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REVIEW

Subacute ruminal acidosis (SARA) challenge, ruminal condition and cellular immunity in cattle

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Abstract

Subacute ruminal acidosis (SARA) is characterized by repeated bouts of low ruminal pH. Cows with SARA often develop complications or other diseases, and associate physiologically with immunosuppression and inflammation. Ruminal free lipopolysaccharide (LPS) increases during SARA and translocates into the blood circulation activating an inflammatory response. Ruminal fermentation and cellular immunity are encouraged by supplementing hay with calf starter during weaning. SARA calves given a 5-day repeated administration of a bacteria-based probiotic had stable ruminal pH levels (6.6–6.8). The repeated administration of probiotics enhance cellular immune function and encourage recovery from diarrhea in pre-weaning calves. Furthermore, the ruminal fermentation could guard against acute and short-term feeding changes, and changes in the rumen microbial composition of SARA cattle might occur following changes in ruminal pH. The repeated bouts of low ruminal pH in SARA cattle might be associated with depression of cellular immunity.

Key Words: cattle, cellular immunity, cytokine mRNA, SARA, ruminal pH

Introduction

Subacute ruminal acidosis (SARA) is characterized by repeated bouts of low ruminal pH[7,8,25)]. Cows with SARA often develop complications or other diseases, such as laminitis, reduced and erratic feed intake, low body condition score (BCS), low milk fat syndrome, abomasal displacement and ulceration and ruminitis[7,25)]. Furthermore, physiological associations of SARA with immunosuppression[15)] and inflammation[13,14,25)] have also been reported. Ruminal free lipopolysaccharide (LPS) increases during SARA[5,10)] and increased LPS translocates from the rumen into the blood circulation activating an inflammatory response[13,14)]. Several symptoms of SARA in cattle, including liver abscesses and laminitis, may be due to the translocation of free LPS from the rumen into the circulation[5,23)]. LPS in the systemic circulation

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could engage pattern recognition receptors, affecting leukocyte populations and triggering the production of pro-inflammatory cytokines and acute phase proteins.

Innate immune mechanisms and transferred maternal antibodies play an important role in the defense mechanisms against infection in calves. The passive immunity provided by an adequate uptake of colostrum is very important for protection against infections during the neonatal period. It is recommended that calves maximize intake of calf starter, which is readily fermented in the rumen, before weaning to foster rumen development. However, rapid ruminal fermentation lowers ruminal pH if proton production outweighs proton removal. Ruminal pH of dairy calves is lower than in cows. Feeding calf starter lowers the ruminal pH in dairy calves[18] and fermentation of calf starter in the rumen will promote papillae development due to the enhanced production of butyrate and propionate. While lower ruminal pH has been associated with detrimental health effects in mature ruminants[23], the effects of lower ruminal pH on the immunity, health and growth of pre-weaning calf starter fed calves are unknown.

Further, certain bacteria-based probiotics were reported to have immune-stimulating effects in cattle[6,12,17] and the beneficial effects of probiotics have been recognized in improving animal health and protecting calves against infection[32,33]. *Lactobacillus* strains, alone or in combination with other probiotics, may reduce diarrhea in neonatal calves, increase weight gain and maintain health[11,12]; however, the effects of these probiotics on ruminal condition and cellular immunity in SARA calves and in diarrheal calves are unknown. The purpose of this paper is to give an outline of the relationship between nutrition and cellular immunity, especially between ruminal condition and cellular immunity in SARA cattle. The results of our research on the effect of hay feeding in weaning calves, effects of bacteria-based probiotics in SARA and diarrheal calves and changes in ruminal condition and cellular immunity in cattle with repeated induced SARA are described.

1. Effects of hay feeding on ruminal pH, VFA and peripheral leukocyte subpopulations in weaning calves

Highly fermentable diets stimulate ruminal microbial proliferation and volatile fatty acid (VFA) production in pre-weaned calves, followed by initiation of ruminal development[21]. Rapid fermentation of ingested calf starter causes an increased concentration of VFA and lactic acid and decreased pH in the incompletely developed rumen[1,19,29]. It is recommended that calves maximize calf starter intake before weaning, but fiber is also necessary because it improves ruminal development and calf health. If calves are fed calf starter before weaning, their ruminal pH should be lower than in cows. However, neither the effect of feeding on ruminal pH nor the relationship between ruminal pH and diarrhea in weaning calves has been studied. The ruminal pH, VFA level and peripheral leukocyte subpopulations in hay-fed and non-hay-fed Holstein calves at the time of weaning were investigated to determine the effects of hay feeding on ruminal pH and peripheral leukocyte function.

Eight rumen-cannulated Holstein bull calves, aged 4 weeks, were used. The calves were fed a standard milk replacement (MR; 24% crude protein and 20% crude fat), calf starter (CS; 22% crude protein and 3% crude fat) and orchardgrass and timothy hay until 7 days before weaning, and thereafter divided into two groups of four calves each. The hay-fed calves were given a combination of MR, CS and hay, while the control calves were given MR and CS only until weaning, after which no calves in either group were given MR. A wireless radio-transmission pH-sensor (YCOW-S; DKK-Toa Yamagata, Yamagata, Japan)[31] was placed in the rumen and the pH was measured every 10 min for 21 days after weaning. Ruminal

fluid and blood specimens were collected at −7, 0, 7 and 21 days after weaning. VFAs (acetic acid, propionic acid and butyric acid) were separated and quantified by gas chromatography (Model 135, Hitachi). Flow cytometry analysis was performed using FACScan analyzer (Becton Dickinson, Franklin Lakes, NJ, USA).

