VETERINARY MEDICINE SCIENCES

IMMUNOGLOBULINS AND LACTOFERRIN CONCENTRATION IN MILK AND BACTERIA CAUSING SUBCLINICAL MASTITIS IN DAIRY COWS

IVETA KOCIŅA, VITA ANTANE, IVARS LŪSIS
Latvia University of Agriculture
e-mail: iveta.kocina@pvd.gov.lv

Abstract

Natural defence mechanisms of the mammary gland tissues play a vital role in protecting the gland from infections. The progress of mammary infection depends on the ability of bacterial pathogens to adapt to milk and udder tissues, and on the various virulence factors they activate, as well as on the cow's response. The levels of immunoglobulins (Ig) and lactoferrin (Lf) concentration in the milk from dairy cows with and without subclinical mastitis were determined. In this investigation it was stated that 82.5% of samples were negative, but 17.5% of samples were positive for pathogens. Cows had subclinical mastitis caused by *Coagulase negative* staphylococci observed in 48.60%, *Streptococcus uberis* – 32.10%, *Staphylococcus aureus* – 18.90% of cases in milk samples. There were no significant differences between all classes of immunoglobulins concentration in the milk without pathogens and with pathogens. The lactoferrin concentration was significantly increased for 40.33% in the milk with pathogens.
Key words: milk, somatic cell count, immunoglobulins, lactoferrin.

Introduction

The udder inflammation is one of most widespread diseases in dairy cows in Latvia. In total 6% of cows are affected with clinical mastitis, but with subclinical mastitis - up to 30% and more of all dairy cows [5]. The most important mastitis causing bacteria, isolated from investigated samples in the State Veterinary Medicine Diagnostic Center, Latvia were *Staphylococcus aureus* in 54% samples, *Streptococcus uberis* – 14%, *Esherichia coli* – 10%.

One of the means of decreasing the impact of mastitis on dairy industry is to increase the natural ability of the cow to resist infection. The immune system of the mammary gland consists of both humoral and cellular components. Immunoglobulins, which contain a specific antibody activity against antigenic stimuli, form the humoral component. The cellular component consists of several different cell groups, the most important of which are the macrophages and various lymphocyte subsets. These cell groups are called somatic cells. Somatic cell count is used for characterising mammary gland health status. The cell count of a healthy udder is about 100.000 cells ml^{-1} [4, 7, 10].

Immunoglobulins (Ig) function as the soluble effectors of specific or humoral immune response. Four classes of Ig are known to influence mammary gland defence against bacteria causing mastitis: IgG1, IgG2, IgA, and IgM.

Concentration of the Ig classes varies with stage of lactation and health of the gland. In cow's milk, the concentration (mg ml^{-1}) of IgA = 0.08, IgG1 = 0.58, IgG2 = 0.06, IgM = 0.09. In the inflamed lactating gland, the concentration of Ig increases 2–3 times [9, 12].

The other antibacterial factor of cow's mammary gland is lactoferrin. It is synthesized in polymorphonuclear leukocytes and udder epithelial cells.

In cows milk, lactoferrin is found at concentration of 0.02–0.35 mg ml^{-1}, depending on the time of lactation and mammary gland health status. Maximum lactoferrin concentration is about 20 mg ml^{-1} obtained after 3 to 4 weeks of parturition. In the cases of udder inflammation, the concentration of lactoferrin in milk can increase up to 100 times [4, 10].

The purpose of this study was to evaluate and compare immunoglobulins and lactoferrin concentration in normal milk and milk with bacterial pathogens.

Materials and Methods

Samples for investigations were taken from a dairy herd consisting of 75 Latvian Brown and Holstain cows at different lactation stages and in different lactation cycles. Cows were kept in an uninsulated shed and were milked two times a day. Average milk yield from a lactating cow was 21 liter per day.

A total of 16 cows were selected to represent all the herd. Samples were taken from each quarter 2 times during the shed keeping period and 2 times during the pasture period.

Mammary gland health status was detected by California Mastitis Test (CMT) and by clinical observation. If the CMT results were positive, bacteorological investigations were performed.

