Effect of high-concentrate corn grain diet-induced elevated ruminal lipopolysaccharide levels on dairy cow liver function

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ABSTRACT

A high-concentrate diet destroys gram-negative bacteria in the cattle rumen, leading to elevated ruminal lipopolysaccharide (LPS) levels. LPS causes liver inflammation through the hepatic portal vein but little is known about the effects of rumen-derived LPS on liver function and the reproductive organs. In this study, we determined the effect of increasing rumen fluid LPS levels on liver function and genital LPS levels. Cows were assigned to control (CON; n = 5) and high-concentrate diet (HC; n = 7) groups. We observed that the ruminal LPS and haptoglobin (Hp) levels were significantly higher and albumin levels were lower in the HC group than in the CON group. In the HC group, the Hp levels and aspartate transaminase (AST) activity were significantly higher and the total cholesterol levels were significantly lower after high-concentrate diet feeding than before feeding. No differences were observed in LPS levels in the peripheral veins, hepatic veins, hepatic portal vein, uterine perfusate, and follicular fluids between the groups. In all samples, the LPS level in the hepatic portal vein blood positively correlated with the AST activity and serum amyloid A level. In conclusion, our results indicate that high-concentrate diets do not have a direct effect on the reproductive organs upon a moderate ruminal LPS level increase. However, an increased ruminal LPS influx into the liver might affect negatively liver function.

Keywords: high-concentrate diet, lipopolysaccharide, liver, reproductive organs, rumen
INTRODUCTION

In order to compensate for the energy shortage in high-lactating cows, the amount of their concentrated feed has been increased over the years. However, feeding large amounts of a high-concentrate diet leads to rapid fermentation in the rumen and a lower pH due to an increase in hydrogen ions caused by elevated levels of volatile fatty acids (VFAs) and lactic acid [27]. The decreasing pH changes the microbiota in the rumen and kills gram-negative bacteria, resulting in the release of large amounts of the endotoxin lipopolysaccharide (LPS) from the surface of the dead cells [16, 17, 26, 27]. In high-concentrate dietary trials, subacute ruminal acidosis (SARA) reportedly caused an increase in ruminal LPS levels [10-12, 16, 17, 19]. The LPS in the rumen fluid is mainly absorbed by the rumen wall, then it passes into the ruminal veins and reaches the liver via the portal vein [12, 16, 17]. However, the intestinal environment may also affect LPS translocation into the bloodstream [16]. A previous study focusing on postpartum cows with uterine Escherichia coli infection reported on increased circulating prostaglandin E₂ (PGE₂) but not prostaglandin F metabolite (PGFM), and that the uterine LPS shifted endometrial epithelial cell secretion from prostaglandin F₂α (PGF₂α) to PGE₂, which prolonged the luteal phase [14]. However, Lüttgenau et al. [20] reported that repeated intrauterine LPS infusions at the onset of the estrous cycle promoted premature luteolysis and altered gene expression in the endometrium and corpus luteum. Previous studies also reported on high LPS levels in the follicular fluid of repeat breeder cows [24] and follicular cyst cows [34], and that LPS in the follicular fluid suppresses steroidogenesis in the follicle granulosa cells in vitro [21]. In addition, LPS administration to cattle before ovulation altered the pulsatile secretion of luteinizing hormone, decreased the blood estradiol-17β (E₂) levels, and suppressed follicular development [36]. These findings also suggested that the transfer of LPS or tumor necrosis factor-α (TNF-α) into the bloodstream might directly affect the genital tract. However, it is still unclear to what extent rumen fluid-derived LPS migrates into the portal vein and affects the genital tract. Hence, in this study, we investigated the effect of increased LPS concentration in the rumen on the LPS level in the genital tract of high-concentrate diet-fed cows.
MATERIALS AND METHODS

Animals, experimental design, diet: A total of 13 non-pregnant, dry Holstein cows were used for the study. The animals were kept at the Department of Veterinary Medicine, Nihon University. Their average body weight was 650 kg. The cows were tied in a stall during the study period. All experiments were conducted according to the Nihon University Guidelines for Management of Animal Experiments (AP17B048-1).

