

Effects of oral supplementation with organically modified clinoptilolite during prepartum period on colostrum quality in primiparous dairy cows

Research Article

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Author for correspondence:

Natalija Fratrić,
Email: nataly@vet.bg.ac.rs

Milica Stojić¹, Vesna Ilić², Marijana Kovačić², Dragan Gvozdić³, Silvana Stajković⁴, Branislav Vejnović⁵, Olivera Savić⁶ and Natalija Fratrić¹

¹Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia; ²Institute for Medical Research, University of Belgrade, Belgrade, Serbia; ³Department of Pathophysiology, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia; ⁴Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia; ⁵Department of Economics and Statistics, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia and ⁶Institute for Blood Transfusion of Serbia, Belgrade, Serbia

Abstract

This research paper addresses the hypothesis that an oral supplementation with organically modified clinoptilolite will improve colostrum quality in primiparous dairy cows whilst having no adverse effects on the cows' health. A total of 36 pregnant Holstein primiparous dairy cattle were randomly assigned to receive daily oral drenching, two hours following morning feeding, with 1 l of water containing either 0 g/l ($n = 16$) or 150 g/l ($n = 20$) of clinoptilolite. Treatment lasted from 24 ± 4 d prior to expected parturition until two days postpartum (pp). Colostrum was collected at 2 to 3 h, 12, 24 and 36 h pp and blood samples were collected at 24 ± 4 and 4 ± 2 d prior to parturition and 1, 2 and 7 d pp. Overall mean dry matter, fat and total protein percentage as well as IgG concentration and mass were significantly greater in colostrum collected from cattle drenched with clinoptilolite (total protein increased by 15% and IgG concentration and mass by 21 and 38% respectively at first sampling and further at second sampling). Total γ globulin and most other blood serum biochemistry parameters did not differ between cattle treated and not treated with clinoptilolite, the only exception being the fast anionic γ globulin fraction that was 17% greater at 4 ± 2 d prior to parturition and 10% lower on the 1st day pp in treated cattle. These results showed that organically modified oral clinoptilolite supplementation at 150 g/d significantly increases the IgG concentration in colostrum and has no adverse effects on the energy status, protein, lipid, and mineral metabolism in primiparous dairy cattle during prepartum period.

The intake of an adequate amount of good quality colostrum within two hours after birth is of utmost importance for the health and survival of newborn calves (Weaver *et al.*, 2000). Colostrum is not just a source of nutrients and growth factors, but also provides immune protection to calves in the early stages of extrauterine life. The complex multilayer structure of the bovine placenta (syndesmochorial) effectively prevents transfer of large molecules between dam and foetus, and bovine calves are born agammaglobulinemic (Weaver *et al.*, 2000) or might have only traces of immunoglobulin G (IgG) in their blood (Chigerwe *et al.*, 2008). This makes newly born calves totally dependent on passive immune protection provided by the intestinal absorption of colostrum immunoglobulin during the first hours of life (Weaver *et al.*, 2000).

The mass of colostrum IgG transferred to the peripheral blood of the calf is affected by the timing of feeding, amount offered and the quality of the colostrum (Kertz *et al.*, 2017). Numerous interconnected factors, such as the breed or age of dam, nutrition, season of calving, volume of colostrum produced, periparturient vaccination of the dam, length of the dry period and delayed colostrum collection affect colostrum quality (Godden, 2008). As a consequence, there is much variation in results reported in studies dealing with factors affecting the quality of colostrum and there is an ongoing need to study these factors.

Among zeolites (minerals and chemical compounds within the group of silicates), clinoptilolite is the most studied substance for use in the human and veterinary medicine, and it is the best known as a feed additive in animal nutrition (Papaioannou *et al.*, 2005). Clinoptilolite has been shown to improve production performance, acting as antioxidant, anti-diarrhoeic and growth-promoting agent (Papaioannou *et al.*, 2004; Pourliotis *et al.*, 2012; Wu *et al.*, 2015). During the last decade, its utilization as a mycotoxin-binding adsorbent has been a topic of considerable interest (Daković *et al.*, 2007; Katsoulos *et al.*, 2016). There are also

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reports indicating its role in the prevention of metabolic diseases in dairy cows (milk fever, ketosis) as well as positive effects on general health (Papaioannou *et al.*, 2005; Katsoulos *et al.*, 2006). However, the effect of its supplementation on the colostrum quality in primiparous dairy cows has not been reported to date.

Starting from the aforementioned positive effects of clinoptilolite on cows' health and production, and its role as a reservoir of ammonium which increases the availability of nitrogen in the rumen (White and Ohlrogge, 1974) and favours the rumen microbe protein synthesis (Hartinger *et al.*, 2018), we hypothesized that an oral supplementation of cows with clinoptilolite, in the period of colostrumgenesis, might improve the quality of colostrum, without showing any adverse effect on the cows' health. This study has investigated the effects of the supplementation of primiparous dairy cows with organically modified clinoptilolite (EP Patent No. 1363854A1 and B1; Milosevic and Tomasevic-Canovic, 2003, 2009) in the period from 24 ± 4 d before the expected parturition to two days after calving on colostrum quality parameters (total protein, IgG, and fat content). Additionally, the effect of the supplementation on the energy status, protein, lipid, and mineral metabolism of these cows was assessed by the analysis of biochemistry parameters of their peripheral blood serum.

