Effects of long term feeding dairy cows on a diet supplemented with clinoptilolite on certain haematological parameters

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ABSTRACT: The effect of the dietary inclusion of clinoptilolite on the haematological parameters of dairy cows has not been studied yet; however in mice, prolonged zeolite administration causes disturbances in the adaptation of erythropoiesis in periods of increased demands. The aim of the present study was to investigate whether the long term supplementation of two levels (1.25 and 2.5%) of clinoptilolite in the concentrate feed of dairy cows has any effect on their haematological parameters. Fifty-two clinically healthy Holstein cows were randomly assigned to one of three groups according to their age and parity. The first group (group A, n = 17) was offered a concentrate feed supplemented with 1.25% clinoptilolite. The second group (group B, n = 17) was offered a concentrate feed supplemented with 2.5% clinoptilolite. The third group (group C, n = 18), which served as control, was offered the same concentrate feed without clinoptilolite supplementation. The experiment started 30 days before the expected parturition and lasted up to the end of lactation. Blood samples from individual animals were collected just before the start of experiment, at the day of calving and, thereafter, at monthly intervals. All samples were tested for packed cell volume (PCV), hemoglobin (Hb) and leukocyte count (WBC) values. The results showed that the 1.25 and 2.5% supplementation of clinoptilolite had no adverse effect on the haematological parameters tested.

Keywords: clinoptilolite; PCV; Hb; WBC; cows

Natural zeolites are characterized by the ability to lose and gain water reversibly, to absorb molecules of appropriate diameter or acting as molecular sieves, and to exchange their constituent cations without major change of their structure (ion-exchange property) (Mumpton and Fishman, 1977). Developing their physical and chemical properties zeolites have been used in animal nutrition since 1960's, mainly to improve animals' performance and to protect them against mycotoxin intoxication (Mumpton, 1999; Trckova et al., 2004).

One of the major concerns that arise from their use as feed additives is whether their long term supplementation in the animals' rations has any effect on their haematological parameters. Such effects are not expected to be direct, as clinoptilolite, due to its stability to gastrointestinal pH of the animals (Pond et al., 1984) and its particle size is not absorbed from the gut. However it is possible to interfere with the absorption of iron and cause alterations to PCV and Hb concentration, or to irritate intestinal mucosa and increase WBC. Experiments conducted in mice suggest that long term effects of clinoptilolite on erythropoiesis (Kartashev and Baskurin, 1995) and changes on WBC cannot be excluded (Martin-Kleiner et al., 2001).

Former studies in steers and sheep indicated that the short-term addition of clinoptilolite in the ration has no major effects on the haematological parameters tested (Bartko et al., 1983; Hutcheson, 1984). So far, despite the fact that the addition of zeolite in dairy rations has extended progressively, there are no references concerning the long term effect of clinoptilolite supplementation on blood parameters of dairy cows.

The objectives of the present study were to determine whether the long-term supplementation of two levels (1.25 and 2.5%) of clinoptilolite in the concentrate feed of dairy cows has any effect on the haematological parameters, the treatment beginning the last month before the expected day of calving and lasting until the end of lactation.

MATERIAL AND METHODS

Animals. Fifty-two clinically healthy Holstein dairy cows, having completed at least one period of lactation, were included in the study. They were randomly assigned to one of three groups that were similar in age and parity. The number of cows per group, their age and parity numbers (mean ± SD), as well as the levels of clinoptilolite (% of concentrates) added in the concentrate feed of each group were as follows:

Group A: comprised of 17 cows aged 5.53 ± 1.80 years and parity 3.71 ± 1.53. They were fed a concentrate supplemented with 1.25% clinoptilolite.

Group B: comprised of 17 cows aged 5.29 ± 2.39 years and parity 3.70 ± 1.99. They were fed a concentrate supplemented with 2.5% clinoptilolite.

Group C: comprised of 18 cows aged 5.05 ± 2.23 and parity 3.80 ± 1.89. They were fed the basal concentrate, without clinoptilolite supplementation, and served as control.

Zeolitic material. The zeolitic material used in the experiment had particle size <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 is SiO_2 68.9%, Al_2O_3 11.27%, CaO 3.02%, MgO 0.6%, Na_2O 0.75%, K_2O 2.23%, Fe_2O_3 0.11% and LOI (Loss on Ignition) 13.05%.

Experimental design. The experiment started 30 days before the expected day of calving. Until parturition, all cows were offered 20 kg corn silage, 2 kg molasses and 2 kg concentrates per animal, per day. During lactation, each cow was offered per day 30 kg corn silage, 2 kg molasses together with 300 g concentrates per liter of milk produced. The concentrate feed was split in two equal portions that were offered twice a day. The concentrates of all three groups were isonitrogenous, isoenergetic and had the same macroelements content (Table 1).

