



The Effects of Fasting and Grazing on Na-glucose Cotransporter-1 (SGLT-1) Gene Expression of Rectal Epithelia in Beef Cattle*

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ABSTRACT : The expression of SGLT-1 mRNA has been reported in the small intestine of mammals and the rectum of chickens. However, the expression and functional significance of SGLT-1 in bovine rectum is not known. In this study, we studied the effects of fasting and grazing on SGLT-1 gene expression in biopsy epithelial tissue of bovine rectum. In Japanese Black beef cattle, i) SGLT-1 gene expression was measured by quantitative real-time PCR in the biopsy rectal epithelia samples obtained through an endoscope, ii) SGLT-1 gene expression in the rectal epithelial tissues increased at 48 and 72 h after fasting correlating with a decrease in body weight. iii) SGLT-1 gene expression decreased after one month from the start of grazing (May to June) and then stabilized until the end of the grazing period (June to October) in the rectal epithelial tissues of grazing cattle. In conclusion, it is clear that SGLT-1 gene expression in the rectal epithelial tissue is increased by a restricted dietary condition. (**Key Words** : Rectum, SGLT-1, Cattle, Epithelia, Graze, Fasting)

INTRODUCTION

Glucose is a very important energy source for mammals. Sodium dependent glucose transporter-1 (SGLT-1) is a carrier protein encoded by the solute carrier family 5 member 1 (SLC5A1) gene. SGLT-1 is a high affinity transporter for glucose uptake in the gastrointestinal tract of many species (Ferraris 2001; Drozdowski and Thomson, 2006). It has been reported that SGLT-1 gene expression was regulated by the type and concentration of the carbohydrate-diet in the gastro-intestinal tract of rat and mouse (Ferraris and Diamond, 1992; Miyamoto et al., 1993; Kishi et al., 1999). In addition, it was shown that sugar directly influences the expression of the SGLT-1 gene via cAMP signaling in epithelial cells of the ovine small intestine (Vayro et al., 2001; Dyer et al., 2003).

In the case of ruminants, it is believed that glucose

absorption is not important in the gastro-intestinal tract because much of the glucose taken up is fermented to short chain fatty acids (SCFAs: acetate, propionate, butyrate) by microorganisms in rumen, and glucose is produced by gluconeogenesis from propionate in liver. However, it was shown that the SGLT-1 gene expression was higher in the small intestine of lactating cows (Zhao et al., 1998; 2005). When a large quantity of cereal grain was fed to cattle, about 50% of the starch escaped from rumen fermentation and flowed into the small intestine (Hill et al., 1991). In addition, the injection of glucose and/or starch into intestine led to an increase in SGLT-1 expression in sheep and beef steers (Lescale-Matys et al., 1993; Shirazi-Beechey et al., 1994; Rodriguez et al., 2004). These results suggested that SGLT-1 gene expression is regulated by a carbohydrate-diet and SGLT-1 has a physiological function in the intestinal tracts of ruminant.

The grazing cattle in the pasture are unable to consume enough nutrients from pasture forage to fulfill the requirement for production because forage quality and availability change by season and nature conditions. Additional supplemental and/or replacement feeding is necessary to meet the required production under conditions of low nutrient level in the pasture. However, exact adjunct food is not accurately supplied to the grazing cattle since

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Table 1. Nutrient composition of Orchard-grass silage fed *ad libitum* in animal house

Item ¹	
DM (%)	26.7
CP (% of DM)	14.1
ADF (% of DM)	34.6
NDF (% of DM)	53.5

¹ DM basis except DM content expressed on as-diet basis.

the understanding of food intake and nutrient condition is insufficient in the grazing cattle.

The grazing cattle might adapt to different nature environments using physiological homeostasis. If a physiological factor which indicates the nutrient status is identified in grazing cattle, the grazing environment in pasture can be improved for the production more effectively. It has been reported that low food intake upregulates GLUT1 and GLUT4 gene expression in porcine muscle (Katsumata et al., 2007). In addition, it has been reported that the fasting increased glucose absorption in small intestinal tracts of dog (Galassetti et al., 1999) and SGLT-1 gene expression in jejunum of rat (Habold et al., 2005). On the other hand, the fasting decreased lipoprotein lipase (LPL) activity and LPL gene expression in adipocytes of rat (Lee et al., 2008). It is thought that fasting (negative energy condition) induces an increase in SGLT-1 gene expression through several hormones and metabolic factors in whole body, and its mechanism may be different from carbohydrate-substance induced SGLT-1 gene expression.

