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Influence of Natural Zeolites Supplemented with Inorganic Selenium on the Productive Performance of Dairy Cows

Monica Paula Marin[1], Elena Narcisa Pogurschi[1]*, Iuliana Marin[2] and Carmen Georgeta Nicolae[1]

[1]*University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania*
[2]*University Politehnica of Bucharest, Romania*

ABSTRACT

Milk quality is the most important criterion for the selection done by consumers. The natural, less expensive methods for improving the milk quality are the most studied by farmers and researches. Clinoptilolite, natural zeolite added to dairy cows diets, has been proven to be an efficient and economic solution. Starting from the fact that natural zeolites are recognized as having beneficial effects on the animal organism, being detoxifiers, immunomodulators, regulators of the internal pH, we studied the effect of using volcanic stuff as a food additive in dairy cows during the precalving, postcalving periods and in the first part of lactation. Experiments were carried out on 90 Holstein–Friesian cows, divided into 3 homogenous groups based on age and number of lactation. In diet of the two experimental groups were added 150 g and 300 g natural zeolite/head/day and we provided 0.28 mg selenium/kg feed dry matter, as opposed to the control group, which received 0.15 mg selenium/kg feed dry matter. The diets supplemented with natural zeolite based on clinoptilolite were administrated one month before parturition and continued for 16 other weeks. The zeolite consumption of 150 g/head/day has determined a decrease in the content of milk in heavy metals, increasing the concentration of unsaturated acids (oleic, linoleic) and immunoglobulins in colostrum, as well as reducing the diarrheal gastric disease of cattle. By adding zeolite in the cow ration during the precalving period has determined a significant increase in blood serum calcium during the last week of precalving period, as well as in the calving and postcalving periods, having as consequence fewer recorded milk fever cases. The results obtained can convince the farmers to use volcanic tuff as a food additive in the feeding of dairy cows during the precalving, postcalving and lactation periods, in parallel with ensuring the selenium requirements, because the natural zeolite can improve the reproductive capacity and productive performance of cows, the health status of the resulting calves.

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Authors' Contribution
MPM and ENP conceived and designed the project, performed the experiments, analyzed the data and wrote the article. IM curated the data and provided software. ENP provided resources.

Key words
Clinoptilolite, Milk, Heavy metals, Physico-chemical parameters, Immunoglobulins

INTRODUCTION

In modern human nutrition more attention is being given to the quality of food in close connection with how to obtain it. Milk and dairy milk products occupy an important place in everyday food, especially of the more sensitive categories as children and the elderly. Several numbers of studies support the use of zeolites in cow's nutrition to improve the milk quality, the daily average growth rate and the calf feed conversion index (Papaioannou *et al.*, 2005). The effectiveness of zeolites depends on the type of zeolite used, the purity and physico-chemical properties, and the level of supplementation in rations (Marin *et al.*, 2018). The Romanian zeolites have a high content of clinoptilolite, which give them a high value. In Romania there are generous zeolite sources, only Rupea (Brasov County, Romania) mine has a production capacity of 5000 tons monthly, this capacity being able to provide zeolites for 100 years (Pogurschi *et al.*, 2016). The effects of zeolites on milk production have been studied extensively during the past years. The evolution of milk production greatly depended on the dose of zeolites administrated to dairy cows. By using less than 300 g zeolites/cow/day, it has been reported a tendency to increase milk production, while doses exceeding 400 g zeolites/cow/day have led to a decrease in milk production (Khachlouf *et al.*, 2018). The mean milk yield of cows fed with 2.50% of clinoptilolite in diet was significantly higher ($p<0.05$) than that of cow fed with classical forages and it was significant than that of cow fed with 1.25% of clinoptilolite during the first five months of experiment (Katsoulos *et al.*, 2006). Statistically significant differences of milk fat were observed between the cows fed with 4% clinoptilolite in diet and the cows fed with 2% clinoptilolite in diet (Dokovic *et al.*, 2011). Few authors (Stojic *et al.*, 1995; Nikkhah *et al.*, 2002; Shahzad *et al.*, 2019) reported an improvement in the