Blood samples were taken on the first day of the experiment, at the day of calving and were repeated at monthly intervals until the end of lactation. The samples were taken by jugular vein puncture with a 20-gauge needle from each cow after the afternoon milking, in vacuum glass tubes containing sodium citrate 3.8% as anticoagulant, supplied with the diagnostic kit (IDEXX, Bovine Sample Preparation Kit, QBC® VetAutoread™ Hematology Analyzer, Sample Preparation Procedures for Bovine Venous Blood. Idexx LaBor. Inc.).

Table 1. Ingredient composition (%) of the concentrate feed used in the three groups of cows throughout the experiment

Ingredient	Group A (%)	Group B (%)	Group C (%)
Soybean meal	34.47	35.42	34.00
Maize grains	20.00	21.00	17.00
Wheat bran	17.69	14.33	22.41
Sunflower meal	8.00	8.00	8.00
Barley grains	8.00	8.00	8.00
Carob fruits	8.00	8.00	8.00
Salt	0.80	0.80	0.80
Limestone	1.00	1.00	1.00
Dicalcium phosphate	0.60	0.76	0.60
Zink oxide	0.04	0.04	0.04
Mixture of vitamins and trace minerals	0.15	0.15	0.15
Clinoptilolite	1.25	2.50	–

Blood samples analysis. Blood analysis included the determination of packed cell volume (PCV), hemoglobin (Hb) and leukocyte count (WBC) with the aid of the veterinary hematology analyzer IDEXX QBC®, using the specific procedure for bovine samples.

Statistical analysis. The statistical analysis was performed using the statistical program SPSS version 12.0 for Windows. The long-term effect of the treatment was evaluated by the method of Repeated Measures Analysis. The normality of the data was tested with Kolmogorov-Smirnov test and the homogeneity of variances with Leven's test. The data at each individual sampling were also analysed by using ANOVA procedure. Tukey's multiple range test was run to determine the statistical significance of differences among the treatment means. A significance level of $P < 0.05$ was used.

RESULTS

The mean values for PCV, Hb and WBC are presented in Table 2.

Repeated Measures Analysis revealed that PCV values were unaffected by clinoptilolite supplementation ($P > 0.05$), as there were not statistically significant differences between the three groups (means ± s.e.: 27.448 ± 0.513, 27.646 ± 0.513, 27.209 ± 0.498, for groups A, B and C, respectively). No significant differences among groups were noted in each individual sampling as well ($P > 0.05$).

The Hb concentration was not significantly affected ($P > 0.05$), in long term basis, by treatment (means ± s.e.: 9.360 ± 0.181, 9.482 ± 0.181 and 9.316 ± 0.176 for groups A, B and C, respectively) and the average values at the monthly samplings were not significantly different between groups ($P > 0.05$).

The long term supplementation of clinoptilolite in the ration did not affect WBC (means ± s.e.: 6.357 ± 0.241, 6.793 ± 0.241 and 6.638 ± 0.234 for groups A, B and C respectively, $P > 0.05$) and no significant difference between the mean values of the three experimental groups was recorded at the individual samplings ($P > 0.05$).

DISCUSSION

The aim of the present study was to investigate whether the long-term supplementation of two levels (1.25 and 2.5%) of clinoptilolite in the concentrate feed of dairy cows has any effect on their haematological parameters.

The PCV values throughout the experiment remained within the reference range in Greece (Panousis et al., 2001) and were not significantly affected by the addition of clinoptilolite, either in the long term or during the monthly samplings, suggesting that erythropoiesis was not impaired by clinoptilolite. These results are similar to those obtained in former studies in steers that were receiving 3% clinoptilolite for 56 days (Hutcheson, 1984) as well as in swine when clinoptilolite was used at the levels of 5% (Vrzgula and Bartko, 1984) or 2% (Kyriakis et al., 2000). In contrast to the other animal species, it seems that in mice the effects of zeolite on PCV values are closely related to the duration of the supplementation. Martin-Kleiner et al. (2001) found that the inclusion of clinoptilolite for 6 weeks does not cause significant alterations on PCV values, whereas Kartashev and Baskurin (1995) observed reduced adaptability of erythropoiesis to increased demands in mice supplied with zeolite, for longer periods of time. Additionally, female rats supplied with kaolin, an aluminosilicate with ion exchange properties like zeolites, for several weeks before and during pregnancy, developed iron deficient anaemia affecting the offspring as well (Patterson and Staszak, 1977).

It seems that the long term supplementation of clinoptilolite does not affect the Hb formation in dairy cows, as blood Hb concentrations followed the same trend with PCV. Similar results have been recorded after the administration of clinoptilolite in sheep at the rate of 0.15 g/kg of body weight (Bartko et al., 1983) and in swine at the level of 5% (Vrzgula and Bartko, 1984). In contrast, Petkova et al. (1982), observed significant increase of Hb concentration in newborn calves that were receiving 2% potassium-calcium zeolite with the colostrum, at first, and the milk afterwards, for the first 15 days after calving; however, there is lack of such references in mature cows.

