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NOTE

Physiology

Effect of intramammary lipopolysaccharide infusion on milk pH of uninfused udder in goat

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ABSTRACT. The change in milk composition in response to intramammary infusion of *Escherichia coli* lipopolysaccharide (LPS) was investigated. Four clinically healthy goats were infused with LPS (100 μg) by intramammary administration to the left udder. Clinical manifestations (rectal temperature and physical activity), selected blood parameters (pH and white blood cell count) and milk compositions (somatic cell count and pH) were evaluated at 0 hr (just before challenge) and at multiple time points over the first 24 hr post-challenge. After intramammary LPS challenge, the pH of milk from both udders increased. Thus, this study revealed that LPS-induced mastitis in goat can result in increased pH in milk from the unchallenged (contralateral) udder.

KEY WORDS: goat, lipopolysaccharide, mastitis, pH

Mastitis is a common disease in the worldwide dairy industry; the disease has large economic impacts [7]. Acute coliform mastitis (ACM), caused primarily by *Escherichia coli*, is associated with problematically high culling rates because the disease results in serious systemic symptoms. Given the rapid progression of the disease, it is important to evaluate the condition of the acute phase for appropriate diagnosis and treatment. Serious systemic symptoms are caused by inflammatory mediators following excessive response to lipopolysaccharide (LPS), a bacterial cell wall component [2, 4]. Although several studies have evaluated changes in milk components of infused udder after intramammary LPS challenge [1, 9], there have been (to the authors' knowledge) no reports of changes in the components of milk obtained from unchallenged (contralateral) udders. Dose-dependent effects of LPS on the immune response in milk cells and mammary tissue have been reported after intramammary administration of LPS [12]. In recent work, we reported the acute-phase response of mastitis induced by LPS intramammary infusion [8]. However, the LPS amount was too small (10 μg) to affect the milk and blood components significantly. Therefore, the present study was designed to determine the effects of high-dose LPS (100 μg) on the pH response in the unchallenged udder of the dairy goat.

This study was approved by the ethics committee of Hiroshima University (No. C14-5). The four goats used for the additional studies were clinically healthy animals of the Tokara breed with the following properties: 25.0 to 30.2 kg each; 133 to 144 days in milk; parity 2 to 3. Notably, the udders of these goats were confirmed to be free from mastitis pathogens. LPS (*Escherichia coli* O111; Wako Pure Chemical, Osaka, Japan) was infused into mammary gland as described previously [13, 14]. LPS was formulated by suspending LPS powder to 2% (100 μg in 5 m*l*) in sterile isotonic saline (0.9% NaCl). The resulting suspension was infused into the left mammary gland of each animal at 5 m*l*/goat; these animals were designated as the high-dose LPS (LPS-H) group. The dose of LPS was selected to be 10 times that used in the previous study [8]; this higher LPS dose was expected to result in severe mastitis. The time of infusion was designated 0 hr. Systemic and local signs and blood collection were performed immediately prior to infusion ("0 hr") and at 1, 2, 3, 4, 6, 8, 12, and 24 hr post-challenge. Rectal temperature, heart rate, respiratory rate, rumen contraction rate, and general attitude were evaluated as systemic signs. Blood samples were collected from the jugular vein into disposable syringes (whole blood, no anticoagulant) and thence into anticoagulant (EDTA) -containing tubes (Venoject®, Terumo Corp., Tokyo, Japan). The pH of the whole blood (no anticoagulant) was measured immediately upon collection using a handheld i-STAT®1 analyzer and CG8+ cartridges (Abbot Laboratories, Princeton, NJ, U.S.A.). White blood cell counts (WBCs) were determined for the EDTA blood samples using a Partice Counter (PCE-170, ERMA Inc., Tokyo, Japan). Separate milk samples were collected aseptically from each mammary gland immediately prior to infusion ("0 hr") and at 4, 8, 12, and 24 hr post-challenge. Each milk sample was collected into a sterilized tube and stored at 4°C pending determination of somatic cell counts

