Abstract

This research paper addresses the hypothesis that an oral supplementation with organically modified clinoptilolite will improve colostrum quality in primiparous dairy cows whilst having no adverse effects on the cows’ health. A total of 36 pregnant Holstein primiparous dairy cattle were randomly assigned to receive daily oral drenching, two hours following morning feeding, with 11 l of water containing either 0 g/l ($n = 16$) or 150 g/l ($n = 20$) of clinoptilolite. Treatment lasted from $24 ± 4$ d prior to expected parturition until two days postpartum (pp). Colostrum was collected at 2 to 3 h, $12$, $24$ and $36$ h pp and blood samples were collected at $24 ± 4$ and $4 ± 2$ d prior to parturition and $1$, $2$ and $7$ d pp. Overall mean dry matter, fat and total protein percentage as well as IgG concentration and mass were significantly greater in colostrum collected from cattle drenched with clinoptilolite (total protein increased by 15% and IgG concentration and mass by $21$ and $38$% respectively at first sampling and further at second sampling). Total γ globulin and most other blood serum biochemistry parameters did not differ between cattle treated and not treated with clinoptilolite, the only exception being the fast anionic γ globulin fraction that was 17% greater at $4 ± 2$ d prior to parturition and 10% lower on the 1st day pp in treated cattle. These results showed that organically modified oral clinoptilolite supplementation at 150 g/d significantly increases the IgG concentration in colostrum and has no adverse effects on the energy status, protein, lipid, and mineral metabolism in primiparous dairy cattle during prepartum period.

The intake of an adequate amount of good quality colostrum within two hours after birth is of utmost importance for the health and survival of newborn calves (Weaver et al., 2000). Colostrum is not just a source of nutrients and growth factors, but also provides immune protection to calves in the early stages of extrauterine life. The complex multilayer structure of the bovine placenta (syndesmochorial) effectively prevents transfer of large molecules between dam and foetus, and bovine calves are born agammaglobulinemic (Weaver et al., 2000). The mass of colostral IgG transferred to the peripheral blood of the calf is affected by the timing of feeding, amount offered and the quality of the colostrum (Kertz et al., 2017). Numerous interconnected factors, such as the breed or age of dam, nutrition, season of calving, volume of colostrum produced, periparturient vaccination of the dam, length of the dry period and delayed colostrum collection affect colostrum quality (Godden, 2008). As a consequence, there is much variation in results reported in studies dealing with factors affecting the quality of colostrum and there is an ongoing need to study these factors.

Among zeolites (minerals and chemical compounds within the group of silicates), clinoptilolite is the most studied substance for use in the human and veterinary medicine, and it is the best known as a feed additive in animal nutrition (Papaioannou et al., 2005). Clinoptilolite has been shown to improve production performance, acting as antioxidant, anti-diarrhoeic and growth-promoting agent (Papaioannou et al., 2004; Pourriotis et al., 2012; Wu et al., 2015). During the last decade, its utilization as a mycotoxin-binding adsorbent has been a topic of considerable interest (Daković et al., 2007; Katsoulos et al., 2016). There are also...
reports indicating its role in the prevention of metabolic diseases in dairy cows (milk fever, ketosis) as well as positive effects on general health (Papaioannou et al., 2005; Katsoulos et al., 2006). However, the effect of its supplementation on the colostrum quality in primiparous dairy cows has not been reported to date.

Starting from the aforementioned positive effects of clinoptilolite on cows’ health and production, and its role as a reservoir of ammonium which increases the availability of nitrogen in the rumen (White and Ohlrogge, 1974) and favours the rumen microbe protein synthesis (Hartinger et al., 2018), we hypothesized that an oral supplementation of cows with clinoptilolite, in the period of colostrogenesis, might improve the quality of colostrum, without showing any adverse effect on the cows’ health. This study has investigated the effects of the supplementation of primiparous dairy cows with organically modified clinoptilolite (EP Patent No. 1363854A1 and B1; Milosevic and Tomasevic-Canovic, 2003, 2009) in the period from 24 ± 4 d before the expected parturition to two days after calving on colostrum quality parameters (total protein, IgG, and fat content). Additionally, the effect of the supplementation on the energy status, protein, lipid, and mineral metabolism of these cows was assessed by the analysis of biochemistry parameters of their peripheral blood serum.

