APPARENT EFFICIENCY OF IMMUNOGLOBULIN ABSORPTION IN NEWBORN CALVES ORALLY TREATED WITH ZEOLITE

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Sixty Holstein newborn calves, which were immediately after parturition separated from their dams and placed in individual pens, were randomly assigned to 4 treatment groups (15 calves per group). All calves were bottle-fed twice a day during the first 48 hours after partus, with their mothers' colostrum in 12 hour intervals, starting two hours after partus, according to the following model: i) group 1 (G1) received 0.75 L of colostrum (C) per meal, with 20 mL of 25% zeolite (clinoptilolite) suspension in distilled water; ii) group 2 (G2) received 1.5L of C per meal, with 20 mL of 25% zeolite suspension in distilled water; iii) group 3 (G3) received 0.75 L of C per meal, and iv) group 4 (G4) received 1.5 L of C per meal. Blood serum immunoglobulin G (IgG) concentrations at 0h (before C ingestion) 6h, 24h and 48h after partus and colostrum IgG concentrations were determined using commercial sRID plates (INEP, Zemun, Serbia). Apparent efficiency of absorption (AEA%) was calculated for individual calves at 6h after partus. Calf blood serum IgG concentrations at all investigated time intervals in G1 were increased compared to G3, with a significant increase at 6h after partus (15±4:10±5 g/L, p<0.05). The same relationship was evident between G2 and G4, with significant mean IgG blood serum differences evident at 6, 24. and 48. hour after partus. Calf blood serum IgG concentration was significantly higher in G2 compared to all experimental groups at 6, 24 and 48h after partus. Apparent efficiency of absorption (AEA%) at 6h after partus was significantly higher in G1, compared to all other experimental groups.

Key words: Immunoglobulin G, zeolite, calves, apparent efficiency of absorption.

INTRODUCTION

Immune protection after parturition in newborn mammals is one of the major survival problems. Colostrum is the only source of immunoglobulin (Ig) for newborn farm animals, since the placenta does not allow transport of Ig from maternal to foetal circulation (Quigley *et al*., 1998). Colostral Ig concentration is

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very high (up to 100 g/L), and Ig molecules are readily resorbed from the intestine of newborn animals (Stott *et al*., 1979a, 1979b, 1979c, 1983; Cabello *et al*., 1980; Kruse, 1983). In unsuckled newborn calves blood immunoglobulin G (IgG) concentration is extremely low (<0.1 g/L) (Arthington *et al*., 2000). However, measurable blood serum IgG concentrations in newborn calves can be detected before colostrum intake (Vukotić *et al*., 1976; Vukotić *et al*., 1979; Edvards *et al*, 1982). Not just calf morbidity and mortality (Besser *et al*., 1994), but also their long-term performance is directly related to low blood Ig concentrations (Wittum *et al*., 1995). Failure of passive immunity transfer (FPT) may be attributed to an inadequate amount of IgG in the colostrum, insufficient quantity of ingested colostrum and inefficient IgG absorption (Lee *et al*., 1983; Abel Francisco *et al*., 1993; Rea *et al*, 1996; Quigley *et al*, 1998). Recent investigations in Canada concerning risk factors associated with FPT revealed that 19% of 225 newborn calves from 45 herds had serum IgG concentrations less than 10 g/L (Filteau *et al*, 2003). Jaster (2005) reported that over 40% of dairy heifer calves sampled by the National Dairy Heifer Evaluation Project in the USA had serum IgG concentrations below 10 g/L, and more than 25% of calves had levels below 6.2 g/L.

Numerous attempts have been made to artificially augment the ability of calves to attain passive immune protection, such as feeding stored surplus colostrum (Foley *et al*., 1978), injecting Ig solutions (Quigley *et al*., 1996), feeding dried colostrum (Morin *et al*., 1997; Garry *et al*., 1996), concentrated milk whey (Mee *et al*., 1996), and supplementing dried bovine serum protein (Arthington *et al*., 2000). Furthermore, addition of trypsin inhibitor to maternal colostrum increased serum concentrations of IgG by 16%, and IgM by 30%, in Jersey calves, regardless of their age at the time of first feeding (0. or 12. hours after birth) (Quigley *et al*., 1995). Bovine antiserum injection at birth also can induce an increase in the apparent efficacy of absorption of colostral Ig by 42% in calves at 24. hours after treatment (Pedersen *et al*., 2000). However the antiserum injection could possibly somehow trigger the active synthesis of IgG.

