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# Anti-lipopolysaccharide antibody mitigates ruminal lipopolysaccharide release without acute-phase inflammation or liver transcriptomic responses in Holstein bulls

**Hitoshi Mizuguchi** [ID] [1], **Keiichiro Kizaki** [ID] [2], **Atsushi Kimura** [ID] [2], **Shiro Kushibiki** [ID] [3], **Kentaro Ikuta** [ID] [4], **Yo-Han Kim** [ID] [2,5,*], **Shigeru Sato** [ID] [2,*]

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

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**\*Corresponding authors:**

**Shigeru Sato**
Cooperative Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, 3 Chome-18-8 Ueda, Morioka 020-8550, Japan.
E-mail: sshigeru@iwate-u.ac.jp

**Yo-Han Kim**
Cooperative Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, 3 Chome-18-8 Ueda, Morioka 020-8550, Japan.
E-mail: haneey@iwate-u.ac.jp

**ORCID iDs**
Hitoshi Mizuguchi [ID]
https://orcid.org/0000-0002-5684-0987

## ABSTRACT

Anti-lipopolysaccharide (LPS) antibody administration has the potential benefits of neutralizing and consequently controlling rumen-derived LPS during subacute ruminal acidosis. Four Holstein bulls were used in this crossover study with a 2-week wash-out period. Anti-LPS antibody (0 or 4 g) was administered once daily for 14 days. Significantly lower ruminal LPS and higher 1-h mean ruminal pH were identified in the 4 g group. However, blood metabolites, acute-phase proteins, cytokines, and hepatic transcriptomes were not different between the two groups. Therefore, anti-LPS antibody administration mitigated ruminal LPS release and pH depression without accompanying responses in acute-phase inflammation or hepatic transcriptomic expression.

**Keywords:** Anti-lipopolysaccharide antibody; cattle; liver transcriptome; rumen fermentation; subacute ruminal acidosis

## INTRODUCTION

The occurrence of ruminal acidosis or subacute ruminal acidosis (SARA) in cattle fed a high-grain diet causes higher acidity, resulting in higher lipopolysaccharide (LPS) activity in the rumen [1]. The increased ruminal LPS translocates to the bloodstream and can provoke inflammatory and acute-phase protein (APP) responses in cattle [2]. Furthermore, transcriptomic analysis of liver tissue revealed that the metabolic consequences of uncontrolled inflammation induced by LPS challenge can be particularly harmful during the early stages of lactation, when there is a marked degree of body fat mobilization [3]. Therefore, LPS neutralization and related roles of liver cells are important in cattle fed a high-grain diet.

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Keiichiro Kizaki (iD)
https://orcid.org/0000-0002-2012-8533
Atsushi Kimura (iD)
https://orcid.org/0000-0003-0541-0327
Shiro Kushibiki (iD)
https://orcid.org/0000-0003-0031-5080
Kentaro Ikuta (iD)
https://orcid.org/0000-0002-9236-2409
Yo-Han Kim (iD)
https://orcid.org/0000-0003-2472-820X
Shigeru Sato (iD)
https://orcid.org/0000-0002-5641-7687

**Conflict of Interest**
The authors declare no conflicts of interest.

Previously, *in vitro* (peptide-bound bead method) [4] and *in vivo* (mouse model) [5] studies using LPS-binding peptides were performed to neutralize LPS. However, there has been little research regarding anti-LPS antibody administration in cattle despite the potential benefits of neutralizing and consequently controlling rumen-derived LPS. Therefore, we investigated the effects of ruminal anti-LPS antibody administration on rumen fermentation and LPS activity, as well as hepatic transcriptomic adaptation during SARA challenge.

## MATERIALS AND METHODS

### Anti-LPS antibody preparation
Anti-LPS antibody was produced under patented and proprietary procedures (EW Nutrition Japan, Japan) as described previously [6]. Briefly, 1 mL antigen ($1 \times 10^9$ CFU/g inactivated whole *Escherichia coli* O139) was injected intramuscularly into egg-laying hens (Hy-Line W36). Then, the egg yolk was separated to prepare the product, yielding 1 g of the product bound to 0.25 g purified LPS from *E. coli* O111. We determined the amount of anti-LPS antibody based on previously reported ruminal LPS concentrations (up to 5 μg/mL) in growing Holstein steers (body weight 330–380 kg) with a rumen volume of approximately 100 L [7].

