The Journal of Veterinary Medical Science

FULL PAPER
Internal Medicine

Anti-lipopolysaccharide antibody administration mitigates ruminal lipopolysaccharide release and depression of ruminal pH during subacute ruminal acidosis challenge in Holstein bull cattle

Hitoshi MIZUGUCHI[1)], Tomoki IKEDA[2)], Yumi WATANABE[2)], Shiro KUSHIBIKI[3)], Kentaro IKUTA[4)], Yo-Han KIM[2,5)]* and Shigeru SATO[2)]*

[1)]DKK-Toa Yamagata Co., Yamagata 996-0053, Japan
[2)]Cooperative Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, Morioka, Iwate 020-8550, Japan
[3)]Institute of Livestock and Grassland Science, NARO, Tsukuba, Ibaraki 305-0901, Japan
[4)]Awaji Agricultural Technology Center, Minami-Awaji, Hyogo 656-0442, Japan
[5)]Department of Animal Resources Science, Dankook University, 119 Dandae-ro, Cheonan 31116, Republic of Korea

ABSTRACT. The effects of anti-lipopolysaccharide (LPS) antibody on rumen fermentation and LPS activity were investigated during subacute ruminal acidosis (SARA) challenge. Eleven Holstein cattle (164 ± 14 kg) were used in a 3 × 3 Latin square design. Cattle were fed a roughage diet on days −11 to −1 (pre-challenge) and day 2 (post-challenge), and a high-grain diet on days 0 and 1 (SARA challenge). For 14 days, 0-, 2-, or 4-g of anti-LPS antibody was administered once daily through a rumen fistula. Ruminal pH was measured continuously, and rumen fluid and blood samples were collected on days −1, 0, 1, and 2. Significantly lower ruminal LPS activity on day 1 was observed in the 2- and 4-g groups than those in the 0-g group. In addition, significantly higher 1-hr mean ruminal pH on SARA challenge period (days 0 and 1) was identified in the 4-g group than in the 0-g group. However, rumen fermentation measurements (total volatile fatty acid [VFA], VFA components, NH_3-N and lactic acid) and peripheral blood metabolites (glucose, free fatty acid, beta-hydroxybutyrate, total cholesterol, blood urea nitrogen, aspartate aminotransferase and gamma-glutamyl transferase) were not different among the groups during the experimental periods. Therefore, anti-LPS antibody administration mitigates LPS release and pH depression without the depression of rumen fermentation and peripheral blood metabolites during SARA challenge in Holstein cattle.

KEY WORDS: anti-lipopolysaccharide antibody, cattle, lipopolysaccharide, rumen fermentation, subacute ruminal acidosis

J. Vet. Med. Sci.
83(6): 905–910, 2021
doi: 10.1292/jvms.21-0037

Received: 20 January 2021
Accepted: 8 April 2021
Advanced Epub:
21 April 2021

Various types of probiotic or microbial supplementations have been used to improve rumen fermentation and to prevent subacute ruminal acidosis (SARA) incidence in cattle [16, 20]. For example, administration of a probiotic consisting of *Lactobacillus plantarum*, *Enterococcus faecium*, and *Clostridium butyricum* reduced the decrease in ruminal pH in Holstein calves fed a high-concentrate diet [16]. In addition, active dried *Sacharomyces cerevisiae* supplementation in calves produce more butyric and lactic acids as energy source during ruminal acidosis challenge [20]. Although probiotic or microbial supplementations may improve rumen fermentation, they were not able to entirely prevent the occurrence of SARA. Furthermore, strategies for controlling free ruminal lipopolysaccharide (LPS) have not been considered extensively.

In the cattle rumen, the bacterial community under the high-forage diet is composed of an approximately one-to-one ratio of *Firmicutes* and *Bacteriodetes* at a phylum level [11, 14]. However, higher acidity caused by a high-grain diet, as seen in ruminal acidosis or SARA, decreases the proportion of *Bacteriodetes* due to death or lysis of gram-negative bacteria, resulting in higher LPS activity in the rumen [1, 20]. Consequently, increased ruminal LPS can translocate to the blood-stream, triggering inflammatory [9] and acute-phase protein responses [4] in cattle. However, LPS bound to lipoproteins is removed from circulation by liver hepatocytes [7]. Therefore, LPS neutralization and related roles of liver cells are important in cattle fed a high-grain diet.