The cows were allocated to a control group (CON; n = 5) and a high-concentrate diet group (HC; n = 7). The CON group was mainly raised on timothy hay. The HC group received a reduced roughage (timothy hay)-content feed in the dry matter, lowered weekly in consecutive steps (Week 1 to 4: 40, 30, 25, and 20%, respectively); the high-concentrate portion of the diet included compressed corn and soybean meal-based feed (Table 1). The feeding regimens ensured that all cows received 100% of the total digestible nutrient and crude protein requirement according to the Nutrient Requirements of Dairy Cattle [29]. All cows were fed twice per day at 8:00 and 16:00, and water was given ad libitum.

To synchronize the estrous cycle, all cows received 100 µg of gonadotropin-releasing hormone (GnRH, fertirelin acetate; Conceral®, MSD Animal Health, Tokyo, Japan) through intramuscular injection, and a controlled intravaginal drug-releasing device (CIDR device, containing 1.9 g of P₄; CIDR® 1900, Zoetis Japan, Tokyo, Japan) was inserted into their vagina. Seven days later, the CIDR device was removed, and the animals were administered 25 mg of prostaglandin F₂α (PGF₂α, dinoprost, Pronalgon® F, Zoetis Japan, Tokyo, Japan) through intramuscular injection. A 2nd GnRH dose was administered 2 days after the CIDR device removal and PGF₂α administration. All samples were collected one day after the 2nd GnRH injection (after 4 weeks of high-concentrate diet feeding in the HC group), when the dominant follicle was present, right before ovulation in order to prevent follicular fluid blood contamination during sampling. Furthermore, in the HC group, peripheral blood was collected before feeding the high-concentrate diet in order to compare blood parameters before and after feeding.

Peripheral blood samples

Peripheral blood was collected from the coccygeal vein. Samples were collected into vacuum tubes.
with glass granules coated with a clot activator for serum collection, and into heparinized vacuum tubes for plasma collection. The serum separator tubes were left to clot, and plasma separator tubes were placed in a cooler box. The tubes were then centrifuged at $1,700 \times g$ at 4 °C for 15 min, and the serum and plasma samples were stored frozen at -20 °C until the assay.

**Blood samples from the portal vein and hepatic veins**

After collecting peripheral blood, samples were taken from the portal vein and the hepatic veins, according to the method described by Ono et al. [30]. Briefly, sterile 14-G catheters (ZENOAQ, Fukushima, Japan) were inserted near to one side of the ultrasonography probe, and blood was collected under ultrasonographic guidance. The portal vein was distinguished from the hepatic veins based on the vessel wall thickness and confirmed by the ammonia levels in the collected blood [25]. The portal blood samples from 1 cow in the CON group and 4 cows in the HC group could not be obtained, excluding these cows from the hormone analysis.

**Rumen fluid samples**

The ruminal fluid collection was performed by transcrotaneous direct puncture 3 hr after feeding, using the procedure reported by Garrett et al. with modifications [9]. Briefly, a 5-cm square area was identified, located 12 to 15 cm caudoventral to the costochondral junction of the last rib on a line parallel with the top of the stifle. The area was clipped, scrubbed with a povidone-iodine scrub, and wiped with 70% alcohol. A sterile 14-G catheter (ZENOAQ was inserted into the ventral rumen, and a 20-ml syringe was used to aspirate a minimum of 3 ml of fluid. The collected rumen solution was immediately filtered through double gauze to prepare a sample stock solution, and after pH measurements, stored frozen at −20 °C.

**Uterine perfusate collection**

The uterine perfusate was collected by a simplified method described by Kaneko et al. [15]. A balloon catheter (16 Fr; Fujihira Industry Co., Ltd., Tokyo, Japan) was advanced into the uterus using the recto-vaginal method, then the balloon was inflated. Sterile physiological saline (50 ml) was infused into
the uterus through the balloon catheter and recovered by gently massaging the uterus. The collected uterine perfusate samples were transferred to sterile tubes and stored at -20 °C until the assay.