* Corresponding author: elena.pogurschi@gmail.com
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immunity at the newborn calves which received colostrum from cows fed with clinoptilolite in diet. The addition of 1 g clinoptilolite/kg body weight to colostrum and milk administrated to newborn calves is appropriate dose for reducing incidence of diarrhea in newborn calves (Sadeghi and Shawarng, 2008). The benefits of zeolite feeding are primarily improved cow welfare by preventing clinical and subclinical parturient hypocalcaemia and associated production diseases (Thilsing-Hansen and Jorgensen, 2001). Thanks to its filtering properties zeolite has been using for centuries as a natural remedy for heavy metal reducing. Few experiments have targeted the use of zeolite, in particular clinoptilolite, to demonstrate its ability to reduce the heavy metal content of milk from cows that have consumed zeolite. Zeolites raise the adsorption of heavy metals in the gastrointestinal tract, connecting and transferring them into insoluble compounds, which together with the undigested remnants are excreted from the organism (Butsjak and Butsajk, 2014). The positive results reported in recent years have led to the investigation of natural Romanian zeolites, the development of a nutritional strategy for obtaining high quality milk by setting optimal doses of clinoptilolite that can be used in dairy cows diet.

MATERIALS AND METHODS

In the present work, the natural Romanian zeolite added to dairy cows diet in different doses has been investigated in order to demonstrate its ability to improve milk quality as well as health status of newborn calves and dairy cows. Chemical composition of the used natural Romanian zeolite was determined by x-ray diffraction, consisting of: 65.2% clinoptilolite, 7.5% quartz, 13.4% biotite, 9.5% feldspar and 4.4% calcite (Table I).

Regarding the chemical composition of the principal oxides, it was also determined based on the x-ray diffraction method and was: 69.4% SiO_2, 5.25% Al_2O_3, 1.26% Fe_2O_3, 4.2% CaO, 2.89% K_2O, 0.75% Na_2O, 0.05% TiO_2, 0.1% MnO.

Table I. Chemical composition of the natural Romanian zeolite.

The minerals	%
Clinoptilolite	65.2
Quatz	7.5
Biotite	13.4
Feldspar	9.5
Calcite	4.4

Experiments were carried out on 90 Holstein–Friesian cows, divided in 3 homogenous groups as age and number of lactation, respectively one control group and two experimental groups. All the cows have been fed with the same total mixed ratio composed of corn silage (55.50%), alfalfa hay (10%), maize (8%), barley (4%), soya meal (8%), beer residues (8%), molasses (1.80%), urea (1%), calcium carbonate (1.20%), dicalcium phosphate (1.00%), salt (0.5%), vitamino-mineral premix (1%). In the diet of the cows from the experimental groups were added 150 g (experimental group E1) and 300 g natural zeolite/cow/day (experimental group E2). Also, for the experimental groups, a level of 0.28 mg selenium/kg feed dry matter (DM) was provided, as opposed to the control group, for which was provided 0.19 mg selenium/kg feed dry matter, the difference being covered by the administration of sodium selenite (0.29 mg sodium selenite/kg feed DM) together with the volcanic tuff. The selenium levels provided to the dairy cows complied with the NRC Recommendations (2001) regarding the requirements of the dairy cows. The diets supplemented with natural zeolite based on clinoptilolite were administrated one month before the expected parturition and continued for 16 other weeks (Table II).

From each group milk samples were collected 2 times/day (in the morning and in the evening), 2 days/week. The concentration of heavy metals in the samples of forages, milk, urine and fecal masses were investigated by spectrophotometry, using the mode of adsorption of air-acetylene flame in atomic adsorption spectrophotometer, after calcination, according to SR EN 14082: 2003. The proportion of lead in the analyzed samples was achieved by vapor absorption spectrometry (CVAAS) after pressure digestion according to the standard SR EN 13806: 2003. The protein content of colostrum and milk was determined by the Kjeldahl method (SE 8968-1: 2014), the fat by the gravimetric method - SR ISO 1211: 2010 and the lactose content of milk was determined by high-performance liquid chromatography based on SR ISO 22662: 2008. Fatty acids in milk have been established by the gas chromatographic method according to SR ISO 15885:2002.

