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## BRIEF COMMUNICATION: Host-defence related bioactive proteins in cows' milk during mastitis and after drying-off

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### Introduction

Besides the caseins and major whey proteins, cows' milk contains a wide range of less abundant proteins that have functions associated with host defence, and are thought to play a role in protection against microbial infections (Stelwagen et al. 2009; Wheeler et al. 2007). Microarray analysis has shown that complex changes in gene expression occur in the bovine mammary gland in response to drying-off, including up-regulation of host-defence associated genes (Singh et al. 2008). The abundance of at least some proteins encoded by these genes can be altered in response to milking frequency and at different stages of the lactation cycle (Farr et al. 2002; Hurley & Rejman 1993; Rejman & Hurley 1988). Although some of these proteins have current or potential commercial value as high-value extracts of milk, the extent of these changes in abundance have not been well characterised. In particular, it is not clear how rapidly and to what extent the drying-off process increases the abundance of particular host-defence proteins and how such responses compare with those observed during intra-mammary infection.

The aim of this study was to compare changes in the abundance of host-defence proteins in response to drying-off and mastitis, so as to provide a basis for estimating the value of on-farm strategies to maximise their extraction during commercial processing. The results for two of these proteins, lactoferrin and cartilage glycoprotein 39 kDa (CG39) are reported here.

### Materials and methods

Milk was collected from three different groups of cows. The first group comprised eight Friesian dairy cows at various stages of lactation with naturally occurring mastitis caused by a variety of pathogens including *Staphylococcus aureus* (n = 1), Coagulase-negative staphylococci (CNS) (n = 2), *Pseudomonas aeruginosa* (n = 2), *Streptococcus uberis* (n = 1), *Escherichia coli* (n = 1), and *Streptococcus dysgalactae* (n = 1). The second group comprised eight cows in early lactation inoculated with *Streptococcus uberis* via the teat to experimentally induce mastitis as previously described (Smolenski et al. 2011). For both groups, milk was collected at the time when clinical symptoms first appeared and before antibiotic treatment had commenced. Somatic cell counts (SCC) in milk from infected quarters were  $12 \pm 3$  (standard error) and  $10 \pm 3$  million cells/mL in cows with

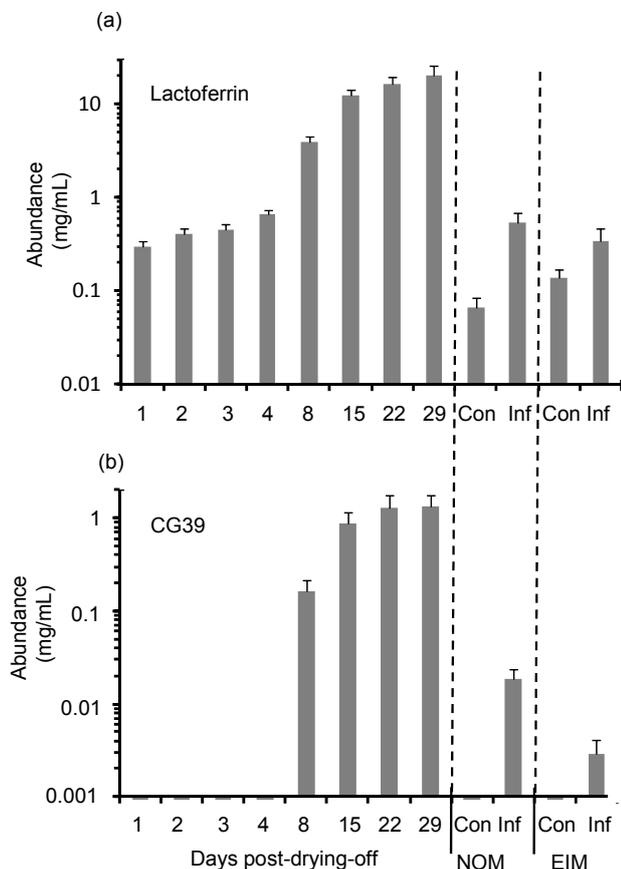
naturally occurring and experimentally induced mastitis, respectively. Milk was also collected from an uninfected quarter exhibiting a SCC of <100,000 cells/mL and a negative result for bacteriology, of each cow. The third group comprised eight cows that were dried-off and milk collected from one of their quarters at Days 1, 2, 3, 4, 8, 15, 22 and 29 thereafter. All milk samples were collected aseptically by hand stripping in accordance with National Mastitis Council guidelines as described previously (Smolenski et al. 2011).

