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Metabolism features and milk microbiota of cows with mastitis in the Amur Oblast

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SUMMARY

The effective management practices of dairy farming are inextricably linked with the production of high quality milk, while the mastitis is one of the causes of reductions in milk yields and quality. The aim of the work was to study the microbiological profile of milk, the sensitivity of isolated microorganisms to antibiotics and metabolism features of cows with mastitis in the Amur Oblast. The following microorganisms were identified in the milk samples from cows with mastitis: *Staphylococcus epidermidis* (34.69%); microbial associations: *Streptococcus agalactiae* + *Escherichia coli* (32.65%) and *Staphylococcus saprophyticus* + *Streptococcus agalactiae* + *Escherichia coli* (30.61%); *Staphylococcus haemolyticus* (2.05%). Microorganisms isolated from the milk of mastitis-affected cows were susceptible to the following antimicrobials: *Escherichia coli* to cefotaxime (28.00 ± 2.00 mm) and ceftriaxone (27.50 ± 0.35 mm); *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus* to cefotaxime (35.50 ± 0.18 mm) and amoxicillin (35.10 ± 0.35 mm); *Streptococcus agalactiae* to tetracycline (27.60 ± 1.17 mm) and gentamicin (26.40 ± 0.99 mm). Metabolic disorders were observed in cows with mastitis. The albumin-globulin ratio was reduced (0.41), which is typical for various inflammatory processes; and a low albumin levels (29.00 ± 0.89%) suggested a decreased protein synthesis in hepatocytes. A moderate increase in gamma globulins (47.60 ± 1.05%) was associated with stimulation of the phagocytic mononuclear system. Water and mineral metabolism disorders were confirmed by low levels of calcium (1.80 ± 0.03 mmol/L) and magnesium (0.70 ± 0.02 mmol/L), which is a sign of many pathological conditions, and is associated with heavy lactation. Calcium-phosphorus ratio was reduced (0.82). Hematological indicators suggested hypochromic anemia (hemoglobin level – 100.60 ± 1.28 g/L, globular value – 0.60 ± 0.01). The leukogram was indicative of lymphocytopenia (36.90 ± 2.60%) and neutrophilia (rod-shaped neutrophils – 1.80 ± 0.13%, segmented neutrophils – 51.80 ± 2.51%).

Keywords: Amur Oblast, mastitis, cattle, mammary gland, opportunistic microorganisms, microbiological test, antibiotics, hematological tests

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Метаболические особенности и микрофлора молока при маститах у коров Амурской области

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РЕЗЮМЕ

Эффективное ведение молочного скотоводства неразрывно связано с получением молока высокого санитарного качества, одной из причин снижения производства которого являются маститы. Цель работы заключалась в исследовании микробиологического профиля молока, определении чувствительности выделенных микроорганизмов к антибиотикам и изучении метаболических особенностей при маститах у коров Амурской области. В животноводческих хозяйствах Амурской области в пробах молока от больных маститом коров в 34,69% случаев выявляли *Staphylococcus epidermidis*; в 32,65 и 30,61% проб обнаруживали ассоциации микроорганизмов: *Streptococcus agalactiae* + *Escherichia coli* и *Staphylococcus saprophyticus* + *Streptococcus agalactiae* + *Escherichia coli* соответственно; доля *Staphylococcus haemolyticus* составила 2,05%. Выделенные из молока от больных маститом коров *Escherichia coli* были чувствительны к цефотаксиму (28,00 ± 2,00 мм) и цефтриаксону (27,50 ± 0,35 мм); *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus* – к цефотаксиму (35,50 ± 0,18 мм) и амоксициллину (35,1 ± 0,35 мм); *Streptococcus agalactiae* – к тетрациклину (27,60 ± 1,17 мм) и гентамицину (26,40 ± 0,99 мм). У больных маститом коров отмечали нарушения метаболизма. Был снижен альбумин-глобулиновый коэффициент (0,41), что характерно для воспалительных процессов различной локализации, а низкий уровень альбуминов (29,00 ± 0,89%) свидетельствовал о снижении протеинсинтетической функции гепатоцитов. Умеренное увеличение гамма-глобулинов (47,60 ± 1,05%) было связано с раздражением системы фагоцитирующих мононуклеаров. Нарушения водно-минерального обмена характеризовались низким уровнем кальция (1,80 ± 0,03 ммоль/л) и магния (0,70 ± 0,02 ммоль/л), что отмечается при многих патологических состояниях, а также при усиленной лактации. Соотношение кальция и фосфора было

