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Effects of Prolonged Consumption of Water with Elevated Nitrate Levels on Certain Blood Serum Trace and Macro Elements of Dairy Cattle and Use of Clinoptilolite for their Amelioration

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Abstract

Elevated levels of nitrates in feed and water can pose a significant risk for dairy cattle, due to their cumulative action. The effect of prolonged consumption of water naturally contaminated with nitrates on the serum concentrations of calcium (Ca), phosphorus (P), magnesium (Mg), copper (Cu), iron (Fe) and zinc (Zn) was investigated at the present study. Concurrently, it was evaluated whether the combination of in-feed inclusion of clinoptilolite and of consumption of water naturally contaminated with nitrates, could have any effect on these elements. Two experiments were run simultaneously in two farms each. In the first trial (Trial 1), farm 1 and farm 2 were assigned into two groups according to the nitrate levels in the borehole water. The first group (nitrate group; NG) consisted of the farm 1that had nitrate levels >40 ppm and the second one (control group; CG) of the farm 2 that had water nitrate levels <40 ppm. Similarly, in the second trial (Trial 2) the first group (nitrate – clinoptilolite group; NC) consisted of the farm 3 that had nitrate levels >40p pm and used clinoptilolite as feed additive at the rate of 2.5% of concentrates. The second one (control group; CCG) consisted of the farm 4that had water nitrate levels <40 p pm and did not incorporate clinoptilolite in the ration. The results indicate that the prolonged consumption of water with increased nitrate levels either alone or in combination with clinoptilolite feeding does not have any adverse effects on the serum concentrations of the macro and trace elements evaluated in dairy cattle.

Keywords: Nitrates; Dairy cattle; Serum; Macro elements; Trace elements; Clinoptilolite

Introduction

Ruminants are especially vulnerable to nitrate toxicosis because ingested nitrates, before their final conversion to ammonia by the rumen flora, are converted to nitrites which are toxic substances that result to methemoglobinemia formation [1]. Although this rumen conversion is an adaptive process depending on the nitrate content in the rumen [1] and despite the ruminal flora's capability to respond to a wide range of nitrate levels, this compensation mechanism often gets overloaded, resulting in nitrite build up and methemoglobinemia [2]. Depending on the amount of intake and the degree of ruminal flora's adaptation, nitrate toxicosis might be acute, subacute or chronic.

In intensive dairy herds both feed and water can be a significant source of nitrates [3]. However, water possesses the highest risk because, apart from the initial contamination that might appear, the prolonged retention in the water troughs and the contamination with organic material significantly increase nitrate levels [4]. It is generally accepted that water nitrate concentrations below 40ppm are absolutely safe for dairy cattle, whereas higher concentrations of up to 132ppm are considered as acceptable if their diet is low in nitrates and nutritionally balanced [5].The detection of nitrate levels between 40ppm-132ppm is not uncommon in routine water analyses in dairy farms. The prolonged consumption of water with such nitrate levels has been recently proven to be associated with impairment of protein and glucose metabolism dairy cattle [6]. It was further observed at the same study that these effects could be ameliorated by the use of a natural zeolite, clinoptilolite.

In the available literature there is a lack of information concerning possible interactions of ingested nitrates with the major macro elements such as calcium, magnesium and phosphorus and important trace elements such as copper, iron and zinc that are supplied to the animals via feed. It is well known that copper, magnesium and iron are involved to the complete reduction of nitrates to ammonia [3]; so, a potential consumption of a proportion of these elements during the reduction process cannot be excluded. Furthermore, all the cations could possibly form complexes with the nitrates that might impair their bioavailability.

Taking into account the high risk for subclinical nitrate intoxication in dairy cattle, the objective of the present study was to evaluate under field conditions whether the prolonged-six months-consumption of water naturally contaminated with nitrates affects the blood serum concentrations of certain macro and trace elements of dairy cattle. A secondary objective was to investigate whether the dietary inclusion of clinoptilolite that is proposed as a preventing measure for the amelioration of the impact of nitrates on the protein and energy metabolism has any effect on these parameters.

Materials and Methods

Farms and experimental design

Four cubicle with earthen paddocks dairy farms located at Thessaloniki region were used in this study. Farm 1 consisted of 136 dairy cows and had mean daily milk yield 35Kg/cow/day. Farm 2 consisted of 115 dairy cows with 36Kg/cow/day average milk yields. Both farms were of the same owner and were using the same total mixed ration. Farm 3 had 162 dairy cows with average milk yield 31Kg/cow/day and farm 4 had 153 dairy cows with 29Kg/cow/day average milk yield. All farms had calving severy month throughout the year so, at each time point, similar numbers of cows were at dry period, early and late lactation. The rations were offered to the animals twice a day, after morning and afternoon milking, and water was provided ad libidum in several automatic plastic drinking bowls. The health status of the mals in all herds was monitored at scheduled farm visits by the attending veterinarians and detailed health and reproductive records were kept electronically. Nitrate levels in borehole water were routinely monitored (4 times per year) in certified laboratories in all four farms.

