Title: Sodium butyrate administration modulates the ruminal villus height, inflammation-related gene expression, and plasma hormones concentration in dry cows fed a high-fiber diet

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Running Head: BUTYRATE ON RUMEN EPITHELIUM AND METABOLISM

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ABSTRACT

The objectives of this study were to evaluate the effects of sodium butyrate on the ruminal villus morphology, mRNA expression associated with nutrient metabolism and inflammation in the ruminal epithelium, and plasma concentrations of metabolites and hormones in non-lactating cows fed a high-fiber diet. Four Holstein cows with a rumen cannula were assigned to 2 treatments in a crossover design. The treatments were ruminal administration of sodium butyrate premix or control premix before feeding to cows fed the same total mixed ration mainly composed of glass silage once a day. Sodium butyrate was provided at a butyrate dose of 0.04% per kg body weight. The control premix was made by replacing sodium-butyrate with wheat bran. The plasma β-hydroxybutyrate concentration increased 3 to 6 h after the butyrate premix administration but returned to a concentration similar to that of the control before feeding. After continuous administration, increases in the ruminal villus height and plasma concentration of glucagon-like peptide-2, and lower gene expression of TNF-α, IL-1β, and TLR-2 in the rumen epithelium were observed in cows supplied with the butyrate premix. These results showed that sodium butyrate affects rumen epithelial morphology and plasma concentrations of hormones even under a low fermentable diet.

Keywords: butyrate, GLP-2, inflammation-related gene, rumen papillae, dairy cow

INTRODUCTION

In recent years, highly fermentable diets have been fed to dairy cattle to meet the nutritional requirements of high lactating ability. Microorganisms utilize high starch diets to produce large quantities of volatile fatty acids (VFA) in the rumen, causing subacute rumen acidosis. Feeding diets with a high starch content also promote posterior intestinal fermentation (Reynolds, 2006) and may induce hindgut acidosis (Li et al., 2012). These have symptoms such as decreased feed intake, diarrhea, and laminitis in dairy cattle, leading to decreased milk yield (Abdela, 2016), and are thus a non-negligible issue in dairy farms. Highly fermentable diets produce large quantities of VFA and accumulation in the rumen, causing damage to the rumen epithelium (Steele et al., 2009). Additionally, low pH in the rumen kills gram-negative bacteria and increases the proinflammatory lipopolysaccharide (LPS) within the rumen (Gozho et al., 2005; 2007). The rumen acidification causes damage and lowering of the barrier function of rumen epithelial cells, and LPS migrates into the blood, binding to lipopolysaccharide-binding protein and causing inflammatory reactions such as laminitis (Nocek, 1997). To prevent these harms, it is important to enhance the functions of nutrient absorption, pH buffering and immunity in the ruminal epithelium. Adaptation of gastrointestinal morphology and function to high-grain diets takes several weeks (Górka et al., 2017). Therefore, in conventional perinatal management, it is recommended to increase the grain ingestion from the close-up period in order to adapt to a highly fermentable diet after calving, but excessive energy intake before calving is not appropriate because it promotes negative energy balance (Hirabayashi et al., 2017). Therefore, an approach that adapts the gastrointestinal tract during the dry period without supplying more

energy than necessary to respond quickly to postpartum highly fermentable diets may be effective in preventing the health harms associated with rumen and hindgut acidosis.

Butyrate is one of the VFA produced by the ruminal fermentation of carbohydrates and is effective in strengthening the structures and functions of the gastrointestinal tract (Górka et al., 2018). In the ruminal epithelium, butyrate promotes cell proliferation and the development of ruminal papillae (Górka et al., 2011; Kowalski et al., 2015; Malhi et al., 2013). In addition to morphological changes, several studies have reported that butyrate affects the nutrition-related gene expression in the rumen. Regarding genes that affect intracellular pH regulation, the increase in ruminal butyrate concentration increases the expression levels of short-chain fatty acid membrane transport (MCT) in calves, lambs, and goats (Laarman et al., 2012; Liu et al., 2019; Yan et al. 2014), and proton membrane transport (NHE) in calves and goats (Laarman et al., 2012; Yan et al., 2014). Simmons et al. (2009) found that the gene expression of urea transport-B1 (UT-B1) in the rumen epithelium of steers was increased by a concentrate-based diet. From these studies, they speculated that the increased ruminal butyrate fermentation due to high concentrate diets or exogenous butyrate may modulate the expression of nutrition-related factors. Therefore, butyrate increases pH buffering capacity and urea utilization in the rumen. Butyrate is also effective in suppressing the inflammatory response by reducing NF-κB activity involved in the transcriptional regulation of cytokine genes such as tumor necrosis factor (TNF), and enhancing barrier function by strengthening the connection of rumen epithelial cells (Zhang et al., 2018).

