrogen and stored at −80°C until RNA extraction. After slaughter, the carcasses were chilled at 0°C for 24 h. Their dressing percentage, and the rib eye area, rib thickness, subcutaneous fat thickness, beef marbling standard (BMS), beef fat color standard (BFS), and beef color standard (BCS) of the section between the sixth and seventh ribs were evaluated according to the Japanese New Beef Carcass Grading Standards [3]. Skeletal muscles tissues from the LL and *semitendinosus* (ST) muscles were obtained for analyses of the nutrient elements, drip loss, cooking loss, and Warner–Bratzler (WB) shear force. Samples of the muscle tissues were minced to determine their crude protein, crude fat, and vitamin content. The minced meat samples were vacuum-packed in an aluminum bag and then stored at −80°C until analysis. The muscles were processed into 2.5-cm (thickness) steaks, vacuum-packed, stored in a refrigerator at 2°C for 2 and 30 days after slaughter, and then frozen at −80°C until analysis.

Measurement of Gene Expression
Total RNA was extracted from muscle tissues using the Trizol reagent according to the manufacturer's protocol. The first strand complementary DNA (cDNA) was synthesized from 3 μg of total RNA according to previous report [4]. After reverse transcription, analysis of gene expression of myosin heavy chain (MyHC) and myostatin was performed by real-time PCR using an ABI 7500 detection system. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a normalization control.

Statistical Analysis
All measurements are presented as means. The significance of between-group differences was analyzed using one-way analysis of variance with a post-hoc Fisher's least significant difference test. P values of < 0.05 were considered to be statistically significant.

III. RESULTS AND DISCUSSION

The growth performance and feed intake of the RS and CT groups are shown in Table 1. The TDN intake and DG decreased in the RS group in comparison with the CT group during the experimental period, whereas the dressed carcass weight did not differ between the groups because no change occurred in the final BW. Previous study showed that steers fed a small amount of rWCS with concentrate showed no change in slaughter BW and dressed carcass weight compared with steers fed rice grass hay with concentrate [5] or grass hay with concentrate [6]. These results were consistent with the present study. Results of the present study suggest that feeding an rWCS-based diet may be able to maintain quantitative productivity as well as feeding a concentrate-based diet.

Table 1 Growth performance and feed intake of steers

	RS	CT
Body weight (BW), kg		
Initial (10 mo)	300	299
Before rWCS feeding (16 mo)	503	512
Final (28 mo)	707	770
TDN intake, kg/day		
Control period	6.31	6.54
WCS feeding period (16-28 mo)	5.23 *	6.31
Daily gain (DG), kg/day		
Control period	1.04	1.08
WCS feeding period (16-28 mo)	0.60 *	0.72

mo: months, TDN: total digestible nutrients.
Values are expressed as means. *$P < 0.05$

The carcass characteristics of the RS and CT groups are shown in Table 2. The BMS, rib eye area, and other carcass characteristics except for the rib thickness did not differ between the RS and CT groups, although the rib thickness was significantly less in the RS group than in the CT group. Mandell et al. (1998) reported that the marbling score and Longissimus muscle area of high-roughage (alfalfa silage) fed Limousin-cross steers were lower than those of the high-grain (high-moisture corn) fed steers [7]. Takahira et al.

(2011) reported that the BMS, rib eye area and rib thickness of Japanese Black steers showed no difference between the feeding of rWCS with concentrate and the feeding of grass hay with concentrate [6]. These results suggest that the difference between the previous studies and the present study may be due to the difference in the roughage source and/or in the breeds.

Table 2 Carcass characteristics

	RS	CT
Dressed carcass weight, kg	439	485
Dressing percentage, %	71.6	71.7
Rib eye area, cm^2	44.7	52.7
Rib thickness, cm	6.05 *	7.37
Subcutaneous fat thickness, cm	2.55	3.78
Beef marbling standard (BMS), No.	4.7	5.2
Beef color standard (BCS), No.	4.7	4.0
Beef fat color standard (BFS), No.	2.8	2.8

Values are expressed as means. *$P < 0.05$

Nutrient content, water-holding capacity, and the WB shear force of muscle samples from the RS and CT groups are shown in Table 3. A huge concentration of α-tocopherol and β-carotene in the muscles was detected in the RS group compared with the CT group. High α-tocopherol concentration in meat minimized drip loss from frozen meat upon thawing, because α-tocopherol may preserve the fluidity of cell membranes [8]. In the present study, the drip loss in the muscle decreased in the RS group compared with the CT group. Results of the present study suggest that a decrease in the drip loss in muscle of the RS group is due to stabilization of cell membranes by the large amount of α-tocopherol and β-carotene present in the rWCS. Cooking loss and the WB shear force were not different between the two groups.

