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Full Research Article

IMPACT OF GARLIC POLYSULFIDE SUPPLEMENTATION ON BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN DAIRY COWS

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Abstract

In recent years, there have been numerous research activities related to the use of different herbal agents, especially those originating from chestnuts and garlic, as alternatives to synthetic growth promoters like antibiotics. Garlic and its active ingredients have been studied for their potential benefits, such as improving feed intake, production performance, rumen fermentation, and udder health. The aim of our study was to investigate the influence of garlic polysulfides, including diallyl polysulfide and dipropyl polysulfide, on hematological and biochemical parameters in dairy cows, as well as their possible effect on subclinical mastitis therapy.

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Eighteen late lactation Holstein cows were divided into three numerically equal groups (n=6): CON (non-supplemented cows), ALL (supplemented with 1000 mg/day of diallyl polysulfide), and PRP (supplemented with 1000 mg/day of dipropyl polysulfide). Blood sampling for hematological and biochemical analyses as well as the California mastitis test (CMT) test were performed before and after the 15-day study.

The results showed that garlic supplementation significantly increased blood leukocyte count and blood glucose concentration ($p<0.05$, respectively), and significantly decreased blood urea concentration ($p<0.05$) after the 15-day trial in both ALL and PRP groups compared with the day 0. In the supplemented groups, the CMT score was significantly lower ($p<0.05$, respectively) after the 15-day treatment compared with the CON group. The use of different mixtures of biologically active garlic polysulfides in the diet could improve immune and metabolic status, as well as support mastitis treatments in dairy cows.

Key Words: dairy cows; garlic polysulfides; blood parameters; CMT score

INTRODUCTION

Herbal supplements in animal feed are widely used and are being intensively studied for their potential beneficial effects on animal health and production, especially after the European Union banned the use of antibiotics as growth promoters in 2006 due to the risk of developing antibiotic resistance in bacteria (Windisch et al., 2008). Certain plant extracts, such as tannins and polyphenols, were found to improve nutrient utilization in the rumen (Wallace, 2004) and can have a positive influence on some blood parameters, such as glucose concentration and insulin levels (Prodanović et al., 2021). They can also improve dairy cows' antioxidant and immunological status (Prodanović et al., 2023; 2024). Similarly, the use of garlic and its bioactive compounds is well-known in humans and has a very broad spectrum of pharmacological effects and low toxicity (Chen et al., 2021; Lue and Sing, 2011). When administered as a dietary supplement in animals, garlic has a number of positive effects, including antifungal, antimicrobial, antiviral, anti-inflammatory, hepatoprotective, anticancer, and immunostimulatory properties (Chen et al., 2021). Hashemzaden-Cigari et al. (2014) showed that supplementing cattle feed with garlic can enhance feed intake, production performance, rumen fermentation, and udder health. Additionally, there is a study related to the potential of garlic to reduce methane emissions in dairy cows (Panthee et al., 2017).

Garlic contains many sulfur compounds, but the main effect is largely attributed to allicin and its metabolites (allylpropyl, diallyl polysulfide, S-allylcysteine, vinyldithiin) (Chen et al., 2021). The degradation of allicin, as the main garlic compound, leads to the production of 2-propenyl sulfenic acid, which can bind free radicals, demonstrating its antioxidant effect (Chen et al., 2021). It is known that organosulfur compounds of garlic with a higher number of sulfur groups are associated with significantly stronger pharmacological effects (Arsenijević et al., 2021). However, the biological effects of

these organosulfur compounds in garlic are poorly investigated in animals. The aim of our study was to investigate the effects of two different active organosulfur compounds from garlic saturated with a higher number of sulfur groups on hematological and biochemical parameters in dairy cows, as well as their potential effect in the therapy of subclinical mastitis.