Ruminal pH in hay-fed (FF) calves increased gradually and appeared to have marked circadian changes after hay feeding. Whereas, ruminal pH in starter-fed (SF) calves had very low values and clear circadian changes were not seen until 3 weeks after weaning (Fig. 1). The 24-h mean pH values of the ruminal fluids increased gradually after weaning in the FF calves and were significantly higher than those in the SF calves from 5 to 21 days after weaning. The value at 21 days in the FF calves was significantly higher than that in the SF calves (n = 4, mean ± SE: 6.35 ± 0.30 vs. 5.72 ± 0.28). The 1-h mean pH values decreased after morning feeding in the FF calves, but remained stable at a low level in the SF calves. The pH values in the FF calves were higher overall than those in the SF calves. Ruminal propionic acid was slightly lower and both butyric acid and the A/P ratio were slightly higher in FF calves than in SF calves. No difference was observed in the blood components of either group at weaning. Whereas, the numbers of $CD21^+$ and $WC1^+$ γδ T cell subsets in the FF calves were significantly higher than those in the SF calves from 0 to 7 d after weaning (Fig. 2).

Our results indicate that ruminal fermentation and some cellular immunity are encouraged by supplementing hay with calf starter during weaning and that the 24-h mean pH increased after circadian decrease and recovery of pH after feeding.

2. Effects of a bacteria-based probiotic on ruminal condition and cellular immunity in calves

A probiotic consisting of lactic-acid-producing bacteria (LAB) promotes stability of the rumen flora[2,4,9,35], resulting in increased dry matter intake and weight gain and improved health

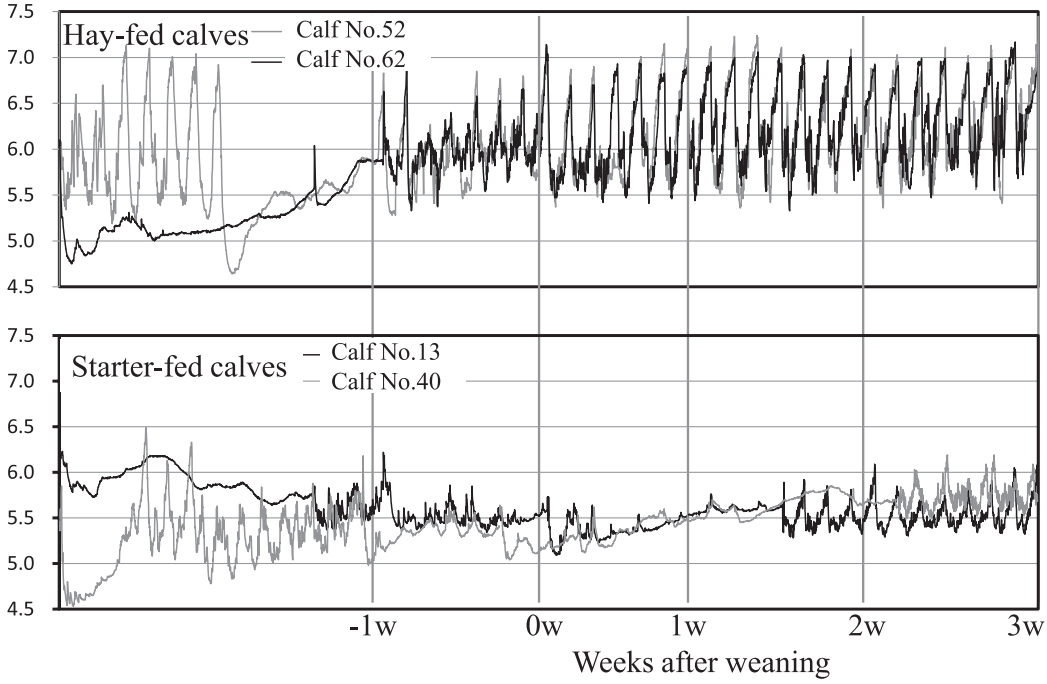

Fig. 1. Continuous changes in ruminal pH of hay-fed and starter-fed calves at weaning period. Hay-fed calves were fed hay and starter, starter-fed calves were fed starter only from 1 week before weaning.