Materials and methods

Experimental design

The use of animals was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Belgrade in accordance with the National Regulations on Animal Welfare. A total of 40 healthy pregnant Holstein primiparous dairy cows, grown at an open stall farm (Padinska Skela, PKB Corporation, Belgrade, Serbia), were randomly selected 30 d prior to the expected date of parturition, and placed in a separate facility, in tie stalls, and kept under the same hygienic and dietetic conditions. All cows were enrolled into the study between the beginning of September and the end of November 2016, and received access to the same total mixed ration on an ad libitum basis throughout the study. At 25 d before expected parturition date the cows were offered a close-up transition diet ($NE_L = 1.60$ Mcal/kg; Table S1, online Supplementary File) and following parturition were transferred to a lactating diet ($NE_L = 1.71$ Mcal/kg; Table S1 online Supplementary File) that was provided for all animals until 30 d into lactation. All offered diets were initially formulated to either meet or exceed the NRC (2001) requirements and the mixed rations were offered in two equal portions at 07.00 and 18.00 h daily. Cows had ad libitum access to clean, fresh water throughout the study.

The cattle were randomly divided into two groups and received each of the two treatments simultaneously: daily oral drenching, of either 1 l of water with 0 g/l ($n = 19$; C0 group) or 150 g/l ($n = 21$; C150 group) of clinoptilolite (Minazel Plus[®], Patent Co., Serbia; Milosevic and Tomasevic-Canovic, 2003, 2009). Water and clinoptilolite suspension were administered using a glass drenching bottle, and given orally in one portion, two hours following morning feeding (09.00), from 24 ± 4 d prior to expected parturition until two days postpartum (pp). Routine mastitis diagnostics was completed following calving and four cows were treated for mastitis, one from the C150 and three from the C0 group, and as such their data were excluded from the study results. The remaining data from a total number of 36 animals

Table 1. Mean quality parameters measured in colostrum^a collected from primiparous dairy cows drenched orally with a supplementation of water (1 l) with either 0 or 150 g/head/d of organically modified clinoptilolite

Variable	Clinoptilolite, g/d		SEM	P value ^b	
	0	150		T	T × H
Solids (%)	19.25	21.54	0.98	0.026	<0.001
Protein (%)	8.07	9.45	0.50	0.009	0.004
Fat (%)	5.76	6.57	0.40	0.049	<0.001
pH	6.30	6.25	0.03	0.120	0.370
Brix (%)	16.7	18.8	0.8	0.008	<0.001
Volume (l)	4.5	5.1	0.6	0.500	0.530
IgG (g/l)	55	73	7	0.016	0.008
IgG (g)	214	300	31	0.001	0.330

^aOverall last square means for 1st, 2nd, 3rd and 4th colostrum.

SEM, standard error of mean; ^bT, Clinoptilolite treatment; H, colostrum sampling time: 2, 12, 24 and 36 h pp.

were included in further data analysis (C0 group $n = 16$, and C150 group $n = 20$).

Colostrum and blood serum sampling

Colostrum was collected at 2 to 3 h, 12, 24 and 36 h pp (referred to as 1st, 2nd, 3rd and 4th colostrum, respectively) and the volume of colostrum collected was recorded. The total collected volume was mixed and representative 200 ml samples were taken. The fresh colostrum was analysed, within 1 h of collection, using digital Brix refractometer (Atago Co. Ltd., Tokyo, Japan) and stored frozen at -20°C until further analysis. Blood samples (9 ml) were collected 24 ± 4 and 4 ± 2 d prepartum, and at 1st, 2nd and 7th day pp, by jugular venepuncture using sterile plastic vacutainer tubes without anticoagulant. The blood serum was separated by centrifugation ($800 \times g$, 15 min) which was completed within 2 h of sample collection, and serum samples (0.5 ml) aliquoted and stored at -20°C until the further analysis.

Analysis of colostrum

The frozen colostrum samples were thawed in a water bath at 37°C and heated to a temperature of 20°C and then mixed by twisting the bottle several times. Colostrum samples were analysed for total dry matter, protein and fat content (%), and pH value (described in online Supplementary File). The Brix refractometry, radial immunodiffusion (RID) assay (using a bovine IgG RID test plate; The Binding Site Group Ltd, Birmingham, UK) were completed as described in Stojić *et al.* (2017).

