Propionate and butyrate induce gene expression of monocarboxylate transporter 4 and cluster of differentiation 147 in cultured rumen epithelial cells derived from preweaning dairy calves¹

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ABSTRACT: Short-chain fatty acids (SCFAs) are the main source of energy for postweaning ruminants. The monocarboxylic acid transporters, MCT1 and MCT4, are thought to contribute to the absorption of SCFAs from the surface of the rumen following weaning. The present study measured changes in MCT1 and MCT4 expression in ruminal epithelial cells isolated from male preweaning (22 to 34 d old, $n = 6$) and postweaning (55 to 58 d old, $n = 8$) calves after euthanasia and sought to examine whether SCFAs stimulate the expression of these transporters. In the current study, cluster of differentiation 147 (CD147) gene expression in the rumen was also investigated since CD147 has been considered to act as ancillary protein for MCT1 and MCT4 to express their correct function. The gene expression levels of MCT1, MCT4, and CD147 in the rumen were found to be significantly higher in postweaning calves than in preweaning calves. Strong MCT1 immunoreactivity was detected in both the stratum basale (SB) and the stratum spinosum (SS) in postweaning ruminal epithelium. Expression

of MCT1 in preweaning calves was localized to a specific region of the SB and of the SS. MCT4-immunopositive cells were detected in the stratum corneum (SC) of the ruminal epithelium in postweaning calves. However, only a low level of signal was detected in the SC of preweaning animals. Furthermore, in vitro experiments, ruminal epithelial cells were incubated for 24 h with acetate (0.04, 0.4, and 4 mM), propionate (0.2, 2, and 20 mM), butyrate (0.1, 1, and 10 mM), or β -hydroxybutyrate (BHBA; 0.1, 1, and 10 mM), respectively. Both propionate and butyrate induced an increase in the gene expression levels of MCT4 and CD147, but did not affect MCT1 gene expression. There are no significant effects of acetate and BHBA treatment on these gene expressions. Taken together, these results suggest that an increase in MCT4 and CD147 gene expression in the ruminal epithelium of postweaning calves is likely to be due to the effects of propionate and butyrate derived from a solid-based diet, which may contribute to ruminal development following weaning.

Key words: calves, MCT1, MCT4, VFA

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INTRODUCTION

Short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate, are the main energy sources (70% to 80%) utilized by

postweaning ruminants (Bergman, 1990). SCFAs are produced within the rumen by microbial fermentation of dietary carbohydrates. These molecules appear to have a significant effect on rumen epithelial growth, blood flow, and the secretion of insulin and glucagon (Bergman, 1990). SCFAs are directly absorbed from rumen epithelial cells and butyrate is intracellularly metabolized to produce end-products β -hydroxybutyrate (BHBA), which are then transported into the bloodstream. The transportation of SCFAs across the plasma membrane is partly mediated by proton-linked monocarboxylate transporters (MCTs) (Muller et al., 2002; Kirat et al., 2006; Aschenbach et al., 2011). Two subtypes of MCT have previously been described in rumen epithelial cells. Previous reports have shown that MCT1 is localized on the basolateral surface of the rumen epithelial layer in cows (Kirat et al., 2005; Graham et al., 2007) and goats (Kirat et al., 2006), and appears to be responsible for the transportation of SCFAs metabolites into the bloodstream (Muller et al., 2002; Dengler et al., 2015). MCT4 is present on the luminal side of rumen epithelial cell layer in cows (Kirat et al., 2007) and is thought to contribute to the absorption of SCFAs from the lumen. The cell surface glycoprotein, cluster of differentiation 147 (CD147), which is also called EMMPRIN, i.e., extracellular matrix metalloproteinase inducer, is specifically associated with MCT1 and MCT4 by binding to the transporter to form heterodimer (Kirk et al., 2000). In mammalian cell lines, transfection with either MCT1 or MCT4 resulted in a failure of cells to express these transporters on their surface. In contrast, cotransfection of cells with both MCT and CD147 successfully induced cell surface expression of these transporters (Kirk et al., 2000). Kirat et al. (2006, 2007) reported a colocalization of CD147 with both MCT1 and MCT4 in the rumen of goats (Kirat et al., 2006) and cows (Kirat et al., 2007) by immunohistochemistry. Therefore, it is possible that CD147 may act as a chaperone to facilitate proper expression of MCT1 and MCT4 on the surface of rumen epithelial cells.

