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Concentrations of antimicrobial components in milk at dry off and postpartum and their relationships to new high somatic cell counts at quarter level in dairy cows

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Research Article

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Abstract

We investigated the antimicrobial components in cow milk at dry off and postpartum and their contribution in preventing new high SCC at quarter level. Milk samples from 72 quarters of 19 lactating cows were collected at last milking before dry off and at 7 d after parturition. Milk yield of each cow was recorded and SCC, IgG, IgA, lactoferrin, lingual antimicrobial peptide (LAP), and S100A7 concentrations in each quarter milk sample were measured. The postpartum milk yield was significantly higher than that at dry off. The IgG, IgA and lactoferrin concentrations in milk at dry off were significantly higher than those at postpartum, whereas the LAP concentration was lower. Quarters with SCC < 300 000 cells/ml at both dry off and postpartum were classified as persistent low SCC (PL) whereas those that rose above that same threshold postpartum were classified as new high SCC (NH). At dry off, IgG and LAP concentrations in milk were significantly higher in PL than in NH. These results suggest that high LAP concentrations during the dry period may contribute toward the prevention of new high SCC.

Appropriate management of udder hygiene during dry periods is important to prevent mastitis in subsequent lactations (McMullen *et al*., 2021). New high SCC postpartum is considered an indicator of a new intramammary infection during the dry period, and the percentage of cows that develop a high SCC after the dry period out of those with a low SCC before the dry period is used as an indicator of key performance at herd level (Krattley-Roodenburg *et al*., 2021).

Antimicrobial components such as immunoglobulins (Ig) and some antimicrobial proteins and peptides play a pivotal role in the primary immune response against mastitic pathogens in milk of dairy cows. The milk of cows contains several antimicrobial components such as Ig, lactoferrin, lingual antimicrobial peptide (LAP) and S100A7 and these components defend from the teat to the alveolus of the mammary glands against pathogens (Cakebread *et al*., 2015; Isobe, 2017). LAP is a β-defensin family protein secreted from mammary epithelial cells (Isobe *et al*., 2009a). High concentrations of IgG and lactoferrin in milk at dry off are important to control against new high SCC in subsequent lactations (Vilar and Rajala-Schultz, 2020). However, the role of other antimicrobial components in milk during the dry period has not been evaluated. Here, we investigate the concentrations of antimicrobial components in milk at dry off and postpartum, and examine their relationship to the changes in SCC at the quarter level.

Materials and methods

Animals and experimental design

This study was approved by Hiroshima University Animal Research Committee (Approval number: E19-3). A total of 19 lactating Holstein Friesian cows housed in the same commercial farm were enrolled in this study. The sampling period (July 2019 – January 2020) was blindly determined, and 19 cows started drying-off during that period. Seventy-two lactating quarters were enrolled for the study because one quarter each from four cows had not lactated for 6 months before the experiment due to mastitis.

Quarter milk samples were collected at the last milking before dry off and 7 d after parturition. Postpartum milk samples in this study were collected 7 d postpartum to avoid the effect of colostrum on the concentrations of antimicrobial components in milk (Cakebread *et al*., 2015). All milk samples were collected during the morning milking (0600 h). SCC was counted within 12 h of sampling using an automated cell counter (DeLaval cell counter DCC; DeLaval, Tumba, Sweden) and milk samples were centrifuged at 500 × ***g*** for 10 min at 4 °C and separated skimmed milk was collected into tubes and stored at −30 °C for further measurement.

Enzyme immunoassay

The concentrations of IgG, IgA and lactoferrin in milk were measured by ELISA quantification following a commercial protocol (Bethyl Laboratories Inc., TX, USA). The concentrations of LAP and S100A7 were measured as described previously (Isobe *et al.*, 2009b; Zhang *et al.*, 2014).