MATERIALS AND METHODS

Animals

The study was conducted on Kovilovo commercial dairy farm, owned by Al Dahra Corporation, with 1300 dairy cows and an average milk production of 8000 liters per 305 days. All of the cows included in the study were aged 5 to 7 years and were in the late lactation period. For this study, a group of 18 Holstein cows were chosen. The cows were kept in an identical tie-stall housing system and were fed twice daily, at 07:00 and 18:00 h, with a total mixed ration (TMR) that met National Research Council (NRC, 2001) requirements. Selected animals were randomly divided into three numerically equal groups. The first group included animals that did not receive dietary supplementation, marked as a control group (n = 6; CON). Two experimental groups received different mixtures of biologically active garlic polysulfides, which were synthesized according to the recipe of the Center for New Technologies Belgrade in cooperation with the Faculty of Chemistry of the University of Belgrade, at the Department of Analytical Chemistry (Arsenijević et al. 2021). One experimental group received a supplement of diallyl polysulfide, marked as an allyl group (n = 6, ALL), while the other one received a supplement of dipropyl polysulfide, marked as a propyl group (n = 6, PRP). Both supplements were in capsule form, and each contained 1000 mg of the active ingredient. The capsules were administered to the cows for 15 days and given by a bolus applicator (Albert Kerbl GmbH, Germany), ensuring that each cow received one capsule of the indicated supplement at the same time each day after the morning meal. At the beginning (day 0) and at the end (day 15) of the study, a California Mastitis Test (CMT) was performed on all cows according to the instructions given by Rice (1981).

Blood sampling, hematological, and biochemical analyses

Blood samples were taken from all cows before (day 0 of the study) and one day after the last administration of supplements (day 15 of the study) by venipuncture of the jugular vein. Blood samples for hematologic analyses were collected in 3.0 mL vacuum tubes (BD Vacutainer, Plymouth, UK) with EDTA as an anticoagulant. For biochemical analysis, blood samples were collected with 10.0 mL vacuum tubes (BD Vacutainer, Plymouth, UK) containing a clot activator. The collected samples were kept in a refrigerator and sent to the laboratory of the Department of Ruminants

and Swine Diseases at the Faculty of Veterinary Medicine, University of Belgrade for analysis.

Hematologic examinations were promptly performed with an automated hematology analyzer (NCC-Vet 30, Neomedica, Niš, Serbia). The blood samples were kept at room temperature for not more than 1 h to allow for spontaneous coagulation, after which they were centrifuged at a speed of 3000 revolutions per minute for 5 minutes to collect the serum. The obtained serum was placed into 1.5-mL polypropylene tubes (Eppendorf AG, Hamburg, Germany) and then kept at a temperature of – 20 °C until biochemical analyses were performed.

Biochemical analysis was conducted on the BioSystems A15 automatic biochemical analyzer (BioSystems, Barcelona, Spain) using diagnostic kits from the same manufacturer. The analysis included measuring concentrations of total protein, albumin, glucose, calcium, phosphorus, urea, bilirubin, cholesterol, and triglycerides, as well as the activity of the enzymes aspartate aminotransferases (AST), lactate dehydrogenase (LDH), and gamma-glutamyl transferase (g-GT) following the procedure described in the work of Bošnjaković et al. (2023).

The Ministry of Agriculture, Forestry, and Water Management of the Republic of Serbia issued an ethical permit (permit number 323-07-11720/2020-05/4) in accordance with the National Regulation on Animal Welfare.

Statistical analysis

The obtained results were analyzed using the statistical software STATISTICA 8 (StatSoft, Inc., Tulsa, OK, USA). The results are presented as the mean ± standard error (SE). The normality of the data distribution was tested using the Shapiro-Wilk test, and all data were normally distributed ($p>0.05$). An independent-samples t-test was used to compare results between the groups examined, while a paired-samples t-test was used to compare results within the same groups at different sampling periods. A value of $p<0.05$ was considered statistically significant.