During the weaning process, the main site for nutrient digestion and subsequent metabolism shifts from the abomasum to the rumen in ruminants (Baldwin et al., 2004). Therefore, a system for ruminal fermentation should be established for postweaning ruminants (Khan et al., 2016). A recent study reported that gene expression related to rumen development shows dynamic change between preweaning and postweaning calves (Kato et al., 2016). Flaga et al. (2015)

showed that age-dependent increase in MCT1 gene expression was detected in the rumen of calves before weaning (Flaga et al., 2015). Age-dependent changes of MCT4 and CD147, however, have not been investigated in calves even if those are significant for absorption of SCFAs. A number of studies have explained that butyrate acts as an important stimulator of rumen epithelial function (Penner et al., 2011; Gorka et al., 2018) and is involved in the regulation of rumen MCT1 gene expression in goats (Malhi et al., 2013) and sheep (Dengler et al., 2015). Although the development of ruminal papillae is thought to be stimulated by acetate and propionate as well in calves (Sander et al., 1959; Tamate et al., 1962), little has been reported on the effect of those SCFAs on MCTs and CD147 gene expression.

The aim of the present study is to elucidate whether the expression of ruminal MCTs and CD147 increases during the weaning process and whether those expressions are directly regulated by SCFAs. First, we examined the gene expression profiles of MCT1, MCT4, and CD147 in the rumen of preweaning and postweaning calves. We subsequently compared the localization of those transporters in the rumen in preweaning and postweaning animals. We then isolated rumen epithelial cells from preweaning calves and cultured them in order to determine whether SCFAs are directly involved in the gene expression of MCT1, MCT4, and CD147.

MATERIALS AND METHODS

Animals and Animal Care

Preweaning (22 to 34 d old, $n = 6$) and postweaning (55 to 58 d old, $n = 8$) male Holstein calves were used in this study. All animals were kept under natural conditions at the Institute of Livestock and Grassland Science, National Agriculture and Food Research Organization (NARO) and provided with water ad libitum. The calves were separated from dams following colostrum intake and provided with a milk replacer. Postweaning animals were weaned at approximately 50 d of age and were then fed grass silage and starter. Animals were euthanized with an overdose of sodium pentobarbital and potassium chloride solution in order to collect the ruminal tissue. The ruminal tissues were used for gene expression analyses, *in vitro* culture, and histology. All procedures for animal experiments were approved by the Committees of the Care and Use of

Experimental Animals at the National Agriculture and Food Research Organization Institute of Livestock and Grassland Science.

SCFA Treatment of Rumen Epithelial Cells In Vitro

Rumen tissues were collected from 4 pre-weaning calves (22 to 26 d old) and washed with saline on ice. Dissociated rumen cells were prepared as described previously (Galfi et al., 1981) with minor modifications. Briefly, the tissues were rinsed 5 times in PBS (pH 7.0) containing amphotericin B (9.0 µg/mL; Sigma-Aldrich, St. Louis, MO) on ice and then incubated in PBS for 1 h at 4 °C. Ruminal tissues were washed twice with PBS and digested by treatment with PBS-0.3% trypsin (Life Technologies, Palo Alto, CA)-0.02% EDTA for 30 min at 37 °C in order to collect rumen epithelial cells. Next, the digested tissues were filtered through a 70-µm pore-size nylon mesh (BD Biosciences, San Jose, CA). The dissociated cells were seeded into collagen IV (Cellmatrix; Nitta Gelatin, Osaka, Japan)-coated flasks and incubated with Dulbecco's Modified Eagle Medium (DMEM, high glucose; Thermo Fisher Scientific, Waltham, MA) containing amphotericin B and 10% fetal bovine serum (FBS; Thermo Fisher Scientific) in 5% CO₂ in air at 37 °C. The medium was replaced every 2 to 3 d.

