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THE EFFECT OF A CLINOPTILOLITE BASED MINERAL ADSORBER ON CONCENTRATIONS OF IMMUNOGLOBULIN G IN THE SERUM OF NEWBORN CALVES FED DIFFERENT AMOUNTS OF COLOSTRUM

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The aim of the study was to investigate the influence of added natural mineral adsorber zeolite (clinoptilolite) to the colostrum on the degree of absorption of colostral IgG in 60 Holstein newborn calves fed different amounts of colostrum during the first two days of life. Calves were divided in four groups: 1) experimental group I received 0.75L of colostrum with 5g/L of clinoptilolite in 12h intervals (0.75+); 2) experimental group II received 1.5L of colostrum with 5g/L of zeolites in 12h intervals (1.5+); 3) control group I received 0.75L of colostrum in 12h intervals (0.75-); 4) control group II received 1.5L of colostrum in 12h intervals (1.5-).

In blood sera from experimental group I, concentrations of IgG were 15.79±6.53 g/l; 21.81±9.29 g/L and 20.30±8.64 g/L respectively 6, 24 and 48 hours after birth. IgG concentrations in the sera of experimental group II were 22.21±8.79; 35.20±8.43 and 30.51±9.86 respectively 6, 24 and 48 hours after birth. In the blood sera of the first control group concentrations of IgG were 9.89±4.97; 18.07±5.32 and 15.84±4.92 respectively 6, 24 and 48 hours after birth. IgG concentrations of the second control group were 14.53±7.19; 25.39±11.57 and 22.88±8.63 respectively 6, 24 and 48 hours after birth. The statistical significance of the difference in mean IgG concentrations between experimental group I and the first control group was high after 6 ($p<0.05$) hours after birth. In this groups, the difference was not statistically significant 24 and 48 hours after birth. The statistical significance of the difference in levels of IgG between the experimental group II and the second control group was high after 6 ($p<0.001$), 24 ($p<0.01$) and 48 ($p<0.05$) hours. It can be seen that the absorption rate of colostral IgG in calves from the experimental groups I and II was approximately 40% higher than in the respective control groups.

The mean concentrations of IgG in the colostrum samples used to feed the calves were almost equal during the observed period.

Agarose-gel electrophoresis of serum proteins 48 hours after birth showed in the gamma electrophoretic zone a more intesively colored fraction for the treated animals compared to the analogous

fraction on the electrophoresogram of serum proteins in the control group.

Imunoglobulin G concentrations in the sera of calves who received 0.75 l of colostrum with clinoptilolite were almost equal to imunoglobulin G concentrations in the sera of calves who received 1.5 l of colostrum at 6 hours after birth (this effect is present at 24 and 48 hours after birth). It can be seen that calves which received an insufficient amount of colostrum with clinoptilolite had the same concentrations of serum IgG compared to the calves which received the full ration of colostrum.

The data from this investigation show that a concentration of 5 g/l of clinoptilolite based mineral adsorber in the colostrum leads to a significantly higher degree of absorption of colostral IgG in newborn calves.

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

INTRODUCTION

In animals with epitheliochorial type of placenta, such are lows, transplacental passage of immunoglobulin molecules is totally prevented. Calves are born with almost no serum Ig under physiological conditions and aquire passive immunity by absorbing immunoglobulins from the colostrum (Stott *et al*., 1979; Stott and Fellah, 1983; Weaver *et al*., 2000). The importance of colostrum for growth and health of newborn offspring is well known (Hammon and Blum, 1998; Blum and Hammon, 2000)