Normal and subclinical mastitis milk from 212 quarters was classified as follows: 175 quarter milk samples, which were CMT negative and negative for pathogens, were regarded as normal milk, i.e. milk without pathogens; 37 quarter milk samples, which were CMT positive and positive for different pathogens were grouped as bacteria causing mastitis milk.

The immunoglobulins and lactoferrin in milk were measured using radial immunodiffussion method in the laboratory of the Riga Reproduction Center.

Somatic Cell Count was detected by "Somocaunt" in the laboratory of Riga Dairy Plant.

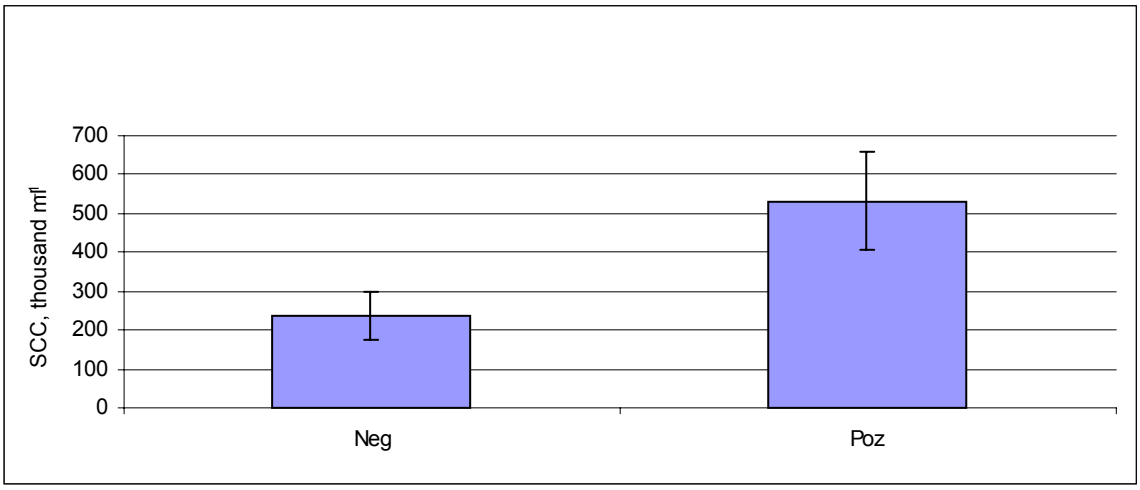

Fig. 1. Comparison of SCC in milk from cows with (Poz) and without (Neg) bacterial pathogens.

Results and Discussion

During the investigations no cases of clinical mastitis were detected. The average somatic cell count (SCC) in the dairy herd was about 355 thousand cells ml^{-1}. The investigation gives evidence that 82.5% of cows mammary gland health status without pathogens was rather good – SCC was 236 ± 278.1 thousand cells ml^{-1}, 17.5% of cows had subclinical mastitis with bacteria presence in the mammary gland and increased SCC – up to 532 ± 120.6 thousand cells ml^{-1} (Figure 1).

The difference between average SCC in milk samples without pathogens and with pathogens was significant (P<0.001).

The most common pathogen in the mammary gland, isolated from infected quarters, was *Coagulase negative staphylococci* found in 48.60% of cases (SCC was increased up to 625 ± 159.3 thousand cells ml^{-1}), *Streptococcus uberis* infection was found in 18.90%, (SCC was increased up to 656 ± 93.0 thousand cells ml^{-1}), and *Staphylococcus aureus* was found in 32.10% of cases (SCC was increased up to 410 ± 110.9 thousand cells ml^{-1}) (Table 1).

It is well reported by different authors that in the case of mammary gland inflammation, somatic cell count in the mammary gland and milk increases significantly [2, 3, 4, 11].

The concentration of each Ig **class** in mammary secretion varies depending on the stage of lactation and infection status of the mammary gland. In healthy glands, the concentration of Ig is low during lactation but increases during inflammation. The inflammatory process reflects the mammary gland immunological response against the invading pathogens [10].

There are different conflicting results of studies about correlation between Ig classes in healthy glands and pathogen affected glands, but this data were based only on milk from acutely infected glands, not for subclinical mastitis, which was the subject of this study.