*Correspondence to: Sato, S.: sshigeru@iwate-u.ac.jp, Kim, Y.-H.: haneey@iwate-u.ac.jp

Previously, studies using LPS-binding peptides to neutralize LPS were performed *in vitro* using the phage display method [12] and peptide-bound beads [18], and *in vivo* studies were performed with mice [3, 22]. However, to the best of our knowledge, no research on anti-LPS antibody administration has been conducted in cattle despite the potential benefits of neutralizing and controlling rumen-induced LPS. Therefore, we investigated the effects of ruminal anti-LPS antibody administration on rumen fermentation and LPS activity during SARA challenge. We hypothesized that the use of an anti-LPS antibody in cattle fed a high-grain diet might mitigate the adverse effects of rumen-derived LPS activity.

MATERIALS AND METHODS

Anti-LPS antibody preparation

The anti-LPS antibody was produced under patented and proprietary procedures (EW Nutrition Japan., Gifu, Japan) as described elsewhere [23]. Briefly, the vaccine containing 1 ml of antigen (1×10^9 colony forming unit/g of inactivated whole *Escherichia coli* O139) with oil adjuvant was injected intramuscularly into egg-laying hens (Hy-Line W36), and the second injection was performed 8 weeks after the first injection. After the 2 weeks after the second injection, eggs were collected and stored at 4°C. The separated egg yolk from the collection was homogenized thoroughly, filtered to eliminate other components, and spray-dried (140 to 72°C) to prepare the product in a powder form. The result was 1 g of the product bound to 0.25 g of purified LPS from *E. coli* O111 as tested by in house ELISA method using the anti-*E. coli* O111:B4 LPS rabbit IgG capture antibody, anti-*E. coli* O111:B4 LPS guinea pig IgG primary antibody, and horseradish peroxidase conjugated anti-guinea pig IgG secondary antibody. We determined the amount of anti-LPS antibody based on the previously reported ruminal LPS concentration (up to 5 µg/ml) in growing Holstein steers (330 to 380 kg body weight) with approximately 100 l of rumen [13].

Table 1. Composition of the roughage and high-grain diets on percentage and dry matter (DM) bases

Item	Roughage diet	High-grain diet
Amount (%)		
Orchard and timothy hay	100	0
Concentrate	0	50
Soybean flakes	0	50
DM basis[1] (%)		
TDN	60.9	80.5
CP	13.0	15.7
ADF	40.5	12.8
NDF	68.0	25.7
NFC	8.0	48.7
Ca	0.5	0.4
P	0.3	0.4

[1]TDN, total digestible nutrients; CP, crude protein; ADF, acid detergent fiber; NDF, Neutral detergent fiber; NFC, non-fiber carbohydrate.

Animals and experimental design

The experimental protocol was approved by the Iwate University Laboratory Animal Care and Use Committee (A201453-1; Morioka, Japan). Eleven fistulated Holstein bull cattle (5–6 months of age) were used in a 3 × 3 Latin square design without a wash-out period. Cattle were fed a roughage (orchard and timothy mixed hay; 5.6–7.0 kg/day) diet during the first 11 days (days −11 to −1; pre-challenge), a high-grain (50% concentrate and 50% soybean flakes; 3.0–3.6 and 3.0–3.8 kg/day, respectively) diet for 2 days (days 0 and 1; SARA challenge), and then a roughage diet for 1 day (day 2; post-challenge) (Table 1). The cattle were administered 0 (control group), 2, or 4 g immunoglobulin yolk containing the anti-LPS antibody (EW Nutrition Japan) per head once daily through the rumen fistula for 14 consecutive days. The diets were supplied daily at 0800 hr and 1630 hr in two equal portions. Feed composition and amounts were based on the requirements of the Japanese Feeding Standard for Dairy Cattle, and all feeds offered to animals were consumed. No abnormal changes in body conditions and behaviors were observed daily throughout the study period.

Sampling and measurements

Ruminal pH was measured continuously every 10 min during the experimental days using a radio transmission system (YCOW-S; DKK-TOA, Shinjo, Japan), as described previously [17]. Rumen fluid and blood samples were collected, right before the morning feeding, at 0800 hr and 1400 hr on days −1, 0, and 1, and at 0800 hr on day 2 to analyze the total volatile fatty acid (VFA), VFA components, NH_3-N and lactic acid concentrations, and LPS activity. The fluid samples were immediately filtered through two layers of cheesecloth and stored at −80°C until use. Blood samples were immediately centrifuged (1,500 × *g*, 15 min, 4°C) to separate the plasma and then preserved at −80°C until analysis.

