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Effect of temporary cessation of milking on the innate immune components in goat milk

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ABSTRACT

Temporary cessation of milking is widely used during the dry period of dairy cows. Temporary cessation of milking induces an increase in the somatic cell count (SCC) and level of several inflammatory components of milk, which is believed to be a local adaptation and defense mechanism of the mammary gland. In Japan, temporary cessation of milking combined with antibiotic administration is widely used to treat mastitis. The present study aimed to elucidate the role of the innate immune system during temporary cessation of milking in a goat model by investigating the concentration of several innate immune components in milk during and around the temporary cessation. In experiment 1, 6 goats were subjected to cessation of milking for 3 d in both udder halves, whereas in experiment 2, 6 other goats were subjected to cessation of milking for 3 d only in 1 udder half. In experiment 1, the milk yield was lower on d 5 and 6, whereas the mean SCC was higher on d 5 compared with d 0 before temporary milking cessation. The concentrations of goat DEFB1, S100A7, cathelicidin-2 and 7 (CATHL-2 and 7), IgA, and lactoferrin were increased after temporary cessation of milking. In experiment 2, the milk yield was lower between d 5 and 7, whereas the mean SCC was higher between d 4 and 7 compared with d 0. The concentrations of CATHL-2, IgA, and lactoferrin were increased after temporary cessation of milking only in the udder half subjected to milking cessation. These results suggest that temporary cessation of milking increase the SCC and concentration of several innate immune components in milk without infection, which may contribute to mastitis treatment.

Key words: milk cessation, innate immunity, mastitis

INTRODUCTION

Mastitis is defined as the inflammation of the mammary gland, usually referred to as an intramammary inflammatory reaction, caused by an infectious agent, primarily bacteria (Zhao and Lacasse, 2008; Zadoks et al., 2011). The annual incidence of clinical mastitis in dairy cows and small ruminants is generally lower than 5%; however, the prevalence of subclinical mastitis has been estimated at 5 to 30% or above (Bergonier and Berthelot, 2003; Contreras et al., 2003).

Due to the predominance of bacterial causes in mastitis, the use of antimicrobial agents in the treatment and control continues to be an important subject of investigation. Antibiotic therapy is the primary strategy for mastitis treatment at present. However, the extensive use of antibiotics in mastitis treatment is not always effective as the bacteria develop resistance and consequent nonresponsiveness to antibiotic therapy (Erskine, et al., 2003). Moreover, the overuse and misuse of antibiotics on dairy farms may represent a severe problem, not only with respect to bacterial resistance, but also regarding the potency of resistant bacteria entering the food chain and the increase of antibiotic residues in milk (White and McDermott, 2001).

Several methods have been proposed to increase antibiotic treatment efficacy, such as the co-administration of different supportive medicines. The administration of antioxidants (vitamins C and E, and β-carotene), lysozyme dimer, or nonsteroidal anti-inflammatory drugs has been reported in improving antibiotic efficiency in clinical mastitis in dairy cows (Smulski et al., 2020). Furthermore, several nonantibiotic methods following antibiotic treatment have been suggested to treat clinical mastitis, such as frequent milking-out (**FMO**), a popular recommendation for clinical mastitis that involves milking the affected quarters out several times a day. Theoretically, the FMO of affected quarters helps remove the infectious agents and toxic products of the infection. A few studies regarding FMO report that a combination of intramammary antibiotic therapy and

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FMO may benefit clinical mastitis treatment (Morin, et al., 1998; Roberson, et al., 2004).

Conversely, in Japan, temporary cessation of milking for 3 d following intramammary infusion of antibiotics is a new treatment method for mastitis in dairy cows. The temporary cessation of milking method was established nationwide, particularly to treat mastitis caused by streptococci and coagulase-negative *Staphylococcus* (Kondo et al., 2013). In dairy cows with streptococcal mastitis, the treatment with antibiotic administration accompanied by cessation of milking for 3 consecutive days resulted in a higher cure rate (69.2%) than that of the control group (40%), which was only treated by antibiotic administration (Kondo et al., 2013). Moreover, the combination of Cephem antibiotic treatment and cessation of milking has successfully cured mastitis caused by *Streptococcus uberis* in 21 of 22 dairy cows (Kondo et al., 2013). These results suggest that cessation of milking combined with antibiotic administration could prolong the effect of the antibiotics relative to infusion of antibiotics without cessation of milking. However, the physiological role of cessation of milking in the mammary gland is unknown.

In the mammary gland, the innate immune system is the first line of defense against pathogens once they have penetrated the physical barrier of the teat canal (Rainard and Riollet, 2006). A wide variety of components related to the innate immune responses have been identified in milk, including antimicrobial peptides (Ezzat Alnakip et al., 2014). Antimicrobial peptides are synthesized in the alveolar epithelial cells and leukocytes and are secreted into milk (Isobe, 2017). Therefore, the present study aimed to elucidate the role of innate immune function in the mammary gland during treatment with temporary cessation of milking. Generally, as both udder halves do not suffer from mastitis at the same time, only the half with mastitis is treated with the temporary cessation of milking method. However, it is not clear whether one or both udder halves should be subjected to cessation of milking. Therefore, the innate immune function was analyzed by applying the cessation of milking method in only one udder half and in both udder halves, and the results were compared.

MATERIALS AND METHODS

Animals

Twelve Tokara goats (BW: 20–25 kg, parity: 1–4, mid-lactation stage, milk yield: 300–800 mL/d) were used. Goats were fed 0.6 kg of hay cubes and 0.2 kg of barley per day with free access to water and trace mineralized salt block. Feed was provided twice per day at 0800 and 1500 h.