### Animals and experimental design
The experimental protocol was approved by the Iwate University Laboratory Animal Care and Use Committee (A201453-1; Japan). Four rumen-fistulated Holstein bulls (5–6 months of age; 162 ± 9 kg) were used in a crossover study with a 2-week washout period. Cattle were fed a roughage diet (orchard and timothy mixed hay: 5.6–7.0 kg/day) during the first 11 days (days −11 to −1; pre-challenge), followed by a high-grain diet (50% concentrate and 50% soybean flakes: 3.0–3.6 and 3.0–3.8 kg/day, respectively) for 2 days (days 0 and 1; SARA challenge), and then a roughage diet for 1 day (day 2; post-challenge). The high-grain diet contained 80.5% total digestible nutrients, 15.7% crude protein, 12.8% acid detergent fiber, and 25.7% neutral detergent fiber.

The cattle were administered 0 (control group) or 4 g anti-LPS antibody per head once daily via the rumen fistula for 14 consecutive days. The diets were supplied daily at 800 and 1,630 h in 2 equal portions. The feed composition and amounts were based on the requirements of the Japanese Feeding Standard for Dairy Cattle.

### Sampling and measurements
Ruminal pH was measured using a radio transmission system (YCOW-S; DKK-TOA, Japan) as described previously [8]. Rumen fluid and blood samples were collected at 800 and 1,400 h on days −1, 0, and 1 and at 800 h on day 2. Then, fluid and blood samples were prepared for further analysis as described previously [9].

The concentrations of total volatile fatty acids (VFAs), $NH_3$-N, lactic acid, and individual VFAs (acetic, propionic, and butyric acids) were determined [9]. Rumen LPS activity was measured by kinetic limulus amebocyte lysate assay (Pyrochrome with Glucashield; Seikagaku, Japan) [9]. For APP analyses, the plasma concentrations of LPS binding protein (LBP), haptoglobin (HP), and serum amyloid A (SAA) were measured using commercial kits [9]. Concentrations of plasma tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin-6 (IL-6) were measured by sandwich enzyme-linked immunosorbent assay [9].

### Transcriptome analysis of liver tissue

Liver tissue was biopsied at 800 h on day 2 in the 0 and 4 g groups. Preparation of RNA samples for microarray analysis and data processing were performed as described previously [10]. The entire microarray data set has been deposited in the Gene Expression Omnibus database with the following accession numbers: platform, GPL22091; samples, GSM 3901089 to GSM 3901115; series, GSE133152.

### Statistical analysis

The normality of the distributions of variables was assessed using the Shapiro-Wilk test. The significance of differences among groups was evaluated using the unpaired $t$-test for normally distributed variables and the Mann-Whitney $U$ test for non-normally distributed variables (Prism ver. 8.10; GraphPad Software, USA). Mixed-model repeated-measures analysis of variance, using time as a fixed effect, followed by Dunnett's multiple comparison method was performed to determine the significance of within-group differences. The microarray data were analyzed using the unpaired $t$-test with Benjamini-Hochberg false discovery rate (FDR) multiple testing correction (FDR corrected $p < 0.10$) (GeneSpring 12.0; Agilent Technologies, USA). In all analyses, $p < 0.05$ was taken to indicate statistical significance.

# RESULTS

### Ruminal LPS, pH, VFAs, and blood metabolites

The ruminal LPS activity (0 g anti-LPS antibody) changed significantly ($p < 0.05$) during the SARA challenge. The ruminal LPS activity was significantly ($p < 0.05$) increased on day 2 (800 h) compared with day −1 (800 h). Significantly ($p < 0.05$) lower LPS activity was identified on day 2 (800 h) in the 4 g group compared with the 0 g group (**Fig. 1**).
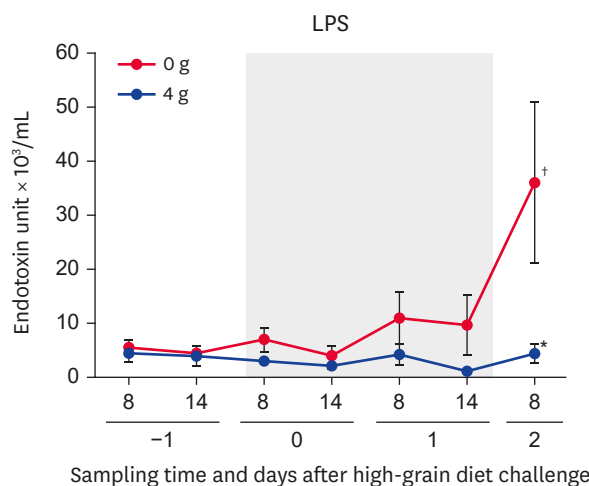


