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KONSTANTINOS POURLIOTIS

MARIA KARATZIA

EFTERPI CHRISTAKI

PANAGIOTIS KATSOULOS

HARILAOS KARATZIAS`

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Effects of dietary inclusion of clinoptilolite in colostrum and milk on certain haematological parameters of calves

Konstantinos POURLIOTIS[1], Maria KARATZIA[1], Efterpi CHRISTAKI[2],
Panagiotis KATSOULOS[1,*], Harilaos KARATZIAS[1]

[1]Clinic of Farm Animals, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki,
546 27, Thessaloniki – GREECE

[2]Laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki,
541 24, Thessaloniki – GREECE

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Abstract: The administration of a natural zeolite, clinoptilolite, in newborn calves via colostrum and milk has been proven to be beneficial in enhancing the intestinal absorption of immunoglobulins and in reducing the incidence and the severity of diarrhoea syndrome. The aim of this study was to investigate whether a 2-month-long supplementation at 2 levels (1 g/kg body weight (BW) and 2 g/kg BW) of clinoptilolite in the colostrum and milk of dairy calves had any effect on their haematological parameters. A total of 84 clinically healthy Holstein calves were assigned to 1 of 3 groups immediately after calving. Group A (n = 28) received clinoptilolite at the rate of 1 g/kg BW per day, initially via colostrum and later via milk. Group B (n = 28) received clinoptilolite at the rate of 2 g/kg BW per day via colostrum and milk, and group C (n = 28), which served as the control, received colostrum and milk without clinoptilolite supplementation. The experiment started at the day of parturition and lasted up to the 60th day after calving. Blood samples were collected from each calf at birth; 12 h, 24 h, and 48 h after calving; and at the age of 15, 30, 45, and 60 days. All samples were tested for packed cell volume (PCV), haemoglobin (Hb) concentration, and the number of red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs). The results showed that the addition of both levels of clinoptilolite to the colostrum and milk significantly increased PCV, RBCs, and the concentration of Hb, but did not have a significant effect on WBC and PLT counts. Overall, clinoptilolite administration via colostrum and milk appeared to enhance the haemopoiesis in newborn calves without having any adverse effect on WBC and PLT values.

Key words: Clinoptilolite, packed cell volume, red blood cells, haemoglobin, platelets, calves

Introduction

The use of zeolites in animal nutrition has increased in recent years, mainly to protect animals against mycotoxin intoxication but also to improve their performance (1). Zeolites such as clinoptilolite are crystalline hydrated aluminosilicates of alkali and alkaline earth cations that have unique properties. They are characterised by the ability to lose and gain water reversibly, to adsorb molecules of appropriate diameters, to act as molecular sieves, and to exchange their constituent cations without a major change in their structure (2). The exploitation of these properties underlies their use in animal nutrition. Recently, the European Commission has provisionally authorised the use of a natural zeolite, clinoptilolite, as an additive in feedstuffs for farm animals (3).

* E-mail: pkatsoul@vet.uth.gr, katsoulosp@yahoo.com

Clinoptilolite is stable at the acidic gastrointestinal pH (4) and, due to its particle size, is not absorbed from the gastrointestinal tract of the animals. However, when administered to the animals for a long period of time, it is possible for it to cause alterations to their haematological profile. For example, clinoptilolite could irritate the intestinal mucosa and increase the number of white blood cells (WBCs) or, due to its cation exchange capacity, it could interfere with the absorption of iron and cause alterations to haematocrit results and the concentration of haemoglobin (Hb). Research results in mice that received clinoptilolite for a prolonged period of time indicated that such alterations in WBC count (5) and erythropoiesis cannot be excluded (6).

Concerning ruminants, it has been proven in previous reports that the addition of clinoptilolite in the feed of adult animals has no major effects on their haematological parameters (7–9). Until now, although the administration of clinoptilolite in newborn calves has been proven to be beneficial in enhancing the intestinal absorption of colostral antibodies and in reducing the incidence and the severity of diarrhoea syndrome, there are no references concerning the long-term effect of clinoptilolite supplementation on the blood parameters of these animals. Evidence in the literature suggests that the short-term administration of clinoptilolite to calves, initially via colostrum and later via milk, has a significant effect on the blood concentration of Hb and the differential cell count (10).

The objective of this study was to determine whether a 2-month-long supplementation of 2 levels (1 g/kg and 2 g/kg body weight [BW]) of clinoptilolite in the colostrum and milk of dairy calves had any effect on their haematological parameters.