The experiments were conducted using rumen epithelial cells at passage 2 in duplicate, which were seeded into 24-well plates coated with collagen IV. Confluent cells were incubated in DMEM-amphotericin B-FBS containing acetate (0.04, 0.4, and 4 mM, Wako Pure Chemical, Osaka, Japan), propionate (0.2, 2, and 20 mM; Wako Pure Chemical), butyrate (0.1, 1, and 10 mM, Wako Pure Chemical) or BHBA (0.1, 1, and 10 mM; Sigma-Aldrich), respectively, in 5% CO₂ at 37 °C. The supernatant was aspirated and 100 µL of lysis solution (Thermo Fisher Scientific) was added at 24 h following treatment in order to recover total RNA from the epithelial cells. The samples were then stored at -80 °C until use in gene quantification analyses. Cell viabilities were measured at 24 h after each treatment by automated cell counter (TC20, Bio-Rad, Hercules, CA) using trypan blue dye (Bio-Rad). Mean cell viabilities even in higher concentrations of each treatment were 83.3% in acetate, 85.3% in propionate, 88.3% in butyrate, 90.8% in BHBA, and 87.0% in vehicle.

Quantification of Gene Expression Levels in the Ruminal Tissues and Cultured Cells

Ruminal tissue samples were placed into 200 µL of QIASol Lysis Reagent (QIAGEN, Hilden, Germany) immediately following their isolation. The samples were then homogenized on ice and stored at -80 °C until total RNA extraction. Total RNA was extracted by RNeasy Plus Universal Mini Kit (QIAGEN). Genomic DNA was removed by gDNA Eliminator solution included in the kit. RNA concentration and absorbance ratio ($A_{260/280}$ and $A_{260/230}$) were measured by spectrophotometer (NanoDrop 1000, Thermo Fisher Scientific). cDNA in a total volume of 20 µL was synthesized from 1 µg of RNA from each sample with 0.5 µg of Oligo deoxythymidine (dT)₁₂₋₁₈ primer at 42 °C for 50 min using Super Script II Reverse Transcriptase (200 U/µL, cat. #: 18064014, Thermo Fisher Scientific) in the presence of 40 units RNaseOUT (cat. #: 10777019, Thermo Fisher Scientific) in accordance with the manufacturer's instructions. The cDNA was incubated with 1 µL of RNase H (2 U/µL) at 37 °C for 20 min to remove RNA complementary to the cDNA. Cultured rumen epithelial cells were lysed with Lysis solution 24 h following treatment. Total RNA was then extracted from the cells using the RNAqueous-Micro Total RNA Isolation Kit (Thermo Fisher Scientific) followed by 1 µL of DNase I treatment for 20 min at 37 °C. RNA concentration and absorbance ratio were measured by spectrophotometer. cDNA in a total volume of 20 µL was synthesized from 1 µg of RNA with 2.5 µM of Oligo (dT)₂₀ primer at 50 °C for 60 min using Thermo Script reverse transcriptase (15 U/µL, cat. #: 12236-014, Thermo Fisher Scientific) in the presence of 40 units RNaseOUT and subsequently incubated with 1 µL of RNase H (2 U/µL) for 20 min at 37 °C in accordance with the manufacturer's instructions. Real-time PCR analysis with Brilliant II SYBR Green QPCR Master Mix (Agilent, CA) was performed in duplicate using Mx4000 (Agilent). Each reaction was performed in the final volume of 20 µL. The following primers were used at the final concentration of 0.25 µM for real-time PCR: MCT1 (*SLC16A1*, NM_001037319, 148 bp) forward (exon2), 5'-TTA ATG CCA CCA CCA GTG AA-3'; reverse (exon3), 5'-AAG CCA CTG CCT GAC AAG AT-3'; MCT4 (*SLC16A3*, NM_001109980, 85 bp) forward (exon4), 5'-TCC AGC TCT ACC TCA CCA CA-3'; reverse (exon5), 5'-TAG CGG TTG AGC ATG ATG AG-3'; CD147 (*BSG*, NM_001075371, 63 bp) forward

