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The Effectiveness of *Helianthus tuberosus* in the Correction of Subclinical Mastitis in Cows

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ABSTRACT

Subclinical mastitis is a common disease in dairy cattle and is frequently associated with impaired metabolic status, reduced productivity, and deterioration of udder health. This study evaluated the biological properties and adjunctive use of *Helianthus tuberosus* phytogranules in lactating cows with subclinical mastitis. A total of 127 Simmental cows were screened using the California Mastitis Test (CMT), and 28 cows showed positive reactions, corresponding to a prevalence of 22.0%. Diagnosis was based on CMT screening only; somatic cell count and bacteriological culture of milk samples were not performed. Both groups received Maxivit and udder massage, while the experimental group additionally received *Helianthus tuberosus* phytogranules at 220-250 g/day for 20 days. Antimicrobial activity was evaluated by agar diffusion against reference bacterial strains, and antiradical activity was assessed using gallic acid as a positive reference compound. The phytogranules showed weak to moderate dose-dependent antibacterial activity, with the highest inhibition at 10% concentration, but demonstrated minimal antiradical activity. Clinically, cows receiving phytogranules showed faster improvement, with negative CMT results by day 6, whereas 90% of control cows became CMT-negative by day 8. Favorable changes were observed in selected hematological and biochemical parameters, including hemoglobin, erythrocytes, leukocytes, glucose, and total protein. These preliminary findings suggest that *H. tuberosus* phytogranules may support metabolic recovery when used as an adjunctive feed additive in cows with subclinical mastitis, rather than acting as a direct antimicrobial or antioxidant treatment.

Keywords: Dairy cattle, Subclinical mastitis, Phytogranules, *Helianthus tuberosus*, Pyrolysis, Blood, Metabolism.

INTRODUCTION

Subclinical mastitis is one of the most important diseases affecting dairy cattle because it develops without visible inflammatory signs in the udder or obvious changes in milk appearance. Despite its latent course, it can reduce milk yield, alter milk quality, increase treatment costs, and contribute to culling losses (Yerzhanova et al. 2023; Kolech et al. 2025). Early detection and appropriate management of subclinical mastitis are therefore essential for maintaining udder health and productivity in dairy herds. The development of mastitis is influenced by pathogen exposure, milking hygiene, housing conditions, lactation stage, parity, milk yield, and the general immune and metabolic status of the cow (Mushtaq et al. 2018; Khasanah et al. 2021; Nissanova et al. 2025). The risk is particularly high during the transition and early lactation periods, when the energy requirement for colostrum and milk production increases sharply while feed intake may be insufficient (Argaw 2016; Fernandes et al. 2021).

Negative energy balance, impaired carbohydrate and protein metabolism, altered mineral status, and reduced resistance can predispose cows to inflammatory disorders, including mastitis (Gutiérrez et al. 2019). These metabolic disturbances may also be reflected in hematological and biochemical blood parameters, including glucose, total protein, leukocytes, erythrocytes, and liver enzyme activity (Uyarlar et al. 2018; Seisenov et al. 2023; Kulpiisova et al. 2024). Although antimicrobial agents remain important in mastitis control, their prolonged or inappropriate use may contribute to antimicrobial resistance and milk residues (Adkins and Middleton 2018).

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In subclinical mastitis, treatment of cows is aimed at suppressing pathogenic microflora, removing the accumulated exudate from the damaged mammary gland, restoring its function, and general strengthening of the organism (Shkromada et al. 2022). According to some authors, the main types of microorganisms causing mastitis are *Staphylococcus aureus* and *Escherichia coli* (Galfi et al. 2017; Kaseke et al. 2023a). Chemotherapeutic agents containing antibiotics, sulfonamides, or nitrofuran preparations have been most widely used for the treatment of this disease. However, prolonged and unsystematic use of antimicrobial agents has led to a significant decrease in the effectiveness of mastitis treatment due to the development of resistance in the microorganisms causing the disease (Jakupov et al. 2023; Kukeyeva et al. 2023). In addition, intracisternal administration of antibacterial drugs reduces the resistance of the mammary gland (Yang et al. 2019). Moreover, antibiotics and other chemotherapeutic drugs, being excreted with milk, are toxic to humans and can cause allergic reactions and dysbiosis in people (Benedet et al. 2020; Kukeyeva et al. 2023). A promising direction in the development of preparations for the treatment and prevention of mammary gland diseases in cows is the use of medicinal plants (Hashemzadeh-Cigari et al. 2014; Hogeveen et al. 2019). Plant-derived antibacterial agents can serve as important sources of new antimicrobial drugs, efflux pump inhibitors, compounds targeting bacterial virulence, or can be used in combination with existing medicinal products (Amber et al. 2018)

Helianthus tuberosus L., commonly known as Jerusalem artichoke, is rich in inulin-type fructans, carbohydrates, fiber, minerals, and other biologically active substances. Inulin and related fructans have prebiotic properties and may influence intestinal fermentation, nutrient utilization, carbohydrate metabolism, and mineral absorption (Tian et al. 2019; Hughes et al. 2022). These properties make *Helianthus tuberosus* a potentially useful feed component for supporting metabolic balance in lactating animals, especially during periods of increased physiological demand (Ersahince and Kara 2017). In Kazakhstan, Jerusalem artichoke can be cultivated under local climatic conditions and may serve as a locally available source of plant-based feed additives.