The expression of SGLT-1 in the chicken rectum was studied earlier (Bindslev et al., 1997; Garriga et al., 1999). It is easy to obtain a sample of biopsy epithelial tissue in grazing cattle because rectum is the terminus of gastrointestinal tract. If SGLT-1 gene expression in the rectum is influenced by the nutrient condition in the grazing cattle, SGLT-1 gene expression in rectum might be used as a marker of the nutrient condition.

Moreover, the effect of fasting on SGLT1 gene expression in the ruminant rectum has not been investigated so far. In addition, the influence of grazing period on the changes in SGLT-1 gene expression in the biopsy rectal epithelia is not known. Therefore we undertook the present investigation.

In this report, we studied i) the measurement of SGLT-1 gene expression in the biopsy epithelial tissue of rectum, ii) the effect of the fasting condition to understand the physiological function of SGLT-1, and iii) the changes in

gene expression under the grazing condition in the pasture, of SGLT-1 in the rectal epithelia of Japanese black beef cattle.

MATERIALS AND METHODS

Animals

Female Japanese Black beef cattle were allowed access to water and orchard grass silage *ad libitum* and were maintained under these conditions for three weeks in the pen (50 m²/cattle) of an animal house before the experiment. The orchard grass silage contained 14.1% CP, 34.6% ADF and 53.5% NDF of a dry matter basis (Table 1).

Five cattle were used to sample the epithelial tissue and the whole tissue in rectum. After the biopsy sampling of rectal epithelial tissue by an endoscope, they were euthanized by an intravenous overdose injection of sodium pentobarbital (100 mg/kg of BW) to excise the rectum. For fasting experiments using five cattle, food was removed at 13:00 h and fasting was continued for 72 h. The biopsy sampling of rectal epithelial tissue and body weight measurement was carried out at 13:00. For the grazing experiment, five cattle were transported to the experimental pasture connecting animal house in our institute, and were grazed in a pasture from May to October. The biopsy sampling was done once a month during the experimental period (before and during period).

Present experiments were approved by animal ethical committee of our institute (NARCH), and were carried out in accordance with the guidelines on handling of laboratory animals of our institute, which basically conform with the guidelines for the care and use of laboratory animals of the National Institutes of Health, USA (NIH publication No.86-23, revised 1985).

The experimental pasture

This grazing study was conducted in Sapporo, National Agricultural Research Center for Hokkaido region (42° 59' N, 141° 24' E). The experimental pasture contains a flatland paddock (2.6 ha) and a slope land paddock (5.0 ha) including shelter woods (0.6 ha). The pasture has been used for the grazing experiment of cattle for 30 years. The flatland paddock of the pasture was regenerated by overseeding of perennial ryegrass with fertilizer in 2000. In the case of present experiment, ten cattle including five experimental cattle have been stocked from May to October

Table 2. Seasonal changes in DM, CP, NDF and standing crop of forage in the pasture¹

Item	June	July	August	September	October
DM (%)	24.2	29.4	28.9	27.9	29.3
CP (% of DM)	13.8	13.9	13.3	13.5	14.5
NDF (% of DM)	50.5	51.4	53.0	52.8	51.8
Standing crop (g of DM/m ²)	141.1	121.1	161.5	186.2	102.2

¹ Forage samples were collected randomly twice a month from ten sites in the pasture.

in 2006. The monthly mean temperature from May to October is 7.1°C to 22.8°C. To understand the quality of forage in the pasture, forage samples were collected randomly twice a month from ten sites in the paddock (Table 2). Samples were dried at 70°C for 48 h and ground with a Wiley mill to pass 0.5-mm screen. Standing crop was expressed as weight of dry matter per area (g of DM/m²). Crude protein (CP) was measured by the Kjeldahl's method (Agriculture, Forestry, and Fisheries Research Council Secretariat, MAFF 1995) using the DK20 Heating Digester and Distillation Unit UDK140 (VELP Scientifica, Milan, Italy). Neutral detergent fiber (NDF) was measured by a modified version of the Van Soest procedure for fiber analysis (Van Soest and Wine, 1967) using ANKOM 220 Fiber Analyzer (ANKOM techno. Corp., Fairport, NY, USA). The mean values of 20 samples in one month are shown as the monthly mean value.