For the VFA analyses, total VFA and individual VFAs (acetic, propionic, and butyric acids) were separated and quantified by gas chromatography (GC-2014; Shimadzu, Kyoto, Japan) as previously described [15]. For lactic acid analyses, the concentration in the supernatant was determined using a commercial F-kit (D-lactate/L-lactate) (J.K. International, Tokyo, Japan) as described previously [15]. To measure rumen LPS activity, a kinetic Limulus amebocyte lysate assay (Pyrochrome with Glucashield; Seikagaku, Tokyo, Japan) was used as previously described [10]. Serum and plasma were separated by centrifugation at 1,500 × *g* for 15 min at 4°C, and biochemical analysis was performed using an automated biochemistry analyzer (Accute, Toshiba, Tokyo, Japan).

Statistical analyses

The normality of the distributions of variables was assessed using the Shapiro–Wilk test. Significant differences among groups were evaluated using unpaired *t*-tests for normally distributed variables and the Mann–Whitney *U*-test for non-normal variables (Prism ver. 8.10; GraphPad Software, La Jolla, CA, USA). A mixed-model ANOVA (accounting for repeated measures), using time as a fixed effect, followed by Dunnett's multiple comparison method was used to determine within-group differences. Significant differences were determined at a threshold of $P<0.05$.

RESULTS

Ruminal LPS activity

In all groups, no significant change in ruminal LPS activity was identified (P>0.10) during SARA challenge. However, significantly (P<0.05) lower LPS activities were identified on day 1 (1400 hr) in the 2-g and 4-g groups compared with the 0-g group (2.32 and 3.34 vs. 6.59 endotoxin unit $\times 10^3$/ml, respectively) (Fig. 1).

Ruminal pH and VFA

The 24-hr mean ruminal pH was significantly (P<0.05) changed during SARA challenge period, and the durations and area under curves (for pH <5.6) were also significantly (P<0.05) changed during the same period (Table 2). The 24-hr minimum, mean, and maximum pH were significantly (P<0.05) decreased on day 0, 1, and 2 compared with day −1. On day 0, the 24-hr minimum ruminal pH was significantly (P<0.05) higher, and the duration under pH <5.6 and area under pH 5.6 were significantly (P<0.05) lower in the 4-g group compared with those in the 0-g group. Furthermore, the 1-hr mean ruminal pH was significantly (P<0.05) higher in the 4-g

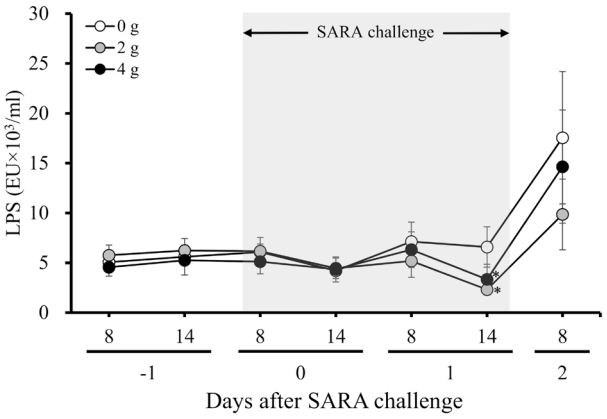

Fig. 1. Changes in the ruminal lipopolysaccharide (LPS) activity in Holstein bulls (n=11) after 0, 2, and 4 g of anti-lipopolysaccharide antibody were administered once daily. Days −2, −1, 0, 1, and 2 denote observations during the pre-challenge (day −2 and −1), subacute ruminal acidosis (SARA) challenge (day 0 and 1; gray square), and post-challenge (day 2) periods. *Denotes significant difference (P<0.05) compared with the 0-g group at that time point. Values represent mean ± SE.

Table 2. Changes in 24-hr mean ruminal pH, duration of time, and area under the curve (for pH <5.6) in Holstein bulls (n=11) administered 0, 2, and 4 g of anti-lipopolysaccharide antibody once daily