снижено (0,82). Гематологические показатели свидетельствовали о гипохромной анемии (уровень гемоглобина – 100,60 ± 1,28 г/л, цветового показателя – 0,60 ± 0,01). Лейкограмма указывала на лимфоцитопению (36,90 ± 2,60%) и нейтрофилию (палочкоядерных нейтрофилов – 1,80 ± 0,13%, сегментоядерных нейтрофилов – 51,80 ± 2,51%).

Ключевые слова: Амурская область, мастит, крупный рогатый скот, молочная железа, условно-патогенные микроорганизмы, микробиологическое исследование, антибиотики, гематологические исследования

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INTRODUCTION

The effective management practices of dairy farming are inextricably linked with the production of high quality milk, while the mastitis is one of the causes of reductions in milk yields and quality [1–3]. The disease can develop during all periods of the cow's udder functionality, but most often during lactation (36%) and drying off (23%). The critical periods include a dry period (16%) and a period immediately after calving (25%) [4].

Microorganisms can enter the mammary gland using different ways: galactogenic (most often), hematogenic and lymphogenic ways [5]. The galactogenic infection of the mammary gland with microorganisms occurs in high yielding dairy cows suffering from metabolic disorders and decreased immunity.

Feeding and maintenance conditions contribute to the bacterial manifestation of pathogenic and opportunistic microorganisms most often detected in case of mastitis: *Staphylococcus aureus*, *Staphylococcus xylosus*, *Staphylococcus epidermidis*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Escherichia coli*, *Pseudomonas aeruginosa* [1, 6–11].

Different generations of antibiotics are used to treat mastitis in cattle, but their misuse can lead to antimicrobial resistance [1].

Metabolomic analysis of cows with mastitis, hematological tests, bacteriological test of milk and antimicrobial susceptibility testing will contribute to correct and timely determination of treatment strategy.

The aim of the work is to study the microbiological profile of milk, the susceptibility of microorganisms to antibiotics and metabolic features in cows with mastitis in the Amur Oblast.

MATERIALS AND METHODS

The experiments were performed in livestock farms of the Amur Oblast using tie-stall housing. 84 milk samples from 21 Holstein cross-bred cows were tested during lactation (2–4 lactations).

Samples of alveolar milk were taken from each quarter of the udder using sterile procedure for testing. The preliminary diagnosis of mastitis and differentiation by types of mastitis was made using the rapid test-kit "Masttest" (LLC NPP "Agrofarm", Russia).

The bacteriological test was performed in accordance with the "Manual on the diagnosis, treatment and prevention of mastitis in cows"[1], "Recommended practices of bacteriological test of cow milk and udder secretion"[2], GOST 32901-2014 "Milk and dairy products. Methods of microbiological testing"[3].

Kessler and Endo media were used to isolate and identify *E. coli*. Smears from the isolated colonies were Gram stained. When staphylococci were detected in smears, the isolated culture was checked for catalase activity (gas production in response to hydrogen peroxide). To isolate streptococci from milk, a Kartashova solid medium was used. Streptococci were differentiated from staphylococci by the presence of catalase activity.

The susceptibility of bacteria to the following antimicrobials was tested on solid nutrient media using disc diffusion[4] susceptibility test: ampicillin, amoxicillin, novobiocin, neomycin, benzylpenicillin, cefotaxime, kanamycin, ceftriaxone, doxycycline, polymyxin, gentamicin, tetracycline.