RESULTS

The results of the hematological parameters are shown in Table 1. There was no significant difference in the leukocyte count between the examined groups, both at the beginning or at the end of the study. Nevertheless, in both the ALL and PRP groups, there was a significant increase in leukocyte counts on day 15 of the study, compared with counts on day 0. Additionally, it was observed that the PRP group experienced a greater leukocyte count increase than the ALL group on day 15. There was no significant difference in the other white blood cell parameters between or within the groups.

The erythrocyte count was significantly higher only in the PRP group compared to the CON group at the end of study. Other hematological parameters showed no significant difference between or within the studied groups, with the exception of MCHC in the ALL group, which was significantly higher on day 15 compared to on day 0 of the study.

Table 1. Hematological parameters (mean±SE) in the examined groups of cows before and after treatment

Parameters	CON	ALL	PRP
Leukocytes ($\times10^9$/L)			
Day 0	7.90±0.63^{a}	7.08±0.66^{a*}	8.43±0.66^{a*}
Day 15	8.78±0.97^{a}	9.90±1.10^{a*}	11.77±1.41^{a*}
Lymphocytes (%)			
Day 0	46.70±5.20^{a}	56.50±0.66^{a}	49.72±2.53^{a}
Day 15	48.00±4.39^{a}	53.62±1.10^{a}	56.05±4.31^{a}
MID (%)			
Day 0	8.68±1.98^{a}	9.33±1.05^{a}	9.80±1.36^{a}
Day 15	10.98±0.69^{a}	10.42±1.11^{a}	10.18±2.02^{a}
Granulocytes (%)			
Day 0	44.62±4.53^{a}	34.17±6.19^{a}	40.48±3.58^{a}
Day 15	37.02±6.15^{a}	36.00±5.12^{a}	32.27±3.80^{a}
Erythrocytes ($\times10^{12}$/L)			
Day 0	5.28±0.27^{a}	5.71±0.16^{a}	5.87±0.18^{a}
Day 15	5.10±0.40^{a}	5.46±0.22^{a,b}	5.84±0.21^{b}
Hemoglobin (g/dL)			
Day 0	7.87±0.32^{a}	8.38±0.12^{a}	7.98±0.10^{a}
Day 15	7.95±0.24^{a}	8.35±0.14^{a}	8.02±0.25^{a}
Hematocrit (%)			
Day 0	24.03±1.08^{a}	23.88±0.66^{a}	23.35±0.63^{a}
Day 15	24.47±1.40^{a}	23.37±1.06^{a}	23.42±0.94^{a}
MCV (fL)			
Day 0	42.22±2.23^{a}	43.12±1.06^{a}	40.12±1.83^{a}
Day 15	42.18±2.32^{a}	42.88±1.03^{a}	40.37±1.88^{a}
MCH (pg)			
Day 0	14.23±0.61^{a}	14.53±0.26^{a}	13.62±0.40^{a}
Day 15	14.48±0.81^{a,b}	15.32±0.44^{a}	13.72±0.48^{b}
MCHC (g/dL)			
Day 0	34.03±0.64^{a}	33.92±0.63^{a*}	34.23±0.63^{a}
Day 15	34.48±0.34^{a}	35.97±1.27^{a*}	34.28±0.55^{a}
Platelets ($\times10^9$/L)			
Day 0	132.33±8.30^{a}	146.00±28.99^{a}	142.00±9.99^{a}
Day 15	186.33±20.94^{a}	188.50±19.44^{a}	170.50±13.36^{a}

CON – control group; **ALL** – group received 1 g/day of diallyl polysulfide during 15 days; **PRP** – group received 1 g/day of dipropyl polysulfide during 15 days;
MID – less frequently occurring and rare cells correlating to monocytes, eosinophils, basophils, blasts and other precursor white cells; **MCV** – mean corpuscular volume; **MCH** – mean corpuscular hemoglobin; **MCHC** – mean corpuscular hemoglobin concentration.
^{a,b} – different lowercase letters in the same row indicate a significant difference between groups ($p<0.05$);
***** – indicates a significant difference at the beginning and at the end of the experiment within each group ($p<0.05$)

The results of the analysis of biochemical parameters are shown in Table 2.

