

THE EFFECT OF MINERAL ADSORBENT IN CALF DIET COLOSTRUM ON THE LEVELS OF SERUM IMMUNOGLOBULIN G, PROTEIN AND GLUCOSE

FRATRIĆ NATALIJA, STOJIĆ V, RAJČIĆ S and RADOJIČIĆ BILJANA

Faculty of Veterinary Medicine, Belgrade

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The aim of the study was to investigate the influence of added natural mineral adsorbent zeolite (clinoptylolite) on the degree of absorption of immunoglobulin G and on glucose and protein concentration in the blood of calves during the neonatal period.

Sixty calves were randomly divided in to four groups (15 calves per group). The quality and timing of colostrum feeding was as follows: group I was fed only with 1.5 L of colostrum at 2nd, 12th, 24th and 36th hour after birth; group II was fed with 1.5 L of colostrum with the mineral adsorbent added at a 5% concentration in the same time intervals; group III was fed with 1.5 L of colostrum together with the mineral adsorbent added at a 5% concentration, but at 2nd and 12 hour after birth it was fed with the first colostrum, and 24 and 36 hours after birth it was fed with the second colostrum. Group IV of calves was fed the same way as the third one only without the addition of the mineral adsorbent.

Mean serum concentration of IgG was 15.14 ± 7.43 ; 22.22 ± 8.15 ; 23.65 ± 9.93 ; 20.81 ± 4.19 g/L in groups I to IV respectively at 6h of age. IgG concentration in the sera of all groups were 29.10 ± 15.25 ; 45.46 ± 13.45 ; 50.72 ± 21.57 ; 41.60 ± 15.42 g/L respectively at 16h after birth. At 30 hours of age, concentration of IgG was 33.29 ± 14.70 ; 47.05 ± 12.38 ; 55.00 ± 19.71 ; 51.59 ± 13.82 g/L in groups I to IV respectively. IgG concentration in the sera of all groups were 29.82 ± 12.69 ; 44.10 ± 13.50 ; 51.88 ± 20.14 ; 47.68 ± 13.18 g/L respectively at 40 hours after birth.

For the calves which were fed with the full colostrum without and with the addition of the mineral adsorbent (Group I and II) the statistical significance of the difference in the concentration of IgG was determined during all the tested intervals. Concentration of IgG in the sera of group II calves (fed colostrum with zeolite added) was approximately 30% higher than in the group I.

The statistical significance of the difference in levels of IgG between group I and groups III and IV was determined during all tested intervals, also. Concentration of IgG in the sera of group III calves was 40% higher than in group I.

Results suggest that calves should receive feedings of high quality colostrum with added mineral adsorbent in order to maximize colostrum IgG absorption.

Determining the proteinemia in the neonatal period of calves is a reliable indicator of the impact of the mineral adsorbent on the degree of absorption of colostrum IgG.

The concentration of glucose for all the tested groups increased significantly with the increase of the quantity of ingested colostrum, with no evident influence of the added mineral adsorbent.

Key words: colostrum, newborn calves, immunoglobulins, IgG, clinoptilolite

INTRODUCTION

Bovine colostrum contains higher amounts of proteins and peptides, fats, fat-soluble vitamins, and various enzymes, hormones, growth factors, cytokines, minerals and nucleotides than mature milk, and except lactose, the levels of those compounds rapidly decrease during the first 3 days of lactation to those typical for mature milk (Campana and Baumrucker, 1995; Blum and Hammon, 2000a, 2000b). Colostrum intake in neonatal calves is essential for passive immunity and influences metabolism, endocrine system and nutritional state (Guilloteau *et al.*, 1997). Ingested immunoglobulins, some proteins and enzymes are absorbed in particular during the first hours after birth (Baumrucker *et al.*, 1994b; Hadorn and Blum, 1997; Hammon and Blum, 1998).