Item	Day −1[1]	Day 0	Day 1	Day 2	SEM	P-value[2]
24-hr mean pH						
Minimum						
0 g	6.48	5.69[a]	5.15[a]	5.76[a]	0.12	<0.001
2 g	6.10	5.25[a]	4.93[a]	5.38[a]	0.11	<0.001
4 g	6.12	5.39[ab]	4.95[a]	5.30[a]	0.10	<0.001
Mean						
0 g	6.12	4.96[a]	4.30[a]	4.99[a]	0.03	<0.001
2 g	6.52	5.92[a]	5.33[a]	6.08[a]	0.02	<0.001
4 g	6.44	5.99[a]	5.36[a]	6.07[a]	0.03	<0.001
Maximum						
0 g	6.80	6.65[a]	5.93[a]	6.35	0.08	<0.001
2 g	6.81	6.80	5.95[a]	6.70	0.05	<0.001
4 g	6.71	6.60[a]	6.02[a]	6.86	0.05	<0.001
Duration of ruminal pH (min/day)						
pH <5.6						
0 g	0	650[a]	1,170[a]	615	83.2	<0.001
2 g	0	413[a]	1,078[a]	248	75.6	<0.001
4 g	2.5	148[ab]	1,120[a]	395[a]	55.7	<0.001
Area under curve (pH × min/day)						
pH <5.6						
0 g	0	27.4[a]	87.6[a]	34.3	5.99	<0.001
2 g	0	11.7	46.6[a]	9.90	4.32	<0.001
4 g	0	1.63[ab]	41.2[a]	14.4	4.44	<0.001

[a]Denotes significant difference (P<0.05) compared with day −1 in each group. [b]Denotes significant difference (P<0.05) between the 0- and 4-g groups at the same time point. [1]Days −1, 0, 1, and 2 denote observations during the pre-challenge (day −1), high-grain diet challenge (day 0 and 1), and post-challenge (day 2) periods. [2]Mixed effects model ANOVA, followed by Dunnett's multiple comparison method, was used to determine within-group differences.

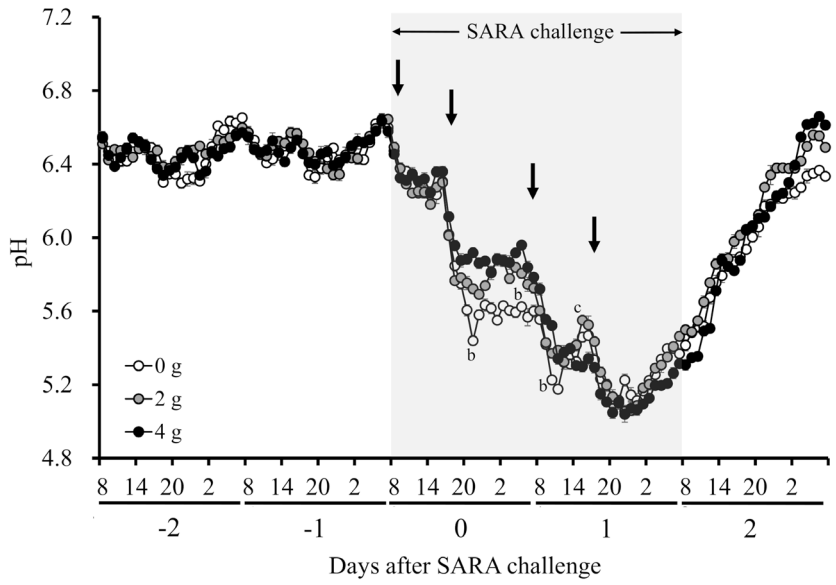

Fig. 2. Diurnal changes in the 1-hr mean ruminal pH in Holstein bulls (n=11) after 0, 2, and 4 g of anti-lipopolysaccharide antibody were administered once daily. Days −2, −1, 0, 1, and 2 denote observations during the pre-challenge (day −2 and −1), subacute ruminal acidosis (SARA) challenge (day 0 and 1; gray square), and post-challenge (day 2) periods. Vertical arrows indicate feeding of a high-grain diet (0800 hr and 1630 hr). [b,c]Denote significant differences (P<0.05) between the 0- and 4-g groups and between the 2- and 4-g groups at that time point. Values represent mean ± SE.

group during SARA challenge compared with the 0-g group (Fig. 2).

The total VFA concentration, proportion of acetic, propionic, and butyric acids (0- and 4-g groups), and ratio of acetic acid-to-propionic acids (2- and 4-g groups), and NH_3-N (2- and 4-g groups) and lactic acid (4-g group) concentrations were significantly (P<0.05) changed during SARA challenge (Table 3). The NH_3-N concentration was significantly (P<0.05) decreased during the pre- (1400 hr on day −1 in the 0-g group) and SARA challenge in each group compared with pre-challenge (0800 hr).