(bridging exon2-3 junction), 5'-CGG AGT ATG AGG TGG ACT CAG AA-3', reverse (exon3), 5'-GCT CCG GAA GGA AGA TGC A-3'; *GAPDH* (NM_001034034, 120 bp) forward (exon3), 5'-AGT TCA ACG GCA CAG TCA AG-3'; reverse (exon4), 5'-CAT ACT CAG CAC CAG CAT CA-3'. Quantitative PCR was carried out using the following program: 95 °C for 10 min, 40 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 90 s, followed by dissociation program (95 °C for 1 min, 55 °C to 95 °C with 0.5 °C interval, 30 s per interval). The melting curve was analyzed to check the specificity of the PCR products. The assay performance was assessed with MxPro QPCR Software ver.3.20 (Agilent). Standards were prepared from 7-points serial dilutions of plasmid containing each target. Linear dynamic range defined as the lowest to the highest quantifiable copy number is from 25 to 10⁷ copies. Reaction efficiency and correlation coefficients were as follows: 106.8% and 0.991 (*GAPDH*), 96.8% and 0.990 (*MCT1*), 95.6% and 0.997 (*MCT4*), and 104.6% and 0.999 (*CD147*). Three housekeeping genes, such as *GAPDH* (Glyceraldehyde-3-phosphate dehydrogenase), *PGK* (Phosphoglycerate kinase), and *ATP5A1* (ATP synthase F1 subunit alpha), were tested to determine optimal reference gene for the quantitative PCR data from rumen epithelial cells by Normfinder software (Andersen et al., 2004). Since *GAPDH* showed a higher stability value (0.028) compared with *PGK* (0.077) and *ATP5A1* (0.067), *GAPDH* gene was used for normalization in the current study.

Immunohistochemistry

Ruminal tissues were fixed with 4% paraformaldehyde overnight at 4 °C and then submerged in 20% sucrose prior to sectioning. The tissues were then cut into 6-µm sections using a cryostat (CM1850; Leica, Wetzlar, Germany) and Tissue-Tek O.C.T. compound (Sakura Finetek Japan, Tokyo, Japan). The sections were then mounted onto silane-coated slide glasses. Slides were boiled in 0.01 M Tris-EDTA buffer (pH 9.0) for 20 min to retrieve antigens for use in immunohistochemistry. Sections were then washed with PBS with 0.1% Tween 20 (PBST) following a 30-min incubation in 0.3% hydrogen peroxide to eliminate endogenous peroxidase activity. Next, sections were incubated with a blocking solution of PBST containing 10% ImmunoBlock (DS Pharma Biomedical Co., Osaka, Japan) for 1 h, followed by an overnight incubation with primary antiserum, such as chicken polyclonal anti-MCT1

antiserum (1:5000, ab1286, Chemicon, Germany) or mouse monoclonal anti-MCT4 antibody (1:50, sc-376140, Santa Cruz Biotechnology, Santa Cruz, CA) at room temperature. The immunoreactivities of MCT1 and MCT4 were visualized using the TSA Biotin System (PerkinElmer, Branchburg, NJ) and Alexa 555-conjugated streptavidin (1:400; Life Technologies). The sections were then incubated with mouse-monoclonal antivimentin antibody (1:100; clone VIM 3B4, Boehringer Mannheim, Mannheim, Germany), followed by incubation with Alexa488 conjugated goat anti-mouse IgG (1:400, ab150113, abcam, Cambridge, UK) to stain the lamina propria. Finally, the sections were embedded with Prolong Gold containing 4',6-diamidino-2-phenylindole dihydrochloride (DAPI; Life Technologies) for nuclear staining. Removal of primary antibodies from the immunohistochemical protocol was found to result in a complete absence of signals. Micrographs were obtained using the fluorescent microscope (FSX100, Olympus, Tokyo, Japan).