Materials and methods

Experimental design

The use of animals was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Belgrade in accordance with the National Regulations on Animal Welfare. A total of 40 healthy pregnant Holstein primiparous dairy cows, grown at an open stall farm (Padinska Skela, PKB Corporation, Belgrade, Serbia), were randomly selected 30 d prior to the expected date of parturition, and placed in a separate facility, in tie stalls, and kept under the same hygienic and dietetic conditions. All cows were enrolled into the study between the beginning of September and the end of November 2016, and received access to the same total mixed ration on an ad libitum basis throughout the study. At 25 d before expected parturition date the cows were offered a close-up transition diet (NE\(_{\text{L}}\) = 1.60 Mcal/kg; Table S1, online Supplementary File) and following parturition were transferred to a lactating diet (NE\(_{\text{L}}\) = 1.71 Mcal/kg; Table S1 online Supplementary File) that was provided for all animals until 30 d into lactation. All offered diets were initially formulated to either meet or exceed the NRC (2001) requirements and the mixed rations were offered in two equal portions at 07.00 and 18.00 h for total dry matter, protein and fat content (%), and pH value. All offered diets were finally formulated to either meet or exceed the NRC (2001) requirements and the mixed rations were offered in two equal portions at 07.00 and 18.00 h for total dry matter, protein and fat content (%), and pH value. All offered diets were initially formulated to either meet or exceed the NRC (2001) requirements and the mixed rations were offered in two equal portions at 07.00 and 18.00 h. Cows had ad libitum access to clean, fresh water throughout the study.

The cattle were randomly divided into two groups and received each of the two treatments simultaneously: daily oral drenching, of either 11 of water with 0 g/l (n = 19; C0 group) or 150 g/l (n = 21; C150 group) of clinoptilolite (Minazel Plus®, Patent Co., Serbia; Milosevic and Tomasevic-Canovic, 2003, 2009). Water and clinoptilolite suspension were administered using a glass drenching bottle, and given orally in one portion, two hours fol-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clinoptilolite, g/d</th>
<th>SEM</th>
<th>T</th>
<th>P value(^{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solids (%)</td>
<td>19.25</td>
<td>21.54</td>
<td>0.98</td>
<td>0.026</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>8.07</td>
<td>9.45</td>
<td>0.50</td>
<td>0.009</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>5.76</td>
<td>6.57</td>
<td>0.40</td>
<td>0.049</td>
</tr>
<tr>
<td>pH</td>
<td>6.30</td>
<td>6.25</td>
<td>0.03</td>
<td>0.120</td>
</tr>
<tr>
<td>Brix (%)</td>
<td>16.7</td>
<td>18.8</td>
<td>0.8</td>
<td>0.008</td>
</tr>
<tr>
<td>Volume (l)</td>
<td>4.5</td>
<td>5.1</td>
<td>0.6</td>
<td>0.500</td>
</tr>
<tr>
<td>IgG (g/l)</td>
<td>55</td>
<td>73</td>
<td>7</td>
<td>0.016</td>
</tr>
<tr>
<td>IgG (g)</td>
<td>214</td>
<td>300</td>
<td>31</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^{a}\)Overall last square means for 1st, 2nd, 3rd and 4th colostrum.

\(\text{SEM}\) standard error of mean; \(\text{T}\), Clinoptilolite treatment; \(H\), colostrum sampling time: 2, 12, 24 and 36 h pp.