Butyrate is currently available as a feed supplement. In calves, the effects in the forestomach and on small intestine development by adding sodium-butyrate to the diet have been summarized by Górka et al. (2018). In addition, sodium-butyrate coated with

fats was developed for slow dissolution and widespread absorption in the gastrointestinal tract (Fernández-Rubio et al., 2009), and is available as a feed additive. In the lower gastrointestinal tract, L cells present in the terminal ileum and colon are stimulated by VFA, particularly butyrate, to stimulate the secretion of glucagon-like peptide-2 (GLP-2) (Tappenden et al., 2003). Glucagon-like peptide-2 enhances the development of intestinal epithelial cells (Taylor-Edwards et al., 2010; Connor et al., 2013) and the barrier function of the ileum and colon (Walker et al. 2015). Our previous study has shown that the supplementation of sodium-butyrate coated with fats increases the plasma GLP-2 concentration in lactating cows (Fukumori et al., 2020), and mitigated rectal acidosis after ruminal starch infusion (Fukumori et al., 2021). Therefore, butyrate affects both the rumen and lower gastrointestinal epithelial tissues. In addition, butyrate is a potent stimulator of insulin secretion and is itself converted to BHBA in the rumen for use as an energy substrate in the body (Elsabagh et al., 2017), so its supply may help improve insulin resistance during the parturition transition. However, studies on the effects of these feed additives containing butyrate on the gastrointestinal morphology and function have been conducted under the condition of feeding moderately to highly fermentable diets such as for lactating cows and weaning calves, and the effects under high-fiber diets such as those for dry cows have not been investigated. If butyrate supplementation can affect the rumen and lower gastrointestinal tract even in cows fed a high-fiber diet, it can be expected to be used as a feed additive suitable for the transition period from the dry period to postpartum. Therefore, the objectives of the present study were to determine the effect of ruminal administration of sodium-butyrate on the ruminal villus height and thickness, inflammation-related gene

expression, and the plasma GLP-2 concentration in non-lactating cows fed a high-fiber diet.

MATERIALS AND METHODS

Cows used in this study were housed at the Rakuno Gakuen Field Education and Research center (Ebetsu, Hokkaido, Japan). All procedures of this study were approved by the Animal Experiment Committee of Rakuno Gakuen University (approval #VH19C5).

Experimental Design, Animals, and Treatments

Four Holstein Friesian cows (body weight 763 kg (SD 10), 51 mo. of age (SD 12), non-pregnant, non-lactating), each fitted with a rumen cannula, were used in this study. Cows were housed in individual tie stalls laid with a rubber mat, and shredded paper. The cows were randomly assigned to 2 treatments in a crossover design. The treatment groups were the butyrate group (BUT) and the control group (CON). This study consisted of two 28-d experimental periods, with a 21-d washout between them. The data used in this study were collected through d 22, and later data are used in the companion paper (Fukumori et al., 2021). The BUT cows were supplemented with sodium-butyrate premix (Gustor BP70: 70% sodium butyrate and 30% other fatty acids: Norel S.A., Madrid, Spain), while the CON cows were supplemented with a control premix consisting of 70% wheat bran and 30% fatty acid mixtures. Gustor BP70 is a commercially available feed additive, but in this experiment, treatment materials were administered in the rumen to allow the prescribed amount to be ingested. The reason for choosing wheat bran as a control is that the high fiber by-product is predominantly

acetate fermentation, and the effect of replacement is considered to be remarkable. The dose quantity of butyrate was 0.04% of the body weight of each cow based on the increased ruminal butyrate concentration in the study by Elsabagh et al. (2017), who demonstrated ruminal infused butyrate increased plasma GLP-2 concentration in sheep. Each premix was ruminally administered once a day just before feeding at 0900 h through the rumen cannula. Cows were fed a total mixed ration (TMR), composed of 45.9% grass silage, 45.3% beet pulp pellets, 8.5% soybean meal, and 0.3% mineral and vitamin premix [dry matter (DM) basis]. The nutrient components of TMR were 15.1% crude protein, 45.9% neutral detergent fiber, and 28.9% non-fiber carbohydrate (as DM). The diet was fed daily at 0900 h *ad libitum* to allow for approximately 8% refusals. The feed refusals were removed at 0800 h and measured feed intake. All cows had free access to water and mineral blocks. The eating time was measured with a load cell attached to the feed box, and the rumination time was measured with an automated rumination tag-monitoring system (HR-Tag and DataFlow II; SCR Engineers, Netanya, Israel) equipped with a cow collar as validated by Schirmann *et al.* (2009).

Sample Collection

Samples of rumen papillae were collected from the rumen abdominal sac by grasping 10-15 villi by hand immediately before feeding on d 1 and 22 of each period. The samples were visually sorted to ensure that they were taken completely from the base and excluded those that were torn off in the middle. They were divided into samples for measuring the morphology and gene expression. The sample for measuring morphology was washed with phosphate-buffered saline (PBS) and then stored in tubes containing 10% formalin solution at room temperature until analysis. The sample for measuring

gene expression was washed with PBS and then immersed in RNA later stabilization solution (Life Technologies, Carlsbad, CA, USA) in an RNAase-free tube and was stored at –30℃ until analysis.

Blood samples were collected by tail venipuncture on d 1, 8, and 15 before feeding, and at 0, 3, and 6 h relative to feeding on d 22 of the collection period. Heparinized tubes (Terumo, Tokyo, Japan) were immediately placed on ice. The tubes were then centrifuged at 1,940 × *g* for 15 min at 4℃. The harvested plasma was stored at -80℃ until analysis to determine concentrations of β-hydroxybutyric acid (BHBA), glucose, urea nitrogen (UN), insulin, and GLP-2.