Blood metabolites

The concentrations of glucose (0- and 2-g groups), free fatty acid (0- and 2-g groups), beta-hydroxybutyrate (4-g group), and blood urea nitrogen (0- and 2-g groups) were significantly (P<0.05) changed during the SARA challenge period (Table 4). However, no significant differences (P>0.10) among group comparisons in the peripheral blood metabolites were found.

DISCUSSION

In the present study, SARA, a condition characterized by ruminal pH <5.6 for an extended period [9], was successfully induced during the SARA challenge. Moreover, the anti-LPS antibody did not affect rumen fermentation and blood metabolites but did suppress ruminal LPS release during SARA challenge. For example, significantly lower LPS activities observed on day 1 (1400 hr) in the 2-g and 4-g groups compared with the 0-g group were likely due to high binding affinity of anti-LPS antibody to ruminal LPS. Furthermore, the total VFA concentration and major VFA components (acetic, propionic, and butyric acids) showed the same direction of change during SARA challenge, and these results were consistent with the general features of a high-grain diet in cattle studies [8, 19, 21]. In addition, NH_3-N and lactic acid concentrations among the groups were changed in the same direction. Therefore, the administration of anti-LPS antibody did not cause significant changes in the rumen fermentation and blood metabolite profiles, preferably leading the selective suppression of ruminal LPS activity in the present study.

Once rumen-derived LPS translocates to the circulation, activated Kupffer cells release more pro-inflammatory cytokines such as TNF-α, IFN-γ, and IL-6 in the systemic circulation, triggering secretion of APPs such as LBP, HP, and SAA [7, 21]. However, administration of anti-LPS antibody alleviated ruminal pH depression and ruminal LPS suppression during SARA challenge in the present study, the effects of anti-LPS antibody administration on pro-inflammatory cytokines of the peripheral blood and transcriptome expression of the liver were unknown. Therefore, further studies need to reveal the effects of anti-LPS antibody on systemic immune responses, and liver functions due to rumen derived LPS in SARA cattle.

In the present study, an administration of anti-LPS antibody alleviated ruminal pH depression during SARA challenge. Cattle in the 4-g group showed significantly lower 1-hr mean pH on days 0 and 1, simultaneously with significantly lower duration of ruminal pH and area under curve (pH <5.6) in the 4-g group those compared with the 0-g group. The duration of time and area under the pH curve (for 5.6) on day 0 was likely due to significantly higher 24-hr minimum pH compared with that of the 0-g group. However, we could not provide plausible evidence that the 1-hr mean ruminal pH was less depressed in the anti-LPS antibody administration groups, and temporally higher minimum ruminal pH on day 0 in the 0-g group than the 4-g group or higher 1-hr mean ruminal pH on day 1 (1500 hr) in the 4-g group than the 2-g group due to limited information on physiological responses to lowered ruminal pH [2, 5]. In addition, the anti-LPS antibody showed a high binding affinity to LPS that might have possible impacts on living Gram-negative bacteria in accordance with other studies using avian-derived polyclonal antibodies against *Fusobacterium necrophorum* and *Streptococcus bovis* in crossbred steers [6] and anti-LPS-enriched colostrum in a mouse

Table 3. Total volatile fatty acid (VFA), individual VFA proportions, acetic acid to propionic acid (A/P) ratio, NH_3-N, and lactic acid concentrations in Holstein bulls (n=11) administered 0, 2, and 4 g of anti-lipopolysaccharide antibody once daily