Table 1. Mean quality parameters measured in colostrum\(^{a}\) collected from primiparous dairy cows drenched orally with a supplementation of water (1 l) with either 0 or 150 g/head/d of organically modified clinoptilolite

Colostrum was collected at 2 to 3 h, 12, 24 and 36 h pp (referred to as 1st, 2nd, 3rd and 4th colostrum, respectively) and the volume of colostrum collected was recorded. The total collected volume was mixed and representative 200 ml samples were taken. The fresh colostrum was analysed, within 1 h of collection, using digital Brix refractometer (Atago Co. Ltd., Tokyo, Japan) and stored frozen at −20°C until further analysis. Blood samples (9 ml) were collected 24 ± 4 and 4 ± 2 d prepartum, and at 1st, 2nd and 7th day pp, by jugular venepuncture using sterile plastic vacuum tubes without anticoagulant. The blood serum was separated by centrifugation (800 × g, 15 min) which was completed within 2 h of sample collection, and serum samples (0.5 ml) aliquoted and stored at −20°C until the further analysis.

Analysis of colostrum

The frozen colostrum samples were thawed in a water bath at 37°C and heated to a temperature of 20°C and then mixed by twisting the bottle several times. Colostrum samples were analysed for total dry matter, protein and fat content (%), and pH value (described in online Supplementary File). The Brix refractometry, radial immunodiffusion (RID) assay (using a bovine IgG RID test plate; The Binding Site Group Ltd, Birmingham, UK) were completed as described in Stojic et al. (2017).

Blood serum biochemistry analyses

The total protein, albumin, beta-hydroxybutyric acid (BHB), urea, glucose, triglyceride, cholesterol, calcium, magnesium, phosphorus, and potassium concentration in blood serum samples of C150 and C0 group were determined using commercial test packages (BHB, Randox, UK; urea, glucose, cholesterol and phosphorus, Bioanalitika, Serbia; triglycerides, calcium and magnesium; BioSystem, Spain). All biochemistry analyses were performed using automatic analyzer (Analyzer A15, BioSystem,
Barcelona, Spain). Agarose gel protein electrophoresis was completed as described in Stojić et al. (2017).

### Statistical analysis

Data from cows that had mastitis were excluded from this analysis. Statistical analysis of the data results was carried out using GraphPad Prism v6 (GraphPad, San Diego, CA, USA) software. Given that some data were not normally distributed (Shapiro–Wilk normality test \( P < 0.05 \), Table 2, Table S2 online Supplementary File), several transformations of data have been attempted until adequate transformation was found. For the data of biochemical analyses of blood plasma following transformations were applied:

- For triglycerides, BHB and magnesium: \( y = x + 1 \)
- For urea: \( y = x + 2 \)
- In colostrum:
  - For fat: \( y = x + 1 \)
  - For volume: \( y = x + 6 \)
  - For the IgG concentration (g/l): \( y = x + 50 \)
  - For IgG mass (g): \( y = x + 700 \)

After transformations all data were normally distributed (Shapiro–Wilk normality test \( P > 0.05 \)). Groups were compared

### Table 2. Mean of peripheral blood biochemistry parameters of primiparous dairy cows drenched orally with a supplementation of water (1 l) with either 0 or 150 g/head/d of organically modified clinoptilolite