The weight of feed offered and feed refusals were recorded daily. Samples of feed ingredients and orts were collected from d 19 to 21 in each experimental period to determine dry matter intake (DMI). These samples were stored in a refrigerator, then composited for each period, and dried in a forced-air oven at 55˚C for 48 h to determine dry matter (DM) content.

Sample Analyses

Rumen papillae samples used for microscopic observation were only those that were definitely cut from the base [number of samples: 10.0 (SD 2.7)]. The samples were immersed in PBS for 1 h after two 1 h immersions in distilled water. Thereafter, dehydration was performed using a series of graded ethanol until 100% and immersion for 1 h using 100% xylene. Samples were embedded in paraffin, and slices 5-μm thick were made with a sliding microtome (SAKURA, IVS-400, Saitama, Japan). The sections were floated in warm water at 40˚C, scooped up with glass slides, and dried at 36˚C. Deparaffinized samples were treated with Mayer's hematoxylin for 10 min.

Thereafter, they were washed with running water for 10 min and treated with distilled water for 1 min. The samples were then immersed in an eosin solution for 3 min, then in a graded series of ethanol (50%, 70%, 90% once each, and 100% three-times; each time for 5 min) for dehydration and clearance with xylene I and xylene II for 2 min each before encapsulation with Marinol. The height and thickness of the villi were measured using an optical microscope (ECLIPSECi type; Nikon Corp., Tokyo, Japan).

Plasma concentrations of metabolites were measured using an automatic analyzer (AU680; Olympus Corp., Tokyo, Japan) with commercially available kits for BHBA (Wako Auto Kit 3-HB; Fujifilm Wako Pure Chemical Corp., Osaka, Japan), glucose (Cicaliquid GLU; Kanto Chemical, Co., Inc., Tokyo, Japan), and UN (N-assay BUN-L; Nittobo Medical Co. Ltd., Tokyo, Japan). Plasma insulin concentrations were measured using a solid-phase competition immunoassay with bovine insulin (Sigma-Aldrich Inc.), europium-labeled bovine insulin, and polystyrene microtiter strips coated with anti-guinea pig γ-globulin as described by Masuda et al. (2019). The intra-assay CV was 4.2%, and the detection limit was 0.055 ng/mL. Plasma GLP-2 concentrations were measured using a solid-phase competition immunoassay with synthetic human GLP-2 (Peptide Institute Inc., Osaka, Japan), europium-labeled human GLP-2, and polystyrene microtiter strips. The strips were coated with goat anti-rabbit γ-globulin and anti-rat GLP-2 serum targeting the N-terminal of the GLP-2 moiety (Yanaihara Institute Inc., Shizuoka, Japan) as described by Elsabagh et al. (2017). Intra- and inter-assay CV were 2.7 and 2.2%, respectively, and the detection limit was 0.042 ng/mL.

Total RNA of the rumen papillae was extracted using a Total RNA purification Kit (Jena Bioscience GmbH, Germany). The purity of the extracted RNA was measured using BioSpec-nano (Shimadzu, Kyoto, Japan) and all samples confirmed that

A260/280 was 1.8 or higher. De-DNA treatment used an RNase-Free DNase Set (Qiagen, Duesseldorf, German). Complementary DNAs (cDNA) were synthesized from 500 ng of total RNA using a ReverTra Ace qPCR RT Master Mix kit (Toyobo, Osaka, Japan). The synthesized cDNA was thermally denatured at 94℃ for 30 s, followed by annealing at 60℃ for 30 s, and extension at 72℃ for 30 seconds for 40 cycles using β-actin primers and Taq DNA polymerase (NEB, USA). For each reaction, a parallel negative control reaction was performed in the absence of reverse transcriptase. After PCR-reaction, 10 μL of increasing byproducts mixed with loading buffer was electrophoresed on a 1.5% TAE agarose gel stained with ethidium bromide and were visualized using a UV transilluminator. The reverse-transcribed cDNA was quantitated using THUNDERBIRD SYBR qPCR Mix (Toyobo, Osaka, Japan) and CFX Connect (Bio-Rad Laboratories, Hercules, Calif., USA). Each primer sequence is presented in Table 1. Thermal cycling was performed for 40 cycles with initial denaturation at 95℃ for 5 min, followed by thermal denaturation at 95℃ for 15 s, annealing at 60℃ for 30 s, and extension at 72℃ for 30 s. In the melting curves, after the PCR, the temperature of the reaction solution was raised from 55 to 95℃ by 0.5℃, and a SYBR Green 1 signal was detected. The expression levels of genes in the individual rumen epithelial samples were calculated using the σσCT method and normalized from the copies of the inner target genes β-actin, DBNDD2 (dystrobrevin-binding protein domain containing 2), and UXT (prefoldin-like chaperone).

Statistical Analysis

The daily amount of DMI, and eating and ruminating time were totaled, and the average value from d 19 to d 21 was calculated. The morphological changes in the

rumen papillae associated with the treatments were expressed as a relative ratio of d 22 to d 1. The mRNA expression in BUT was expressed as a relative value when the measurement in CON was 1.0.