Two experiments were conducted. In experiment 1, 6 goats were used where milking of both udder halves was stopped temporarily for 3 d, followed by the resumption of milking once per day. In experiment 2, 6 goats were used where milking of one udder half was stopped temporarily for 3 d, followed by the resumption of milking once per day, whereas the other half of the udder was milked normally once per day during the experimental period. The day of onset of cessation of milking was considered as d 0. All experiments were approved by the Hiroshima University Animal Research Committee and were conducted in accordance with its guidelines (C19–4).

Collection of Milk Samples

In experiments 1 and 2, milk samples were collected from 3 d before the temporary cessation of milking until 7 d after the resumption of milking by hand-milking. During 3 d of cessation of the milking period, 4 mL of milk was collected gently without milking for SCC measurement and enzyme-immunoassay analyses. The milk was then centrifuged at 1,900 × *g* for 5 min at 4°C. The milk fat and skim milk were removed, leaving only the somatic cells (Nishikawa et al., 2018). Skim milk was stored at −20°C for enzyme immunoassay for goat DEFB1 (β-defensin 1), S100A7, CATHL-2 (cathelicidin-2), CATHL-7 (cathelicidin-7), IgA, and lactoferrin (**LF**), and cell pellets were resuspended in PBS for performing the SCC. The SCC measurement was performed using a Countess Automated Cell Counter (Life Technologies Japan Co. Ltd.). Sodium ion concentration was measured using LAQUAtwin (HORIBA).

Enzyme Immunoassay

Enzyme immunoassay was conducted to measure the concentrations of DEFB1, S100A7, CATHL-2, CATHL-7, IgA, and LF in goat milk. The antibodies against DEFB1 (TQGIRSRRSC), S100A7 (CFEKQDKNKDRKID), CATHL-2 (CFRPPVRPPFRPPFRPPF), and CATHL-7 (CRDSLRRGGQ) were generated in rabbits as previously described (Kuwahara et al., 2017; Nishikawa et al., 2018; Isobe et al., 2020). Then, the antibodies were purified from the serum using a HiTrap Protein G High-Performance affinity column (GE Healthcare, Princeton), according to the manufacturer's protocol. These antibodies were used for enzyme immunoassay. We determined DEFB1, S100A7, CATHL-2, and CATHL-7 concentrations in milk as previously described (Zhang et al., 2014a,b; Nishikawa et al., 2018). Briefly, a 96-well microtiter plate was coated with affinity-purified rabbit antibodies against the peptides (1 μg/mL). Milk samples for DEFB1, S100A7,

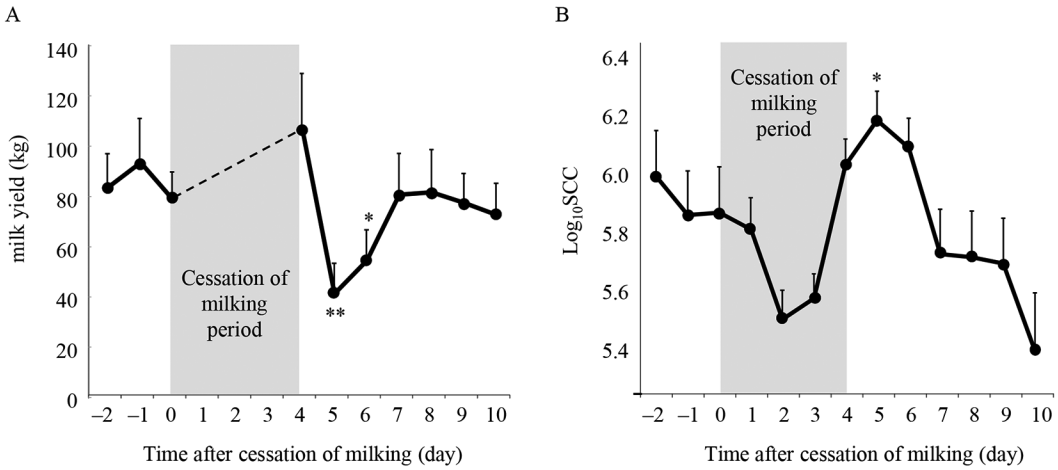

Figure 1. Changes in milk yield (A) and SCC (B) after cessation of milking for 3 d in both udder halves (experiment 1, n = 6). Results are represented as mean ± SEM. *$P < 0.05$; **$P < 0.01$ mean significant difference compared with d 0.

CATHL-2, and CATHL-7 analyses were diluted 1:50, 1:5,000, 1:20, and 1:20, respectively, and then added to the plate. Goat IgA concentrations in milk were measured using rabbit anti-goat IgA antibody (A50–106A, Bethyl). Skim milk was diluted 1:1,000 before measurement. The antibodies for LF were purchased from Life Laboratory. Milk LF concentration was measured as described in Kuwahara et al. (2017). Milk samples for LF measurement were diluted 1:5,000. The optical density was measured using a microplate reader, Thermo Scientific Multiskan FC Microplate type 357 (Thermo Fisher Scientific Co. Ltd.). Intra- and interassay coefficients of variation were 6.8% and 17.7% for DEFB1, 7.5% and 19.6% for S100A7, 11.3% and 17.9% for CATHL-2, 3.7% and 20.0% for CATHL-7, 3.5% and 11.2% for IgA, and 6.5% and 9.3% for LF.

Statistical Analyses

Data were analyzed using JMP Pro14 (SAS Institute Inc.). The Kolmogorov–Smirnov test was used to check the normal distribution of the data. A nonparametric Kruskal Wallis test followed by Steel–Dwass test was used to compare the data among different days. One-way ANOVA, followed by Tukey multiplex analysis, was used to compare SCC among different days. Results are reported as least squares means and standard error of the mean. Differences were considered significant at $P < 0.05$.