The prolonged in-feed inclusion of clinoptilolite appears to have minimal irritating effects for the intestinal mucosa of dairy cows as no alterations in WBC values were noted throughout the experiment. Conversely, it was found that leukocyte count, mainly lymphocytes, was increased in mice supplemented with clinoptilolite, due to the intestinal irritation and inflammation elicited by the rough zeolite particles (Martin-Kleiner et al.,

Table 2. Means and SD of mean PCV values (%), Hb concentrations (g/100 ml) and WBC values (× 10^3/100 ml) of in blood samples of the three groups of cows that were obtained 30 days before the expected calving (blood sampling 1), at the day of calving (blood sampling 2) and subsequently at monthly intervals during lactation and up to the start of the next dry period (blood samplings 3–13, respectively). The cows were offered concentrates that were supplemented with either 1.25% (Group A) or 2.5% (Group B) clinoptilolite, whereas those in Group C were fed the unsupplemented concentrate and served as controls

Blood Sampling	Group	PCV (%)		Hb (g/100 ml)		WBC (× 10^3/100 ml)	
		mean	SD	mean	SD	mean	SD
	A	29.13	2.53	10.12	0.81	4.87	1.21
1	B	29.94	4.76	10.38	1.63	5.07	1.02
	C	28.42	3.29	9.77	1.14	5.40	1.30
	A	29.25	1.78	9.75	0.69	8.04	4.34
2	B	28.70	3.86	9.92	1.43	8.78	4.13
	C	27.22	3.13	9.41	1.24	6.98	2.27
	A	25.04	2.44	8.67	0.75	6.14	1.43
3	B	25.94	3.81	8.90	1.32	6.12	1.45
	C	26.18	2.90	9.01	0.96	7.26	2.15
	A	25.59	3.14	8.76	1.04	5.60	1.99
4	B	26.26	3.12	8.89	0.88	7.20	2.33
	C	26.88	2.56	9.02	0.79	7.11	2.20
	A	26.42	3.43	8.92	1.41	7.22	3.47
5	B	27.08	3.74	9.28	1.19	6.19	1.16
	C	27.79	3.13	9.35	1.00	6.96	1.90
	A	25.74	3.54	8.77	1.26	5.75	1.60
6	B	25.98	3.12	8.95	1.12	6.88	2.33
	C	26.66	3.19	9.14	1.08	6.57	1.26
	A	26.80	1.97	9.09	0.72	6.77	2.58
7	B	27.72	3.81	9.39	1.10	7.36	3.00
	C	27.42	2.52	9.36	1.02	7.06	1.82
	A	27.76	3.43	9.48	1.23	5.95	1.38
8	B	27.69	4.30	9.41	1.44	6.95	1.99
	C	27.27	3.04	9.46	0.80	7.13	1.68
	A	29.27	2.94	9.74	1.03	6.60	1.86
9	B	28.19	3.89	9.72	1.21	6.93	2.21
	C	28.11	3.15	9.63	1.06	5.93	2.05
	A	29.12	2.09	9.90	0.85	6.55	1.38
10	B	27.83	4.87	9.53	1.69	7.64	2.16
	C	27.10	1.95	9.24	1.00	6.69	1.89
	A	28.03	3.53	9.52	1.09	6.31	1.56
11	B	27.93	3.86	9.64	1.24	6.65	1.73
	C	27.48	3.68	9.32	1.31	6.40	1.49
	A	26.88	2.81	9.23	0.93	6.27	1.28
12	B	27.56	4.71	9.55	1.49	6.32	1.57
	C	25.90	3.13	8.97	1.16	6.58	0.84
	A	28.71	1.95	10.04	0.60	6.37	1.71
13	B	30.52	4.65	10.28	1.84	6.70	0.93
	C	24.38	4.24	8.52	1.46	6.18	1.07

2001), and that in newborn calves, the use of 2% potassium-calcium zeolite for 15 days after calving increased the absolute values of granulocytes and lymphocytes (Petkova et al., 1982). Such effects were not observed in dairy cows probably because of the relatively lower ratio between particle size of clinoptilolite and the diameter of the intestinal tract. However, similar results with the present study were obtained by the administration of clinoptilolite in sheep (Bartko et al., 1983) and swine (Vrzgula and Bartko, 1984).

The present study provides the first evidence concerning the effect of the use of clinoptilolite as feed additive on the haematological parameters of dairy cows. The main conclusion is that the long-term supplementation of clinoptilolite at the levels of 1.25 and 2.5% in the concentrates does not have any adverse effect on PCV, Hb and WBC values.

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