Table 2. Biochemical parameters (mean±SE) in investigated groups of cows before and after treatment

Parameters	CON	ALL	PRP
Glucose (mmol/L)			
Day 0	3.31±0.37[a]	3.04±0.20[a]*	2.63±0.14[a]*
Day 15	3.78±0.08[a]	3.62±0.13[a]*	3.51±0.17[a]*
Total proteins (g/L)			
Day 0	92.60±1.72[a]	88.52±2.64[a]	89.02±1.47[a]
Day 15	90.40±2.11[a]	89.40±2.71[a]	87.10±0.55[a]
Albumin (g/L)			
Day 0	37.20±2.77[a]	39.44±0.75[a]*	38.23±1.21[a]
Day 15	34.00±1.79[a]	36.28±1.02[a]*	37.43±0.94[a]
Calcium (mmol/L)			
Day 0	2.87±0.20[a]*	2.63±0.17[a]*	2.90±0.04[a]*
Day 15	2.20±0.10[a]*	2.32±0.04[a]*	2.22±0.05[a]*
Phosphorus (mmol/L)			
Day 0	2.32±0.20[a]	2.09±0.13[a]	2.11±0.09[a]
Day 15	2.18±0.05[a]	1.85±0.17[a]	2.04±0.11[a]
Urea (mmol/L)			
Day 0	4.91±0.49[a]	4.28±0.25[a]*	4.53±0.25[a]*
Day 15	4.19±0.33[a]	3.39±0.30[b]*	2.69±0.20[b]*
Bilirubin (µmol/L)			
Day 0	2.03±0.36[a]	2.50±0.11[a]	2.82±0.29[a]
Day 15	2.33±0.21[a]	2.39±0.48[a]	2.27±0.10[a]
Cholesterol (mmol/L)			
Day 0	5.68±0.41[a]*	6.09±0.28[a]*	5.90±0.70[a]*
Day 15	4.62±0.53[a]*	5.20±0.32[a]*	5.13±0.60[a]*
Triglycerides (mmol/L)			
Day 0	0.15±0.03[a]	0.21±0.04[a]	0.17±0.02[a]
Day 15	0.15±0.02[a]	0.17±0.02[a]	0.19±0.02[a]
AST (U/L)			
Day 0	93.07±5.36[a]	97.63±5.01[a]	82.93±4.28[a]
Day 15	93.76±8.18[a]	93.57±5.37[a]	92.40±5.32[a]
LDH (U/L)			
Day 0	1981.40±24.46[a]*	2079.90±47.53[a]*	2012.60±34.82[a]*
Day 15	1752.60±44.84[a]*	1846.60±24.61[a]*	1831.30±14.35[a]*
g-GT (U/L)			
Day 0	39.48±5.09[a]	43.64±5.24[a]	40.73±4.54[a]
Day 15	36.32±3.15[a]	39.55±4.63[a]	42.75±4.65[a]

CON – control group; **ALL** – group received 1000 mg/day of diallyl polysulfide during 15 days; **PRP** – group received 1000 mg/day of dipropyl polysulfide during 15 days;
AST – aspartate aminotransferase; **LDH** – lactate dehydrogenase; **g-GT** – gamma-glutamyl transferase;
[a,b] – different lowercase letters in the same row indicate significant difference between groups ($p<0.05$);
***** – indicates significant difference at the beginning and at the end of the experiment within each group ($p<0.05$)

There was no significant difference in blood glucose concentrations between the studied groups, both at the beginning or at the end of the study. Nevertheless, a significant increase in glucose concentration was observed in both the ALL and PRP groups 15 days after the application of the supplement compared to day 0. At the beginning of the study (day 0), there was no significant difference in urea concentration between the examined groups. However, after the supplement was applied (day 15), there was a significant decrease in urea concentration in both the ALL and PRP groups compared to the CON group, as well as compared to the concentration before treatment. Furthermore, a significant decrease in calcium and cholesterol concentrations, as well as LDH activity, was found within both the CON and experimental groups on day 15 compared to day 0. Other parameters showed no significant difference between or within the groups.

