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The influence of sunflower oil in meals on the blood mineral profile of dairy cows

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Abstract

Minerals are essential substances with an important function in livestock, and their disbalance negatively affects the health and production, especially of ruminants. The addition of fat to the rations of dairy cows impacts the overall and mineral metabolism, and thus on health and production. The aim of the research was to examine the correlative relationships between balanced production meals and the mineral status of lactating cows with different ration compositions with or without the presence of vegetable oil. The research was conducted on 30 dairy cows of the Holstein breed at the Butmir farm, Sarajevo, Bosnia and Herzegovina. Three groups of 10 animals were formed based on productivity (A, 13-15 kg of milk/day, B, 19-21 kg of milk/day and C, 25-27 kg of milk/day). Samples of feed, blood plasma and milk of animals were collected on three occasions at 3-week intervals. According to the feed analysis results, the rations were standardized for each group, given for three weeks without oil, and then for three weeks with the addition of sunflower oil in the amount of 2.5% of the ration dry matter. After determining the content of basic nutrients, dry matter and ash in the rations, the concentrations of Ca, Mg, Na and K were determined by atomic absorption spectrophotometry, while the colorimetric method was used to analyse P according to Woy and Eggertz-Finkener. Analysis of blood mineral parameters was performed spectrophotometrically. The content of fat, proteins, lactose, and non-fat dry matter in milk samples was determined by infrared spectrometry using an automatic analyser. Based on the results, we observed a similarity in changes of values and the statistical significance of the differences in plasma Ca, P and Mg between the study groups and at sampling intervals. Milk production was positively correlated with the concentration of Mg and Ca with addition of the sunflower oil to the meal, while no correlation coefficient was established for P in any of the samplings. Balanced meals with and without the addition of oil did not significantly affect the content of Ca, P or Mg in the blood plasma of the tested animals, nor were significant differences found between the groups. However, by determining the correlations between milk parameters and blood biochemical parameters, a significant positive correlation was established between the amount of milk and Ca and Mg levels with a diet supplemented by oil. Given that the addition of sunflower oil in the amount of 2.5% of dry matter of the balanced rations for cows had no negative effects on the mineral profile

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and the parameters of the tested animals, we conclude that the introduction of this oil could be useful both from the economic point and improving nutritional composition of milk as an animal food present in the daily human diet.

Key words: *sunflower oil; dairy cows; minerals; blood plasma; milk*

Introduction

Minerals are essential nutrients with an important function in organisms, and their disbalance leads to negative effects on health and production, especially in ruminants. The most important macroelements that can affect the homeostasis of dairy cows are: calcium, magnesium, inorganic phosphorus, potassium and sodium (Folnožić et al., 2019). An imbalance in mineral metabolism during the peripartum period is a prerequisite for the occurrence of disorders such as hypocalcemia, ketosis, retention of foetal membranes, tetany, mastitis and lameness (Vince et al., 2017). The assessment of the mineral needs of animals includes criteria such as milk yield, mutual relationships with other nutrients, representation and relationships of minerals in feed, breed and adaptation of animals. The mineral profile of liquid tissues can be used to assess mineral content in ruminants (Khan et al., 2015). Blood plasma is a good indicator of the status of certain elements, and the levels of these elements in the plasma are important for determining certain pathological changes (Mtui et al., 2007). In addition to the mineral profile, clinical signs of mineral deficiencies and the results of nutrient and soil and water analyses are additional tools for determining mineral deficiencies. Mineral concentrations in animal tissues are often better indicators of animal mineral status than mineral concentrations in forage or soil (Khan et al., 2015).

In ruminant nutrition, in addition to the addition of concentrated feed, much research has investigated the use of oil as an energy supplement, because supplementing energy contributes to meeting the energy needs of high production and physiological disbalance of the energy balance in the early puerperium (Grubić and Adamović, 2007). The addition of fat to meals reduces the loss of body mass during the early stage of lactation, the number of cases of ketosis and ensures better reproductive efficiency (Đorđević et al., 2007). At the same time, fat in the meal adversely affects the development of bacteria in the first three compartments of the ruminant stomach, reducing their ability to break down raw fibre, with an unfavourable effect on the synthesis of milk fat. Likewise, fat binds calcium and magnesium, so their concentrations in the meal should be increased. The addition of fat in the rations of dairy cows certainly has an impact on metabolism, health and production functions, and in this sense, the research of the mineral profile of lactating cows under conditions of different meal composition with the addition of fat has both fundamental and possible applicable significance. The aim of the research was to examine the correlative relationships between balanced production rations and the mineral status of lactating cows with different meal compositions; with or without the presence of vegetable oil in the ration.