Table 3 Nutrient content, water-holding (WH) capacity, and Warner-Bratzler (WB) shear force of LL and ST muscles

	L L		S T	
	RS	CT	RS	CT
Crude protein, %	17.1	16.2	20.2	20.1
Extract lipid, %	25.0	30.3	8.15 *	11.5
Vitamin				
Retinol, μg/100g	26.8	21.8	8.75	8.50
β-CA, μg/100g	19.0	ND	10.3	ND
α-Toc., mg/kg	15.6 *	3.65	12.2 *	2.88
WH capacity				
Drip loss, %	2.50	3.54	5.39 *	6.86
Cooking loss, %	35.5	35.5	40.9	39.0
WB shear force, kg	1.17	1.19	2.83	2.48

LL: *M. longissimus lumborum*, ST: *M. semitendinosus*, CA: Carotene, Toc.: Tocopherol, Values are expressed as means. *$P < 0.05$

Myostatin and MyHC genes expression in the skeletal muscle of steers are shown in Figure 1 Myostatin is a known specific negative regulator of skeletal muscle growth because of studies with myostatin null mice [9]. In the present study, the myostatin gene expression at 19 months of age after switching to rWCS decreased in the CT group compared with the RS group. The muscle in the RS group at the 19 months of age had lower MyHC gene expression than at 25 months of age. These results indicate that the skeletal muscle growth of the steers at 19 months of age would be suppressed by myostatin gene expression. Furthermore, the late fattening period (25 to 28 months) of the RS group showed a decrease in myostatin gene expression compared with 19 months of age, and MyHC gene expression in the muscle at 28 months of age was greater in the RS group than the CT group. These gene expression patterns suggest that activation of skeletal muscle growth may occur at the end of fattening in the RS group because of a decrease in myostatin expression and an increase in MyHC expression at that time.

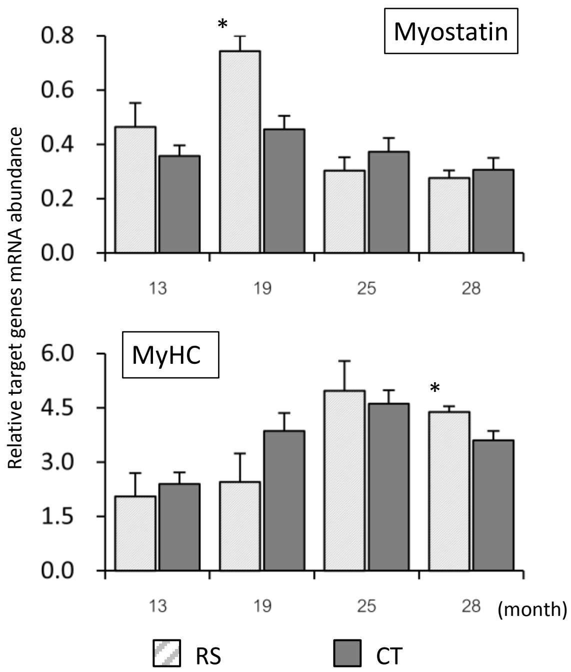

Figure 1. Developmental change in myostatin and myosin heavy chain (MyHC) genes expression in the *longissimus lumborum* (LL) muscle. The data represent the means ± SEM at each point. An asterisk indicates that the means differ significantly between treatments of same age ($P < 0.05$).

IV. CONCLUSION

The present study suggests that feeding a large amount of rWCS-based diet to steers beginning at the middle fattening stage may be able to maintain the quantitative productivity of beef equally as well as feeding a concentrate-based diet. Specifically, these results imply that switching the diet from concentrate to rWCS during the middle fattening stage may be possible without causing a reduction in beef productivity.
However, in terms of meat quality, the present study suggests that the water-holding capacity may be improved in steers fed a large amount of rWCS because of a decrease in drip loss. Furthermore, the α-tocopherol and β-carotene contents in muscle increased in feeding an rWCS-based diet in comparison with feeding a concentrate-based diet to steers.

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