**Follicular fluid collection**

Follicular fluid aspiration was performed using an ultrasound device (MyLab OneVET, Esaote, Genova, Italy) equipped with a transvaginal micro-convex 6 to 10 MHz transducer connected to a needle guide system (SC3123 with ovum pick up attachment, Esaote, Genova, Italy). The dominant follicle was confirmed by ultrasound, the egg collection needle (Disposable cow ova needle, 17G×600 mm, Misawa Medical Industry Co., Ibaraki, Japan) was punctured into the follicle, and aspirated with a 5 ml syringe. The follicular fluid samples were stored frozen at -20°C until the assay. Follicular fluid from 1 cow in the CON group and 3 follicles in the HC group were excluded from the analysis as the follicular fluid was lost due to rupture during collection, and the samples could not be collected.

**Sample analysis**

LPS concentrations in the peripheral blood, hepatic portal vein blood, uterine perfusate, and follicular fluid were measured using the QCL-1000 Chromogenic Limulus Amebocyte Lysate Endpoint Assay Kit (Lonza Walkersville, Inc., Walkersville, MD, USA) as previously described by Magata et al. [21]. Samples were thawed, diluted in endotoxin-free distilled water, and tested for non-specific LAL inhibition by comparing samples spiked with a known LPS concentration to non-spiked samples. Samples with evidence of LAL inhibition were subjected to proteinase K digestion, as described by Petsch et al. [31]. Briefly, samples were treated with proteinase K at a final concentration of 0.4 mg/ml for 18 hr at 37 °C. The reaction was stopped by heat inactivation at 80 °C for 10 min using a heat block, and the measurement was performed according to the manufacturer’s instructions.

Furthermore, commercial kits were used to measure peripheral blood LPS binding protein (LBP, multi-species, HK503 ELISA kit; Hycult Biotech, Uden, Netherlands) and TNF-α (Duoset ELISA Development bovine TNF-α; R&D Systems, Minneapolis, Minn, USA) levels. In addition, peripheral blood and follicular fluid estradiol-17β concentrations were measured using a commercial kit (#
Estradiol ELISA Kit, Cayman Chemical, Ann Arbor, MI, USA). The serum samples were submitted to the Health Sciences Research Institute (Yokohama, Japan) for albumin (ALB), aspartate transaminase (AST), γ-glutamyl transpeptidase (GGT), total cholesterol (T-Chol), and non-esterified fatty acid (NEFA) analysis. VFAs were measured using a gas chromatograph (7890A; Agilent) equipped with a flame ionization detector and a capillary column (Nukol, 15 m, 0.25 mm ID, 0.25 μm df; Supelco / Sigma-Aldrich, St. Louis, MO, USA) as previously described by Asano [4]. Serum amyloid A (SAA) was measured using an automated latex agglutination turbidimetric immunoassay kit (LAT: SAA-1, Eiken Chemical Co., Tokyo, Japan) and an automated clinical chemical analyzer (Hitachi 7170S, Hitachi Ltd., Tokyo, Japan) according to the method described by Tsukano et al. [37]. Furthermore, peripheral blood haptoglobin (Hp) levels were measured using a modified hemoglobin binding assay according to the method described by Nakamura et al. [28].

Statistical analysis: All values are expressed as mean ± standard error. The means of two groups were compared using a significance test, specifically, Student’s t-test for normally distributed parameter values and Wilcoxon rank-sum test for non-normally distributed parameter values. Normality was tested using the Shapiro-Wilk W-test. In order to compare the corresponding samples in the HC group before (week 0) and after (week 4) high-concentrate diet feeding, the Wilcoxon signed-rank test for paired comparisons was performed. The differences between LPS concentrations at different sites were compared using the Kruskal-Wallis test. Univariate correlation analysis among inflammatory markers, blood biochemical parameters indicating the liver function, and LPS concentrations at various sites was performed using the Spearman rank correlation coefficient. A difference was considered significant when the probability value (P) was less than the significance level of 5% (P< 0.05). Statistical analyses were performed using the JMP® 13.2.0 software (SAS Institute Inc., Tokyo, Japan).