Blood was taken from the caudal vein and stabilized with heparin for hematological testing. Metabolic panel serum tests were performed using StatFax 1904+R photometer (Awareness Technology, Inc., USA) and a set of reagents manufactured by company "Vital Development Corporation" (Russia). Hematological tests were performed according to generally accepted methods.

All experiments were carried out in strict accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123).

Statistical processing of the results was performed by standard methods using the MS Excel software package.

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RESULTS AND DISCUSSION

58.33% samples showed a clot of various densities following the reaction of "Masttest" surfactant (sulfonol) with the somatic cell nuclear DNA, which was indicative of the udder inflammation. The interactions of the milk mixture with the diagnosticum differed and gave the following results: 36.90% – inconclusive; 5.95% (+), 15.48% (++) – positive; 41.67% samples showed no reaction (negative result).

The udder examination revealed that most often (28.57%) the inflammatory process was localized in the posterior lobes of the udder. Lesions of the anterior left lobes (24.49%) came second, the affected anterior right lobes (18.37%) came third.

The greatest lesions (++ according to "Masttest" results) were found in the udder posterior lobes: 57.14% – in the left and 35.71% – in the right lobe.

The CFU/g value in the affected udder lobes [$(34.70 \pm 2.46) \times 10^5$, $n = 49$] differed significantly ($p < 0.001$) from the CFU/g value in the healthy udder lobes [$(15.40 \pm 0.86) \times 10^5$, $n = 35$].

Milk samples from cows with mastitis revealed: 1) *Staphylococcus epidermidis* (34.69%); 2) microbial associations *Streptococcus agalactiae* + *Escherichia coli* (32.65%) and *Staphylococcus saprophyticus* + *Streptococcus agalactiae* + *Escherichia coli* (30.61%); 3) *Staphylococcus haemolyticus* (2.05%).

The antimicrobial susceptibility test showed the highest susceptibility to the following antimicrobials ($n = 24$): *Escherichia coli* to cefotaxime (28.00 ± 2.00 mm, $p < 0.001$) and ceftriaxone (27.50 ± 0.35 mm, $p < 0.001$); *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus* to cefotaxime (35.50 ± 0.18 mm, $p < 0.001$) and amoxicillin (35.10 ± 0.35 mm, $p < 0.001$); *Streptococcus agalactiae* to tetracycline (27.60 ± 1.17 mm, $p < 0.001$) and gentamicin (26.40 ± 0.99 mm, $p < 0.001$).

Metabolic panel blood test showed increased levels of total protein (94.70 ± 0.75 g/L, $p < 0.001$) and gamma globulins (47.60 ± 1.05%, $p < 0.001$); decreased albumins (29.00 ± 0.89%, $p < 0.001$) and alpha globulins (10.60 ± 0.72%, $p < 0.001$). The levels of beta globulin (12.80 ± 0.69%), urea (4.40 ± 0.10 mmol/L) and creatinine (67.10 ± 0.87 μmol/L) were within physiological limits. The albumin-globulin ratio was reduced to 0.41 (limits 1.2–1.8).

Bilirubin levels (8.50 ± 0.44 μmol/L, $p < 0.001$) exceeded the normal limits, the aminotransferase activity (ALT and AST, 21.50 ± 0.61 and 97.40 ± 8.48 units/L respectively) was within physiological limits, and glucose concentration (1.00 ± 0.02 mmol/L, $p < 0.001$) was low.

Water and electrolyte metabolism analysis showed that against the normal values of phosphorus (2.20 ± 0.04 mmol/L) and potassium (4.50 ± 0.06 mmol/L), the levels of calcium (1.80 ± 0.03 mmol/L, $p < 0.001$) and magnesium (0.70 ± 0.02 mmol/L, $p < 0.001$) were decreased. The calcium and phosphorus ratio was 0.82.