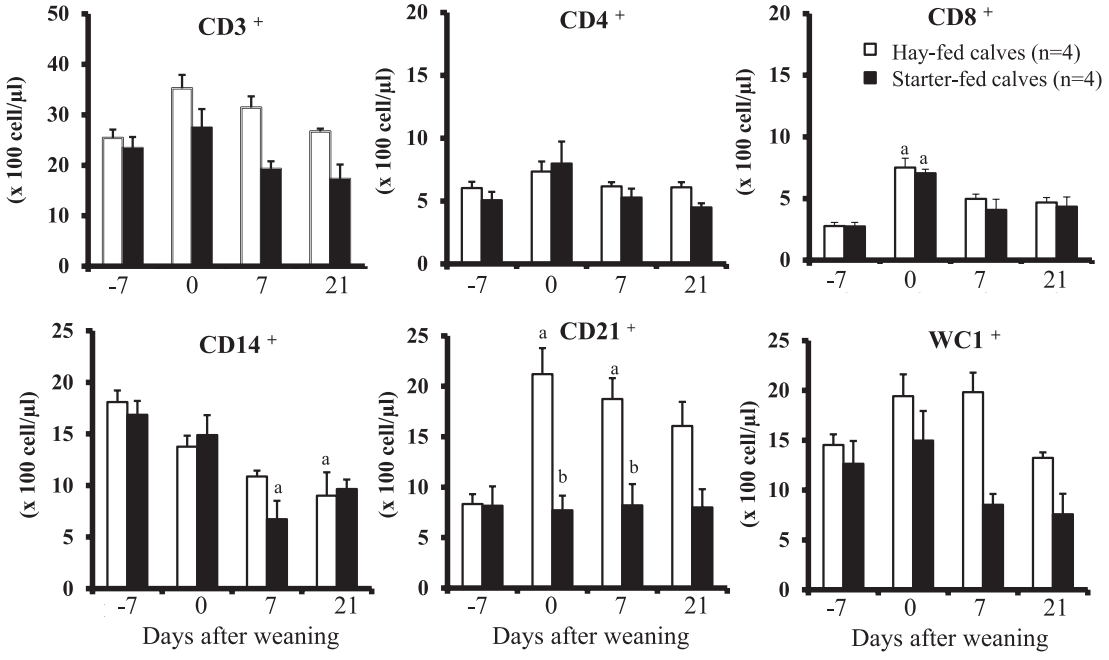

Fig. 2. Number of CD3$^+$, CD4$^+$, CD8$^+$, CD14$^+$, CD21$^+$ and WC1$^+$ cells in the peripheral leukocytes of hay-fed and starter-fed calves. Values represent means ± SE ($n = 4$). [a]Compared to day -7 in the same group ($P < 0.05$), [b]Compared to hay-fed calves in the same day ($P < 0.05$).

in cattle[3,34]. Immune stimulatory effects of probiotics were examined in calves[22,24,32]. It was hypothesized that bacteria-based probiotics might impact the host immune system in a number of ways, such as upregulation of cell-mediated immunity, increased antibody production and epithelial barrier integrity, enhanced dendritic cell-T cell interactions, heightened T cell association and increased Toll-like receptor (TLR) signaling[12,20,36]. Regulation of the immune stimulatory effects of probiotics may contribute to the treatment of diarrhea in calves[24]. However, few reports address the action of bacteria-based probiotics on peripheral blood leukocytes in SARA and diarrheal calves.

(1) Effects on ruminal pH, VFA and bacterial flora in SARA calves

Twelve ruminally cannulated Holstein calves (12 ± 3 weeks) were used to identify the effects of a probiotic comprised of *Lactobacillus plantarum*, *Enterococcus faecium* and *Clostridium butyricum* on ruminal components in SARA calves[28]. The calves were adapted to a diet containing a 50% high-concentrate (standard diet) for 1 week and developed to SARA, then the probiotic was given once daily for 5 days at 1.5 or 3.0 g/100 kg body weight to groups of four calves each. Four additional calves fed the standard diet without the probiotic served as the corresponding control. Ruminal pH was measured continuously throughout the 15-day experimental period. Ruminal fluid was collected via a fistula at a defined time to assess VFA, lactic acid and ammonia-nitrogen (NH_3-N) concentrations, as well as the bacterial community. Bacterial composition of ruminal fluid was assessed using terminal-restriction-fragment length polymorphism (T-RFLP) and real-time PCR, The probiotic at either dose improved the reduced 24-h mean ruminal pH in SARA calves (Fig. 3). The circadian patterns of the 1-h mean ruminal pH were identical between the probiotic doses (Fig. 4). In both probiotic groups, ruminal lactic acid concentrations remained significantly lower than the control. The probiotic did not affect ruminal VFA concentrations. In T-RFLP analysis, *L. plantarum*