Materials and methods

The research was carried out at the Butmir dairy farm, located in the Sara-

Table 1. Results of chemical analysis of feed and additives used on the farm (pretrial)

	Humidity %	Protein %	Fat %	Fibre %	Ash %	Ca %	P %	Mg %	Na %	K %
Hay	11.24	6.82	2.48	28.69	5.11	0.85	0.13	0.17	0.12	0.78
Corn silage	77.20	1.70	0.77	6.36	1.10	0.11	0.05	0.05	0.01	0.17
Wheat barn	13.11	15.21	4.18	10.43	5.24	0.09	1.18	0.44	0.02	1.29
Sunflower meal	12.44	27.59	1.79	24.06	6.02	0.31	1.07	0.55	0.01	1.45
Grain mix 18%	13.28	19.62	2.75	7.05	6.80	1.18	0.77	0.28	0.22	0.90
Soybean meal	12.33	46.35	2.38	5.29	5.75	0.26	0.61	0.27	0.01	2.25
Cornmeal	13.17	7.98	4.39	2.55	1.24	0.03	0.23	0.09	0.01	0.33
MVD[1]	3.99	–[2]	–	–	67.58	14.82	6.07	5.00	8.01	0.31
Calcium carbonate (Chalk)	0.03	–	–	–	98.92	39.12	–	–	–	–
Salt	0.10	–	–	–	99.83	–	–	–	38.74	–

[1] mineral-vitamin supplement Milkmaster, manufacturer Veterina doo, Kalinovica, Zagreb, Croatia
[2] (–) analysis not performed

jevo area, Bosnia and Herzegovina at an altitude of nearly 500 m. Cows were fed forage, hay or silage, mostly grown independently, with the addition of commercial concentrate fodder and nutritional supplements. The study was conducted on 30 cows of the Holstein breed aged between two to seven lactations, in the middle of the production cycle. On the basis of productivity, estimated as the range of the lowest and highest amount of milk produced in the week before sampling, the cows were divided into three groups each of 10 cows. The first group (A) consisted of cows of lower milk production (13–15 kg/day), the second group (B) represented cows of medium production (19–21 kg/day), and the third group (C) of cows had the higher level of production (25–27 kg/day). The cows were kept fixed in stalls with an individual and controlled feeding regimen, while water was provided *ad libitum*. Samples of feed, blood plasma and milk of animals were collected in the winter period on three occasions at intervals of 3 weeks. After the first sampling, on the basis of the results of the chemical analysis of feed and supplements to the rations (Table 1), rations were prepared separately for each group of cows, balanced for the given level of milk yield. Meals were formulated according to the norms of the National Research Council (NRC) or slightly above (Nutrient Requirements of Dairy Cattle, 2021).

In the rationed meals for the next three weeks, the main part was corn silage and meadow hay, and the supplement to the norm was made by adding cornmeal, sunflower meal, wheat bran, soybean meal and ready-made feed mixture, as well as animal salt and a mineral-vitamin supplement (Table 2). The results of the chemical analysis of standard rations are given in Table 3.

After the second sampling, in the following three weeks, the same animals were used according to the experimental plan, fed rationed meals with the addi-

Table 2. Structure of standardized meals stratified according to groups and fed during 3 weeks after first sampling (sampling 0)

	Group A	Group B	Group C
Corn silage, kg	25	30	30
Hay, kg	4	4	4
Cornmeal, kg	0.52	0.81	2.42
Wheat barn, kg	0.50	0.50	0.50
Sunflower meal, kg	1.83	1.08	0.34
Soybean meal, kg	0.51	1.37	1.94
Grain mix, 18%, kg	0.96	2.19	3.75
salt, kg	0.06	0.07	0.08
MVD Milkmaster, kg	0.06	0.05	0.04

Table 3. Chemical composition and nutritive values of the standardised meal during first 3 weeks of the field experiment (sampling 1)

