Milk quality and its suitability for technological processing in cows with metritis

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Abstract. It has been established that the development of metritis in cows after providing obstetric aid as a result of abortion, eversion of the uterus or retention of the placenta is accompanied by an increased microbial and fungal background of the uterus. Without obstetric aid during delivery, only from 5 to 9 days after birth, 35.37% of cows had genitals contaminated with various pathogenic microflora. Already on the third day of puerperia, 14 species of bacteria were isolated from cows that were assisted in delivery, which in 74.5% of cases were contaminated with pathogenic microflora: S. aureus (in 15.5% of cases), E. coli (37%), K. pneumonia (12%), and S. pyogenes (10% of cases). The results of mycological studies revealed that A. fumigatus, C. albicans and C. crusei were isolated from cows after obstetrics. It was found out that the content of somatic cells (SC) r = 0.63, the activity of muramidase (AM) r = 0.84, lactoperoxidase (LPO) r = 0.65 and lactoferrin (LF) r = 0.66 change with a high degree of correlation. Milk from cows with metritis showed 2 times higher total bacterial contamination than milk from clinically healthy animals. Milk from sick cows has a reduced number of lactic acid organisms after the first day of storage. At the same time, acid formation occurred faster by 5.0–15.0% than that in control samples of milk prepared for production of lactic acid products. The acidity in milk fermented with Lactobacillus bulgarus was 12.0-13.3% higher than that in the control sourdough samples, and the cell viability of the symbiotic combination was an order of magnitude lower (2.5×106 versus 2.5×107) compared to the control samples of the lactic acid product.

1. Introduction

Improvement and further application of innovative technologies in dairy farming in all countries of the world are of great socio-economic importance because they provide the world's population with high-grade dairy products [1]. This happens due to the fact that milk producers are concerned about the problem of producing high-quality milk and dairy products, guaranteeing complete safety of finished products for consumption, and the problem of metritis in cows developing after calving [2]. Today, according to [3], it is known that metritis in cows with high productivity is diagnosed in 25.0-65.0% of cases, which significantly causes material and financial damage.

According to the results of studies [4], inflammation of the uterus in cows manifests itself as a
complication of early puerperia. According to [5], metritis of bacterial etiology develops as a result of exogenous infection of the vaginal mucosa, cervical canal and uterus with pathogenic microflora after providing obstetric aid in cows as a result of abortion, uterus eversion or placenta retention. The data published in [6] reveal the fact of a significant spread of treatment-resistant strains of opportunistic microbes in lactating cows on dairy farms of the Russian Federation.

Therefore, the effectiveness of treatment with the use of antimicrobial preparations turns out to be low and ineffective. It clinically presents by prolonged infertility of broodstock females and by a decrease in the quality of the milk with a low coefficient of suitability for its technological processing. According to studies [7], the most common pathogens for the genitals and mammary gland are *E. coli*, *S. uberis*, *S. aureus*, *S. dysgalactiae* and *S. Agalactiae*, and *C. Bovis*.

Thus, the overwhelming majority of metritis are of bacterial origin and in 60 - 75% of cases cause long-term sterility of dairy cows. In 80.0% of cases, they require antibacterial therapy with insufficient therapeutic efficacy and thereby reduce the genetic potential of the broodstock and the quality of dairy products. Cows with metritis give poor quality milk, which causes material and financial damage to producers and processors of milk and dairy products.

2. Materials and methods

There were 1450 cows under observation. The method of work consisted of a clinical study and laboratory assessment of milk from cows with metritis. The sanitary assessment of milk was carried out according to the results of cows’ clinical examination and a laboratory study of the secretion (the secretion reaction according to tests “Ketotest”, “Masttest” and sedimentation test).

Microbiological studies of sterile obtained samples were carried out by inoculation on standard Endo, CODA and Sabouraud media. Species and their identification were determined taking into account the morphological and biochemical properties, guided by the “Bergi Brief Guide to Bacteria” [4]. Fungi were identified by the methods of N.A. Spesivtseva [7]. The type of bacteria and the type of lactic acid bacteria were determined using plates “Diagnostic systems”, Nizhny Novgorod.

Determination of bacteria sensitivity to antibiotics was carried out by the method of diffusion in agar. To evaluate the bacterial milk composition, 149 samples from lactating cows with metritis and from 15 clinically healthy ones were examined. Sampling was carried out according to the method of V.I. Slobodyanik, N.T. Klimov and V.V. Podberezny (cit. [1]). Inoculations of meat infusion agar, meat infusion broth, Sabouraud, Endo media, Giss colored media were taken from these samples.

To assess the milk quality, the peroxidase activity was determined according to B.P. Pleshkov (cit. [1]). The concentration of lactoferrin (Lf) was determined using radial immunodiffusion according to G.A. Manhcini (cit. [1]), free hydroxyproline - spectrophotometrically according to M.A. Osadchuk (cit. [1]).