Items	Day −1[1]		Day 0		Day 1		Day 2	SEM	*P*-value[2]
	0800 hr	1400 hr	0800 hr	1400 hr	0800 hr	1400 hr	0800 hr		
Total VFA (mmol/dl)									
0 g	10.7	9.76	11.0	14.7[a]	14.5	17.0[a]	14.7[a]	0.83	<0.001
2 g	10.3	9.47	10.0	13.4[a]	15.6[a]	16.3[a]	13.5	0.68	<0.001
4 g	10.6	10.1	11.0	13.2	14.5[a]	17.8[a]	14.9[a]	0.60	<0.001
Acetic acid (%)									
0 g	72.9	72.5	72.3	66.4[a]	56.5[a]	53.4[a]	52.0[a]	1.69	<0.001
2 g	72.9	72.5	72.6	67.6[a]	54.8[a]	51.6[a]	56.3[a]	1.79	<0.001
4 g	72.1	71.6	72.3	67.0[a]	57.5[a]	54.3[a]	59.4[a]	1.58	<0.001
Propionic acid (%)									
0 g	17.9	17.6	18.8[a]	21.4[a]	22.9[a]	24.9[a]	22.2	1.12	0.033
2 g	18.3	18.0	18.9	20.9[a]	21.7[a]	22.8[a]	19.9	0.76	0.043
4 g	19.1	18.7	18.9	21.2[a]	23.2[a]	22.7	16.9	0.84	<0.001
Butyric acid (%)									
0 g	7.13	8.15[a]	6.90	10.3[a]	17.9[a]	19.4[a]	22.1[a]	1.29	<0.001
2 g	6.64	7.60[a]	6.45	9.62[a]	20.6[a]	23.1[a]	21.0[a]	1.52	<0.001
4 g	6.99	8.06[a]	6.98	9.94[a]	16.5[a]	20.7[a]	21.1[a]	1.12	<0.001
A/P ratio									
0 g	4.09	4.13	3.87	3.12	2.53	2.31	3.43	0.28	0.104
2 g	4.0	4.04	3.86	3.25[a]	2.63[a]	2.34[a]	3.26	0.20	0.015
4 g	3.83	3.85	3.84	3.17[a]	2.60[a]	2.51[a]	3.99[b]	0.21	0.014
NH_3-N (mg/dl)									
0 g	7.02	4.41	7.07	5.89	4.97	6.41	6.89	0.87	0.173
2 g	8.11	5.73[a]	7.78	6.06	3.59[a]	4.46[a]	6.22	0.85	0.006
4 g	6.28	3.92[a]	6.62	5.14	3.37	4.30	5.70	0.72	0.040
Lactic acid (g/l)									
0 g	0.031	0.027	0.023	0.039	0.898	1.105	1.599	0.304	0.167
2 g	0.029	0.025	0.023	0.023	0.040	0.228	2.519	0.197	0.072
4 g	0.027	0.027	0.026	0.024	0.077	0.416	2.791	0.222	0.048

[a]Denotes significant difference ($P<0.05$) compared with day −1 in each group. [b]Denotes significant difference ($P<0.05$) between the 0- and 4-g groups at the same time point. [1]Days −1, 0, 1, and 2 denote observations during the pre-challenge (day −1), high-grain diet challenge (day 0 and 1), and post-challenge (day 2) periods. [2]Mixed effects model ANOVA, followed by Dunnett's multiple comparison method, was used to determine within-group differences.

Table 4. Biochemical analysis of peripheral blood in Holstein bulls (n=11) administered 0, 2, and 4 g of anti-lipopolysaccharide antibody once daily

Items[3]	Day −1[1]		Day 0		Day 1		Day 2	SEM	*P*-value[2]
	0800 hr	1400 hr	0800 hr	1400 hr	0800 hr	1400 hr	0800 hr		
GLU (mg/dl)									
0 g	88.8	85.0	91.1	96.2	97.4	88.7	90.1	5.57	0.012
2 g	78.9	82.6	85.3	94.1	95.0	88.1	78.8	3.81	0.035
4 g	83.8	73.4	86.2	118.5	93.7	88.1	81.8	8.42	0.060
FFA (μEq/l)									
0 g	119	139	154	74.0	57.4	53.6	83.6	24.8	0.031
2 g	101	93.5	135	67.1	62.4	56.9	64.1	18.0	0.013
4 g	86.3	81.4	84.0	65.0	59.3	53.2	59.4	10.6	0.209
BHB (μmol/l)									
0 g	483	378	370	578	597	635	769	161	0.082
2 g	574	467	532	694	792	951	974	114	0.054
4 g	486	470	516	528	626	862	849	74.1	0.011
T-CHO (mg/dl)									
0 g	75.9	70.9	68.2	64.5	60.2	64.5	63.6	5.03	0.175
2 g	72.8	72.7	69.0	68.4	65.2	64.0	63.6	3.69	0.119
4 g	80.5	74.6	72.5	70.3	68.4	71.1	70.5	7.35	0.461
BUN (mg/dl)									
0 g	5.98	6.58	5.83	4.85	2.73	2.50	3.70	1.24	0.022
2 g	6.10	7.08	6.60	6.73	4.10	3.13	2.98	1.09	0.007
4 g	5.93	5.50	5.55	5.38	3.75	3.65	3.28	1.29	0.059
AST (U/l)									
0 g	64.0	62.1	65.5	62.6	54.0	64.7	67.5	4.40	0.441
2 g	61.0	57.0	56.2	55.3	97.5	111	59.3	17.3	0.544
4 g	56.6	54.0	57.0	64.8	54.6	54.1	59.9	5.03	0.518
GGT (U/l)									
0 g	16.4	15.1	16.0	16.3	15.3	15.2	16.4	1.13	0.761
2 g	17.7	13.7	17.0	16.2	14.2	18.1	17.9	2.81	0.852
4 g	15.6	13.3	15.6	18.6	13.9	15.9	16.2	1.89	0.775