RESULTS

In experiment 1, milking was stopped in both udder halves. The milk yield was significantly lower on d 5 and 6 than on d 0, whereas the mean SCC was significantly higher on d 5 than on d 0 ($P < 0.05$; Figure 1A and B). The concentration of DEFB1 was significantly higher on d 5, and that of S100A7 was significantly higher on d 4 than on d 0, whereas the mean concentration of CATHL-2 was significantly higher on d 4 to 6, and that of CATHL-7 on d 5 to 7 than on d 0 ($P < 0.05$; Figure 2A–D). Furthermore, the mean concentration of IgA was significantly higher on d 5, and that of LF on d 5 to 7 than on d 0 ($P < 0.05$; Figure 3A and B).

In experiment 2, milking was stopped only in one udder half. All parameters (treatment, time, and treatment × time) significantly affected the milk yield, SCC, and the concentrations of sodium ion (Na^+), CATHL-2, IgA, and LF in milk ($P < 0.05$ for all parameters; except for treatment × time of IgA, $P = 0.075$). The milk yield significantly decreased on d 5 followed by a gradual increase. There were significant differences on d 5 to 7 between methods with and without the cessation ($P < 0.05$; Figure 4A). Conversely, the mean SCC, Na, CATHL-2, IgA, and LF concentrations were significantly higher in the udder halves with cessation than those without cessation on d 4 to 8 ($P < 0.05$; Figure 4B-C, 5A-C).

DISCUSSION

This study hypothesized that the innate immune system plays an important role in the mammary gland during treatment with temporary cessation of milking. In experiments 1 and 2, milk yield was significantly lower in the udder halves with the cessation of milking treatment on d 5 to 6 and 4 to 7, respectively, than on d 0. A significant decrease in milk yield was observed in many studies associated with cessation of milking in both abrupt and gradual cessation methods. The

present study demonstrated that milk yield was temporarily reduced after cessation of milking for 3 d and then immediately returned to normal. This result is in agreement with previous studies that demonstrated that milking once per day in dairy cows applied over several days and a single prolonged milking interval (24 h) led to impaired mammary tight junction integrity, subsequently decreasing the milk yield (Stelwagen et al., 1994, 1997; Lakic et al., 2009, 2011).

In goats, tight junctions between mammary epithelial cells act as pressure-induced inhibitors of milk secretions. The loss of tight junction integrity after 18 h of milk accumulation subsequently reduced milk secretion (Knight et al., 1988). In the present study, Na^+ concentration in milk was higher on d 4 to 6 compared with d 0. The characteristics of leaky tight junction in the mammary gland are increased Na^+ concentrations in milk (Stelwagen et al., 1994, 1999). Therefore, the impairment of mammary gland tight junctions during the cessation of milking for 3 d could be one of the causes of decreased milk yield. However, the decrease in milk yield can be affected by other factors, such as changes in cell number and activity, feedback inhibition, apoptosis, and cisternal and alveolar milk storage (Stelwagen, 2001).

To elucidate our hypothesis that the innate immune system in the mammary gland has an important role during treatment with temporary cessation of milking, we evaluated the concentration of several innate immune components in goat milk, such as that of DEFB1, S100A7, CATHL-2, CATHL-7, IgA, and LF during and around the treatment with cessation of milking method. The concentrations of all these components were increased in the milk after temporary cessation of milking (DEFB1, S100A7, CATHL-7, and IgA, 2 times; CATHL-2, 2.5–4 times; LF, 10 times). These components play an important role in killing bacteria such as *Escherichia coli* (β-defensin and S100A7; Isobe et al., 2009; Regenhard et al., 2010), *Strep. uberis* (LF; Swanson et al., 2009), and *Staph. aureus* (β-defensin and LF; Chaneton et al., 2008; Kuwahara et al., 2017). Therefore, the temporary cessation of milking contrib-