<table>
<thead>
<tr>
<th>Days in relation to parturition</th>
<th>Clinoptilolite (g/day)</th>
<th>0</th>
<th>150</th>
<th>0</th>
<th>150</th>
<th>0</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−24 ± 4</td>
<td></td>
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<tr>
<td></td>
<td>24 ± 4</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>( x \ ± se )</td>
<td>66 ± 6</td>
<td>67 ± 4</td>
<td>65 ± 6</td>
<td>65 ± 5</td>
<td>69 ± 5</td>
<td>66 ± 5</td>
</tr>
<tr>
<td></td>
<td>CV %</td>
<td>2</td>
<td></td>
<td>1</td>
<td></td>
<td>2</td>
<td></td>
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<tr>
<td></td>
<td>32 ± 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>( x \ ± se )</td>
<td>33 ± 2</td>
<td>33 ± 2</td>
<td>33 ± 2</td>
<td>32 ± 2</td>
<td>34 ± 3</td>
<td>32 ± 3</td>
</tr>
<tr>
<td></td>
<td>CV %</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
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<tr>
<td></td>
<td>1</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>( x \ ± se )</td>
<td>2.6 ± 1.0</td>
<td>2.2 ± 0.8</td>
<td>2.6 ± 1.1</td>
<td>3.0 ± 1.0</td>
<td>4.1 ± 1.4</td>
<td>3.3 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>CV %</td>
<td>0.3</td>
<td></td>
<td>0.2</td>
<td></td>
<td>0.3</td>
<td></td>
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<tr>
<td></td>
<td>38 ± 35</td>
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<tr>
<td>Glucose (mmol/l)</td>
<td>( x \ ± se )</td>
<td>2.9 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>3.4 ± 0.6</td>
<td>3.3 ± 0.5</td>
<td>3.1 ± 0.8</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>CV %</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>42 ± 32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>( x \ ± se )</td>
<td>2.7 ± 0.4</td>
<td>2.5 ± 0.3</td>
<td>2.3 ± 0.5</td>
<td>2.6 ± 0.5</td>
<td>2.4 ± 0.5</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>CV %</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14 ± 14</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>BHB (mmol/l)</td>
<td>( x \ ± se )</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>CV %</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mmol/l)</td>
<td>( x \ ± se )</td>
<td>2.2 ± 0.3</td>
<td>2.0 ± 0.2</td>
<td>2.3 ± 0.5</td>
<td>2.0 ± 0.4</td>
<td>1.8 ± 0.2</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>CV %</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
<td>( x \ ± se )</td>
<td>2.4 ± 0.3</td>
<td>2.6 ± 0.3</td>
<td>2.5 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>CV %</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Magnesium (mmol/l)</td>
<td>( x \ ± se )</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.3</td>
<td>0.9 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>CV %</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>( x \ ± se )</td>
<td>4.4 ± 0.3</td>
<td>4.5 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>4.4 ± 0.3</td>
<td>4.6 ± 0.4</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>CV %</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

Day, day prepartum (−) or day pp (+); \( x \ ± se \), mean ± standard deviation; se, standard error of mean; CV, coefficient of variation.
Results

Mean values for dry matter (%), total protein (%), fat (%), pH, % Brix and the concentration and mass of IgG in the colostrum samples of C150 and C0 group of cows, for every sampling time (2, 12, 24 and 36 h after calving), are presented in Fig. 1 and in online Supplementary Table S2. Full details of the statistical analysis are given in online Supplementary Table S3. The overall means value of dry matter, fat, protein, % Brix, IgG concentration and IgG mass were significantly higher in colostrum of C150 then in colostrum of C0 group (Table 1). These results showed that the 1st colostrum (collected 2–3 h pp) total protein and %Brix values were higher (by 15 and 11% respectively) in C150 than C0 group. In the 2nd colostrum (collected 12 h pp) the value of colostral solids, total protein, %Brix and fat were higher (by 30, 30, 29 and 45% respectively) in C150 than C0 group. The concentration of IgG in the 1st and 2nd colostrum of C150 group was higher (by 21 and 54% respectively) compared to the C0 group. Despite the fact that there were no differences in the volume of colostrum between C150 and C0 groups, the total mass of colostral IgG in C150 group was higher (by 38%, P = 0.04) in the 1st colostrum.

The blood serum biochemistry results showed that oral clinoptilolite supplementation had no effect on the total protein, albumin, urea, glucose, triglycerides, cholesterol, BHB, phosphorus, calcium, magnesium or potassium concentration (Table 2).

The results of agarose gel electrophoresis showed that clinoptilolite supplementation did not influence the concentration of blood serum γ globulins (mainly IgG; Butler, 1983) (Fig. 2 and online Supplementary Tables S4 and S5). However, analysis of the relative content (percentage) of cationic ‘slow’ and anionic ‘fast’ γ globulin fractions showed that there were significant differences between the groups both before and after parturition. At 4 ± 2 d prepartum we observed a decrease of 7% (P = 0.02) in the relative content of cationic γ globulin and an increase of 17% (P = 0.02) in the relative content of anionic γ globulin in the treated cows. However, the opposite effect was observed on the 1st day after calving, when the relative content of cationic γ globulin was 7% greater (P = 0.02), and the relative content of anionic γ globulins was 10% lower (P = 0.02) in C150 group (Tables S4 and S5, online Supplementary File).