The tissue sampling

The excised whole rectal tissue (about 2.5 g) was

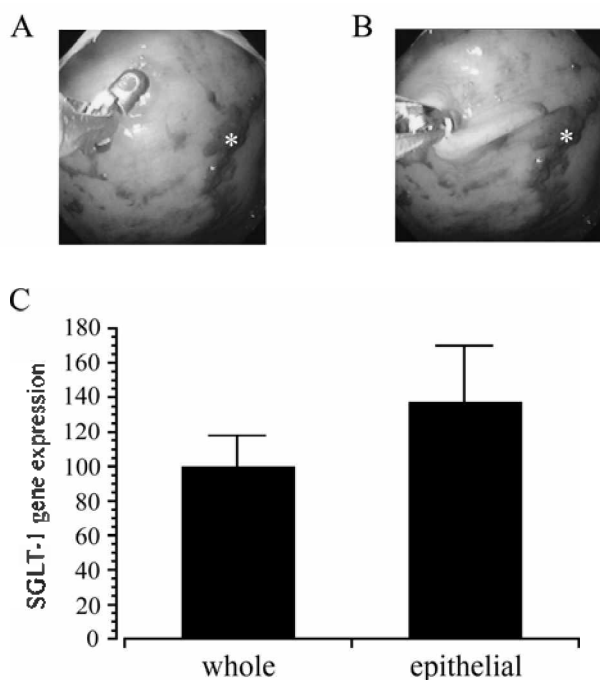


Figure 1. SGLT-1 gene expression in the biopsy epithelial tissue of rectum in female Japanese Black beef cattle fed orchard grass silage freely. (A) The biopsy forceps approaching to rectal epithelia after the removing of feces (*) from the central space. (B) The biopsy forceps pulling out rectal epithelial tissue. (C) SGLT-1 gene expression in the whole tissue and the biopsy epithelial tissue of rectum (n = 5). SGLT-1 mRNA expression levels were measured by real-time PCR using relative standard curve, and calculated by normalizing the expression level of SGLT-1 with that of beta-actin. Results are shown as percentage of mean values of SGLT-1 mRNA expression in the whole tissue (the mean+SEM).

washed with ice-cold PBS and was placed in 50 ml of a commercial RNA extraction solution (Trizol, Invitrogen) on ice. In the case of biopsy sampling, an endoscope system for animal (AVS Co., Olympus VES) was used. The endoscope (AVS Co., VQ-8203A) was inserted until 40-50 cm from the anus after removal of feces (Figure 1A). The epithelial tissues were taken by biopsy forceps under observation of video-camera (Figure 1B). The biopsy sample (about 20 mg) was washed by ice-cold PBS, and was placed in 600 µl of a Trizol solution on ice. Total RNAs were prepared from the tissues immediately after moving to experimental room.

Real-time PCR

The sample cDNA for real-time PCR was obtained by reverse transcriptase reaction of total RNAs. Amplification was carried out by two-step PCR using the TaqMan PCR system. Oligonucleotide primers for bovine SGLT-1 (Genbank accession no. AF508807) were 5'- ATGGTGATG CCAGGGATGAT - 3' (sense) and 5'- TGGTGCCGCAGT ATTTCTCA - 3' (antisense). Oligonucleotide primers for bovine beta-actin (Genbank accession no. AY141970) were 5'- AGGTCATCACCATCGGCAAT - 3' (sense) and 5'- TCGTGAATGCCGCAGGAT - 3' (antisense). The detection probes (Biosearch technologies, USA) for bovine SGLT-1 and beta-actin were FAM - TGTTACAGAGAAAGT TGCCTGCACCG - BHQ and FAM - TTCCGCTGCCCT GAGGCTCTCTTC - BHQ, respectively. Reaction condition was 95°C for 10 min followed by 40 cycles of the amplification step (95°C for 15 s and 62°C for 1 min). The amplification products from mRNAs were predicted to be 91 base pairs (bp) for SGLT-1 and beta-actin. A negative control reaction was performed with no-reverse transcriptase to confirm the specific amplification of cDNA created from the RNA provided in the reaction, and that genomic DNA was not influential in the results. Gene expression rate was obtained by normalizing the amount of SGLT-1 with that of beta-actin using relative standard curve method.