The obtained data were processed by statistical methods that compared the recorded average values, the probability of differences between control group and experimental groups parameters being evaluated using the Student test criteria.

RESULTS AND DISCUSSION

Milk yield, milk chemical composition, colostrum composition

The recorded milk yield among the three experimental groups did not vary significantly ($p>0.05$). The mean value of control group for daily milk yield was found as

Table II. Experimental scheme.

Batch	n	Treatment	Objectives
Control group C	30	Total mixed ratio (TMR)	Milk yield Milk chemical composition Fatty acids content of milk Heavy metal content of milk, feces, urine (Pb, Cd, Hg, Zn) Colostrum composition Incidence of diarrhea in newborn calves and milk fever in cows
Experimental group E1	30	TMR + 150 g natural zeolite/head/day + 0.29 mg sodium selenite/kg feed DM	
Experimental group E2	30	TMR + 300 g natural zeolite/head/day + 0.29 mg sodium selenite/kg feed DM	

28.19±1.05 l/head/day. The dose of 150 g clinoptilolite/cow/day resulted in a mean value for milk production of 29.05±0.99 l/head/day, while the double dose of clinoptilolite resulted in a mean value for milk production of 28.74±1.76 l/head/day as could be observed in Table III, not significant differences ($p>0.05$).

Table III. The effects of clinoptilolite on milk yield and milk quality.

Parameter	Control group C	Experimental group E1	Experimental group E2
Milk production (l/head/day)	28.19±1.05	29.05±0.99	28.74±1.76
Protein (%)	3.61±0.19	3.75±0.31	3.69±0.22
Fat (%)	3.51±0.24	3.62±0.17	3.64±0.32
Density (g/cm^3)	1.030±0.09	1.026±0.10	1.026±0.07

It is known that diet is a major factor of influence of the fat percentage and protein milk content, which is why in the present study milk samples were analyzed to reveal whether there are significant differences ($p<0.05$) of these two chemical constituents of milk, when the dairy cows received daily clinoptilolite (Gradinaru *et al*., 2007). The milk protein content was higher in E1 group (3.75%). Milk protein content of the control group and E2 group was similar, respectively of 3.61% and 3.69%. The differences are non-significant ($p>0.05$). Higher variations were recorded in the milk fat content. The highest fat content in milk was recorded in E2 group (3.64%), followed by E1 group (3.62%) and control group (3.51%), the differences are statistically insignificant ($p>0.05$). Milk density, a key parameter especially for processors, in the present case recorded small variation limits (1.026 g/cm^3 for E1 and E2 and 1.030 g/cm^3 for the control group).

In our experiment the effects of clinoptilolite feed supplementation on milk production were not significant ($p>0.05$). Several previous studies have also not noticed differences in milk production as a result of supplementing feed of dairy cows with clinoptilolite. Milk yield was unaffected by 200 g/cow/day clinoptilolite (Bosi *et al*., 2002). Research on Brown Swiss cattle that received 6% zeolite in concentrated feed (Azman *et al*., 1999) revealed the same fact that zeolite is not a factor that can influence milk production. Contrary to the results recorded in the present paper and other results reported by various authors, a 3% clinoptilolite added to Holstein-Frisian cow's diet for 16 weeks led to a significant increase in milk production from 30.63±0.851 l/day to 33.66±0.756 l/day (Ural, 2014). Supplementing dairy cow's diet with 4 and 2% clinoptilolite led to significant differences in milk fat content, supplementation with 4% clinoptilolite resulted in 4.62% fat in milk (Dokovic *et al*., 2011). The results regarding milk protein content when the clinoptilolite was added in 4 and 2% to dairy cows diet comply with the values registered in our experiment. Milk protein concentration tended to be higher for the zeolite dietary than for the control and for sodium bicarbonate dietary (Dschaak *et al*., 2010). Milk yield, milk fat and milk protein content did not differ between treatments as seen in previous studies (Grabherr *et al*., 2009; Thilsing-Hansen *et al*., 2007).