Most of these attempts are based on the same approach, namely to provide some other source of Ig instead and/or additional to colostrum. Our previous studies have indicated that peroral treatment with zeolite (clinoptilolite) based mineral adsorbent could induce an increase in newborn calf blood serum IgG concentration (Stojić *et al*., 1995; Stojić *et al*., 1998; Fratrić *et al*., 2005). This novel approach has been utilized in this experiment as our objective was to determine the apparent efficiency of absorption (AEA%) in newborn calves treated with zeolite (clinoptilolite) receiving different amounts of colostrum.

MATERIALS AND METHODS

Zeolite. A sample of natural zeolitic rich tuff from the Zlatokop deposit (Vranje, southern Serbia) was used in this study. The mineralogical composition of the natural zeolitic tuff was primarily clinoptilolite/heulandite (HEU) - minimum 85%, with trace amounts of feldspar and quartz, as determined by X-ray powder diffraction (XRPD) analysis. HEU group zeolites have the same framework topology, but clinoptilolite and heulandite are differentiated by their chemical

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composition, thermal stability, and ^{1}H NMR spectra (Mumpton, 1998; Ward and McKague, 1994). Thermal stability study (Mihajlović-Radosavljević *et al*., 2003) and ^{1}H NMR analysis (Daković *et al*., 2007), showed that natural zeolitic tuff from Zlatokop deposit (Serbia) is rich in mineral clinoptilolite.

Chemical composition of the clinoptilolite rich zeolitic tuff (clinoptilolite), determined by atomic absorption spectroscopy, is given in Table 1.

Table 1. Chemical composition of the mineral adsorbent (%)

Component	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	CaO	MgO	Na_2O	K_2O	L.I.
Content, %	64.21	11.48	0.88	0.25	4.55	1.45	1.71	1.29	14.00

The predominant cation associated with clinoptilolite was calcium and the minimum cation exchange capacity (CEC) was 146 mmolM$^+$/100g (Table 2) measured by the ammonium chloride method.

Table 2. CEC and exchanging cations of the mineral adsorbent

Exchangeable cation	Ca^{++}	Mg^{++}	Na^{++}	K^+	Σ
CEC, mmolM$^+$/100g	95	13	22	16	146

Thermogravimetric analysis of the used clinoptilolite (plot is not shown) showed continuous weight loss during heating up to 800°C, due to the loss of hygroscopic water and the loss of water residing in the channels and cavities of the zeolite framework; the total water content was 13.80%. Thermal analysis was performed on a Netzsch STA 409 EP. Sample was heated (20-800°C) in air atmosphere, with a heating rate of 10°C/min.

A fine powder, <10 μm fraction of clinoptilolite, was used in the experiments. The particle size of the material was determined on a Coulter Counter. The average particle size was 4.5 μm (99.9% of the particles <10 mm and 90.0% <7 μm). For experiments, 25% suspension of clinoptilolite in distilled water was prepared.

Animals and treatments. The experiment was carried out on a total number of 60 newborn Holstein calves, both sexes, weighting 35±3 kg (mean±SD), which were immediately after parturition separated from their dams and placed in individual pens. Calves were divided in four experimental groups, with 15 calves each. All calves were bottle-feed twice/day during the first 48 hours after partus, with their mother's colostrum in 12 hour intervals, starting two hours after partus. Calves from experimental group 1 (G1) and 2 (G2) received 0.75 L and 1.5 L of colostrum per meal, respectively, with 20 mL of 25% zeolite (clinoptilolite) suspension in distilled water. Calves from group 3 (G3) and 4 (G4) received 0.75L and 1.5L of colostrum, respectively, without zeolite suspension.

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Blood serum samples. Blood samples were taken from the jugular vein of calves at 6, 24 and 48 hours after birth. After spontaneous coagulation at room temperature blood serum was separated and stored at -20°C until analyzed.