All data were analyzed using the JMP fit model (version 13.2.1; SAS Institute Inc., Cary, NC, USA). For analysis of changes in plasma components with days of administration, the period, treatment, day, and interaction between treatment and day were defined as fixed effects, and cows were defined as random effects. For analysis of plasma component changes before and after feeding on d 22 of each period, the treatment, time, and interaction between treatment and time were defined as fixed effects, and cows were defined as random effects. For the analysis of DMI, eating and ruminating time, and profiles of the rumen papillae, the period and treatment were fixed effects, and cows were random effects. In all statistical treatments, $P < 0.05$ was considered significant and $P < 0.1$ tended to approach significance.

RESULTS AND DISCUSSION

Dry matter intake, and eating and ruminating behavior

The DMI, and eating and ruminating behavior in each group are presented in Table 2. The DMI was 12.3 kg/d in BUT and 12.8 kg/d in CON, and there was no significant difference. Sodium butyrate supplementation to mature cows did not affect DMI in a previous report (Izumi et al., 2019), similar to the present result. The eating and ruminating times were also similar in both treatments, and there was no significant difference. The lack of influence of butyrate on feeding behavior may be due to the fact that there was no inhibitory factor of DMI such as over fermentation in the gastrointestinal tract because cows were fed a high-fiber diet.

Changes in ruminal villus height and thickness, and mRNA expression of rumen papillae

The changes in the rumen papillae after 21 days of continuous treatments are shown in Table 3. The height of the rumen papillae did not change in CON, but increased from d 1 to d 22 in BUT ($P = 0.01$). Butyrate is effective in developing rumen papillae (Górka et al., 2011), and it is thought that the uptake of butyrate in the rumen epithelium directly affected the development of the rumen papillae. Rumen villus thickness decreased from d1 to d22 in both treatments ($P < 0.001$) and did not differ between treatments. It has been observed that feeding highly fermentable diets after calving increases the height of rumen villi but decreases their thickness, which may contribute to the increased efficiency of VFA absorption (Dieho et al., 2016). In the present study, both the control premix and the butyrate premix contained palmitic acid and stearic acid in the same ratio, and it was reported that these saturated fatty acids did not inhibit rumen microbial fermentation, and in particular palmitic acid rather improved NDF digestibility (de Sousa & Lock, 2018, 2019). The effect of these saturated fatty acids on rumen villi has not been known, but they may have reduced thickness through rumen fermentation.

In the present study, gene expressions of mRNA related to nutrient metabolism (MCT-1, NHE-1, and UT-B1) in the rumen epithelium were investigated to verify whether butyrate enhances nutrient absorption in the ruminal epithelium, but they were not affected by treatment (Fig. 1). Urea transporters send urea from the blood into the

rumen and contribute to its reuse for microbial protein synthesis. Simmons et al. (2009) reported an increase in the length of rumen papillae and an increase in urea-transporter UT-B1 gene expression in steers fed a highly fermentable diet and they suggested that the increase in butyrate fermentation in the rumen might have increased the UT-B1 gene expression and the utility of urea. On the other hand, in the study in which butyrate was exogenously infused, butyrate did not affect the recycling of UN (Whitelaw & Milne, 1991). Therefore, butyrate itself might not affect gene expression for urea uptake. Transport of short-chain fatty acids and homeostasis of intracellular pH in the rumen epithelium are regulated by the MCT-and NHE-families (Graham et al., 2007; Kuzinski et al., 2012). Liu et al. (2019) conducted the oral administration of sodium-butyrate for 39 d and observed the increase in MCT-1 gene expression. On the other hand, Laarman et al. (2013) reported that 7-d sodium-butyrate infusion increased MCT-1 protein expression in the rumen epithelium, but it was not enough to make a significant difference from the control group in lactating cows. These result suggests that the duration of administration was associated with gene expression. Increased ruminal butyrate concentration by feeding high concentrate diets (Laarman et al., 2012; Yan et al., 2014), but exogenous butyrate supplement did not increased pH regulation (NHE: Laarman et al., 2013; Liu et al., 2019), suggesting butyrate itself does not stimulate the expression of NHE, and the expression may be influenced by the absolute amount of VFA in the rumen.

The mRNA expression of TNF-α and TLR-2 in BUT was lower than in CON (smaller than 1.0, $P < 0.05$). The mRNA expression of IL-1β in BUT tended to be lower than that in CON ($P < 0.1$). The rumen and intestinal epithelium contain receptors that recognize endotoxins such as TLR2 and TLR4 (Chen & Oba, 2012; Villena et al., 2014)

that stimulate the receptors to produce IL-1β and TNF-α, which induce inflammation (Dai et al., 2017). Dai et al. (2017) reported that administration of butyrate to goats fed a grain-based diet reduced gene expression of TLR-4, IL-1β, and TNF-α. In this study, the depression of ruminal pH was small due to the high NDF diet, and the risk of ruminal acidosis was considered to be low. Under such conditions, whether the effect of butyrate on reducing the expression of inflammation-related mRNA in ruminal epithelium exerted a substantial effect has been unclear.

Because the dose of butyrate in previous studies listed above was equal to or lower than that in this study (0.03%-0.04% of kg BW or 1.0%-2.5% of DMI (our study was 2.4% of DMI)), the luck of effects on mRNA expression was probably not due to the insufficient dose of butyrate. However, the increase in the ruminal butyrate concentration by butyrate supplement in the present study [14.0 vs. 17.2 mM, presented in the companion paper (Fukumori et al., 2021)] was smaller than that in previous study, despite the same dose amount (14.1 vs. 21.4 mM, Dai et al., 2017). This may be because their study used sodium butyrate, whereas we used fat-coated sodium butyrate, so the amount of butyrate dissociated in the rumen and stimulated the ruminal epithelium might be low.