Blood serum biochemistry analyses

The total protein, albumin, beta-hydroxybutyric acid (BHB), urea, glucose, triglyceride, cholesterol, calcium, magnesium, phosphorus, and potassium concentration in blood serum samples of C150 and C0 group were determined using commercial test packages (BHB, Randox, UK; urea, glucose, cholesterol and phosphorus, Bioanalitika, Serbia; triglycerides, calcium and magnesium; BioSystem, Spain). All biochemistry analyses were performed using automatic analyzer (Analyzer A15, BioSystem,

Table 2. Mean of peripheral blood biochemistry parameters of primiparous dairy cows drenched orally with a supplementation of water (1 l) with either 0 or 150 g/head/d of organically modified clinoptilolite

		Days in relation to parturition					
		-24 ± 4		-4 ± 2		2	
		Clinoptilolite (g/day)					
		0	150	0	150	0	150
Total protein (g/l)	$\bar{x} \pm \text{SD}$	66 ± 6	67 ± 4	65 ± 6	65 ± 5	69 ± 5	66 ± 5
	SE	2	1	2	1	1	1
	C _V %	9	6	9	8	7	8
Albumin (g/l)	$\bar{x} \pm \text{SD}$	33 ± 2	33 ± 2	33 ± 2	32 ± 2	34 ± 3	32 ± 3
	SE	1	1	1	1	1	1
	C _V %	7	5	7	6	9	8
Urea (mmol/l)	$\bar{x} \pm \text{SD}$	2.6 ± 1.0	2.2 ± 0.8	2.6 ± 1.1	3.0 ± 1.0	4.1 ± 1.4	3.3 ± 0.9
	SE	0.3	0.2	0.3	0.3	0.4	0.2
	C _V %	38	35	43	35	35	27
Glucose (mmol/l)	$\bar{x} \pm \text{SD}$	2.9 ± 0.3	2.8 ± 0.3	3.4 ± 0.6	3.3 ± 0.5	3.1 ± 0.8	3.2 ± 0.7
	SE	0.1	0.1	0.2	0.1	0.2	0.2
	CV %	10	10	17	16	26	23
Triglycerides (mmol/l)	$\bar{x} \pm \text{SD}$	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.3 ± 0.4	0.2 ± 0.1	0.3 ± 0.4
	SE	0.1	0.1	0.1	0.1	0.1	0.1
	C _V %	42	32	39	45	38	69
Cholesterol (mmol/l)	$\bar{x} \pm \text{SD}$	2.7 ± 0.4	2.5 ± 0.3	2.3 ± 0.5	2.6 ± 0.5	2.4 ± 0.5	2.3 ± 0.4
	SE	0.1	0.1	0.1	0.1	0.1	0.1
	C _V %	14	14	21	19	20	18
BHB (mmol/l)	$\bar{x} \pm \text{SD}$	0.5 ± 0.2	0.4 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.6 ± 0.2	0.6 ± 0.1
	SE	0.1	0.1	0.1	0.1	0.1	0.1
	C _V %	31	29	31	27	35	23
Phosphorus (mmol/l)	$\bar{x} \pm \text{SD}$	2.2 ± 0.3	2.0 ± 0.2	2.3 ± 0.5	2.0 ± 0.4	1.8 ± 0.2	1.6 ± 0.4
	SE	0.1	0.1	0.1	0.1	0.1	0.1
	C _V %	12	10	24	18	13	27
Calcium (mmol/l)	$\bar{x} \pm \text{SD}$	2.4 ± 0.3	2.6 ± 0.3	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.2	2.5 ± 0.4
	SE	0.1	0.1	0.1	0.1	0.1	0.1
	CV %	13	12	8	7	10	15
Magnesium (mmol/l)	$\bar{x} \pm \text{SD}$	0.9 ± 0.2	0.9 ± 0.2	1.0 ± 0.3	0.9 ± 0.3	1.0 ± 0.2	0.9 ± 0.2
	SE	0.1	0.1	0.1	0.1	0.1	0.1
	CV %	20	23	28	31	19	25
Potassium (mmol/l)	$\bar{x} \pm \text{SD}$	4.4 ± 0.3	4.5 ± 0.2	4.4 ± 0.2	4.4 ± 0.3	4.6 ± 0.4	4.8 ± 0.5
	SE	0.1	0.1	0.1	0.1	0.2	0.2
	CV %	7	5	4	6	10	10

Day, day prepartum (-) or day pp (+); $\bar{x} \pm \text{SD}$, mean ± standard deviation; SE, standard error of mean; CV, coefficient of variation.

Barcelona, Spain). Agarose gel protein electrophoresis was completed as described in Stojić *et al.* (2017).

Statistical analysis

Data from cows that had mastitis were excluded from this analysis. Statistical analysis of the data results was carried out using GraphPad Prism v6 (GraphPad, San Diego, CA, USA) software. Given that some data were not normally distributed (Shapiro-Wilk

normality test $P < 0.05$, Table 2, Table S2 online Supplementary File), several transformations of data have been attempted until adequate transformation was found. For the data of biochemical analyses of blood plasma following transformations were applied: $y = x + 1$ for triglycerides, BHB and magnesium; $y = x + 2$ for urea; In colostrum: $y = x + 1$ for fat, $y = x + 6$ for volume, $y = x + 50$ for the IgG concentration (g/l), and $y = x + 700$ for IgG mass (g). After transformations all data were normally distributed (Shapiro-Wilk normality test $P > 0.05$). Groups were compared

using two-way ANOVA with repeated measures in one factor (time of sampling) followed by Tukey's multiple comparisons test within groups over sampling time and Sidak's multiple comparisons test between groups through each sampling time. Untransformed data are presented in Tables and Figures.