RESULTS

Rumen properties

The rumen fluid pH, VFA composition, and LPS concentration of each group are shown in Table 2.
The rumen pH was significantly lower in the HC group than that in the CON group (6.12 ± 0.27 vs. 7.21 ± 0.27, p < 0.05). The assessment of the VFA (mol%) in the rumen fluid showed that acetic acid was significantly lower in the HC group than that in the CON group, whereas butyric acid and isovaleric acid were significantly higher in the HC group. The acetic-acid-to-propionic-acid ratio (A/P ratio) was significantly lower in the HC group than that in the CON group (2.6 ± 0.3 vs. 3.6 ± 0.2, p < 0.05). The rumen LPS concentration was significantly higher in the HC group than in the CON group (36.5 ± 2.4 vs. 21.7 ± 2.7 EU/l, p < 0.05).

Peripheral blood LBP, TNF-α, Hp, SAA, and blood biochemical properties

The LBP, TNF-α, Hp, SAA, and blood biochemical properties of each group are shown in Table 3. The Hp concentration was significantly higher in the HC group than that in the CON group (421.8 ± 130.3 vs. 86.9 ± 28.3 µg/ml, p < 0.05) but the LBP, SAA, and TNF-α did not significantly differ between the two groups. The blood biochemical property analysis showed that ALB was significantly lower in the HC group than that in the CON group (2.63 ± 0.11 vs. 2.90 ± 0.03 g/dl, p < 0.05). The AST, GGT, T-Chol, and NEFA values did not significantly differ between the two groups. In the HC group, the Hp level and AST activity were significantly increased after high-concentrate diet feeding compared with those before feeding (Fig. 1; 421.8 ± 130.3 vs. 69.5 ± 11.3 µg/ml, p < 0.05 and 96.9 ± 24.7 vs. 56.9 ± 5.5 U/l, p < 0.05, respectively). In contrast, the T-Chol level was significantly lower (60.4 ± 7.8 vs. 75.0 ± 6.7 mg/dl, p < 0.05) and the ALB level showed a trend to be lower after treatment (2.63 ± 0.11 vs. 2.79 ± 0.09 g/dl, p = 0.09) than before treatment (Fig. 1).

Estradiol concentration in follicular fluid

The follicular fluid E2 concentrations did not significantly vary between the HC group and the CON group (26.8 ± 2.5 vs. 28.7 ± 7.3 ng/ml).

LPS concentration in peripheral blood, hepatic portal vein blood, uterine perfusate, and follicular fluid

The LPS concentrations in the peripheral vein blood, hepatic portal vein blood, hepatic vein blood,
uterine perfusate, and follicular fluid are presented in Table 4. The LPS concentrations (EU/ml) in the CON and HC groups were 0.28 ± 0.00 (n = 5) and 0.26 ± 0.01 (n = 7) in peripheral vein blood, 0.35 ± 0.10 (n = 4) and 0.31 ± 0.06 (n = 3) in hepatic portal vein blood, 0.20 ± 0.05 (n = 4) and 0.21 ± 0.01 (n = 1) in hepatic vein blood, 0.27 ± 0.03 (n = 5) and 0.27 ± 0.00 (n = 7) in uterine perfusate, and 0.31 ± 0.02 (n = 4) and 0.31 ± 0.03 (n = 4) in follicular fluid, respectively. We did not observe statistically significant differences between the two groups. In a comparison among different sampling sites, we did not observe statistically significant differences in the LPS concentration between the CON and the HC groups.

