Acta Veterinarija (Beograd), Vol.45, No. 2-3, 67–74, 1995.

UDK 619:612.017.11

THE EFFECT OF A CLINOPTILOLITE BASED MINERAL ADSORBER ON COLOSTRAL IMMUNOGLOBULIN G ABSORPTION IN NEWBORN CALVES

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(Received, 24. February 1995.)

The aim of this study was to investigate the influence of the presence of added natural zeolites in colostrum on the degree of absorption of colostral IgG in newborn calves. The experimental group was fed colostrum in which 5g/L of a mineral adsorber preparation, manufactured by processing natural zeolites containing at least 90% of the zeolitic mineral active component clinoptilolite, was added.

In blood sera from the experimental group, concentrations of IgG were 20.9 ± 9.9 g/L; 46.8 ± 14.7 g/L and 44.1 ± 12.3 g/L respectively 6,24 and 48 hours after birth. In blood sera from control group concentrations of IgG were 13.8 ± 9.6 g/L; 23.4 ± 10.5 g/L and 25.2 ± 9.9 g/L respectively 6,24 and 48 hours after birth. The statistical significance of the difference in levels of IgG between the control and experimental group was high after 24 and 48 hours. Calves of the control group had a 50% higher concentration of IgG than the respective experimental group 6 hours after birth. However the difference was not statistically significant.

Mean concentrations of IgG in the colostrum samples used to feed the calves from both groups were almost equal during the observed intervals.

High voltage agarose-gel electrophoresis of serum proteins from the 48 hours after birth showed in the gamma electrophoretic zone the presence of a more intensively colored fraction for the treated animals compared with the analogous fraction on the electrophoregram of serum proteins of the control group.

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, colostral immunoglobulins, IgG immunoglobulins, newborn calves, clinoptilolite.

INTRODUCTION

It has been already demonstrated, that blood serum of newborn calves under physiological conditions does not contain immunoglobulins before colostrum intake. Passive immunity is aquired by newborn calves after colostrum has been

ingested and intact immunoglobulins are absorbed in the intestine (Bush et al. 1971. and Stott et al. 1979.

Absorption of colostral immunoglobulins occurs via pinocytosis by epithelial cells in the intestine. Intact immunoglobulin molecules first enter the lymphatic system and hence the blood system (Logan and Gibson, 1975; Logan, 1978 and Pellerin, 1982). The process of intestinal absorpltion of unaltered colostral immunoglobulin molecules does not last for long and different opinions exist concerning the time when such ab sorption is definitely terminated. Some authors found the period to last for up 8 hours (Selman et al. 1970). Penhale et al. 1970). Penhale et al. (1973) state that immunoglobulins are absorbed for up to 27 hours. Pellerin (1982) found the period to last for up 36 hours. However the majority of authors consider that 38 hours after birth absorption of immunoglobulins from colostrum is ceased.

Since colostral immunoglobulins 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 may be increased. Thus, Stott (1975) examined the effect of the environment and stress, while Cabello and Levieux (1978, 1980) studied the influence of thyroid hormones and climatic factors on the level of absorption of colostral immunoglobulins. A number of authors investigated factors such as the way in which colostrum is fed to calves (suckling their dams or nipple pail feeding), the time at which colostrum was first ingested, the effect of the concentration of immunoglobulins in the colostrum and the extent of concentration of immunoglobulins in the colostrum and the extent of absorption in the digestive system (Sott et al. 1979, Mattel et al. 1982 and Besser et al. 1985). Besides the above mentioned authors there were many more whom we have not cited, but whose results had a great impact in elucidating the problem of intestinal absorption of colostral immunoglobulins in newborn calves.

In this paper the effects of a clinoptilolite based adsorber added to colostrum on the degree of absorption of colostral immunoglobulins G in newborn calves is presented.

MATERIALS AND METHODS

Animals. The experiment was carried out on 15 (7 male and 8 female) newborn calves of a black and white spotted breed and average body weight 37.8 ± 5.3 kg at birth. The control group consisted of 11 (8 male and 3 female) calves with body weight 39.3 ± 4.8 kg.