The present study aimed to evaluate the antimicrobial and antiradical properties of locally produced *Helianthus tuberosus* phytogranules and to assess their adjunctive use for correcting selected hematological and biochemical parameters in lactating cows with subclinical mastitis.

MATERIALS AND METHODS

Ethics statement

This study was performed in compliance with national and international laws and guidelines on animal handling, and the experimental protocol was approved by the Institutional Review Board and Ethics Committee of the S. Seifullin Kazakh Agrotechnical Research University (permit number: 2).

Preparation and composition of phytogranules

The phytogranules were obtained at LLP Scientific and Production Technical Center «Zhalyn» (Almaty, Kazakhstan). The formulation consisted of *Helianthus tuberosus* raw material, carbonized rice husk obtained by pyrolysis, feed salt, and carboxymethylcellulose as a binding component (Fig. 1). The composition of the phytogranules was as follows: Jerusalem artichoke tuber powder, 3 %; Jerusalem artichoke herb powder, 85 %; carbonized rice husk, 5%; feed salt, 5%; and carboxymethylcellulose, 2%. To further characterize the phytogranule product, the main chemical components of the final *H. tuberosus*-based phytogranules were determined. The product contained a high proportion of inulin, moderate levels of protein and crude fiber, and measurable amounts of ash and mineral elements, including potassium, calcium, phosphorus, magnesium, and sodium (Table 1).

A B

Fig. 1: A – *Helianthus tuberosus* in nature; B – phytogranules of *Helianthus tuberosus.*

Table 1: Chemical composition of phytogranules based on *Helianthus tuberosus*

Component	Content, % (w/w)
Inulin	60–80
Protein	7–10
Crude fiber	5–10
Ash	5–7
Potassium	0.4–0.6
Calcium	0.02–0.05
Phosphorus	0.05–0.10
Magnesium	0.02–0.04
Sodium	up to 0.02

The Jerusalem artichoke component was included as the main source of plant carbohydrates, inulin-type fructans, fiber, minerals, and other biologically active substances. Carbonized rice husk was included as a phytosorbent component, feed salt as a mineral component, and carboxymethylcellulose as a binder to improve granule structure.

Rice husk consists of 70–72 % organic compounds and 28–30% inorganic. The composition of organic compounds includes, % (by mass): C = 39.8–41.1; H = 5.7–6.1; O = 0.5–0.6; N = 36.6 - 37.4. The main carbohydrates of rice husk are cellulose (fiber) and hemicellulose containing pentosans.

During pyrolysis of rice husk (heating to temperatures of 300–500°C without air access), a composite of carbon and silicon dioxide is formed, retaining the shape of the original husk, an aqueous distillate of liquid resins, gas, acetic acid, methyl alcohol, acetone, and about 2% carbonates of alkali and alkaline earth metals.

Experimental animals and feeding conditions

The studies were conducted on lactating cows of the Simmental breed, in the amount of 127 heads, kept under the conditions of the farm «Viktor» of Zhaksyn district of Akmola region. The animals selected for screening were mainly multiparous cows in their second to fourth parity. At the time of examination, cows were at different stages of lactation, with days in milk ranging from approximately 35 to 210 days. According to lactation stage, the herd was classified into early lactation, peak lactation, and late lactation groups. Among the 28 cows that tested positive for subclinical mastitis, 4 cows were in early lactation, 22 cows were in peak lactation, and 2 cows were in late lactation. The average daily milk yield of cows included in the treatment trial ranged from 9.5 to 13.0 L/day, with a mean value of approximately 11.0±0.5 L/day. Body condition score was assessed using a 5-point scale and ranged from 2.75 to 3.50, indicating that the animals were generally in moderate body condition. According to farm records and veterinary examination, the selected cows had no visible signs of clinical mastitis, systemic illness, lameness, metabolic disease, or recent antimicrobial treatment before inclusion in the study. Only cows with positive California Mastitis Test results and without visible abnormalities of the udder or milk were included in the experimental and control groups. 50% of the cows' ration by weight consisted of haylage; as a source of energy in the ration of cows in this physiological period, barley, wheat, and corn were added, which provide 25% of dry matter from the total amount. The protein adequacy of the cows' ration is represented by rapeseed meal and sunflower cake, which provide 9% of dry matter from the total amount.

Diagnosis of subclinical mastitis

Detection of subclinical mastitis was carried out by examining cows using mastitis tests with the California mastitis test (manufacturer DeLaval, Sweden). The sedimentation test was used as a control. Milk from each udder quarter was mixed with an equal volume of CMT reagent and evaluated according to the manufacturer's instructions. Samples showing visible thickening or gel formation of the milk-reagent mixture were considered CMT-positive and classified as indicative of subclinical mastitis in the absence of visible udder inflammation or gross milk abnormalities. Out of the examined population, 28 cows showed a positive reaction to the test, that is, 22% of cows had a latent form of mastitis. Somatic cell count and bacteriological culture of milk samples were not performed in the present study.

