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CHANGES IN THE LACTOFERRIN CONCENTRATION AT DRYING OFF PERIOD[1]

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ABSTRACT

In the lactation cycle of dairy cows, the dry off is a critical period of rest for the udder, in order to optimize milk production in the next lactation. Lactoferrin (LF) is an iron-binding protein normally excreted in cow's milk and it is one of the natural protective factors having the ability to inhibit the growth of mastitis-causing pathogens. The objective of this research was to determine whether LF concentration in the milk is increased at drying off period. Quarter milk samples (n=40) from clinically healthy Holstein-Friesian cows were taken. Milk samples were collected one week prior to dry off and on the day of dry off. The milk samples were analyzed for physicochemical properties, somatic cells (SCC) and concentrations of lactoferrin. The mean LF concentration in the milk was found to increase significantly ($p<0,05$) from 2.977,28 μg/mL, one week prior to dry off to 4.529,07 μg/mL on the day of drying off process.

KEY WORDS: lactoferrin, cow's milk, somatic cells, dry off period.

INTRODUCTION

The importance of the dry (nonlactating) period in the dynamics of intramammary infections in dairy cattle is well established (Neave et al.,

[1] original scientific paper

1950; Oliver & Mitchell, 198; Bradley & Green, 2000; Green et al., 2002). The dry period is the period before calving when cows are not milked, which is usually about 6 to 8 weeks. This period has multiple functions. Main functions are to allow the cow a rest period before birth of the next calf (Kok et al., 2017), next function is to allow the cow to produce maximal level of milk in the next lactation. The third function is, renewing of the mammary cells at a faster rate than when cows would be milked up to calving (Capuco et al., 1997). This results in a large concentration of renewed mammary cells at the moment of calving which explains the high peak milk yield in the next lactation after a dry period (Kuhn, Melvin T.; Hutchison, Norman, 2005; Knegsel et al., 2013).

Concentration of natural protective factors (NPFs) which have the ability to inhibit growth of mastitis-causing pathogens increase rapidly following the cessation of milking of dairy cows. One such NPF is lactoferrin (LF), an iron-binding protein present in high concentrations in dry-cow secretions (Newman et al., 2009). On the other side, LF is an iron-binding glycoprotein that is synthesized elsewhere in the body, for example by specific granules in PMNL (Baggiolini et al., 1970) and glandular epithelial cells (Masson et al., 1966). Lactoferrin is present in milk and on mucosal surfaces (Reiter & Oram, 1967). The main role of LF is in the defense mechanisms of the mammary gland of lactating animals. Bacteria require iron for growth, and LF can inhibit bacteria by chelating iron under certain conditions (Weinberg, 1978). In addition to its iron-binding function, LF may directly kill certain bacterial strains (Bellamy et al., 1992) or may weaken bacterial resistance by adhesion to the surface of bacteria (Arnold et al., 1977).

Recently (Singh et al., 2021) has found that a novel hydrolytic molecule from the N-lobe of LF, lactosmart, has been generated using trypsin. Lactosmart has been tested against different strains of bacteria, namely, *E. coli*, *S. aureus*, *S. flexneri*, and *P. aeruginosa*, which confirmed the ability of LF to fight against numerous agents that can cause intramammary infection in cows. The investigation of (Cheng et al., 2008) on LF concentration in the milk from normal lactating cows showed that the stage of lactation, with increase as the end of lactation approaches, and daily milk production contributed the most to it, whereas parity showed no association.

MATERIAL AND METHODS

Milk samples were collected at the end of lactation for physicochemical analysis, somatic cell count and lactoferrin from 10 pregnant and clinically healthy Holstein Friesian cows. Five of the cows were primiparous and five were multiparous. The drying-off process started when the average dairy milk yield was approximately 10 kg per cow. The process was initiated approximately 8 weeks before parturition and the gradual drying–off process lasted for 2 weeks with reduced frequency of milking and changes in feeding. The first milk sample was taken one week prior to dry–off (pre-dry) in this period cows were milked only once a day. The last week prior to dry-off cows was intermittent milked (once every second day) and the second milk sample was taken at the last day of drying off process.

Milk samples were taken for determination of LF. The samples were frozen at -20 °C and LF concentration was measured 2-4 months later. LF concentrations in milk were determined using commercial immunoenzyme test Bovine Lactoferrin ELISA kit (Biopanda Reagents, Belfast, The United Kingdom), following the procedure recommended by the manufacturer's instructions. Milk samples were diluted in proportion 1/10,000 and a standard curve was plotted for each plate separately. Results from the testing were obtained by measurement of optical density of the sample on wavelength λ=450 nm using Microplate reader Model 680 (BioRad, Hercules, California, U.S.A.). The determination of physicochemical properties of the milk (milk fat, solids-non-fat (SNF), protein and lactose) was performed with precise mid-infrared analyzer Lactoscope C4+ (Delta Instruments B.V, Drachten, the Netherlands), according to IDF 9622: 1999 standard. Conductivity (mS/cm) was measured by HANNA HI 98192 EC/TDS/NaCl/Resistivity. Samples intended for examination of somatic cells were conserved with Broad Spectrum Microtabs II (BSM) (Advanced Instruments INC, Norwood, U.S.A.). The determination of the somatic cell count (SCC) was made by fluoro-optical electronic counter Somacount CC 150 (Bentley Instruments, Inc., Chaska, Minnesota, U.S.A.). Milk-enumeration of SCC was in accordance with MK EN ISO 13366-52:2010 standard.