Statistical analysis

The results are represented as the mean+SEM. The mean values were analyzed by student's *t*-test and one-way ANOVA followed by Fisher's LSD test (Zar, 1984). *P* values of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

SGLT-1 gene expression in the biopsy epithelial tissue of rectum

The rectal epithelial tissues were obtained around 40-50 cm from the anus by biopsy forceps using an endoscope

(Figure 1A and 1B). The whole rectal tissue was obtained after euthanasia. Figure 1C shows the gene expression of SGLT-1 in the whole tissue and the biopsy epithelial tissue of the rectum in beef cattle fed orchard grass silage freely ($n = 5$). SGLT-1 mRNA levels were measured by real-time PCR using relative standard curve. SGLT-1 gene expression was shown as percentage of mean values of the whole tissue. SGLT-1 gene expression in the biopsy epithelial tissue (137.5 ± 31.6) was similar to that of the whole tissue (100.0 ± 17.3). There is no significant difference between SGLT-1 gene expression in both tissues (Student's *t*-test, $p = 0.32$). The rectal SGLT-1 gene expression has been not reported in cattle. Although the SGLT-1 gene expression is very low in bovine rectum, we were able to detect the SGLT-1 gene expression in the whole tissue and the biopsy epithelial tissue of bovine rectum. We studied the rectum to measure SGLT-1 gene expression of biopsy epithelial tissue using an endoscopy system because we are able to obtain samples without slaughter. Moreover, it is not known what kind of relationship exists between SGLT-1 gene expression and nutrient condition in bovine rectum. In the next experiment, we studied the effect of restricted dietary condition on SGLT-1 gene expression in rectal epithelia.

The effect of fasting on gene expression

We studied the effect of 72 h-fasting on SGLT-1 gene expression in the rectal epithelia. Results are shown as percentage of mean values of SGLT-1 mRNA expression at 0 day (free-feeding condition).

As shown in Figure 2A, SGLT-1 gene expression at 0 h (100.0 ± 6.4) did not change at 24 h after fasting (88.8 ± 5.3), and increased at 48 and 72 h after fasting (144.0 ± 15.6 , 218.6 ± 46.4). There was a significant difference between the SGLT-1 gene expression at 0 h (before fasting) and that at 48 and 72 h after fasting (Fisher's LSD test, $p < 0.05$).

The animal had lost some BW during the fasting period (for 72 h). Figure 2B shows the relationship between the loss of BW and SGLT-1 gene expression during the fasting. The decrease in BW was shown as percentage of body weight at day 0. We observed a significant correlation between SGLT-1 gene expression and the decrease in BW ($n = 15$; $r = 0.65$, $p < 0.01$). These results suggest that an increase in SGLT-1 gene expression induced by fasting in the rectum is dependent on a decrease in BW.

SGLT-1 will not have a physiological function to absorb glucose in bovine rectum under the normal and the fasting condition because SGLT-1 gene expression in the bovine rectum was lower than that in bovine jejunum (unpublished data, T. Kozakai) and glucose and/or carbohydrate will not flow to rectum under the both conditions. However, SGLT-1 gene expression in the rectum was increased under the fasting condition (Figure 2A). It has been reported that the