Statistical Analysis

Statistical differences ($P < 0.05$) in gene expression levels between preweaning and postweaning animals were determined using the Student's *t*-test. Comparison of each dose group to a control revealed a significant difference ($P < 0.05$) in gene expression levels in vitro using the Steel's test (Steel, 1959).

RESULTS

Comparison of MCT1, MCT4, and CD147 Gene Expression Levels in Ruminal Tissues Isolated Preweaning and Postweaning

The gene expression levels of MCT1, MCT4, and CD147 in the rumens of postweaning calves were found to be significantly higher than those in preweaning animals (Figure 1).

Localization of MCT1 and MCT4 in the Rumens of Preweaning and Postweaning Calves

MCT1 and MCT4 immunoreactivity was detected in rumen epithelial cells. Vimentin was stained the lamina propria of both preweaning and postweaning dairy calves (Figure 2). MCT1 immunoreactivity was detected in a region of the stratum basale (SB) and stratum spinosum (SS, Figure 2B) in preweaning calves. MCT1 immunoreactivity was detected in the SB, SS, and the partial stratum

Figure 1. Gene expression analysis of monocarboxylate transporters, MCT1 and MCT4, and CD147 in preweaning and postweaning calves by quantitative RT-PCR. Values are mean $\pm$ SEM. Asterisks indicate a significant difference between preweaning ($n = 7$) and postweaning ($n = 8$) animals (** $P < 0.01$, * $P < 0.05$, Student's t -test).

granulosum (SG, [Figure 2D](#)) in postweaning animals. MCT4-immunopositive cells localized to a region of the stratum corneum (SC) facing lumen in preweaning calves ([Figure 2F](#)), and this staining was observed in almost all SC region in postweaning animals ([Figure 2H](#)).

Effects of SCFAs and BHBA on Gene Expression of MCT1, MCT4, and CD147 in Cultured Rumen Epithelial Cells In Vitro

Treatment with either SCFA or BHBA had no effect on the gene expression of MCT1 in rumen epithelial cells in vitro. No significant difference was observed between the gene expression levels of MCT1 in the control group and the experimental groups ([Figure 3A](#)). A high dose of propionate (20 mM) induced a significant increase in MCT4 gene expression level compared with that observed in the control group ([Figure 3B](#)). Treatment with middle (1 mM) and high doses (10 mM) of butyrate was also found to stimulate MCT4 gene expression, which was significantly higher than that in control group ([Figure 3B](#)). Similarly, the gene expression levels of CD147 induced by high doses of propionate (20 mM) and butyrate (10 mM) were significantly higher than that in the control group ([Figure 3C](#)). Treatment with either acetate or BHBA had no effect on MCT4 and CD147 gene expression levels ([Figure 3B](#) and [C](#)).

DISCUSSION

The present study demonstrated that the gene expression levels of MCT1, MCT4, and CD147 were upregulated in postweaning animals. The results of in vitro experiments clearly showed that

propionate and butyrate directly stimulate rumen epithelial cells, leading to enhance MCT4 and CD147 gene expression but not MCT1 gene expression. In dairy calves, ruminal infusion of butyrate induced an increase in the bloodstream and absorption of VFA ([Storm et al., 2011](#)). A daily ruminal infusion of butyrate promotes gene expression of MCT4 and increases the absorption rate of SCFA from the luminal side of the rumen in adult goats ([Malhi et al., 2013](#)). Taking these reports into consideration, the present results suggest that the increase in MCT4 and CD147 gene expression in postweaning animals may be due to the effects of diet-derived propionate and butyrate on rumen epithelial cells. To the best of our knowledge, since the report of gene expression changes by propionate has been limited, the present result would provide a notable possibility what propionate directly affect the gene expression of MCT4 and CD147 in the rumen epithelial cells.

