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Effects of short-term supplementation of clinoptilolite in colostrum and milk on hematology, serum proteins, performance, and health in neonatal dairy calves

M. Mohri *, H.A. Seifi, F. Daraei

Department of Clinical Sciences and Center of excellence in Ruminant Abortion and Neonatal Mortality, School of Veterinary Medicine, Ferdowsi University of Mashhad, P.O. Box 91775-1793, Mashhad, Iran

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Abstract

In recent years, the use of both natural and synthetic zeolites in animal nutrition has increased, mainly to improve their performance, health, and to protect against mycotoxins intoxication. Thirty calves were used in the present study for the determination of the effects of clinoptilolite supplementation on hematology, serum proteins, performance, and health. The animals were divided equally into three groups (control, test 1 and test 2). The three groups of calves were homogeneous for parity of dams, sex, and month of birth. For test 1 group, clinoptilolite in the concentration of 2% of each colostrum meal was added for 48 h and for test 2 group, clinoptilolite in the concentration of 2% was added to each colostrum and milk meal for 14 days. Blood samples were taken from all calves 12 h after birth and at the end of the first, second, third, forth, fifth and sixth weeks of life (end of the experiment: 42 days of life) and analyzed for hematology, plasma fibrinogen and for total protein, albumin, beta and gamma globulin measurement. Performance and health of calves were also recorded during the experiment. For statistical analysis of data a repeated measures approach using ANOVA with Mixed linear models, and χ-test was used. Clinoptilolite supplementation had significant effect on the values of hematocrit (HCT), red cell count (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), monocyte (Mono), and albumin (Alb). The values of most above parameters were significantly higher in test group 2, except MCV that was significantly lower in test group 1 than other trial groups ($p < 0.05$). No significant difference was seen for other measured parameters, performance, and health between trial groups.

Keywords: Calves; Clinoptilolite; Health; Hematology; Performance; Serum proteins

1. Introduction

A zeolite is a crystalline, hydrated aluminosilicate of alkali and alkaline earth cations having an infinite, open, three-dimensional structure. It is further able to lose and gain water reversibly and to exchange extra framework cations, both without change of crystal structure. On removal of water by heating at 350–400 °C, small molecules can pass through entry channels, but larger molecules are excluded the so-called "molecular sieve" property of crystalline zeolites. The cation-exchange capacity (CEC) of a zeolite is a function of the amount of aluminum that substitutes for silicon in the framework tetrahedra; the greater the Al content, the more extra framework cations needed to balance the charge (Mumpton and Fishman, 1977).

The application of zeolites in animal husbandry and health was reviewed (Nestorov, 1984; Kyriakis et al., 2002; Trckova et al., 2004). In recent years, the use of both natural and synthetic zeolites in animal nutrition has increased, mainly to improve their performance, and health. Katsoulos et al. (2006) reported the cows with 2.5% clinoptilolite in ration had significantly fewer cases of clinical ketosis during the first month after calving and a higher total milk yield. Thilsing-Hansen and Jorgensen (2001) suggested the addition of synthetic zeolite A to the

* Corresponding author. Tel.: +98 511 6610430; fax: +98 511 6620166.
E-mail address: mohri@ferdowsi.um.ac.ir (M. Mohri).

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daily ration during the last month of pregnancy prevented parturient paresis as well as subclinical hypocalcemia in Jersey cows. The benefit of clinoptilolite addition to ration on health and performance of weaned, growing, and finishing pigs was suggested by Papaioannou et al. (2004).

The effects of zeolite supplementation on hematology and serum biochemistry of dairy cows, sheep, and sows were studied (Verzgula et al., 1982; Bartko et al., 1983; Papaioannou et al., 2002; Thilsing-Hansen et al., 2003; Katsoulos et al., 2005a,b,c,d, 2006). In addition, limited information exist concerning the effects of zeolite supplementation on performance, health, hematology, serum proteins, and immunoglobulin concentration in serum of neonatal dairy calves, and lambs (Petkova et al., 1982a,b, 1983; Verzgula, 1986; Stojic et al., 1995; Fratrić et al., 2005; Gowda et al., 2007) but there is no complete well controlled study in neonatal dairy calves. The objectives of the present study were to determine the effects of short-term clinoptilolite supplementation in colostrum and milk on hematology, serum proteins, performance, and health of neonatal dairy calves.