In bovine colostrum, the antibody (immunoglobulin) provides a major antimicrobial effect against a wide range of microbes and confers passive immunity until the calf's own immune system has matured (Korhonen *et al*., 2000). However, intestinal absorption of maternal Ig from the colostrum plays a vital role during early neonatal life. This ability to absorb whole proteins is limited to the first 24-38 hours of life. Most macromolecules are pinocytosed by intestinal epithelial cells. It has been established that enterocytes, having membrane receptors for the Fc fragment are capable of transfering intact immunoglobulin molecules. In general, permeability of the intestinal epithelium is highest immediately after birth, but 50% lower within six hours, because the intestinal cells that absorb Ig are replaced by a more mature cell population (Tizard, 1996). Absorption of all Ig classes will drop to a relatively low level after approximately 24 hours (Vukotić 1967, 1972; Kiryama *et al*., 1989; Arthington *et al*., 2000; Korhonen *et al*., 2000; Rauprich *et al*., 2000; Franklin *et al*., 2003). Numerous authors consider that 38 hours after birth the absorption of immunoglobulins from colostrum has ceased.

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 (Denise, 1989; Hopkins *et al*. 1997; Davenport *et al*., 2000). A number of authors investigated factors such as the way in which colostrum is fed to calves

(suckling their dams or nipple pail feeding) (Kruse *et al*. 1970), the time at which colostrum was first ingested (Stott *et al*. 1979), the effect of the concentration of immunoglobulins in the colostrum (Stott *et al*. 1981; Pritchett *et al*. 1991) the extent of concentration of immunoglobulins in the colostrum (Devery-Pocius and Larson, 1983) and the efficiency of absorption of Ig by the calf (Matte *et al* 1982; Besser *et al*. 1985; Morin *et al*. 1997).

Stojić *et al*. (1995; 1998), examined the effects of a clinoptilolite based adsorber added to the colostrum on the degree of absorption of colostral immunoglobulin G in newborn animals. They 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. The effects of a mineral adsorbent, based on clinoptilolite, have been widely applied in domestic animals in the last ten years (Tomašević-Čanović *et al*. 1994; Petrović *et al*. 1995; Stankov *et al*. 1992; Rajić *et al*., 1995; Stojić *et al*., (1995; 1998).

The aim of our investigation was to determine the effect of a clinoptilolite based adsorber added to colostrum on the serum concentrations of immunoglobulin G in newborn calves, fed different amounts of colostrum, during the first 48 hours of life.

MATERIAL AND METHODS

Animals.

The experiment was carried out using two experimental groups (n=30) and two control groups of newborn calves (n=30). In the experimental groups 5 g per liter of clinoptilolite was added to each colostrum sample.

Groups were treated as follows:

Groups	colostrum	clinoptilolite
Experimental group I (0.75+) (n=15)	0.75 L	5 g/L
Experimental group II (1.5+) (n=15)	1.5 L	5 g/L
Control group I (0.75–) (n=15)	0.75 L	–
Control group II (1.5–) (n=15)	1.5 L	–

Blood sampling.

Blood samples were taken from the jugular vein of calves immediately after birth, before colostrum intake, and then 6, 24 and 48 hours after birth. After spontaneous cougulation at room temperature, the serum was separated and frozen at - 20^{o}C for subsequent analysis.

Colostrum sampling.

Colostrum samples were collected from the mothers of the newborn calves. Samples of colostrum were taken immediately after birth (before colostrum intake), and at 12 hours intervals. After casein precipitation with 2% acetic acid, colostral serum was separated and stored at - 20°C.

Mineral adsorber. The examined mineral adsorber was obtained by technological preparation of the zeolitic tuff from the Zlatokop (South Serbia, SCG) deposit.

Mineral composition: the basic component is clinoptilolite with the presence of quartz and plagioclase. Clinoptilolite (Minazel –S, ITNMS, Beograd, Serbia and Montenegro) suspension was prepared according to the producer's instructions.

Methods

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

Electrophoresis. Separation of electrophoretic fractions of blood serum proteins was performed using high voltage agarose gel electrophoresis. Fractions were stained with Amido- Black.

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 AND DISCUSSION

Concentrations of serum immunoglobulin G in calves of the experimental and control groups before (0h) and after colostrum intake (6h, 24h, and 48 hours after birth) are shown in Table 1, 2.