Statistical analysis

To elucidate the changes of concentrations of antimicrobial components at dry off and 7 d after parturition, milk yield, SCC, concentrations of IgG, IgA, lactoferrin, LAP and S100A7 were analyzed by generalized linear mixed model. Mixed effect regression models were used to elucidate the correlation between concentrations of IgG, IgA, lactoferrin, LAP and S100A7 concentration at dry off and after parturition. Concentration at 7 d after parturition was included as dependent variable and concentration at dry off was included as fixed effect and individual cows and quarters branched from individual cows were included as random effect. The data from each sampling unit were separately assessed, and time was included as the fixed effect in the models.

To elucidate the potency of concentrations of antimicrobial components at dry off against new high SCC, the quarters with SCC < 300 000 cells/ml at dry off were selected and the Mann–Whitney *U* test was used to compare the following two groups: quarters with persistent low SCC (PL, when SCC were < 300 000 cells/ml in postpartum milk, $n = 29$) and new high SCC (NH, when SCC were > 300 000 cells/ml in postpartum milk, $n = 18$). The threshold SCC for quarter-level mastitis was set at 300 000 cells/ml based on similar sampling procedures carried out previously (Hernández-Castellano *et al.*, 2017; Khatun *et al.*, 2019). Statistical analyses were performed using the JMP Pro15 software (SAS Institute Inc., NC, USA).

Results

The postpartum milk yield was significantly higher than that at dry off ($P < 0.001$). The pattern of changes in SCC between dry off and postpartum as well as the changing concentrations of IgG, IgA, lactoferrin, LAP and S100A7 are given schematically in Fig. 1. Online Supplementary Fig. S1 shows the same data with actual values presented (Fig. S1A), as well as showing the correlations between the different factors (Fig. S1B). The mixed model for repeated measures showed that the concentrations of IgG, IgA, and lactoferrin in milk at dry off were higher than those of milk at postpartum ($P < 0.001$), whereas the reverse was true for LAP (postpartum higher than dry off ($P < 0.01$). The mixed model for correlation of the dry period and postpartum concentrations showed a significant positive correlation ($P < 0.01$) for LAP. The correlations between antimicrobial components and SCC in the same milk at dry off are shown in online Supplementary Table S1, the only significant correlations being for postpartum IgG and lactoferrin (coefficients of 0.38 and 0.61 respectively, both $P < 0.001$)

The comparisons of SCC and antimicrobial components at dry off between PL and NH are shown in online Supplementary Fig. S2. At dry off, the IgG and LAP concentrations in milk of PL were significantly higher than those of NH (the median of IgG and LAP concentration were 1.54 *vs.* 0.79 mg/ml and 3.28 *vs.* 1.39 ng/ml, respectively, both $P < 0.05$). There were no other significant differences.

Discussion

The IgG and IgA and lactoferrin concentrations in postpartum milk were lower than those in milk obtained at dry off. Low milk production increases IgG and IgA concentration in milk (Zhao *et al.*, 2010; Vilar and Rajala-Schultz, 2020). Thus, in the present results, the lower concentration of IgG and IgA in postpartum milk may be a result of high milk production. Similar to the immunoglobulins, high milk yield can decrease lactoferrin concentration (Vilar and Rajala-Schultz, 2020), as supported by our results. Whereas, interestingly, the inverse result was observed for LAP. However, the higher LAP concentration in postpartum milk as compared with that in milk at dry off cannot be explained by the dilution caused by increased milk yield. There was a positive correlation between LAP concentrations in milk collected at dry off and postpartum from the same quarter, and this result was the same when 47 samples were used for comparing SCC and antimicrobial components at dry-off period between persistent low SCC and new high SCC ($R^2 = 0.429$, $P < 0.001$, data not shown). LAP is synthesized by the epithelial cells of the alveoli and milk ducts, however, IgG, IgA, and (to some extent) lactoferrin are plasma proteins produced by cells such as leukocytes, not by the mammary epithelial cells (Pallister *et al.*, 2015; Isobe, 2017). The mechanisms of appearance in milk are thus very different, namely secretion (LAP) or passive transfer. Thus, our results may indicate that the mammary epithelial LAP synthesis and secretion is upregulated postpartum after mammary epithelial regeneration during the dry period. For more detailed analysis,

