

Rapid Communication  
Internal Medicine



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

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## 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.

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

The authors declare no conflicts of interest.

**Author Contributions**

Conceptualization: Mizuguchi H, Sato S; Data curation: Kizaki K, Kimura A; Formal analysis: Kim YH, Kushibiki S, Ikuta K; Validation: Kizaki K, Kimura A, Kushibiki S, Kim YH; Visualization: Kim YH; Writing - original draft: Mizuguchi H, Kim YH, Sato S; Writing - review & editing: Mizuguchi H, Kim YH, Sato S.

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  $\mu\text{g/mL}$ ) 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;  $162 \pm 9$  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),  $\text{NH}_3\text{-N}$ , lactic acid, and individual VFAs (acetic, propionic, and butyric acids) were determined [9]. Rumen LPS activity was measured by kinetic limulus amoebocyte 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- $\alpha$  (TNF- $\alpha$ ), interferon- $\gamma$  (IFN- $\gamma$ ), 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.

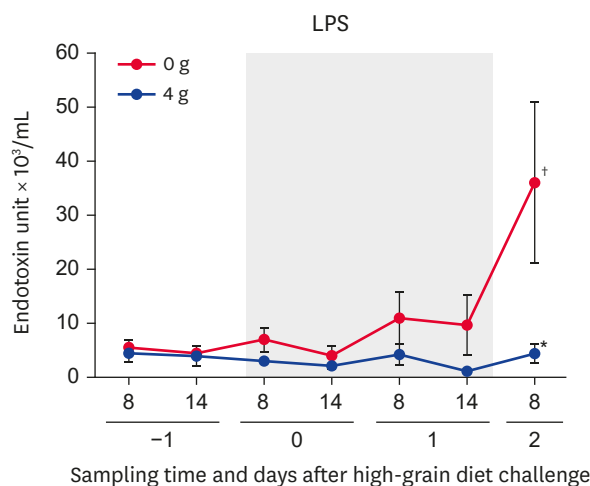
### Statistical analysis

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

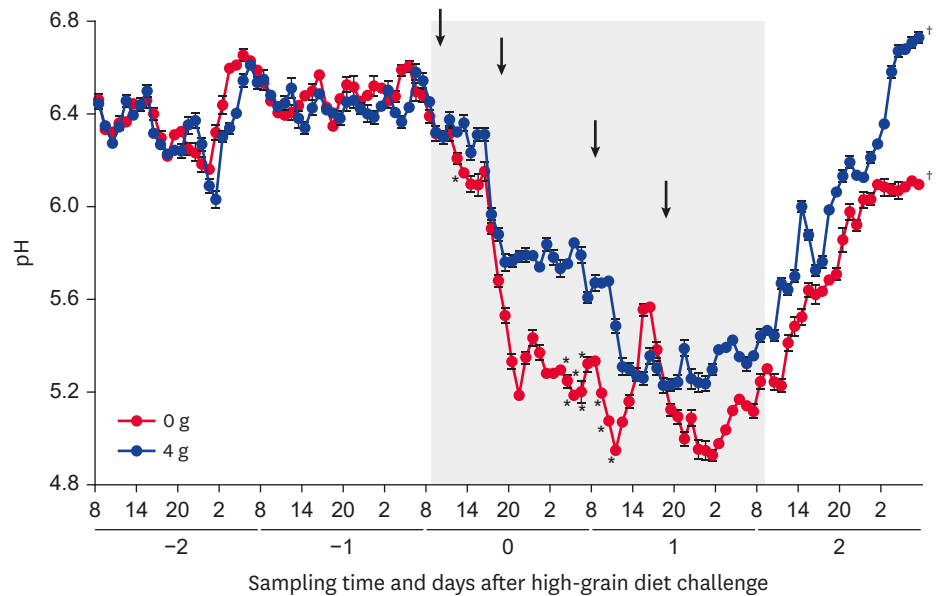
### Ruminal LPS, pH, VFAs, and blood metabolites

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 ( $p < 0.05$ ); <sup>†</sup>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  $\pm$  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  $\text{NH}_3\text{-N}$  or lactic acid concentration was detected during the SARA challenge period.