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

	Experimental group I				Control group I			
	IgG conc (g/L)				IgG conc (g/L)			
	0h	6h a.b.	24h a.b.	48h a.b.	0h	6h a.b.	24h a.b.	48h a.b.
Mean	1.08	15.79	21.81	20.30	0.77	9.89	18.07	15.84
SD	0.94	6.53	9.29	8.64	0.79	4.97	5.32	4.92
SE	0.23	1.58	2.25	2.09	0.20	1.28	1.37	1.27
CV%	87.00	41.35	42.59	42.56	102.59	50.25	29.44	31.06

Legend: a.b. (after birth)

Table 2. Concentrations of serum immunoglobulin G in calves from the experimental group II and control group II before and after colostrum intake

	Experimental group II				Control group II			
	IgG conc (g/L)				IgG conc (g/L)			
	0h	6h a.b.	24h a.b.	48h a.b.	0h	6h a.b.	24h a.b.	48h a.b.
Mean	0.78	22.21	35.20	30.51	0.76	14.53	25.39	22.88
SD	0.71	8.79	8.43	9.86	0.81	7.19	11.57	8.63
SE	0.18	2.19	2.11	2.46	0.21	1.86	2.98	2.23
CV%	91.02	38.67	23.95	32.32	105.26	49.48	45.57	37.72

Legend: a.b. (after birth)

Data in Table 1. reveals the presence of imunoglobulin G in the blood from both experimental groups and control groups before colostrum intake. At birth IgG was detected in most precolostral newborn calves. Mean IgG concentrations in the serum of newborn calves from both groups were low 1.08±0.94 g/L; 0.78±0.71 g/L; 0.77±0.79 g/L and 0.76±0.81 g/L respectively. A large concentration of neonatal immunoglobulin G leads to a lower rate of its absorption in the gut (Vukotić and Movsesijan, 1976). Their common characteristicis was a wide variation interval of values. Our values obtained for both groups correspond to the values found by other authors (Vukotić and Stojić, 1979; Vukotić and Movsesijan, 1976; Edvards, 1982; Kiriyama *et al*. 1989). We did not find significant differences in the mean concentrations of neonatal immunoglobulin G between the groups.

By 6 hours after birth all calves had detectable levels of IgG. Mean immunoglobulin G concentrations in the serum of calves from experimental group I (0.75+ group) and control group I (0.75- group) 6 hours after birth were 15.79±6.53 g/L and 9.89 ± 4.97 g/L (Figure 1).

At the same time, IgG concentrations in the serum of the experimental group II (1,5+ group) and control group II (1.5-group) were 22.21±8.79 g/L and 14.53 ± 7.19 g/L (Figure 2).

It can be seen that the absorption rate of colostral IgG in the experimental groups I and II was approximately 40% higher than in the respective controls.

The mean immunoglobulin G concentration in the serum of calves from experimental group I and control group I 24 hours after birth were 21.81±9.29 g/L and 18.07±5.32 g/L. At the same time, in the serum of the experimental group II and control group II were 35.20±8.43 g/L and 25.39±11.57 g/L. The mean immunoglobulin G concentrations in the serum of calves from experimental group I and control group I 48 hours after birth were 20.30±8.64 g/L and 15.84±4.92 g/L respectively. Concentrations of IgG in the serum of calves from experimental group II and control group II were 30.51±9.86 g/L and 22.88±8.63 g/L, respectively.