Materials and methods

Animals and experimental design

Eighty-four newborn dairy calves that were judged to be healthy according to the results of a clinical examination and haematological and blood serum biochemical tests were used in the experiment. All calves were separated from their dams immediately after calving and, after weighing, were housed in separate boxes with straw bedding. Seven days after calving, they were transferred to larger pens in groups of 3, where they stayed until the end of the experimental period. All calves' pens were in a separate building from the adult animals with controlled environmental conditions (temperature 15–18 °C, relative humidity 80%) in order to avoid environmental stress. During the first 10 days of their life, the calves were fed with colostrum and bulk tank milk, and afterwards, until the end of the experimental period, they were fed with milk replacer in a quantity of 10% of their BW.

The calves were divided equally into 3 groups according to their BW, their sex, and the parity of their dams. The first group (group A, n = 28) consisted of calves that received clinoptilolite at the rate of 1 g/kg BW per day, initially via colostrum and later via milk. The calves of the second group (group B, n = 28) received clinoptilolite at the rate of 2 g/kg BW per day via colostrum and milk, and those of the third group (group C, n = 28) received colostrum and milk without clinoptilolite supplementation and served as controls.

The experiment started at the day of calving and lasted until the age of 60 days. Blood samples were collected from each calf at 8 time points: at birth (before the first colostrum meal); 12 h, 24 h, and 48 h after calving; and at the age of 15, 30, 45, and 60 days. The samples were taken via jugular vein-puncture using a 21-gauge needle into evacuated glass tubes with an anticoagulant (EDTANa). Blood analysis included the determination of packed cell volume (PCV), Hb concentration, and the number of red blood cells (RBCs), WBCs, and platelets (PLTs) with the aid of a veterinary haematology analyser (Micros® OT-CT-OS-ABC Vet-ICOR, ABX Diagnostics), using the specific procedure for bovine samples.

Zeolitic material

The zeolitic material used in the experiment had a particle size of <0.80 mm and contained approximately 92% clinoptilolite, and the admixture was 8% opal ($SiO_2 \times nH_2O$), as determined by X-ray powder diffraction. The material's cation exchange capacity was 220 mEq/100 g and its chemical composition was 68.97% SiO_2, 11.27% Al_2O_3, 3.02% CaO, 0.6% MgO, 0.75% Na_2O, 2.23% K_2O, 0.11% Fe_2O_3, and 13.05% loss on ignition.

Statistical analysis

The data were analysed using SPSS 15. Before analysis, they were tested for normality using the Kolmogorov–Smirnov test, and the homogeneity of variances was tested using Levene's test. The data were analysed using analysis of variance (ANOVA). Post hoc analysis was done using the Bonferroni and Tamhane tests in cases of equal or unequal variances, respectively. In all cases, a significance level of $P \leq 0.05$ was used.

Results

Clinoptilolite supplementation led to some significant effects on the haematological parameters (Table 1, Figure). The average PCV in the control group was significantly lower ($P < 0.05$) than that of group B for all samplings from 48 h after calving until the end of the experiment. Furthermore, it was significantly lower ($P < 0.05$) than that of group A on the last sampling day (60 days). No significant difference ($P > 0.05$) was noted among the groups on the other

Table 1. Means ± standard errors (SEs) of PCV values (%) and Hb concentrations (g/dL) in blood samples that were obtained from the 3 groups of calves at birth (0 h); 12 h, 24 h, and 48 h after calving; and at the age of 15, 30, 45, and 60 days. The experimental calves were fed with colostrum and milk supplemented with either 1 g/kg BW or 2 g/kg BW clinoptilolite per day, whereas the controls were fed with the unsupplemented colostrum and milk. ^{a,b}: Different superscripts in the same column for each sampling day denote a significant difference ($P < 0.05$).

