EFICIENCY OF IMMUNOGLOBULIN ABSORPTION IN NEWBORN CALVES RECEIVING ORAL CLINOPTILOLITE TREATMENT

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Summary

Experiment was carried out on total number of 30 newborn calves, which were immediately after parturition separated from their dams and placed in individual pens. Calves were divided in two experimental groups, with 15 calves each. All calves were bottle-feed two times/day with their mothers colostrum in 12 hours interval during first 48 hours after partus, starting two hours after partus. One group served as a control while second group received 20 mL of oral clinoptilolite suspension per meal during first 48h. Blood samples were taken from the jugular vein of calves at 6h, 24h. and 48h after birth. Colostral sera was obtained from first colostrum after fat and casein removal. Blood serum and first colostral serum immunoglobulin G concentration was determined using single radioimmunodiffusion method (sRID). Apparent efficacy of absorption (AEA%) was calculated for individual calves at 6h after partus, by the method of Husband et al., (1973). The results were subjected to analysis of variance ANOVA with two factors (clinoptilolite x time) and 15 replicates (2x3x15 model). Statistical significance of differences between mean values were calculated using LSD test, at 95% and 99% probability.

Calf blood serum IgG concentration was increased in clinoptilolite treated group of calves at periods of investigation. Significant increase in the AEA value at 6h after partus was also evident in the clinoptilolite-treated compared to the control group of calves. Negative correlation between value of AEA and mass of IgG in the colostrum fed was evident in both experimental groups of calves.

Key words: newborn calves, IgG, AEA

Colostrum is the only source of immunoglobulin (Ig) for newborn farm animals, since placenta does not allow transport of Ig from maternal to the foetal circulation (Quigley et al., 1998). Colostral Ig concentration is very high (up to the 100 g/L), and Ig molecules are readily resorbed from the intestine of newborn animals (Stott et al., 1979a, 1979b, 1979c, 1983). In the unsuckled newborn calves blood immunoglobulin G (IgG) concentration is extremely low (<0.1 g/L) (Arthington et al., 2000). Not just calf morbidity and mortality (Besser et al., 1994), but also their long-term performance is directly related to the low blood Ig concentrations (Wittum et al., 1995). Failure of passive immunity transfer (FPT) may be attributed to inadequate amount of IgG in colostrum, insufficient quantity if ingested colostrum and inefficient IgG absorption (Lee et al., 1983; Abel et al., 1993; Rea et al, 1996; Quigley et al, 1998). Recent investigation in Canada
concerning risk factors associated with FPT revealed that 19% of 225 newborn calves from 45 herds had serum IgG concentration 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 (Foley et al., 1978; Quigley et al., 1996; Morin et al., 1997; Garry et al., 1996; Arthington et al., 2000). Most of those attempts were aimed to provide some other source of Ig instead/additional to the colostrum. Our previous research have indicated that peroral treatment with clinoptilolite based mineral adsorbent could induce an increase in newborn calf blood serum IgG concentration (Fratrić et al., 2005). This novel approach has been utilized in the experiment were our objective was to determine apparent efficiency of absorption (AEA%) in newborn calves treated with clinoptilolite receiving different amounts of colostrum.

Materials and methods

Mineral adsorbent. Clinoptilolite (Minazel-S, ITNMS, Belgrade, Serbia) suspension was prepared in accordance with the producer's instructions. The chemical composition of Minazel-S is mostly SiO$_2$ (66.46%) and Al$_2$O$_3$ (11.77%).

Animals and treatments. The experiment was carried out on total number of 30 newborn calves, which were immediately after parturition separated from their dams and placed in individual pens. Calves were divided in two experimental groups, with 15 calves each. All calves were bottle-feed two times/day during first 48 hours after partus, with their mothers colostrum in 12 hours interval, starting two hours after partus, according to the model presented in table 1.

Table 1. Model of an experiment in newborn calves

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>N</th>
<th>Colostrum intake (L) per meal</th>
<th>Clinoptilolite* intake per meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>15</td>
<td>1.5</td>
<td>20 mL</td>
</tr>
<tr>
<td>Group 2</td>
<td>15</td>
<td>1.5</td>
<td>-</td>
</tr>
</tbody>
</table>

Legend - *25% suspension in distilled water

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 the room temperature blood serum was separated and stored in a deep freeze 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 samples. Casein was precipitated by the 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 removal by aspiration. Colostral serum samples were stored in a deep freeze at –20°C until analyzed.

**Immunoglobulin G analysis.** The quantity of immunoglobulin G, serum and colostrum samples were placed in single radial immunodiffusion (sRID) plates containing monospecific antisera in buffered agarose. Reference standards were pipetted (5µl) into the first four wells of each agarose plate, and serum and colostral whey samples were pipetted (5µl) into the remaining wells of each agarose plate. Diluted serum samples were used for IgG. 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) were 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 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 plated in duplicate. Duplicate analyses of samples gave a repeatability within 5%.

**Apparent efficiency of absorption (AEA%).** Apparent efficacy 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)(0.07BW)(100%)]/[(Colostrum Ig concentration)(L colostrum fed)]. Values of AEA% were calculated using an estimated average weight of 35 kg for each calf. This assumption reduces the accuracy of the AEA% estimate for each individual and tends to reduce the changes of finding a significant relationship between AEA% and colostrum Ig mass fed.