Colostral serum preparation. Colostral sera were obtained from first colostrum after fat and casein removal. Fat removal was performed by centrifugation of colostral samples at 2000 RPM for one hour, when fat was separated in the upper layer and manually removed from the samples. Casein was precipitated by homogenization of 2 mL of 2% acetic acid, added in 1 mL of fat-free casein sample, following centrifugation at 3000 RPM for 10 min. and colostral serum was removed by Pasteur pipette. Colostral serum samples were stored at -20°C until analyzed.

Immunoglobulin G analysis. Blood serum IgG and first colostrum IgG concentrations were determined using single radial immunodiffusion (sRID) plates containing monospecific antisera in buffered agarose. Reference standards were pipetted (5 μL) into the first four wells of each agarose plate, and serum and colostral whey samples were pipetted (5 μL) into the remaining wells of each agarose plate. The IgG serum samples were diluted 1:30 with saline (2.9 mL saline and 0.1 mL sera) before they were added to sRID plates. Colostral whey for IgG (1:60) was diluted with saline (5.9 mL saline and 0.1 mL colostral whey) before placement in sRID plates. The plates were left undisturbed at room temperature for 24 to 48h, and the ring diameters were read using a finescale comparator RID-meter (millimeters). The diameters were plotted on a scale with reference standards to obtain the concentrations serum and colostral IgG (g/L). Each sample was set in duplicate. Duplicate analyses of samples gave a repeatability within 5%.

Apparent efficiency of absorption (AEA%). Apparent efficiency of absorption (AEA%) was calculated for individual calves at 6h after partus, by the method of Husband *et al*., (1973): AEA = [(Peak serum Ig concentration) x (0.07 BW) (100%)] / [(Colostrum Ig concentration)(L colostrum fed)]. Values of AEA% were calculated using an average weight of 35 kg for each calf.

Statistical analysis. The results are expressed as mean values and standard deviation (SD), for each group of animals. The results were subjected to analysis of variance (ANOVA), and statistical significance of differences between mean values was calculated using LSD test, at 95% and 99% probability.

RESULTS AND DISCUSSION

The mean IgG concentrations in the first colostrum of all experimental groups varied between 82 ± 16 to 100 ± 18 g/L (G1 and G4, respectively), and are presented in Figure 1. There were no significant differences between first colostrum IgG concentrations fed to all groups of calves.

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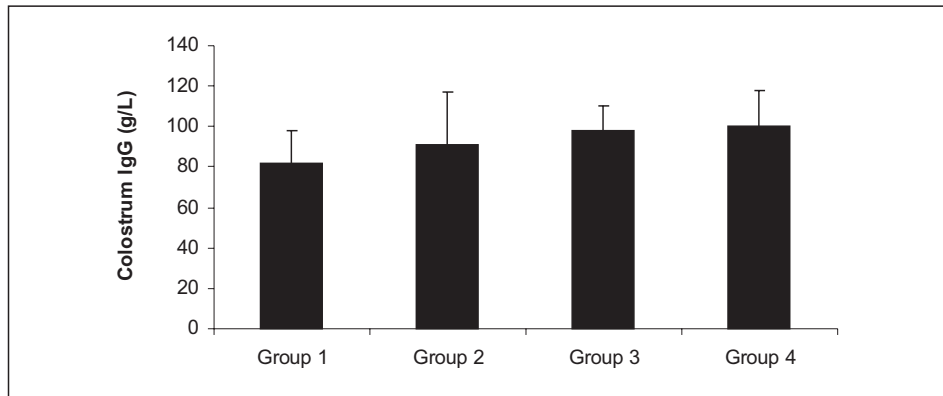absorption in newborn calves orally treated with zeolite

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Figure 1. Mean IgG concentrations (Mean±SD g/L) in the first colostrum

Blood serum IgG concentrations in all four experimental groups of calves at different time intervals and the statistical significance of differences between means in the same time interval are presented in the Table 3.

