Effects of intravenous infusion of lipopolysaccharide on plasma micromineral, magnesium, and cytokine concentrations and serum cortisol concentrations in lactating goats

Jiufeng Wang, DVM, PhD; Lianguo Jiao, DVM, MSc; Jinlei Ma, DVM, MSc; Chunxia Wu, MSc; Kai Wang, DVM, MSc; Ming Wang, DVM, PhD

Objective—To assess the effects of various doses of lipopolysaccharide (LPS) administered IV on plasma microminerals, magnesium, tumor necrosis factor (TNF-α), and interleukin (IL)-6 concentrations and serum cortisol concentrations in lactating goats.

Animals—6 lactating goats.

Procedures—Goats were allotted to 3 LPS-treatment groups: control (0 µg/kg), low LPS (10 µg/kg), and high LPS (50 µg/kg). Rectal temperatures and behaviors of goats were recorded immediately before a 10-minute IV infusion of LPS and at 0.5, 1, 2, 4, 6, 8, and 24 hours after infusion. Blood samples were obtained before IV infusion and at 0.5, 1, 2, 4, 6, 8, and 24 hours after infusion. Plasma zinc, copper, iron, and magnesium concentrations were determined by atomic absorption spectrometry; plasma TNF-α and IL-6 concentrations were measured by use of an ELISA; and serum cortisol concentrations were determined by use of a radioimmunoassay.

Results—A monophasic fever developed in low-LPS and high-LPS groups. In the low-LPS and high-LPS group, plasma zinc concentrations decreased at 6 hours after infusion; compared with control groups. Plasma iron concentrations were lower at 24 hours after infusion in low-LPS and high-LPS groups than in the control group. Plasma TNF-α and IL-6 concentrations were higher in low-LPS and high-LPS groups than in the control group at 1, 2, and 4 hours after infusion. In low-LPS and high-LPS groups, serum cortisol concentrations increased from 0.5 hours onward and peaked at 1 (high-LPS group) and 2 (low-LPS group) hours after infusion.

Conclusions and Clinical Relevance—Following IV infusion of LPS, the immune system is activated, which might affect micromineral homeostatic regulation and, subsequently, the metabolic health of lactating goats. (Am J Vet Res 2007;68:529–534)

Lipopolysaccharide, the major outer membrane component of gram-negative bacteria, is a potent endotoxin that, through the activation of cellular immunity, induces cytokine-mediated, localized, and systemic inflammatory responses in the host.1,2 The inflammatory reaction is a series of complex physiologic events occurring in the host after tissue injury, trauma, or infection. General agreement exists that LPS is the main pathogenic factor involved in infections induced by Escherichia coli.3 Bacterial LPS typically consists of lipid A, a core oligosaccharide, and a distal polysaccharide (or O antigen).4 Lipid A is a glucosamine-based phospholipid that makes up the outer monolayer of outer membranes of most gram-negative bacteria.5 Systemic exposure to LPS has been linked to numerous common diseases such as coliform mastitis, displaced abdomen, and ruminal acidosis.6,8 It is the key molecule in the development of coliform mastitis.8,11 Lipopolysaccharide is released after bacteriolysis or during periods of rapid bacterial proliferation. Lipopolysaccharide is implicated in a wide range of the pathophysiologic responses, from generalized inflammation to severe gram-negative bacterial infections. Pathophysiologic responses are characterized by a number of physiologic changes, including fever, leukocytosis, changes in plasma metal concentrations, and changes in concentrations of hepatocyte-derived plasma proteins and acute-phase proteins.11,12 Biological actions of LPS are mainly caused by triggering the production and release of endogenous mediators of inflammation, including the secretion of cytokines such as TNF-α and IL-6.13,14 Nearly all LPS effects are strictly dose related.14 Study of the physiologic effects of LPS infusion could provide a pathophysiologic paradigm used to extensively explore the mechanisms of manifold systemic signs of infection (fever, anorexia, and emaciation) and the reciprocal

ABBREVIATIONS

LPS Lipopolysaccharide
TNF Tumor necrosis factor
IL Interleukin
HPA Hypothalamic-pituitary-adrenal

Received September 15, 2006.
Accepted November 22, 2006.
From the College of Veterinary Medicine, China Agricultural University, Beijing 100094, People’s Republic of China.
Supported by the National Natural Science Foundation of China (Project No. 30571363) and the China Agricultural University.
Address correspondence to Dr. Jiufeng Wang.
interactions between several macrophage-produced cytokines and HPA hormones. Results of recent studies indicate that infectious toxins, especially LPS, act as immunostimulating factors and potent activators of the HPA axis.