2. Materials and methods

The study was conducted in a dairy herd with approximately 600 calves per year at Mashhad suburb (northeast of Iran). This herd consisted of purebred Holstein cattle and was totally confined in free-stall housing without access to pasture. Dry cows were fed with alfalfa hay, corn silage, a proprietary concentrate containing barley, cottonseed, bran, beetroot, and 1% DM (of concentrate) supplement. Cows were dried 2 months before expected time of parturition and transferred to a single occupation separate stall. The vaccination program and the dry cow therapy were similar in the cows of the experiment. As the time of parturition approached, the cows were moved to straw-bedded maternity pens. In general, Prompt assistance was given to cows with dystocia although no dystocia was recorded in the cows of the experiment. Following parturition, the umbilicus of each calf was treated with Povidone iodine and the calf was weighed and transferred to a separate individual pen. Within the first 6 h of life, 2.5 kg of dam's colostrum was fed by nipple bottle to all calves and colostrum feeding was continued every 12 h for 48 h. Subsequently, herd milk replaced colostrum and was allowed twice daily (2.5 kg every 12 h) together with calf starter including concentrate (90% DM, Table 1) and high quality alfalfa (10% DM) with water available ad libitum. The calves were weaned at 90 days of life. The heifer calves were mainly used as herd replacements.

The zeolitic-tuff used contained approx 92% clinoptilolite (Afrand Tusca Co, Tehran, Iran). The cation-exchange capacity and specific gravity of the zeolite material was 160–180 mEq/100 g and 1 g/cm^3, respectively. Its chemical composition was 66.5% SiO_2, 11.8% Al_2O_3, 3.1% CaO, 0.8% MgO, 2.00% Na_2O, 2.1% K_2O, 1.3% Fe_2O_3, 0.3% TiO, 0.04% MnO, 0.01% P_2O_5, and L.O.I. (loss on ignition) 12%; Granullometry of clinoptilolite was adjusted by crushing and screening the raw material to a size of <1 mm (99% of the particles <0.7 mm).

Table 1
Ingredient composition of concentrate mix fed to calves (DM%)

Ingredients	%	Ingredients	%
Corn	50	Molasses	5.5
Barley	15	D.C.P	0.2
Soybean meal	22	Limestone	0.9
Beet pup	3	Supplement*	0.4
Wheat bran	3		

* Each kg of supplement contain: Vitamin A (50,000 IU), Vitamin D3 (10,000 IU), Vitamin E (0.1 g), Calcium (196 g), Phosphorus (96 g), Sodium (71 g), Magnesium (19 g), Iron (3 g), Copper (0.3 g), Manganese (2 g), Zinc (3 g), Cobalt (0.1 g), Iodine (0.1 g), Selenium (0.001 g).

Thirty calves were used in the present study. The animals were divided equally into three groups (control, test 1 and test 2). The three groups of calves were homogeneous for parity of dams, sex, and month of birth. For test 1 group, clinoptilolite in the concentration of 2% was added to each colostrum meal for 48 h and for test group 2, clinoptilolite in the concentration of 2% was added to each colostrum and milk meal for 14 days. All other aspects of the diet were identical for all groups including the controls. Ten milliliters of jugular blood were taken from all calves 12 h after birth and at the end of the first, second, third, forth, fifth and sixth weeks of life. Two milliliters of blood was anticoagulated with EDTA for hematological analysis, and plain tubes supplied serum for analysis of some biochemical parameters. The serum was separated after centrifugation at 1800*g* for 10 min and stored at −18 °C until analysis.

Anti-coagulated blood was analyzed shortly after collection for: number of red blood cell (RBC), hemoglobin concentration (Hb), hematocrit (HCT), total leukocyte count (WBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV) by an automatic veterinary hematology cell counter (Nihon kohden, Celltac *a*, Tokyo, Japan). Differential leukocyte counts were performed on routinely prepared Giemsa stained blood films using the cross-sectional technique. Fibrinogen concentration was estimated by heat precipitation method (Jain, 1986).

Stored serum samples were analyzed for total protein (TP, Biuret method, Tomas, 1998) and albumin (Alb, Bromcresol green method, Johnson et al., 1999), by commercial kits (Pars Azmoon, Tehran, Iran) using a spectrophotometer (Jenway 6105, Jenway, Felstead, England). Control serum (Randox control sera, Antrim, UK) was used for controlling measurement accuracy. Percentage and absolute levels of beta and gamma globulins were determined by cellulose acetate electrophoresis and densitometry (Helena system).

Disease occurrence and treatment days were also recorded during the experiment. Calves were weighed at birth and weekly until the end of the sixth week of experiment.