3. Results and discussions

After a microbiological study of the uterine contents from 200 cows with metritis, 14 species and 133 izosit were identified, which amounted to 64.63%. Among cows without obstetric aid during delivery, only from 5 ... 9 days after birth, 35.37% of cows had genitals contaminated with various pathogenic microflora. At the same time, they were contaminated with the microflora of *S. aureus* in 15.5% of cases, *E. coli* in 37%, *K. pneumonia* - 12%, and *S. pyogenes* in 10% of cases, respectively which, as a rule, were isolated in associations and were in synergistic relationships.

After obstetric aid as a result of abortion, the proportion of cases is 25%. The share of the uterus reversion or placenta retention is 50.6% and 61.2% of cases, respectively. The share of fungi ranges from 5 to 18% of cases. At the same time, 35.5% of cultures had hemolytic activity, which gave a positive plasma coagulation reaction (18.3% of cultures) and were pathogenic for laboratory animals (51% of cultures).

Results of mycological studies evidences that microscopic fungi were isolated from cows as a result of pathological delivery: *A. fumigatus* in 5.4% of cases, *C. albicans* in 9.6% of cases and *C. Crusei* in 18.2% of cases, respectively.
In the milk of cows with metritis, a decrease in the content of albumin and γ-lactoglobulin is observed, while the percentage ratio of β-lactoglobulin and α-lactoalbumin fractions increases (Figure 1).

![Figure 1. Indicators of albumin fractions in the milk of cows with metritis after pathological delivery.](image1)

According to Figure 2, the general pattern of changes in milk from cows with metritis compared with clinically healthy ones is an increase in the activity of muramidase (lysozyme M), immunoglobulin M and a decrease in the immunoglobulin fraction G. Thus, mammary gland of sick lactating cows is characterized by the activation of humoral defense mechanisms and a factor of nonspecific local resistance of lactoferrin, which is good for drinking milk and bad for processing it into lactic acid products.

The nature of the functional state of the mammary gland determines the features of the dynamics of somatic cells and lactoperoxidase activity of milk (Figures 3, 4). It was found out that the content of somatic cells (SC) r = 0.63, the activity of muramidase (AM) r = 0.84, lactoperoxidase (LPO) r = 0.65 and lactoferrin (LF) r = 0.66 change with a high degree of correlation. Neutrophils and lactocytes, being a source of lactoferrin in the udder secretion, release it from special granules due to degranulation during phagocytosis and destruction of these granules, which causes its high concentration in inflammatory processes in the uterus of sick cows (p < 0.01).
Thus, obtained results make it possible to control the course of the inflammatory process in the uterus of cows and predict the suitability of the milk for technological processing based on informative indicators of mammary gland secretion.

The research results indicate that the number of mesophilic anaerobic lactate-fermenting microorganisms depends on the level of total bacterial contamination of milk and the state of the genitals (p < 0.05). In cows with acute metritis, the total bacterial contamination of milk is 2 times higher than in clinically healthy cows (Table 1). In chronic metritis, the total bacterial contamination of milk was 287.9±19.5 thousand / cm$^3$, which is highly significant (p <0.01).

| Table 1. Microbiological indicators of milk from cows with metritis |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| Indicator                                      | Metritis         |                |                |
|                                                | acute (n = 20)   | occult (n = 12)| apparently healthy (n = 35) |
| Total bacterial number, thousand/cm$^3$       | 478.9±22.8       | 287.9±19.5**   | 227.3±25.4**   |
| Number of mesophilic aerobic and facultative anaerobic microorganisms, CFU/cm$^3$ | (5.1±0.12)×10$^4$ | (4.2±0.09)×10$^4$ | (4.0±0.07)×10$^4$** |
| Number of mesophilic aerobic lactate-fermenting microorganisms, microbial cell/cm$^3$ | 112.7±12.8         | 72.6±10.9**    | 65.6±9.56**    |

Milk from sick cows has a reduced amount of lactic acid organisms after the first day of storage. In the experimental samples, acid formation was faster by 5.0-15.0% than in the control samples. The acidity in the samples fermented with Lactobacillus bulgarus was 12.0 - 13.3% higher than in the control samples. The viability of the cells of the symbiotic combination was an order of magnitude...
lower (2.5×10⁶ versus 2.5×10⁷) than in the control samples of the lactic acid product.

4. Conclusion

It was found out that the uterus of cows that were assisted in delivery was contaminated with pathogenic microflora *S. aureus* in 15.5\% of cases, *E. coli* in 37\%, *K. pneumonia* - 12\%, and *S. pyogenes* in 10 \% of cases, respectively. It was determined that the content of somatic cells (SC) \( r = 0.63 \), the activity of muramidase (AM) \( r = 0.84 \), lactoperoxidase (LPO) \( r = 0.65 \) and lactoferrin (LF) \( r = 0.66 \) change with a high degree of correlation. Milk from sick cows has a reduced amount of lactic acid organisms after the first day of storage. In the experimental samples, acid formation was faster by 5.0-15.0\% than in the control samples. The viability of the cells of the symbiotic combination was an order of magnitude lower than in the control samples of the lactic acid product. The materials of the current work should be taken into account in the future, when studying fresh cows with metritis, as a concept of the milk suitability for technological processing, which correlates with a number authors’ opinion [8, 9].

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