Lactoferrin was measured in milk samples using a commercial ELISA kit (Bethyl Laboratories, Montgomery, TX, USA). Samples were diluted and analysed in duplicate and the results averaged. Purified bovine lactoferrin standard supplied in the assay kit was used to quantify results. A coefficient of variation between duplicate samples of less than 10% was achieved. The concentration of CG39 in milk was determined by quantitative Western blotting using a rabbit polyclonal antibody raised against recombinant bovine CG39. A coefficient of variation between duplicate samples of less than 20% was achieved. Briefly, between 5 and 80  $\mu$ g of skimmed milk was loaded on lanes of a sodium dodecyl sulphate (SDS) polyacrylamide gel and subjected to electrophoresis. A dilution series of a standard sample was included in every gel. Following electroblotting to a nitrocellulose membrane, the membrane was probed with the anti-CG39 IgG followed by goat anti-rabbit IgG-peroxidase conjugate. The signal was visualised by enhanced chemiluminescence and quantified by either film densitometry or directly using a charge-coupled device (CCD) camera-based luminescence detector (ChemiDoc, Bio-Rad, Hercules, CA, USA). The detailed procedure has been described previously (Smolenski et al. 2011).

### Results and discussion

Lactoferrin concentrations increased in skim milk during mastitis infection and drying-off (Fig. 1a). Lactoferrin concentrations in milk from cows with naturally occurring mastitis were  $0.53 \pm 0.14$  mg/mL as compared to  $0.07 \pm 0.02$  mg/mL in uninfected quarters from the same cows, representing a  $7.6 \pm 0.2$  fold increase in lactoferrin concentration in response to infection. Lactoferrin concentrations in cows with experimentally induced mastitis were  $0.35 \pm 0.13$  mg/mL as compared to  $0.14 \pm 0.03$  mg/mL in uninfected quarters from the same cows, representing a

**Figure 1** Changes in concentration of lactoferrin and CG39 in cow's milk post-drying-off and during naturally occurring mastitis (NOM) and experimentally induced mastitis (EIM). Milk was obtained and analysed for lactoferrin (a) and CG39 (b). Data represents the mean  $\pm$  standard error of mean of values from eight cows per condition (Con = Control uninfected quarters. Inf = Infected quarters).



2.5  $\pm$  0.2 fold increase in lactoferrin concentration. Similar concentrations of lactoferrin in mastitic milk have been reported previously (Kawai et al. 1999). Lactoferrin concentrations also increased during involution (Fig. 1a), with levels comparable to those observed during mastitis being detected by Day 3 post-drying-off of 0.45  $\pm$  0.07 mg/mL. The lactoferrin concentration increased substantially thereafter, reaching 20.0  $\pm$  5.0 mg/mL in the fully involuted gland by Day 29 post-drying-off. A similar profile of increased lactoferrin concentration during the time course of involution has been reported previously (Rejman et al. 1989).

The concentration of CG39 in milk also increased in response to mastitis infection and drying-off (Fig. 1b). Concentrations in milk from cows with naturally occurring and experimentally induced mastitis were 0.018  $\pm$  0.005 mg/mL and 0.003  $\pm$  0.001 mg/mL, respectively. No CG39 was detected in milk from uninfected quarters. During involution, no CG39 was detected until eight days post-drying-off. CG39 concentrations rose markedly thereafter to reach 1.35  $\pm$  0.39 mg/mL by Day 29 post-drying-off. Rejman et al.