Considering all of the aforementioned aspects, it is important to better understand how LPS acts as an immunostimulating factor and a potent activator of the HPA axis. The inflammatory response elicited by LPS reduces plasma calcium and phosphorus concentrations in dairy cows and lactating sows. However, whether the immune activation may have effects on micromineral metabolism in animals has not been clarified. A dearth of information exists regarding the influences of infectious disease on the metabolism of lactating goats. Objectives of the study reported here were to investigate the host response of lactating goats following challenge with bacterial endotoxin and to evaluate the changes in plasma Zn, Cu, Fe, Mg, TNF-α, and IL-6 concentrations and in serum cortisol concentrations. We hypothesized that the perturbation of endocrine factors is associated with alterations in micromineral and cytokine concentrations.

**Materials and Methods**

**Animals and experimental protocol**—This study was completed at the animal experimental facility of the College of Veterinary Medicine, China Agricultural University. The experimental protocol was performed in compliance with institutional guidelines for the use and care of animals established by the China Laboratory Animal Care Committee.

A total of 6 nonpregnant lactating goats (Swiss Saanen) with a mean weight of 40 kg (range, 35 to 45 kg), obtained from a healthy herd at a farm in Zunhua City, Hebei province, China, were used in our study. Before the experiment, all goats were considered clinically normal on the basis of physical examination findings. All goats had mammary glands that were free of mastitic pathogens on the basis of milk bacterial cultureings. All goats had mammary glands that were free of clinically normal on the basis of physical examination find...
ated by the testing period, with the interaction of goat, period, and treatment as the error term (the interaction had values of $P < 0.15$ for all variables). Overall effects of treatment and period were also tested, with the interaction of goat, period, and treatment as the error term. When a significant ($P < 0.05$) overall effect of treatment was found, differences between means were compared by use of the Fisher least significant difference. Results are presented as means ± SEM. Values of $P < 0.05$ were considered significant.

Results

Rectal temperature and clinical signs—Control group goats had a rectal temperature of $< 38^\circ$C at all sample collection times throughout the experimental period. In low-LPS and high-LPS groups, however, rectal temperature of goats increased during the LPS infusion from 0.5 hours onward. The low-LPS group reached a maximum rectal temperature of 40.5°C at 4 hours after infusion, and the high-LPS group had a maximum rectal temperature of 40.8°C at 2 hours after infusion. Afterwards, rectal temperatures decreased gradually and returned to preinfusion values at 24 hours after infusion. The rectal temperature was significantly ($P = 0.008$) lower in the control group than in the low-LPS group at 4 hours after infusion. The rectal temperature was significantly ($P = 0.009$) lower in the control group than in the high-LPS group at 2 hours after infusion. No significant differences were seen in the rectal temperature between the low-LPS and high-LPS groups at all sample collection times.

Control group goats behaved normally and remained in good health throughout the experimental period. However, goats in the low-LPS and high-LPS groups had clear signs of general depression (ie, they kept their heads down and moved slowly), and 2 goats within the high-LPS group had tremors of the legs after LPS infusion. These clinical signs were detected during the first 1 to 4 hours after LPS administration, varying between goats and temporally. After 8 hours after infusion, all goats behaved normally.

Effects of LPS infusion on mineral concentrations—Plasma Zn concentrations were significantly lower in the low-LPS and high-LPS groups than in the control group at 0 (6 $P = 0.049$), 8 ($P = 0.046$), and 24 ($P = 0.045$) hours after infusion (Figure 1). No differences were found in plasma Zn concentrations between the low-LPS and high-LPS groups throughout the experiment. Minimal and maximal plasma concentrations of Zn, respectively, were 0.59 mg/L (0.5 hours) and 0.75 mg/L (24 hours) in the control group, 0.44 mg/L (6 hours) and 0.78 mg/L (0.5 hours) in the low-LPS group, and 0.31 mg/L (8 hours) and 0.66 mg/L (0.5 hours) in the high-LPS group. Plasma Zn concentrations appeared to be affected by LPS in a dose-dependent manner, with an increase in the LPS dose resulting in a further decrease in plasma Zn concentrations, but this finding was not significant ($P = 0.12$).