The data were analyzed by SAS 8.2 version statistical package. Because serum parameters measured over time, a repeated measures approach using ANOVA with Mixed linear models in SAS was used (fixed effects of group and covariates, random effect of calf). All outcome variables were screened for normality by visual assessment of the distributions and calculation of kurtosis and skewness. The metabolites without normal distribution were converted using a natural logarithmic transformation to achieve a normal distribution. Several covariance structures were evaluated for each analyzed metabolite and the covariance structure that resulted in Akaike's information criterion (AIC) closest to zero was used (Wang and Goonewardene, 2004). Interactions between treatment and time (days 0, 7, 14, 21, 28, 35, 42) were tested and included in the final model if significant and data were reanalyzed after stratification by sample. Results were shown with least square of means and standard errors. One way ANOVA with Bonferroni *t*-test were used to investigate significant difference between groups for total weight gain, and mean daily gain. χ-test was also used for comparison of disease occurrence between groups. $P < 0.05$ was considered as significant.

3. Results

The results are summarized in Tables 2–4. Group had significant effect on the values of HCT, RBC, hemoglobin, MCV, monocyte, and albumin (Figs. 1–6). The values of most above parameters were significantly higher in test group 2, except for MCV that was significantly lower in test group 1 than other trial groups [($p < 0.05$) (Tables 2 and 3)].

Table 2
Effects of clinoptilolite supplementation on hematological parameters

Parameters	Control	Test 1	Test 2	SE	Age	Group	Age * group
HCT (L/L)	0.26[a]	0.25[a]	0.31[b]	0.01	S	S	NS
RBC (10^{12}/L)	8.00[a]	7.76[a]	9.45[b]	0.32	S	S	NS
Hb (g/L)	97.1[a]	96.7[a]	112.5[b]	3.9	S	S	NS
MCV (fl)	32.67[a]	30.96[b]	32.29[a]	0.52	S	S	NS
MCH (pg)	11.79	10.99	10.51	0.63	S	NS	NS
MCHC (%)	34.13	35.21	34.79	0.56	S	NS	NS
WBC (10^9/L)	9.64	10.09	9.69	0.48	S	NS	NS
Neut (10^9/L)	3.20	3.30	2.80	0.54	S	NS	NS
Lymph (10^9/L)	5.92	5.90	5.66	0.35	S	NS	NS
Mono (10^9/L)	0.39[a]	0.56[b]	0.74[c]	0.10	NS	S	NS
Fib (g/L)	3.46	3.03	2.51	0.44	NS	NS	NS

Means within rows lacking a common superscript differ ($P < 0.05$).
S, significant effect ($P < 0.05$), NS, not significant effect.
HCT (hematocrit); RBC (red cell count); Hb (hemoglobin); MCV (mean corpuscular volume); MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration); WBC (white blood cell count); Neut (neutrophil); Lymph (lymphocyte); Mono (monocyte); Fib (fibrinogen).

Table 3
Effects of clinoptilolite supplementation on serum proteins

Parameters	Control	Test 1	Test 2	SE	Age	Group	Age * group
TP (g/L)	60.8	61.7	61.00	2.60	S	NS	NS
Alb (g/L)	35.2[a]	37.2[b]	37.3[b]	0.3	S	S	S
γ globulin (g/L)	16.9	15.5	13.6	1.3	NS	NS	NS
β globulin (g/L)	13.3	13.4	14.6	1.2	S	NS	NS
A:G ratio	1.52	1.60	1.63	0.22	S	NS	NS

Means within rows lacking a common superscript differ ($P < 0.05$).
S, significant effect ($P < 0.05$), NS, not significant effect.
TP (total protein); Alb (albumin).

Table 4
Mean ± SE of total performance between trial groups

Parameters	Control	Test 1	Test 2	*P* value
Total weight gain (kg)	16.27 ± 0.73	14.85 ± 0.78	15.22 ± 1.24	NS
Mean daily gain (kg)	0.410 ± 0.018	0.364 ± 0.019	0.382 ± 0.092	NS

NS, not significant difference.