(1989) reported a similar profile for the increase of CG39 concentration during the time course of involution, however these researchers were able to detect the presence of CG39 earlier in the involution process.

Although it is well documented that the concentrations of minor milk proteins such as lactoferrin increase during mastitis and involution (Kawai et al. 1999; Rejman et al. 1989), data directly comparing the abundance of these proteins in milk from cows in the same production environment is limited. Results of the current study demonstrate that the concentration of minor milk proteins such as lactoferrin and CG39 increase to a greater extent during drying-off than they do during mastitis.

Lactoferrin is currently extracted from milk on a commercial scale and marketed, based on its reported antimicrobial and immunomodulatory activities (Wakabayashi et al. 2006). Thus, it seems conceivable that value could be added to milk by collecting milk specifically for bioactive processing after a period of not milking, or by reducing milking frequency. However, this would involve some logistical and management challenges. The CG39 protein also has value-adding potential due to its antimicrobial activity (TT Wheeler, Unpublished data), although a market for this protein has not been established to date. The reduced volume of milk obtained after drying-off is an important consideration to be factored into determination of yield and addition of value. In addition to the proteins investigated here, other potentially valuable bioactive proteins may also increase in abundance in milk during the drying-off period, providing further potential value-adding opportunities for milk collected at this time if markets can be established for them.

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**References**

Farr VC, Prosser CG, Clark DA, Tong M, Cooper CV, Willix-Payne D, Davis SR 2002. Lactoferrin concentration is increased in milk from cows milked once daily. *Proceedings of the New Zealand Society for Animal Production* 62: 225–226.

Hurley WL, Rejman JJ 1993. Bovine lactoferrin in involuting mammary tissue. *Cell Biology International* 17: 283–289.

Kawai K, Hagiwara S, Anri A, Nagahata, H 1999. Lactoferrin concentration in milk of bovine clinical mastitis. *Veterinary Research Communications* 23: 391–398.

Rejman JJ, Hurley WL 1988. Isolation and characterization of a novel 39 kilodalton whey protein from bovine mammary secretions collected during the nonlactating period. *Biochemical and Biophysical Research Communication* 150: 329–334.

- Rejman JJ, Hurley WL, Bahr JM 1989. Enzyme-linked immunosorbent assays of bovine lactoferrin and 39-kilodalton protein found in mammary secretions during involution. *Journal of Dairy Science* 72: 555–560.
- Singh K, Davis SR, Dobson JM, Molenaar AJ, Wheeler TT, Prosser CG, Farr VC, Oden K, Swanson KM, Phyn CV, Hyndman DL, Wilson T, Henderson HV, Stelwagen K 2008. cDNA microarray analysis reveals that antioxidant and immune genes are upregulated during involution of the bovine mammary gland. *Journal of Dairy Science* 91: 2236–2246.
- Smolenski GA, Wieliczko RJ, Pryor SM, Broadhurst MK, Wheeler TT, Haigh BJ 2011. The abundance of milk cathelicidin proteins during bovine mastitis. *Veterinary Immunology and Immunopathology* 143(1-2): 125–130.
- Stelwagen K, Carpenter E, Haigh B, Hodgkinson A, Wheeler TT 2009. Immune components of bovine colostrum and milk. *Journal of Animal Science* 87(13) (Supplement): 3–9.
- Wakabayashi H, Yamauchi K, Takase M 2006. Lactoferrin research, technology and applications. *International Dairy Journal* 16: 1241–1251.
- Wheeler TT, Hodgkinson AJ, Prosser CG, Davis SR 2007. Immune components of colostrum and milk—a historical perspective. *Journal of Mammary Gland Biology and Neoplasia* 12: 237–247.