Results

Mean values for dry matter (%), total protein (%), fat (%), pH, % Brix and the concentration and mass of IgG in the colostrum samples of C150 and C0 group of cows, for every sampling time (2, 12, 24 and 36 h after calving), are presented in Fig. 1 and in online Supplementary Table S2. Full details of the statistical analysis are given in online Supplementary Table S3. The overall means value of dry matter, fat, protein, % Brix, IgG concentration and IgG mass were significantly higher in colostrum of C150 than in colostrum of C0 group (Table 1). These results showed that in the 1st colostrum (collected 2–3 h pp) total protein and %Brix values were higher (by 15 and 11% respectively) in C150 than C0 group. In the 2nd colostrum (collected 12 h pp) the value of colostrum solids, total protein, %Brix and fat were higher (by 30, 30, 29 and 45% respectively) in C150 than C0 group. The concentration of IgG in the 1st and 2nd colostrum of C150 group was higher (by 21 and 54% respectively) compared to the C0 group. Despite the fact that there were no differences in the volume of colostrum between C150 and C0 groups, the total mass of colostrum IgG in C150 group was higher (by 38%, $P = 0.04$) in the 1st colostrum.

The blood serum biochemistry results showed that oral clinoptilolite supplementation had no effect on the total protein, albumin, urea, glucose, triglycerides, cholesterol, BHB, phosphorus, calcium, magnesium or potassium concentration (Table 2).

The results of agarose gel electrophoresis showed that clinoptilolite supplementation did not influence the concentration of blood serum γ globulins (mainly IgG; Butler, 1983) (Fig. 2 and online Supplementary Tables S4 and S5). However, analysis of the relative content (percentage) of cationic 'slow' and anionic 'fast' γ globulin fractions showed that there were significant differences between the groups both before and after parturition. At 4 ± 2 d prepartum we observed a decrease of 7% ($P = 0.02$) in the relative content of cationic γ globulin and an increase of 17% ($P = 0.02$) in the relative content of anionic γ globulin in the treated cows. However, the opposite effect was observed on the 1st day after calving, when the relative content of cationic γ globulin was 7% greater ($P = 0.02$), and the relative content of anionic γ globulins was 10% lower ($P = 0.02$) in C150 group (Tables S4 and S5, online Supplementary File).

Discussion

In this study the effect of the organically modified clinoptilolite on colostrum quality in primiparous dairy cows was analysed. This formulation has been produced by adsorption of long-chain organic cations which adsorbs several mycotoxins (aflatoxin B1, ochratoxin A, zearalenone; 99, 96, 94% adsorption, respectively) without influencing adsorption of vitamins, amino acids and trace elements (Milosevic and Tomasevic-Canovic *et al.*, 2003, 2009).

The lactation number is one of the key factors that can affect the IgG concentration and nutrient composition of colostrum (Devery-Pocius and Larson, 1983). Primiparous cows have been exposed to a smaller number of antigens (environmental or

vaccinal), and the antigen exposure time is shorter (Morris, 2011), which might result in lower IgG concentrations in their colostrum (Devery-Pocius and Larson, 1983). However, in our current study, primiparous cows from both control (C0) and clinoptilolite treated (C150) groups produced high quality colostrum (>50 g/l IgG). This is in accordance with the results of Kehoe *et al.* (2011) who reported that, as a result of modern cow management regimes that include greater attention to feeding and vaccination programmes, cows from first lactation can produce high quality colostrum. Starting from an assumption that cows from second and greater lactations might have more diversity of antibodies in their colostrum (exposed to more antigens, for longer period) we chose to focus on primiparous cows due to their 'immunological inexperience' and the greater uniformity of the experimental groups.

Numerous studies confirm that zeolite (clinoptilolite) supplementation improves milk yield (Karatzia *et al.*, 2013) and the level of milk fat (Roussel *et al.*, 1992) and total protein (Dschaak *et al.*, 2010). Previous results showed that oral clinoptilolite supplementation of calves during the period of colostrum nutrition increased blood serum IgG level (Stojić *et al.*, 1995; Fratrić *et al.*, 2005, 2007). The current study has showed that oral supplementation with 150 g/day of organically modified clinoptilolite improved colostrum quality. Although results of several research groups showed a beneficial effect of zeolite supplementation, the results are difficult to compare directly (Papaioannou *et al.*, 2005; Kerwin *et al.*, 2019). It has been reported that the extent of performance enhancement effects is related to the types of zeolite, their physico-chemical properties, purity in terms of the content of clinoptilolite, as well as to the supplementation level used in the diets (Papaioannou *et al.*, 2005). In addition, the particle size of the zeolitic material, crystallite size and the degree of aggregation, as well as the porosity of individual particles strongly affect its ion exchange, adsorption and catalytic properties (Papaioannou *et al.*, 2005). In a recent study it was shown that the use of synthetic zeolite A in the prepartum period of cows in second and greater lactation did not influence colostrum IgG concentration and IgG mass (Kerwin *et al.*, 2019). According to data reviewed in Papaioannou *et al.* (2005) we believe that the discrepancy between our result and results of Kerwin *et al.* (2019) lies in the variations in the experimental approach: use of different zeolites and use of cows with different lactations/parities.