Changes in plasma concentrations of metabolites and hormones.

Changes in plasma parameters with days of administration are shown in Fig. 2. There was no difference between the treatments for plasma BUN concentrations through the experimental period (Fig. 2A). The change in basal plasma BHBA concentration was not observed (Fig. 2B), but plasma BHBA concentration was higher in BUT than in CON at 3 and 6 h after administration ($P < 0.05$, Fig. 3A). When

butyrate is absorbed from the rumen, it is converted to BHBA in the ruminal epithelium, but since there was no difference in the treatments in the basal concentration, the administered butyrate disappeared from the rumen within 24 h after administration of butyrate premix and was metabolized entirely in the blood.

Plasma insulin concentration was higher in BUT than in CON during the experimental period ($P = 0.025$, Fig. 2C). In addition, plasma insulin concentrations in BUT at 0 and 3 h after administration were higher than in CON ($P < 0.05$, Fig. 3B). The potent stimulating effect of butyrate on insulin secretion is well known (DeJong, 1982, Mann & Boda, 1967). However, the increase in plasma insulin concentration induced by butyrate was not observed in studies of lactating dairy cows (Herrick et al., 2018; Izumi et al., 2019). Lactating cows might have different insulin responses to butyrate because they are fed highly fermentable diets or they have different physiological conditions. The butyrate premix administration did not affect basal plasma glucose concentration during the experimental period (Fig. 2D), but resulted in the short-term change associated with administration, that is, plasma glucose concentration 3 h after administration was lower in BUT than in CON ($P < 0.05$, Fig. 3C). The decrease in plasma glucose by butyrate was consistent with previous reports (Herrick et al., 2018; Huthanen et al., 1993), and likely due to an increased plasma insulin concentration. The basal plasma GLP-2 concentration increased with the progress of the day from the start of administration of the butyrate premix, but was not observed in CON, and the GLP-2 concentration was higher in BUT than in CON at d 22 (Fig. 2E, $P < 0.05$). The GLP-2-stimulating effect of butyrate was confirmed in a previous report on intra-ruminal infusion of VFA (ElSabagh et al., 2017). In addition, a similar result was obtained in our previous study in which the same product as in this study was fed to lactating dairy

cows (Fukumori et al., 2020). Interestingly, the butyrate premix increased basal plasma insulin and GLP-2 concentrations, even though the basal BHBA concentration did not change (perhaps, the stimulation of butyrate absorption from the rumen had ended, but postruminal stimulation might be persistent). Since the butyrate supplement used in the present study was coated with fatty acids, it is presumed that part of it was not absorbed in the rumen but was transferred to the small intestine, which might chronically stimulate insulin and GLP-2 secretion. Therefore, we speculated that supplemented butyrate had a lasting effect on the secretion of gut hormones. As a concern in this study, there was a difference in plasma insulin and GLP-2 concentrations at the start (d 0). This study was performed with a crossover method with a 21-day wash-out period between treatments, but may require a longer period considering its sustained effect on the intestinal mucosa. Therefore, in this study, we considered the changes associated with administration.

Regarding the effect of GLP-2 on inflammation, in an LPS-stimulated study in which macrophages were exposed to GLP-2 in vitro the expression levels of IL-1β and TNF-α in macrophages decreased (Xie et al., 2014). Although there are few GLP-2 receptors in the rumen epithelium (Taylor-Edwards et al., 2010), the anti-inflammatory effect of butyrate might be mediated not only by its direct effect on the rumen epithelium, but also by its increased concentration of GLP-2 in the circulating blood. Further studies are required regarding the contribution of GLP-2 to the rumen epithelium.

Based on the responses to our study, continuous administration of sodium butyrate to dry cows increased the height of rumen papillae, decreased inflammatory related gene expressions in the rumen epithelium, and increased the plasma concentrations of insulin

and GLP-2. Administration of sodium butyrate is thus expected to have effects on cows fed a forage-based diet such as that during the dry period.

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Conflict of interest

The authors have no conflicts of interest directly relevant to the content of this article.

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Figures and tables

Table 1. Primers used in real-time PCR analysis

Target gene[1]	Primer sequence (5'-3')	reference
IL-1β	F: AGTGCCTACGCACATGTCTTC R:TGCGTCACACAGAAACTCGTC	Gondaira et al. (2015)
TNF-α	F:TCTTCTCAAGCCTCAAGTAACAAGC R:AATGACAGCGGCGTCTACTT	Gondaira et al. (2015)
TLR-4	F:CATTCCCTGGCAAGTGGATTATC R:GGAATGGCCTTCTTGTCAATGG	Gondaira et al. (2015)
TLR-2	F:CTTCCCGGGGGATGTTTCAA R:CCTGAGGCGGTTTCTACTCG	Gondaira et al. (2015)
NHE-1	F:GAAAGACAAGCTCAACCGGTTT R:GGAGCGCTCACCGGCTAT	Laarman et al. (2012)
MCT-1	F:ATCTACGCGGGATTCTTTGGA R:AAGGTCCATCAGCGTTTCAAA	Laarman et al. (2012)
UT-B1	F:TGCCTAA-CATAACGAGTTC R:GAAGATGC-CCCCTGTCCACGG	Simmons et al. (2009)
β-actin	F:AGCAAGCAGGACTACGATGAG R:ATCCAACCGACTGCTGTCA	Gondaira et al. (2015)