The approximately 400 fatty acids present in cow's milk fat make it the most complex natural fat. Besides the activity of microorganisms in rumen, diet is a major factor that determines the profile of fatty acids in milk. Starting from the premise that clinoptilolite may be part of a long-term nutritional strategy applied in dairy farms, the profile of fatty acids in milk obtained was also analyzed in this paper. In the present experiment there is no variation of the fatty acid content given by the grazing period or the winter season, because the feeding system applied is the same throughout the year with the purpose of not having differences in the fat percentage that dictates the purchase price of milk. The fatty acids content of milk is shown in Table IV. The mean value of control group for butyric acid was found as 3.21±0.11%. The dose of 150 g clinoptilolite/cow/day resulted in a mean value for butyric acid of 3.56±0.24%, while the double dose of clinoptilolite resulted in a mean value for butyric acid of 3.49±0.17%, as could be observe in Table IV. The differences registered between the tested groups are statistically significant ($p<0.05$).

Table IV. Fatty acid concentration in milk (% by weight of total fatty acids).

Fatty acids	Control group C	Experimental group E1	Experimental group E2
Saturated fatty acids			
Butyric acid C4:0	3.21±0.11[a]	3.56±0.24[b]	3.49±0.17[b]
Caproic acid C6:0	1.75±0.03	1.81±0.01	1.83±0.01
Caprylic acid C8:0	0.82±0.04	0.87±0.01	0.89±0.02
Capric acid C10:0	2.03±0.01	1.99±0.02	2.07±0.03
Lauric acid C12:0	3.24±0.09[a]	3.73±0.14[b]	3.68±0.06[b]
Myristic acid C14:0	9.82±0.76	10.92±0.53	11.05±0.49[b]
Palmitic acid C16:0	28.52±2.05	30.12±1.56[b]	30.74±2.11[b]
Stearic acid C18:0	9.36±0.65[a]	12.23±0.39[b]	12.15±0.46[b]
Unsaturated fatty acids			
Oleic acid C18:1	21.67±2.68[a]	24.12±2.11[b]	24.42±2.54[b]
Linoleic acid C18:2	4.09±0.11[a]	5.77±0.08[b]	5.64±0.06[b]

[a,b] significant differences between groups C, E1, E2 (P<0.05)

No statistically significant (p>0.05) differences were observed regarding the level of caproic, caprylic and capric acid in the analyzed milk samples.

For the cow group fed with a normal diet without addition of clinoptilolite, the amount of lauric acid on average was 3.24% by weight of total fatty acids. The highest level of lauric acid in milk, 3.73% by weight of total fatty acids, was noticed in E1 group, treated with 150 g clinoptilolite/head/day, and the differences registered between C and E1 groups are statistically significant (p<0.05). The mean value of E2 group (treated with 300 g clinoptilolite/head/day) for lauric acid was found as 3.68±0.06% and compared to control group the differences are statistically significant (p<0.05).

The milk myristic acid concentration increased proportionally with the dietary clinoptilolite, the highest value (11.05% by weight of total fatty acids) being recorded in E2 group, followed by 10.92% by weight of total fatty acids) in E1 group, significantly different (p<0.05) from 9.82%, in the control group. The same tendency to increase the level with the increase of the amount of zeolite added to diet was also recorded in the case of palmitic acid. The dose of 150 g clinoptilolite/cow/day resulted in a mean value for palmitic acid of 30,12±1.56%, while the double dose of clinoptilolite resulted in a mean value for palmitic acid of 30.74±2.11%, and the differences registered between the tested groups and control group are statistically significant (p<0.05). Milk content of stearic acid varied considerably, the highest value (12.23% by weight of total fatty acids) being recorded in E1 group (treated with 150 g clinoptilolite/head/day), followed by 12.15% by weight of total fatty acids in E2 group, significantly different (p<0.05) from 9.36%, in the control group.