Control intake. Each calf was fed 3 liters of respective maternal colostum via a nipple pail twice a day, the first time 2 hours after birth. In the experimental group 5g per liter of mineral adsorber was added to each colostrum sample (total 15 g).

Mineral adsorber. The examined mineral adsorber was obtained by technological preparation of the zeolitic tuff from the Zlatokop deposit.

Mineral composition: the basic component is clinoptilolite with the presence of quartz and plagioclase.

The particle size distribution determined on a Cyclisizer is presented in Table 1.

Table 1. Particle size distribution of the mineral adsorber

Particle size (m)	M(%)
> 44	1.17
< 44 > 33	2.33
< 33 > 23	5.47
< 23 > 15	7.57
< 15 > 10	6.89
< 10 > 10	76.57

The chemical composition is given in Table 2.

Table 2. Chemical composition of the mineral adsorber (%)

SiO_2	AL_2O_3	Fe_2O_3	TiO_3	MnO	CaO	MgO	Na_2O	K_2O	P_2O_5	L.I.
64.21	11.48	0.88	0.25	0.03	4.55	1.45	1.71	1.29	0.05	14.00

Unit cell composition (Obradović, 1987):

$Na_{0.59}K_{0.75}Ca_{2.14}Mg_{0.96}(Al_{6.05}Si_{29.10}O_{72})14.00H_2O$

The cation exhange capacity, determined by the ammonium acetate method is presented in Table 3.

Table 3. CEC and exchangeable cations of the mineral adsorber

Cation	K+	Na+	Ca++	Mg++	Total
CEC (meq/100g)	17.3	82.6	49.5	16.8	166.2

Blood samples. Blood samples were taken from the jugular vein of the 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 stored in a deep freeze at -200C until analyzed.

Colostral samples. Samples of colostrum were taken at the first nipple pail feeding and then 24 and 36 hours after birth. After casein precipitation with concentrated acetate, colostral serum was separated by centrifugation at 3000 RPM for 10 min. Colostral serum was stored in a deep freeze at -20^{0}C until analyzed.

Immunodifusion. Immunoglobulin G concentration in the blood sera of calves and colostral sera of cows was determined using double immunodiffusion on commercial RID plates (INEP-Zemun, Yugoslavia).

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

Statistical analysis. The significance o 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 and after colostrum intake are shown in Table 4. and Figure 1.

Table 4. Immunoglobulin G concentration in blood sera of calves from the experimental and control group before and after colostrum intake

	Experimental group (n=15)				Control group (n=11)			
	Immunoglobulin G (g/L)				Immunoglobulin G (g/L)			
	0^h	6^h	24^h	48^h	0^h	6^h	24^h	48^h
Mean	0.72	20.90	46,80	44.10	1.80	13.80	23.40	25.20
SD	1.38	9.20	14.70	12.30	5.10	9.60	10.50	9.90
SE	0.36	2.64	3.90	3.30	1.80	3.18	3.48	3.30
CV%	191.70	47.80	31.90	27.90	283.00	69.5	44.90	39.40

Data in Table 4. and Figure 1. reveal the presence of immunoglobulin G in the blood of calves from both experimental and control groups, before colostrum intake. Mean concentrations of this neonantal immunoglobulin G in sera of calves from the experimental and control groups were 0.72 ± 1.38 g/L, and 1.8 ± 5.1 g/L, respectively. There is a large number of data about the concentration of precolostral

