

Dietary zeolite clinoptilolite supplementation influences chemical composition of milk and udder health in dairy cows

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Introduction

Natural, synthetic and modified zeolites, found mainly in sedimentary rocks of volcanic origin, are crystalline, hydrated aluminosilicates and cations clustered to form micro aggregates with three-dimensional structures comprising multiple micro pores. This modulates their unique catalytic properties to absorb gas and water molecules, facilitate ion exchange and act as “molecular sieves” with long-term chemical and biological stability (Kralj and Pavelić, 2003). Among the 140 types of natural zeolites, clinoptilolite (CPL) is the most widespread scientifically studied substance and is best known as a zootechnical and biomedical feed ingredient that is widely used in farm animal nutrition as a candidate agent to replace antibiotics (Papatsiros et al., 2013). To date, CPL has been suc-

cessfully applied in animal biotechnology and veterinary medicine as an agent capable of ameliorating mycotoxicosis, maintaining gut health by acting favourably on intestinal microbiota, reducing, preventing and/or treating diarrheal disease in farm animals, decreasing the level of toxic heavy metals and ammonia, and improving immunity, general health and growth performance in animals of veterinary importance (Collela, 2011; Valpotić et al., 2016). Several toxicological studies, and haematological, biochemical and histopathological analyses of natural CPL have shown that this compound is non-toxic and safe for use in human and veterinary medicine (Šperanda et al., 2006; Valpotić et al., 2017a).

Contemporary studies on the influence of dietary zeolites, particularly

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clinoptilolite, have focused on analyses of the biochemical and haematological parameters in the blood of laboratory animals and cows (Grabherr et al., 2009). In both tested groups of animals, a beneficial effect of the agent was found on blood parameters (Katsoulos et al., 2005; Ipek et al., 2012), and on kidney and liver histology in laboratory animals (Bosi et al., 2002). The administration of dietary zeolites in dairy cows has aimed to achieve several objectives. Due to its highly absorptive capacity, zeolites have been used to bind in-feed mycotoxins and to decrease the ammonia content released into the environment (Laurino and Palmieri, 2015). Considering the well-defined chemical properties of zeolites (Pavelić et al., 2002), beneficial effects on health and productivity of dairy cows have been expected and confirmed (Bosi et al., 2002). Furthermore, CPL is thought to modulate metabolic, endocrine and antioxidative status in dairy cows, thus improving their health, fertility and milk yield (Valpotić et al., 2017b).

Mastitis is the one of the most important diseases in dairy cattle and is responsible for significant economic losses (Santos et al., 2003; Bačić, 2009). Although the diagnosis of clinical mastitis is relatively simple, the subclinical form of mastitis is difficult to diagnose due to the lack of visible clinical signs of inflammation. Increased somatic cell counts (SCC) in milk is the only evidence to facilitate diagnosis. Subclinical mastitis has been recognized as one of the major disease issues for the dairy industry. It is also one of the most costly diseases for dairy farmers (Topolko and BeniĆ, 1997).

Cows with subclinical mastitis have a reduced milk yield (20% in average). Apart from the economic importance, this also has public health significance. The existence of a pathogen within the mammary gland, without evidence of mastitis, is called a latent infection (Benić, 2001; Sears and McCarthy, 2003). In this

form, the inflammatory reaction within the mammary gland is detectable with diagnostic screening tests (mastitis test or SCC) or by isolation and culturing of causative agents from milk samples (Gronlund et al., 2005; Cvetnić et al., 2016). Early detection of subclinical mastitis in the dairy industry is of great significance to prevent economic losses (Ruegg, 2003; Viguiet et al., 2009; Djuricic et al., 2014).

Accordingly, observation of systemic inflammatory and oxidative stress responses in cows with subclinical mastitis could be a useful tool in the early detection of subclinical mastitis. It could also assist in reducing economic losses through the prompt treatment of affected cows (Turk et al., 2012; Turk et al., 2017). More recently, the beneficial effects of dietary zeolites were observed in dairy cows by an analysis of the chemical composition and SCC in milk (Alic Ural, 2014) and in the decreased incidence of milk fever in cows (Jorgensen and Theilgaard 2014). The aim of this preliminary study was to establish the influence of the dietary zeolite CPL on the chemical composition of milk and udder health in dairy cows.