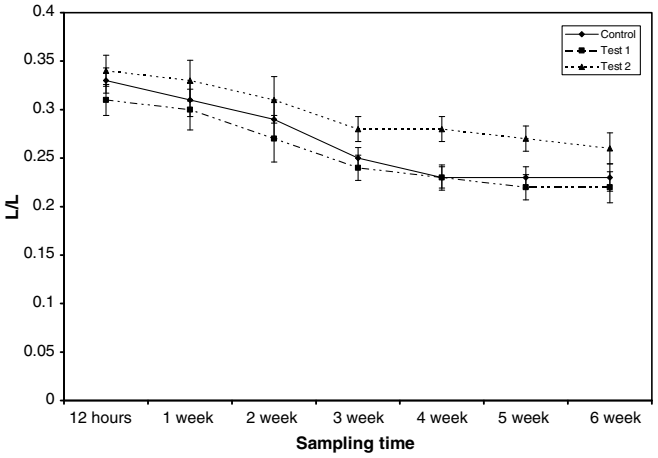

Fig. 1. Mean hematocrit (SE) in clinoptilolite treated and control calves.

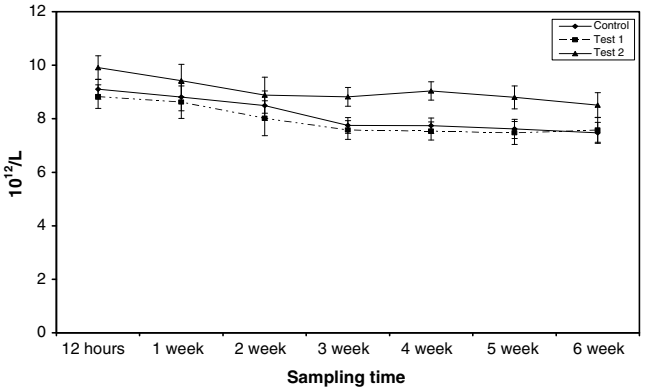

Fig. 2. Mean RBC (SE) in clinoptilolite treated and control calves.

Age (sampling time) had significant effects on the values of most measured parameters except monocyte, fibrinogen, and gamma globulin [($p < 0.05$) (Tables 2 and 3)]. Significant interactions between sampling time and group were only seen for albumin concentration [($p < 0.05$) (Table 3)]. Group had not significant effect on weight of calves (control: 50.35 kg, test 1: 45.75 kg, test 2: 48.78 kg, pooled SE:

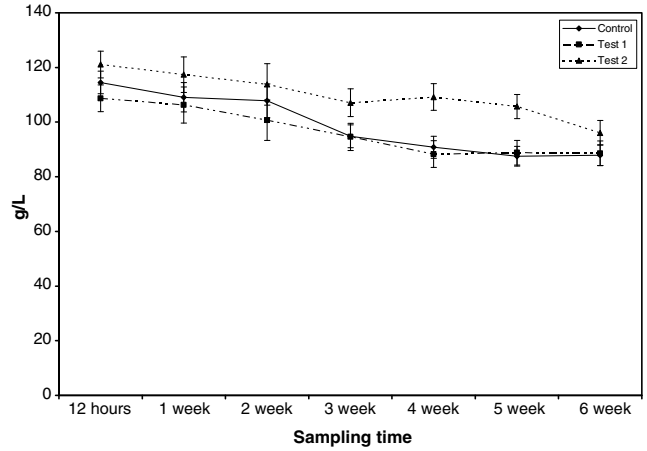

Fig. 3. Mean hemoglobin (SE) in clinoptilolite treated and control calves.

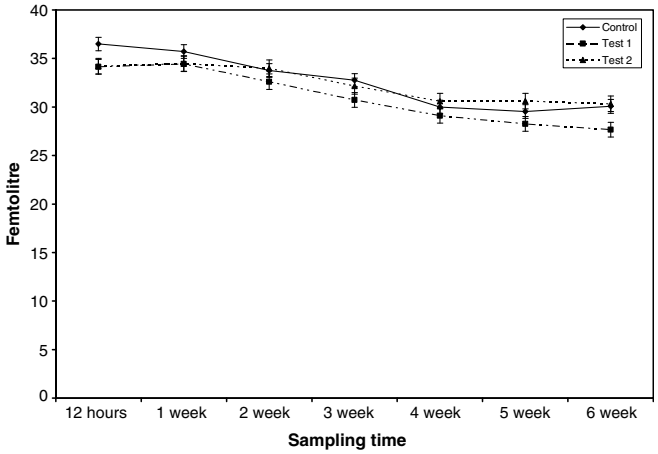

Fig. 4. Mean MCV (SE) in clinoptilolite treated and control calves.