Correlations among inflammatory markers, liver function indicators, and LPS concentrations

Table 5 shows the Spearman correlation coefficient (\( \rho \)) of ALB, AST, Hp, SAA, and TNF-\( \alpha \) relative to the LPS concentration. A significant negative correlation was detected between the LPS concentration in the rumen fluid and the ALB concentration (\( \rho = -0.82, p < 0.01 \)). The LPS concentration in the rumen fluid showed a trend to positively correlate with the AST (\( \rho = 0.51, p = 0.09 \)) and Hp concentration (\( \rho = 0.51, p = 0.09 \)). In contrast, the LPS concentration in the hepatic portal vein blood showed a trend to positively correlate with the AST (\( \rho = 0.67, p = 0.10 \)) and SAA concentration (\( \rho = 0.87, p = 0.10 \)). A significant positive correlation was observed between LPS in the uterine perfusate and SAA (\( \rho = 0.64, p < 0.05 \)), and LPS in the follicular fluid showed a trend to positively correlate with TNF-\( \alpha \) (\( \rho = 0.67, p = 0.07 \)). However, no significant correlation was observed between other LPS levels, inflammatory markers, and blood biochemical parameters indicating liver function.

DISCUSSION

In our study, the pH and A/P ratio in the rumen fluid were significantly lower in the HC group than those in the CON group. We further determined that the LPS concentration in the rumen fluid was significantly higher in the HC group than that in the CON group. These observations were consistent with previous reports [12, 16, 26, 27] describing that a high-concentrate diet lowers the rumen pH and kills gram-negative bacteria, releasing LPS, a proinflammatory component of the gram-negative cell.
envelope, into the rumen fluid supernatant. Nearly 60% of rumen LPS is reportedly generated by the rapid growth of gram-negative bacteria [17]. LPS released in the rumen is absorbed by the rumen wall, released into the ruminal veins, and carried to the liver via the portal vein. Importantly, increased peripheral blood LPS levels could be observed when the endotoxin inflow into the liver exceeds the hepatic detoxification capacity [12, 17]. In addition, it has been reported that a high-concentrate diet-triggered increase in ruminal LPS concentration induces inflammatory gene expression in the uterus of cows [5] and goats [6]. The uterine inflammation-induced intrauterine LPS production was also reported to accumulate in the follicular fluid [14, 22, 23]. For these reasons, in the case of increased concentration in the rumen, LPS could translocate into the blood and affect the reproductive organs. Nevertheless, in our study, the LPS levels in the peripheral vein blood, hepatic portal vein blood, follicular fluid, and uterine perfusate did not significantly vary between the two dietary groups. This result was likely due to the slight ruminal LPS level increase in the HC group. In a previous study, without feed treatment, the rumen LPS concentrations in the healthy and SARA groups in 13 farms were 34,179 and 42,206 EU/ml, respectively [35], which is consistent with the level recorded in our study. In contrast, the ruminal LPS level in grain-induced SARA cows was 12,589-168,391 EU/ml (3.4 to 16.2 times higher than that in the control) [10-12, 33]. In addition, the LPS concentration in the hepatic portal vein of grain-induced SARA cows was reportedly 0.95–1.42 EU/ml (1.8-3.2 times higher than that in the control) [1, 18]. The SARA-induced reduction in rumen pH increases gram-negative bacterial lysis and the LPS level but such a rumen LPS level increase suggests that it depends on the diet fed before the induction of SARA, and thus, the rumen bacterial population at the time of induction [32]. In our study, it might be due to the fact that the test cows were fed dent corn silage in both groups before the start of the study.