Results of the present study showed that the primiparous dairy cows had high quality colostrum (>50 g/l IgG; Godden, 2008), and that the supplementation with organically modified clinoptilolite further improved it. Thus, the supplemented cows (C150 group) have, compared to control ones (C0 group), higher concentrations of IgG in colostrum collected 2 to 3 h as well as 12 h pp, and increased total mass of IgG at 2 to 3 h pp. The exact mechanisms of the positive effects of zeolite supplementation on colostrum quality in primiparous dairy cows are not clear at this time. However, reports on the efficacy of zeolites to reduce ammonia release into the environment, and to contrast dietary mycotoxins' effects on health, production and milk composition of dairy cows can help us in the interpretation of our data. In 1974 it was proven that ammonia ions produced in the rumen by the decomposition of non-protein nitrogen sources are immediately exchanged in zeolites and subsequently released by the regenerative action of sodium ions entering the rumen by the saliva during the after-feeding fermentation period (White and Ohlrogge, 1974). Both *in vivo* and *in vitro* experiments have established that up to 15% of the ammonium ions

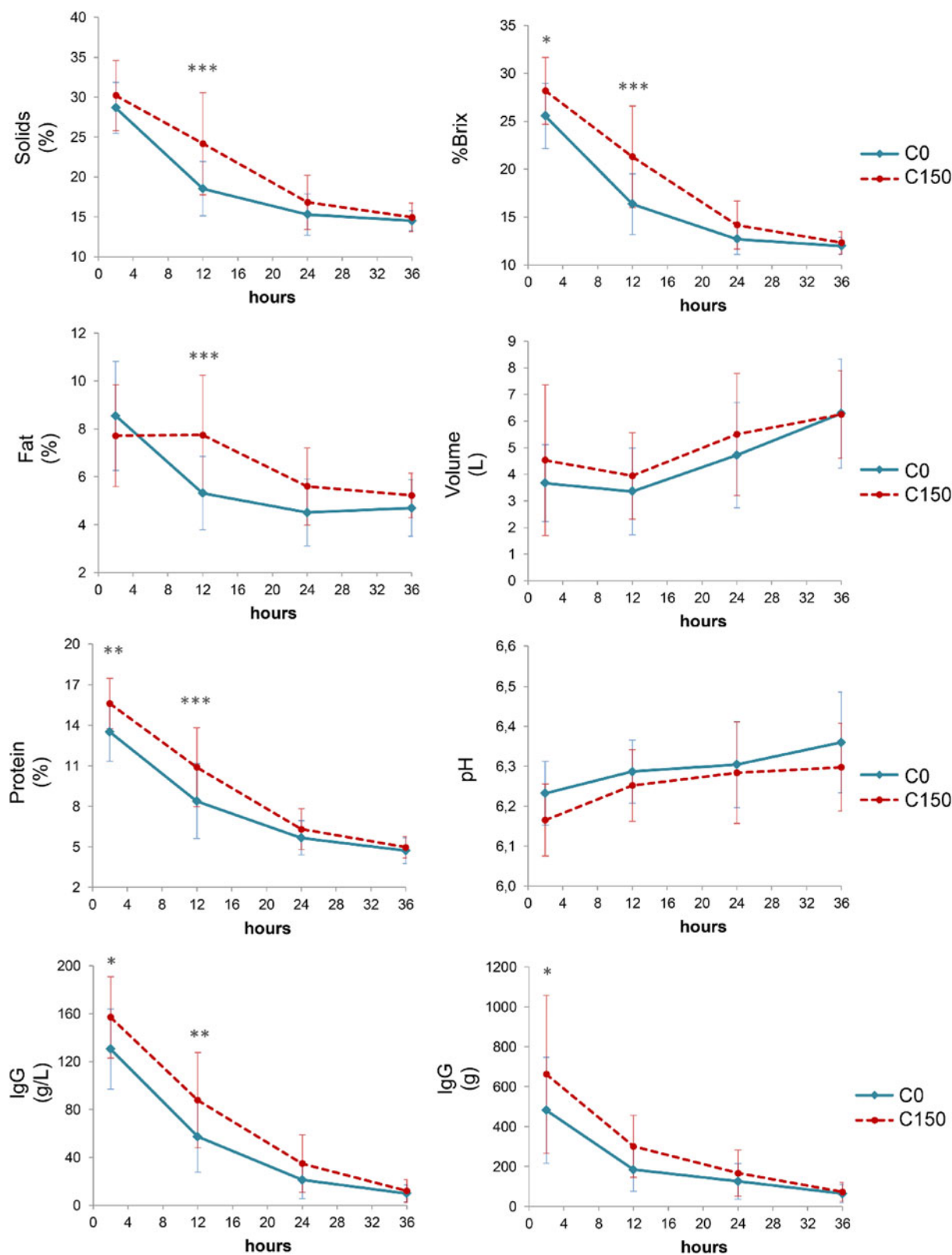


Fig. 1. Quality parameters measured in colostrum collected from primiparous dairy cows drenched orally with a supplementation of water (1 l) with either 0 or 150 g/head/d of organically modified clinoptilolite. C0: 0 g/head/d of the clinoptilolite; C150: 150 g/head/d of the clinoptilolite. Colostrum sampled 2, 12, 24 and 36 h after calving; The results are mean \pm sd; (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$.