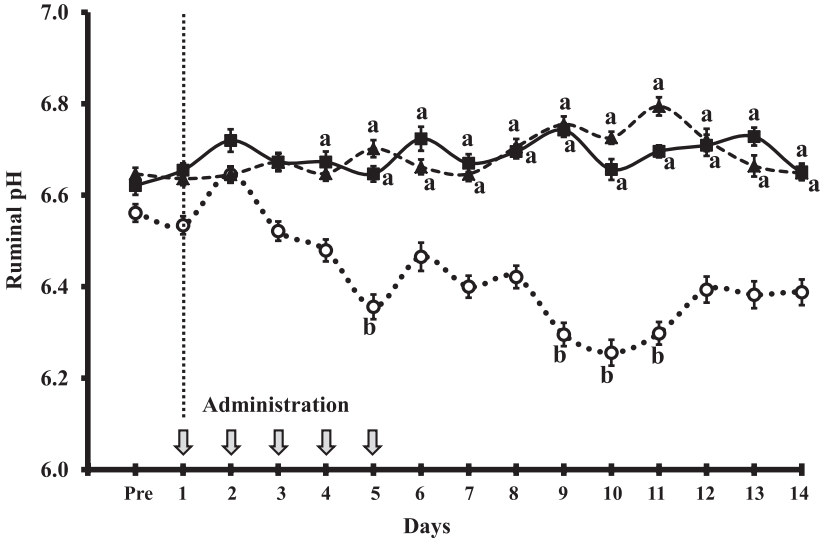

Fig. 3. Changes in 24-hr mean ruminal pH in calves given 1.5 g (n = 4; ▲) or 3.0 g/100 kg BW (n = 4; ■) probiotic for 5 consecutive days, and control calves (n = 4; ○). [a]Compared to the control on the same day ($P < 0.01$). [b]Compared to the pre-dose day (Pre) in the control ($P < 0.01$).

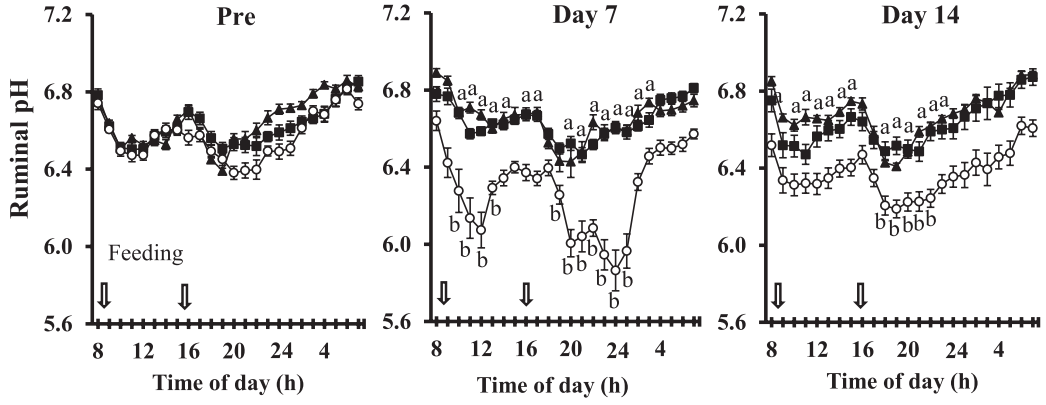

Fig. 4. Circadian changes in 1-hr mean ruminal pH at pre-dose day (Pre) and days 7 and 14 in calves given 1.5 g (n = 4; ▲) or 3.0 g/100 kg BW (n = 4; ■) probiotic for 5 consecutive days, and control calves (n = 4; ○). [a]Compared to the control at the same time ($P < 0.01$). [b]Compared to the time before feeding (8 : 00) in the control ($P < 0.01$).

and *C. butyricum* were not detected in the rumen of calves given the high-dose probiotic, whereas *Enterococcus* spp. remained unchanged. These results suggest that SARA calves given a probiotic had stable ruminal pH levels (6.6–6.8), presumably due to the effects of the probiotic on stabilizing rumen-predominant bacteria, which consume greater amounts of lactate in the rumen.

(2) Effects on peripheral leukocyte subpopulations and cytokine mRNA expression in SARA calves

Few studies have described the effects of probiotics on the peripheral leukocytes in healthy and SARA calves. Therefore, eight Holstein calves (10 ± 3 weeks) were used to examine the interaction between a bacteria-based probiotic and the peripheral leukocyte subpopulations and their cytokine mRNA expression in SARA calves[26]. The calves were induced SARA by feeding 50% high-concentrate (standard diet) for 1 week. The probiotic, consisting of *Lactobacillus*