Two trials were run simultaneously for the purpose of the study. In the first trial (Trial 1) farm 1 and farm 2 used. They were assigned into two groups according to the nitrate levels in the borehole water, based on the results of 4 consecutive measurements of the previous year. The first group (nitrate group; NG) consisted of the farm 1that had nitrate levels >40p pm (average annual levels 80p pm) and the second one (control group; CG) of the farm 2 that had water nitrate levels <40p pm. In the second trial (Trial 2) farm 3 and farm 4 were assigned into two groups according to the nitrate levels in the borehole water, based on the results of 4 consecutive measurements of the previous year and the use of clinoptilolite as follows: the first group (nitrate– clinoptilolite group; NC) consisted of the farm 3 that had nitrate levels >40p pm (average annual levels 73 p pm) and used clinoptilolite as feed additive at the rate of 2.5% of concentrates. The second one (control group; CCG) consisted of the farm 4that had water nitrate levels <40 p pm and did not incorporate clinoptilolite in the ration. Both experiments lasted 6 months, from January to June 2014.

Clinoptilolite

The clinoptilolite-richzeolitic material (Vivolith 85, S & B, Greece) used in the second trial had particle size <0.15mm in diameter and contained approximately 85% clinoptilolite; the admixtures were feldspar, micas and clays as determined by x-ray powder diffraction. The cation exchange capacity of the material used was 160mEq/100 g and its chemical composition was as follows: SiO_2 68.3%, Al_2O_3 12.8%, Fe_2O_3 1.1%, CaO 3.2%, MgO 0.7%, K_2O 3.4%, Na_2O 1.0% and LOI 8.5%.

Animal feeding

Table 1: Composition (kg) of the total mixed ration (TMR) and of concentrates (%) fed to cows of the farms 3 and 4 during lactation and dry period in Trial 2.

	Farm 3		Farm 4	
TMR (kg)	Lactation	Dry period	Lactation	Dry period
Maize silage	35	22	30	20
Alfalfa hay	3	2	4.5	2
Wheat straw	0.5	1.5	-	1.5
Molasses	2	-	-	-
Concentrates	8	3	8	3
Concentrates (%)				
Maize grains	55		30	
Soybean meal	26		30	
Wheat bran	14		20	
Carob fruits	-		17	
Mixture of vitamins, macro- & trace minerals	2.5		3	
Clinoptilolite	2.5		-	

In Trial 1, lactating animals in both farms were fed the total mixed ration (TMR) consisting of 25kg maize silage, 3.5kg alfalfa hay, 0.5kg wheat straw, 3.5kg soybean meal, 2kg maize grains, 2kg distiller's dried grains with soluble (DDGS), 1.5kg rape cake, 0.2 kg fat and 0.3kg mixture of vitamins, macro and trace minerals. The TMR provided 19.99mg of calcium, 8.11mg of phosphorus, 124.99mg of iron, 38.49mg of copper and 19.95mg of zinc per kg. Dry cows were offered the same total mixed ration mixed with wheat straw at a rate 80:20 and fed libitum. In Trial 2 lactating and dry cows were fed the rations presented in (Table 1). In farm 3 the basic ration provided 1.57 mg of calcium, 0.41 mg of phosphorus, 43.41mg of iron, 1.29mg of copper and 5.60mg of zinc per Kg and the concentrates 9.53mg of calcium, 3.81mg of phosphorus, 141.37mg of iron, 191.75mg of copper and 24.51mg of zinc per kg. In farm 4 the basic ration provided1.47mg of

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calcium, 0.34 mg of phosphorus, 41.43mg of iron, 1.01mg of copper and 4.26mg of zinc per Kg and the concentrates 10.55mg of calcium, 3.94mg of phosphorus, 142.06mg of iron, 193.85 mg of copper and 25.99mg of zinc per kg.

Farm visits-animals' selection

Each farm was visited 3 times, at the onset of the experiment (January) and then at 3-month intervals (March and June). All farms were visited after the morning milking. At each visit, 15 dairy cows, 5 early lactation cows (less than or equal to 4 months of lactation), 5 late lactation cows (equal to or more than 5 months of lactation)and 5 dry cows that were expected to deliver within the next 30 days were selected for blood sampling. The selection of the animals was random; the first 5 animals trapped in the feeding troughs from each category that were judged to be healthy after thorough clinical examination was used.