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(SCCs) using a DeLaval cell counter (DCC: DeLaval International AB, Tumba, Sweden) according to the method of Kawai *et al.* [6]. Milk pHs were measured using a Digital pH meter (D-71, Horiba Scientific, Kyoto, Japan) at 37°C. The data in the previous study [8] were used for the control (saline 5 m*l*, n=5) and low-dose (10 μg/goat) LPS (LPS-L, n=5) groups. SCCs were converted to common logarithm values. A two-tailed paired *t*-test was used to compare each measured value in the respective animal and udder before and after LPS challenge. Significance was considered at $P<0.05$. All statistical analyses were performed with EZR (ver. 1.27; Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R foundation for Statistical Computing, Vienna, Austria). This is a modified version of R commander designed to add statistical functions frequently used in biostatistics [5].

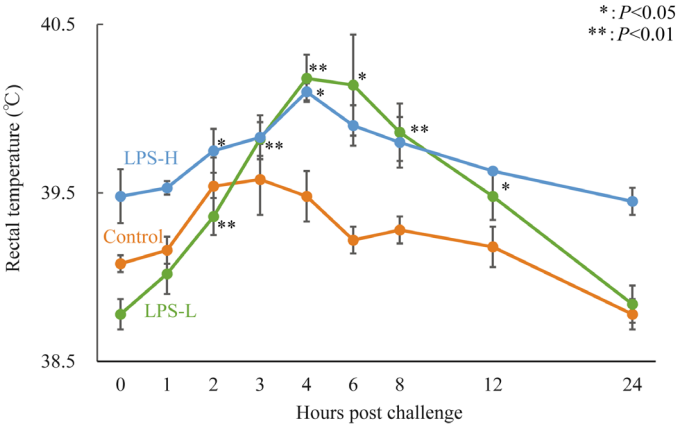

Fig. 1. Rectal temperature after intramammary challenge with saline (red: n=5), LPS-L (green: n=5), and LPS-H (blue: n=4). Values are means ± SEMs. Asterisks indicate significant differences compared with 0 hr. Statistical significance was determined using a two-tailed paired *t*-test. Data for the Control and LPS-L groups are taken from Shinozuka *et al.* (2018).

In both the LPS-L and LPS-H groups, all goats showed up to 8 hr post-dose loss of appetite and signs of fever post-challenge, but the animals subsequently recovered, such that general symptoms were no longer seen by 24 hr post-challenge. Rectal temperature in both LPS groups initially increased significantly at 2 hr post-challenge compared to baseline that at 0 hr, peaked at 4 hr post-challenge, and returned to normal by 24 hr post-challenge, when physical activity and appetite also recovered (Fig. 1). The respiratory rate, pulse rate, and ruminal motility did not differ significantly among the three groups (data not shown). Although blood pH showed no significant changes (compared to baseline) in the three groups (Fig. 2a), the WBCs in LPS groups decreased significantly from 2 hr post-challenge (compared to the respective baseline values); while values started to rise by 12 hr, values at 12 and 24 hr were still significantly lower than those at 0 hr in respective animals (Fig. 2b). In milk testing, SCCs of milk from LPS-challenged udders (in both the LPS-H and LPS-L groups) were significantly increased (compared to baseline) at 4 through 12 hr post-challenge and unrecovered by 24 hr (Fig. 3a). Milk from unchallenged udders showed no changes (compared to baseline) in SCC in the LPS-challenged groups (Fig. 3b). In the LPS-L group, the pH of milk from the challenged udders was significantly increased (compared to baseline) from 4 hr post-challenge (Fig. 3c). In the LPS-H group, the pH of milk from the challenged udder could not be measured due to the marked decrease of milk production in these udders. In contrast, pH of milk from the unchallenged udders of LPS-L animals was not significantly changed (compared to baseline) within 24 hr of the challenge, while the pH of milk from the unchallenged udders of LPS-H animals was significantly elevated (compared to baseline) at 4 to 12 hr post-challenge (Fig. 3d).