Hp, SAA, and LBP are representative bovine inflammatory markers [8, 40]. Many previous studies [10, 11, 13, 16, 17, 41] described significantly increased blood Hp, SAA, and LBP levels in SARA cows that were associated with high levels of inflammation. In our study, Hp levels were significantly higher in the HC group than those in the CON group, but no significant variations could be detected in the SAA and LBP levels. The discrepancy in the findings between our study and previous ones was probably influenced by the slight ruminal LPS level increase in the HC group and the differences in
the SAA and LBP assay methods. However, our results still suggest that increased LPS concentrations in the rumen induce Hp due to inflammation. Khafipour et al. [16] reported that a grain-based SARA challenge increased the LPS to 0.52 EU/ml. Our study did not show elevated levels of peripheral blood LPS in the HC group, and this discrepancy might be due to the varying degrees of ruminal wall damage. It has been reported that ruminal acidosis, induced by lowering the effective neutral digestive fiber in the feed, increases ruminal LPS but cows without colonic acidosis do not have elevated blood LPS levels [19]. If inflammation does not occur in the rumen wall or the mucosa of the large intestine, LPS absorption is blocked. However, if the intestinal mucosa is damaged, LPS absorption will likely occur at this site. In addition, ruminal LPS that enter the hepatic portal circulation can be detoxified in the liver before reaching the peripheral blood circulation [2]. However, the majority of cytokine (TNF-α and interleukin-6) receptors that induce inflammatory proteins such as Hp and SAA are found in liver Kupffer cells [7], and therefore, proinflammatory cytokines might be expressed prior to detoxification. Some studies have shown that experimentally induced SARA increases inflammatory markers without increasing LPS in the peripheral blood [12].

LPS induces cytokine production, such as TNF-α [39], which causes liver damage [38]. Hepatic lipidosis is associated with a decreased capacity for endotoxin clearance in the liver, as demonstrated by Andersen et al. [3]. By measuring the liver function biochemical indicators, T-Chol, ALB, and AST, we found that the ALB level was lower and AST activity was higher in the HC group than those in the CON group. Similarly, in the HC group, the AST activity increased, and the T-Chol and ALB levels decreased after feeding the high-concentrate diet compared with those before feeding. Furthermore, in our feeding study, increased rumen fluid LPS levels were associated with reduced ALB levels, and higher portal blood LPS levels were linked to increased blood AST activity and SAA level. These results suggested that a large LPS influx into the liver might reduce liver function.

In conclusion, these results indicate that high-concentrate diets do not have a direct effect on the reproductive organs following a moderate rumen LPS level increase. However, an increased ruminal LPS influx into the liver might affect negatively liver function. Further studies, including analyses in cows with a high LPS influx into the liver, are needed to clarify the effect of increased ruminal LPS on the genital tract.
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**Figure legends**

Fig. 1. Haptoglobin (Hp), albumin (ALB), aspartate transaminase (AST) and total cholesterol (T-Chol), before (week 0) and after treatment (week 4) in high-concentrate diet (HC) cows
Table 1. Ingredient dry matter content (%) in each treatment week (weeks 1-4) in the high-concentrate diet group

| Ingredients | Week 1 | Week 2 | Week 3 | Week 4 |
|-------------|--------|--------|--------|--------|
| Timothy     | 40     | 30     | 25     | 20     |
| Corn        | 55.2   | 64.4   | 69.0   | 73.6   |
| Soybean     | 4.8    | 5.6    | 6.0    | 6.4    |

The dry matter amount was calculated assuming that any feed comprised of 89% of it, and that 8% of the concentrated feed consisted of soybean meal. The concentration of roughage in the feed was reduced in a stepwise manner by substituting it with corn and soybean meal-based feed in the HC group (weekly fed proportion of roughage from Week 1 to 4: 40, 30, 25, and 20%, respectively).
Table 2. Rumen fluid pH, volatile fatty acid composition (mol%), and LPS concentrations in each group

| Item                  | CON (n = 5) |         | HC (n = 7) |         |
|-----------------------|-------------|---------|------------|---------|
| pH                    | 7.21 ± 0.27 |         | 6.12 ± 0.27* |         |
| acetic acid (mol %)   | 69.0 ± 1.5  |         | 54.5 ± 2.2** |         |
| propionic acid (mol %)| 19.4 ± 0.8  |         | 23.1 ± 2.6  |         |
| A/P ratio             | 3.6 ± 0.2   |         | 2.6 ± 0.3*  |         |
| butyric acid (mol %)  | 8.7 ± 0.8   |         | 16.2 ± 2.0* |         |
| isovaleric acid (mol %)| 1.1 ± 0.1   |         | 3.3 ± 0.4** |         |
| valeric acid (mol %)  | 1.4 ± 0.3   |         | 1.9 ± 0.3   |         |
| LPS (EU/l)            | 21.7 ± 2.7  |         | 36.5 ± 2.4* |         |