**Fig. 1.** Changes in ruminal LPS activity in Holstein bulls. Anti-LPS antibody was administered once daily at a dose of 0 or 4 g. Days −1, 0, 1, and 2 denote observations during the pre-challenge (day −1), subacute ruminal acidosis challenge (days 0 and 1; gray squares), and post-challenge (day 2) periods. Sampling times of day are shown as 8 (800 h) and 14 (1,400 h). Values represent means ± SE.
*Significant difference between the 0 and 4 g groups at that time point ($p < 0.05$); †Significant changes during the SARA challenge period ($p < 0.05$).
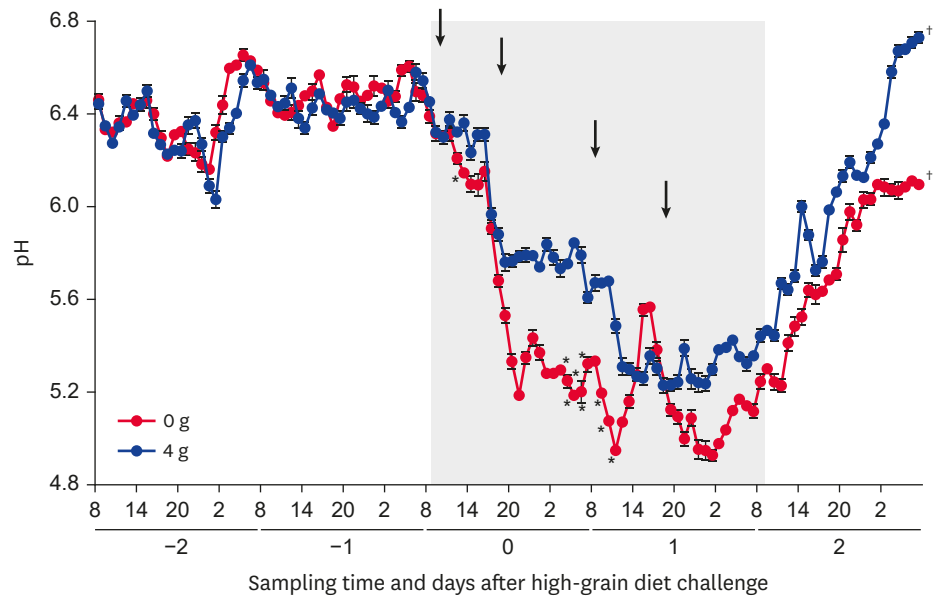LPS, lipopolysaccharide.

**Fig. 2.** Diurnal changes in the 1-h mean ruminal pH in Holstein bulls. Anti-lipopolysaccharide antibody was administered once daily at a dose of 0 or 4 g. Days −2, −1, 0, 1, and 2 denote observations during the pre-challenge (days −2 and −1), subacute ruminal acidosis challenge (days 0 and 1; gray squares), and post-challenge (day 2) periods. Arrows indicate feeding of a high-grain diet (800 and 1,630 h). Sampling times of day are shown as 8 (800 h), 14 (1,400 h), 20 (2,000 h), and 2 (200 h). Values represent means ± SE.
*Significant difference between the 0 and 4 g groups at that time point ($p < 0.05$); †Significant changes during the SARA challenge period ($p < 0.05$).

The 1-h mean ruminal pH (0 and 4 g anti-LPS antibody) changed significantly ($p < 0.05$) during the SARA challenge. The 1-h mean ruminal pH was significantly ($p < 0.05$) higher on days 0 (1,300 h) and 1 (300–700 h and 900–1,100 h) in the 4 g compared with 0 g group (**Fig. 2**).