	Group A	Group B	Group C
Dry matter, kg	13.10	15.70	18.30
In dry matter:			
protein %	13.30	14.60	15.20
Fat %	3.09	3.17	3.35
fibre %	25.41	23.05	19.79
ash %	6.23	6.17	5.93
NEL MJ/kg[1]	6.32	6.49	6.61
Calcium %	0.67	0.68	0.67
Phosphorus %	0.45	0.43	0.43
Magnesium %	0.29	0.27	0.25
Sodium %	0.28	0.27	0.27
Potassium %	0.98	1.00	0.98

[1] Net Energy Level – NEL standardised according to milk yield

tion of edible sunflower oil in the amount of 2.5% of the dry matter of the meal, with minimal corrections of the amounts of other nutrients (Tables 4 and 5). During the entire experiment, cows were fed twice daily, at 07:00 and 15:00, with half of the meal in each feeding. Silage and hay were also given by groups, while the concentrate part of the meal was dosed to each cow individually in the amount that ensures the consumption of individual components as stated in Tables 2 and 4.

Cows were milked twice daily, at 08:00 and 18:00. Milking control and milk

Table 4. Structure of standardised mails stratified according to groups of animals with added oil

	Group A	Group B	Group C
Corn silage, kg	25	30	30
Hay, kg	4	4	4
Cornmeal, kg	0.10	0.20	1.32
Wheat barn, kg	0.50	0.50	0.50
Sunflower meal, kg	2.59	1.56	0.63
Soybean meal, kg	0.17	1.14	1.94
Grain mix, 18%, kg	1.10	2.31	3.81
Sunflower oil, kg	0.34	0.40	0.46
Salt, kg	0.06	0.07	0.08
MVD Milkmaster, kg	0.06	0.05	0.04

Table 5. Chemical composition and nutritional value of meals with added oil

	Group A	Group B	Group C
Dry matter, kg	13.60	15.90	18.10
In dry matter:			
protein %	13.20	14.40	15.40
Fat %	5.42	5.53	5.69
fibre %	25.75	23.37	20.24
ash %	6.24	6.20	6.04
NEL MJ/kg[1]	6.65	6.82	6.94
Calcium %	0.67	0.68	0.69
Phosphorus %	0.48	0.45	0.44
Magnesium %	0.31	0.28	0.26
Sodium %	0.27	0.27	0.27
Potassium %	0.97	1.00	0.99

[1] Net Energy Level – NEL standardised according to milk yield

sampling were performed at the beginning, middle and end of the experiment, on day 1 (sampling 0), day 21 (sampling 1) and day 42 (sampling 2). For this purpose, a graduated measuring cup of 600 mL (Mark 5 Milk Meter, Alfa Laval Agri GmbH) was used. An average sample of morning and evening milk, proportional to the amount of the daily milk yield, was collected in a plastic bottle from which an aliquot was separated for chemical analysis. Determination of milk fat, protein, lactose and fat-free dry matter of milk was carried out by the method of infrared spectrometry on the MILKOSCAN automatic analyser.

The content of nutrients in the forage was determined by the Weende method; protein content on an automatic analyser (Kjeltec Auto 1030, Tecator, Sweden), and others (fat, fibre, ash and dry matter) by classical methodology. For the analysis of calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na) and potassium (K), the samples were mineralised using a dry process. The content of Ca, Mg, Na and K was determined by atomic absorption spectrophotometry (AAnalyst 300, Perkin Elmer Corp., Norwalk CT, USA) with a previous dilution of the sample with strontium chloride in a ratio of 1:50 (for Na and K with deionised water in the same ratio). Analysis of P was carried out calorimetrically according to Woy and Eggertz-Finkener.

Blood samples were taken from the jugular vein using the same dynamics as milk samples. All relevant standard professional and bioethical measures were taken to avoid harming and endangering the well-being and health of animals during the research (Decision by Ethics Committee of the Faculty of Veterinary Medicine, University of Sarajevo 07-03-768-2/23). From each cow, 2 x 15 ml of blood were taken into heparinised vacuum tubes (Becton Dickinson VACUTAINER Systems, USA). Analysis of blood minerals was done spectrophotometrically using the "ALCYON ABBOTT 300".

Statistical analysis of the obtained data was carried out by analysis of variance (ANOVA), one-factor for milk, and two-factor for blood mineral parameters.