in the rumen could be taken up by the zeolite, allowing gradual release of excess nitrogen into the rumen (White and Ohlogge, 1974). This gradual release contributes to capturing the nitrogen

into microbial protein, which is then assimilated into the animals' digestive system. The ability of zeolite to act as a reservoir of ammonium ions protects the animal against the production of

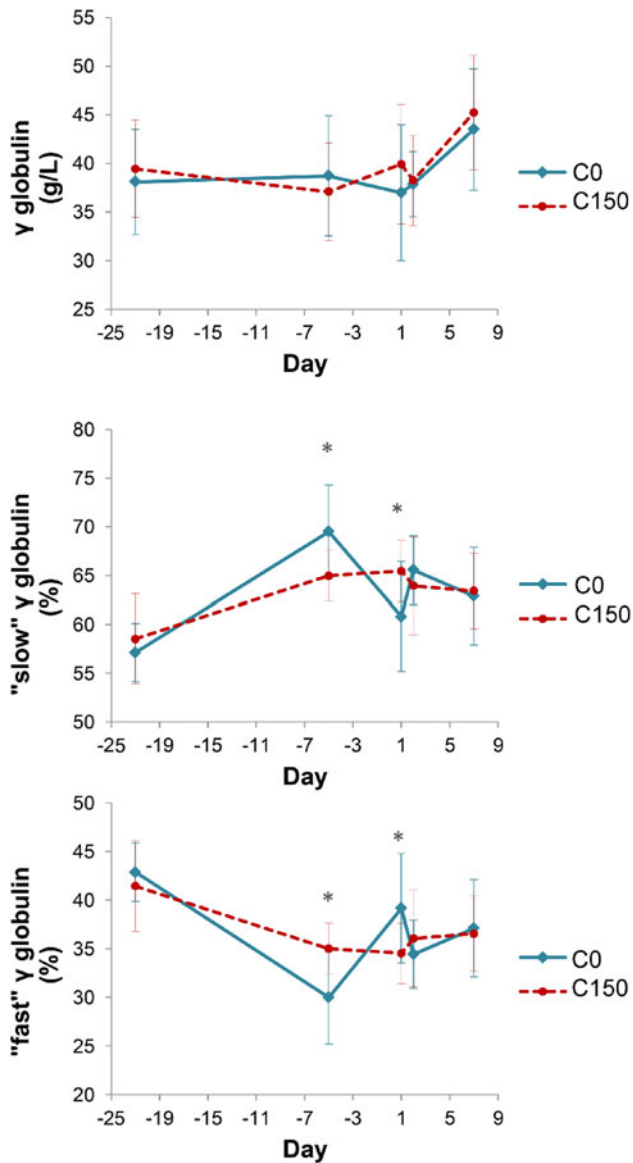


Fig. 2. Peripheral blood serum γ globulin in primiparous dairy cows drenched orally with a supplementation of water (1 l) with either 0 or 150 g/head/d of organically modified clinoptilolite. C0: 0 g/head/d of the clinoptilolite; C150: 150 g/head/d of the clinoptilolite. The blood sampled 24 ± 4 and 4 ± 2 d before, and on day 1, 2 and 7 after calving; the results are mean \pm SD; (*) $P < 0.05$.

toxic levels of ammonia in the rumen and enables the addition of supplemental nitrogen to the animal feed (White and Ohlrogge, 1974). Hemken *et al.* (1984) showed that supplementation of 6% clinoptilolite, in the ration of dairy cows containing urea, significantly reduced the rumen ammonia concentration. Furthermore, clinoptilolite was effective in reducing steers rumen ammonia concentration even when no urea was present, and this reduction was linearly associated with the percentage of clinoptilolite inclusion (McCullum and Galyean, 1983). Nestorov (1984) referred that simultaneous administration of clinoptilolite and urea in sheep protects rumen flora from toxic effects of ammonia by inhibiting the reduction of microbiota population. It is possible that, with the release of ammonia subsequent to each meal, clinoptilolite absorbs high levels of ammonia of rumen, and releases it when its concentration is reduced, and when it is required for bacteria, as a main

source of nitrogen to synthesize microbial protein. Because proteolysis occurs rapidly in the rumen, it usually exceeds the rate of utilization by microorganisms resulting in an accumulation of ammonia (Dschaak, 2012; Hartinger *et al.*, 2018). Ammonia, in excess of that used by the microorganisms, is absorbed across the rumen wall, converted to urea in the liver, released as blood urea nitrogen, and excreted into urine or recycled back to the rumen *via* saliva or diffusion through the rumen wall (Dschaak, 2012). The results of the present study showing an increase in the percentage of total protein and colostral IgG concentration in the clinoptilolite treated group, might be explained by the positive effect of the clinoptilolite on increasing the availability of nitrogen in the rumen, and thus by favouring the microbe protein synthesis process in the cows' rumen.