In summary, although both the LPS-L and LPS-H groups exhibited mastitis with systemic signs, animals of these two groups did not differ significantly (compared to each other) in clinical signs. This observation suggested that LPS-L intramammary infusion was sufficient to induce systemic inflammatory responses, such that the strength of LPS stress could not be estimated by goat-side clinical findings alone. In milk testing, the SCCs exhibited an apparently LPS-dose-dependent increase; milk pH was also elevated in the LPS-L group, but a block in milk production in the LPS-H animals precluded pH assessment in the latter group. Increased pH in mastitic milk has been attributed to permeability of the blood capillaries due to inflammation of the mammary gland, permitting entry into the milk of alkaline blood constituents (Na^+ and bicarbonate). In the unchallenged udders, although neither inflammatory local clinical signs nor increased milk SCCs were observed, the pH of the corresponding milk from the LPS-H animals was significantly elevated. Since LPS infusion had no effect on blood pH, it was hypothesized that the high-dose LPS induced a stronger systemic inflammatory response than the low-dose LPS, yielding increased vascular permeability even in the unchallenged (contralateral) udder.

Evaluation of the severity of episodes of ACM in dairy cows is important for determining the appropriate treatment; several studies have proposed severity classification systems for ACM [10, 11] based on clinical symptoms. The modified California mastitis test (CMT) is a system that combines the CMT (an indirect method for measurement of SCCs using formation of a "gel-like" matrix [3]) with a bromothymol blue-based assay of milk pH. In Japan, the modified CMT is commonly used for the detection of mastitis; this system has the advantage of being quick, cheap, simple to perform, and amenable for use as a "cow-side" test, facilitating veterinary use of this system to determine the milk pH of all quarters. Our results showed that changes in the pH of milk from the unchallenged udder may be useful information for evaluating the severity of LPS-induced mastitis in goats. This finding could be applied as a diagnostic index for severity evaluation of acute coliform mastitis in dairy cows, and is a new interpretation of the modified CMT.

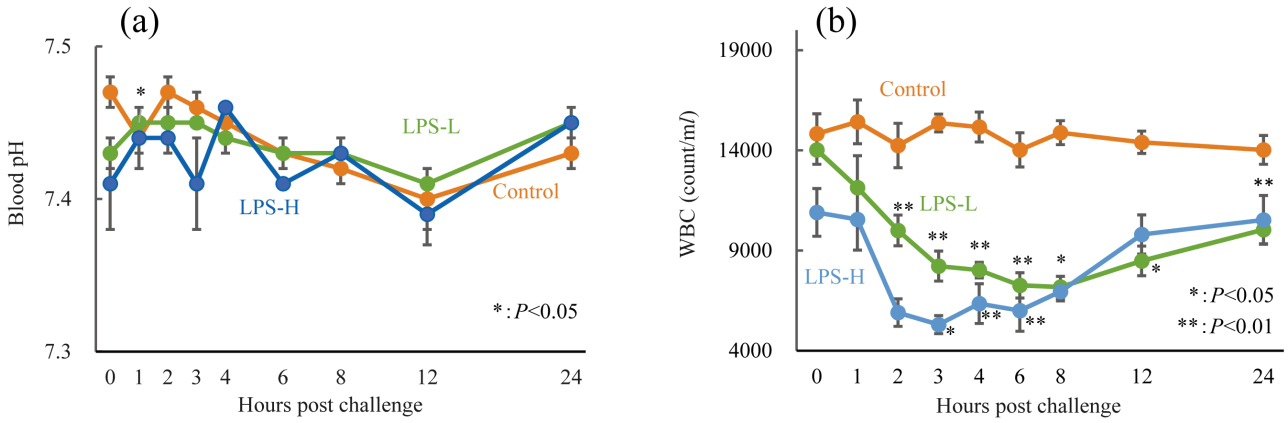

Fig. 2. Blood pH (a) and white blood cells counts (b) after intramammary challenge with saline (red: n=5), LPS-L (green: n=5), and LPS-H (blue: n=4). Values are means ± SEMs. Asterisks indicate significant differences compared with 0 hr. Statistical significance was determined using a two-tailed paired *t*-test. Data for the Control and LPS-L groups are taken from Shinozuka *et al.* (2018).