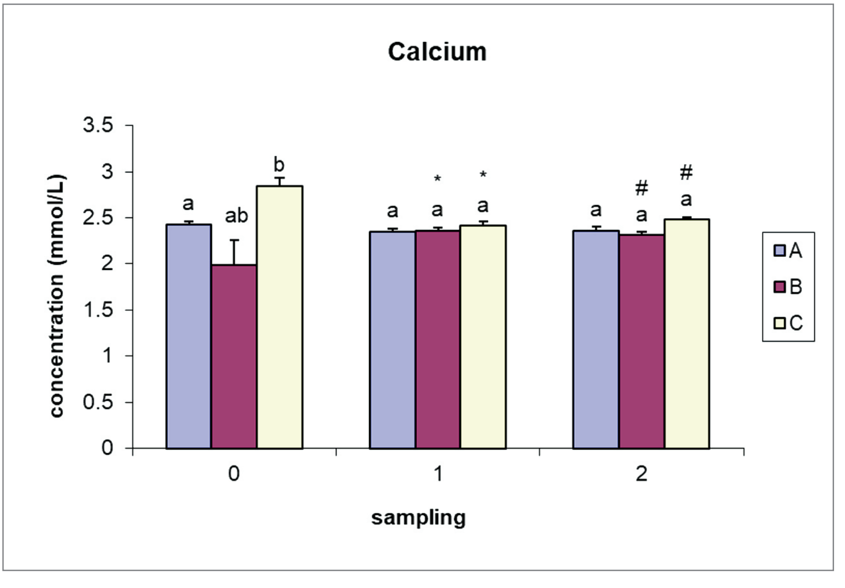

Figure 1. The concentration Ca (mmol/L) in the plasma expressed as the mean with standard error of the mean for each group of animals (A, B and C) at the onset of the study (sampling 0), after 21 days (sampling 1) and after 42 days (sampling 2).

a, b = values for each sampling that have a different letter are statistically significant ($P<0.05$)
* = statistically significant difference ($P<0.05$) between sampling 1 and 0 of the same group
# = statistically significant difference ($P<0.05$) between sampling 2 and 0 of the same group

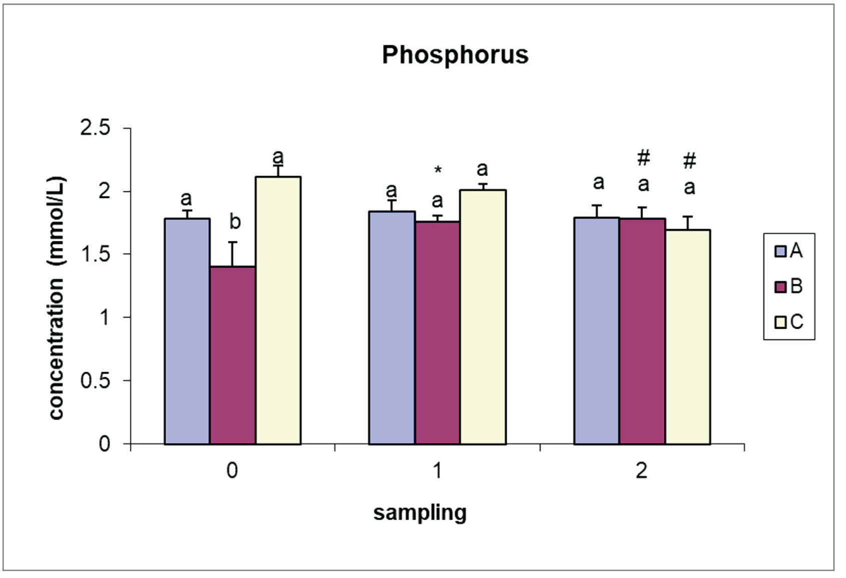

Figure 2. The concentration of P (mmol/L) in the plasma expressed as the mean with standard error of the mean for each group of animals (A, B and C) at the onset of the study (sampling 0), after 21 days (sampling 1) and after 42 days (sampling 2).

a, b = values for each sampling that have a different letter are statistically significant ($P<0.05$).
* = statistically significant difference ($P<0.05$) between sampling 1 and 0 of the same group
# = statistically significant difference ($P<0.05$) between sampling 2 and 0 of the same group

The differences of the obtained mean values were further analysed by Duncan's multiple test at a significance level of P<0.05. To determine correlations between milk parameters and blood mineral parameters under conditions of feeding with and without added oil, aggregated data for all three groups of cows were used, especially for samplings 1 and 2. Pearson's correlation coefficients were calculated using the Microsoft Office Excel 2003 software package, and their statistical significance was determined at the level of P<0.05.