The CMT score results (Figure 1) indicated that all cows had subclinical or, in some cases, clinical mastitis. Notably, there was a significant decrease in CMT scores in the majority of udder quarters in the two experimental groups on day 15 of the study compared with the day 0 scores, especially in the PRP group.

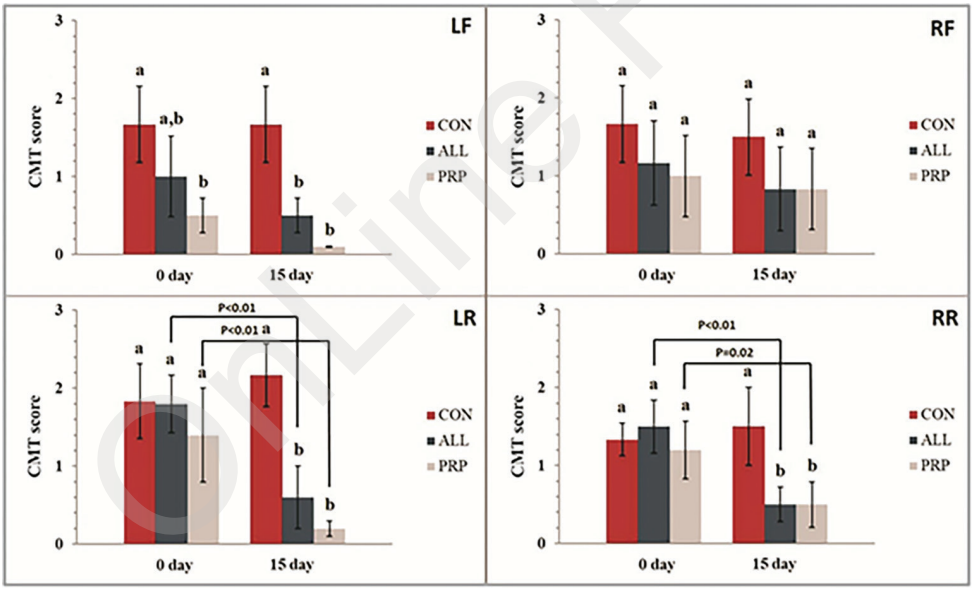

Figure 1. Mean California mastitis test **(CMT)** scores in individual udder quarters before and after treatment, grouped according to treatment.

LF – left front; **RF** – right front; **LR** – left rear; **RR** – right rear; **CON** – control group; **ALL** – group received 1000 mg/day of diallyl polysulfide during 15 days; **PRP** – group received 1000 mg/day of dipropyl polysulfide during 15 days;
a,b – different lower case letters indicate a significant difference ($p<0.05$) between groups tested on the same day

DISCUSSION

The most important aspect of our study is its focus on using active compounds derived from garlic that possess a higher concentration of sulfur groups, such as diallyl polysulfide and dipropyl polysulfide. Our study results suggest that administering different mixtures of biologically active garlic polysulfides may have a beneficial effect on some blood parameters and metabolites in dairy cows. Namely, the results showed that both experimental groups had increased total leukocyte counts after treatments. However, the findings of our study are not consistent with the research conducted by Yang et al. (2007) on cows, or Amin et al. (2014), and Thamer et al. (2020) on lambs, which reported no changes in total leukocyte count after the use of garlic extract and oil. In a study by Granados-Echegoyen et al. (2015) conducted on rats, leukopenia was observed when garlic extract was used in different concentrations, although Adebayo et al. (2010) mentioned that an increase in leukocyte count is a normal response of rats to the administration of foreign substances as a defense mechanism. The explanation for this discrepancy may be associated with the immunomodulatory potential of the garlic products used, as validated by Kik et al. (2001). Furthermore, we found no change in hemoglobin concentration or the red blood cell and platelet counts after treatments, suggesting that garlic extract does not have any adverse impact on erythropoiesis. This finding aligns well with Thamer et al. (2020), who reported no effect of garlic oil on hematological indices in sheep. However, El-Sebaey et al. (2019) showed the erythropoietic potential of garlic in rats.