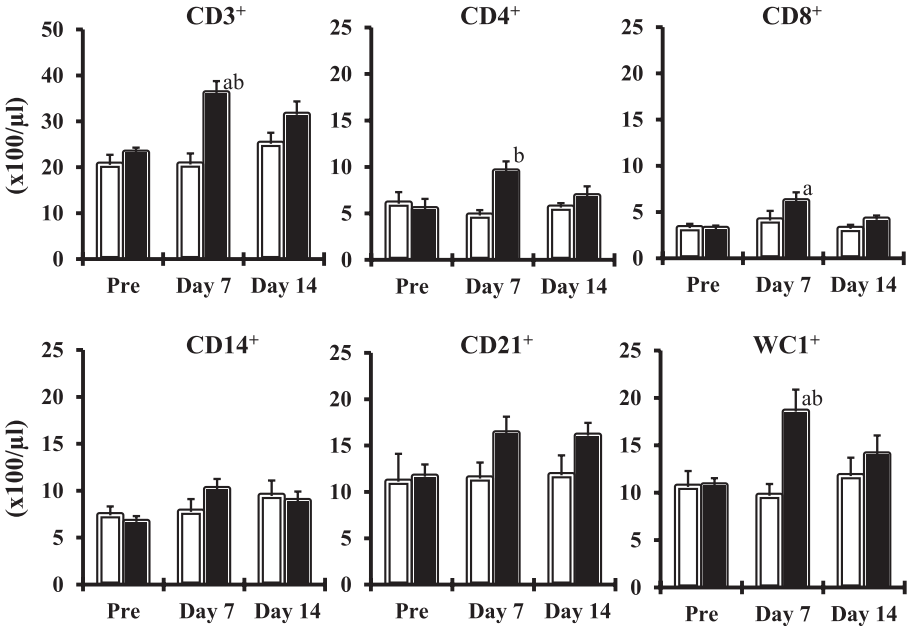

Fig. 5. Number of CD3$^+$, CD4$^+$, CD8$^+$, CD14$^+$, CD21$^+$ and WC1$^+$ cells in the peripheral leukocytes of calves given 3.0 g/100 kg BW of probiotic once daily for 5 consecutive days (black columns; n = 8), and control calves (white columns; n = 8). [a]Compared to the pre-dose day (Pre) in the same group ($P < 0.05$). [b]Compared to the control on the same day ($P < 0.05$).

plantarum, *Enterococcus faecium* and *Clostridium butyricum*, was administered orally at 3.0 g/100 kg body weight to calves once daily for 5 consecutive days. Calves given the vehicle alone with no probiotic served as the control. Total RNA extraction from peripheral leukocytes and cDNA synthesis, and real-time PCR assay were performed as described previously[26]. In the treatment group, increased numbers of CD282$^+$ (TLR2) monocytes, CD3$^+$ T cells and CD4$^+$, CD8$^+$ and WC1$^+$ γδ T cell subsets were noted on day 7 post-placement compared to the pre-dose period and the control group (Fig. 5). Expressions of interleukin (IL)-6, interferon-gamma (INF-γ) and tumor necrosis factor-alpha (TNF-α) were elevated in peripheral leukocytes on days 7 and 14 (Fig. 6). These results suggest that peripheral blood leukocytes in SARA calves may be stimulated via the gastrointestinal microbiota, which was increased by the oral probiotic treatment, with overall stability of the rumen bacterial flora. The 5-day repeated administration of a bacteria-based probiotic may enhance, cellular immune function in SARA calves.

(3) Immune-stimulatory effects on peripheral leukocyte subpopulations and cytokine mRNA expression in diarrheal calves

Diarrheal Holstein calves (10 ± 5 days; n = 42) treated with a probiotic consisting of *Lactobacillus plantarum*, *Enterococcus faecium* and *Clostridium butyricum* were used to evaluate the immune-stimulatory effects of the probiotic on peripheral leukocyte subpopulations and their cytokine mRNA expression[27]. The calves were assigned to the diarrhea or healthy group and then subdivided into pathogen-positive treated (n = 8), pathogen-positive control (n = 8), pathogen-negative treated (n = 6), pathogen-negative control (n = 6), healthy treated (n = 6), and healthy control (n = 8) groups. A single dose per day of the probiotic (3.0 g/100 kg BW) was given to each calf in the treatment groups for 5 days. Blood samples were collected on the first day of diarrhea occurrence (day 0) and on day 7. In the diarrheal calves, smaller peripheral leukocyte subpopulations and lower cytokine mRNA expression levels were noted on day 0. The numbers of CD3$^+$ T cells, CD4$^+$, CD8$^+$ and WC1$^+$

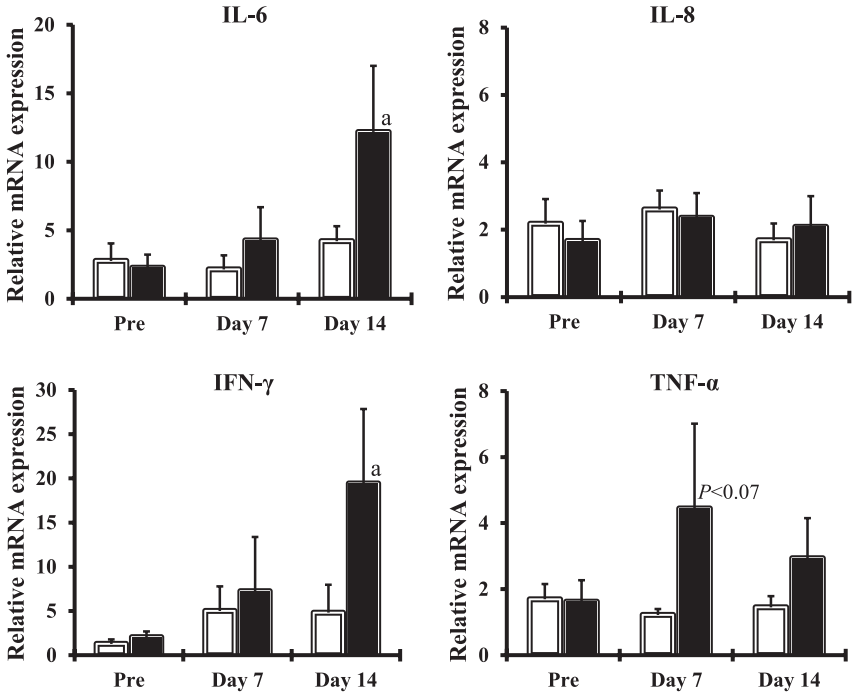

Fig. 6. Relative mRNA expression of IL-6, IL-8, IFN-γ and TNF-α in the peripheral leukocytes of calves given 3.0 g/100 kg BW of probiotic once daily for 5 consecutive days (black columns; n = 8), and control calves (white columns; n = 8). [a]Compared to the pre-dose day (Pre) in the same group ($P < 0.05$).