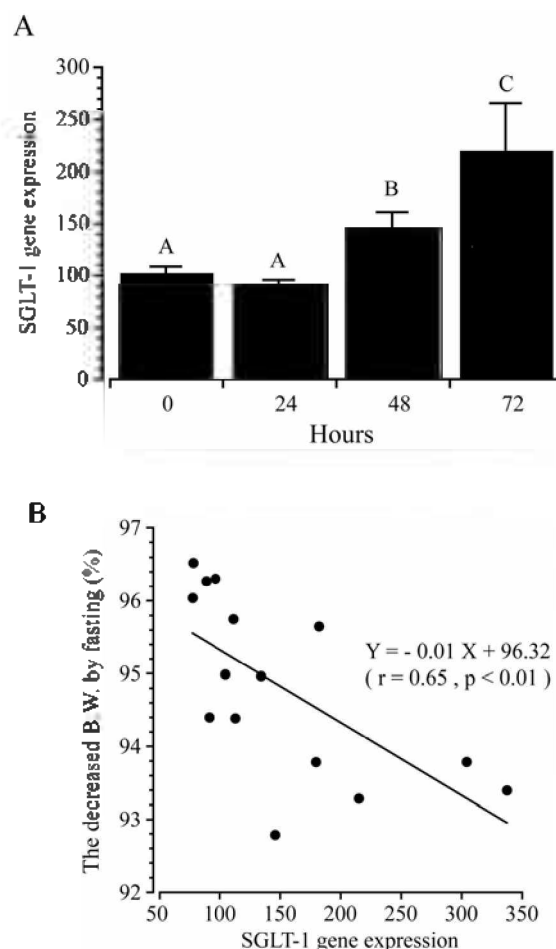


Figure 2. The effect of fasting on the gene expression of SGLT-1 in bovine rectal epithelia. Animals were allowed to orchard grass silage *ad libitum* before fasting (0 h). The biopsy epithelial tissues were taken at 24, 48 and 72 h after fasting. mRNA expression levels were measured by real-time PCR using relative standard curve, and calculated by normalizing the expression level of SGLT-1 with that of beta-actin. (A) The fasting-induced SGLT-1 gene expression in rectal epithelial tissue ($n = 5$). Results of gene expression are shown as percentage of mean values of SGLT-1 mRNA expression before fasting (the mean \pm SEM). Means not sharing a common letter (A, B and C) are significantly different ($p < 0.05$; Fisher's LSD test). (B) A significant correlation between the SGLT-1 gene expression and fasting-induced decrease in body weight at 24, 48 and 72 h after fasting ($n = 15$, $r = 0.65$, $p < 0.01$). Results of decrease in body weight are shown as percentage of mean values of body weight before fasting (at 0 h).

glucose absorption and SGLT-1 gene expression in the small intestine increased in fasting rats and dogs (Galassetti et al., 1999; Habold et al., 2005). The fasting-induced SGLT-1 gene expression might be enhanced to get a little energy although SGLT-1 will not have a physiological function to absorb glucose from the rectal digesta. The present results suggest that the measurement of SGLT-1 gene expression in the rectal epithelial tissue might indicate the nutrient condition in ruminants. The understanding of

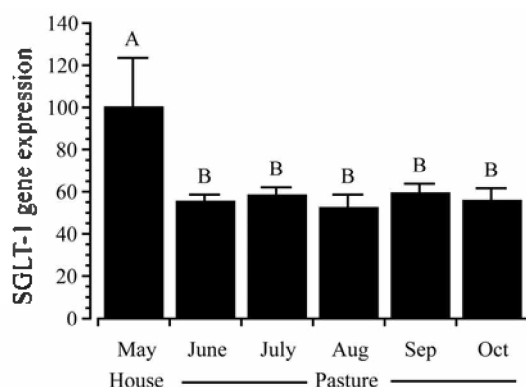


Figure 3. Changes in SGLT-1 gene expression in rectal epithelial tissue during the grazing period ($n = 5$). Animals were allowed to orchard grass silage *ad libitum* before grazing (in animal house in May), and were maintained in an experimental pasture from May to October. The biopsy sampling was done once a month in the pasture. SGLT-1 mRNA expression levels were measured by real-time PCR using relative standard curve, and calculated by normalizing the expression level of SGLT-1 with that of beta-actin. Results are shown as percentage of mean values of SGLT-1 mRNA expression before the grazing (the mean \pm SEM). Means not sharing a common letter (A and B) are significantly different ($p < 0.05$; Fisher's LSD test).

food intake and/or nutrient condition is difficult in the grazing cattle. We hypothesized that we might be able to understand the status of nutrient condition in grazing, if we elucidate the SGLT-1 gene expression in the rectum as physiological nutrient marker. In the next experiment, we studied whether if the gene expression in rectum is influenced by grazing in the pasture.