Inadequate or improper colostrum feeding and management causes a significant portion of calf morbidity and mortality on dairy farms. The importance of ingestion of adequate colostrum of suitable quality is critical in the first 24h of life (Matte *et al.*, 1982; Hammon and Blum, 1998; Blum and Hammon, 2000). In animals with epitheliochorial type of placenta such as pigs, sheep, cattle, transplacental passage of immunoglobulin molecules is totally prevented. Calves are born with almost no serum Ig under physiological conditions (hypogammaglobulinemic state) and acquire passive immunity by absorbing immunoglobulins from the colostrum (Stott *et al.*, 1979; Stott and Fellah, 1983; Weaver *et al.*, 2000). Colostrum contains high levels of immunoglobulins. There are 3 types of Ig (IgG, IgM, IgA) in the colostrum, which typically account for about 85% to 90%, 5%, and 7%, respectively, of total Ig in colostrum (Roy, 1980). These Ig provide the calf with passive immunity (immunity provided by the cow) until the calf's own active immunity develops (Korhonen *et al.*, 2000).

Immunoglobulin intake depends on colostrum intake and its Ig concentration. Immunoglobulin ingested by the calf is taken up by epithelial cells of the small intestine and passes into the lymph spaces and then into the blood circulation. This transport mechanism starts to decline approximately 12 to 24 hours after birth (Vukotić 1976, 1972; Kiryama *et al.*, 1989; Arthington *et al.*, 2000; Korhonen *et al.*, 2000; Rauprich *et al.*, 2000; Franklin *et al.*, 2003). Although the level of Ig that provides adequate protection will vary with exposure to infectious

organisms, stress, environment, and temperature, a management target of 10 mg/mL has been suggested as a minimum level of IgG in the serum of calves by approximately 24 h of age to prevent failure of passive transfer (Bovine Alliance on Management and Nutrition, 1995).

Numerous authors consider that 38 hours after birth the absorption of immunoglobulins from colostrum has ceased. It has been proposed that gut closure is associated with postnatal replacement of fetally produced intestinal enterocytes with cells incapable of internalizing macromolecules (Smith, 1988; Rou-Xu, 1996). Postnatal enterocyte differentiation may also contribute to the gut closure process.

Since colostral Ig are the key factor of humoral immunity in newborn calves and during the first few weeks of their life, many authors have studied conditions under which the degree of absorption of colostral immunoglobulins (Ig) may be increased, such as: the way in which colostrum is fed to calves, the time at which colostrum was first ingested, the effect of the concentration of immunoglobulins in the colostrum, and the efficiency of absorption of Ig by the calf (Kruse *et al.*, 1970; Stott *et al.*, 1979; Stott *et al.*, 1981; Besser *et al.*, 1985; Pritchett *et al.*, 1991; Morin *et al.*, 1997; Hopkins *et al.*, 1997; Davenport *et al.*, 2000).

The effects of a mineral adsorbent, based on clinoptilolite, have been widely applied in domestic animals in the last ten years (Stankov *et al.*, 1992; Tomašević-Čanović *et al.*, 1994; Petrović *et al.*, 1995; Rajić *et al.*, 1995; Stojić *et al.*, 1995; 1998).

Stojić *et al.* (1995, 1998), examined the effects of a clinoptilolite based adsorbent added to colostrum on the degree of absorption of colostral immunoglobulin G in newborn animals. They showed that a clinoptilolite based mineral adsorbent in the colostrum leads to a significantly higher absorption of colostrum IgG in newborn calves and piglets.

The aim of our investigation was to determine the effect of prolonged clinoptilolite enriched first colostrum feeding on absorption and serum concentration of immunoglobulin G in newborn calves, during the first 48 hours of life.

MATERIAL AND METHODS

Animals.

The experiment was carried out on 60 calves, divided in four groups (15 calves per group). *Experimental group I.* Each of the 15 calves received 1.5 L of colostrum at 2, 12, 24 and 36 hours after birth; *Experimental group II.* Each of the 15 calves received 1.5 L of colostrum with the mineral adsorbent in a 5% concentration in the same time intervals; *Experimental group III.* Each of the 15 calves received 1.5 L of colostrum together with the mineral adsorbent in a 5% concentration, but 2 and 12 hours after birth it was fed with the first colostrum and at 24 and 36 hours it was fed with the second colostrum; *Experimental group IV.* Each of the 15 calves was fed in the same way as the third group but without the addition of the mineral adsorbent.

Blood sampling.

Blood samples were taken from the jugular vein at 6, 16, 30, and 40 hours after birth. After spontaneous coagulation at room temperature, the serum was separated and frozen at -20°C for subsequent analysis.