γδ T cell subsets, and $CD14^+$, $CD21^+$ and $CD282^+$ (TLR2) cells were significantly increased in the diarrheal and healthy treated calves on day 7 (Fig. 7). IL-6, INF-γ and TNF-α mRNA expressions were also elevated in the peripheral leukocytes of the diarrheal and healthy treated calves on day 7 (Fig. 8). The diarrheal calves given the probiotic recovered by day 7. A significantly smaller number of peripheral CD^+ leukocytes and lower cytokine mRNA expression levels might be induced by the occurrence of diarrhea in calves. Repeated probiotic administration might stimulate cellular immunity and encourage recovery from diarrhea in pre-weaning Holstein calves.

3. Changes in ruminal pH, VFA and peripheral leukocyte subpopulations in cattle with repeated induced SARA

Molecular techniques have shown that the rumen bacterial community is highly diverse, but the factors influencing its composition remain unknown. The bacterial community in SARA cows has been reported, but the relationship between the ruminal pH, VFA and microbial composition in SARA cattle is unclear. The relationships among ruminal pH, VFA and microbial composition, and peripheral leukocyte subpopulations in cattle with repeated induced SARA were investigated to reveal ruminal adaptation against acute and short-term changes in feeding.

Eight rumen-cannulated Holstein steers (age, 8–10 months; weight, 180–200 kg) were used and fed hay or SARA-inducing diet (hay : concentrate, 2 : 8) for 7 days[16,30]. The experiment was continuously performed four times. A wireless radio-transmission pH-sensor (YCOW-S; DKK-Toa Yamagata) was placed in the ventral sac of the rumen, and the pH was measured every 10 min. Ruminal fluid and blood specimens were collected three times (08 : 00, 14 : 00 and 20 : 00) 7 days after each feeding. Total VFA and VFA components were separated and quantified

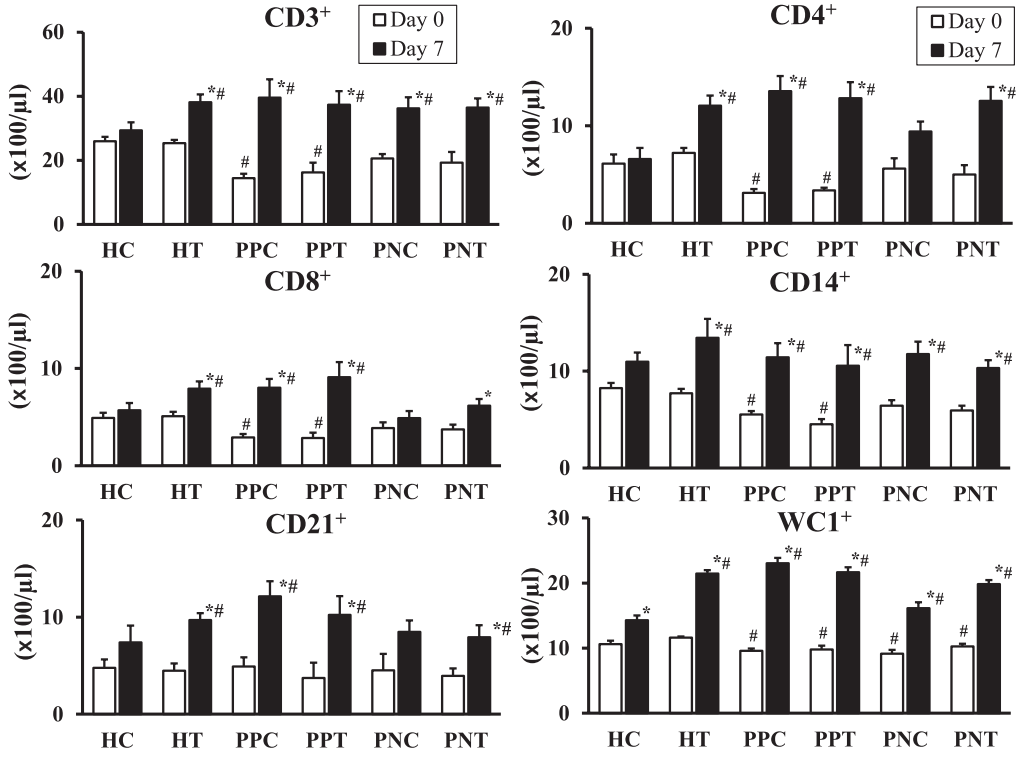

Fig. 7. Number of CD3$^+$, CD4$^+$, CD8$^+$, CD14$^+$, CD21$^+$ and WC1$^+$ cells in the peripheral leukocytes of the healthy control (HC; n = 8) and treated (HT; n = 6), diarrheal pathogen positive control (PPC; n = 8) and treated (PPT; n = 8), pathogen negative control (PNC; n = 6) and treated (PNT; n = 6) groups. Calves in the treated groups were given 3.0 g/100 kg BW of the probiotic once daily for 5 days. *Compared to day 0 in the same group ($P < 0.05$). #Compared to the HC group ($P < 0.05$).