RESULTS AND DISCUSSION

Mean LF concentrations in milk gradually increased during the sampling period from one week prior to dry off to the day of drying off period

(Table 1). Similar results were presented by Kutila T., et al., (2003) where lactoferrin concentration in mammary secretion gradually increased from the last milking to days 2 and 6 of the dry period. According to Welty et al. (1976) the LF concentration was increased during involution of the mammary gland, and some cows have greater concentration from 20.000 to 100.000 µg/ml. According to Kutila et al., (2003) LF concentration varied greatly between the udder quarters of the cow. The lowest concentrations of LF were 2.217,76 µg/ml and 3.471,83 µg/ml in pre dry and on the day of drying off, respectively. On the other hand, the highest concentrations of LF were 4.080,99 µg/ml and 6.335,10 µg/ml in pre dry and on the day of drying off, respectively. The individual difference in concentration of LF may be as a result of infection in this period. Cow quarters infected with major pathogens synthetize greater concentration of lactoferrin compared to minor pathogens (Newman K.A., 2008). The highest LF concentration in our research was observed in cows at first lactation 4.211,28 µg/ml compared with cows from second lactation 3.295,07 µg/ml ($p<0,05$) (Table 1). These results differ from those published by Krol et al. (2010, 2012), where primiparous cow secrete significantly lower values of lactoferrin compared to multiparous cows and the concentration of lactoferrin is 2-3 times higher compared to primiparous.

Table 1 Mean lactoferrin (LF) concentration in milk one week prior to dry off (pre-dry) and on the day of drying off process (dry-off) and lactation number of the cow

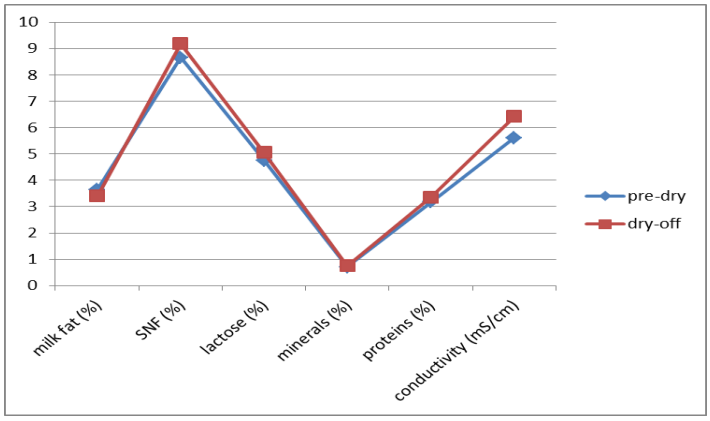
	Dry period		**Lactation number of the cows**	
Mean ± SD	**Pre-dry**	**Dry-off**	**Primiparous**	**Multiparous**
LF (µg/ml)	2.977,28 ± 798,65[a]	4.529,07 ± 1.283,25[b]	4.211,28 ±1.683,42[a]	3.295,07±484,73[b]
SCC/ml	1.268.333 ± 1.291.040[a]	1.530.750 ± 990.699,8[b]	1.743.750 ± 845.957[a]	984.333,3 ±1.315.690[b]
Correlation (r)	0,911; $p<0,05$	0,14; $p>0,05$	0,31; $p<0,05$	0,71; $p<0,05$

*The differences in values with different superscripts in same row are statistically significant at level: a:b ($p<0.05$)

According to Sordillo et al., (1987) somatic cells increased significantly during the first seven days of the udder involution. In our study the

somatic cell count increased from 1.268.33 SCC/ml to 1.530.750 SCC/ml. Very high degree of correlation was observed between lactoferrin and somatic cells ($r = 0,911$; $p<0,05$) one week prior to dry off process. On the day of drying off process slight correlation was notices ($r=0,14$; $p>0,05$). Similar results were obtained by Harmon et al., (1975) and Newman K.A., (2008) who observed a positive correlation between somatic cells and lactoferrin. The correlation between somatic cells and the concentration of lactoferrin in the dry period is due to the changes that occur in the mammary tissue, the reduced milk yield, and the earlier mastitis infections in this period. According to Pantoja JCF et al., (2009) quarters with SCC > or =200.000 cells/ml at dry-off and postcalving were 2,7 times more likely to experience a first case of mastitis than quarters with SCC <200.000 cells/mL at both periods.

Figure 1 Physicochemical changes in milk during dry off period



Electrical conductivity in milk (EC) is dependent on Na+, K+, and Cl- concentrations and varies between 4,0-5,5 mS/cm at uninfected cows (Kitchen et al., 1980). In our study the milk conductivity was 5,62 mS/cm pre dry and 6,42 mS/cm on the day of drying off ($p<0,05$). This result was in agreement with increased LF concentration and somatic cell count in milk. No statistical significance was noticed in other physicochemical parameters in milk pre dry and on the day of drying off process (Figure 1).

CONCLUSION

Results from our study indicate that the concentration of lactoferrin increased significantly ($p<0,05$) from 2.977,28 μg/mL, one week prior to dry off (pre-dry) to 4.529,07 μg/mL on the day of drying off process (dry-off) and plays a significant role in the defense mechanisms of the mammary gland of lactating animals. The average number of somatic cell count was high at sampling period, and this was probably due to intramammary infections later in lactation. Also, a positive degree of correlation was noticed between concentration of LF, and somatic cell count one week prior to dry off process ($r=0,911$; $p<0,05$). Concentration of natural protective factors, like lactoferrin have the ability to inhibit growth of mastitis causing pathogens during drying off period.

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