The data are expressed as mean ± SEM. Asterisks indicate significant differences between the high-concentrate diet (HC) and control (CON) groups (* p < 0.05, ** p < 0.01).
Table 3. Blood parameter concentrations in control (CON) and high-concentrate diet (HC) cows

| Item          | CON (n = 5) | HC (n = 7) |
|---------------|-------------|------------|
| LBP (ng/ml)   | 128.3 ± 4.2 | 129.7 ± 6.9 |
| TNF-α (pg/ml) | 81.4 ± 49.5 | 275.3 ± 214.4 |
| Hp (µg/ml)    | 86.9 ± 28.3 | 421.8 ± 130.3* |
| SAA (µg/ml)   | 1.42 ± 0.53 | 1.44 ± 0.13 |
| ALB (g/dl)    | 2.90 ± 0.03 | 2.63 ± 0.11* |
| AST (U/l)     | 54.2 ± 3.1  | 96.9 ± 24.7 |
| GGT (U/l)     | 26.6 ± 0.7  | 33.6 ± 9.5 |
| T-Chol (mg/dl)| 75.0 ± 5.6  | 60.4 ± 7.8 |
| NEFA (mEq/l)  | 0.24 ± 0.10 | 0.15 ± 0.02 |

The blood lipopolysaccharide-binding protein (LBP), tumor necrosis factor-α (TNF-α), haptoglobin (Hp), serum amyloid A (SAA), albumin (ALB), aspartate transaminase (AST), γ-glutamyl transpeptidase (GGT), total cholesterol (T-Chol), and non-esterified fatty acids (NEFA) levels were evaluated. The data are expressed as mean ± SEM. Asterisks indicate significant differences between the HC and CON groups (* p < 0.05).
Hp Conc. (µg/ml)

ALB Conc. (g/dl)

AST Conc. (U/l)

T-Chol Conc. (mg/dl)

ALB Conc. (g/dl)

week 0 vs week 4:
p < 0.05

week 0 vs week 4:
p = 0.09
| Sample type                    | CON       | HC        |
|-------------------------------|-----------|-----------|
| peripheral vein (EU/ml)       | 0.28 ± 0.00 (n = 5) | 0.26 ± 0.01 (n = 7) |
| portal vein (EU/ml)           | 0.35 ± 0.10 (n = 4) | 0.31 ± 0.06 (n = 3) |
| hepatic vein (EU/ml)          | 0.20 ± 0.05 (n = 4) | 0.21 (n = 1) |
| uterine perfusate (EU/ml)     | 0.27 ± 0.03 (n = 5) | 0.27 ± 0.00 (n = 7) |
| follicular fluid (EU/ml)       | 0.31 ± 0.02 (n = 4) | 0.31 ± 0.03 (n = 4) |

CON: control. HC: high-concentrate diet. The data are expressed as the mean ± SEM.
Table 5. albumin (ALB), aspartate transaminase (AST), haptoglobin (Hp), serum amyloid A (SAA), and tumor necrosis factor-α (TNF-α) spearman correlation coefficients (ρ) relative to the lipopolysaccharide (LPS) concentration

| LPS parameter       | indices  | ρ      | p       |
|---------------------|----------|--------|---------|
| rumen LPS           | ALB      | -0.82  | p < 0.01|
| rumen LPS           | AST      | 0.51   | p = 0.09|
| rumen LPS           | Hp       | 0.51   | p = 0.09|
| portal vein LPS     | SAA      | 0.87   | p = 0.10|
| portal vein LPS     | AST      | 0.67   | p = 0.10|
| uterine perfusate LPS | SAA    | 0.64   | p < 0.05|
| follicular fluid LPS | TNF-α   | 0.67   | p = 0.07|