Experimental design

Two groups were formed from animals positively reacting to the test, the first (experimental) and the second (control) (n=14 in each), selected according to the principle of paired analogues. Cows of the first group received phytogranules; animals of the second group were not fed phytogranules. Both groups received Maxivit, an injectable multivitamin supportive preparation containing vitamins A, D3, E, and B-group vitamins, including thiamine, riboflavin, nicotinamide, pyridoxine, cyanocobalamin, D-panthenol, and biotin, at a dose of 5 mL per animal by single intramuscular administration. No antibiotics or intramammary antimicrobial preparations were administered during the study. Before adding phytogranules to the cows' ration, they were dosed and weighed per head, taking into account the daily milk yield and live weight of the cows. The average live weight of cows was 475–485 kg; based on this, cows received 220–250 g of phytogranules daily. Phytogranules were added to the ration in the morning, once a day. The duration of supplementation of the cows' ration with phytogranules was 20 days. Animals were monitored daily. Before the beginning and after completion of the study, blood samples were taken from cows of both groups for hematological and biochemical analysis using automatic analyzers Hemax 330 Vet and BioChem FC 200.

Antiradical activity

Antiradical activity of the *Helianthus tuberosus* phytogranules was determined spectrophotometrically using gallic acid as the positive reference compound. Briefly, the phytogranules were ground into a fine powder, and 1.0 g of the sample was mixed with 10 mL of distilled water, shaken for 30 min at room temperature, and filtered through filter paper. The obtained filtrate was used to prepare test solutions at concentrations of 0.1, 0.25, 0.5, 0.75, and 1.0 mg/mL. Gallic acid solutions at the same concentrations were prepared and used as the positive control. The blank consisted of distilled water and reagent without the test sample, while the control contained reagent solution without antioxidant sample. After mixing, the reaction mixtures were incubated for 30 min at room temperature in the dark. Optical density was measured at 517 nm using a spectrophotometer. All measurements were performed in triplicate. Antiradical activity was calculated using the following formula:

$$\text{Antiradical activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control solution and A_{sample} is the absorbance of the tested phytogranule or gallic acid solution.

Antimicrobial activity

Evaluation of antimicrobial activity by the agar diffusion method. The antimicrobial activity of the medicinal form of *Helianthus tuberosus* in 2.5%, 5% and 10% concentrations on reference test microorganisms: facultative anaerobic gram-positive cocci, *Staphylococcus aureus* ATCC 6538 B-RKM 0470, facultative anaerobic gram-negative rods *Escherichia coli* ATCC 25922 B-RKM 0447, and *Serratia marcescens* B-RKM 0832 was studied by the agar diffusion method (wells). The strains of microorganisms used in the study were taken from the Republican collection of standard cultures of microorganisms. The reference preparation is benzylpenicillin sodium salt. The agar diffusion method is based on the evaluation of the inhibition of the growth of test microorganisms by a certain concentration of the studied substance.

For the study, pure cultures of the studied strains were obtained, which were grown on shaken meat-peptone agar in a liquid medium with pH 7.3±0.2 at a temperature of 30–37°C for 24–48 hours. A standard bacterial suspension was prepared by diluting the culture in sterile dimethyl sulfoxide (DMSO) in a ratio of 1:1000. 1.0 mL of the corresponding bacterial suspension was introduced into plates with appropriate selective nutrient media for the studied strains and inoculated by the “lawn” method. After drying on the agar surface, wells of 6.0 mm size were formed, into which 20 μL of solutions of crushed granules *of Helianthus tuberosus* in 3 concentrations (2.5%, 5% and 10%) were added. Water for injections, with which the samples were diluted in equal amounts, was used as a control. The inoculated bacteria were incubated for 24 hours at 37 °C. The antimicrobial activity of the samples was evaluated by the diameter (mm) of the zones of growth inhibition of the studied strains around the wells, including the well diameter: absence of a growth inhibition zone – the studied culture is not sensitive to this concentration of the sample; diameter of growth inhibition zones less than 10 mm and continuous growth in the plate were evaluated as absence of antibacterial activity, 10–15 mm – weak activity, 15–20 mm – moderately expressed activity, more than 20 mm – expressed activity. Each sample was tested in three parallel experiments at concentrations of 2.5, 5, and 10, respectively. For comparative characterization of antimicrobial activity, an antibiotic solution was used: benzylpenicillin sodium salt.

Statistical analysis

Data were entered into a Microsoft Excel spreadsheet, edited, and subsequently analyzed using the Python programming environment (Jupyter Notebook, v 3.9.7). Data distribution was assessed using the Shapiro-Wilk test. For normally distributed paired data, within-group before-and-after comparisons were performed using the paired t-test; for non-normally distributed paired data, the Wilcoxon signed-rank test was used. Between-group comparisons were performed using the independent-samples t-test or Mann-Whitney U test, depending on data distribution. Antimicrobial inhibition zones were compared using one-way ANOVA, followed by post hoc analysis. Recovery time was compared between groups using the Mann-Whitney U test. A P-value<0.05 was considered statistically significant.

