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**UDC 636.084:636.088.31** **Research article**

# Assessment of the milk produced by mastitis-affected cows using an extruded feed supplement containing phytogenics

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**Abstract**

Background and Aim. For farmers and dairy producers, producing safe and high-quality milk is a top priority. Despite the enormous number of animals, there are several causes for low milk yield, such as the prevalence of livestock-borne diseases, widespread livestock farming techniques, and a low realization of the genetic potential of animals. Mastitis is a common infectious condition in dairy production that is the primary source of antibiotic residues in milk and has a substantial financial impact. The study aimed to investigate the effects of an extruded polyfunctional feed supplement called «BioFeed-P» which contains bitter wormwood extract on the physico-chemical and organoleptic characteristics of mastitic cow milk.

Materials and Methods. The research material included samples of cow's milk and feed additives made of extruded components that were created by barothermal treatment of grain forage, specifically barley, and oats, with the addition of the extruded BioFeed-P additive and wormwood bitter extract. The additive was created and manufactured at the NCJSC «S.Seifullin Kazakh Agrotechnical Research University» in the manufacturing and testing workshop of «NFT-KATU» LLP. Standard organoleptic and physicochemical techniques were used to study milk.

Results. It was demonstrated that feed additives improved the physicochemical characteristics of milk. There was a 3.1% increase in protein content and a 10% increase in milk fat content. Following the application of extruded additives with phytogenics, a 68% reduction in the number of somatic cells in the milk of mastitis-affected cows was noted.

Conclusion. When combined with bitter wormwood extract, the extruded BioFeed-P additive improves physicochemical parameters and dramatically lowers the number of somatic cells in milk from mastitis-affected cows.

**Keywords:** extrusion; phytogenics; BioFeed-P; bitter wormwood extract; cow mastitis; veterinary and sanitary expertise.

## Introduction

Numerous factors influence the quality and hygienic standards of cow's milk as both a food product and a raw material. These factors include the individual traits of the animals [1], the characteristics of their feed ration [2, 3, 4, 5], and the farm’s adherence to primary milk processing technology and cleanliness.

Statistically, non-communicable udder disorders are among the most common pathologies that cause significant economic harm to dairy farming. These disorders result in both quantitative and qualitative losses in raw milk. Moreover, milk from cows affected by mastitis not only loses quality but also becomes

contaminated with microorganisms, including pathogens that can cause food-borne illnesses in humans. Therefore, it is essential to have veterinary and sanitary expertise, as well as to conduct scientifically based sanitary assessments of milk from cows with mastitis, due to the potential risks to human health.

Antibiotics are commonly used to treat mastitis, but their unwarranted use raises concerns about the development of resistant microorganisms. This resistance can lead to the transfer of resistant bacteria from animals to humans [6]. Some farmers have found ways to circumvent regulatory controls by using antibiotic formulations that are not listed in the official guidelines. As stated by *Stein,* and *Chirilă* [7], we are currently facing a medical and public health crisis known as the post-antibiotic era. All antimicrobial medications used in clinical practice can lead to microbial resistance, often occurring almost immediately after new antibiotics are introduced. The extensive use of antibiotics in agriculture, including aquaculture, livestock, and poultry farming, as well as targeted contamination during food manufacturing, particularly in meat and sausage products, is a major contributor to the rise of antimicrobial-resistant bacteria.

In response to these concerns, Sweden became the first country to ban the use of certain antibiotics in animal feed in 1986 [8]. Following this, in 2006, European Union member states enacted Regulation No. 1831/2003, which prohibited the use of all growth stimulants and antibiotics in animal feed.

Alternatives to antibiotics, such as plant-based remedies, are gaining attention for preventing various types of mastitis. Recently, many researchers focusing on farm animals have investigated biologically active compounds known as probiotics and phytogenics [9]. These compounds, derived from plants, include tannins, flavonoids, essential oils, phytoncides, and other beneficial substances [10]. Studies have shown [11, 12] that phytobiotics can enhance immune function, significantly benefit animal health, and improve productivity. It is worth noting that phytobiotics are used in phytogenetics but typically for animals that are fed solely on these compounds.

*Sultanayeva* et.al. [13] examined the effects of the developed the extruded BioFeed-Padditive, on the quality and safety of goat milk. This additive contains various components, including resins, essential oils, glycosides such as salicin and populin, and flavonoids like pinostrobin. Additionally, it comprises phenolic carboxylic acids, phenolic glycosides, chalcones, leucoanthocyanins, organic acids (such as benzoic, ascorbic, and malic acids), and fatty oils essential for animal health. The aim of this study was to evaluate the effect of several feed additives on the milk productivity of Saanen goats.