Most research investigating the addition of garlic to ruminant diets has focused on the effect on blood glucose, urea, and cholesterol concentrations (Amin et al., 2014; Zhong et al., 2019). In line with this, our study showed a significant increase in blood glucose concentration in both treated groups, which is in contrast with the findings of Amin et al. (2014), who reported no change in blood glucose concentrations in sheep. Additionally, a significant decrease in glucose concentration in response to garlic supplementation was observed in buffalo calves and explained by the effect of allyl-propyl disulfide on increasing insulin concentration (Duvvu et al. 2018). According to Castillo-Lopez et al. (2021), addition of garlic with feed reduces the acetate-to-propionate ratio in the rumen while leaving the electrochemical response of the rumen contents unaffected. Also, the study conducted by Zhong et al. (2019) showed that the use of garlic powder leads to an increase in the level of propionic acid while decreasing the levels of acetate. Therefore, the higher blood glucose concentrations we found in both treated groups further support studies regarding the positive effect of garlic supplements on propionate formation in the rumen. Research has also shown that addition of plant supplements to the diet of ruminants has an impact on nitrogen utilization in the rumen, resulting in a beneficial effect on the environment (Panthee et al., 2017). Our study showed a significant reduction in blood urea concentration after treatments in the ALL and PRP groups. These results are consistent with the findings of Prodanović et al. (2021), who determined that the dietary addition of

tannins from chestnut extract leads to a reduction in blood urea concentration due to suppressed protein degradation in rumen. Additionally, sheep that are fed with garlic straw and leaves exhibit enhanced nitrogen and energy utilization without negative effects on rumen fermentation (Kamruzzaman et al., 2011). Moreover, Wanapat et al. (2008) revealed the increased digestibility of raw proteins and/or decreased blood urea concentration in cattle supplemented with garlic powder. Thus, the reduction of ammonia in the rumen under the influence of garlic results in a decreased blood urea concentration and/or an increase in protein availability in the small intestine (Wanapat et al., 2008).

The serum concentrations of calcium, cholesterol, and LDH activity significantly decreased on day 15 of the study (compared with the start) in all examined groups. While Amin et al. (2014) propose that garlic supplements could reduce blood cholesterol levels, our findings indicate that the observed results may be attributed more to physiological fluctuations rather than the direct biological action of garlic. Duvvu et al. (2018) found similar results in buffalo studies, attributing them to physiological variations and the inability of garlic to influence calcium and phosphorus regulation.

The quantity of somatic cells is a reliable indicator of the udder's health status. A rise in somatic cell count signifies an immune response to bacteria (Bochenek and Kuczynska, 2019). Healthy udders typically include 75% epithelial cells among their somatic cells. However, in cases of mastitis, the percentage of epithelial cells drops to 20–30% in favor of more leukocytes. The enhanced CMT score observed in our study in both experimental groups (ALL and PRP) of cows after treatment could be related to the antibacterial activity of garlic (Gull et al., 2012), which results in a decrease in the quantity of somatic cells, namely leukocytes, in the milk. The findings of our study align with the studies conducted by Mussarat et al. (2014), who investigated the use of garlic in treating mastitis, and Amber et al. (2018), who provided evidence that garlic facilitates the recovery of cows, buffaloes, sheep, and goats affected by mastitis. Additionally, our results suggest that garlic has a positive effect on the immune system's defense mechanisms against infections, as evidenced by the increased leukocyte counts in the blood of the experimental groups. Animals with a low leukocyte count are at higher risk of infection, while those with a high leukocyte count have greater resistance to infection due to their ability to produce antibodies and higher phagocytic capacity (Soetan et al., 2013). The role of garlic in enhancing the immune system's functions is reflected in its activation of NK cells, T lymphocytes, and macrophage phagocytosis, leading to increased lysozyme activity (Ndong and Fall, 2011) and increased IL-2 levels (Ozougwu, 2011). The effects of garlic on the immune system can also be attributed to the action of its sulfur compounds (S-alylcysteine, *N*-acetylcysteine, lectin, and pectin) and allicin, which hydrolyze into various sulfur compounds responsible for garlic's antibacterial and bactericidal effects (Khalil et al., 2014). Considering the aforementioned research, we consider that the addition of garlic extract to the diet of cows with subclinical mastitis could improve their health and increase the quality