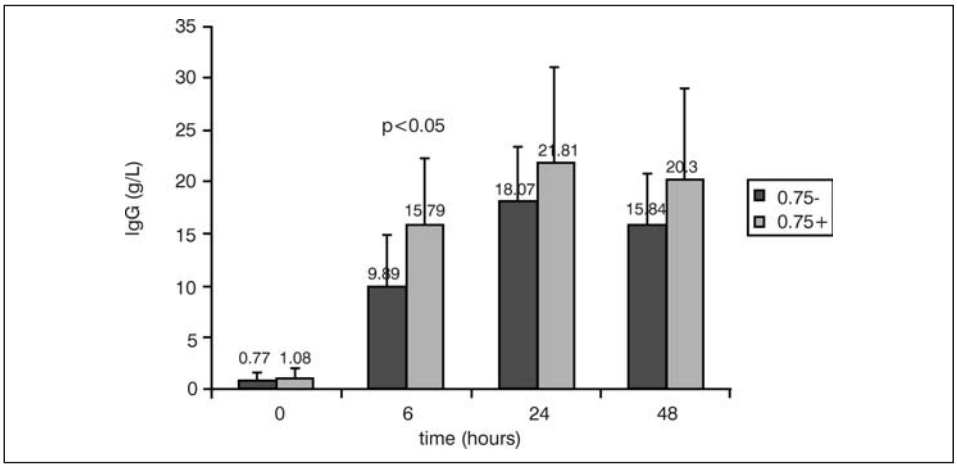

Figure 1. Concentrations of IgG in calf blood sera before and after colostrum intake experimental group I (0.75+), control group I (0.75–)

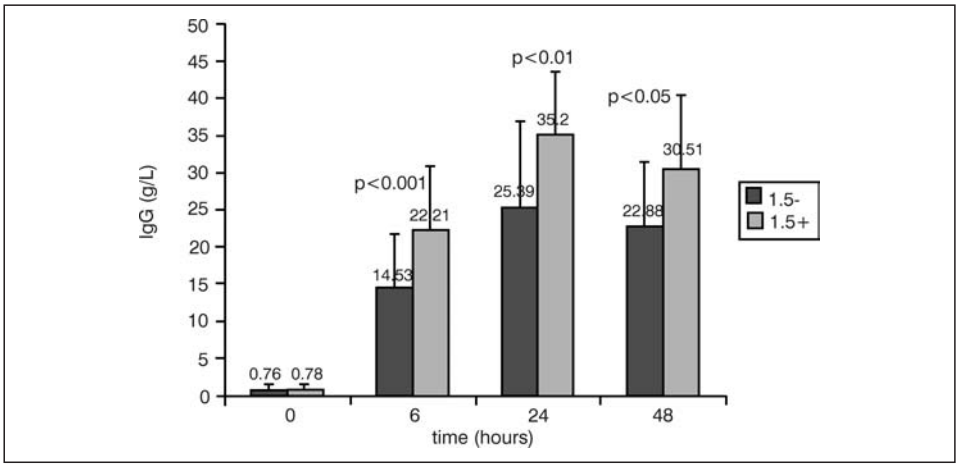

Figure 2. Concentrations of IgG in calf blood sera before and after colostrum intake experimental group II (1.5 +), control group II (1.5–)

Significantly higher concentrations of serum IgG were found in calves from the experimental group II (1.5+ group) compared to the control group II (1.5- group) 6 ($p<0.001$), 24 ($p<0.01$) and 48 ($p<0.05$) hours after birth. The difference in the mean concentration of IgG between experimental group I and control group I was higher at 6 hours after birth ($p<0.05$). Significantly higher concentrations of serum IgG were found in calves from both experimental groups.

The results clearly indicate that a clinoptilolite based mineral adsorber in the colostrum led to a significant increase of IgG concentrations in the blood of newborn calves. Our data for IgG concentrations are in accordance with previous studies by Stojić *et al*. (1995, 1998). They found that a 5 g/L clinoptilolite suspension in the colostrum leads to a significantly higher absorption of colostrum IgG in newborn calves. In those experiments calves received the full ration of colostrum in all experimental groups. An experiment carried out on piglets showed similar results (Stojić *et al*., 1998). Our values obtained for the control group, correspond to the values found by other authors (Stott *et al*.1979; Logan, 1981; Matte *et al*.1982; Korhonen *et al*. 2000).