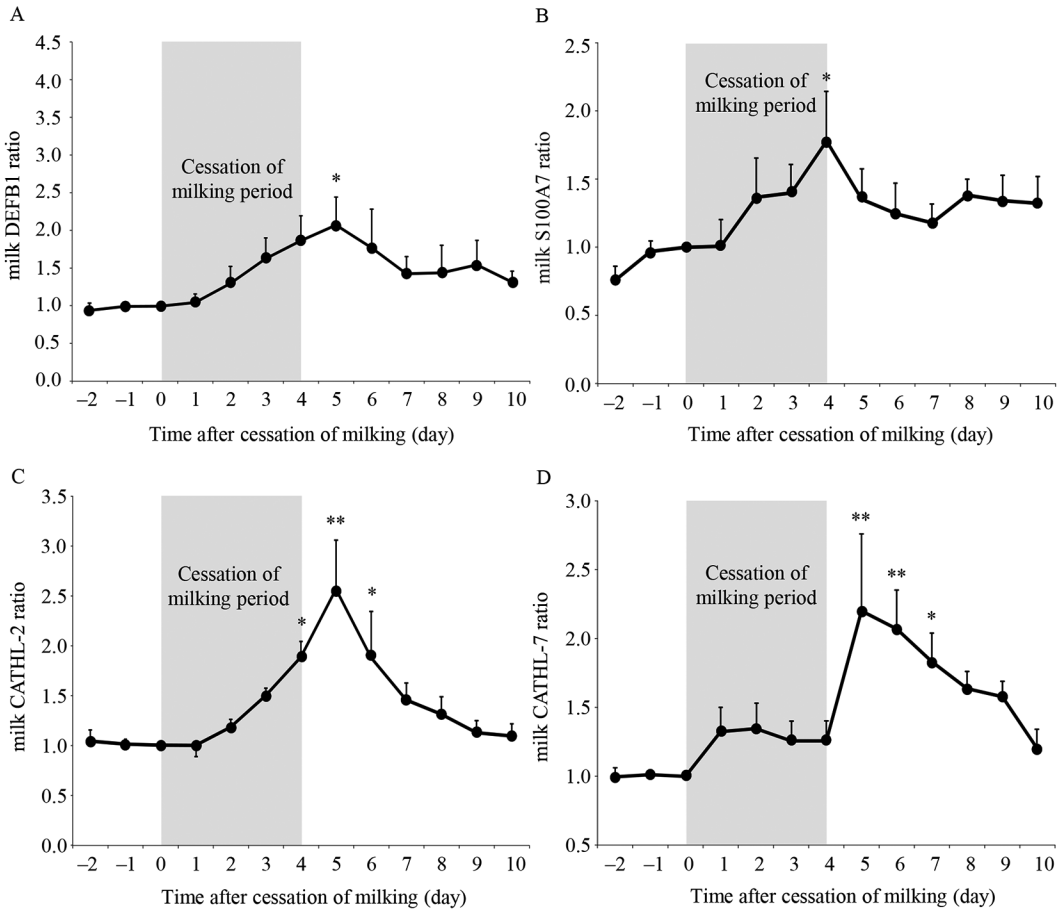

Figure 2. Changes in concentration of goat β defensin-1 (DEFB1; A), S100A7 (B), cathelicidin-2 (CATHL-2; C), and cathelicidin-7 (CATHL-7; D) in milk after cessation of milking for 3 d in both udder halves (experiment 1, n = 6). The vertical axis displays the ratio where d 0 (cessation of milking) is set as 1. Results are represented as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$ mean significant difference compared with d 0.

utes to bacterial removal by increasing the concentration of innate immune components. The antimicrobial component with the highest increase was LF and could have a special role for mammary defense systems. It is reported that the concentration of innate immune components such as that of DEFB1 (Kuwahara et al., 2017), S100A7 (Zhang et al., 2014a), CATHL-2 (Zhang et al., 2014b), CATHL-7 (Nishikawa et al., 2018), and LF (Huang et al., 2012) were increased after LPS infusion into the mammary gland. This response suggests the crucial role of the defense system in the mammary gland (Isobe, 2017). Because there are other antimicrobial components related to the defense system of the mammary gland, further studies are required.

Conversely, milk yield was reduced just after the temporary cessation of milking in both experiments 1 and 2. Therefore, one of the reasons for increased innate immune components is attributable to the reduced milk yield. This is consistent with a previously reported finding (Purba et al., 2020). The Na^+ concentration was increased after temporal cessation of milking in experiment 2, suggesting a downregulated tight junction. However, mammary epithelial cells synthesize and secrete DEFB1, S100A7, cathelicidins, and LF (Hurley and Rejman, 1993; Isobe et al., 2009; Zhang et al., 2014a; Cubeddu et al., 2017), which indicates that mammary epithelial cells can maintain the ability to secrete innate immune components even when tight junction is downregulated. Also, CATHL-2, CATHL-7, and LF are produced by leukocytes alongside mammary epithelial cells. Because SCC was increased by temporal cessation, the increase in the concentration of these innate immune components must be at least partially due to the increase in SCC.

In this study, temporary cessation of milking was similar to the involution process after the dry period of dairy cows. During involution, LF concentration increased (Welty et al., 1976). The LF disrupts the outer membrane of gram-negative bacteria and binds to iron, making the iron unavailable for iron-dependent bacteria (Ellison et al., 1988). Therefore, LF has an important role in inhibiting the growth of bacteria during the dry period in cows. Concentrations of other innate immune components were also increased after temporary cessation of milking, suggesting the important role of these components, similar to that reported for LF during the dry period. However, it is not clear if the functions of these components are the same as that of LF.

Immunoglobulin A plays a dominant role in mammary gland defense against bacterial pathogens (Ezzat Alnakip et al., 2014). The IgA is thought to be involved in toxin neutralization and bacterial agglutination, thereby hindering bacterial spread and colonization (Korhonen et al., 2000). Natural IgA is also involved in innate immunity (de Klerk et al., 2015). This type of IgA can bind to pathogens nonspecifically. In this study, IgA concentration was increased after temporal cessation of milking, suggesting that IgA also played a role in the innate immune function together with other components.

In experiment 2, the increase in concentration of several innate immune components in milk occurred in the udder halves subjected to cessation of milking, whereas these changes were not seen in the udder halves without temporary cessation of milking. Thus, milking stimulation in one udder half does not prevent the increase in the level of innate immune components in the other udder half with temporary cessation of milking. Milking stimulates prolactin secretion in the pituitary, then prolactin exerts milk production in the mammary epithelial cells. However, the milk yield was decreased in the udder halves with cessation of milking.