Results

The measurements of the mineral profile of plasma samples are shown in the graphs (Figures 1, 2 and 3) separately for each macroelement evaluated (Ca, P and Mg) and for each sampling, stratified according to the groups of cows in the experiment (A, B and C).

Comparisons between groups for the results obtained at first sampling showed statistically significant differences that were lost in the subsequent samplings. Statistical significance of differences was established between sampling 1 and 0 and 2 and 0 for groups B and C.

From the results presented in Figure 3, it can be observed that levels of P behaved similarly to the levels of Ca in terms of the statistical significance of differences between groups and sampling, with the exception of group C, which did not show a statistical significance between sampling 1 and 0.

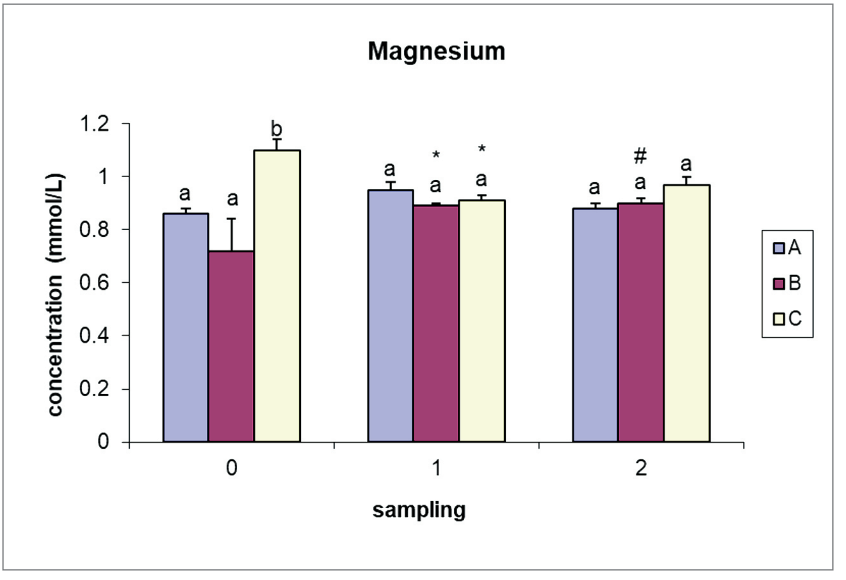

Figure 3. The concentration of Mg (mmol/L) in the plasma expressed as mean with standard error of the mean for each group of animals (A, B and C) at the onset of the study (sampling 0), after 21 days (sampling 1) and after 42 days (sampling 2)

a,b = values for each sampling that have a different letter are statistically significant ($P<0.05$)
* = statistically significant difference ($P<0.05$) between sampling 1 and 0 of the same group
# = statistically significant difference ($P<0.05$) between sampling 2 and 0 of the same group

Regarding the statistical significance of the differences in mean concentration levels, Mg did not differ significantly from the evaluated differences for Ca and P levels. Statistical significance for Mg was established between sampling 2 and 0 only for group B, while for P it was also established for group C.

Discussion

Khan et al. (2015) showed that the concentrations of Ca, Mg, zinc, copper and iron in the plasma affect the physiological functions of dairy cows and that the concentrations of Ca and copper in the plasma were higher, while the levels of Mg, zinc and iron in plasma were lower in dry cows compared to lactating cows. Low plasma Ca concentrations in lactating animals may be due to the physiological status of the animals; since the concentration of Ca in the blood is under the control of hormones, such as calcitonin and parathormone, affecting mechanisms of Ca regulations of the kidneys, intestines and bones, and in dry cows, the levels of vitamins in the blood affected by these hormones increase with the onset of lactation (Caldari-Torres et al., 2011; Palamarević, 2021). In the lactation period, the concentration of Ca in the plasma decreases due to the high need for milk production, following the stabilisation of Ca levels due to the balance of milk production (Jawor et al., 2012). In addition to Ca deficit, and related disorders, Mg and P homeostasis disorders are often associated.