No differences were found in plasma Cu concentrations among treatment groups throughout the experiment. Minimal and maximal concentrations of plasma Cu concentrations, respectively, were 1.66 mg/L (6 hours) and 1.91 mg/L (0.5 hours) in the control group, 1.74 mg/L (4 hours) and 1.90 mg/L (0.5 hours) in the low-LPS group, and 1.69 mg/L (0.5 hours) and 2.02 mg/L (1 hour) in the high-LPS group. At 24 hours after infusion, plasma Fe concentrations were significantly lower in the low-LPS and high-LPS groups than those in the control group (Figure 2). Minimal and maximal concentrations of plasma Fe, respectively, were 0.72 mg/L (0 hours) and 0.88 mg/L (24 hours) in the control group, 0.48 mg/L (24 hours) and 0.88 mg/L (0.5 hours) in the low-LPS group, and 0.45 mg/L (24 hours) and 0.78 mg/L (0.5 hours) in the high-LPS group.

No differences in plasma Mg concentrations were observed among treatment groups throughout the experiment. Minimal and maximal concentrations of plasma Mg, respectively, were 23.98 mg/L (4 hours) and 29.40 mg/L (8 hours) in the control group, 21.08 mg/L (4 hours) and 28.47 mg/L (0.5 hours) in the low-LPS group, and 22.68 mg/L (4 hours) and 31.03 mg/L (0 hours) in the high-LPS group.

Effects of LPS infusion on cortisol concentrations—Serum cortisol concentrations in the control group remained stable at all sample collection times. (Figure 3). In the low-LPS group, serum cortisol concentrations significantly increased from 0.5 hours onward and reached a maximum (178.8 ng/mL) at 2
hours after infusion. At 4 hours after infusion, serum cortisol concentrations were still high (98.4 ng/mL) and returned to within baseline at 24 hours after infusion. Concentrations of serum cortisol were significantly higher in the low-LPS group than in the control group. Serum cortisol concentrations were significantly higher in the high-LPS group than in the low-LPS and high-LPS groups.

Effects of LPS infusion on TNF-α concentrations—Plasma TNF-α concentrations were significantly higher in the low-LPS and high-LPS groups than in the control group at 0.5 (P = 0.045), 1 (P = 0.006), 2 (P = 0.005), and 4 (P = 0.046) hours after infusion (Figure 4). At 6 and 8 hours after infusion, plasma TNF-α concentrations were significantly higher in the low-LPS group than in the control group. Peak plasma TNF-α concentrations were measured at 1 hour after infusion in the high-LPS group and at 2 hours after infusion in the low-LPS group. No differences in plasma TNF-α concentrations were observed between the low-LPS and high-LPS groups at all sample collection times.

Effects of LPS infusion on IL-6 concentrations—Plasma IL-6 concentrations were higher in the low-LPS and high-LPS groups than in the control group at 1 (P = 0.005), 2 (P = 0.008), and 4 (P = 0.007) hours after infusion (Figure 3). Peak plasma IL-6 concentrations were measured at 1 hour after infusion in the low-LPS and high-LPS groups. From 6 hours after infusion onward, plasma IL-6 concentrations in the low-LPS and high-LPS groups were similar to that of the control group.

Discussion

Lipopolysaccharide isolated from gram-negative bacteria is recognized by immune cells as a pathogen-associated molecular pattern and activates innate immune responses. Lipopolysaccharide is a potent inducer of inflammation. Inflammatory cytokines such as TNF-α and IL-6 are key regulators of innate immunity and are also directly responsible for the characteristic set of clinical signs that accompany infections. A biphasic fever is the usual reaction of the body to a moderate dose of LPS. However, a mono-, bi-, and triphasic febrile response to LPS doses of 10, 100, and 1,000 ng/kg, respectively, was observed in a study by Jacobsen et al. In comparison, relatively higher doses (10 and 50 µg/kg) of LPS were used in our study, resulting in a monophasic increase in rectal temperature in the low-LPS and high-LPS groups and peak rectal temperature at 2 (40.8°C; high-LPS group) and 4 hours (40.5°C; low-LPS group), which indicated that LPS doses of 10 and 50 µg/kg resulted in a monophasic febrile response in goats. A fever in response to LPS is presumably caused by the prostaglandin-mediated action of cytokines on the hypothalamus.