Our results showed too, that the mean concentration of IgG between experimental group I (0.75+ group) and control group II (1.5-group) was not statistically significant. Therefore, imunoglobulin G concentrations in the sera of calves which received 0.75 L of colostrum with clinoptilolite were almost equal to imunoglobulin G concentrations in sera of calves which received 1.5 L of colostrum at 6 hours after birth (this effect being present at 24 and 48 hours after birth) (Figure 3.).

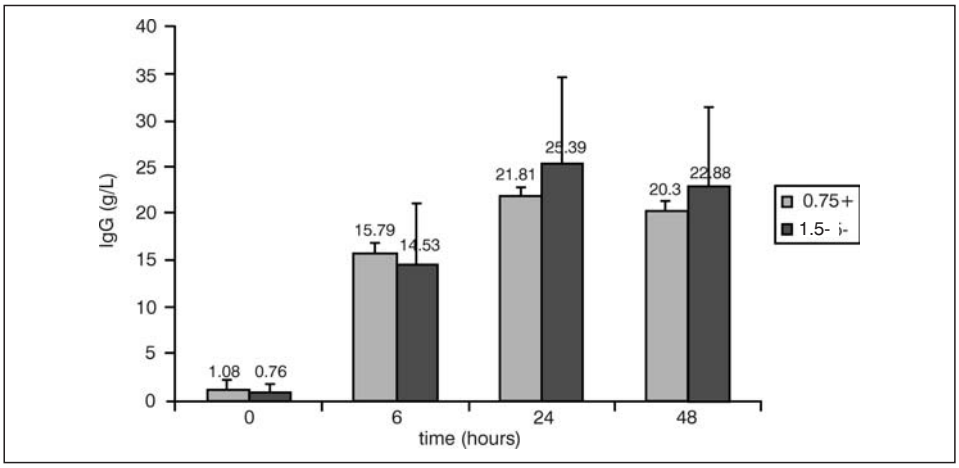

Figure 3. Concentrations of IgG in calf blood sera before and after colostrum intake experimental group I (0.75+), control group II (1.5–)

It can be seen that calves which received an insufficient amount of colostrum with clinoptilolite had the same concentrations of serum IgG compared with the calves which received the full ration of colostrum. These data additionally support the above mentioned opinion about the possiibble mode of clinoptilolite action to increase the absorption of colostral IgG. Calves absorbing high amounts of maternal antibodies are at a significantly lower risk of neonatal septicemia and death than those receiving inadequate amounts (Donovan *et al*. 1987; Weaver *et al*. 2000).

The IgG concentrations found in the colostrum in both groups (experimental and control groups) involved in our experiment are presented in table 3.

Table 3. The mean serum levels of IgG (g/L) in the colostrum from experimental and control group

Group N=15	Experimental group I	Experimental group II	Control group I	Control group II
	Colostrum g/L		Colostrum g/L	
Mean	83.11	93.70	93.91	96.75
SD	20.25	27.55	18.51	24.07
SE	4.91	6.89	4.78	6.21
CV (%)	24.36	29.40	19.71	24.87

From the data presented in Table 3. it is obvious that there was no significant difference in the mean IgG concentrations between the colostrums ingested by calves in the experimental and control groups.

We confirmed our results using agarose gel electrophoresis of the serum (Figure 4.)