Oleic acid, the fatty acid that represents most of the unsaturated fatty acids in milk, increased proportionally with the clinoptilolite dose added to dairy cows diet, the highest value (24.42% by weight of total fatty acids) being reported for E2 group, treated with 300 g clinoptilolite/head/day, 12.69% higher than for the control group. On the other hand, the lowest milk linoleic acid concentration (4.09% by weight of total fatty acids) was also reported for control group, 29.11% lower than for E1 group and 27.48% than for E2 group, the differences registered between the experimental groups and control group are statistically significant (p<0.05).

A general analysis of the data presented in Table IV shows an increase in the concentration of unsaturated fatty acids in milk as the dose of clinoptilolite increases, while the saturated fatty acid concentration had the tendency to increase, but no statistically significant differences (p>0.05) were observed, except butyric acid and long-chain fatty acids (C12:0, C16:0, C18:0).

Notably, we observed that the diet of dairy cows supplemented with clinoptilolite does not significantly influence (p>0.05) the level of saturated fatty acids in milk; this result is consistent with the reports of several previously conducted experiments (Olteanu *et al*., 2019; Kerwin *et al*., 2019). As the dose of zeolite increased, we observed an improvement in the level of unsaturated fatty acids.

Knowing that heavy metals in foodstuffs have a negative influence on human health, we proposed in this paper to evaluate their concentration in milk. The content of heavy metals in milk and dairy products is limited by certain regulations. The main source of heavy metals accumulated in the animal body is the ingested food, the water usually having a negligible content in heavy metals. The excretion pathways are: milk, urine and feces. The heavy metal content of the feed ingredients is presented in Table V.

The corn silage, which is the main ingredient of basal diet, had: 4.36 mg Pb/kg, 0.26 mg Cd/kg, 0.14 mg Hg/kg and 59.12 mg Zn/kg. Alfalfa hay, the second ingredient of proportion in TMR, had: 64.29 mg Zn/kg, 0.08 mg Hg/kg, 0.32 mg Cd/kg and 5.02 mg Pb/kg. The highest lead content of the feed ingredients was found in the soybean meal (7.35 mg Pb/kg), but this ingredient does not represent the ingredient that brings the highest proportion of lead in the total mixed ration, the inclusion rate in the basal diet being below 10%.

Table V. Heavy metal content of feed ingredients and total mixed ratio (mg/kg).

Fodder	Lead (Pb)	Cadmium (Cd)	Mercury (Hg)	Zinc (Zn)
Corn silage	4.36±0.22	0.26±0.03	0.14±0.005	59.12±3.16
Alfalfa hay	5.02±0.17	0.32±0.02	0.08±0.002	64.29±5.63
Maize	6.11±0.35	0.35±0.03	0.06±0.002	55.75±3.98
Barley	4.76±0.10	0.22±0.05	0.10±0.003	60.42±4.11
Soya meal	7.35±0.42	0.41±0.02	0.15±0.002	57.37±4.29
TMR	5.21±0.25	0.33±0.04	0.11±0.003	54.38±3.98

The concentration of heavy metals in milk, urine and fecal masses is presented in Table VI.

Table VI. The concentration of heavy metals in fecal masses, urine and milk (mg/kg).

Batch	Heavy metal	Fecal masses	Urine	Milk
Control group C	Pb	5.63^{a}	0.855^{A}	0.218^{a}
	Cd	0.41^{a}	0.052	0.027
	Hg	0.16	0.047	0.005
	Zn	57.02^{a}	3.37	2.05^{a}
Experi-mental group E1	Pb	6.45^{b}	0.745^{B}	0.191^{b}
	Cd	0.50^{b}	0.048	0.022
	Hg	0.18	0.044	0.003
	Zn	58.03^{b}	3.28	2.24^{b}
Experi-mental group E2	Pb	7.12^{B}	0.710^{B}	0.165^{B}
	Cd	0.51	0.049	0.020
	Hg	0.21	0.043	0.003
	Zn	57.76	2.97^{b}	2.01