[1]Days −1, 0, 1, and 2 denote observations during the prechallenge (day −1), high-grain diet challenge (day 0 and 1), and postchallenge (day 2) periods. [2]Mixed effects model ANOVA, followed by Dunnett's multiple comparison method, was used to determine within-group differences. [3]GLU, glucose; FFA, free fatty acid; BHB, β-hydroxybutyrate; T-CHO, total cholesterol; BUN, blood urea nitrogen; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

model [3]. Therefore, further studies are required to clarify the detailed regulatory mechanism of anti-LPS antibody in the rumen, including its potential effect on physiological responses and bacterial community structures.

In conclusion, anti-LPS ruminal administration mitigated LPS release and pH depression without any depression of rumen fermentation and blood metabolites among the 0-, 2-, and 4-g groups. Further studies are required to elucidate the effects of anti-LPS antibody on systemic immune responses, and liver transcriptomic adaptations, and the regulatory mechanism of ruminal pH through analysis of the rumen bacterial community.

CONFLICT OF INTEREST. The authors have nothing to disclose.

ACKNOWLEDGMENTS. The authors appreciate EW Nutrition Japan (Gifu, Japan) who kindly provided the immunoglobulin yolk containing anti-LPS antibody.

REFERENCES

1. Abaker, J. A., Xu, T. L., Jin, D., Chang, G. J., Zhang, K. and Shen, X. Z. 2017. Lipopolysaccharide derived from the digestive tract provokes oxidative stress in the liver of dairy cows fed a high-grain diet. *J. Dairy Sci.* **100**: 666–678. [Medline] [CrossRef]
2. Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *J. Dairy Sci.* **80**: 1447–1462. [Medline] [CrossRef]
3. Ben Ya'acov, A., Lichtenstein, Y., Zolotarov, L. and Ilan, Y. 2015. The gut microbiome as a target for regulatory T cell-based immunotherapy: induction of regulatory lymphocytes by oral administration of anti-LPS enriched colostrum alleviates immune mediated colitis. *BMC Gastroenterol.* **15**: 154. [Medline] [CrossRef]
4. Danscher, A. M., Thoefner, M. B., Heegaard, P. M., Ekstrøm, C. T. and Jacobsen, S. 2011. Acute phase protein response during acute ruminal acidosis in cattle. *Livest. Sci.* **135**: 62–69. [CrossRef]
5. DeVries, T. J., Beauchemin, K. A., Dohme, F. and Schwartzkopf-Genswein, K. S. 2009. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: feeding, ruminating, and lying behavior. *J. Dairy Sci.* **92**: 5067–5078. [Medline] [CrossRef]
6. DiLorenzo, N., Diez-Gonzalez, F. and DiCostanzo, A. 2006. Effects of feeding polyclonal antibody preparations on ruminal bacterial populations and ruminal pH of steers fed high-grain diets. *J. Anim. Sci.* **84**: 2178–2185. [Medline] [CrossRef]
7. Eckel, E. F. and Ametaj, B. N. 2016. Invited review: Role of bacterial endotoxins in the etiopathogenesis of periparturient diseases of transition dairy cows. *J. Dairy Sci.* **99**: 5967–5990. [Medline] [CrossRef]
8. Golder, H. M., Celi, P., Rabiee, A. R., Heuer, C., Bramley, E., Miller, D. W., King, R. and Lean, I. J. 2012. Effects of grain, fructose, and histidine on ruminal pH and fermentation products during an induced subacute acidosis protocol. *J. Dairy Sci.* **95**: 1971–1982. [Medline] [CrossRef]
9. Gozho, G. N., Plaizier, J. C., Krause, D. O., Kennedy, A. D. and Wittenberg, K. M. 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. *J. Dairy Sci.* **88**: 1399–1403. [Medline] [CrossRef]
10. Hirabayashi, H., Kawashima, K., Okimura, T., Tateno, A., Suzuki, A., Asakuma, S., Isobe, N., Obitsu, T., Kushibiki, S. and Sugino, T. 2017. Effect of nutrient levels during the far-off period on postpartum productivity in dairy cows. *Anim. Sci. J.* **88**: 1162–1170. [Medline] [CrossRef]