by gas chromatography. The microbiota of the ruminal fluids was analyzed by denaturing gradient gel electrophoresis (DGGE) and 16S rRNA arrangement analysis using bacterial DNA extraction from fluids collected at 20 : 00.

The 24-h mean ruminal pH values in the SARA cattle were significantly lower than those in the hay-fed cattle and were slightly higher in the third and fourth experimental periods compared to the first period (Fig. 9). The 1-h mean pH values of the ruminal fluids decreased gradually following morning and evening feeding in both the hay-fed and SARA cattle and the values in the SARA cattle were lower overall than those in the hay-fed cattle. Ruminal acetic acid levels in the hay-fed cattle and butyric acid levels in the SARA cattle were slightly higher than those in the respective other groups (Fig. 10). In the SARA cattle, ruminal acetic acid, butyric acid and NH_3-N levels were significantly higher in the fourth experiment than in the first experiment. DGGE analysis showed that the rumen microbial composition was simpler in the SARA cattle than in the hay-fed cattle. The 16S rRNA arrangement analysis of the bacterial components yielded results similar to those of DGGE and showed that some genera (*Prevotella* and *Eubacterium*) were decreased and several genera (*Clostridium*, *Butyrivibrio* and *Ruminococcus*) were increased in SARA cattle. No significant difference was observed in the numbers of CD3$^+$ T cells, CD4$^+$, CD8$^+$ and WC1$^+$ γδ T cell subsets, and CD14$^+$ and CD21$^+$ cells between SARA cattle and hay-fed cattle.

Both ruminal pH and bacterial diversity were significantly lower and the microbial composition was simpler in the SARA cattle. Changes in the rumen microbial composition of SARA cattle might be related to decreases in the number of ruminal bacteria that occur following

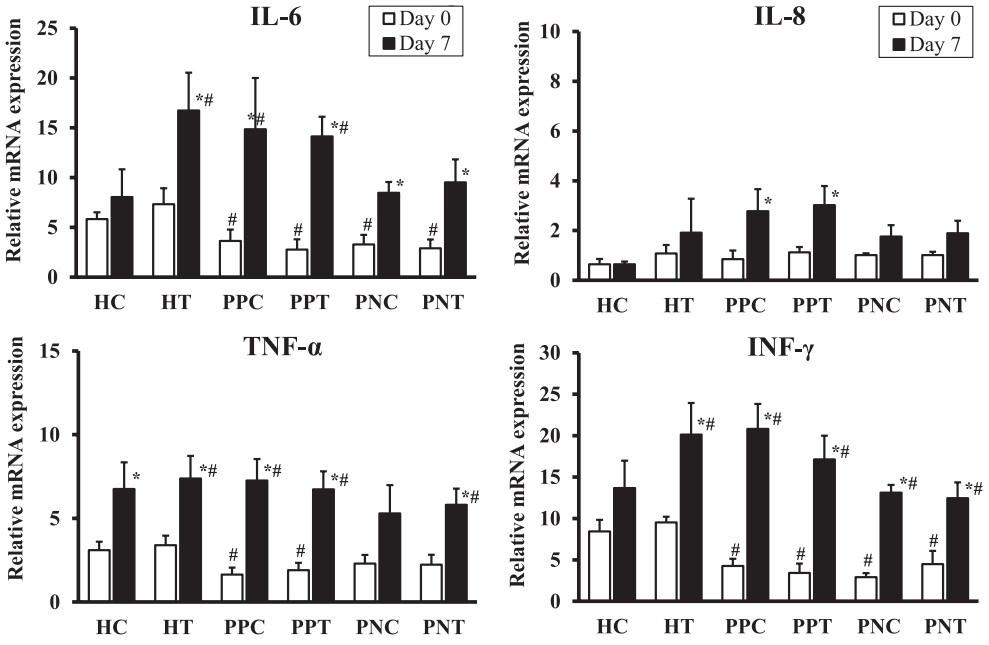

Fig. 8. Relative mRNA expression of IL-6, IL-8, TNF-α and IFN-γ in the peripheral leukocytes of the healthy control (HC; n = 8) and treated (HT; n = 6), diarrheal pathogen positive control (PPC; n = 8) and treated (PPT; n = 8), pathogen negative control (PNC; n = 6) and treated (PNT; n = 6) groups. Calves in the treated groups were given 3.0 g/100 kg BW of the probiotic once daily for 5 days. *Compared to day 0 in the same group ($P < 0.05$). #Compared to the HC group ($P < 0.05$).