**Statistical analysis.** The results are expressed as mean values (X) and standard error (SE), for each group of animals. The results were subjected to analysis of variance (ANOVA) with three factors (clinoptilolite x colostrum x time) and 15 replicates (2x2x3x15 model). Statistical significance of differences between mean values was calculated using LSD test, at 95% and 99% probability.

**Results**

**Colostral immunoglobulin G.** Colostral immunoglobulin G concentration of in individual colostrum fed to the calves varied from 54.8 to 149.3 g/L. The mean IgG concentrations in first colostrum for all experimental groups are presented in figure 1.
Colostral serum immunoglobulin analysis confirmed that there were no significant differences between mean IgG concentrations from colostrum fed to all four groups of calves.

**Calf blood serum IgG concentration.** Blood serum IgG concentrations in all four experimental groups of calves at different time intervals and statistical significance of differences between means in the same time interval are presented in the Table 2.

**Table 2. Calf blood serum IgG concentrations (X±SE; g/L) at different time intervals after partus**

<table>
<thead>
<tr>
<th>Experimental group&lt;sup&gt;1&lt;/sup&gt;</th>
<th>6. hour&lt;sup&gt;*&lt;/sup&gt;</th>
<th>24. hour</th>
<th>48. hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>22±2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35±2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31±2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G2</td>
<td>15±2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25±3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23±2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>**</sup>Means in the same column without common superscript are significantly different (p<0.05)
<sup>*</sup>hours postpartum
<sup>1</sup>Calves in G1 received oral clinoptilolite suspension

Calf blood serum IgG concentrations at all investigated time intervals in group G1, receiving clinoptilolite are increased compared to the group G2, receiving only colostrum.
Apparent efficiency of absorption (AEA%). Apparent efficiency of absorption (AEA%) at 6h after partus and statistical significance of differences between means for all groups of calves are presented in figure 2.

![Figure 2](image)

**Figure 2.** Apparent efficiency of absorption (X±SE, %) at 6 hours after partus for all groups of calves in experiment and statistical significance (** p<0.01).

Apparent efficiency of absorption at 6h after partum was significantly higher in the G1 treated with clinoptilolite and receiving .15L of colostrum, compared to the group of calves receiving only colostrum. After segregation of calves on the basis of colostral IgG concentration we have determined correlation between value of AEA% and mass of IgG in the colost rum fed. A correlation between value of AEA% and mass of IgG in the colost rum fed for G1 and G2 of calves is presented figures 3. and 4, respectively.

**Group 1**

![Figure 3](image)

**Figure 3.** A correlation between between value of AEA% and mass of IgG in the colostrum fed for G1 of calves.
Negative correlation between value of AEA% and mass of IgG in the colostrum fed was evident in both experimental groups of calves.

**Discussions**

Success of the 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 BW (Roy, 1990), amount of the IgG consumed (Besser et al., 1985), colostral quality (Hough et al., 1990), method of colostrum feeding (Brignole et al., 1980). Oral clinoptilolite treatment induced an increase in calf blood serum IgG concentration in group G1 at 6h after partus, the effect being evident also at 24h and 48h after partus. Intake of colostral IgG has a marked influence on blood IgG concentrations (Hopkins et al., 1997), with linear positive relationship. However, Jaster (2005) reported similar serum IgG concentrations at 12h after 2L or 4L of high IgG1 colostrum fed to the Jersey calves, but a significant increase in the serum IgG1 concentrations at 24h in calves fed 2 L of colostrum with high IgG1 concentrations. It has been recommended that Holstein calves should be fed 1.98L of colostrum in the first few hours of life, followed by an additional 1.89L within 12h (Roy, 1980). Recommendation given by Besser et al., (1985) and Gay (1994) is 3.78L of colostrum by oesophageal feeder immediately after birth, with the second feeding of 1.89L 12h latter. Bovine Alliance on Management and Nutrition (1995) concluded that 2.84L of colostrum feed soon after birth is adequate. Furthermore, if quality of colostrum can be determined, this group recommends feeding only 1.89L at birth. All those recommendations are aimed to the management target of at least
10 g/L calf serum Ig concentration at 48h of age. Our results show that feeding 1.5L of high quality colostrum with oral clinoptilolite treatment could produce calf blood serum IgG concentration well above recommended value at 6h of age. 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; Basser et al., 1985). Stott et al. (1983) reported a linear response, where calves fed 1L of colostrum were more efficient in absorbing IgG than were calves fed the same mass of IgG in 2L. Basser et al. (1985) reported a significant negative correlation between AEA% and mass of IgG fed to the calves. Stott et al., (1979c) suggested that relationship between AEA% and IgG intake is curvilinear, and that excessive amount of colostrum may cause inhibition of Ig absorption, particularly with increasing age. Our results indicate that oral clinoptilolite treatment could induce an increase in AEA% value at 6h after partus, and there was strong negative correlation between AEA% and mass of IgG in the colostrum fed in both experimental groups of calves.

Acknowledgements

The Ministry of Science and Technology, Republic of Serbia, Project Grant No. 101816 supported this research.

References