Blood sampling and analysis, water nitrate levels

After selection, a blood sample was obtained by tail vein puncture into a vacuum glass tube (BD, Franklin Lakes, NJ, USA) with an 18-g needle. After clotting, serum was separated by low-speed centrifugation, transferred to plastic vials and stored at -20 oC until analysis. Serum calcium and inorganic phosphorus concentrations were determined by colorimetric methods in an automatic biochemical analyzer (Vitalab Flexor E, Vital Scientific N.V, The Netherlands) using a commercial diagnostic kits (Thermo Fisher Scientific Inc. VA, USA). The determination of serum concentrations of magnesium, copper, iron and zinc were done using a Perkin-Elmer A Analyst 300 flame atomic absorption spectrophotometer (Perkin-Elmer Corp. Norwalk, Conn.) according to the instructions of manufacturer.

At the end of the experiment, the data concerning the nitrate levels at the borehole water for this period were obtained from the records of each farm.

Ethics

The procedures and the experiments were done according to the ethical standards in the Helsinki Declaration of 1975, as revised in 2000, as well as the national law and the guidelines of our Institutional Animal Care and Use Committee.

Statistical analysis

Data were analyze dusing the statistical program SPSS® 21. Normality of data distribution was assessed with Kolmogorov-Smirnov test and homogeneity of variances was evaluated with Levene test. For each production category of animals, the effect of the increased water nitrate levels at the Trial 1 and the effect of the combination of increased water nitrates and clinoptilolite supplementation at the Trial 2 on the serum concentrations of the macro and trace elements evaluated were tested with Univariate Analysis of Variances, with group and time of sampling as fixed factors. The main effects of group and of time of sampling as well as their interaction (group x sampling) at each experiment were tested with Bonferroni test. A value of $P \leq 0.05$ was considered significant in all comparisons.

Results and Discussion

The mean±SD of water nitrate levels for the 4 farms during the experimental period according to their records were 87±12.5ppm for farm 1, 4±1.5ppm for farm 2, 13±2.4ppm for farm 3 and 76±14.2ppm for farm 4 were measured. In both trials, the measured nitrate levels in the boreholes were similar to those recorded at the previous year and according to which farms were assigned into groups. The values were within the limit of 40ppm to 132ppm in both contaminated farms. Such levels are frequently detected in dairy farms but are considered acceptable for cows if their diet is low in nitrates and nutritionally balanced [5].

Table 2: Mean±SE of the serum concentrations and reference range (RR) of calcium (Ca), phosphorus (P) and magnesium (Mg) in early lactation, late lactation and dry cows that were receiving water with elevated (NG) and normal (CG) nitrate levels in Trial 1.

			Ca (mgl/dl) RR: 7.4 – 10.2	P (mg/dl) RR: 3.9 – 7.7	Mg (mg/dl) RR: 1.8 – 2.2
Early lactation cows	NG		8.99±0.18	6.11±0.38	1.94±0.03
	CG		9.20±0.22	6.04±0.25	1.88±0.04
	P	Group	NS	NS	NS
		Sampling	NS	NS	NS
		Group x sampling	NS	NS	NS
Late lactation cows	NG		9.93±0.18	5.58±0.21	1.91±0.05
	CG		8.78±0.20	5.42±0.35	1.98±0.06
	P	Group	NS	NS	NS
		Sampling	NS	NS	NS
		Group x sampling	NS	NS	NS
Dry cows	NG		8.85±0.19	5.67±0.33	1.93±0.03
	CG		9.06±0.17	5.76±0.40	1.92±0.04
	P	Group	NS	NS	NS
		Sampling	NS	NS	NS
		Group x sampling	NS	NS	NS

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In order to assess the main objective of the present study which was to investigate whether of prolonged consumption of nitrates has any effect on the serum concentration of the main macro and trace elements of dairy cattle the Trial 1 was conducted. The selected farms were considered as ideal for such an evaluation because they had the same management practices, similar milk production and the animals were fed with the same ration but had different nitrate levels in the supplied water. As it is shown in Table 2 the average concentrations of Ca, P and Mg remained within the reference range throughout the experimental period and were not significantly affected either by nitrate consumption (group; P>0.05) or by sampling (P>0.05) or their interactions (group x sampling; P>0.05) in dry, early lactation and late lactation cows. Similar results were obtained for Cu, Fe and Zn (Table 3). Their average values were also within the reference range and were unaffected by group (P>0.05), sampling (P>0.05) and their interactions (group x sampling; P>0.05) in all three cow categories. These findings provide the first evidence that the consumption of water with elevated but within the acceptable limits nitrate levels is not associated with adverse effects on the serum concentrations of the main macro and trace elements. The results further indicate that quantity of Mg, Fe and Cu that are used during the reduction process of nitrates to ammonia is negligible and does not cause any imbalance to these elements [3].

Table 3: Mean±SE of the serum concentrations and reference range (RR) of copper (Cu), iron (Fe) and zinc (Zn) in early lactation, late lactation and dry cows that were receiving water with elevated (NG) and normal (CG) nitrate levels in Trial1.