Table 3. Calf blood serum IgG concentrations ($\overline{X}$±SD; g/L) at different time intervals after partus

Experimental group[1]	0. hour*	6. hour	24. hour	48. hour
G1	1±1	15±4[b]	22±10[bc]	21±9[bc]
G2	1±1	21±8[a]	35±9[a]	30±10[a]
G3	1±1	10±5[c]	18±5[c]	16±5[b]
G4	1±1	15±7[b]	25±12[b]	23±9[c]

[abc]Means in the same column without common superscript are significantly different ($p < 0.05$);
*hours postpartum;
[1]Calves in G1 and G2 received oral clinoptilolite suspension; calves in G1 and G3 received 0.75 L of colostrum; calves in G2 and G4 received 1.5 L of colostrum.

Detectable blood serum IgG concentrations in newborn calves were evident before colostral intake, and similar results have been reported by many other researchers (Vukotić *et al*., 1976; Vukotić *et al*., 1979; Edvards *et al*., 1982). Since blood serum IgG concentrations before colostral intake had great individual variations there were no significant differences between the four experimental groups.

Calf blood serum IgG concentrations at all investigated time intervals in G1, receiving zeolite treatment with colostrum were higher compared to G3, receiving only colostrum. The differences in mean blood serum IgG concentrations between G1 and G3 were statistically significant at 6. hour postpartum

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(15±4:10±5 g/L, p<0.05). The same relationship is evident between G2 and G4, while the differences in mean blood serum IgG concentrations were statistically significant at all time intervals except at the begining of the experiment (0. hour). An increase in the amount of colostral intake from 0.75L to 1.5L induced a significant increase in calf blood serum IgG concentration at 6h, 24h and 48h, irrespective to the zeolite treatment.

Apparent efficiency of absorption (AEA%) at 6h after partus and statistical significance of differences between means for all four groups of calves are presented in Figure 2.
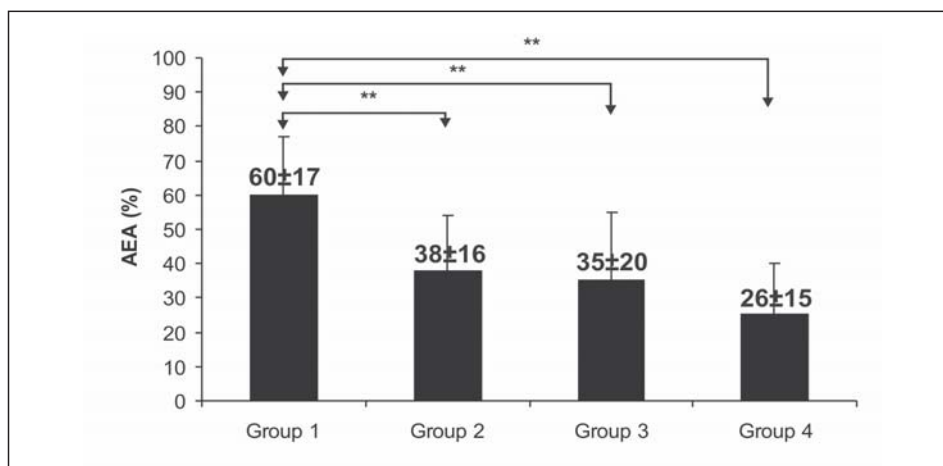

Figure 2. Apparent efficiency of absorption (X±SD, %) at 6. hours after partus (** - p<0.01)

Apparent efficiency of absorption at 6h after partus was significantly higher in G1 treated with zeolite and receiving 0.75L of colostrum, compared to all other experimental groups. Value of AEA% in G2, receiving zeolite treatment and 1.5 L of colostrum was not significantly different compared to G3 (38±16:35±20, p= 0.601), but the statistical significance of difference between AEA% means for G2 and G4 (38±16:25±15) was p=0.051.

Success of passive immunity transfer is typically assessed by measuring calves blood serum IgG concentrations at 24 to 48 hours after partus. Blood serum IgG concentration in calves may be influenced by many factors, including the age at the first feeding (Rajala *et al*., 1995), sex of the calf and body weight (Roy, 1990), amount of IgG consumed (Besser *et al*., 1985), colostrum quality (Hough *et al*., 1990) and method of colostrum feeding (Brignole *et al*., 1980). Oral zeolite treatment induced an increase in calf blood serum IgG concentration in G1 and G2 (Table 3), at 6h after partus. This effect was evident later on at 24h and 48h after partus. The treatment effect on the time scale was more evident in G1, receiving 0.75 L of colostrum than in G2, that received 1.5 L of colostrum. Besser