CD147 is considered to play a significant role for MCT4 to express on cell surface ([Kirk et al., 2000](#)), because the transporter fails to express on cell surface even if the gene and protein successfully expressed when CD147 gene expression is suppressed ([Gallagher et al., 2007](#)). [Wilson et al. \(2002\)](#) showed that an active transporter complex consists of two transporter molecules and two CD147 molecules on plasma membrane ([Wilson et al., 2002](#)), suggesting that an increase in CD147 gene expression correlated with an elevation of MCT4 gene expression would be important for the transporters to show their correct function on cell surface. SCFAs, especially butyrate, are considered to act as inhibitor of histone deacetylase ([Waldecker et al., 2008](#)), which would induce an increase in acetylation

Figure 2. Representative images of ruminal tissue samples stained with monocarboxylate transporters, MCT1 and MCT4, vimentin (Vim), and 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) in the rumen of preweaning and postweaning calves. Nuclear staining of ruminal sections with DAPI (cyan blue; A, C, E, and G). Immunostaining of MCT1 (red) and Vim (green) in the rumen of preweaning and postweaning calves (B and D). Immunostaining of MCT4 (red) and Vim (green) in the rumen of preweaning and postweaning animals (F and H). SC = stratum corneum; SG = stratum granulosum; SS = stratum spinosum; SB = stratum basale; LP = lamina propria; Scale bar = 50 μ m.

of histones (New et al., 2012). This epigenetic regulation of SCFAs would open the chromatin structure to access transcription factors to the promoter

region of genes. Previous studies have shown that MCT4 and CD147 gene expression can be stimulated by factors including hypoxia-inducible factor

Figure 3. Effects of the administration of each short-chain fatty acid (SCFA) (acetate, propionate, or butyrate) or β -hydroxybutyrate (BHBA) on gene expression levels of monocarboxylate transporter 1 (MCT1; A), MCT4 (B), and CD147 (C) in cultured rumen epithelial cells. Values are mean $\pm$ SEM. Asterisks indicate a significant difference comparing each dose group to a control ($n = 4$, ** $P < 0.01$, * $P < 0.05$, Steel's test).

1 α (Ullah et al., 2006) and Sp1 (Kong et al., 2010). These factors are known to be activated by butyrate in human colon cell lines (Nakano et al., 1997; Kelly et al., 2015). These findings raise a possibility that SCFAs might switch the MCT4 and CD147 gene from an inactive to an active by epigenetic regulation and affect the activity of transcriptional factors to promote the gene expressions. Interestingly, SCFAs

are reported as a ligand of GPR41 and GPR43 as well (Brown et al., 2003; Le Poul et al., 2003). Those G-protein coupled receptors (GPCRs) express in rumen epithelial cells of cows (Wang et al., 2009). The GPCR-mediated signaling induces a decrease in cAMP, which induces abundance of MCTs expression on cell surface (Borthakur et al., 2012). Further studies focusing on acetylation statement are needed to better understand the intercellular mechanism underlying the regulation of MCT4 and CD147 gene expression, and the research for protein expression and function mediated by the GPCR signaling of SCFAs in the rumen would be required as well.