RESULTS

Prevalence of subclinical mastitis

Diagnosis of subclinical mastitis was carried out using the California Mastitis Test (manufacturer DeLaval, Sweden). The highest percentage of positive tests was observed in the group of cows at peak lactation – 78.6% (Fig. 2).

Screening for biological activity included the determination of antimicrobial and antiradical activity (Fig. 3). The results of the study of the antimicrobial activity of *Helianthus tuberosus* at concentrations of 2.5, 5 and 10% using the agar diffusion method are presented in Table 2.

Antimicrobial activity of *Helianthus tuberosus*

As a result of the study of antimicrobial activity, it was established that *Helianthus tuberosus* at a dilution of 2.5% (11.5±0.2) and the extract at a concentration of 5% (12±0.2) exhibited weak antibacterial activity against the gram-negative test strain Escherichia coli. Slightly higher activity was observed against *Staphylococcus aureus* and *Serratia marcescens* at a 5% dilution. The highest antibacterial activity of *Helianthus tuberosus* was detected at a 10% dilution and amounted to 20±0.4. Thus, *Helianthus tuberosus* phytogranules demonstrated weak to moderate in vitro inhibitory activity against the tested reference bacterial strains; however, no milk-derived field isolates were examined in this study.

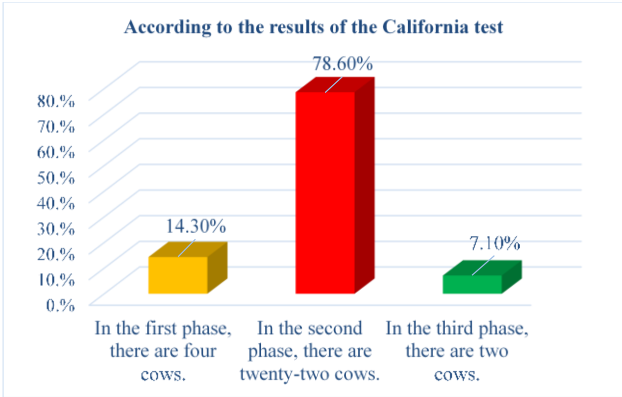

Fig. 2: Degree of prevalence of subclinical mastitis in dairy cows (n=127) in different phases of lactation (according to the results of the California test).

Helianthus tuberosus, at dilution concentration 10%

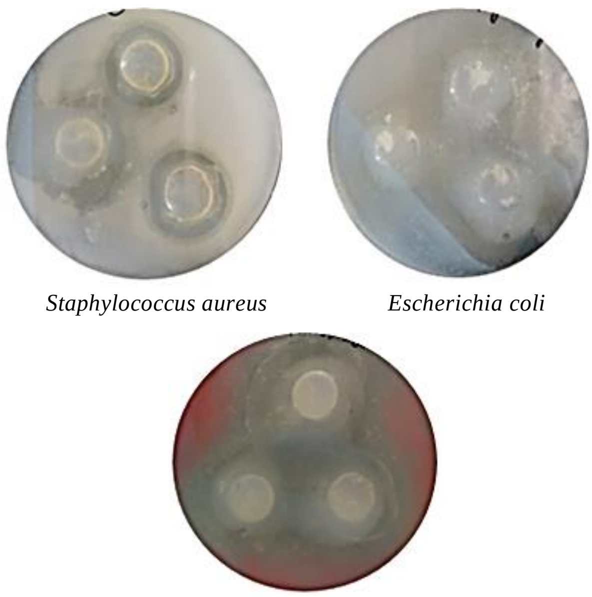

Fig. 3: Antimicrobial activity of *Helianthus tuberosus.*

The detection of the antiradical activity of *Helianthus tuberosus* was required to assess its antioxidant potential and possible practical value in veterinary pharmacology, since high antiradical activity indicates that the plant contains antioxidants and can protect the body's cells from lipid peroxidation and premature aging. Metabolic disorders in animals and, against this background, the occurrence of such diseases as mastitis and ketosis may lead to oxidative stress in the body; therefore, the search for plants with high antiradical activity is necessary for prevention or treatment. However, in our studies, the dosage form of the investigated plant, *Helianthus tuberosus*, did not demonstrate such an effect.

Table 2: Antimicrobial activity of dosage forms of *Helianthus tuberosus* at the tested concentrations

Sample codes	*Staphylococcus aureus* ATCC 6538 B-RKM 0470	*Escherichia coli* ATCC 25922 B-RKM 0447	*Serratia marcescens* B-RKM 0832
1	2	3	4
Negative control water for injection/DMSO	0.0±0.0	0.0±0.0	0.0±0.0
Helianthus tuberosus at a dilution concentration 2.5 %	11±0.3*	11.5±0.2*	14±0.2*
Helianthus tuberosus at a dilution concentration 5 %	13±0.1*	12 ±0.2*	15±0.1*
Helianthus tuberosus at a dilution concentration 10 %	14±0.3*	13±0.1*	20±0.4*
Benzylpenicillin sodium salt	15±0.1*	14±0.1*	16±0.2*

Note: Values are presented as mean±SEM. The negative control consisted of the solvent/diluent used for preparing the phytogranule solutions and showed no inhibition zones. * indicates a statistically significant difference compared with the negative control, P<0.005.