^{a,b} significant differences between groups C, E1, E2 (P<0.05); ^{A,B} distinct significant differences between groups C, E1, E2 (P<0.01)

The highest content of lead was recorded in milk obtained from the control group cows, fed only with the basal diet, respectively 0.218 mg Pb/kg. The addition of clinoptilolite greatly reduced the amount of lead in the milk. The lowest milk lead content was recorded in E2 group, where the addition of clinoptilolite was 300 g/head/day, 24.31% lower than the control group, where distinct significant differences were noted (p<0.01). The dose of 150 g clinoptilolite/cow/day resulted in a milk content of lead of 0.191 mg/kg, 12.38% lower than the control group, significant differences were noted (p<0.05). The amount of lead excreted through the feces extended by increasing the dose of clinoptilolite. The highest amount of lead excreted by feces was recorded in E2 group (7.12 mg Pb/kg), 26.46% higher than control group (5.63 mg Pb/kg), the differences between groups C, E2 being distinct significant (p<0.01). The lead content of feces collected from E1 group was intermediate (6.45 mg Pb/kg) between the control group (5.63 mg Pb/kg) and E2 group, 14.56% higher than in the control group, significant differences were noted (p<0.05).

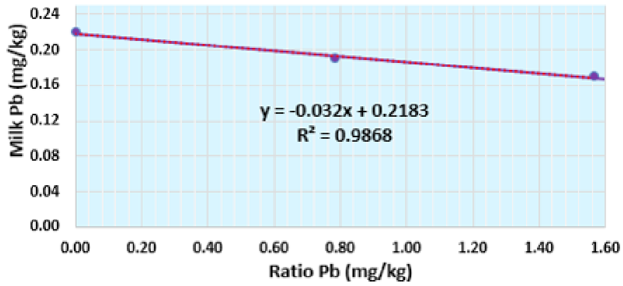

Fig. 1. Correlation between the lead from the total mixed ratio (TMR) and the lead milk content.

Figure 1 shows the correlation between the Pb from the total mixed ratio (TMR) and the Pb milk content, according to equation y=-0.032x ± 0.2183 and coefficient R^2= 0.9868.

The level of mercury excreted through feces had the same tendency, having a proportional increase with the amount of clinoptilolite supplied. The highest value (0.21 mg Hg/kg) recorded in E2 group, followed by 0.18 mg Hg/kg, in E1 group and 0.16 mg Hg/kg in control group; statistically the differences were not significant (p>0.05). We also did not see any significant differences in mercury level excreted through urine and milk. Significant differences (p<0.05) were noted in the case of cadmium and zinc excreted through feces between E1 group and control group. The amount of cadmium excreted through feces recorded by E1 group (0.50 mg Cd/kg) was 21.95% higher than cadmium amount recorded by control group (0.41 mg Cd/kg).

We found distinct significant differences (p<0.01) between the lead content of cow milk in the control group (0.218 mg Pb/kg) and the E2 group (0.165 mg Pb/kg), where the addition of clinoptilolite was 300 g/head/day and significant differences (p<0.05) were noted between control group and E1 group (0.191 mg Pb/kg), where the addition of clinoptilolite was 150 g/head/day. This fact certifies that zeolite has the capacity to bind certain toxic elements in the gastrointestinal tract, reducing absorption and increasing excretion of heavy metals. The same tendency had mercury excretion through feces, but the level of milk mercury was similar in the experimental groups, slightly lower compared to the control group, the differences having no statistical support. The obtained values were compared with those established by the European Commission Regulation, within the recommended limits. Certain mineral elements incorporated in enzymes with antioxidant properties has been linked to improve cow immunity during the transition period (Spears and Weiss, 2008). The influence of clinoptilolite on the level of cadmium and lead were studied *in vitro* in ruminants, being observed to cause the reduction of the levels of these elements in rumen and the abomasal fluid by 91% of Pb and 99% of Cd in the rumen fluid within 24 of hours and 94% of Pb in less than 1 hour in the abomasal fluid (Vrzgula and Seidel, 1989). To our knowledge, previous studies (Sadeghi and Shawarng, 2008; Kerwin *et al*., 2019; Mohri *et al*., 2008) have evaluated the ability of zeolite to influence colostrum composition and further the immunity of calves only when clinoptilolite was introduced into colostrum at different doses.