Discussion

In this study the effect of the organically modified clinoptilolite on colostrum quality in primiparous dairy cows was analysed. This formulation has been produced by adsorption of long-chain organic cations which adsorbs several mycotoxins (aflatoxin B1, ochratoxin A, zearalenone; 99, 96, 94% adsorption, respectively) without influencing adsorption of vitamins, amino acids and trace elements (Milosevic and Tomasevic-Canovic et al., 2003, 2009).

The lactation number is one of the key factors that can affect the IgG concentration and nutrient composition of colostrum (Devery-Pocius and Larson, 1983). Primiparous cows have been exposed to a smaller number of antigens (environmental or vaccinal), and the antigen exposure time is shorter (Morril, 2011), which might result in lower IgG concentrations in their colostrum (Devery-Pocius and Larson, 1983). However, in our current study, primiparous cows from both control (C0) and clinoptilolite treated (C150) groups produced high quality colostrum (>50 g/l IgG). This is in accordance with the results of Kehoe et al. (2011) who reported that, as a result of modern cow management regimes that include greater attention to feeding and vaccination programmes, cows from first lactation can produce high quality colostrum. Starting from an assumption that cows from second and greater lactations might have more diversity of antibodies in their colostrum (exposed to more antigens, for longer period) we chose to focus on primiparous cows due to their ‘immunological inexperience’ and the greater uniformity of the experimental groups.

Numerous studies confirm that zeolite (clinoptilolite) supplementation improves milk yield (Karatzia et al., 2013) and the level of milk fat (Roussel et al., 1992) and total protein (Dschaak et al., 2010). Previous results showed that oral clinoptilolite supplementation of calves during the period of colostral nutrition increased blood serum IgG level (Stojić et al., 1995; Frazier et al., 2005, 2007). The current study has showed that oral supplementation with 150 g/day of organically modified clinoptilolite improved colostrum quality. Although results of several research groups showed a beneficial effect of zeolite supplementation, the results are difficult to compare directly (Papaioannou et al., 2005; Kerwin et al., 2019). It has been reported that the extent of performance enhancement effects is related to the types of zeolite, their physico-chemical properties, purity in terms of the content of clinoptilolite, as well as to the supplementation level used in the diets (Papaioannou et al., 2005). In addition, the particle size of the zeolitic material, crystallite size and the degree of aggregation, as well as the porosity of individual particles strongly affect its ion exchange, adsorption and catalytic properties (Papaioannou et al., 2005). In a recent study it was shown that the use of synthetic zeolite A in the prepartum period of cows in second and greater lactation did not influence colostral IgG concentration and IgG mass (Kerwin et al., 2019). According to data reviewed in Papaioannou et al. (2005) we believe that the discrepancy between our result and results of Kerwin et al. (2019) lies in the variations in the experimental approach: use of different zeolites and use of cows with different lactations/parities.

Results of the present study showed that the primiparous dairy cows had high quality colostrum (>50 g/l IgG; Godden, 2008), and that the supplementation with organically modified clinoptilolite further improved it. Thus, the supplemented cows (C150 group) have, compared to control ones (C0 group), higher concentrations of IgG in colostrum collected 2 to 3 h as well as 12 h pp, and increased total mass of IgG at 2 to 3 h pp. The exact mechanisms of the positive effects of zeolite supplementation on colostrum quality in primiparous dairy cows are not clear at this time. However, reports on the efficacy of zeolites to reduce ammonia release into the environment, and to contrast dietary mycotoxins’ effects on health, production and milk composition of dairy cows can help us in the interpretation of our data. In 1974 it was proven that ammonia ions produced in the rumen by the decomposition of non-protein nitrogen sources are immediately exchanged in zeolites and subsequently released by the regenerative action of sodium ions entering the rumen by the saliva during the after-feeding fermentation period (White and Ohlrogge, 1974). Both in vivo and in vitro experiments have established that up to 15% of the ammonium ions...
in the rumen could be taken up by the zeolite, allowing gradual release of excess nitrogen into the rumen (White and Ohlrogge, 1974). This gradual release contributes to capturing the nitrogen into microbial protein, which is then assimilated into the animals' digestive system. The ability of zeolite to act as a reservoir of ammonium ions protects the animal against the production of