of their milk. This is due to the fact that garlic extracts have been shown to decrease somatic cell counts and possess potent antibacterial properties.

CONCLUSION

The supplementation of different mixtures of biologically active garlic polysulfides in the diet of dairy cows could have a positive impact on the immune response and, thereby, aid in the therapy of mastitis. Additionally, the positive influence on the metabolism of glucose and urea, as key molecules on which productive indicators depend, justifies the use of active garlic ingredients as supplements in the diet of dairy cows.

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Authors' contributions

Conceptualization, SN, IV; methodology, DB, SN; validation, DK, LJ; formal analysis, MG, SN, SA, AM; synthesis and encapsulation of diallyl polysulfides and dipropyl polysulfides: DM, MM; investigation, SN, SA, RP, IV; writing—original draft preparation, MG, SN; writing—review and editing, DB, SN, JB, LJ, DK, RP. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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UTICAJ PRIMENE POLISULFIDA BELOG LUKA NA BIOHEMIJSKE I HEMATOLOŠKE PARAMETRE MLEČNIH KRAVA

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Kratak sadržaj

Poslednjih godina spovedena su brojna istraživanja o upotrebi različitih aktivnih jedinjenja poreklom iz biljaka, naročito onih koji potiču iz kestena i belog luka kao zamena za sintetske promotore rasta, naročito antibiotike. Beli luk i njegovi aktivni sastojci dosta su proučavani zbog njihovih potencijalnih korisnih efekata, kao što su: poboljšanje unosa hrane, proizvodnih performansi, fermentacije u buragu kao i očuvanja zdravlja vimena.

Cilj našeg rada bio je da se ispita uticaj polisulfida belog luka, uključujući dialil polisulfid i dipropil polisulfid na hematološke i biohemijske parametre kod mlečnih krava, kao i njihov potencijalni efekat u terapiji supkliničkih mastitisa.

Osamnaest krava holštajn rase u periodu kasne laktacije podeljene su u tri numerički jednake grupe (n=6): KON (bez suplementacije), ALL (suplementacija sa 1000 mg dialil polisulfida) i PRP (suplementacija sa 1000 mg dipropili polisulfida). Krave ALL i PRP grupa dobijale su preparate belog luka tokom 15 dana ogleda. Uzimanje uzoraka krvi za hematološke i biohemijske analize kao i Kalifornija mastitis test (CMT) obavljeni su pre (0. dan) i nakon tretmana (15. dan).

Dobijeni rezultati ukazuju da primena belog luka dovodi do statistički značajnog povećanja broja leukocita i koncentracije glukoze ($p<0,05$, pojedinačno), kao i smanjenja koncentracije uree u krvi ($p<0,05$) kod ALIL i PROPIL grupa nakon 15 dana tretmana u poređenju sa KON grupom. U grupama sa suplementacijom, ocena CMT testa je bila značajno niža ($p<0,05$, pojedinačno) nakon 15 dana tretmana u

poređenju sa KON grupom. Upotreba različitih mešavina biološki aktivnih polisulfida belog luka u ishrani krava može imati pozitivan uticaj na imunitet, metabolički status, i terapiju supkliničkih mastitisa.

Ključne reči: mlečne krave, polisulfidi belog luka, parametri krvi, CMT test