Antiradical activity of *Helianthus tuberosus*

Based on the analysis of the values presented in Fig. 4 and 5, the activity of gallic acid showed very high antiradical activity at concentrations of 0.1–1.0 mg/mL at 80.22–83.88%, respectively.

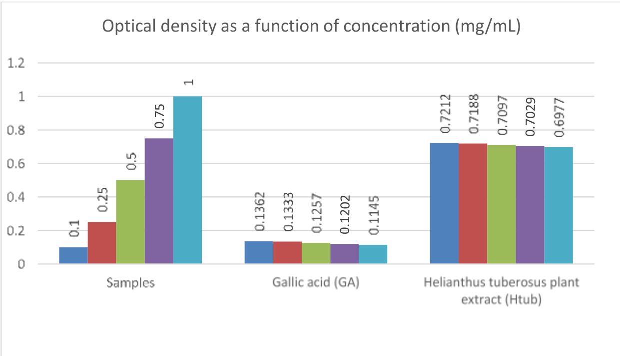

Fig. 4: Change in the optical density of solutions depending on concentration.

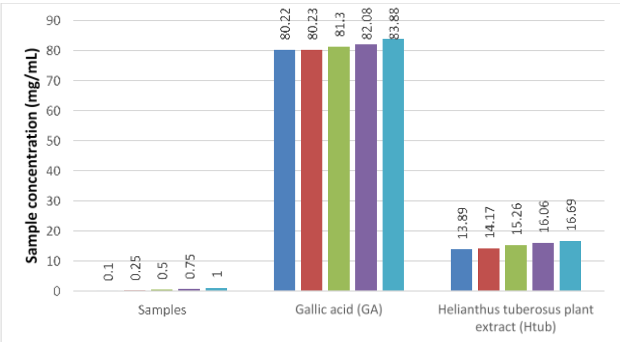

Fig. 5: Antiradical activity of *Helianthus tuberosus.*

Further, in order to establish the pharmacological activity of the granulated form of *Helianthus tuberosus*, a scheme for the correction of subclinical mastitis in cows was developed (Table 3).

Clinical recovery dynamics

Phytogranules of *Helianthus tuberosus* were added to the diet of animals in the first group in the morning, once a day, at a dose of 220–250 g per head. The duration of supplementation of the cows' diet with phytogranules was 20 days. Manual massage was applied externally, in an upward direction from bottom to top, twice a day. In addition, cows of both groups received the vitamin-mineral complex «Maxivit» intramuscularly, at a dose of 5 mL per head, once.

As a result of the measures carried out, it was established that in cows suffering from the subclinical form of mastitis, improvement in condition was observed at different time points. Thus, in animals of the first group, inflammatory processes in the mammary gland subsided much earlier than in animals of the second group. Test evaluation during the early treatment period. In the experimental group, the first positive clinical response was observed on day 3.0±0.4, and CMT became negative by day 6.0±0.5. In the control group, improvement was observed later, on day 4.0±0.5, and 90% of cows became CMT-negative by day 8.0±0.6. The mean time to negative CMT was significantly shorter in the experimental group than in the control group (P<0.05) (Fig. 6). The test for subclinical mastitis, conducted 8 days after the start of treatment, showed a negative result in 90% of the animals; 10% of cows in the control group did not respond to supportive treatment and showed worsening udder condition; therefore, they were removed from the trial and sent for forced slaughter according to farm veterinary decision.

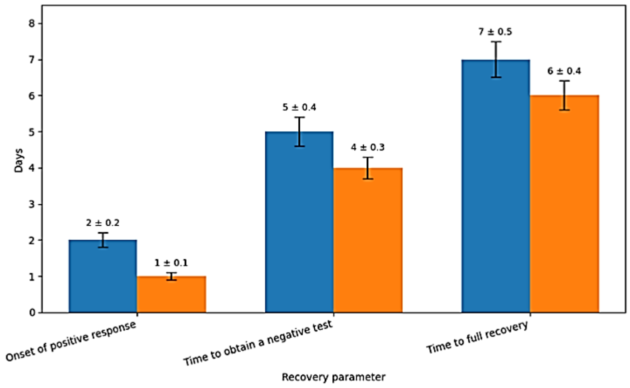

Fig. 6: Dynamics of recovery in cows of the control and experimental groups (days).