The analysis of the influence of clinoptilolite on the chemical composition and concentration in the immunoglobulins of colostrum was another major objective of the present experiment. Table VII data show that the addition of clinoptilolite to dairy cows basal diet does not influence the major components of colostrum, such as fat and protein, similar to the situation of milk. In accordance with the recorded results, the mean value of protein in colostrum was: 12.44±0.79% for control group, 12.95±1.12% for E1 group and 12.75±1.37% for E2 group. No statistically significant differences ($p>0.05$) were observed regarding the level of fat and lactose in the colostrum samples analyzed.

Table VII. The effects of clinoptilolite on colostrum composition and immunoglobulin concentration.

Parameter	Control group C	Experimental group E1	Experimental group E2
Protein (%)	12.44±0.79	12.95±1.12	12.75±1.37
Fat (%)	6.72±0.19	6.83±0.52	6.95±0.46
Lactose (%)	5.63±0.35	5.51±0.22	5.73±0.52
IgG (mg/ml)	35.15±1.79[a]	37.28±2.03[b]	37.45±1.95[b]

[a,b] significant differences between groups C, E1, E2 ($P<0.05$).

Colostrum IgG concentration was significantly higher ($p<0.05$) for the experimental groups than the control group, which is why we can say that the introduction of clinoptilolite in cows ratio increases the concentration of immunoglobulins from colostrum, which means better immunity of newborn calves. It is known that the neonatal calves are born with no immunoglobulins in the blood and the immediate administration of colostrum is mandatory.

IgG absorption from colostrum is mediated by intestinal pinocytosis, which continues only 24 hours after birth. It is known that increased Se content in colostrum can activate the secretion of pinocytosis by intestinal epithelial cells (Kamada *et al*., 2007). The highest mean value of colostrum IgG concentration was noticed in colostrum obtained from the E2 group cows, treated with 300 g clinoptilolite/head/day, respectively 37.45±1.95 mg/ml, followed by E1 group with 37.28±2.03 mg/ml and control group, 35.15±1.79 mg/ml, were noticed significant differences ($p<0.05$) between C, E1, E2 groups.

Considering that the nutritional strategy based on the use of clinoptilolite in the feeding of dairy cows will be applied on an industrial scale, the introduction of clinoptilolite in colostrum or milk would diminish the efficiency of work in dairy cows farms. For the proposed objective, the efficacy of clinoptilolite added to dairy cows basal diet on the IgG concentration of colostrum is satisfactory. Also, zeolite in combination with a source of selenium contributes to the development of the immune system of calves, selenium being an essential mineral trace element for animals, and in particular, for newborn calves.

Incidence of diarrhea in newborn calves and milk fever in cows

High blood immunoglobulin concentration has been linked to a good heath status, less cases of diarrhea and mortality in newborn calves.

According to the records of the farm, the cases of diarrhea in newborn calves are presented in Table VIII.

Table VIII. The cases of diarrhea in newborn calves.

Parameter	Control group C	Experimental group E1	Experimental group E2
Neonatal dairy calves with diarrhea (capita)			
0-7 days	4	2	2
7-14 days	8	3	4
Mortality (capita)			
0-7 days	1	0	0
7-14 days	0	1	0

The newborn calves were closely monitored and any signs of disease were well analyzed. In the first postpartum week, the control group recorded 4 cases of diarrhea while in the experimental groups were recorded only 2 cases of diarrhea. In the second postpartum week the incidence of diarrhea has increased. Diarrhea cases have doubled in the control and E2 groups. During the second postpartum week in the E1 group there were 3 cases of diarrhea. In the first two weeks from the parturition there were no deaths in E2 group. In the control group, 1 dead neonatal dairy calve was registered in the first week postpartum and in the E1 group in the second week postpartum 1 dead neonatal dairy calve was also registered.