11. Kim, Y. H., Nagata, R., Ohtani, N., Ichijo, T., Ikuta, K. and Sato, S. 2016. Effects of dietary forage and calf starter diet on ruminal pH and bacteria in Holstein calves during weaning transition. *Front. Microbiol.* **7**: 1575. [Medline] [CrossRef]
12. Matsumoto, M., Horiuchi, Y., Yamamoto, A., Ochiai, M., Niwa, M., Takagi, T., Omi, H., Kobayashi, T. and Suzuki, M. M. 2010. Lipopolysaccaride-binding peptides obtained by phage display method. *J. Microbiol. Methods* **82**: 54–58. [Medline] [CrossRef]
13. Motoi, Y., Oohashi, T., Hirose, H., Hiramatsu, M., Miyazaki, S., Nagasawa, S. and Takahashi, J. 1993. Turbidimetric-kinetic assay of endotoxin in rumen fluid or serum of cattle fed rations containing various levels of rolled barley. *J. Vet. Med. Sci.* **55**: 19–25. [Medline] [CrossRef]
14. Nagata, R., Kim, Y. H., Ohkubo, A., Kushibiki, S., Ichijo, T. and Sato, S. 2018. Effects of repeated subacute ruminal acidosis challenges on the adaptation of the rumen bacterial community in Holstein bulls. *J. Dairy Sci.* **101**: 4424–4436. [Medline] [CrossRef]
15. Ogata, T., Makino, H., Ishizuka, N., Iwamoto, E., Masaki, T., Kizaki, K., Kim, Y. H. and Sato, S. 2020. Long-term high-grain diet alters ruminal pH, fermentation, and epithelial transcriptomes, leading to restored mitochondrial oxidative phosphorylation in Japanese Black cattle. *Sci. Rep.* **10**: 6381. [Medline] [CrossRef]
16. Qadis, A. Q., Goya, S., Ikuta, K., Yatsu, M., Kimura, A., Nakanishi, S. and Sato, S. 2014. Effects of a bacteria-based probiotic on ruminal pH, volatile fatty acids and bacterial flora of Holstein calves. *J. Vet. Med. Sci.* **76**: 877–885. [Medline] [CrossRef]
17. Sato, S., Kimura, A., Anan, T., Yamagishi, N., Okada, K., Mizuguchi, H. and Ito, K. 2012. A radio transmission pH measurement system for continuous evaluation of fluid pH in the rumen of cows. *Vet. Res. Commun.* **36**: 85–89. [Medline] [CrossRef]
18. Suzuki, M. M., Matsumoto, M., Omi, H., Kobayashi, T., Nakamura, A., Kishi, H., Kobayashi, S. and Takagi, T. 2014. Interaction of peptide-bound beads with lipopolysaccharide and lipoproteins. *J. Microbiol. Methods* **100**: 137–141. [Medline] [CrossRef]
19. Wang, D. S., Zhang, R. Y., Zhu, W. Y. and Mao, S. Y. 2013. Effects of subacute ruminal acidosis challenges on fermentation and biogenic amines in the rumen of dairy cows. *Livest. Sci.* **155**: 262–272. [CrossRef]
20. Watanabe, Y., Kim, Y. H., Kushibiki, S., Ikuta, K., Ichijo, T. and Sato, S. 2019. Effects of active dried Saccharomyces cerevisiae on ruminal fermentation and bacterial community during the short-term ruminal acidosis challenge model in Holstein calves. *J. Dairy Sci.* **102**: 6518–6531. [Medline] [CrossRef]
21. Zhao, C., Liu, G., Li, X., Guan, Y., Wang, Y., Yuan, X., Sun, G., Wang, Z. and Li, X. 2018. Inflammatory mechanism of Rumenitis in dairy cows with subacute ruminal acidosis. *BMC Vet. Res.* **14**: 135. [Medline] [CrossRef]
22. Zhou, X., Wang, P., Chen, Y. and Ma, S. Y. 2019. Intact anti-LPS IgY is found in the blood after intragastric administration in mice. *FEBS Open Bio* **9**: 428–436. [Medline] [CrossRef]
23. Zúñiga, A., Yokoyama, H., Albicker-Rippinger, P., Eggenberger, E. and Bertschinger, H. U. 1997. Reduced intestinal colonisation with F18-positive enterotoxigenic Escherichia coli in weaned pigs fed chicken egg antibody against the fimbriae. *FEMS Immunol. Med. Microbiol.* **18**: 153–161. [Medline] [CrossRef]