Hematological tests revealed increased red blood cells [$(8.80 \pm 0.14) \times 10^{12}$/L, $p < 0.001$], low hemoglobin levels (100.60 ± 1.28 g/L, $p < 0.001$) and color index (0.60 ± 0.01, $p < 0.001$), which suggested anemia. White blood cells [$(7.70 \pm 0.40) \times 10^9$/L, $p < 0.001$] were within physiological limits, but the leukogram showed decreased lymphocytes (36.90 ± 2.60%, $p < 0.001$) and rod-shaped neutrophils (1.80 ± 0.13%, $p < 0.001$), as well as higher segmented neutrophil levels (51.80 ± 2.51%, $p < 0.001$).

As a result of the tests performed, it was found that the following microorganisms are identified in milk of cows with mastitis: *Staphylococcus haemolyticus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae* and *Escherichia coli*, which is consistent with the publications of other researchers [12–16].

Milk of cows with mastitis contained one representative of opportunistic microbiota (*Staphylococcus epidermidis* – 34.69% and *Staphylococcus haemolyticus* – 2.05%) and also microbial associations (*Streptococcus agalactiae* + *Escherichia coli* – 32.65% and *Staphylococcus saprophyticus* + *Streptococcus agalactiae* + *Escherichia coli* – 30.61%).

During their lifetime the isolated staphylococci and streptococci, depending on the type of secreted toxins, damage the membranes of erythrocytes, leukocytes, hepatocytes, cardiomyocytes, connective tissue cells; they suppress phagocytosis and dissolve torus demarcationis fibrin in the process of inflammation; disturb osmotic pressure and facilitate cell lysis; separately and together with enterobacteria they can cause exogenous infections [17].

The susceptibility testing of microorganisms isolated from milk of cows with mastitis revealed that the largest zones of inhibition of enterobacteria and staphylococci were formed in response to cefotaxime, and streptococci were sensitive to tetracycline and gentamicin. Therefore, in case of mammary gland inflammation caused by microbial associations, it is necessary to use several antimicrobials.

The analysis of protein metabolism in cows with mastitis showed lower albumin-globulin ratio (0.41), which is consistent with various inflammatory processes, and a low level of albumins (29.00 ± 0.89%) suggested a decrease in the protein synthesis in hepatocytes. A moderate increase in gamma globulins (47.60 ± 1.05%) was associated with stimulation of the phagocytic mononuclear system [18].

Disorders in water and mineral metabolism were characterized by low levels of calcium and magnesium, which is characteristic for many pathological conditions, as well as for heavy lactation. Calcium-phosphorus ratio, equal to 0.82, is typical for cows in dry period. For lactating cows, this value should be 1.2–1.8 [18].

General blood test showed hypochromic anemia, lymphocytopenia and neutrophilosis in animals, which is typical for inflammatory processes [19].

CONCLUSION

In livestock farms of the Amur Oblast, *Staphylococcus epidermidis* was detected in 34.69% of milk samples from cows with mastitis; microbial associations were revealed in 32.65 and 30.61% of samples: *Streptococcus agalactiae* + *Escherichia coli* and *Staphylococcus saprophyticus* + *Streptococcus agalactiae* + *Escherichia coli*, respectively; *Staphylococcus haemolyticus* was detected in 2.05% of samples.

Escherichia coli isolated from milk of mastitis-affected cows were sensitive to cefotaxime and ceftriaxone; *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus* to cefotaxime and amoxicillin; *Streptococcus agalactiae* to tetracycline and gentamicin.

Metabolic disorders were observed in cows with mastitis. The albumin-globulin ratio was reduced, which suggests the development of inflammatory processes, and low albumin levels were indicative of impaired protein synthesis in hepatocytes. A moderate increase in gamma

globulins was associated with stimulation of the phagocytic mononuclear system. Disorders in water and mineral metabolism were characterized by low levels of calcium and magnesium, which is characteristic for many pathological conditions, as well as for heavy lactation. Calcium-phosphorus ratio was low.

Hematological indicators suggested hypochromic anemia, and the leukogram was indicative of lymphocytopenia and neutrophilia.

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