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# Effects of clinoptilolite addition to colostrum on the concentration of serum proteins, minerals, enzyme activities in neonatal calves

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## ABSTRACT

The objective of our research was to determine the effects of colostrum supplementation with clinoptilolite on concentrations of serum proteins, minerals and enzyme activities in newborn calves. Twenty newborn calves that were divided into control group (n=10) which received colostrum and experimental group (n=10) that received colostrum with 0.5% clinoptilolite added in the first three colostrum meals were studied. Blood samples were collected from jugular vein in vacutainer tubes from all calves prior to colostrum intake and after that, at 24 and 48 h after birth. Samples were analyzed for total protein, albumin, α1-globulin, α2-globulin, ß-globulin, γ-globulin, calcium, phosphorus, magnesium, iron, gamma glutamyltransferase, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Clinoptilolite supplementation had significant effect ($p<0.0006$) on serum iron concentrations in experimental (27.64±3.78 µmol/l) vs control group (8.93±1.26 µmol/l) after 48h. GGT values were also significantly higher ($p<0.04$) in E (163.60±26.67 U/l) than in C group (84.01±19.77 U/l) at 48h after parturition. Other parameters analyzed were unaffected by clinoptilolite treatment. Obtained data revealed that colostrum supplemented with clinoptilolite had positive effects on some mineral parameters.

## Indexing terms/Keywords

Clinoptilolite; Calves; Enzyme activities; Minerals; Serum proteins

## Academic Discipline And Sub-Disciplines

Veterinary Medicine, Biochemistry

## SUBJECT CLASSIFICATION

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## TYPE (METHOD/APPROACH)

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## INTRODUCTION

Colostral period represents an important moment for the newborns; especially in ruminants. In these species acquiring passive immunity is achieved exclusively through ingestion and absorption of adequate amounts of colostral immunoglobulins (Ig)[21]. Starting a good protection against neonatal diseases depends on how this period is managed. Due to many factors that affect the level of colostrum immunoglobulins absorbed, the incidence of passive immunity failure is still high in young ruminants, clinical seen in high incidence of neonatal morbidity and mortality, with reducing the average daily gain and increased frequencies of treatments and economic costs [2, 6, 21].

One natural method for reducing the failure passive transfer (FPT) by improving the absorption of Ig can be done with clinoptilolite, a natural zeolite [3, 4, 19]. Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, having three-dimensional structures capable of trapping molecules of proper dimensions for further release or elimination [10, 11]. Biological effects of zeolites make use of one or more of their properties, such as ion exchange capacity, adsorption and related molecular sieve [10, 11]. Due to their unique properties, zeolites are used in a wide range of biological processes where they have beneficial effects both in veterinary and human medicine [10, 11, 12]. Enhancement of passive immunity in newborn calves leads to other positive effects like reducing the incidence of diarrhea and higher average weight [17, 18].

Effects of zeolites in animal nutrition could be influenced by different length of administration, different type, particles size and purity of the zeolitic material and by different physiological processes involved in digestion of feed in young and adult animals [9, 12].

Literature data regarding positive effects of zeolite supplimentation on passive immunity [3, 4, 17] and on biochemical parameters [9, 18, 19] in newborns calves exist, with zeolites used in different doses and for different time interval, but not in higher quantity of colostrum. From this reason our objective was to determine the effects of short term clinoptilolite added in colostrum (3 l) in Holstein calves on the level of resorption of colostrum immunoglobulin, values of some minerals and enzymatic parameters.

## MATERIALS AND METHODS

The study was carried out on 20 newborn Holstein calves in a farm with 540 dairy cows, in Western part of Romania. All procedures used in animal experiments were in compliance with local ethics committee. Calves were managed according to the farm procedures. Briefly, calves were separated from the dams within 10-20 min after parturition in individual pens, without nursing. They were fed nipple bottle 2 h after birth with 3 l of mother's colostrum, at 12 h interval, during the first 5 days postpartum. After colostrum period, calves received herd milk. From first week of life, calves were offered high quality starter and water ad libitum. The individual pens were cleaned daily. For experimental design, the calves were assigned in two groups based on the birth order. Control (C) group (n=10) were fed nipple bottle 2 h after birth with colostrum and experimental (E) group (n=10) with colostrum containing 0.5% of clinoptilolite, in the first 3 meals. The blood samples were collected from jugular vein in vacutainer tubes prior to colostrum intake, at 24 and 48 h after giving first colostrum. Serum was obtained after centrifugation the samples at 3000 rpm for 5 minutes and stored at -20$^0$C until analysis.