**Materials and Methods**

The extruded polyfunctional feed additive was produced at the feed room «NFT-KATU» LLP based in the NCJSC «S.Seifullin Kazakh Agrotechnical Research University», Faculty of Veterinary and Livestock Technologies. The extrusion line at the production and testing shop includes the following machinery made in the Kostanay region (Republic of Kazakhstan). Kostanay (LLP «Agrotekhservice-12»): PD-2000 pneumatic hammer crusher, SG-800 kg mixer with load cells and cyclone, frequency-controlled feed hopper, PE-350 extruder, and PG-600 flat matrix pelletizer. Under the circumstances of LLP «Agrofirm Rodina» in the Akmola region, a scientific and financial study was carried out to determine the efficacy of using extruded polyfunctional feed additives in feeding dairy cows.

The study included 60 Holstein dairy cows with high yields, both black and brown. In total, 120 milk samples from mastitis-afflicted animals were collected. Cows in the mastitis group were given the extruded BioFeed-P additive for 15 days during treatment. Dairy production, as well as milk quality and safety, were examined and assessed.

Organoleptic properties of milk were determined by me and the student according to the standard Republic of Kazakhstan 1732-2007 «Milk and dairy products. Organoleptic method of determining quality indicators».

Dairy milk yield was studied by control milking for 15 days. Milk sampling was performed into sterile disposable bottles before the addition of the extruded BioFeed-P additive and during the subsequent 15-day experiment. The qualitative parameters of milk were determined using a milk analyzer «Expert Super Plem Kombo» (percentage of fat, protein, skimmed milk powder and lactose, acidity, density, temperature, freezing point, conductivity), the number of somatic cells on the analyzer «Ekomilk Scan».

Latent mastitis of animals was preliminarily determined using the California test in LLP «Agrofirm Rodina» in the Akmola region.

The animal experiments were approved by the local ethical committee of the Faculty of Veterinary Science and Technology of Animal Husbandry of NCJSC «S.Seifullin Kazakh Agrotechnical Research University», protocol №6 from 28.03.2022.

Statistical analyses were performed with SAS, version 9.4 (SAS Institute, Cary, NC). Normality of distribution of measured variables was tested with the Kolmogorov–Smirnov test. Since the observed values showed significant deviation from normal distribution, a logarithmic transformation was applied to all variables, expect for freezing point. After transformation, the differences between pre- and post-treatment were evaluated with the General Linear Model (GLM), with treatment as a fixed, and individual animal as a random factor to account for between-animal variability.

**Results**

*Organoleptic tests of milk*

Organoleptic examination of milk from cows with mastitis showed that the samples met the established standards both before and after using the extruded BioFeed-Padditive (Table 1). The taste was not determined because the milk was obtained from cows with mastitis. Most of the animals had latent mastitis, which was pre-determined by the California test.

Table 1 – Organoleptic indicators of milk before and after using the extruded BioFeed-P additive

| Indicator | Observed characteristics (n=120) | |
|---|---|---|
| | pre-treatment | post-treatment |
| consistency | uniform, non-sticky | homogeneous, not thick, non-sticky |
| bloom | Milky white to creamy, without impurities | milky white to creamy, free of foreign matter |
| smell | typical for cow's milk | characteristic of cow's milk, without extraneous odors, including those of applied phytopreparations |

The results of physicochemical tests of milk samples collected from mastitic cows before and after using the extruded BioFeed-P additive are summarized in Table 2.

Table 2 – Physico-chemical parameters of milk of mastitic cows using the extruded BioFeed-P additive