Zinc is a trace element that is essential for immune functions. Zinc is involved in the regulation of cellular functions and maintenance of immune functions, and its concentration in blood is low in endotoxemia. Zinc may normalize T-cell function suppressed by LPS, stimulate natural killer cells, and exert a mitogenic influence on lymphocytes. In endotoxemia, bacterial LPS affects monocytes and macrophages, which sequentially contribute to the production of proinflammatory cytokines such as TNF-α and IL-6. Plasma Zn
concentration is used as an assessment parameter of gram-negative infections\(^{30}\), Zn selectively enhances cytokine induction by LPS in a dose-dependent fashion.\(^{31}\)

In our study, LPS doses of 10 and 50 µg/kg resulted in decreased plasma Zn concentrations that were consistent with hypozincemia (ie, < 0.55 mg/L), whereas plasma Cu and Mg concentrations did not change. This suggests that redistribution of Zn within the body following LPS infusion occurred. Immune function is greatly attenuated in endotoxemia. In our study, each LPS dose resulted in an increase in the plasma cortisol concentration and a decrease in the plasma Zn concentrations. Gaetke et al\(^2\) postulated that LPS-stimulated hypozincemia might be mediated, at least partly, by a cytokine-directed internal redistribution of Zn. More recently, results of a study\(^4\) performed with mice indicate that the Zn transporter Zip14 in liver most likely plays a major role in the mechanism responsible for hypozincemia that accompanies the acute-phase response to inflammation and infection. High serum Zn concentrations restore impaired immune response.\(^13\)

At 24 hours after LPS infusion in our study, LPS doses of 10 and 50 µg/kg resulted in plasma Fe concentrations that were considered hypoferremia, which is consistent with findings in earlier studies.\(^3,4,33\) This suggests that redistribution of Fe within the body following LPS infusion occurred and that plasma Fe concentrations were lowered. In our study, no changes in plasma Cu and Mg concentrations were found following LPS infusion throughout the experimental period, implying that plasma Cu and Mg concentrations are relatively stable in goats following LPS infusion.

In our study, an increase in cortisol concentration was detected in goats following LPS infusion. This implies that administration of LPS increased the activity of the HPA axis. This finding is consistent with those of numerous studies in pigs\(^4,21,36,37\) and cattle\(^26,38\) following intramammary or IV administration of \textit{E. coli} or LPS.

Substantial evidence exists to indicate that the metabolic influences characterizing immunologic challenge are important for maintaining homeostasis during infection.\(^3\) These responses are attributed to cytokines released by activated macrophages. Cytokines (eg, TNF-α and IL-6) play an important role and have profound metabolic effects during various acute and chronic inflammatory processes.\(^40,41\) Tumor necrosis factor-α has been implicated as an important mediator of inflammatory processes and clinical manifestations in acute infectious diseases. In essence, the immune system uses cytokines such as TNF-α and IL-6 to convey information to other physiologic systems.\(^42\) For instance, TNF-α induces adipocytes to produce leptin, which acts centrally to reduce food intake and increase energy expenditure.\(^43\) As observed in our study, an increase in plasma TNF-α and IL-6 concentrations was found following LPS infusion and the kinetic data of plasma IL-6 concentrations were similar to that of plasma TNF-α concentrations. Intramammary or IV infusion of LPS to lactating cows induces many systemic responses, including an increase in the plasma cortisol concentration,\(^38\) and these responses result from an LPS-induced release of cytokines (eg, TNF-α) from peripheral blood monocytes and liver Kupffer cells.\(^39\) Tumor necrosis factor-α stimulates the HPA axis in rats, mainly through an effect on the hypothalamus.\(^53\)

Results of our study on goats indicate that immune activation via IV infusion of LPS decreases plasma concentrations of Zn and Fe and increases plasma TNF-α and IL-6 concentrations and serum cortisol concentrations in lactating goats. Following LPS infusion, the redistribution of Zn and Fe within the body occurred. However, plasma Cu and Mg concentrations were not influenced by immune activation. The cause of the hypozincemia and hypoferremia following immune activation is not fully understood and requires further study.

a. Fossomatic Model 90, Foss Food Technology, Hillerød, Denmark.

b. \textit{Escherichia coli} 026:B13, Sigma-Aldrich Co, St Louis, Mo.

c. Perkin-Elmer Corp, Norwalk, Conn.

d. ELISA kit specific for goat TNF-α, Bionewtrans Pharmaceutical Biotechnology Co Ltd, Franklin, Mass.

e. ELISA kit specific for goat IL-6, Bionewtrans Pharmaceutical Biotechnology Co Ltd, Franklin, Mass.

f. 1:21,11 radioimmunoassay kit validated for use on goat serum cortisol, ICN Biomedicals ICN Biomedicals, Costa Mesa, Calif.

g. GLM procedure of SAS, SAS Institute Inc, Cary, NC.

References

16. Carroll EJ, Schalm OW, Lasmanis J. Experimental coliform infection and endotoxin in dairy α α 32 monocytes and liver Kupffer cells. α

AJVR, Vol 68, No. 5, May 2007 533

Unauthenticated | Downloaded 11/27/23 03:09 AM UTC