The current study demonstrated that neither VFA nor BHBA treatment had any effect on MCT1 gene expression in cultured rumen epithelial cells in vitro. However, in vivo, gene expression levels increased in postweaning calves compared with that observed in preweaning animals. Our inconsistent results of MCT1 gene expression between in vitro and in vivo would uncover that MCT1 gene expression in the rumen epithelial cells is regulated by complex factors other than SCFAs in vivo. Intraruminal infusion of butyrate had no effect on MCT1 expression in adult goats (Malhi et al., 2013) and cows (Baldwin et al., 2012), suggesting that the effect of SCFA on MCT1 expression is likely to be small in the rumen. Pfannkuche et al. (2014) suggested that the presence of factors that control MCT1 expression under non-SCFA conditions in calves as ruminal MCT1 expression was found to change dramatically within 4 d following the birth (Pfannkuche et al., 2014). Previous studies raise the possibility that rumen epithelial development may be affected by a variety of factors including hormones and growth factors secreted by other organs or tissues. Insulin, insulin-like growth factor I, and epidermal growth factor (Neogady et al., 1989; Baldwin, 1999) are known to stimulate ruminal cell proliferation in vitro. Furthermore, signaling via peroxisome proliferator-activated receptor α (PPAR α) upregulates the gene expression levels of MCT1 in the rumen epithelial cells of sheep (Benesch et al., 2014). However, the endogenous ligand that stimulates MCT1 expression via PPAR α is still unknown. These results suggest that MCT1 expression in the ruminal epithelium may be modulated by body-derived factors other than SCFAs. By contrast, MCT1 levels were shown to be stimulated by butyrate in human colonic epithelial cells (Cuff et al., 2002) and intestinal cells (Borthakur et al., 2008). Therefore, further studies are needed to identify the cause of the increase in MCT1 gene

expression in the rumen observed following weaning in calves.

In this study, we investigated the localization of MCT1 and MCT4 in the rumen of preweaning and postweaning calves. The results of immunohistochemistry demonstrate that the immunoreactivities of MCT1 and MCT4 are mainly observed in the SB and SC of the ruminal epithelium, as shown in a previous study (Kirat et al., 2007). Strong MCT1-immunoreactivities were detected in several layers of tissue containing SB, SS, and SG derived from postweaning calves. However, those signals were observed in only a single layer, or at most, in 2 to 3 layers of the tissue in preweaning animals. The immunoreactivities of MCT4 were detected in only a single layer of cornified cells in postweaning calves at 60 d old. In much more senior cows at 4 to 5 yr old, those animals showed MCT4-immunopositive cells in both SC and SB (Kirat et al., 2007). Functional significance of MCT4 in SC is controversial since SC is assembled from keratinized cells. The increases in MCT-immunoreactivities in SC following weaning may not coincide with the promotion of MCT function. At a little while after weaning, SCFAs across the ruminal epithelium might be dependent on the permeability of undissociated form of SCFAs to the lipid bilayer (Walter et al., 1982) or exchange of SCFAs anion for bicarbonate via transporter protein (Aschenbach et al., 2009). It also should be noted that the intracellular immunoreactivities of MCTs were detected. This distribution of those transporters in rumen epithelial cells could be partly due to the storage of those proteins in the endoplasmic reticulum (Kirk et al., 2000). Furthermore, MCTs might not only be stored at intracellular but also act as lactate transporter in mitochondria (Kirat et al., 2007; Hussien and Brooks, 2011). The functional significance of the transporters at a little while after weaning would be investigated by the future study.

In conclusion, the current study demonstrates that the gene expression of MCT4 and CD147 observed in the ruminal epithelium following weaning would be due to the effects of propionate and butyrate derived from the animals' diet. MCT1 gene expression was upregulated in postweaning calves but the expression levels of MCT1 were not affected by SCFAs in vitro, suggesting that body-derived factor may primarily control MCT1 expression. Further studies are needed to obtain a better understanding of the mechanisms involved in ruminal growth and development. Devising a specific diet to enhance the ruminal development of calves may help shorten the weaning period of

calves. Since it is noted that gene expression does not necessarily imply protein expression and/or activity, studies focusing on protein expression and function would be required in the future.

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