Mineral adsorber. The examined mineral adsorber was obtained by technological preparation of the zeolitic tuff from the Zlatokop (South Serbia) deposit. Mineral composition: the basic component is clinoptilolite with the presence of quartz and plagioclase. Clinoptilolite (Minazel –S, ITNMS, Beograd, Serbia) suspension was prepared according to the producer's instructions.

Methods

Immunodiffusion. Immunoglobulin G concentrations in the blood sera of calves and colostrum sera were determined using double immunodiffusion on commercial RID plates (INEP-Zemun, SRB).

Total protein concentration was determined by the colorimetric method

Serum enzyme activities (AST, ALT) were measured by commercially available kits and a clinical biochemical analyzer.

Glucose was measured using kits from Hoffman-LaRoche (Basle, Switzerland) and an automatic analyzer (Secomam CE, BP 106). All assays were performed in duplicate and all samples were analyzed within the same run of an assay.

Statistical analysis. The results are expressed as mean (M), standard deviation (SD), standard error (SE) and variation coefficient (CV%). The significance of differences between groups was calculated using Students t-test.

RESULTS

Concentrations of serum immunoglobulin G in calves of experimental groups I-IV at 6, 16, 30 and 48 hours after birth are shown in Table 1, 2.

Table 1. Concentrations of serum immunoglobulin G in calves from experimental group I and group II after colostrum intake

	Experimental group I				Experimental group II (zeolite)			
	IgG conc (g/L)				IgG conc (g/L)			
	6h	16h	30h	40h	6h	16h	30h	40h
Mean	15.14	29.10	33.29	29.82	22.22	45.46	47.05	44.10
SD	7.43	15.25	14.70	12.69	8.15	13.45	12.38	13.50
SE	1.92	3.93	3.79	3.27	2.10	3.47	3.19	3.48
CV%	49.07	52.40	44.15	43.45	36.67	29.58	26.31	30.61

Table 2. Concentrations of serum immunoglobulin G in calves from experimental group III and group IV after colostrum intake

	Experimental group III (zeolite)				Experimental group IV			
	IgG conc (g/L)				IgG conc (g/L)			
	6h	16h	30h	40h	6h	16h	30h	40h
Mean	23.65	50.72	55.00	51.88	20.81	41.60	51.59	47.68
SD	9.93	21.57	19.71	20.14	4.19	15.42	13.82	13.18
SE	2.65	5.57	5.09	5.20	1.08	3.98	3.56	3.40
CV%	41.98	42.52	36.83	38.82	20.13	37.06	26.78	27.64

Results indicated that the concentration of IgG in the serum increased significantly after colostrum intake. Mean immunoglobulin G concentrations in the serum of calves from experimental group I and II 6 hours after birth were 15.14 ± 7.43 g/L and 22.22 ± 8.15 g/L, respectively.

At the same time, IgG concentrations in the serum of the experimental groups III and IV were 23.65 ± 9.93 g/L and 20.81 ± 4.19 g/L, respectively.

The mean immunoglobulin G concentration in the serum of calves from experimental groups I and II 16 hours after birth were 29.10 ± 15.25 g/L and 45.46 ± 13.45 g/L. At the same time, in the serum of the experimental groups III and IV were 50.72 ± 21.57 g/L and 41.60 ± 15.42 g/L, respectively.

The mean immunoglobulin G concentrations in the serum of calves from experimental groups I and II 30 hours after birth were 33.29 ± 14.70 g/L and 47.05 ± 12.38 g/L respectively. Concentrations of IgG in the serum of calves from experimental groups III and IV were 55.00 ± 19.71 g/L and 51.59 ± 13.82 g/L, respectively.

The mean immunoglobulin G concentrations in the calf sera from experimental groups I and II 40 hours after birth were 29.82 ± 12.69 g/L and 44.10 ± 13.50 g/L respectively. Concentrations of IgG in the serum of calves from experimental groups III and IV were 51.88 ± 20.14 g/L and 47.68 ± 13.18 g/L, respectively.

Significantly higher concentrations of serum IgG were found in calves from experimental group II compared to group I, 6 h ($p < 0.05$), 16h ($p < 0.01$), 30h ($p < 0.01$) and 40h ($p < 0.01$) after birth.