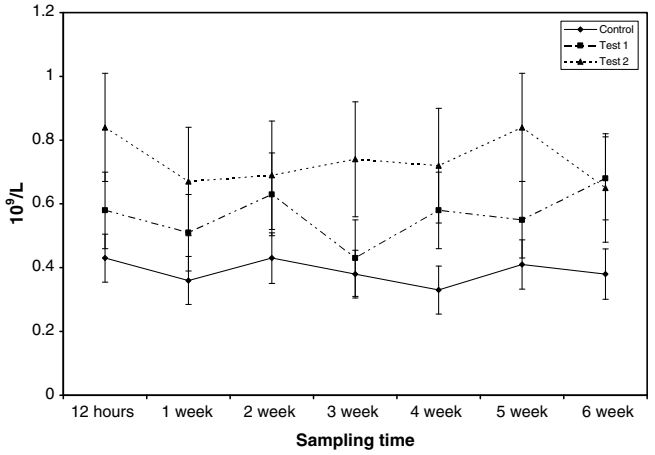

Fig. 5. Mean monocyte (SE) in clinoptilolite treated and control calves.

3.15). No significant differences were seen for total weight gain, and mean daily gain between trial groups (Table 4). χ-test revealed no significant difference for the incidence and the days of treatment between groups.

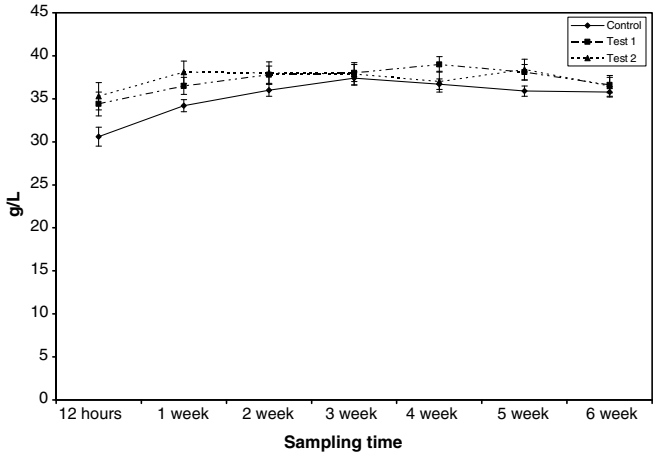

Fig. 6. Mean albumin (SE) in clinoptilolite treated and control calves.

4. Discussion

Hematology of food and laboratory animals following short or long-term zeolite supplementation was studied. Hutcheson (1984) suggested no significant difference for PCV amount in calves supplemented with 3% of diet with clinoptilolite for 56 days and control calves. In another study, Katsoulos et al. (2005c) reported no significant effect of long-term clinoptilolite supplementation at a rate of 1.25% and 2.5% in the concentrate feed of dairy cows on the values of RBC and PCV. Bartko et al. (1983) suggested that addition of clinoptilolite at a rate of 0.15 g/kg of live weight daily for three months in sheep had no significant effect on RBC. Martin-Kleiner et al. (2001) suggested that the addition of clinoptilolite for 6 weeks did not cause significant alterations on PCV values in mice. In contrast with previous reports and in consistency with the results of the present study, Petkova et al. (1982a), revealed significant increase of Hb concentration in newborn calves that supplemented with 2% potassium–calcium zeolite with the colostrum at first, and the milk afterward, for first 15 days of life. Similar results were also obtained by supplementation of colostrums and milk of the newborn calves with clinoptilolite at a rate of 1 g/kg body weight for the first 15 days of life (Verzgula, 1986). In the present study, the exact mechanisms of increased RBC in clinoptilolite supplemented test 2 calves are not clear, but it could be attributed to a possible increase of iron concentration due to clinoptilolite supplementation for 14 days to these calves. Hutcheson (1984) believed that zeolite might possibly supply a significant amount of iron to the diets of cattle, providing that the iron is present in a form that can be used by the body. The exact cause of significant decreasing of MCV values in calves of test 1 group was not clear. The only published paper which found by us concerning to effects of clinoptilolite on RBC indices was related to Martin-Kleiner et al. (2001). They reported no changes in RBC indices of mice after clinoptilolite supplementation.

In the present study, clinoptilolite supplementation had no significant effect on WBC, neutrophil, and lymphocyte count but a significant effect was seen on monocyte count.