Fig. 1. Quality parameters measured in colostrum collected from primiparous dairy cows drenched orally with a supplementation of water (1 l) with either 0 or 150 g/head/d of organically modified clinoptilolite. C0: 0 g/head of the clinoptilolite; C150: 150 g/head/d of the clinoptilolite. Colostrum sampled 2, 12, 24 and 36 h after calving. The results are mean ± SD; (*) \( P < 0.05; \) (** \( P < 0.01; \) (*** \( P < 0.001. \)

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McCollum and Galyean, 1983). Nestorov (1984) referred that sim-
linearly associated with the percentage of clinoptilolite inclusion
centration even when no urea was present, and this reduction was
clinoptilolite was effective in reducing steers rumen ammonia con-
cantly reduced the rumen ammonia concentration. Furthermore,
clinoptilolite, in the ration of dairy cows containing urea, signifi-
cantly decreases the rumen ammonia concentration. This was
results of the present study showing an increase in the percentage
of total protein and colostral IgG concentration in the clinop-
tilitolite treated group, might be explained by the positive effect of
the clinoptilolite on increasing the availability of nitrogen in the
rumen, and thus by favouring the microbe protein synthesis
process in the cows’ rumen.

The relative content of dry matter in colostrum of treated
group was higher in 2nd colostrum, with relatively higher content
of total protein and fat. Considering that colostrum fat is primar-
ily derived from the free fatty acids absorbed in the rumen, the
results showed increasing percentage of fat in the colostrum of
clinoptilolite supplemented (C150) group, are comparable to the
results of Karatzia et al. (2011) who reported an increase in the
acetic acid concentration in cows supplemented with zeolite. In
the study by Khachlouf et al. (2018) the addition of zeolite also
resulted in the highest rumen molar percentage of acetate and
higher acetate: propionate ratio. Similar to the results of this
study, Roussel et al. (1992) observed a significant increase in
milk fat content, feeding cows a diet containing 1% of sodium
zeolite-A.

Khachlouf et al. (2019) have recently reported that the use of
zeolite in periparturient period of cows can be effective, with posi-
tive influence on milk production and its components, and have
do deleterious effects on milk composition or blood biochemistry
parameters. This study confirmed that, besides positive effect on
colostrum quality, oral supplementation of the organically modi-
fied clinoptilolite has no apparent adverse effects on the protein
metabolism (total protein, albumin and urea level), energy status
(glucose and BHB), lipid (triglycerides and cholesterol) and min-
eral (calcium, phosphorus, magnesium and potassium) metabol-
ism in the primiparous dairy cows during the experimental
period. The applied supplementation did not influence the abso-
lute concentration of γ globulins in blood serum of experimental
animals. However, our results indicate that oral supplementation
with 150 g/day organically modified clinoptilolite could have a
beneficial effect on the relative content of anionic γ globulin frac-
tions (mostly IgG1) (Kickofen et al., 1968) with increased relative
content on day 4 ± 2 before calving and decreased relative content
on 1st day after calving, reflecting their increased synthesis before
calving and transport to the colostrum at the day of calving.

In conclusion, the oral supplementation of primiparous dairy

cows with 150 g per day of organically modified clinoptilolite
during the prepartum period resulted in the secretion of colos-
trum with increased concentration of IgG, total proteins and
lipid, and had no adverse effects on the cows energy status,
protein, lipid, and mineral metabolism.

**Supplementary material.** The supplementary material for this article can
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