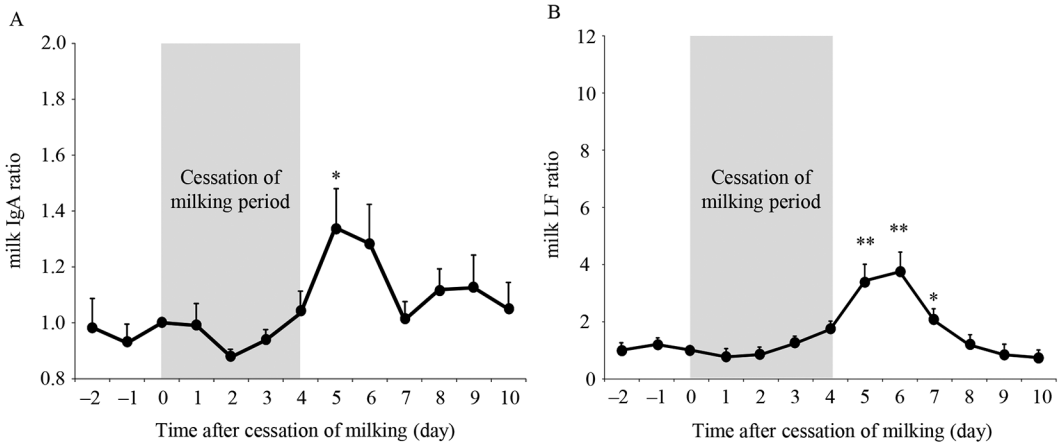

Figure 3. Changes in concentration of IgA (A) and lactoferrin (LF; B) in milk after cessation of milking for 3 d in both udder halves (experiment 1, n = 6). The vertical axis displays the ratio where d 0 (cessation of milking) is set as 1. Results are represented as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$ mean significant difference compared with d 0.

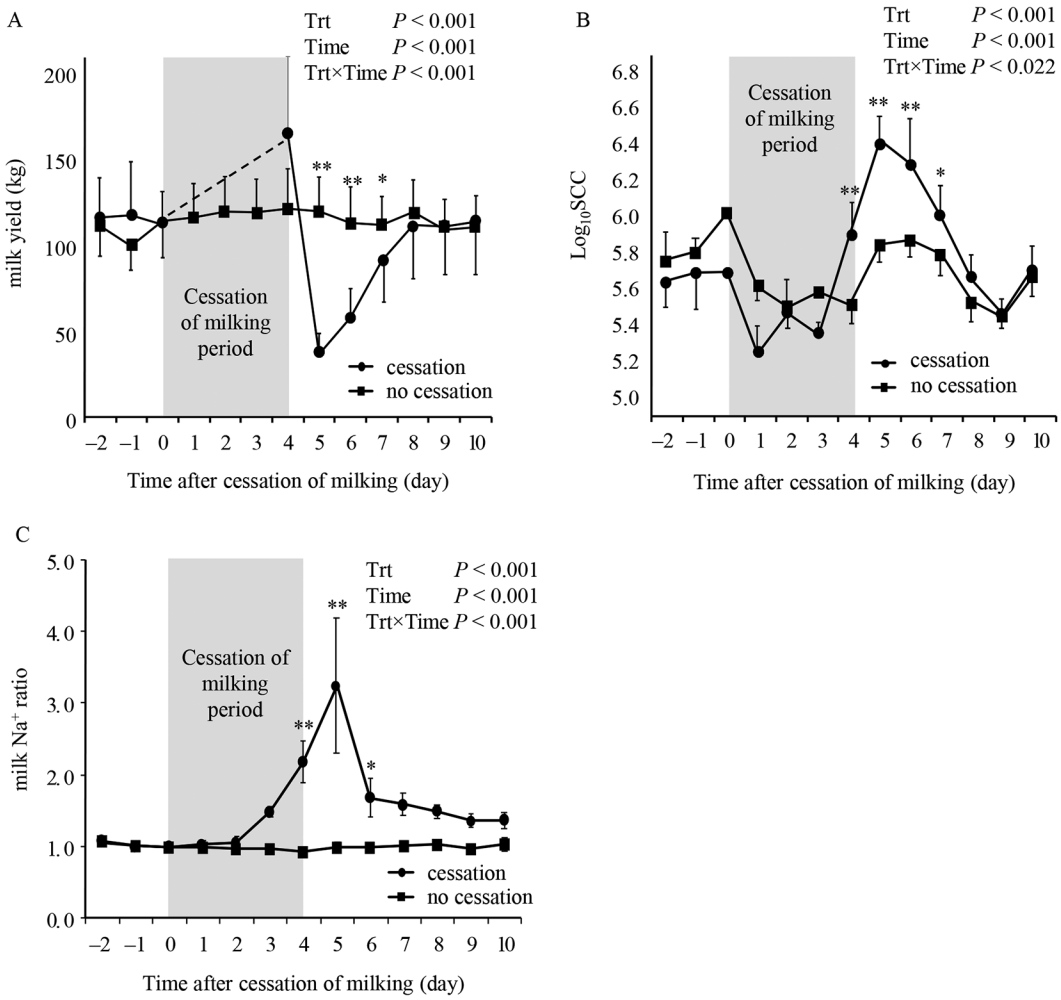

Figure 4. Changes in milk yield (A), SCC (B), and Na^+ concentration (C) in milk after cessation of milking for 3 d only in one udder half (experiment 2, n = 6). The vertical axis of (C) displays the ratio where d 0 (cessation of milking) is set as 1. Results are represented as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$ mean significant difference between cessation and no cessation group on the same day. Trt = treatment; Trt × Time = interaction of treatment and time.

Therefore, mammary epithelial cells in the udder halves with cessation of milking did not respond to prolactin. In this sense, it has been reported that decreased milking frequency reduced both milk yield and prolactin receptor expression (Bernier-Dodier et al., 2010; Toledo et al., 2020). Hence, it is possible that milk removal or cessation of milking affects the responsiveness of the mammary gland to prolactin.

Many studies have reported that the increase in concentration of inflammatory components in milk after cessation of milking is associated with mammary gland defense against subsequent intramammary infection (Sordillo et al., 1997; Sordillo and Streicher, 2002; Rajala-Schultz et al., 2005; Newman et al., 2009). However, a previous study also demonstrated that cessation of milking in dairy cows, particularly in those with high milk production (25–35 L/d), causes distress and ineffective antibacterial immune response (Silanikove et al., 2013). Therefore, the degree of stress during temporary cessation of milking should be considered.