The difference in the mean concentrations of IgG between experimental groups I and IV was higher 6 hours after birth ($p < 0.05$), 16h ($p < 0.05$), 30h ($p < 0.01$), and 40h ($p < 0.001$) after birth. Significantly higher concentrations of serum IgG were found in calves from experimental group IV compared with experimental group I.

Our results showed that the mean concentration of IgG between experimental group II and group III and IV was not statistically significant. Also the difference was not statistically significant for the levels of IgG between groups III and IV.

Concentration of IgG in sera of calves group II where fed colostrum with added zeolite was approximately 30% higher than in the group I (which was fed with colostrum without zeolite). Concentration of IgG in sera of calves in group III was 40% higher than in group I. Although, no significant statistical difference was found between the groups which were fed with the mineral adsorbent (II and III group), but the concentration of IgG in group III was 10-20% higher than in group II.

Results suggest that calves should receive feedings of high quality colostrum with mineral adsorbent added in order to maximize the colostrum IgG absorption.

Concentration of IgG in sera of group II calves (fed colostrum with added zeolite) was higher at 6h and 16h compared to the concentration of IgG in group IV in the same period, but at 30h and 40h the concentration of IgG in group IV was higher than in group II.

These results show the importance of clinoptilolite on IgG absorption, especially in the first 24 hours of postnatal life. Our results suggest that feeding high quality colostrum with added mineral adsorbent in the first two days after birth has an influence on high IgG concentrations in blood sera.

The data from this investigation show that clinoptilolite based mineral adsorbent added to colostrum leads to a significantly higher degree of absorption of colostrum IgG in newborn calves, which has medical and economic effects.

The protein concentration is increased significantly after intake colostrum (Table 3, 4). Determining proteinemia in the neonatal period of calves is a reliable indicator of the impact of the mineral adsorbent on the degree of the absorption of colostrum IgG.

The glucose concentration for all the tested groups increased significantly with the increase of the quantity of ingested colostrum, without larger influence of the mineral adsorbent (Table 5, 6).

Table 3. Concentrations of total protein (g/L) in serum of calves from experimental group I and group II after colostrum intake

	Experimental group I				Experimental group II (zeolite)			
	protein conc (g/L)				protein conc (g/L)			
	6h	16h	30h	40h	6h	16h	30h	40h
Mean	47.89	59.59	64.44	61.56	57.0	83.7	87.3	81.2
SD	13.89	14.60	19.84	15.77	12.50	15.59	14.18	17.52
SE	3.58	3.77	5.12	4.07	3.23	4.02	3.66	4.52
CV%	29.00	24.50	30.78	25.61	21.92	18.62	16.24	21.57

Table 4. Concentrations of total protein (g/L) in serum of calves from experimental group III and group IV after colostrum intake

	Experimental group III				Experimental group IV (zeolite)			
	protein conc (g/L)				protein conc (g/L)			
	6h	16h	30h	40h	6h	16h	30h	40h
Mean	55.80	84.22	86.32	83.64	58.95	76.74	85.2	96.8
SD	12.99	24.39	23.11	27.88	7.31	17.80	16.57	19.54
SE	3.35	6.29	5.96	7.19	1.88	4.59	4.27	5.04
CV%	23.27	28.95	26.77	33.33	12.40	23.19	19.44	20.18

Table 5. Concentrations of glucose (mmol/L) in serum of calves from experimental group I and group II after colostrum intake

	Experimental group I				Experimental group II (zeolite)			
	glucose (mmol/L)				glucose (mmol/L)			
	6h	16h	30h	40h	6h	16h	30h	40h
Mean	3.02	4.84	5.34	5.68	2.70	5.24	5.61	6.21
SD	0.50	1.09	0.96	0.71	0.76	1.69	1.20	0.81
SE	0.13	0.30	0.24	0.19	0.19	0.43	0.31	0.21
CV%	16.5	22.5	17.97	12.5	28.14	32.25	21.39	13.04

Table 6. Concentrations of glucose (mmol/L) in serum of calves from experimental group III and group IV after colostrum intake