DBNDD2	F:GTGGAGCTTATCGACCTGGG R:GGAGTTGGTGGAGGGTCTTC	Die et al. (2017)
UXT	F:CACATGTTGCTAGAGGGGCT R:TCAGTGCTGAGTCTCTGGGA	Die et al. (2017)

[1] IL-1β = interleukin-1β, TNF-α = tumor necrosis factor-α; TLR-4 = toll like receptor-4; TLR-2 = toll like receptor-2; NHE-1 = NA+/H+ exchanger isoforms 1; MCT-1 = Monocarboxylate transporter isoform1; UT-B1 = Urea transporter-B1; DBNDD2 = dystrobrevin-binding protein domain containing 2; UXT = prefoldin-like chaperone.

Table 2. Effects of sodium butyrate on DMI, eating and ruminating behavior[1]

	Treatment[2]		SEM	*P*-value
	CON	BUT		
DMI, kg/d	12.3	12.8	1.03	0.517
Eating time, min/d	206	229	21.2	0.385
Ruminating time, min/d	458	466	72.7	0.867

[1]The average value from d 19 to d 21 was calculated. Values are least squares means (LSMEAN).

[2]CON =control group; BUT =butyrate group (n = 4, cross-over).

Table 3. Effects of sodium butyrate on the development of rumen papillae[1]

	d 1		d 22			*P*-value [3]		
	CON[2]	BUT[2]	CON	BUT	SEM	Butyrate	Time	Butyrate ×Time
Height								
mm	6.17 [ab]	5.29 [b]	5.64 [ab]	6.97 [a]	0.71	0.63	0.24	0.04
% [4]	100 [b]	100 [b]	94.7 [b]	133.9 [a]	9.80	0.01	0.03	0.01
Tickness								
μm	112	103	76.6	82.0	5.40	0.74	<0.001	0.19

%	100	100	68.7	80.1	5.00	0.31	0.001	0.31

[1] Values are least squares means (LSMEAN; n = 4, cross-over).

[2] CON = control group; BUT = butyrate group.

[3] Effect of butyrate administration (Butyrate), timely change (Time), and their interaction (Butyrate×Time).

[4] Data are presented as the relative value of d 22 against that of d 1.

Figure 1. The effects of sodium butyrate on messenger RNA expression of genes in the rumen epithelial after 21-d administration. The data are presented as values relative to CON. The value are means ± SEM, n = 4. MCT-1 = Monocarboxylate transporter isoform1; NHE-1 = NA+/H+ exchanger isoforms 1; UT-B1 = Urea transporter-B1; TLR-2 = toll like receptor-2; TLR-4 = toll like receptor-4; IL-1β = interleukin-1β, TNF-α = tumor necrosis factor-α.
Differences in tendency ($P < 0.1$) are indicated by *, and significant differences ($P < 0.05$) are indicated by **.

Figure 2. Effects of sodium butyrate on plasma concentrations of BUN (A), BHBA (B), insulin (C), glucose (D), and GLP-2 (E). The Values are LSMEAN, $n = 4$. The solid line shows CON and the dashed line shows BUT. Significant differences between treatments at the same time point are indicated by asterisks.

Figure 3. Temporal changes in plasma concentrations of BHBA (A), insulin (B), and glucose (C) in cows after administration of CON and BUT premix. Values are LSMEAN, $n = 4$. The solid line shows CON and the dashed line shows BUT. Significant differences between treatments at the same time point are indicated by asterisks.

Figure 1.