Comparing Ig concentrations in the milk negative for pathogens and milk positive for pathogens, the following results were obtained (Figure 2). In milk samples without pathogens, **IgG concentration** was 2.12 ng ml^{-1}, and with pathogens – 2.18 ng ml^{-1}. The increase of IgG was not significant – 2.75% (p>0.05).

A.Saran, G.Leitner (2000) and G.Leitner, B.Yadlin (2000) reported that no significant differences in IgG were found among the quarters of the same cow, whether the quarter was infected or not [11, 6]. However, D.L. Watson (1980) reported that specific immunological protection of mammary tissue may be mediated through blood-derived antibody (particularly IgG) and locally synthesized antibody (particularly IgA) or phagocytic cells. This cytophilic antibody (IgG and IgA) can play an important role in enhancing the phagocytic capacity of neutrophils in the mammary gland [13]. Investigations of M.B. Barrio, P.Rainard et al. (2003) suggested that IgG is not opsonic for bovine immune cells

Table 1

Summary of pathogens isolated from cow's quarter milk

Pathogens	Number of samples	% of cases	Average SCC, thousand cells ml^{-1}
Coagulase negative staphylococci	18	48.60	625 ± 159.3
Staphylococcus aureus	7	32.10	410 ± 110.9
Streptococcus uberis	12	18.90	656 ± 93.0
Total	37	100%	532 ± 120.6

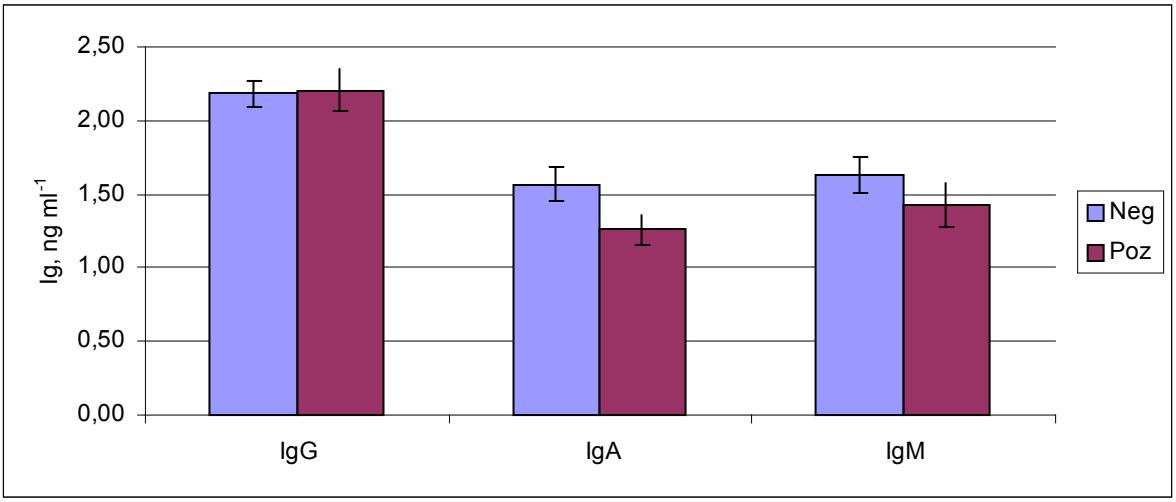

Fig. 2. Comparison of Ig concentrations in milk from cows with and without bacterial pathogens.

except IgG2, and actually milk is relatively low in immunoglobulin compared with sera and other body fluids [1].

In milk without pathogens, **IgA concentration** was 1.57 ng ml^{-1}, and in milk samples with pathogens – 1.26 ng ml^{-1}; the decrease of IgA was not significant – 17.74% ($p>0.05$).

The results of D.P. Mackie and E.F. Logan (1986) indicated that the IgA and IgM antibodies increase in infected quarter milk during the acute stages of inflammation [9]. M.Sandholm et al. (1995) reported that activity of IgA in mammary gland decreases due to fat globules, and suggested that mostly the production of IgA antibodies is local while IgG evolves the systemic immune system. It is known that IgA is not a good opsonion for phagocytosis and therefore has no significance in bacterial elimination [10].