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et al. (1985) suggested that the prevalence of failure of passive transfer in dairy herds could be minimized by artificially feeding calves large volumes (3 to 4 L) of fresh or refrigerated colostrum within the first 24 h. Intake of colostral IgG has a marked influence on blood IgG concentrations (Hopkins *et al*., 1997), with a positive linear relationship. When less than 2L of first milking colostrum are fed the concentration of Ig in the colostrum may be insufficient to ensure the transfer of an adequate quantitiy of Ig (Quigley *et al*., 1998). However, our results are similar to the results of Jaster (2005), who reported a significant increase in serum IgG_1 concentrations at 24h in calves fed 2 L of colostrum with high IgG_1 concentrations. Using reduced amounts of colostrum (0.75 L in G1 and G3, and 1.5 L in G2 and G4), we have tried to minimize the effect of the colostrum amounts on serum Ig concentrations, which could mask an influence of zeolite treatment. Not surprisingly, the increase in the amount of ingested colostrum (from 0.75 L to 1.5 L) induced an increase in serum IgG concentrations (Table 3), but interestingly, the effect of zeolite treatment was similar to the effect of increased amount of colostrum fed.

It has been recommended that Holstein calves should be fed 1.98 L of colostrum in the first few hours of life, followed by an additional 1.89 L within 12h (Roy, 1980). The recommendation given by Besser *et al*., (1985) and Gay (1994) is 3.78 L of colostrum by oesophageal feeder immediately after birth, with the second feeding of 1.89 L 12h later on. Bovine Alliance on Management and Nutrition (1995) concluded that 2.84 L of colostrum fed soon after birth is adequate. Furthermore, if the quality of colostrum can be determined, this group recommends feeding only 1.89 L at birth. All those recommendations are aimed to the target of at least 10 g/L calf serum Ig concentration at 48h of age. Our results show that feeding 0.75 L of high quality colostrum with oral zeolite treatment could produce calf blood serum IgG concentration well above the recommended value at 6h of age. Furthermore, increasing the amount of colostral intake to 1.5 L, combined with oral zeolite, could also increase serum IgG concentration above 15 g/L at 6h of age, which is probably more desirable as a management target to reduce calf morbidity and mortality (Davis *et al*., 1998).

Apparent efficiency of absorption helps to better understanding of the nature of IgG absorption. The AEA% measures the efficiency with which Ig are absorbed, not the total Ig absorption. Mean AEA% from maternal colostrum typically averages 20 to 35% (Quigley *et al*., 1998), and may be influenced by the concentration of IgG in the colostrum (Stott *et al*., 1979c; Stott *et al*., 1983; Besser *et al*., 1985). Stott *et al*. (1983) reported a linear response, where calves fed 1 L of colostrum were more efficient in absorbing IgG than were calves fed the same mass of IgG in 2 L. Besser *et al*. (1985) reported a significant negative correlation between AEA% and mass of IgG fed to the calves. Stott *et al*. (1979c) suggested that the relationship between AEA% and IgG intake is curvilinear, and that an excessive amount of colostrum may cause inhibition of Ig absorption, particularly with increasing age. It seems that reduction in absorption efficiency leads to 2 L of colostrum as an optimum to be fed to the average large-breed calves at birth up to 16h (Stott *et al*., 1979c). The most significant increase in mean AEA% value at 6h after partus in our experiment was obtained with oral zeolite treatment in calves