The relative content of dry matter in colostrum of treated group was higher in 2nd colostrum, with relatively higher content of total protein and fat. Considering that colostrum fat is primarily derived from the free fatty acids absorbed in the rumen, the results showed increasing percentage of fat in the colostrum of clinoptilolite supplemented (C150) group, are comparable to the results of Karatzia *et al.* (2011) who reported an increase in the acetate concentration in cows supplemented with zeolite. In the study by Khachlouf *et al.* (2018) the addition of zeolite also resulted in the highest rumen molar percentage of acetate and higher acetate: propionate ratio. Similar to the results of this study, Roussel *et al.* (1992) observed a significant increase in milk fat content, feeding cows a diet containing 1% of sodium zeolite-A.

Khachlouf *et al.* (2019) have recently reported that the use of zeolite in periparturient period of cows can be effective, with positive influence on milk production and its components, and have no deleterious effects on milk composition or blood biochemistry parameters. This study confirmed that, besides positive effect on colostrum quality, oral supplementation of the organically modified clinoptilolite has no apparent adverse effects on the protein metabolism (total protein, albumin and urea level), energy status (glucose and BHB), lipid (triglycerides and cholesterol) and mineral (calcium, phosphorus, magnesium and potassium) metabolism in the primiparous dairy cows during the experimental period. The applied supplementation did not influence the absolute concentration of γ globulins in blood serum of experimental animals. However, our results indicate that oral supplementation with 150 g/day organically modified clinoptilolite could have a beneficial effect on the relative content of anionic γ globulin fractions (mostly IgG1) (Kickofen *et al.*, 1968) with increased relative content on day 4 ± 2 before calving and decreased relative content on 1st day after calving, reflecting their increased synthesis before calving and transport to the colostrum at the day of calving.

In conclusion, the oral supplementation of primiparous dairy cows with 150 g per day of organically modified clinoptilolite during the prepartum period resulted in the secretion of colostrum with increased concentration of IgG, total proteins and lipid, and had no adverse effects on the cows energy status, protein, lipid, and mineral metabolism.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0022029920001077>