		PCV (%)	Hb (g/dL)
Blood sampling (age of calves)	**Group**	**Mean**	**Mean**
	1 g	31.90 ± 1.20^{a}	9.40 ± 0.36^{a}
0 h	2 g	34.80 ± 1.32^{a}	10.10 ± 0.42^{a}
	Control	32.60 ± 0.97^{a}	9.80 ± 0.32^{a}
	1 g	30.50 ± 0.95^{a}	9.20 ± 0.29^{a}
12 h	2 g	31.30 ± 1.42^{a}	9.50 ± 0.35^{a}
	Control	29.00 ± 1.26^{a}	9.20 ± 0.29^{a}
	1 g	31.20 ± 1.08^{a}	9.50 ± 0.35^{a}
24 h	2 g	32.80 ± 1.12^{a}	9.90 ± 0.34^{a}
	Control	29.90 ± 1.08^{a}	9.40 ± 0.34^{a}
	1 g	30.30 ± 1.00^{ab}	9.10 ± 0.32^{a}
48 h	2 g	31.80 ± 0.97^{a}	9.80 ± 0.27^{a}
	Control	28.70 ± 0.90^{b}	8.90 ± 0.27^{a}
	1 g	29.90 ± 1.04^{ab}	9.10 ± 0.32^{ab}
15 days	2 g	30.80 ± 1.12^{a}	9.60 ± 0.26^{a}
	Control	27.30 ± 0.84^{b}	8.60 ± 0.24^{b}
	1 g	30.10 ± 0.90^{ab}	9.00 ± 0.32^{ab}
30 days	2 g	31.40 ± 0.84^{a}	9.50 ± 0.24^{a}
	Control	27.70 ± 0.77^{b}	8.50 ± 0.23^{b}
	1 g	30.40 ± 0.94^{ab}	9.00 ± 0.35^{ab}
45 days	2 g	32.30 ± 0.89^{a}	9.70 ± 0.25^{a}
	Control	28.10 ± 0.60^{b}	8.50 ± 0.23^{b}
	1 g	31.00 ± 0.92^{a}	9.50 ± 0.26^{a}
60 days	2 g	33.00 ± 0.89^{a}	9.80 ± 0.26^{b}
	Control	27.90 ± 0.60^{b}	8.40 ± 0.21^{b}

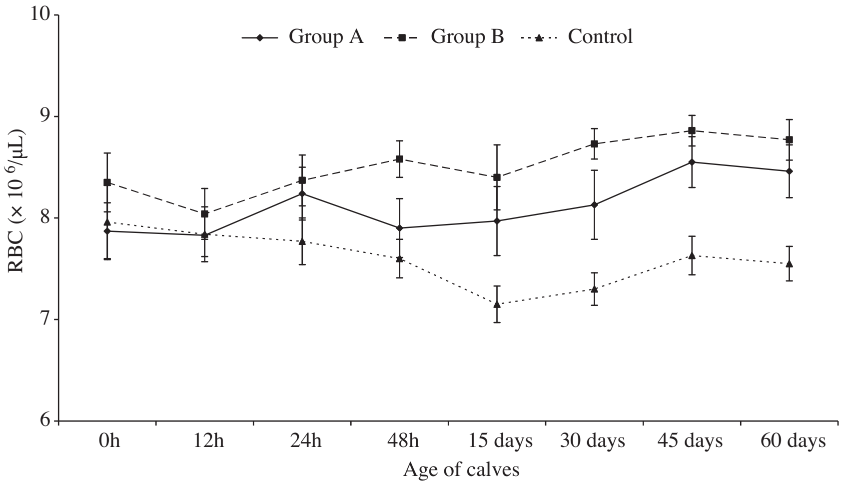

Figure. Means ± SEs of RBC values (× 10^6/μL) in blood samples that were obtained from the 3 groups of calves at birth (0 h); 12 h, 24 h, and 48 h after calving; and at the age of 15, 30, 45, and 60 days. The experimental calves were fed with colostrum and milk supplemented with either 1 g/kg BW (Group A) or 2 g/kg BW (Group B) clinoptilolite per day, whereas those in Group C were fed with the unsupplemented colostrum and milk and served as controls.

sampling days. Mean RBC count followed the same trend and was significantly lower (P < 0.05) in the control group than in group B from 48 h after calving until the end of the experiment, and significantly lower than in group A on the last 3 sampling days (30, 45, and 60 days). Mean Hb concentration was also affected by the addition of clinoptilolite to the colostrum and milk and was significantly lower (P < 0.05) in the control group than in group B from day 15 until the end of the experiment and significantly lower (P < 0.05) than in group A on the last sampling day (60 days). No other significant differences (P > 0.05) were recorded on the other sampling days.

As is shown in Table 2, WBC and PLT values were not significantly affected by the addition of clinoptilolite to the colostrum and milk since there was no significant difference (P > 0.05) among the groups on any sampling day.

Discussion

The aim of this study was to investigate whether a 2-month-long supplementation of 2 levels (1 g/kg BW and 2 g/kg BW) of clinoptilolite in the colostrum and milk of dairy calves had any effect on haematological parameters.