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receiving 0.75 L of high quality colostrum (60%). This increase of mean AEA% value resulted in calf blood serum IgG concentration similar than IgG concentration in calves receiving 1.5 L of high quality colostrum. Doubling the amount of colostrum obviously reduced AEA% value in zeolite treated calves (from 60% in G1 to 38% in G2), the effect also being apparent in calves receiving only their mothers colostrum, but on a much smaller scale (from 35% in G3 to 26% in G4). Our results for AEA% value in G2 and G4 are similar to the results reported by Qugley *et al.*, (1998), but this is the first report we know about the possible modification of AEA% in newborn calves. It seems that oral clinoptilolite treatment could modify the process of IgG absorption in two ways: 1) increasing AEA%, and 2) making it's dynamics similar to the situation in which calves were fed with more than 2 L of high quality colostrum. In his recent extensive rewiew Papaioannou *et al*., (2005) summarized proposed mechanisms involved in animals performance promoting properties of the dietary use of natural and synthetic zeolites as feed additives. Among those mechanisms two could have impact on the AEA% value in newborn calves: 1) ammonia binding effect, which eliminates toxic effects of $NH4^+$ produced by intestinal microbial activity, and 2) slowing the passage rate of digesta trough the intestine. Regarding the first mechanism of ammonia binding it could eliminate negative effects of microbial destruction of proteins which are abundant in colostrum, and could be further increased thanks to the lower rate of proteolytic enzyme activity in newborn calves intestine (Hardy, 1969; James *et al*., 1978). Reduction of ammonia toxic effects could also contribute to the increased survival rate and pinocytotic activity of early postnatal enterocyte population that has the unique ability to absorb intact macromolecules. The uptake of macromolecules by the intestinal epithelial cells could be non-specific and receptor mediated endocytosis. Selective immunoglobulin absorption in rats involves binding of the molecules to the Fc recetors of the microvillous membrane of the epithelial cells in the jejunal region, and non-specific absorption occurs in the ileal region of the small intestine (Weaver *et al*., 1989). It seems that IgG absorption in calves is meadiated mostly by non-specific mechanisms, since radiolabeld γ-globuline uptake is greatest in the caudal parts of the ileum (James *et al*., 1978). However, both mechanisms suggested by Papaioannou *et al*, (2005) could be responsible for the increase of AEA%, but the exact explanation of zeolite effect on efficiency of IgG absorption in newborn calves still remains to be elucidated.

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RELATIVNA EFIKASNOST RESORPCIJE IMUNOGLOBULINA KOD NOVOROĐENE TELADI ORALNO TRETIRANE ZEOLITOM

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SADRŽAJ

Šezdeset novorođenih teladi Holštajn rase, koja su neposredno nakon rođenja odvojena od majki i stavljena u individualne bokseve, su metodom slučajnog izbora podeljene u 4 ogledne grupe (15 teladi po grupi). Sva telad su za vreme prvih 48 sati života hranjena dva puta dnevno (u intervalu od 12 sati) kolostrumom majki, počevši od drugog sata nakon partusa prema sledećem eksperimentalnom modelu: i) grupa 1 (G1) dobijala je 0.75 L kolostruma (C) po obroku, sa dodatkom 20 mL suspenzije 25% zeolita (klinoptilolita) u destilovanoj vodi; ii) grupa 2 (G2) dobijala je 1.5 L C po obroku, sa dodatkom 20 mL suspenzije 25% zeolita (klinoptilolita) u destilovanoj vodi; iii) grupa 3 (G3) dobijala je 0.75L C po obroku; iv) grupa 4 (G4) dobijala je 1.5 L C po obroku. Koncentracija imunoglobulina G (IgG) određivana je komercijalnim radijalnim imunodifuzionim testom (sRID, INEP, Zemin) u krvnom serumu dobijenom iz uzoraka krvi uzetih punkcijom *v. jugularis* pre uzimanja kolosruma (0. sat), 6-og, 24-og i 48-og sata nakon rođenja, dok je relativna efikasnost apsorpcije imunoglobulina (AEA%) određena 6-og sata po rođenju. Koncentracija IgG u krvnom serumu kod teladi iz grupe G1 u odnosu na G2 bila je viša 6-og, 24-og i 48-og sata, dok je statistička značajnost razlika srednjih vrednosti ustanovljena 6-og sata po rođenju ($15\pm4:10\pm5$ g/L, $p<0.05$). Isti odnos srednjih vrednosti koncentracije IgG u krvnom serumu novorođenih teladi je zabeležen između grupe G2 i G4, gde su statistički značajno više srednje vrednosti utvrđene kod grupe G2 u odnosu na G4 u periodu 6, 24. i 48. sati po rođenju ($p<0.01$ za sve navedene vremenske periode). Relativna efikasnost apsorpcije 6-og sata po rođenju je bila statistički vrlo značajno viša ($p<0.01$) kod grupe G1 u odnosu na ostale ogledne grupe teladi.