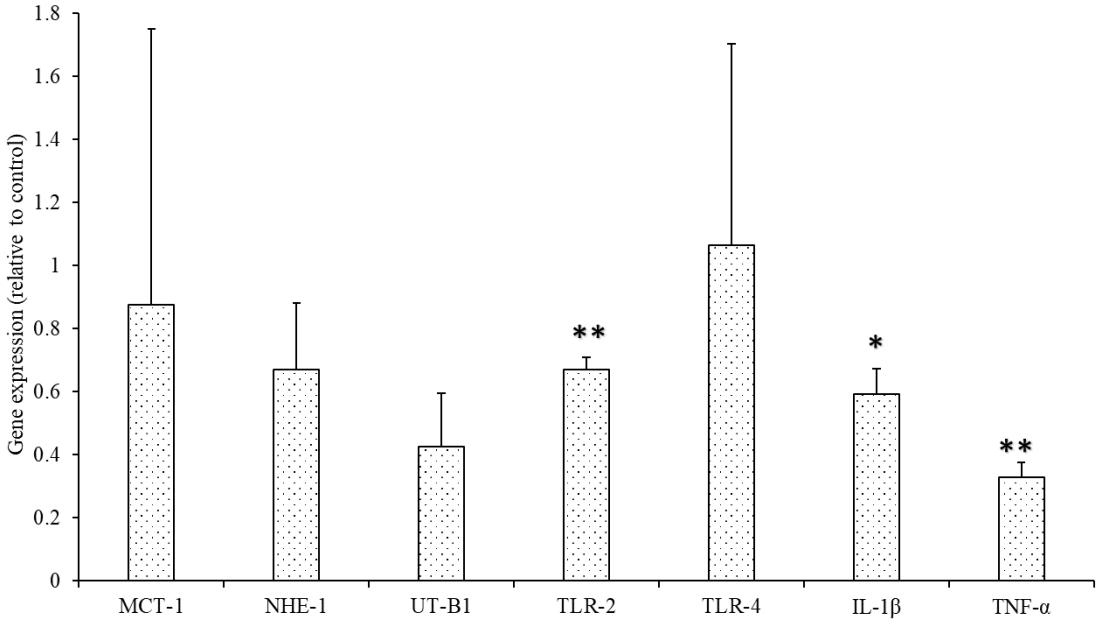

Gene expression (relative to control)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
*
**
MCT-1
NHE-1
UT-B1
TLR-2
TLR-4
IL-1β
TNF-α

Figure 2.

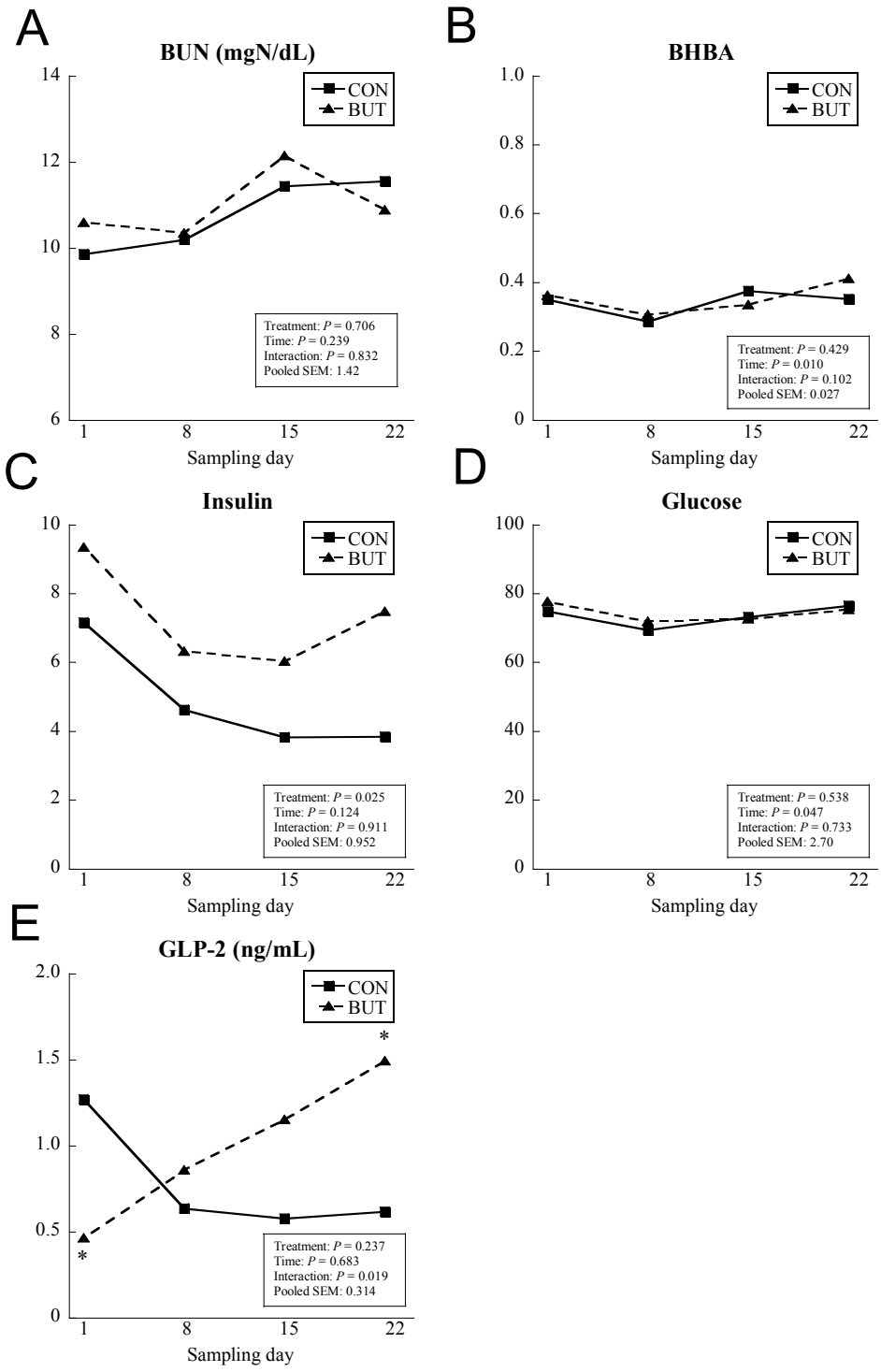
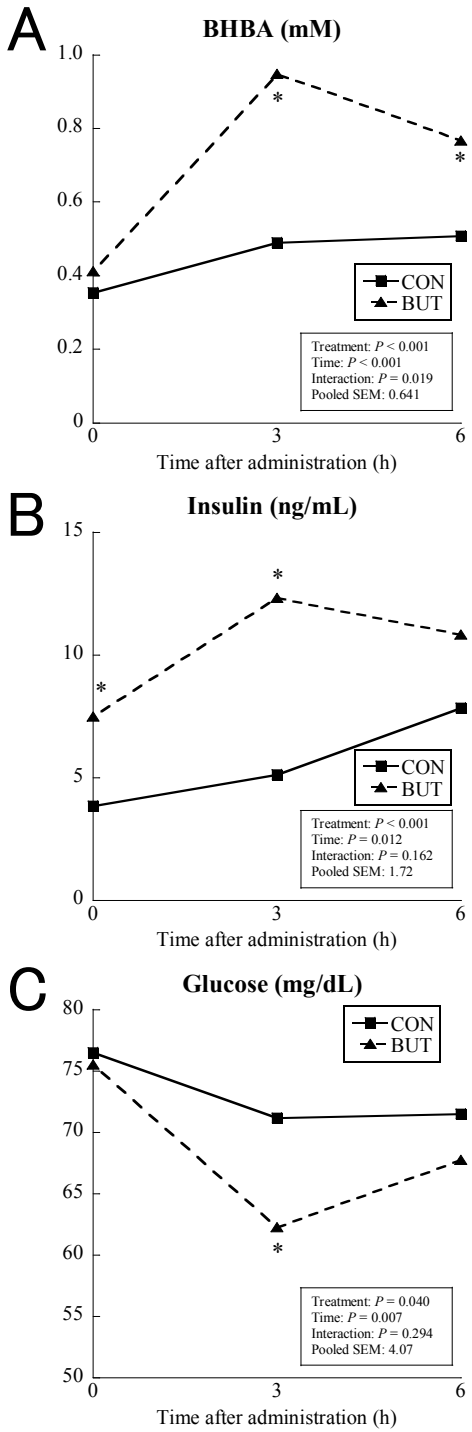