In the study of Katsoulos et al. (2005c), prolonged in-feed inclusion of clinoptilolite at two levels (1.25% and 2.5%) in dairy cows ration had no significant effect on WBC. Similar results were reported with clinoptilolite supplementation in sheep (Bartko et al., 1983). In contrast, Petkova et al. (1982a) suggested significant changes with increased numbers of granulocytes and lymphocytes in newborn calves following 15 days supplementation of clinoptilolite in milk. Increased leukocyte count, mainly lymphocytes was reported in mice supplemented with clinoptilolite (Martin-Kleiner et al., 2001). In the present study, the monocyte count was significantly higher in clinoptilolite supplemented than the control group, especially in test group 2 where the duration of supplementation was longer. This increased monocyte count in the two test groups may be attributed to alimentary tract irritation elicited by zeolite particle.

Clinioptilolite supplementation had no significant effect on the concentrations of serum TP, gamma, and beta globulin in the present study; only Alb concentrations were significantly affected by supplementation. Verzgula (1986) suggested that following clinoptilolite supplementation at a rate of 1 g/kg body weight for 15 days in milk, Alb concentrations were significantly higher in supplemented calves. Higher levels of Alb were also reported in calves following 15 days of zeolite supplementation at a rate of 1–3% (Petkova et al., 1982a). In the study of the effects of long-term feeding of a diet supplemented with clinoptilolite at a rate of 1.25% and 2.5% to dairy cows, no apparent effects were seen on the concentrations of serum total protein (Katsoulos et al., 2006). In growing lambs, clinoptilolite supplementation did not affect the concentration of serum total protein (Pond et al., 1984). The exact mechanisms by which zeolite affect the Alb concentration are not clear; a possible explanation might be that the dietary protein is utilized better, favouring the albumin synthesis by the liver. White and Ohlrogge (1974) found that zeolites could sequester and release 15% of the ammonium ions in the rumen and affects the availability of nitrogen and then the metabolism of proteins. In neonatal calves supplemented with zeolite, the better digestion of milk protein and the higher absorption of amino acids may be possible reasons of higher albumin levels.

In contrast to our results, clinoptilolite supplementation in colostrum or colostrum and milk resulted in higher IgG concentrations in serum of supplemented calves (Petkova et al., 1982a; Stojic et al., 1995; Fratrić et al., 2005). Higher absorption of immunoglobulins of colostrum and/or absorption of antigens in the intestine may be the causes of higher levels of immunoglobulins in the serum of zeolite supplemented calves. In the present study, the cause of non significant effect of clinoptilolite supplementation on gamma and beta globulin concentrations was not clear.

In accordance to our result, Hutcheson (1984) reported the average daily gains of steers fed clinoptilolite ores tended to be reduced compared to those of control animals but the differences were not significant. On the other hand, Petkova et al. (1983) suggested about 19% greater average daily gain in lambs supplemented with 4% zeolite in ration for 45 days. In addition, Pajovic et al. (1998) reported better average daily gain and total gain in zeolite supplemented suckling calves for 75 days. Weight gain may be caused by the zeolite acting as an ammonium reservoir in the gastrointestinal tract, thereby allowing the animal to use ingested nitrogen more efficiently but it seems this effect created after long-term supplementation.

Petkova et al. (1982a) studied the effects of addition of 1.2% or 3% of zeolite in colostrum or milk for 15 days in calves. Zeolite improved the general health of the calves and stimulated their resistant to disease. In other study, the supplementation of natural zeolite at a dose rate of 1 g/kg of body weight revealed the effectiveness of 68.7% compared to 18% in preventing of diarrhea in supplemented versus control calves, respectively (Bartko et al., 1995). Similar results were reported following zeolite supplementation in piglets (Petkova et al., 1982b). Bartko et al. (1983) suggested no differences in the health condition of sheep fed zeolite at a rate of 0.15 g/kg of live weight daily for three months. The prevention or minimization of scours and other intestinal diseases, however, is more baffling. An NH_4^+-containing zeolite may support the growth of nitrogen-loving bacteria that contribute to the health of the animals; the zeolite may take up deleterious heavy metals, or it may simply regulate pH in the gut system, resulting in fewer or less severe stomach ailments. These reactions await serious physiological and biochemical examination (Mumpton, 1999).

In conclusion, short-term supplementation of clinoptilolite in colostrum and milk could significantly affected hematological parameters especially RBC parameters and serum albumin concentration in neonatal dairy calves. Since our results were not in accordance with previous reports, it seems further studies for elucidation of effects of clinoptilolite supplementation (with different amounts and duration of supplementation) on immunoglobulin concentrations, performance, and health could be useful.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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