			Cu (mg/l) RR: 0.5-1.1	P (mg/dl) RR: 3.9 – 7.7	Mg (mg/dl) RR: 1.8 – 2.2
Early lactation cows	NG		0.69±0.09	0.78±0.03	0.88±0.09
	CG		0.74±0.07	0.72±0.04	0.91±0.08
	P	Group	NS	NS	NS
		Sampling	NS	NS	NS
		Group x sampling	NS	NS	NS
Late lactation cows	NG		0.76±0.06	0.77±0.09	1.08±0.09
	CG		0.76±0.05	0.78±0.08	1.10±0.07
	P	Group	NS	NS	NS
		Sampling	NS	NS	NS
		Group x sampling	NS	NS	NS
Dry cows	NG		0.68±0.07	0.80±0.02	0.92±0.09
	CG		0.73±0.06	0.82±0.04	1.02±0.07
	P	Group	NS	NS	NS
		Sampling	NS	NS	NS

Table 4: Mean±SE of the serum concentrations and reference range (RR) of calcium (Ca), phosphorus (P) and magnesium (Mg) in early lactation, late lactation and dry cows that were fed clinoptilolite and receiving water with elevated nitrate levels (NC) and normal (CCG) nitrate levels in (Trial 2).

			Ca (mgl/dl) RR: 7.4 – 10.2	P (mg/dl) RR: 3.9 – 7.7	Mg (mg/dl) RR: 1.8 – 2.2
Early lactation cows	NG		8.78±0.19	5.13±0.16	1.93±0.04
	CCG		8.93±0.15	5.32±0.15	1.99±0.03
	P	Group	NS	NS	NS
		Sampling	NS	NS	NS
		Group x sampling	NS	NS	NS
Late lactation cows	NG		8.57±0.22	5.88±0.30	2.00±0.05
	CCG		8.72±0.14	6.14±0.65	1.93±0.03
	P	Group	NS	NS	NS
		Sampling	NS	NS	NS
		Group x sampling	NS	NS	NS
Dry cows	NG		9.14±0.24	4.94±0.20	1.94±0.05
	CCG		9.17±0.13	5.25±0.18	1.94±0.03
	P	Group	NS	NS	NS
		Sampling	NS	NS	NS
		Group x sampling	NS	NS	NS

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Table 5: Mean±SE of the serum concentrations and reference range (RR) of copper (Cu), iron (Fe) and zinc (Zn) in early lactation, late lactation and dry cows that were fed clinoptilolite and receiving water with elevated nitrate levels (NC) and normal (CCG) nitrate levels in (Trial 2).

			Cu (mg/l) RR: 0.5 – 1.1	Fe (mg/l) RR: 0.6 – 1.0	Zn (mg/l) RR: 0.8 – 1.3
Early lactation cows	NG		0.80±0.05	0.78±0.04	1.04±0.06
	CCG		0.77±0.03	0.81±0.04	1.06±0.04
	P	Group	NS	NS	NS
		Sampling	NS	NS	NS
		Group x sampling	NS	NS	NS
Late lactation cows	NG		0.79±0.05	0.72±0.03	1.03±0.05
	CCG		0.79±0.04	0.78±0.05	1.03±0.04
	P	Group	NS	NS	NS
		Sampling	NS	NS	NS
		Group x sampling	NS	NS	NS
Dry cows	NG		0.87±0.08	0.69±0.02	1.09±0.05
	CCG		0.81±0.05	0.73±0.02	1.07±0.04
	P	Group	NS	NS	NS
		Sampling	NS	NS	NS
		Group x sampling	NS	NS	NS

It was suggested, based on the findings on a previous research, that the dietary administration of clinoptilolite could be used as a preventive measure for the amelioration of the effects of nitrates on protein and glucose metabolism. Trial 2 was designed in order to evaluate whether such a dietary intervention affects the serum concentration of the main macro (Table 4) and trace elements (Table 5). The serum values of macro (Ca, P and Mg) and trace elements (Cu, Fe and Zn) were unaffected by nitrates and clinoptilolite feeding (group; P>0.05), sampling (P>0.05) and their interactions (group x sampling; P>0.05). Furthermore, their average values remained within the reference range in all three categories of cattle evaluated. These results denote that clinoptilolite feeding in cattle receiving water with elevated nitrate levels does not impair the availability of these elements. It is well documented in former researches that the dietary administration of clinoptilolite at dose rates similar to those offered at the present study, does not have any adverse effect on the serum concentrations of the major macro [7] and trace elements in dairy cattle [8] and heifers [9].

Conclusion

Based on these findings it can be concluded that the prolonged consumption of water with increased nitrate levels either alone or in combination with clinoptilolite feeding does not have any adverse effects on the serum concentrations of the macro and trace elements evaluated in dairy cattle.

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