In milk samples without pathogens, **IgM concentration** was 1.43 ng ml^{-1}, and in samples with pathogens – 1.69 ng ml^{-1}; the decrease of IgM was not significant - 15.38% ($p>0.05$).

Experiments from M.B. Barrio et al. (2003) indicated that IgA, like IgG1 and unlike IgM or IgG2, can not be considered as a major opsonion for phagocytosis [1].

In the investigated dairy heard, the changes in all classes of Ig concentration in milk with bacterial pathogens and without them were not significant (Figure 2). It could be due to the lack of acute infected glands. We suggest it is the reason why IgG and IgM did not transfer from blood to udder and milk, and IgA production did not increase in mammary gland tissue.

M.R. Williams and R.Halliday (1980) reported that in antibody production there is a balance between quality and quantity; cows with normally high serum immunoglobulin concentrations generally produce more antibody in response to challenge but this increase only compensates for the relatively lower avidity of their antibody for the antigen [14].

Comparison of Lf concentrations in negative for pathogen and positive for pathogen milk is given in Figure 3.

Lf concentration in samples without pathogens

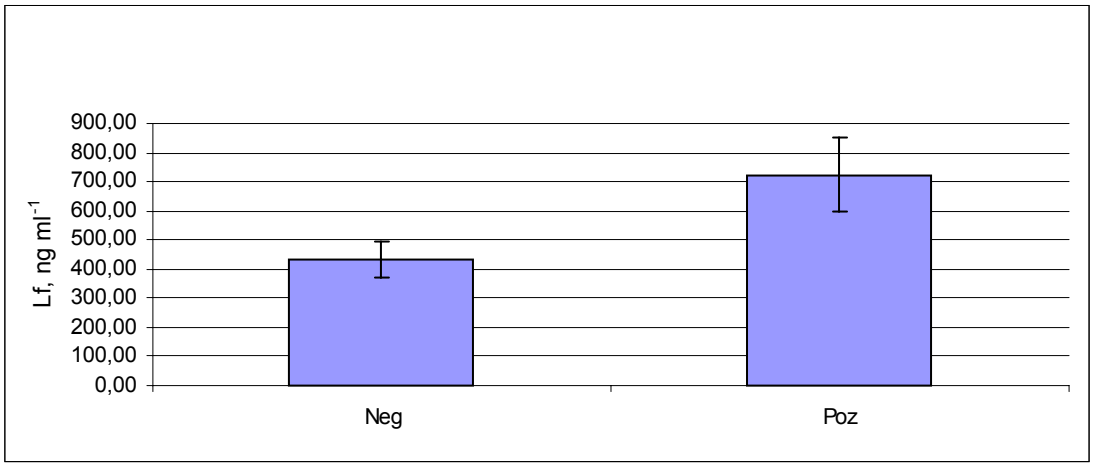

Fig. 3. Comparison of Lf concentrations in milk from cows with and without bacterial pathogens.

was 432 ng ml^{-1}, and in the pathogen causing subclinical mastitis samples – 724 ng ml^{-1}. The changes in Lf concentrations in milk without pathogens and with pathogens was significant – 40.33% ($p<0.05$). The same results are reported by other authors [2, 4, 8, 10, 11].

Traditionally, Lf has been considered one of the non-specific antibacterial factors in bovine milk. The Lf mediated antibacterial effect is due to its sequestration of iron from the environment where the bacteria reside so that the bacteria are deprived of iron for their growth. However, streptococci, including *S. uberis*, are more resistant to the antibacterial effects of Lf than Gram-negative bacteria, probably because of their low requirement for iron [13].

Results of this investigation show that major role for protection of mammary gland from being infected is played by the cellular immunity and antibacterial factor – Lactoferrin.

Conclusions

1. In the investigated dairy herd, 17.5% of cases were subclinical mastitis caused by C*oagulase negative staphylococci* (48.6%), *Streptococcus uberis* (32.10%) *and Staphylococcus aureus* (18.90%).

2. The sommatic cell count was significantly increased (55.55%) in milk with the presence of pathogens ($p<0.001$).

3. There were no significant changes in all classes of immunoglobulin concentrations in milk without pathogens and with pathogens.

4. The lactoferrin concentration was significantly increased (40.33%) in milk with pathogens ($p<0.05$).

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