Materials and methods

Animals

Twenty cows of the Holstein-Frisian breed were included in the study. Cows were aged between 3 to 5 years, were pregnant for 3 months and kept at a small family farm in the vicinity of the town of Đurđevac, Croatia.

Study design

The cows were assigned into two groups, control (n=10) and CPL-fed group (n=10). The CPL group received 100 g zeolite in the ratio for dairy cows on a daily basis. Initial milk sampling was performed prior to adding CPL to feed.

Four additional sampling sessions were carried out on a monthly basis, up to the 7th month of pregnancy, *i.e.*, dry period.

Milk sampling and analysis

Individual quarter samples for bacteriological analysis were taken prior to the morning milking. The initial streams were discarded; teat ends were disinfected with 70% ethanol. Samples were collected in sterile tubes for microbiological examination. The samples were cooled and transported in a portable refrigerator to the Laboratory for Mastitis and Raw Milk Quality of the Croatian Veterinary Institute (Zagreb, Croatia) for further analysis. Somatic cell counts and chemical analysis (milk fat, proteins, lactose, solids non-fat and urea) were conducted during routine monthly control of the performance of dairy cows in the Central Laboratory for Milk Control (Križevačka Poljana, Croatia). Data on soma-

tic cell counts and the chemical composition of milk were kindly provided by the Croatian Agricultural Agency.

Statistical analysis

The data were analysed using the program Stata 13.1 (Stata Corp., USA). The values of milk fat, protein, lactose and solids non-fat (SNF), as well as SCC, were measured five times during the trial in both groups of cows. Average values of milk components were compared by Student *t*-test between two groups for each sampling point. The values for a milk component obtained in repeated samplings within the same group of cows were compared using the *repeated* ANOVA. Post hoc analysis of values within the group was performed by the paired *t*-test. The values obtained for SCC were log transformed prior to statistical analysis in order to normalize distribution. The number of infected

Table 1. The influence of dietary clinoptilolite (CPL) supplementation on the chemical composition of milk in dairy cows as determined in five consecutive monthly samplings between the 3rd and 7th months of pregnancy

Milk component ^a	Group: control (n=10)/CPL-fed (n=10)	No. of milk samplings:					P values
		1	2	3	4	5	
Milk fat (%)	No	3.71±0.97	4.15±1.14	4.71±0.86	4.65±0.88	4.13±1.00	0.0243 ^c
	Yes	3.60±0.84	3.73±0.52	4.31±0.75	4.01±0.67	3.92±0.56	0.0176 ^b
Proteins (%)	No	3.72±0.47	3.83±0.49	3.94±0.54	3.88±0.48	3.61±0.47	0.0117 ^c
	Yes	3.60±0.53	3.53±0.36	3.72±0.36	3.66±0.35	3.38±0.32	0.1002
Lactase (%)	No	4.37±0.20	4.40±0.20	4.40±0.19	4.36±0.19	4.55±0.15	0.0139 ^c
	Yes	4.39±0.27	4.38±0.15	4.37±0.19	4.36±0.18	4.52±0.15	0.1495
Solids non-fat (%)	No	9.06±0.52	9.21±0.59	9.34±0.48	9.23±0.46	9.14±0.58	0.1050
	Yes	8.83±0.58	8.84±0.35	9.03±0.29	8.98±0.28	8.79±0.30	0.3749
Urea (mg/mL)	No	18.5±7.13	11.7±3.59	29.5±6.90	24.3±6.99	21.9±7.93	0.0000 ^c
	Yes	23.5±6.09	14.3±6.18	26.0±4.44	22.8±6.25	21.8±3.64	0.0008 ^b

^aNone of the milk components tested differed significantly ($P>0.05$) between the CPL-fed and control cows;

^bSignificant differences were recorded at particular samplings for milk fat and urea values in the CPL-fed cows ($P<0.05$),

^cSignificant differences were recorded at all particular samplings for the values of all ingredients tested, except for the value of solids non-fat in the control cows ($P<0.05$)

udder quarters was compared between the groups of cows by the Fisher exact test. The Odds Ratio (OR) of intramammary infection was calculated by logistic regression.