	Experimental group III				Experimental group IV (zeolite)			
	glucose (mmol/L)				glucose (mmol/L)			
	6h	16h	30h	40h	6h	16h	30h	40h
Mean	2.93	4.66	5.52	5.70	3.92	5.96	6.41	7.01
SD	0.75	1.05	1.06	1.00	0.80	1.27	1.35	1.23
SE	0.19	0.27	0.27	0.25	0.20	0.32	0.34	0.31
CV%	25.59	22.53	19.20	18.18	20,40	21.3	21.06	17.54

DISCUSSION

Colostrum intake in neonatal calves is essential for passive immunity. It is critically important to feed colostrum immediately after birth. The intestine of a newborn is capable of absorbing large intact protein molecules (such as

immunoglobulins-Ig) within the first 24 hours of life, resulting in an increase in circulating IgG concentration in the calf's blood (Stott *et al.*, 1979; Morin *et al.*, 1997). Our results show that concentrations of IgG were much higher after colostrum intake, which is in accordance with many authors (Rajala and Castren, 1995; Gu-Hyun Suh *et al.*, 2003; Jaster, 2005).

Since colostral Ig are the key factor of humoral immunity in newborn calves and during the first few weeks of their life, authors have studied conditions under which the degree of absorption of colostral immunoglobulins (Ig) may be increased (Denise, 1989; Hopkins *et al.*, 1997; Davenport *et al.*, 2000). Authors investigated factors such as the way in which colostrum is fed to calves, the time at which colostrum was first ingested, the effect of the concentration of immunoglobulins in the colostrum, the extent of concentration of immunoglobulins in the colostrum and the efficiency of absorption of Ig by the calf (Matte *et al.*, 1982; Besser *et al.*, 1985; Morin *et al.*, 1997).

The effects of a mineral adsorbent, based on clinoptilolite, have been widely applied in domestic animals in the last ten years (Stankov *et al.*, 1992; Tomašević-Čanović *et al.*, 1994; Petrović *et al.*, 1995; Kovačević, 2000, Janković, 2004; Bojković, 2005; Fratrić *et al.*, 2005; Stojić *et al.*, 2005). Stojić *et al.* (1995; 1998), showed that a clinoptilolite based mineral adsorbent in the colostrum 5 g/L suspension leads to a significantly higher absorption of colostrum IgG in newborn calves and piglets.

Our results also clearly indicate that a clinoptilolite based mineral adsorbent in the colostrum led to a significant increase of IgG concentrations in the blood of newborn calves. These data additionally support the above mentioned opinion about the possible mode of clinoptilolite action to increase the absorption of colostral IgG. Our data for IgG concentrations are in accordance with previous studies by a number of authors (Kovačević, 2000; Janković, 2004; Fratrić *et al.*, 2005; Stojić *et al.*, 2005).

The question on the mechanism of influence of clinoptilolite on increased IgG adsorption is still open. However, since it has been shown that this mineral adsorbent efficiently binds aflatoxins B1 and G1, we can speculate that in conditions of high protein intake and absence of digestive enzymes, it may bind some degradation products of colostral proteins in the gut, thus preventing their negative effects on mucosal epithelial cells designated for immunoglobulin absorption. We can assume that clinoptilolite based mineral adsorbent could be of influence on a longer life time of mucosal epithelial cells which have more Fc receptors for IgG compared with the next generation of enterocytes. The resorption capacity of enterocytes for IgG exceed than the overall IgG concentration in 1.5 L of colostrum, which shows that calves can ingest more than 1.5 L of colostrum.

Changes in neonatal calf proteinemia are a consequence of abundant absorption of colostral proteins, specially immunoglobulins in the first hours of life. Our data for protein concentrations were similar to those previously reported by many other authors (Vukotic, 1972; Mao *et al.*, 1994; Adams *et al.*, 1992; Kuhne *et al.*, 2000). The significantly higher serum protein concentration in the groups which consumed colostrum with added zeolite indicates that proteinemia in the

neonatal period in calves is a reliable indicator of the impact of the mineral adsorbent on the degree of the absorption of colostrum IgG. The significantly higher serum protein concentration in the groups which consumed colostrum with zeolite indicates that the quality of colostrum consumed during the first 40 hours of postnatal life had a prolonged effect on the concentration of serum proteins, probably as a consequence of the long half-life of circulating immunoglobulins.

At 40 hours of age, calves fed colostrum with higher a concentration of total ingested IgG (group IV) had the highest serum protein concentrations which indicate that the quality of colostrum consumed during the first 40 hours of postnatal life had a prolonged effect on the concentration of serum proteins. This is an agreement with reports by Hammon and Blum (1998) and Jaster (2005).