Colostrum IgG were analyzed by radial immunodifussion method (INEP ZEMUN, Belgrad, Serbia) and concentrations of fat, protein, lactose, solid non-fat extract were determinated with automatic milkanalyser (LactoScan, Delta Intrumentes).

The commercial product used in experiment was *Min-a-Zel S*, from PATENTKOMERC, Belgrad, Serbia. The composition of clinoptilolite was 64.21% $SiO_2$; 11.48% $Al_2O_3$; 0.88% $Fe_2O_3$; 0.25% $TiO_2$; 0.03% MnO; 4.55% CaO; 1.45% MgO; 1.71% $Na_2O$; 1.29% $K_2O$ and 14.00% L.O.I. (loss on ignition). The chemical analysis of the product was determined at Institute for the Application of Nuclear Energy (ITNMS), Serbia.

The concentrations of total protein (TP), calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe) and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT) and alkaline phosphatase (ALP) in serum samples were determinated with commercially available kits (Hospitex Diagnostics, Italy) using biochemical automatically analyzer (EOS Bravo Forte, Hospitex, Italy). HD calibrator serum, HD normal serum and QC serum (Hospitex, Italy) was used for controlling measurement accuracy. The automated system was regularly monitored for accuracy and precision in accordance with "Good laboratory practice" guidelines.

Protein fraction (albumin, α1-globulin, α2-globulin, ß-globulin and γ-globulin) were analyzed by electrophoresis on cellulose acetate with kits for determining serum protein (Interlab, Italy) with Genio electrophoresis device.

Serum concentrations of protein fraction (g/l) were obtained according to relation:

$$\text{Concentration of protein fraction (g/l)} = \frac{\text{total protein} \cdot \text{protein fraction}}{100}$$

Albumin/globulins ratio (A/G) was obtain according to relation:

$$\text{A/G ratio} = \frac{\text{albumin concentration}}{\sum \text{globulin concentrations}}$$

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The apparent efficiency of IgG absorption (AEA) was calculated according to the next equation for individual calves at 24 hours after parturition, where plasmatic volume was expressed as 9.1% of body weight (16):

$$AEA\ (\%) = \frac{\text{serum IgG concentration X plasmatic volume}}{\text{IgG consumed}} \cdot 100$$

Values are expressed as means with standard error. One way analysis of variance (ANOVA) was used to test for statistically differences ($p<0.05$) between the groups of calves and inside the group.

## RESULTS

The mean values of total protein, globulin fractions, mineral and enzymatic parameters in blood serum of calves at parturition and after birth at different intervals are shown in Table 1 and 2.

**Table 1. Total protein and protein fractions concentrations in blood serum of calves at different time interval**

| Parameters | Group | At parturition | 24h | 48h |
|---|---|---|---|---|
| Total protein (g/l) | C | 46.84±1.72 | 67.97±5.11* | 71.21±5.02* |
| | E | 50.81±1.23 | 72.07±5.59* | 70.65±4.35* |
| Albumin (g/l) | C | 34.55±1.45 | 37.27±2.45 | 36.72±2.28 |
| | E | 35.70±1.76 | 40.68±1.67* | 39.39±2.16 |
| α1-globulin (g/l) | C | 4.60±1.76 | 6.49±1.25 | 6.76±1.74 |
| | E | 4.79±1.16 | 4.91±1.13 | 5.71±1.14 |
| α2-globulin (g/l) | C | 2.35±0.69 | 5.87±1.32 | 6.06±1.49 |
| | E | 4.83±1.45 | 8.12±2.20* | 7.01±1.47* |
| ß-globulin (g/l) | C | 4.14±0.68 | 5.68±0.64 | 6.57±1.16 |
| | E | 4.40±0.55 | 5.36±0.51 | 6.12±0.49* |
| γ -globulin (g/l) | C | 1.21±0.26 | 12.79±1.78* | 15.44±1.85* |
| | E | 1.07±0.30 | 13.00±2.28* | 12.42±1.37* |
| A/G | C | 3.19±0.41 | 1.26±0.10* | 1.20±0.18* |
| | E | 2.66±0.34 | 1.53±0.22* | 1.39±0.14* |