Hematological changes

Before pharmacocorrection, some morphophysiological blood parameters of animals in the first and second groups exceeded standard values or were within ranges bordering on minimal physiological parameters (Table 4). According to the results obtained, it should be noted that the hemoglobin level in the blood after correction was significantly higher in the animals of the experimental group, by 19.8%, compared to the control group. The hemoglobin content in animals of both groups was at the level of the minimum normative values. A slight decrease in the number of erythrocytes was observed in the blood of cows of both the experimental and control groups before the beginning of the experiment; after the correction, this indicator increased to normative values in the cows of the first group. The leukocyte content in cows with

Table 3: Scheme for correction of subclinical mastitis in cows during the lactation period

Animal groups (n=14)	Name of treatment agents	Dose (g, mL)	Method of administration
1 First	1) Phytogranules of *Helianthus tuberosus*	220.0 – 250.0	Once a day, mixed with feed, for 20 days
	2) Maxivit	5 mL	Single administration, intramuscularly
	3) Udder massage	–	Twice a day, for 5 days
2 Second	1) Maxivit	5 mL	Single administration, intramuscularly
	2) Udder massage	–	Twice a day, for 5 days

Table 4: Hematological blood parameters in cows with subclinical mastitis before and after the use of *Helianthus tuberosus*

No.	Parameter	Reference values	Experimental group before treatment	Experimental group after treatment	Control group before treatment	Control group after treatment
1	Hemoglobin, g/L	80–150	88±0.96	121±0.23*,†	86±0.32	97±0.95*
2	Erythrocytes, 10^6/µL	5–10	4.1±0.57	6.79±0.78*	4.41±1.20	5.2±0.78*
3	Leukocytes, 10^3/µL	4–12	13.82±0.98	7.3±1.23	14.18±0.58	9.34±0.68*
4	Lymphocytes, %	45–75	51.4±0.56	58.0±0.89*,†	63.0±0.96	53.6±1.33*
5	Monocytes, %	2–7	5.9±0.09	1.0±0.023†	0.0±0.00	4.4±0.12*
6	Platelets, 10^3/µL	100–800	470±15.3	484±19.0	410±12.2	498±21.6
7	ESR, mm/h	0.5–1.5	6.0±0.034	2.0±0.066†	6.0±0.033	3.0±0.045*

Note: Values are presented as mean±SEM. * indicates a significant within-group difference compared with the corresponding pre-treatment value, P<0.05. † indicates a significant post-treatment difference between the experimental and control groups, P<0.05. Absence of a symbol indicates no statistically significant difference.

Table 5: Biochemical blood parameters of cows before and after the use of *Helianthus tuberosus*

No.	Parameter	Reference values	Experimental group before treatment	Experimental group after treatment	Control group before treatment	Control group after treatment
1	Total protein, g/L	60–120	71.05±0.56	83.15±0.74†	79.5±0.63	80.3±0.43*
2	Glucose, mmol/L	2.4–2.8	1.90±0.07	2.66±0.23*	1.79±0.04	2.45±0.52
3	Creatinine, mmol/L	56–162	58.5±0.99	121±8.96	57.0±1.30	113.0±7.99
4	Calcium, mmol/L	1.62–3.37	2.01±0.11	2.36±0.48*	2.15±0.22	2.40±0.01*
5	Phosphorus, mmol/L	0.81–2.72	2.07±0.13	1.77±0.32*	1.92±0.37	2.16±0.14*
6	ALT, µkat/L	6.9–35	34.9±1.11	33.1±0.45*	32.0±0.96	32.9±0.98
7	AST, µkat/L	45–110	104.45±0.88	84.15±0.63†	96.60±0.78	93.2±1.23

Note: Values are presented as mean±SEM. * indicates a significant within-group difference compared with the corresponding pre-treatment value, P<0.05. † indicates a significant post-treatment difference between the experimental and control groups, P<0.05. Absence of a symbol indicates no statistically significant difference.

subclinical mastitis before correction slightly exceeded physiological values by 13.2% in the experimental group and by 15.3% in the control group; after correction, the leukocyte level shows a pronounced tendency to decrease in both groups, reaching the upper normative limits.

Biochemical changes

The initial indicators of total protein in both groups were within the normal limits. After pharmacological correction, the concentration of total protein in the blood of the experimental cows increased by 14.55%, whereas in the control animals, this indicator increased insignificantly by 0.99%. In the dynamics of changes in glucose content, before correction, the values were below the average minimum values by 20.8% in cows of the first group and by 25.4% in cows of the second group (Table 5). After correction, the glucose content in the blood of animals of both groups increased by 28.6 and 26.93%, respectively, reaching physiological values.

Creatinine increased markedly after treatment in both groups, from 58.5±0.99 to 121±8.96 mmol/L in the experimental group and from 57.0±1.3 to 113.0±7.99 mmol/L in the control group. However, the post-treatment values remained within the stated reference range of 56–162 mmol/L. Therefore, this increase was not interpreted as evidence of renal dysfunction, but rather as a possible physiological change associated with altered protein metabolism, hydration status, or recovery-associated metabolic shifts.