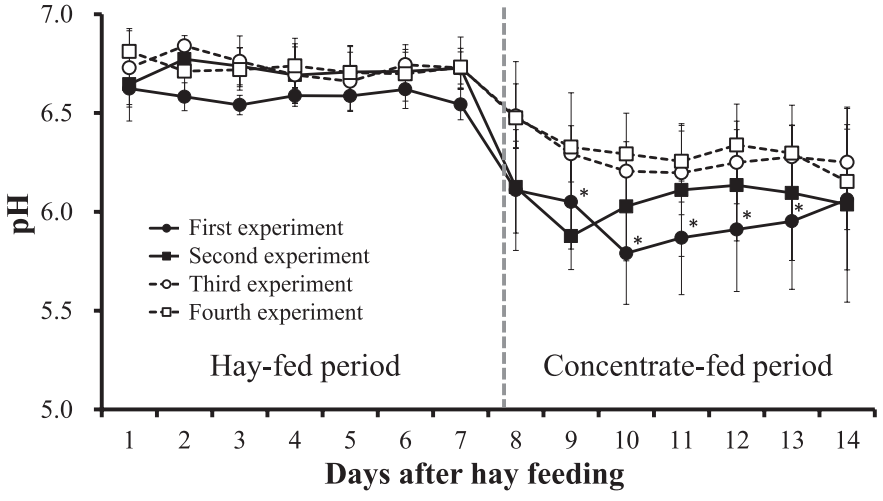

Fig. 9. Changes in 24-hr mean ruminal pH of cattle with repeated induced SARA at hay-fed and concentrate-fed periods (n = 8). *Compared to day 7 in the same group ($P < 0.05$).

marked changes in ruminal pH.

Conclusion

Ruminal fermentation and cellular immunity may be encouraged by supplementing hay with calf starter during weaning. SARA calves given 5-day repeated administration of a bacteria-based probiotic had stable ruminal pH levels (6.6–6.8), presumably due to the effects of the probiotic on stabilizing rumen-predominant bacteria, which consume greater lactate in the rumen. The repeated administration of probiotics

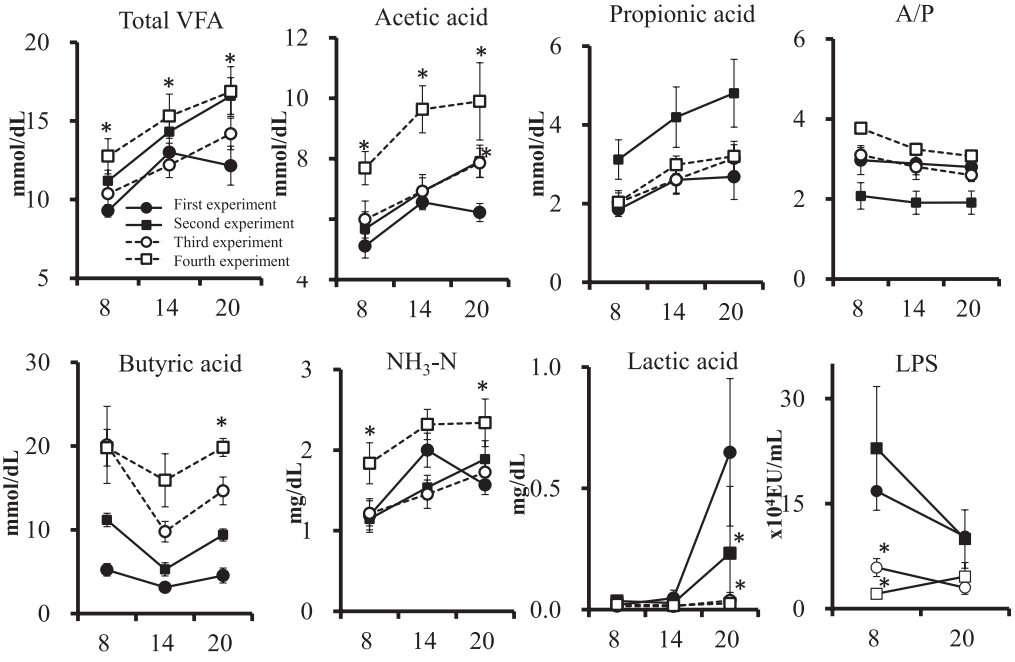

Fig. 10. Changes in VFAs, ammonia-nitrogen (NH_3-N), lactic acid level and LPS activity in the ruminal fluid of cattle with repeated induced SARA at 7 days after concentration feeding (n = 8). *Compared to the first period of feeding in the same time ($P < 0.05$).

may enhance cellular immune function and encourage recovery from diarrhea in pre-weaning calves. Furthermore, the ruminal fermentation could guard against acute and short-term feeding changes, and changes in the rumen microbial composition of SARA cattle might occur following changes in ruminal pH. The repeated bouts of low ruminal pH in SARA cattle might be associated with depression of cellular immunity. Increasing ruminal and systematic LPS levels might be related to cellular immunity in SARA cattle, however, further research is need to reveal the relation between feeding strategy, ruminal condition and cellular immunity.

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