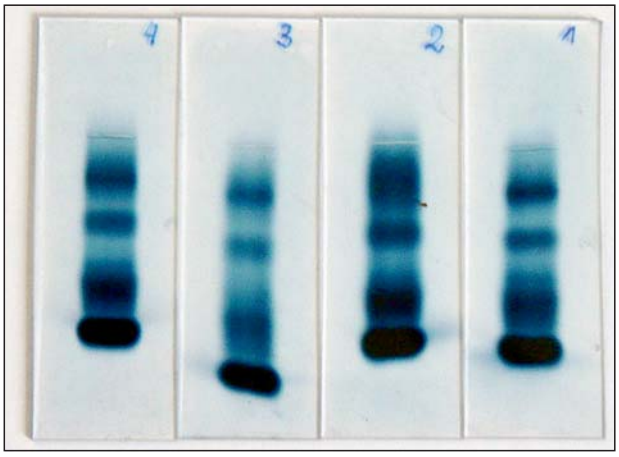

Figure 4. Elektrophoregrams, 1-blood serum proteins of experimental group I, 2- blood serum proteins of experimental group II, 3- blood serum proteins of control group I, 4- blood serum proteins of control group II

In the gamma electrophoretical zone of the serum electrophoregram of calves from the experimental groups, the analysis revealed in this protein fraction

a higher stain intensity compared to the corresponding electrophoretical zone of the control groups serum.

The results indicate that the presence of clinoptilolite in the digestive tract of newborn calves positively influences the absorption of colostral IgG in all casses.

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EFEKAT DAVANJA RAZLIČITE KOLIČINE KOLOSTRUMA NA KONCENTRACIJU IMUNOGLOBULINA G U SERUMU NOVOROĐENE TELADI NAPAJANE RAZLIČITOM KOLIČINOM KOLOSTRUMA

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SADRŽAJ

U ogledu je ispitivan uticaj mineralnog adsorbera na bazi klinoptilolita na stepen resorpcije IgG kod 60 novorođene teladi napajane različitom količinom kolostruma. Telad su bila podeljena u četiri grupe: 1) prva eksperimentalna grupa napajana je sa 0,75 L kolostruma u intervalima od 12 sati i tretirana klinoptilolitom (0,75+), 2) druga eksperimentalna grupa napajana je sa 1,5 l kolostruma u intervalima od 12 sati i tretirana klinoptilolitom (1,5+), 3) prva kontrolna grupa teladi napajana je sa 0.75 L kolostruma u intervalima od 12 sati (0,75–), 4) a druga kontrolna grupa teladi napajana je sa 1.5 L kolostruma u intervalima od 12 sati (1,5–). Uzorci krvi uzeti su odmah nakon rođenja a zatim 6., 24. i 48. sata života.

Između teladi koja su primila dvostruko manju količinu kolostruma (0,75 l) sa zeolitom i bez zeolita statistički značajna razlika u koncentraciji IgG ($p<0,05$) utvrđena je samo u 6-om satu nakon rođenja, dok u ostalim satima (24 i 48 sati) nije bilo statistički značajne razlike.

Između teladi koja su primala pun kolostrum (1,5 l) sa zeolitom i bez zeolita statistički značajna razlika u koncentraciji IgG utvrđena je u svim ispitivanim vremenima: 6-og sata ($p<0,001$), 24-og sat ($p<0,01$) i 48-og sata ($p<0,05$). Pokazalo se da je u uzorcima uzetim 6, 24 i 48 sati posle rođenja prisustvo IgG otkriveno u značajno većoj koncentraciji kod grupe teladi koja je dobijala kolostrum sa zeolitom nego bez njega.

Koncentracija IgG u serumu teladi koja su dobijala pola (0,75 L) kolostruma sa zeolitom jednaka je koncentraciji IgG u serumu teladi koja su dobijala pun (1,5 L) kolostrum bez zeolita (u 6-om satu). Taj trend je uočljiv i u ostalim satima (24 i 48 sat). Ovi rezultati jasno ukazuju da se davanjem dvostruko manje količine kolostruma sa zeolitom postiže nivo koncentracije IgG u krvnom serumu kao da je dat pun kolostrum (bez zeolita).

Dobijeni rezultati jasno ukazuju da prisustvo mineralnog adsorbera u kolostrumu koji sadrži više od 90% klinoptilolita značajno povećava stepen resorpcije kolostralnih imunoglobulina G kod novorođene teladi.