In conclusion, our results indicated that cessation of milking for 3 d induced an increase in the concentration of several innate immune component present in milk, which might be involved in preventing and treating mastitis. This finding has important immunological implications toward controlling the problem of mastitis in the dairy industry.

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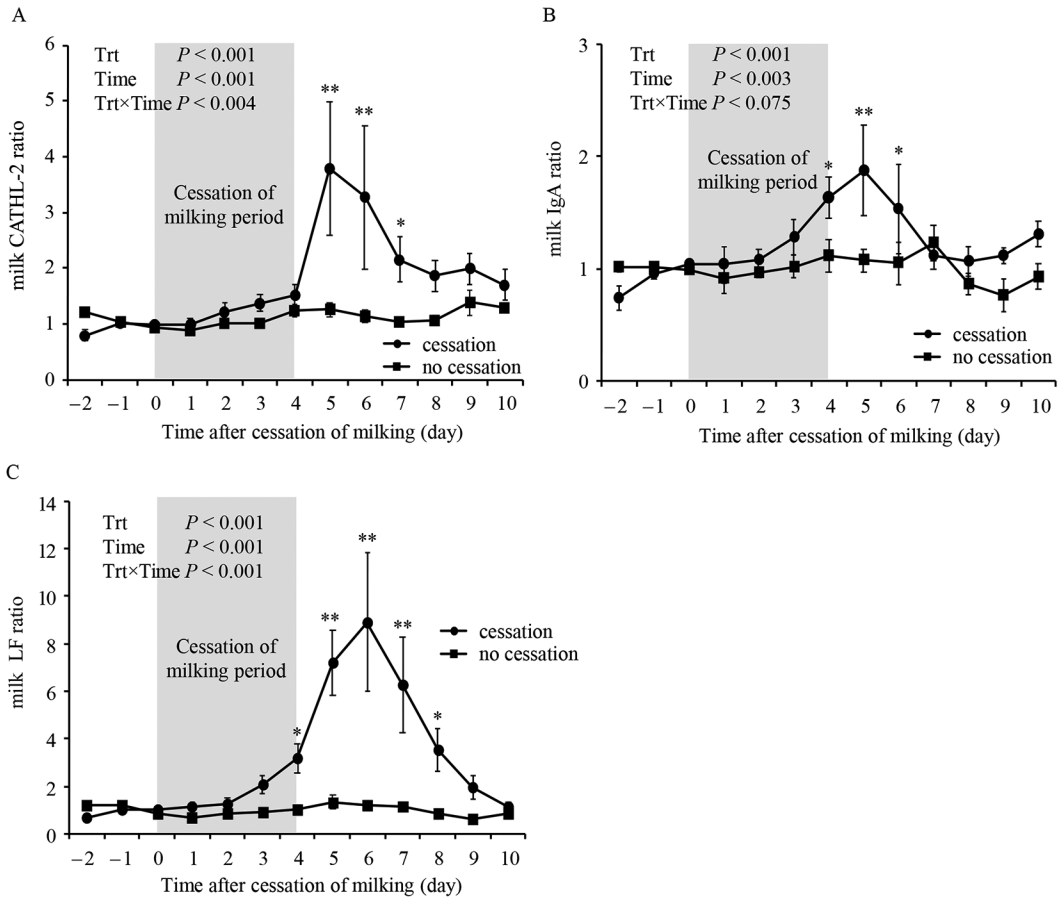

Figure 5. Changes in concentration of cathelicidin-2 (CATHL-2; A), IgA (B), and lactoferrin (LF; C) in milk after cessation of milking for 3 d only in one udder half (experiment 2, n = 6). The vertical axis displays the ratio where d 0 (cessation of milking) is set as 1. Results are represented as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$ mean significant difference between cessation and no cessation group on the same day. Trt = treatment; Trt × Time = interaction of treatment and time.

Naoki Isobe. The authors have not stated any conflicts of interest.

REFERENCES

Bergonier, D., and X. Berthelot. 2003. New advances in epizootiology and control of ewe mastitis. Livest. Prod. Sci. 79:1–16. https://doi.org/10.1016/S0301-6226(02)00145-8.

Bernier-Dodier, P., L. Delbecchi, G. F. Wagner, B. G. Talbot, and P. Lacasse. 2010. Effect of milking frequency on lactation persistency and mammary gland remodeling in mid-lactation cows. J. Dairy Sci. 93:555–564. https://doi.org/10.3168/jds.2009-2320.

Chaneton, L., L. Tirante, J. Maito, J. Chaves, and L. E. Bussmann. 2008. Relationship between milk lactoferrin and etiological agent in the mastitic bovine mammary gland. J. Dairy Sci. 91:1865–1873. https://doi.org/10.3168/jds.2007-0732.

Contreras, A., C. Luengo, A. Sánchez, and J. C. Corrales. 2003. The role of intramammary pathogens in dairy goats. Livest. Prod. Sci. 79:273–283. https://doi.org/10.1016/S0301-6226(02)00172-0.

Cubeddu, T., C. Cacciotto, S. Pisanu, V. Tedde, A. Alberti, M. Pittau, S. Dore, A. Cannas, S. Uzzau, S. Rocca, and M. F. Addis. 2017. Cathelicidin production and release by mammary epithelial cells during infectious mastitis. Vet. Immunol. Immunopathol. 189:66–70. https://doi.org/10.1016/j.vetimm.2017.06.002.

de Klerk, B., B. J. Ducro, H. C. Heuven, I. den Uyl, J. A. van Arendonk, H. K. Parmentier, and J. J. van der Poel. 2015. Phenotypic and genetic relationships of bovine natural antibodies binding keyhole limpet hemocyanin in plasma and milk. J. Dairy Sci. 98:2746–2752. https://doi.org/10.3168/jds.2014-8818.