Significant differences ($p<0.05$) inside the group: *vs parturition

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**Table 2. The concentration of minerals and enzymatic activites in blood serum of calves**

| Parameters | Group | At parturition | 24h | 48h |
|---|---|---|---|---|
| Ca (mmol/l) | C | 3.38±0.26 | 3.40±0.08 | 3.50±0.13 |
| | E | 4.13±0.17$^{a}$ | 3.92±0.17$^{b}$ | 4.19±0.13$^{c}$ |
| P (mmol/l) | C | 2.44±0.52 | 2.61±0.29 | 3.32±0.54 |
| | E | 2.65±0.48 | 3.02±0.53 | 3.32±0.62 |
| Mg (mmol/l) | C | 1.34±0.08 | 1.33±0.08 | 1.38±0.14 |
| | E | 1.48±0.07 | 1.42±0.07 | 1.34±0.05 |
| Fe (µmol/l) | C | 13.91±3.16 | 14.24±2.24 | 8.93±1.26$^{**}$ |
| | E | 12.55±2.48 | 20.03±3.12 | 27.64±3.78$^{*c}$ |
| GGT (U/l) | C | 10.95±3.93 | 82.91±10.46$^{*}$ | 84.01±19.77$^{*}$ |
| | E | 12.84±4.94 | 126.57±37.78$^{*}$ | 163.60±26.67$^{*c}$ |
| ALP (U/l) | C | 463.70±79.78 | 789.82±109.10$^{*}$ | 642.87±119.47 |
| | E | 473.69±63.89 | 729.96±90.25$^{*}$ | 692.30±67.74$^{*}$ |
| AST (U/l) | C | 42.40±14.22 | 83.53±13.32$^{*}$ | 74.01±9.44 |
| | E | 55.85±10.63 | 98.97±7.58$^{*}$ | 88.90±26.10 |
| ALT (U/l) | C | 5.67±1.41 | 13.66±4.19 | 8.65±2.63 |
| | E | 5.04±0.91 | 7.11±1.88 | 8.42±2.39 |

Significant differences (p<0.05) inside the group: $^{*}$vs parturition; $^{**}$vs 24h
Significant differences (p<0.05) between the groups: $^{a}$vs parturition; $^{b}$vs 24h; $^{c}$vs 48h

Group had significant effect on the values of GGT, Ca and Fe (Table 2). These values were significantly higher in group E than C group (p<0.04 for GGT; p<0.002 for Ca and p<0.0006 for Fe) at 48h postpartum. GGT level was significantly increased in group E at 48 hours (E/C: +94.73%, p<0.04). Inside the groups from parturition till 48h postpartum GGT increased significantly both in control group and in experimental group: 7.67 times in group C (p<0.009) and 12.74 times in group E (p<0.008). The difference between groups regarding Ca values was not relevant for discussion because a significant difference between groups was also found at parturition (p<0.02). The differences between groups for ALP were: at parturition E/M: +2.15%, p<0.92; at 24h E/M: -7.57%, p<0.67; at 48 hours E/M: +7.68%, p<0.71. At 48 h postpartum albumin/globulin ratio (A/G) decreased in both group with 60.50% (p<0.0003) in C group and with 42.48% (p<0.01) in group E, with no differences between groups.

The chemical composition of colostrum administrated to calves from both groups was similar for all parameters: (C group: 64.78±1.45g/l IgG; 8.46±1.17% fat; 17.94±1.14% protein; 2.23±0.19% lactose and 28.65±2.15% solid non-fat; E group: 62.55±3.86g/l IgG; 7.52±1.12% fat; 17.49±1.46% protein; 2.31±0.12% lactose and 27.34±2.06% solid non-fat).

## DISCUSSION

Different studies on calves fed with colostrum supplemented with clinoptilolite gave positive results on Ig levels and on reducing diarrhea. According to Fratrić et al. [3] the IgG concentration during the first 48 h of life in newborn calves that received 5g/l clinoptilolite in 0.75l/1.5l colostrum at 12 h intervals was up to 40% higher than in control groups. In stead Sadeghi et al. [17] did not achieve a significant increase in passive immunity to calves that received clinoptilolite at various concentrations for 45 days (0.5/1/1.5/2g clinoptilolite/kg body weight), but clinically they reported fewer cases of diarrhea in the group that received clinoptilolite (1g/kg body weight). In our study clinoptilolite supplementation had no significant effect on γ globulin fraction, but increased significantly in both groups after colostrum administration, being more than 10 g/l at 24 h; the level that is generally considered to be adequate for succesful transfer of passive immunity [21]. A reason of these results could be the high quantity of colostrum administrated. It is known that Ig absorption efficiency is inversely proportional to the amount of Ig in colostrum, suggesting that there is a peak saturation of the transport mechanism for macromolecules in the intestine [16]. Apparent efficiency of absorption (AEA), the efficiency with which Ig are absorbed, was calculated for understanding the dynamics of Ig absorption. The AEA in calves from both groups at 24h was low (24.02±3.61% in C group and 25.89±3.78 % in E group), this could be due to high quantity of colostrum fed that may not improve absorption.

Albumin, α1, α2 globulin and ß globulin fractions recorded variations with no significant differences between groups. Different from our results, Mohri et al. (2007) observed increased serum albumin levels following supplementation with 2% clinoptilolite, probably due to length of administration (14 d)[8].