In animals of the first group, the activity of the enzyme ALT was at the borderline of the maximum permissible level in the blood; however, after correction, this indicator slightly decreased by 5.2% in cows of the first group, while in the blood of cows of the second group, ALT activity did not undergo significant changes. At the same time, ALT and AST activities showed a tendency towards improvement, especially in the experimental group. ALT slightly decreased in the experimental group, while AST decreased from 104.45±0.88 to 84.15±0.63 µkat/L. However, AST values were already within the stated reference range before correction; therefore, these changes were described as improvement in enzyme activity rather than normalization. Calcium and phosphorus remained within the stated physiological ranges throughout the study. In the dynamics of all studied biochemical parameters in the blood of cows of the first group after correction with *Helianthus tuberosus*, a pronounced tendency toward improvement and stimulation within physiological parameters was noted.

DISCUSSION

The present study evaluated the biological activity and adjunctive use of *Helianthus tuberosus* phytogranules in cows with subclinical mastitis. Because cows in both groups received Maxivit and udder massage, the observed effects should be interpreted as the result of phytogranules used as an adjunctive feed additive rather than as a standalone treatment. The main findings were faster CMT improvement in the experimental group and favorable changes in selected hematological and biochemical parameters. These results suggest that the phytogranules may have supported metabolic recovery in cows with subclinical mastitis, rather than acting as a proven antimicrobial or antioxidant therapy (Ijaz et al. 2021; Rahman et al. 2024).

The metabolic profile of blood serum is a widely used method for monitoring the health and nutritional status of dairy cows; at the same time, the content of the main blood metabolites may be influenced by environmental factors (Abd El-Mola and Aboul-Fotouh 2018; Ebrahimie et al. 2018; Lopreiato et al. 2020). The improvement in glucose and total protein values in the experimental group may indicate better energy and protein metabolism during recovery (Di Meo et al. 2023). Subclinical intramammary infection and inflammation have been associated with changes in blood biochemical traits, including energy-related metabolites, liver-function indicators, inflammatory parameters, oxidative stress markers, innate immunity, and mineral status in dairy cows (Pegolo et al. 2023; Jung et al. 2025). *Helianthus tuberosus* contains inulin-type fructans, carbohydrates, fiber, minerals, and other biologically active compounds, which may support gastrointestinal fermentation, nutrient utilization, and metabolic balance in lactating cows (Cheng and Han 2020; Wang et al. 2020). Dietary inulin supplementation has also been reported to influence rumen fermentation, lactation performance, serum biochemical indices, antioxidant status, and immune-related responses in dairy cows (Wang et al. 2021; Zhao et al. 2022). Thus, the possible beneficial effect of the phytogranules in the present study may be related to support of carbohydrate and protein metabolism. However, inulin and other active compounds were not directly measured in the final phytogranule product; therefore, this mechanism should be considered hypothetical (Abd El-Mola and Aboul-Fotouh 2018; Caroprese et al. 2020; Alem 2024).

The antimicrobial results should be interpreted cautiously. The phytogranules showed weak to moderate inhibitory activity against reference strains of *Staphylococcus aureus*, *Escherichia coli*, and *Serratia marcescens*. Plant-derived compounds and extracts may show in vitro activity against mastitis-associated bacteria, and such activity has been reported for several medicinal plants and natural agents (Ananda Baskaran et al. 2009; Kovačević et al. 2021; Bari et al. 2022; Kaseke et al. 2023b). However, milk samples from affected cows were not cultured in the present study, and no field isolates were tested. Therefore, these findings demonstrate only preliminary in vitro antibacterial activity and cannot be used as evidence of bacteriological cure in cows with mastitis (Yuan et al. 2012; Long et al. 2016; Kulpiisova et al. 2024).

The study of the effect of Jerusalem artichoke extract as a feed additive on productivity, at doses of 400 mg/L and 200 mg/L, improved productivity, antioxidant capacity, biochemical and immunological blood parameters, and intestinal microflora during the rearing of Japanese quails (Antanaitis et al. 2021; Abdel-Wahab et al. 2023). The antiradical activity of the phytogranules was low compared with gallic acid. Therefore, strong antioxidant-related therapeutic claims are not supported by the present data. Previous studies have shown that antioxidant activity of *Helianthus tuberosus* may vary depending on plant part, extraction method, processing technology, and final formulation (Wang et al. 2020; Abdel-Wahab et al. 2023). The low activity observed in the present study may therefore be related to the use of a granulated plant-based feed form rather than a concentrated extract. In addition, total phenolic content, flavonoid content, moisture, microbiological safety indicators, heavy metals, and mycotoxins were not quantified in the final phytogranule product. Therefore, the proposed metabolic-support mechanism is based on the known biological properties of *H. tuberosus* components and should be considered hypothetical.

In our studies, cows in the first and second phases of lactation had low glucose levels (hypoglycemia) simultaneously with high activity of ALT and AST enzymes, which indicates a disturbance of energy metabolism and changes in liver function, which, in turn, is associated with metabolic deviations in the body of cows after calving. Our data are consistent with the data of Necasova et al. (2021), obtained in the study of the relationships between subclinical mastitis and dysfunctions in the rumen and, apparently, are also associated with the onset of destructive changes in the liver. A negative energy balance in the body is indicated by the fact that hypoglycemia is observed against the background of increased hepatic enzymes. This circumstance requires further clarification, in particular, the study of β-hydroxybutyrate in the blood and the establishment of a correlation relationship.