The concentrations of glucose for all the tested groups increase significantly with the increase of quantity of ingested colostrum, without significant influence of the mineral adsorbent. Our data on glucose concentrations is in agreement with published data of other investigators (Grutter *et al.*, 1991; Kurz and Willett, 1991; Kuhne *et al.*, 2000).

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Address for correspondence:
Natalija Fratrić PhD
Faculty of Veterinary Medicine
Department of Physiology and Biochemistry
Bul Joslobodenja 18, 11000 Beograd
Serbia
e-mail:nataly@vet.bg.ac.yu

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EFEKAT MINERALNOG ADSORBENTA NA NIVO SERUMSKIH IMUNOGLOBULINA G, PROTEINA I GLUKOZE KOD TELADI NA KOLOSTRALNOJ ISHRANI

FRATRIC NATALIJA, STOJIC V, RAJČIĆ S i RADOJIČIĆ BILJANA

SADRŽAJ

Cilj ovog rada bio je da se ispita uticaj mineralnog adsorbenta na bazi klinkoptilolita na stepen resorpcije IgG i koncentraciju nekih biohemijskih parametara u krvnom serumu teladi u neonatalnom periodu.

Telad su bila podeljena u četiri grupe (po 15 teladi u svakoj grupi). I grupa teladi napajana je sa kolostrumom 2h, 12h, 24h i 36h posle rođenja, II grupa teladi napajana je sa kolostrumom u koji je dodat mineralni adsorbent u koncentraciji od 5% u istim vremenskim intervalima, III grupa napajana je sa 1,5 L kolostrumom u koji je dodat mineralni adsorbent u koncentraciji od 5% ali 2h i 12h posle rođenja napajana su prvim kolostrumom, a 24h i 36h dugim kolostrumom, IV grupa teladi hranjena je na isti način kao i III grupa samo što u kolostrum nije bio dodat mineralni adsorbent.

Koncentracija IgG u krvnom serumu teladi od I do IV grupe, 6 sata iznosila je pojedinačno $15,14 \pm 7,43$; $22,22 \pm 8,15$; $23,65 \pm 9,93$; $20,81 \pm 4,19$ g/L. U krvnom serumu teladi kod svih ispitivanih grupa koncentracija IgG u 16h iznosila je pojedinačno $29,10 \pm 15,25$; $45,46 \pm 13,45$; $50,72 \pm 21,57$; $41,60 \pm 15,42$ g/L. Koncentracija IgG 30sata kod ispitivanih grupa teladi (I do IV) bila je pojedinačno $33,29 \pm 14,70$; $47,05 \pm 12,38$; $55,00 \pm 19,71$; $51,59 \pm 13,82$ g/L. Koncentracija IgG u krvnom serumu svih grupa 40h iznosila je $29,82 \pm 12,69$; $44,10 \pm 13,50$; $51,88 \pm 20,14$; $47,68 \pm 13,18$ g/L pojedinačno.

Kod teladi koja su napajana punim kolostrumom bez i sa dodatkom mineralnog adsorbenta (I i II grupa) utvrđena je statistička značajnost razlike u koncentraciji IgG u svim ispitivanim intervalima. Koncentracija IgG u krvnom serumu teladi koja su napajana kolostrumom sa dodatkom zeolita (II grupa) bila je oko 30% veća u odnosu na I grupu. Statistički značajna razlika u koncentraciji IgG utvrđena je i poređenjem I grupe sa III i IV u svim ispitivanim intervalima. Koncentracija IgG u krvnom serumu kod III grupe teladi bila je za 40% veća nego u I.

Rezultati su ukazali da kod teladi koja su napajana kolostrumom visokog kvaliteta, u koji je dodat mineralni adsorbent, dolazi do maksimalne adsorpcije IgG. Određivanje proteinemije kod teladi u neonatalnom periodu je pouzdan pokazatelj uticaja mineralnog adsorbenta na resorpciju kolostralnih IgG. Koncentracija glukoze kod svih oglednih grupa se značajno povećava sa povećanjem broja uzezih kolostruma, bez većeg uticaja mineralnog adsorbenta.