| Indicator | Norms* | Measured values (n=15) | | P-value |
|---|---|---|---|---|
| | | pre-treatment | post-treatment | |
| Fat, % | ≤2.8 | 4.2±0.49 | 4.56±0.37 | 0.440 |
| SMP, % | ≤ 8.2 | 8.75±0.09 | 8.73±0.12 | 0.975 |
| Density, °A | ≤ 27 | 29.6±0.69 | 29.24±0.71 | 0.780 |
| Lactose, % | 4.0-5.5 | 4.78±0.05 | 4.77±0.07 | 0.956 |
| Salts, % | 0.6-0.9 | 0.73±0.01 | 0.73±0.01 | 0.954 |
| Protein, % | ≤ 2.8 | 3.22±0.01 | 3.21±0.03 | 0.954 |
| Temperature, °C | - | 21.01±0.09 | 22.92±0.28 | <0.001 |
| Freezing point, °C | ≤ -0.505 | -0.56±0.01 | -0.56±0.01 | 0.478 |
| Added water, % | 0 | 0 | 0 | |
| pH | 6.4-6.7 | 7.31±0.03 | 7.35±0.02 | 0.361 |
| Somatic cell counts in 1 $cm^3$ | >750 000** | 402.11±106.68 | 127.0±6.36 | 0.017 |

Notes: * CU TR 033/2013 «On safety of milk and dairy products».
** are not in force in the Republic of Kazakhstan. The applicable standard for top-grade milk is 200,000 units.
Data are presented as the Microsoft Excel 2021 program for calculations.

It was shown that latent mastitis has no significant effect on the physico-chemical parameters of milk in the context of averaged parameters for the group, except for an increase in the content of somatic cells in milk. It is considered that milk with somatic cell content of 500 000 units in 1 ml and higher is obtained from cows with mastitis. At present, the norms for Kazakhstan are 200,000 somatic cells in 1 ml for top-grade milk and 1,000,000 cells for first and second-grade milk, as set out in CU TR 033/2013 «On the safety of milk and dairy products» in the edition before the changes of 31.12.2019 (CU TR 033/2013 «On the safety of milk and dairy products», approved by Decision of the Commission of the CU from 28.05.2010 №299).

The results showed no significant changes in majority indicators between pre-treatment and post-treatment values. SMP remained nearly constant with pre-treatment at 8.75 ± 0.09 and post-treatment at 8.73 ± 0.12, both close to the norm of ≤8.2%. Similarly, density showed no significant change, with pre-treatment at 29.6 ± 0.69 and post-treatment at 29.24 ± 0.71, both slightly above the norm of ≤ 27°. Lactose and salt levels also remained stable, with pre-treatment values of 4.78 ± 0.05 and 0.73 ± 0.01, respectively, and post-treatment values of 4.77 ± 0.07 and 0.73 ± 0.01, showing no significant differences. Temperature, however, increased significantly from 21.01 ± 0.09 °C pre-treatment to 22.92 ± 0.28 °C post-treatment ($P<0.001$). Other indicators, including protein, freezing point, pH, and added water, also showed no significant differences, while somatic cell count decreased significantly.

After application of the extruded BioFeed-P additive, the number of somatic cells in the experimental group of cows decreased by 68%, so the average values before the experiment were 402.11±106.68 units/ml, after the treatment measures – 127.0 ± 6.36 units/ml, which corresponds to the required standards for milk of the highest grade. These changes indicate a decrease in the level of udder inflammation and improved milk safety, which in turn proves the effectiveness of phytogenics application in mastitis of dairy cows. The decrease in the number of somatic cells is the main proof that the developed feed additives containing phytogenics are effective in the treatment of mastitis, as somatic cells are an indicator of udder inflammation in animals. The decrease in their number indicates that the treatment was effective and reduced the inflammatory process in the udder of the experimental group of animals.

Addition of BioFeed-P additive also resulted in slight, but not significant changes in the fat index by 10% from 4.2 ± 0.49 to 4.56 ± 0.37, and in protein index by 3.1%, from 3.21 ± 0.03 to 3.22 ± 0.01%.

**Discussion and Conclusion**

Milk obtained from cows suffering from mastitis does not meet the organoleptic, physicochemical, and sanitary standards outlined in regulatory documentation, rendering the product unsuitable for food purposes. At the production facility of «Agrofirma Rodina» LLP, significant attention is dedicated to ensuring the quality and safety of milk. Daily, during the milking process, specialists perform individual checks on the animals for signs of mastitis and carry out the California mastitis test, which helps identify the latent form of the disease. If a positive result is obtained, affected cows are separated into distinct groups for treatment, and their milk is collected in a separate tank.

Our experiments demonstrated that after two weeks of administering the extruded BioFeed-Padditive and an extract of bitter wormwood to the cows with mastitis, the organoleptic and physicochemical parameters of the milk improved significantly across almost all key indicators.