Ellison, R. T. 3rd, T. J. Giehl, and F. M. LaForce. 1988. Damage of the outer membrane of enteric gram-negative bacteria by lactoferrin and transferrin. Infect. Immun. 56:2774–2781. https://doi.org/10.1128/iai.56.11.2774-2781.1988.

Erskine, R. J., S. Wagner, and F. J. DeGraves. 2003. Mastitis therapy and pharmacology. Vet. Clin. North Am. Food Anim. Pract. 19:109–138. https://doi.org/10.1016/S0749-0720(02)00067-1.

Ezzat Alnakip, M., M. Quintela-Baluja, K. Böhme, I. Fernández-No, S. Caamaño-Antelo, P. Calo-Mata, and J. Barros-Velázquez. 2014. The immunology of mammary gland of dairy ruminants between healthy and inflammatory conditions. J. Vet. Med. 2014:659801. https://doi.org/10.1155/2014/659801.

Huang, Y. Q., K. Morimoto, K. Hosoda, Y. Yoshimura, and N. Isobe. 2012. Differential immunolocalization between lingual antimicrobial peptide and lactoferrin in mammary gland of dairy cows. Vet. Immunol. Immunopathol. 145:499–504. https://doi.org/10.1016/j.vetimm.2011.10.017.

Hurley, W. L., and J. J. Rejman. 1993. Bovine lactoferrin in involuting mammary tissue. Cell Biol. Int. 17:283–290. https://doi.org/10.1006/cbir.1993.1064.

Isobe, N. 2017. Control mechanisms for producing antimicrobial factors in ruminant mammary gland. Anim. Sci. J. 88:937–943. https://doi.org/10.1111/asj.12808.

Isobe, N., S. Matsukawa, K. Kubo, K. Ueno, T. Sugino, T. Nii, and Y. Yoshimura. 2020. Effects of oral administration of colostrum whey in peripartum goat on antimicrobial peptides in postpartum milk. Anim. Sci. J. 91:e13365. https://doi.org/10.1111/asj.13365.

Isobe, N., J. Nakamura, H. Nakano, and Y. Yoshimura. 2009. Existence of functional lingual antimicrobial peptide (LAP) in bovine milk. J. Dairy Sci. 92:2691–2695. https://doi.org/10.3168/jds.2008-1940.

Knight, C. H., C. J. Wilde, and M. Peaker. 1988. Manipulation of milk secretion. Pages 3–14 in Nutrition and Lactation in the Dairy Cow. P. C. Garnsworthy, ed. Elsevier Ltd.

Kondo, Y., T. Matsui, M. Miura, S. Kakinuma, S. Miyoshi, T. Enomoto, H. Akamatsu, H. Goto, T. Sato, K. Konaka, T. Uchida, and K. Kawai. 2013. Temporal cessation of milking for the cows with udder infected with *Streptococcus*. Japanese Journal of Animal Hygiene 39:130–131. (In Japanese).

Korhonen, H., P. Marnila, and H. S. Gill. 2000. Milk immunoglobulins and complement factors. Br. J. Nutr. 84(S1):S75–80. https://doi.org/10.1017/S0007114500002282.

Kuwahara, K., Y. Yoshimura, and N. Isobe. 2017. Effect of steroid hormones on the innate immune response induced by *Staphylococcus aureus* in the goat mammary gland. Reprod. Domest. Anim. 52:579–584. https://doi.org/10.1111/rda.12948.

Lakic, B., K. Svennersten-Sjaunja, L. Norell, J. Dernfalk, and K. Östensson. 2011. The effect of a single prolonged milking interval on inflammatory parameters, milk composition and yield in dairy cows. Vet. Immunol. Immunopathol. 140:110–118. https://doi.org/10.1016/j.vetimm.2010.11.022.

Lakic, B., E. Wredle, K. Svennersten-Sjaunja, and K. Ostensson. 2009. Is there a special mechanism behind the changes in somatic cell and polymorphonuclear leukocyte counts, and composition of milk after a single prolonged milking interval in cows? Acta Vet. Scand. 51:4. https://doi.org/10.1186/1751-0147-51-4.

Morin, D. E., R. D. Shanks, and G. C. McCoy. 1998. Comparison of antibiotic administration in conjunction with supportive measures versus supportive measures alone for treatment of dairy cows with clinical mastitis. J. Am. Vet. Med. Assoc. 213:676–684.

Newman, K. A., P. J. Rajala-Schultz, J. Lakritz, and F. J. DeGraves. 2009. Lactoferrin concentrations in bovine milk prior to dry-off. J. Dairy Res. 76:426–432. https://doi.org/10.1017/S0022029909990033.

Nishikawa, M., T. Nii, Y. Yoshimura, and N. Isobe. 2018. Investigation of the binding of goat cathelicidin-7 to lipopolysaccharide and leucocidal suppression of pro-inflammatory cytokines. Small Rumin. Res. 168:101–106. https://doi.org/10.1016/j.smallrumres.2018.10.006.

Purba, F. Y., T. Nii, Y. Yoshimura, and N. Isobe. 2020. Translocation of intrauterine-infused bacterial lipopolysaccharides to the mammary gland in dexamethasone-treated goats. Reprod. Domest. Anim. 55:1688–1697. https://doi.org/10.1111/rda.13820.