During the SARA challenge, the total VFA concentration (4 g group) was significantly ($p < 0.05$) increased on days 1 (1,400 h) and 2 (800 h) compared with day −1 (800 h) (**Fig. 3**). However, no significant change in the $NH_3$-N or lactic acid concentration was detected during the SARA challenge period.

The levels of peripheral blood APPs (LBP, HP, and SAA) were increased in the 0 g group, compared with the 4 g group, during the later part of the SARA challenge period (**Fig. 3**). The LBP and SAA concentrations in the 0 g group increased significantly ($p < 0.05$) during the SARA challenge. However, no significant changes in the levels of HP or cytokines (TNF-α, IFN-γ, and IL-6) were detected during the SARA challenge period.

### Microarray analysis of liver tissue
There were no significantly differentially expressed genes (FDR corrected $p < 0.10$), including genes encoding APPs and cytokines, between the 0 and 4 g groups.

## DISCUSSION

In the present study, ruminal LPS release and pH depression were alleviated by administration of anti-LPS antibody during SARA challenge. It is plausible that the anti-LPS antibody,
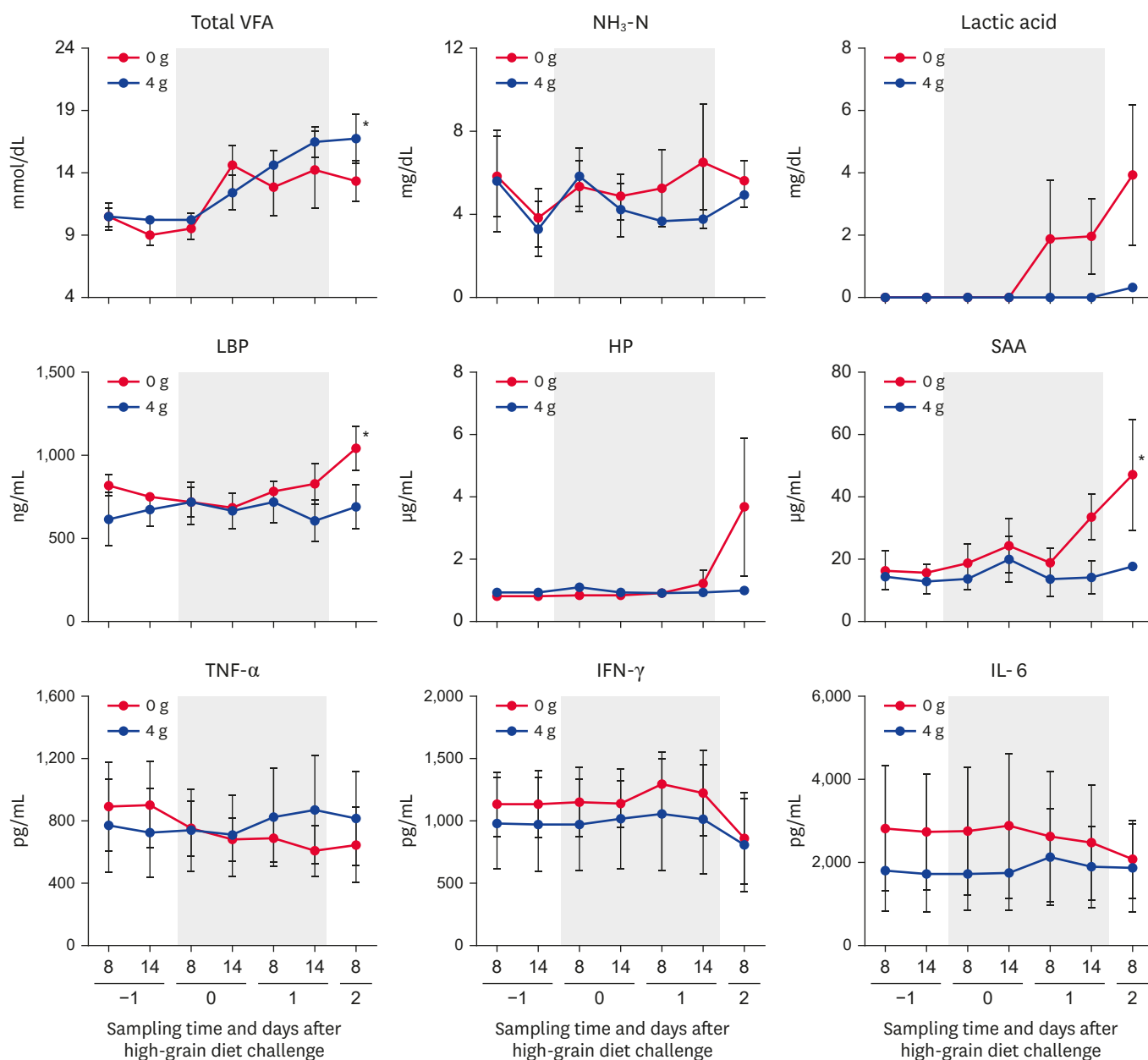
**Fig. 3.** Changes in the levels of rumen fermentation parameters, peripheral blood APPs, and cytokines. The levels of rumen fermentation parameters (total VFAs, $NH_3$-N, and lactic acid), peripheral blood APPs (LBP, HP, SAA), and cytokines (TNF-$\alpha$, IFN-$\gamma$, IL-6) were determined in the groups administered 0 and 4 g of anti-lipopolysaccharide antibody. Days −1, 0, 1, and 2 denote observations during the pre-challenge (day −1), subacute ruminal acidosis challenge (days 0 and 1; gray squares), and post-challenge (day 2) periods. Sampling times of day are shown as 8 (800 h) and 14 (1,400 h). Values represent means ± SE.
VFA, volatile fatty acid; LBP, lipopolysaccharide binding protein; HP, haptoglobin; SAA, serum amyloid A; TNF-$\alpha$, tumor necrosis factor-$\alpha$; IFN-$\gamma$, interferon-$\gamma$; IL-6, interleukin-6; APP, acute-phase protein.
*Significant changes during the SARA challenge period ($p < 0.05$).