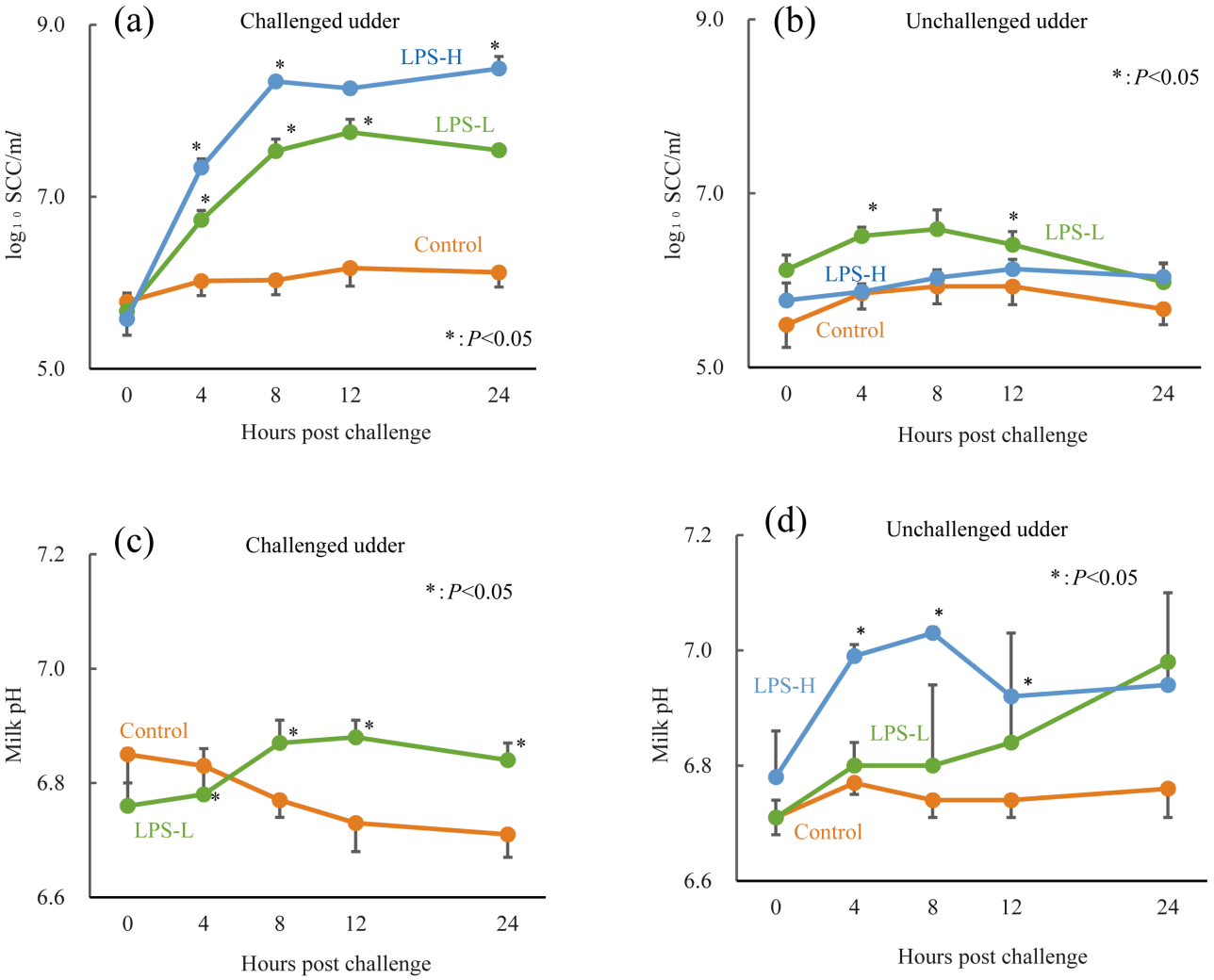

Fig. 3. Milk pH of LPS-challenged udder (a) and unchallenged udder (b). Somatic cell counts of LPS-challenged udder (c) and unchallenged udder (d) in milk after intramammary challenge with saline (red: n=5), LPS-L (green: n=5), and LPS-H (blue: n=4). Values are means ± SEMs. Asterisks indicate significant differences compared with 0 hr. Statistical significance was determined using a two-tailed paired *t*-test. Data for the Control and LPS-L groups are taken from Shinozuka *et al.* (2018).

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