The effects of zeolites on serum minerals in dairy cows, sows/gilts, mice have been studied [7, 13, 20]. In agreement with the results of Mohri et al. [9] and different from Step et al. [19], our study suggested significant increase of iron level at 48h ($p<0.0006$) in calves that received 0.5% clinoptilolite in colostrum. A possible explanation could be that in duodenum and in anterior part of jejunum, where iron absorption take place, clinoptilolite influence iron absorption due to ion exchange properties; altering in this way the pH or by reducing intestinal transit of digesta, that could lead to a better utilization of nutrients [7, 12, 15]. It is known that low intestinal motility and acid pH promotes iron absorption and that in the bovine neonate the pH of the whole intestinal content ranges from 5.5 to 6.5, also the motility of the gastrointestinal tract become well organized only after first 2-3 days of postnatal life [5, 15]. This benefice could be important in preventing iron deficiency anemia (Fe<14.32 µmol/l) especially in veal calves fed exclusively with milk [15].

Other important parameters for monitoring the physiology of newborns are enzymes. Increased GGT values after colostrum ingestion in this experiment underlines the useful of this biochemical parameter in predicting the efficiency of passive transfer in newborns, due to its absorption from colostrum. A limit value of <50 U/l GGT in the first 2 weeks of life had been established as predicting failure of passive transfer (<10 g/l $IgG_1$)[14]. ALP activity was higher before consuming colostrum and increased significantly in all calves at 24 and 48 h postnatal. Factors responsible of this high activity of ALP in the first month of life are: intense digestion that take place in the gut after the consumption of colostrum rich in nutrients which can stimulates growing the activity of intestinal ALP; intense activity of osteoblasts in calves during the first month of life, which implies that existence of bone izoenzimatic form in greater quantity (B-ALP, bone alkaline phosphatase)[1]. Enzyme activities AST and ALT in calves who received clinoptilolite added in colostrum were not significantly different from control group which means that clinoptilolite added in colostrum has no influence on parenchymatous organs; similar results to previous studies [7, 18]. Most biochemical parameters in newborn animals are different from those of adults due to their special needs for adapting to the new environment and due to colostrum consumption.

## CONCLUSION

The results of our study show that short-time supplementation with clinoptilolite in high quantity of colostrum increased the iron level in the first 2 days after birth, did not improve absorption of immunoglobulins from colostrum probably due to high quantity of colostrum and had no negative effects on other biochemical parameters; which means that morphofunctional processes that take place in the newborns, necessary for adapting to the new environment, were not affecting.

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Scientific activity: published 31 scientific works, in 9 like first author.

*List of the most significant achievements:*

1. Simona Marc Zarcula, Camelia Tulcan, Mircu Călin, Godja Gabriel and Gheorghe Bonca, *Ovum pick-up (OPU) utilisation in cattle, teoretical aspects,* Lucrările celui de al XV- lea Simpozion Internaţional Tinerii şi Cercetarea Multidisciplinară, 14-15 noiembrie **2013**, Timişoara, România, pp. 133-138.

2. Simona Marc Zarcula, Camelia Tulcan, Danijela Kirovski, H. Samanc, C. Mircu, GH. Bonca, G. Otavă, D. Chirilă, H. Cernescu, *Effects of short term addition of clinoptilolite to colostrum on some biochemical parameters in newborn calves,* Lucr. St. Med. Vet, Vol XLVI (3), 167-172, (**2013**)**.**
3. Camelia Tulcan, Violeta Igna, Mirela Ahmadi, Simona Zarcula, Marilena Motoc, Claudia Borza, *Quantitative Analysis of Superoxide Ions, Hydroperoxide and Lipoperoxidation Products in Characterizationof Fresh Organic Fluid Quality*", REV. CHIM., vol.64, no.2, pp. 195-197, **2013**.
4. Simona Marc Zarcula, Horia Cernescu, Gabriel Godja, Violeta Igna, *Effects of recovering bovine oocyte methods on quantity and quality of cumulus-oocyte complexes,* The 1 st Virtual International Conference „Advanced Research in Scientific Areas", Slovak Republica, 3-7.12.**2012**, (http://www.arsa-conf.com/ )
5. V. Ardelean, Violeta Igna, Simona Marc Zarcula, GH. Bonca, C. Mircu, G. Otavă, H. Cernescu, G. Godja, M.G. Muresan, A. Ardelean, *Registered constants of mobility parameters after thawing of cryopreserved semen in Holstein Friesian breed bulls,* Lucr. St. Med. Vet, Vol XLVI, **2013**.

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