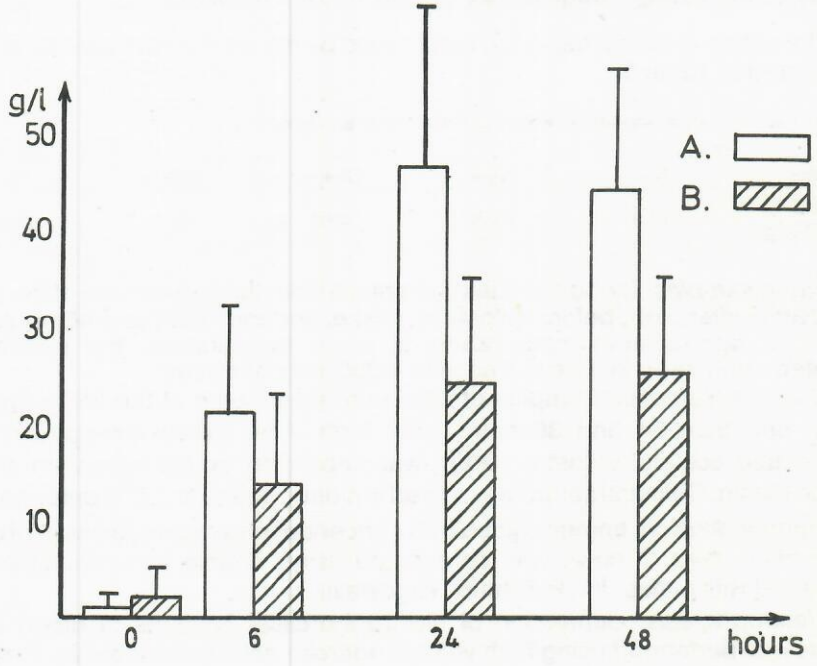

Figure 1. The immunoglobulin G concentration in calf blood sera before and after colostral intake. A-experimental group, B-control group

immunoglobulin G in the serum of newborn calves. Their common characteristic is a wide range of variation of individual values. Hiroko and Kariyama (1989) found that individual values ranged from 0.13 - 7.60 g/L; whereas according to Vukotić (1976), the range was from 0.12 -5.70 g/L. We measured the levels of precolostral immunoglobulin G in the sera of both groups of calves to determine if there were significant differences between them, being aware of the fact that a larger concentration of neonatal immunoglobulin G leads to a lower rate of its absorption in the gut (Vukotić and Movsesijan, 1976). We did not find significant differences in mean concentrations of neonatal immunoglobulin G betweeen the experimental and control groups. However, large variation parameters (SD, SE, CV%) indicate that the individual values of neonatal IgG concentration may not fallow a normal distribution of frequenceies, so that these values cannot be destribed using parameters derived from the normal distribution. This was first postulated by Vukotić and Stojić (1979) who found that, distribution took the exponential form.

Mean immunoglobulin G concentration in serum of calves from the experimental group 6 hours after birth, and 4 hours after colostrum intake was 20.9 ± 9.2 g/L. At the same time, in the serum of the control group it was 13.8 ± 9.6 g/L. It can be seen that the absorption rate of colostral IgG in the calves from the experimental group was approximately 50% higher than in the control, but the difference was not statistically significant due to high variance parameters. Significantly higher concentrations of serum IgG were found in calves from the experimental group compared to the control 24 ($p<0.001$) and 48 hours (p.001) after birth. Our values obtained for the control group correspond to the values found by other authors (Stott et al. 1975, 1979; Logan, 1978; Robinson et al. 1988). On the other hand, mean serum IgG concentrations in calves from the experimental group 24 and 48 hours after birth were much higher than those of the mentioned authors. Similar values are few in the literature.

Table 5. Mean IgG concentration in samples of colostrum used for nipple pail feeding of calves from the experimental and control group

	Experimental group			Control group		
	Colostrum g/L			Colostrum g/L		
	0^h	24^h	36^h	0^h	24^h	36^h
Mean	110.80	45.00	11.20	116.20	38.60	8.00
SD	25.10	24.60	8.20	17.50	20.30	6.90
SE	6.90	6.80	2.30	5.80	6.80	2.90
CV%	22.70	54.10	73.00	15.10	52.80	87.30

From the data presented in Table 5. it is obvious that there was no significant difference in IgG concentrations in the samples of colostrum used for nipple pail feeding of the calves, as demonstrated by statistical analysis ($p>0.05$). It is widely accepted among the authors studying the problem of colostral protection of calves that intact IgG molecules, not their fragments, are absorbed in the gut after colostrum intake. We confirmed this finding using agarose gel electrophoresis of the serum. (Figure 2.)