A
BUN (mgN/dL)
CON
BUT
Treatment: P = 0.706
Time: P = 0.239
Interaction: P = 0.832
Pooled SEM: 1.42
Sampling day
B
BHBA
CON
BUT
Treatment: P = 0.429
Time: P = 0.010
Interaction: P = 0.102
Pooled SEM: 0.027
Sampling day
C
Insulin
CON
BUT
Treatment: P = 0.025
Time: P = 0.124
Interaction: P = 0.911
Pooled SEM: 0.952
Sampling day
D
Glucose
CON
BUT
Treatment: P = 0.538
Time: P = 0.047
Interaction: P = 0.733
Pooled SEM: 2.70
Sampling day
E
GLP-2 (ng/mL)
CON
BUT
Treatment: P = 0.237
Time: P = 0.683
Interaction: P = 0.019
Pooled SEM: 0.314
Sampling day

Figure 3.


A
BHBA (mM)
1.0
0.8
0.6
0.4
0.2
0
0
3
6
Time after administration (h)
CON
BUT
Treatment: P < 0.001
Time: P < 0.001
Interaction: P = 0.019
Pooled SEM: 0.641
B
Insulin (ng/mL)
15
10
5
0
0
3
6
Time after administration (h)
CON
BUT
Treatment: P < 0.001
Time: P = 0.012
Interaction: P = 0.162
Pooled SEM: 1.72
C
Glucose (mg/dL)
80
75
70
65
60
55
50
0
3
6
Time after administration (h)
CON
BUT
Treatment: P = 0.040
Time: P = 0.007
Interaction: P = 0.294
Pooled SEM: 4.07

タイトル：高繊維質飼料給与下において非泌乳牛への酪酸塩の投与はルーメン絨毛長、炎症関連遺伝子発現量、血漿ホルモン濃度を変化させる

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抄録

本研究の目的は、高繊維質飼料給与下の非泌乳牛において、酪酸塩の補給がルーメン絨毛の形態、ルーメン絨毛の栄養吸収ならびに炎症関連遺伝子発現量、血漿ホルモンおよび代謝産物濃度に及ぼす影響を解析することであった。ルーメンカニューレが装着されたホルスタイン種乳牛（非妊娠・非泌乳）を 4 頭用い、2 処理区反転法にて試験を実施した。処理区には、酪酸塩を含むプレミックスを投与する酪酸区と、酪酸塩部分を小麦フスマで置き換えたプレミックスを投与する対照区の 2 処理区を設け、いずれも同一の基礎飼料（グラスサイレージ主体混合飼料）を給餌する直前にルーメン内へ単回投与した。酪酸塩

投与量は酪酸として体重当たり 0.04%に設定した。投与開始 22 日目において、酪酸区のルーメン絨毛長が対照区と比較して長く、血漿 GLP-2 濃度が高く、ルーメン絨毛における TNF-α、IL-1β および TLR-2 遺伝子発現量が低いことが確認された。酪酸区の血漿 BHBA 濃度は投与後 3 および 6 時間目において対照区と比較して高値を示したが、翌日の投与直前には対照区と同レベルに戻っていた。これらの結果から酪酸塩は高繊維質飼料のような低発酵性飼料を給与している条件下においてもルーメン上皮や血中ホルモン濃度に影響を及ぼすことが示された。