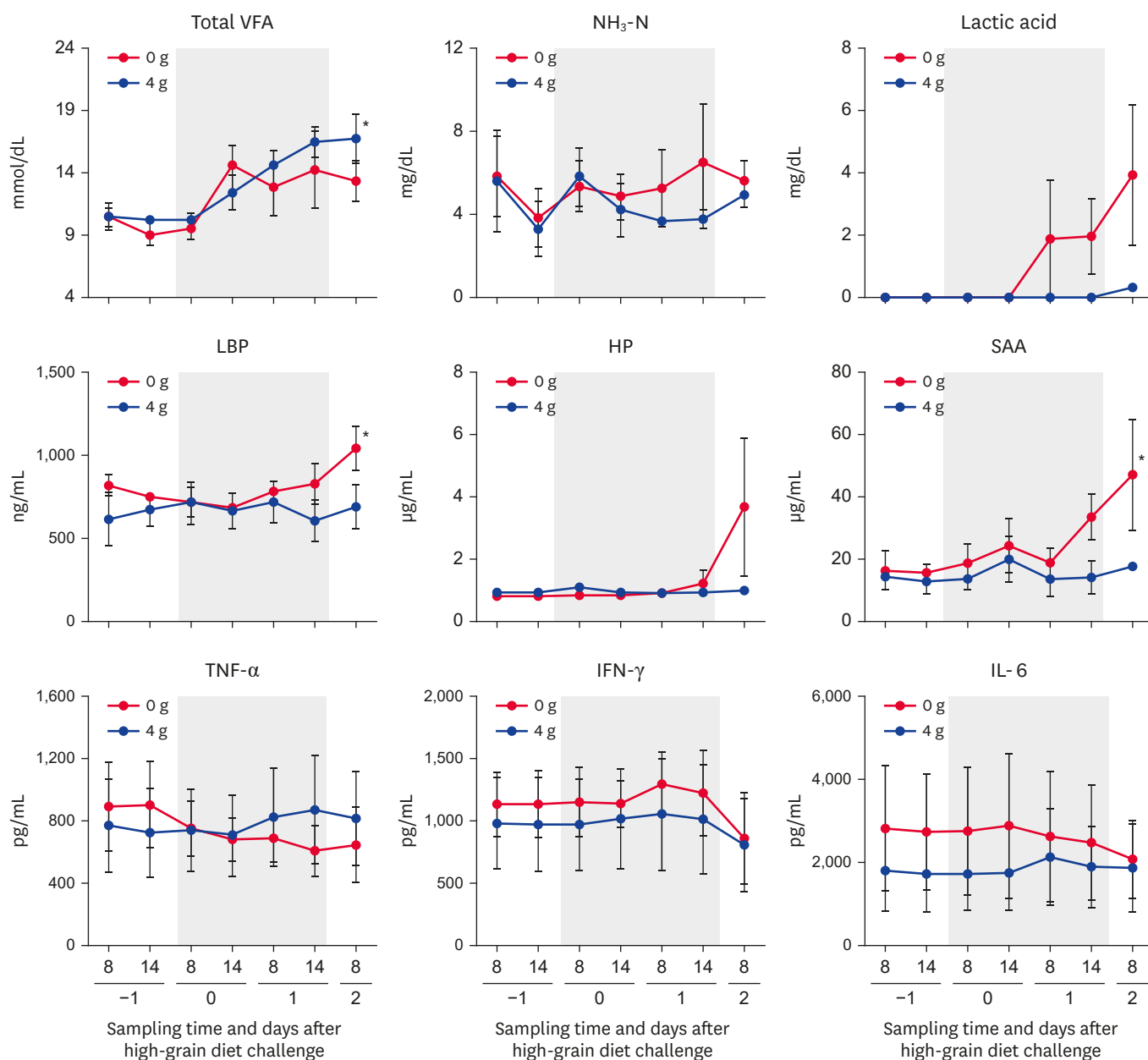
The levels of peripheral blood APPs (LBP, HP, and SAA) were increased in the 0 g group, compared with the 4 g group, during the later part of the SARA challenge period (**Fig. 3**). The LBP and SAA concentrations in the 0 g group increased significantly ( $p < 0.05$ ) during the SARA challenge. However, no significant changes in the levels of HP or cytokines (TNF- $\alpha$ , IFN- $\gamma$ , 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,  $\text{NH}_3\text{-N}$ , and lactic acid), peripheral blood APPs (LBP, HP, SAA), and cytokines ( $\text{TNF-}\alpha$ ,  $\text{IFN-}\gamma$ , 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  $\pm$  SE.

VFA, volatile fatty acid; LBP, lipopolysaccharide binding protein; HP, haptoglobin; SAA, serum amyloid A;  $\text{TNF-}\alpha$ , tumor necrosis factor- $\alpha$ ;  $\text{IFN-}\gamma$ , 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- $\alpha$ , IFN- $\gamma$ , 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  $\gamma$ -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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