The values of calcium in the blood plasma of dairy cows at each sampling in all three experimental groups were within the physiological limits, although they significantly differed between groups after the first sampling. In group B (19-21 kg/day) at the first sampling, average Ca values were at the lower physiological limit with a large standard error of the mean. The rationed meal (sampling 1) led to the loss of the statistical significance of differences between groups in terms of plasma Ca concentration. The meal to which we added sunflower oil in the amount of 2.5% of dry matter (sampling 2) did not affect the Ca values and the concentration remained approximately the same as in the previous sampling. Between groups, there was also no statistically significant difference in Ca concentrations between the second and third sampling. Statistical significance of the differences was established between samplings 1 and 0 and 2 and 0 only for groups B and C (Chart 1).

Phosphorus is the second major mineral required after calcium in the diet of ruminants (Jain and Mudgal, 2021). Adequate levels of Ca and P depend on three factors: sufficient supply of both and their appropriate ratio, with the presence of vitamin D for proper absorption in the body. Literature data indicate that P concentration values in large agglomerations are often below physiological

Table 6. Correlation between milk parameters and mineral elements of the blood of cows fed without addition the oil, expressed by Pearson's correlation coefficient

Blood mineral concentrations / Milk parameters	Ca	P	Mg
Amount of milk produced, kg	0.26	0.29	-0.02
% milk fat	-0.19	-0.06	-0.01
% milk protein	-0.27	-0.22	0.03
% lactose in milk	-0.23	-0.21	-0.08

Table 7. Correlation between milk parameters and mineral elements of the blood of cows fed with addition the oil, expressed by Pearson's correlation coefficient

Blood mineral concentrations / Milk parameters	Ca	P	Mg
Amount of milk produced, kg	0.38[a]	-0.03	0.38a
% milk fat	-0.04	0.25	-0.15
% milk protein	-0.24	0.21	-0.29
% lactose in milk	-0.30	0.08	-0.23

a = statistically significant difference correlation coefficient parameters milk and blood mineral elements for standardised diet with and without oil supplementation, $P<0.05$

limits in different production categories of cows, and hypophosphatemia most often occurs during lactation in cows with the highest milk production and in the peripartum period (Hussein Awad et al., 2022). The results of this research show that the level of production and feeding have an impact on phosphorus levels, since in cows with the highest milk production (group C) at the beginning and on the 21st day of the experiment, the average concentration of inorganic P was slightly higher compared to the groups of cows with lower milk yield at the same sampling (Figure 2). With the addition of sunflower oil, the average concentration of P in all three groups of dairy cows included in the experiment was approximately the same, with a slightly lower value in the group with the highest milk yield, but without any statistically significant differences.

The reasons for the low concentration of Mg in plasma may be the low absorption capacity of Mg in the gastrointestinal tract of animals and the low concentration of Mg in feed and mineral supplements (Khan et al., 2015). The concentration of Mg in the body is maintained by resorption from the digestive system and excretion by the kidneys, which means that there is no primary hormonal regulation of the concentration of Mg in the plasma, and the body's needs can only be met with food. The values of Mg concentration in the blood plasma of dairy cows in our study were within the physiological limits with statistically significant differences between groups and samplings. Statistically significant differences were found between the groups at the beginning of the experiment. A statistically significant (P<0.05) difference was found between samplings 1 and 0 for groups B and C, and between samplings 2 and 0 only for group B (Figure 3).

Based on these results, we established the similarity of changes in the values and the statistical significance of the differences for plasma Ca, P and Mg. On the other hand, Stojković et al. (2007) showed that the concentration of Mg did not significantly change in dairy cows of the Simmental breed, in contrast to P and Ca, where they found a decrease of these elements in the period of high lactation in cows with lower milk production. In our study, milk production was positively correlated with the concentration of Mg and Ca, also when sunflower oil was added to the ration, while no correlation was established for P in any of the samplings (Tables 6 and 7). Bearing in mind that feeding, lactation, pregnancy and age of the animal are the most significant factors affecting plasma concentrations of Ca and Mg. Simultaneously these factors directly affect the level of milk production, thus a positive correlation between these parameters for meals with the addition of oil is not surprising, bearing in mind the influence of the fat on the resorption of Ca and Mg in certain parts of the digestive tract.