The results of the study of cows (n=127) for subclinical mastitis using the California test revealed the presence of a true positive result in 28 cows, which is 22.0%; the highest percentage was found in the group of cows at peak lactation (4–6 months after calving) – 78.6%. Our data are consistent with Uyarlar et al. (2018) and indicate that even when metabolic disorders are not clinically detected, against the background of reduced immunity the risk of mastitis increases.

In the present study, cows of both groups exceeded physiological reference values or were close to the lower reference limits (Table 4). After correction, hemoglobin increased in both groups, with a more pronounced increase in the experimental group. Erythrocyte counts also increased numerically in the experimental group, from 4.1±0.57 to $6.79\pm0.78\times10^6/\mu L$, reaching the stated reference range; however, because this change was not marked as statistically significant in Table 4, it was interpreted as a positive tendency rather than a confirmed significant effect. Leukocyte counts decreased in both groups after treatment, indicating a reduction in inflammatory response. ESR also decreased in both groups, but remained above the stated reference range after treatment; therefore, these changes indicate partial improvement rather than complete normalization. Monocytes decreased to 1.0±0.023% in the experimental group, which was below the stated reference range of 2–7%; this finding was retained as reported and interpreted cautiously as a possible shift in leukocyte distribution during recovery. Platelet counts remained within the physiological range in both groups; therefore, no specific pathological interpretation was made. Overall, *Helianthus* *tuberosus* phytogranule supplementation was associated with improvement of selected hematological parameters, but the results should be interpreted cautiously

Although cows receiving *Helianthus tuberosus* phytogranules showed earlier CMT improvement than control cows, the study was not designed as a prevention trial. The medicinal form of *Helianthus tuberosus* in all tested dilutions exhibited very low activity compared to the antiradical activity of gallic acid. This is probably due to the fact that not an extractive form of the plant was tested, but a powder from the tubers and aerial parts of Jerusalem artichoke. Therefore, the phytogranules should not be claimed to prevent subclinical mastitis. The findings only suggest that supplementation may support recovery and improve selected blood metabolic parameters when used together with supportive management.

This study has several limitations. First, the number of treated animals was small, and detailed randomization procedures were not described. Second, subclinical mastitis was diagnosed using CMT without somatic cell count confirmation. Although CMT is a practical cow-side screening test for subclinical mastitis, stronger confirmation generally requires SCC determination and/or bacteriological culture to assess inflammation and identify causative pathogens (Kandeel et al. 2018; Ramuada et al. 2024). Third, milk samples from affected cows were not cultured, so bacteriological cure and pathogen-specific effects could not be assessed. Fourth, milk yield and milk composition were not measured during the trial, and no long-term follow-up was performed to evaluate relapse or sustained recovery. Fifth, both groups received Maxivit and udder massage, making it difficult to completely separate the specific effect of phytogranules from the supportive treatment. Finally, inulin and other active compounds were not quantified in the final phytogranule product. Consequently, the proposed mechanism of metabolic support remains hypothetical and should be confirmed by chemical characterization of the final phytogranule product in future studies. Further randomized controlled studies with larger sample sizes, SCC data, milk bacteriology, milk-yield records, chemical characterization of phytogranules, and long-term follow-up are needed to confirm these preliminary findings.

Thus, the use of *H. tuberosus* phytogranules as an adjunct to Maxivit and udder massage was associated with faster CMT improvement and favorable changes in selected blood metabolic parameters in cows with CMT-positive subclinical mastitis. However, the study was not designed as a prevention trial, because all enrolled cows were already CMT-positive at the start of the experiment. Therefore, the phytogranules should not be claimed to prevent subclinical mastitis. They may be considered as an adjunctive feed additive for supporting metabolic recovery in cows with subclinical mastitis, but their preventive effect requires separate evaluation in a properly designed preventive study.

Conclusion

The present study showed that *Helianthus tuberosus* phytogranules, used as an adjunct to Maxivit and udder massage, were associated with faster CMT improvement and favorable changes in selected hematological and biochemical parameters in cows with subclinical mastitis. The phytogranules demonstrated weak to moderate in vitro inhibitory activity against reference bacterial strains but showed low antiradical activity; therefore, their effect should be interpreted mainly as metabolic support rather than direct antimicrobial or antioxidant treatment. These findings suggest that *Helianthus tuberosus* phytogranules may be useful as an adjunctive feed additive for supporting recovery in cows with subclinical mastitis. Further randomized studies including somatic cell count, milk bacteriology, milk-yield data, active-compound analysis, and long-term follow-up are required to confirm these preliminary results.

DECLARATIONS

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Data Availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author's Contribution: K.A., G.Y., L.K.: supervision, writing—original draft, writing—review and editing; Z.B., T.A., T.B., and Zh.Z.: formal analysis, visualization, validation, resources, conceptualization, methodology, investigation. All authors have read and agreed to the published version of the manuscript.

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