Recent studies have shown that the administration of zeolite in the feeding of lactating dairy cows during suckling can make it possible to prevent the risk of milk fever, as well as hypocalcemia, the zeolite having the ability to fix calcium in the intestinal tract and make it unavailable for absorption. The blood calcium serum concentration recorded during the precalving, calving and postcalving period and farm-recorded milk fever cases are presented in Table IX.

Table IX. The effects of clinoptilolite on blood serum calcium concentration and the cases of milk fever.

Parameter	Control group C	Experimental group E1	Experimental group E2
Blood serum calcium concentration (mg/dl):			
2 weeks before calving	7.14±0.07	7.28±0.03	7.35±0.05
1 week before calving	6.99±0.09[a]	7.46±0.05[b]	7.67±0.04[b]
Calving	6.71±0.03[a]	7.69±0.02[b]	7.85±0.05[b]
1 week after calving	7.55±0.07[a]	7.98±0.03[b]	8.25±0.09[b]
Milk fever cases:			
Capita	7	3	2
% total group	23.33	10.00	6.67

[a,b] significant differences between groups C, E1, E2 ($P<0.05$).

On average, in the first week before caving, cows belonging to the experimental group E2, treated with 300 g clinoptilolite/head/day, had blood serum calcium concentration approximately 0.68 mg/dl greater than those belonging to the control group and 0.21 mg/dl greater than those belonging to the experimental group E1, treated with half the dose of clinoptilolite, respectively 150 g/head/day; we noticed significant differences between groups C, E1, E2 ($p<0.05$). The same tendency of significant increase in blood serum calcium was also recorded during the calving and post calving period. The mean value of calcium in blood serum was: 6.71±0.03 mg/dl for control group, 7.69±0.02 mg/dl for group E1 and 7.85±0.05 mg/dl for E2 group. One week after calving, the mean value of blood serum calcium level recorded by E2 group was 8.25±0.09 mg/dl, which means that given for a longer period of time clinoptilolite leads to higher values of the calcium blood serum level. The recorded milk fever cases were considerably less in the case of E2 group, respectively only 2, compared to the control group, where 7 cases were diagnosed.

A previous study reported that supplementation with 2.5% clinoptilolite of dairy cows basal diet reduced the incidence of parturition paresis. As a result, the addition of clinoptilolite to dairy cows diet during the suckling period could be used as a preventive treatment for parturient paresis (Mohri *et al*., 2008). On the other hand, the administration of 0.5 and 1.0 kg of zeolite daily in the dairy cows during the last 2 to 4 weeks of the weaning period caused a significant increase in the average level of calcium in the blood serum on the day of calving. As a result, an average efficiency of zeolite supplementation of cows' feed was established in order to prevent hypocalcemia on the day of calving of 58% (Thilsing-Hansen *et al*., 2003). A novel nutritional strategy to prevent milk fever is based on the effect of zeolite on blood calcium level (Wilson, 2001). Milk fever is an important disease in dairy farms. If there are more than 10% cases diagnosed in the farm, a specific control program to prevent this disease must be implemented (Radostits *et al*., 2000) and form our results we can conclude that the addition of zeolite to dairy cow diet could be part of this program.

CONCLUSIONS

The use of natural zeolites in the feeding of dairy cows during the precalving, postcalving periods and in the first part of lactation plays the role of promoter of productive performances, improving the chemical composition of colostrum and milk, but also had the role of reducing the proportion of heavy metals in milk. By improving the proportion of colostrum immunoglobulins and blood calcium in cows, zeolites, in combination with a source of selenium, have favorable effects in preventing diarrhea in cattle, as well as milk fever in cows. As a result, the use of zeolites in dairy farms can be a solution for the control and prevention of the problems related to the productivity and health of the animals, constituting an easy solution to integrate in the production chain of the farm to maintain its profitability.

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Statement of conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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