Balanced meals with and without the addition of oil did not significantly affect the content of macroelements (Ca, P and Mg) in the blood plasma of the tested animals, nor were significant differences found between the groups. The similar fluctuations and trends established for these three parameters are a likely consequence of the high and precise homeostatic regulation in healthy animals. However, a significant positive correlation was found between the amount of skimmed milk and calcium and magnesium levels in conditions of nutrition with the addition of oil. Interdependence of milk production and the level of blood macroelements can be linked to the influence of the fat component of food on their resorption in certain parts of the digestive tract and their significant excretion through milk. Based on the fact that the addition of sunflower oil in the amount of 2.5% dry matter of a balanced ratio had no negative effects on the mineral profile and the measured production parameters of the tested animals, we can conclude that the introduction of this oil can be a useful energy supplement to rations for dairy cows both from an economic perspective and with a view to improving the nutritional composition of milk as an animal food present in the daily human diet.

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Utjecaj suncokretovog ulja u obrocima na mineralni profil krvi muznih krava

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Minerali su esencijalne tvari s važnom funkcijom u organizmima, a njihova neravnoteža negativno utječe na zdravlje i proizvodnju mlijeka, posebice preživača. Dodatak masti u obroke mliječnih krava ima utjecaj na sveukupni i metabolizam minerala, a time i na zdravlje i proizvodne funkcije.

Cilj istraživanja bio je istražiti korelativne odnose između izbalansiranog proizvodnog obroka i mineralnog statusa krava u laktaciji pri različitim sastavima obroka sa ili bez prisustva biljnog ulja.

Istraživanje je provedeno na 30 muznih krava crno-šare pasmine na farmi "Butmir" u Sarajevu. Formirane su 3 skupine od po 10 grla na osnovu proizvodnosti (A, 13-15 kg mlijeka/dnevno, B, 19-21 kg mlijeka/ dnevno i C, 25-27 kg mlijeka/ dnevno). Uzorci krmiva, krvne plazme i mlijeka životinja prikupljani su tri puta u razmaku od 3 tjedna. Prema rezultatima analize krmiva normirani su obroci za svaku skupinu, davani tri tjedna bez ulja, a zatim tri tjedna s dodatkom suncokretovog ulja u količini od 2,5 % suhe tvari obroka. Nakon određivanja sadržaja osnovnih nutrijenata, suhe tvari i pepela u obrocima krava, koncentracija Ca, Mg, Na i K određena je atomskom apsorpcionom spektrofotometrijom (AAnalyst 300, Perkin Elmer Corp., Norwalk CT, SAD), dok je za analizu P korištena kolorimetrijska metoda prema Woyu i Eggertz-Finkeneru. Analiza mineralnih parametara krvi provedena je spektrofotometrijski (ALCYON ABBOTT 300). Određivanje sadržaja mliječne masti, proteina, laktoze i bezmasne suhe tvari mlijeka provedeno je metodom infracrvene spektrometrije na automatskom analizatoru MILKOSCAN. Na temelju dobivenih rezultata može se uočiti sličnost u kretanju vrijednosti i statističke značajnosti razlika za plazmatski Ca, P i Mg između skupina u istraživanju, odnosno redoslijeda uzorkovanja. Proizvodnja mlijeka pozitivno je korelirala s koncentracijom Mg i Ca dodavanjem u obrok suncokretovog ulja, dok korelacioni koeficijent nije ustanovljen za P niti u jednom od uzorkovanja. Balanisrani obroci sa i bez dodatka ulja nisu značajno utjecali na sadržaj Ca, P i Mg u krvnoj plazmi ispitivanih životinja, niti su uočene značajne razlike između skupina. Međutim, određivanjem korelacija između parametara mlijeka i biokemijskih parametara krvi, utvrđena je značajna pozitivna korelacija između količine izmuženog mlijeka i kalcemije i magnezijemije u uvjetima ishrane s dodatkom ulja. S obzirom da dodatak suncokretovog ulja u količini 2,5 % suhe tvari uravnoteženog obroka u hranidbi krava nije imao negativne učinke na mineralni profil i mjerene proizvodne parametre ispitivanih životinja, preporuka je da uvođenje ovog ulja može biti korisno s ekonomskog kao i sa stanovišta poboljšanja nutritivnog sastava mlijeka kao animalne namirnice svakodnevno prisutne u ljudskoj hrani.

Ključne riječi: *suncokretovo ulje, mliječne krave, minerali, krvna plazma, mlijeko*