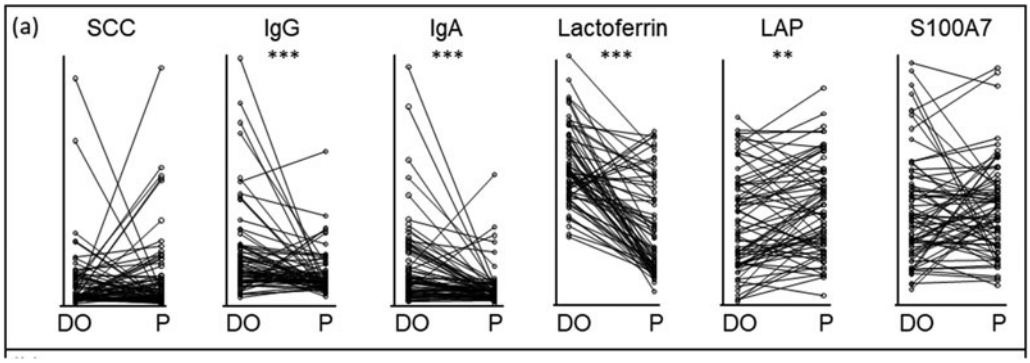

Figure 1. SCC and IgG, IgA, lactoferrin, lingual antimicrobial peptide (LAP) and S100A7 concentrations in milk at last milking prior to dry off (DO) and at 7 d after parturition (P) from 72 quarters. ** and *** indicate significant difference between dry off and postpartum ($P < 0.01$ and $P < 0.001$, respectively).

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investigation of quarter level relationships between milk yield and antimicrobial components is needed. Day to day variations of lactoferrin and LAP concentration in bovine milk is thought to be negligible (Isobe, 2017), whereas that of other antimicrobial proteins is unclear. The dynamics of the concentration of antimicrobial components during the dry period are not clear from our data because only single samples were obtained at dry-off and postpartum. An increased number of sampling days are required in any further study.

SCC and the concentrations of IgA, lactoferrin and S100A7 in milk at dry off showed no significant difference between persistent low SCC and new high SCC quarters, whereas IgG and LAP were both higher in the former. Thus, our results indicate that the IgG and LAP concentrations at dry off may be important to prevent a new infection and thus high SCC. In addition, when the cut-off value between high and low SCC was 200 000 cell/ml, IgG and LAP concentrations in quarters with persistent low SCC were again significantly higher than those in quarters with new high SCC (data not shown). Vilar and Rajala-Schultz (2020) indicated that the high IgG and lactoferrin concentrations in milk at dry off are important to prevent intramammary infection during the dry period. Our results confirm this finding for IgG, but also point to LAP as another important factor. However, our results also indicated that the lactoferrin concentration at dry off may only make a small contribution toward preventing new high SCC at quarter level. The bactericidal activity of lactoferrin may be rather limited (Kawai *et al.*, 2007), whereas β-defensins, such as LAP, have direct bactericidal activity against mastitic pathogens (Gurao *et al.*, 2017). The changes in LAP concentration between dry-off and postpartum were generally an increase, however, a decrease was observed in some quarters. The proportion of quarters showing a decrease was 41.4% in PL and only 5.9% in NH (data not shown). This may be partly due to a higher starting value in PL, but it could also be the case that intramammary infection during the dry period could cause increased postpartum LAP concentration. Bacteriological examination was not included in this study, so it is still unclear whether the potential of LAP for preventing new high SCC is due to its antimicrobial activity. In addition, more detail dynamics of antimicrobial components in milk during dry period should be investigated to clarify when antimicrobial components act during the dry period.

In conclusion, we have confirmed that IgG concentration at dry off is associated with a reduced risk of high SCC postpartum, and in addition have shown that the same is true for LAP.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029924000050

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