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References

- Butler JE (1983) Bovine immunoglobulins: an augmented review. *Veterinary Immunology and Immunopathology* **4**, 43–152.
- Chigerwe M, Tyler JW, Nagy DW and Middleton JR (2008) Frequency of detectable serum IgG concentrations in precolostral calves. *American Journal of Veterinary Research* **69**, 791–795.
- Daković A, Tomašević-Čanović M, Rottinghaus GE, Matijašević S and Sekulić Ž (2007) Fumonisin B1 adsorption to octadecyldimethylbenzyl ammonium-modified clinoptilolite-rich zeolitic tuff. *Microporous and Mesoporous Materials* **105**, 285–290.
- Devery-Pocius JE and Larson BL (1983) Age and previous lactations as factors in the amount of bovine colostral immunoglobulins. *Journal of Dairy Science* **66**, 221–226.
- Dschaak CM (2012) *Use of rumen modifiers to manipulate ruminal fermentation and improve nutrient utilization and lactational performance of dairy cows* (PhD thesis). Utah State Univ, Utah.
- Dschaak CM, Eun JS, Young AJ, Stott RD and Peterson S (2010) Effects of supplementation of natural zeolite on intake, digestion, ruminal fermentation, and lactational performance of dairy cows. *The Professional Animal Scientist* **26**, 647–654.
- Fratric N, Stojić V, Janković D, Šamanc H and Gvozdić D (2005) The effect of a clinoptilolite based mineral adsorbent on concentrations of immunoglobulin G in the serum of newborn calves fed different amounts of colostrum. *Acta Veterinaria-Beograd* **55**, 11–21.
- Fratric N, Stojić V, Rajčić S and Radojčić B (2007) The effect of mineral adsorbent in calf diet colostrum on the levels of serum immunoglobulin G, protein and glucose. *Acta Veterinaria-Beograd* **57**, 169–180.
- Godden S (2008) Colostrum management for dairy calves. *Veterinary Clinics of North America: Food Animal Practice* **24**, 19–39.
- Hartinger T, Gresner N and Südekum KH (2018) Does intra-ruminal nitrogen recycling waste valuable resources? A review of major players and their manipulation. *Journal of Animal Science and Biotechnology* **9**, 33.
- Hemken RW, Harmon RJ and Mann LM (1984) Effect of clinoptilolite on lactating dairy cows fed a diet containing urea as a source of protein. In Pond WG and Mumpton FA (eds), *Zeo-agriculture. Use of Natural Zeolites in Agriculture and Aquaculture*. Boulder: Westview Press, pp. 175–181.
- Karatzia MA, Pourliotis K, Katsoulos PD and Karatzias H (2011) Effects of in-feed inclusion of clinoptilolite on blood serum concentrations of aluminium and inorganic phosphorus and on ruminal pH and volatile fatty acid concentrations in dairy cows. *Biological Trace Element Research* **142**, 159–166.
- Karatzia MA, Katsoulos PD and Karatzias H (2013) Diet supplementation with clinoptilolite improves energy status, reproductive efficiency and increases milk yield in dairy heifers. *Animal Production Science* **53**, 234–239.
- Katsoulos PD, Panousis N, Roubies N, Christaki E, Arsenos G and Karatzias H (2006) Effects of long-term feeding of a diet supplemented with clinoptilolite to dairy cows on the incidence of ketosis, milk yield and liver function. *Veterinary Record* **159**, 415–418.
- Katsoulos PD, Karatzia MA, Boscoc C, Wolf P and Karatzias H (2016) In-field evaluation of clinoptilolite feeding efficacy on the reduction of milk aflatoxin M1 concentration in dairy cattle. *Journal of Animal Science and Technology* **58**, 24.
- Kehoe SI, Heinrichs AJ, Moody ML, Jones CM and Long MR (2011) Comparison of immunoglobulin G concentrations in primiparous and multiparous bovine colostrum. *The Professional Animal Scientist* **27**, 176–180.
- Kertz AF, Hill TM, Quigley III JD, Heinrichs AJ, Linn JG and Drackley JK (2017) A 100-year review: calf nutrition and management. *Journal of Dairy Science* **100**, 10151–10572.
- Kerwin AL, Ryan CM, Leno BM, Jakobsen M, Theilgaard P, Barbano DM and Overton TR (2019) Effects of feeding synthetic zeolite A during the prepartum period on serum mineral concentration, oxidant status, and performance of multiparous Holstein cows. *Journal of Dairy Science* **102**, 5191–5207.
- Khachlouf K, Hamed H, Gdoura R and Gargouri A (2018) Effects of zeolite supplementation on dairy cow production and ruminal parameters – a review. *Annals of Animal Science* **18**, 857–877.
- Khachlouf K, Hamed H, Gdoura R and Gargouri A (2019) Effects of dietary zeolite supplementation on milk yield and composition and blood minerals status in lactating dairy cows. *Journal of Applied Animal Research* **47**, 54–62.
- Kickofen B, Hammer DK and Scheel D (1968) Isolation and characterisation of γ G type immunoglobulins from bovine serum and colostrum. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* **349**, 1755–1773.
- McCollum FT and Galyean ML (1983) Effects of clinoptilolite on rumen fermentation, digestion and feedlot performance in beef steers fed high concentrate diets. *Journal of Animal Science* **56**, 517–524.
- Milosevic S and Tomasevic-Canovic M (2003) Processes of tribochemical obtaining organozeolite adsorbent of mycotoxins, procedure for production and application. EP Patent No. 1363854A1.
- Milosevic S and Tomasevic-Canovic M (2009) Tribochemical process for obtaining organozeolite adsorbent of mycotoxins. EP Patent No. 1363854B1.
- Morril KM (2011) *Modifying current laboratory methods for rapid determination of colostral IgG concentration and colostral IgG absorption in the neonate* (PhD thesis). Iowa State Univ, USA.
- National Research Council (2001) *Nutrient Requirements of Dairy Cattle*, 7th rev. ed., Washington, DC: Natl. Acad. Press.
- Nestorov N (1984) Possible applications of natural zeolites in animal husbandry. In Pond WG and Mumpton FA (eds), *Zeo-agriculture. Use of Natural Zeolites in Agriculture and Aquaculture*. Boulder: Westview Press, pp. 167–174.
- Papaioannou DS, Kyriakis CS, Alexopoulos C, Tzika ED, Polizopoulou ZS and Kyriakis SC (2004) A field study on the effect of the dietary use of a clinoptilolite-rich tuff, alone or in combination with certain antimicrobials, on the health status and performance of weaned, growing and finishing pigs. *Research in Veterinary Science* **76**, 19–29.
- Papaioannou D, Katsoulos PD, Panousis N and Karatzias H (2005) The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: a review. *Microporous and Mesoporous Materials* **84**, 161–170.
- Pourliotis K, Karatzia MA, Florou-Paneri P, Katsoulos PD and Karatzias H (2012) Effects of dietary inclusion of clinoptilolite in colostrum and milk of dairy calves on absorption of antibodies against *Escherichia coli* and the incidence of diarrhea. *Animal Feed Science and Technology* **172**, 136–140.
- Roussel JD, Thibodeaux JK, Adkinson RW, Toups GM and Goodeaux LL (1992) Effect of feeding various levels of sodium zeolite A on milk yield, milk composition and blood profiles in thermally stressed Holstein cows. *Journal International de Vitaminologie et de Nutrition* **62**, 91–98.
- Stojić V, Šamanc H and Fratric N (1995) The effect of a clinoptilolite based mineral adsorbent on colostral immunoglobulin G absorption in newborn calves. *Acta Veterinaria-Beograd* **45**, 67–74.
- Stojić M, Fratric N, Kovačić M, Ilić V, Gvozdić D, Savić O, Djoković R and Valčić O (2017) Brix refractometry of colostrum from primiparous dairy cows and new-born calf blood serum in the evaluation of failure of passive transfer. *Acta Veterinaria-Beograd* **67**, 508–524.
- Weaver DM, Tyler JW, VanMetre DC, Hostetler DE and Barrington GM (2000) Passive transfer of colostral immunoglobulins in calves. *Journal of Veterinary Internal Medicine* **14**, 569–577.
- White JL and Ohlrogge AJ (1974) Ion exchange materials to increase consumption of non-protein nitrogen in ruminants. Canadian patent 939186.
- Wu QJ, Wang YQ, Zhou YM and Wang T (2015) Dietary clinoptilolite influences antioxidant capability and oxidative status of broilers. *Journal of Applied Poultry Research* **24**, 99–104.