showing high binding affinity to LPS, may affect living gram-negative bacteria, consistent with other studies using avian-derived polyclonal antibodies against *Fusobacterium necrophorum* and *Streptococcus bovis* in crossbred steers [11] and anti-LPS-enriched colostrum in a mouse model [5]. However, the present study verified the selective suppression of ruminal LPS activity, without significant changes in the rumen fermentation or blood metabolite profiles.

Once rumen-derived LPS enters the circulation, it activates Kupffer cells to release proinflammatory cytokines, such as TNF-α, IFN-γ, and IL-6, into the systemic circulation and triggers the secretion of APPs, such as LBP, HP, and SAA [12,13]. However, oral administration of anti-LPS-enriched colostrum alleviated immune-mediated colitis by lessening bowel inflammation in a mouse model, suggesting that the gut microbiome may serve as a target for regulatory T-cell-based immunotherapy [5]. In the present study, we found significant changes in the LBP and SAA concentrations only in the 0 g group. Furthermore, gradual increases in LBP, HP, and SAA concentrations during the later parts of the SARA challenge period were observed in the 0 g compared with 4 g group, suggesting the need for longer-term observations. Therefore, we postulated that the significantly lower ruminal LPS levels on day 2 in the administration groups may play a suppressive role in the acute-phase inflammatory responses of the peripheral blood in comparison with the 0 g group during SARA challenge.

In the present study, no differences in hepatic gene expression were detected between the 0 and 4 g groups although the ruminal LPS activity was significantly lower in the 4 g group. This was consistent with previous reports showing no significant effects of a single and mild episode of SARA on the ruminal epithelial barrier function immediately after the episode [14] and with the lack of significant differences in peripheral blood hepatic enzyme levels (aspartate aminotransferase and γ-glutamyltransferase) in the present study. That is, ruminal LPS release by SARA challenge may have a limited effect on hepatic responsiveness, likely due to relatively low LPS activity compared with a previous study (36.0 endotoxin units [EU] $\times 10^3$/mL on day 2 in the present study vs. 47.17 and 79.04 EU $\times 10^3$/mL in low- and high-grain-fed dairy cows, respectively [15]). Furthermore, the present study was performed over a short period to evaluate liver adaptation to both SARA challenge and anti-LPS antibody administration. Therefore, further studies are required to verify the effects of anti-LPS antibody administration on alterations in the hepatic transcriptome using longer-term challenge models.

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