The PCV, Hb, and RBC values were significantly affected by the supplementation of clinoptilolite, initially to the colostrum and later to the milk, suggesting that erythropoiesis was enhanced by clinoptilolite, especially when added at a rate of 2 g/kg BW. These results are similar to those obtained in former studies in calves. Petkova et al. (10) found that clinoptilolite, when supplemented at a rate of 2% of the daily milk ration from day 1 to day 15 after calving, significantly increased the concentration of haemoglobin. Mohri et al. (11) observed that the addition of clinoptilolite to the colostrum and milk of calves at the rate of 2% for the first 14 days of their life significantly increased the PCV, the concentration of Hb, and the number of RBCs. In contrast to supplementation for newborn calves, the long-term dietary supplementation of clinoptilolite in adult cattle has no significant effect on either PCV or Hb concentration (9). The exact mechanism by which clinoptilolite may enhance the erythropoiesis in newborn calves is currently unknown. Given that clinoptilolite contains Fe, a possible explanation might be that clinoptilolite provides a considerable amount of Fe that can be used for erythropoiesis. In agreement with this point of view, Hutcheson (8) supported the idea that clinoptilolite, when used in

Table 2. Means ± SEs of WBC values (× 10^3/μL) and PLT (× 10^3/μL) values in blood samples that were obtained from the 3 groups of calves at birth (0 h); 12 h, 24 h, and 48 h after calving; and at the age of 15, 30, 45, and 60 days. The experimental calves were fed with colostrum and milk supplemented with either 1 g/kg BW or 2 g/kg BW clinoptilolite per day, whereas the controls were fed with the unsupplemented colostrum and milk.

		WBC (× 10^3/μL)	**PLT (× 10^3/μL)**
Blood sampling (age of calves)	**Group**	**Mean**	**Mean**
	1 g	10.5 ± 0.70[a]	681.5 ± 50.2[a]
0 h	2 g	9.82 ± 0.64[a]	695.4 ± 60.4[a]
	Control	9.04 ± 0.61[a]	652.0 ± 57.1[a]
	1 g	10.65 ± 0.76[a]	641.6 ± 45.6[a]
12 h	2 g	9.52 ± 0.62[a]	674.8 ± 46.5[a]
	Control	11.03 ± 0.75[a]	678.7 ± 42.2[a]
	1 g	10.76 ± 0.70[a]	814.4 ± 38.5[a]
24 h	2 g	9.74 ± 0.63[a]	826.5 ± 43.7[a]
	Control	10.82 ± 0.66[a]	813.3 ± 34.4[a]
	1 g	10.84 ± 0.72[a]	771.8 ± 38.9[a]
48 h	2 g	10.23 ± 0.32[a]	842.1 ± 40.3[a]
	Control	11.30 ± 0.73[a]	848.9 ± 34.4[a]
	1 g	10.27 ± 0.39[a]	835.9 ± 32.5[a]
15 days	2 g	9.81 ± 0.40[a]	871.1 ± 43.8[a]
	Control	11.06 ± 0.47[a]	860.7 ± 26.6[a]
	1 g	9.70 ± 0.49[a]	788.2 ± 41.8[a]
30 days	2 g	11.52 ± 0.62[a]	862.2 ± 49.5[a]
	Control	11.29 ± 0.59[a]	886.5 ± 38.4[a]
	1 g	10.77 ± 0.66[a]	865.9 ± 44.9[a]
45 days	2 g	11.27 ± 0.64[a]	887.6 ± 41.1[a]
	Control	11.46 ± 0.66[a]	822.4 ± 23.8[a]
	1 g	9.33 ± 0.64[a]	883.3 ± 33.2[a]
60 days	2 g	11.55 ± 0.71[a]	882.0 ± 46.4[a]
	Control	11.63 ± 0.69[a]	879.2 ± 36.3[a]

the diet of cattle, can supply a significant amount of Fe that can be assimilated by the body.

The long-term administration of clinoptilolite appears to have an insignificant effect on WBCs in newborn calves as no alterations in WBC values were noted throughout the experiment. This is in accordance with the results of former studies conducted on calves (11) and dairy cows (9). Increased leukocyte counts, mainly lymphocytes, have been reported in mice receiving clinoptilolite and were attributed to the intestinal irritation and inflammation caused by the rough zeolite particles (5). Such effects were not observed in the calves in the present study, indicating that clinoptilolite does not irritate the intestinal mucosa.

The PLT values were not significantly affected by the administration of clinoptilolite via colostrum and milk. To our knowledge, the potential effect of clinoptilolite feeding on the PLT values of animals has not yet been investigated. The present study provides the first evidence that PLT production by the bone marrow is not impaired by the dietary administration of clinoptilolite.

Overall, the results of the present study indicate that the prolonged administration of clinoptilolite in calves at the levels of 1 or 2 g/kg BW per day, initially via colostrum and later via milk, enhances haemopoiesis and appears to have no adverse effect on WBC and PLT values.

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