Rainard, P., and C. Riollet. 2006. Innate immunity of the bovine mammary gland. Vet. Res. 37:369–400. https://doi.org/10.1051/vetres:2006007.

Rajala-Schultz, P. J., J. S. Hogan, and K. L. Smith. 2005. *Short communication:* Association between milk yield at dry-off and probability of intramammary infections at calving. J. Dairy Sci. 88:577–579. https://doi.org/10.3168/jds.S0022-0302(05)72720-X.

Regenhard, P., W. Petzl, H. Zerbe, and H. Sauerwein. 2010. The antibacterial psoriasin is induced by *E. coli* infection in the bovine udder. Vet. Microbiol. 143:293–298. https://doi.org/10.1016/j.vetmic.2009.11.035.

Roberson, J. R., L. D. Warnick, and G. Moore. 2004. Mild to moderate clinical mastitis: Efficacy of intramammary amoxicillin, frequent milk-out, a combined intramammary amoxicillin, and frequent milk-out treatment versus no treatment. J. Dairy Sci. 87:583–592. https://doi.org/10.3168/jds.S0022-0302(04)73200-2.

Silanikove, N., U. Merin, F. Shapiro, and G. Leitner. 2013. Early mammary gland metabolic and immune responses during natural-like and forceful drying-off in high-yielding dairy cows. J. Dairy Sci. 96:P6400–6411. https://doi.org/10.3168/jds.2013-6740.

Smulski, S., M. Gehrke, K. Libera, A. Cieslak, H. Huang, A. K. Patra, and M. Szumacher-Strabel. 2020. Effects of various mastitis treatments on the reproductive performance of cows. BMC Vet. Res. 16:99. https://doi.org/10.1186/s12917-020-02305-7.

Sordillo, L. M., K. Shafer-Weaver, and D. Derosa. 1997. Immunobiology of the mammary gland. J. Dairy Sci. 80:1851–1865. https://doi.org/10.3168/jds.S0022-0302(97)76121-6.

Sordillo, L. M., and K. L. Streicher. 2002. Mammary gland immunity and mastitis susceptibility. J. Mammary Gland Biol. Neoplasia 7:135–146. https://doi.org/10.1023/A:1020347818725.

Stelwagen, K. 2001. Effect of milking frequency on mammary functioning and shape of the lactation curve. J. Dairy Sci. 84:E204–E211. https://doi.org/10.3168/jds.S0022-0302(01)70219-6.

Stelwagen, K., S. R. Davis, V. C. Farr, C. G. Prosser, and R. A. Sherlock. 1994. Mammary epithelial cell tight junction integrity and mammary blood flow during an extended milking interval in goats. J. Dairy Sci. 77:426–432. https://doi.org/10.3168/jds.S0022-0302(94)76969-1.

Stelwagen, K., V. C. Farr, and H. A. McFadden. 1999. Alteration of the sodium to potassium ratio in milk and the effect on milk secretion in goats. J. Dairy Sci. 82:52–59. https://doi.org/10.3168/jds.S0022-0302(99)75208-2.

Stelwagen, K., V. C. Farr, H. A. McFadden, C. G. Prosser, and S. R. Davis. 1997. Time course of milk accumulation-induced opening of mammary tight junctions, and blood clearance of milk components. Am. J. Physiol. 273:R379–386. https://doi.org/10.1152/ajpregu.1997.273.1.R379.

Swanson, K. M., K. Stelwagen, J. Dobson, H. V. Henderson, S. R. Davis, V. C. Farr, and K. Singh. 2009. Transcriptome profiling of *Streptococcus uberis*-induced mastitis reveals fundamental differences between immune gene expression in the mammary gland and in a primary cell culture model. J. Dairy Sci. 92:117–129. https://doi.org/10.3168/jds.2008-1382.

Toledo, I. M., X. Zhao, and P. Lacasse. 2020. Effects of milking frequency and domperidone injections on milk production and prolactin signaling in the mammary gland of dairy cows. J. Dairy Sci. 103:1969–1981. https://doi.org/10.3168/jds.2019-17330.

Welty, F. K., K. L. Smith, and F. L. Schanbacher. 1976. Lactoferrin concentration during involution of the bovine mammary gland. J. Dairy Sci. 59:224–231. https://doi.org/10.3168/jds.S0022-0302(76)84188-4.

White, D. G., and P. F. McDermott. 2001. Emergence and transfer of antibacterial resistance. J. Dairy Sci. 84:E151–E155. https://doi.org/10.3168/jds.S0022-0302(01)70209-3.

Zadoks, R. N., J. R. Middleton, S. Mcdougall, J. Katholm, and Y. H. Schukken. 2011. Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. J. Mammary Gland Biol. Neoplasia 16:357–372. https://doi.org/10.1007/s10911-011-9236-y.

Zhang, G. W., S. J. Lai, Y. Yoshimura, and N. Isobe. 2014a. Messenger RNA expression and immunolocalization of psoriasin in the goat mammary gland and its milk concentration after an intramammary infusion of lipopolysaccharide. Vet. J. 202:89–93. https://doi.org/10.1016/j.tvjl.2014.06.013.

Zhang, G. W., S. J. Lai, Y. Yoshimura, and N. Isobe. 2014b. Expression of *cathelicidins* mRNA in the goat mammary gland and effect of the intramammary infusion of lipopolysaccharide on milk cathelicidin-2 concentration. Vet. Microbiol. 170:125–134. https://doi.org/10.1016/j.vetmic.2014.01.029.

Zhao, X., and P. Lacasse. 2008. Mammary tissue damage during bovine mastitis: Causes and control. J. Anim. Sci. 86(suppl_13):57–65. https://doi.org/10.2527/jas.2007-0302.

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