Changes in SGLT-1 gene expression during grazing period in pasture

The pasture experiment was done in our institute as described in materials and methods. Results are shown as percentage of mean values of SGLT-1 mRNA expression before grazing. SGLT-1 gene expression was high in May before the start of grazing (100.0 ± 22.9), and decreased after one month from the start of grazing in June (55.2 ± 2.9). The decrease in gene expression was maintained until the end of the grazing period in October (from 52.3 ± 5.8 to 59.2 ± 4.1). There is a significant difference between mean values before and during the grazing period (Fisher's LSD test, $p < 0.05$). This result suggests that the nutrient condition during the grazing period is better than that before the grazing period since SGLT-1 gene expression is increased by the low nutrient condition (Figure 2A). It is possible that the animals might be able to select and feed on the high-quality grass *ad libitum* because they could move freely in the pasture. In addition, it is known that the nutrient utilization of raw grass is high because TDN (% of DM) of the raw grass is higher than that of silage. The decrease in SGLT-1

gene expression after the start of the grazing might be caused by the changing of food from silage to raw grass. On the other hand, SGLT-1 gene expression in the grazing period (June to October) remained stable from 52.3 ± 5.8 to 59.2 ± 4.1 (Figure 3). There were no significant differences between mean values during the grazing period. The quality of grass was stable in the experimental pasture because CP and NDF of monthly samples of the grass obtained from 10 randomly selected area in the experimental pasture were from 13.3% to 14.5% and from 50.5 to 53.0%, respectively (Table 2). These results suggest that nutrient condition in the grazing cattle was stable during the grazing period in present study.

SGLT-1 gene expression in rectal epithelia might be useful as the acute nutrient marker because SGLT-1 gene expression of biopsy rectal epithelial tissue increased at 48 and 72 h after fasting in the daily sample (Figure 2A) and decreased after one month from the start of grazing (Figure 3). For example, it might be useful for the judging the nutrient condition in the transport cattle over long distances. However, it might be difficult to understand the nutrient condition in the pasture by the measuring of SGLT-1 gene expression of rectal epithelia in grazing cattle since SGLT-1 gene expression in the biopsy rectal epithelial tissue remained constant without drastic changes in monthly sample of animals fed freely the grazing grass in the pasture (Figure 3).

In this study, fasting increased SGLT-1 gene expression of the rectal epithelial tissue in beef cattle (Figure 2). In the rat, fasting increased SGLT-1 gene expression in the jejunum (Hobold et al., 2005). Fasting-induced SGLT-1 gene expression might be increased in the not only rectal but also jejunum in ruminants because it has thought that the effects of fasting was mediated by several hormones and metabolic factors in whole body. On the other hand, there are glucose induces SGLT-1 expression in the small intestine of ruminants (Lescale-Matys et al., 1993; Shirazi-Beechey et al., 1994; Rodriguez et al., 2004). Also, sugar substance influenced directly SGLT-1 gene expression via cAMP signaling in epithelial cells of the ovine small intestine (Vayro et al., 2001; Dyer et al., 2003). There might be two different mechanisms (directly and indirectly stimulations) to regulate SGLT-1 gene expression for the effective absorption of glucose in not only the monogastric-animals but also in ruminants.

In conclusion, we show here that, i) in the rectal epithelia samples obtained by biopsy forceps and the endoscope system, SGLT-1 gene expression was reliably measured by the quantitative real-time PCR. ii) SGLT-1 gene expression in the rectal epithelial tissues increased at 48 and 72 h after fasting dependent on the decrease in body weight. iii) SGLT-1 gene expression decreased after one month from start of grazing (May to June) and then

stabilized to the end of grazing period (June to October) in the rectal epithelial tissues.

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