Previously, we examined the effectiveness of the phytobiotic feed additive BioFeed-P in enhancing animal productivity. This preparation is a source of biologically active compounds, primarily flavonoids, which include pinostrobin, tectochrysin, pinocembrin, and chrysin. Flavonoids have a broad spectrum of effects on the body, including anti-inflammatory, analgesic, and anti-allergic properties. They are also characterized by hepatoprotective, cytostatic, apoptotic, estrogenic, and anti-estrogenic effects. Following the application of BioFeed-P to boost productivity, the animals exhibited increased immunity, improved metabolic processes, and enhanced quality indicators in goat milk (protein levels increased by 1.3%, lactose by 3.32%, and somatic cell count decreased by up to 40%). The average daily weight gain of fattening steers in the experimental group surpassed that of the control group by 36%.

Enriching feed with phytobiotic supplements further enhances the immune status of animals, which contributes to increased milk production. In earlier studies, Balji and Knicky used balsamic poplar bud extract to enhance the meat productivity of young steers. The effects of various phytobiotics on animal performance were reviewed.

Another phytogenic used in our experiments was an extract of bitter wormwood. According to international scholars, bitter wormwood contains various biologically active substances that produce specific physiological effects when introduced into the body. These substances include bitter glycosides, essential oils, flavonoids, phytoncides, alkaloids, organic acids, vitamins, tannins, and resins. Mugwort notably contains the essential oil absintol, which ranges from 0.12% to 2%.

The substance includes oxygen derivatives of bicyclic terpenes, sesquiterpenes, and monocyclic terpenes, featuring 10-25% thujol (C6H18O) and up to 10% thujone (C10H14O). Other components include pinene (C10H16), pellandrene, cineole, borneol, β-caryophyllene, β-sepinene, bisabolene, and hamazulene. Among the bicyclic sesquiterpenes is cadinene (C15H24), along with monocyclic catolactones such as ketopelanolide A, ketopelanolide B, and oxipelanolide. The bitter glycosides are represented by ten sesquiterpene lactones: absintin, anabsintin, guaianopides, artabsin, arborescin, and others, which contribute to the herb's distinctive bitter flavor. The aglycone of the glycosides, artaboin, can be processed to yield hamazulene. Additionally, the flavonoid artemetin (artemisinin) has been isolated from the herb, while inulin (a phytoncide) has been identified in the roots. The organic acids present include succinic and malic acids, along with esters of thujol alcohol with acetic, isovaleric, and palmitic acids, as well as vitamins C, K, B6, and provitamin A [14, 15].

Mugwort is recognized for its antimicrobial properties, demonstrating the ability to inhibit the growth of pathogens associated with mastitis, including Escherichia coli and Staphylococcus aureus. This effect is attributed to the essential oils, which disrupt bacterial metabolism and cell wall integrity, thereby hindering reproduction [16, 17]. Moreover, wormwood bitter enhances feed intake, subsequently improving the overall condition of the animal.

BioFeed-P phytobiotic comprises various compounds, including salicylates known for their potent anti-inflammatory properties. These compounds aid in reducing inflammation within the mammary gland and help prevent further tissue damage.

Overall, both BioFeed-P phytobiotic and wormwood extract possess a rich chemical composition, and their combined properties make them effective auxiliary agents in treating latent mastitis in cows. The use of extruded granules as a carrier for phytogenics minimizes the digestive burden and enhances metabolic processes during the animal's illness, positively influencing the recovery of cows. The use of the phytobiotic preparation «BioFeed-P» and wormwood extract in extruded granule form has been suggested to enhance the health, milk quality, and productivity of cows suffering from mastitis. Furthermore, these additives facilitate a swift recovery and boost immunity following illness.

Dairy farms, as well as private subsidiary farms, instead of antibiotic drugs, it is desirable to use grain fodder extruded and containing phytobiotic feed additives, such as BioFeed-P, extract of bitter wormwood or others. Phytobiotics used in our experiments contribute not only to the improvement of qualitative and quantitative indicators of milk, but also have therapeutic and preventive properties in mastitis of cows.

**Authors' Contribution**

GA: conducted laboratory research, prepared the initial text of the article; YuB: developed the aim and objectives of the work, as well as determined the methodology of the experiment, carried out the production of phytogenics; LS: participated in the experimental part of the work, responsible for the final revision of the text and design of the article in accordance with the requirements of the publication; GZ: performed statistical analysis and reviewed the manuscript. All authors read, reviewed and approved the final manuscript revision.

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**Conflicts of Interest**

The authors declare no conflicts of interest.

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