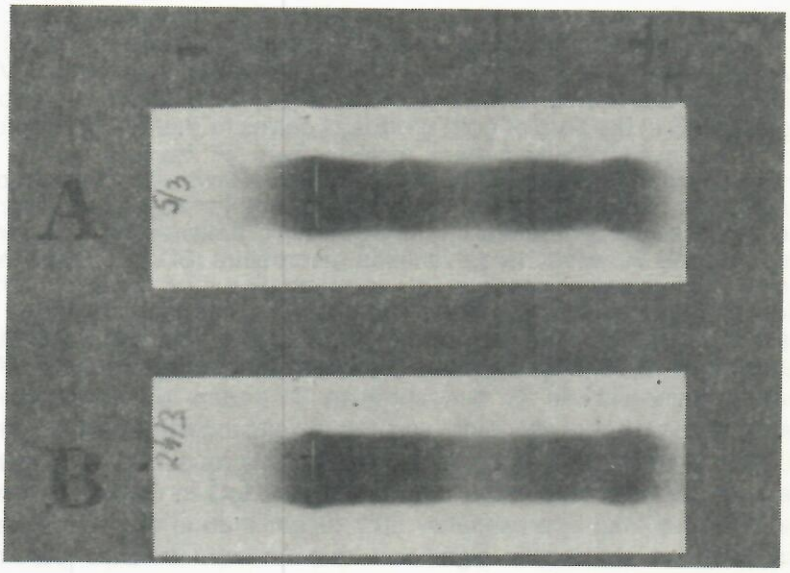

Figure 2. Electrophoregrams. A-blood serum proteins of experimental calves, B-blood serum proteins of control calves

In the gamma electrophoretical zone of the serum electrophoregram of calves from the experimental group, the analysis revealed a higher stain intensity in this protein fraction than in the corresponding electrophoretical zone of the control group serum. If the significant increase of IgG level in the serum of the experimental group was the result of increased absorption of fragments instead of whole molecules, this finding would not have taken place because of the higher electrophoretic mobility of fragments over whole IgG molecules.

The results presented in Table 1, and Figures 1. and 2. clearly indicate that the addition of mineral adsorbent to colostrum causes a significant increase of IgG absorption rate in the gut of the newborn calf.

At the present moment we do not have a satifactory explanation for this surprising phenomenon. However, since it has been shown that this mineral adsorbent efficiently binds aflatoxins B1 and G1 (Tomašević-Čanović et al. 1994), we can speculate that in contitions of high protein intake and the absence of digestive enzymes, it may bind some degradation product of colostral proteins in the gut, thus preventing their negative effect on the mucosal epithelial cells designated for immunoglobulin absorption.

Since this is a new finding of the sort, we did not hesitate to publish in the belief that it is acceptable with some certainty. Our future engagement will be directed to confirmation of the present data using a much larger number of animals,

with a simultaneous survey of the states of health of the calves in the first weeks of their lives.

Acknowledgments

This work was supported by the Serbian Fund for Scientific Research.

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Acta Veterinaria (Beograd), Vol. 45, No. 2–3, 67–74, 1995
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obulin G absorption in newborn calves

UTICAJ MINERALNOG ADSORBERA NA BAZI KLINOPTILOLITA NA RESORPCIJU KOLOSTRALNIH IMUNOGLOBULINA G KOD NOVOROĐENE TELADI

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SADRŽAJ

U ogledu na 15 novorođene teladi ispitivan je uticaj prisustva mineralnog adsorbera na bazi klinoptilolita, na stepen resorpcije imunoglobulina G kod novorođene teladi. Novorođena telad su napajana sa po 3 litra kolostruma u koji je dodato 15g zeolitskog preparata. U uzorcima krvi uzetim 6h, 24h i 48h posle rođenja otkriveno je prisustvo imunoglobulina G u značajno većoj koncentraciji nego kod kontrolne grupe teladi. Na osnovu dobijenih rezultata zaključeno je da se prisustvo mineralnog adsorbera u kolostrumu koji sadrži više od 90% klinoptilolita, značajno povećava stepen resorpcije kolostralnih imunoglobulina G kod novorođene teladi.