Results

The influence of dietary CPL on the chemical composition of milk in dairy cows is presented in Table 1, and its influence on the SCC in milk of dairy cows is shown in Table 2. The milk fat content, proteins, lactose, non-fatty dry matter and urea and SCC in both groups of dairy cows are presented in Figures 1 to 6.

The observed differences in the content of a particular milk component between the groups of cows did not differ significantly in any of sampling points. Statistically significant differences between single samplings in CPL-fed cows were found for milk fat and urea. Moreover, significant statistical differences were found between single sampling points for all milk ingredients tested, except non-fatty dry matter in milk of the control group of cows. Hence the chemical composition of milk was more stable in cows fed by CLP supplement.

The SCC in milk from both groups of cows did not differ significantly between the groups in any of the sampling points. Recorded differences of SCC between single samplings did not differ significantly in either the control group or CPL-fed groups of cows.

The content of milk fat measured in the first sampling in milk of the control group differed significantly from the third ($P=0.028$) and the fourth consecutive sampling ($P=0.037$). The lactose content measured in the fifth sampling in milk of the control group was significantly different from the first ($P=0.045$) and the fourth samplings ($P=0.036$). The urea content in milk of control group was significantly different between the majority of samplings, with the exception

of the fourth and fifth sampling in relation to the first sampling, between the fourth and fifth sampling and between the fourth and the third sampling. The milk fat content measured at the first sampling in milk of CPL-fed cows differed statistically from the values obtained in the third sampling ($P=0.026$). The urea content measured at the first sampling in milk of CPL-fed cows differed statistically from the values obtained at all other sampling points.

Statistically significant differences in the number of infected udder quarters between the cow groups was found after the third sampling ($P=0.03$). During the

Figure 1. The milk fat content in milk of the control group [0] and CPL-fed group [1] as determined in five consecutive monthly samplings between the 3rd and 7th months of pregnancy

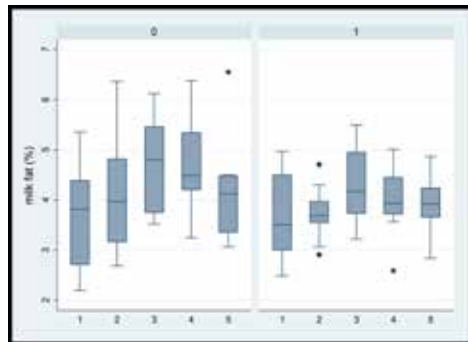


Figure 2. The protein content in milk of the control group [0] and CPL-fed group [1] as determined in five consecutive monthly samplings between the 3rd and 7th months of pregnancy

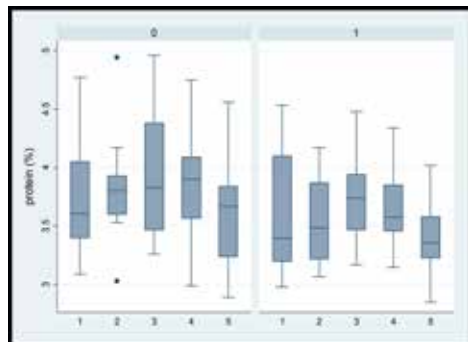


Figure 3. The lactose content in milk of the control group (0) and CPL-fed group (1) as determined in five consecutive monthly samplings between the 3rd and 7th months of pregnancy

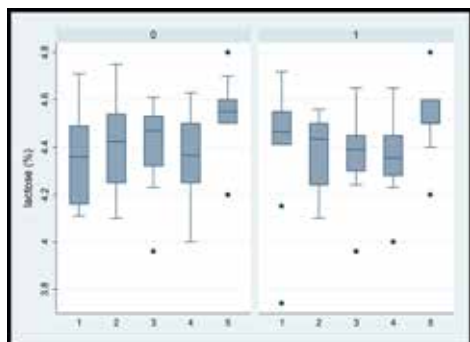


Figure 4. The solids non-fat content in milk of the control group (0) and CPL-fed group (1) as determined in five consecutive monthly samplings between the 3rd and 7th months of pregnancy

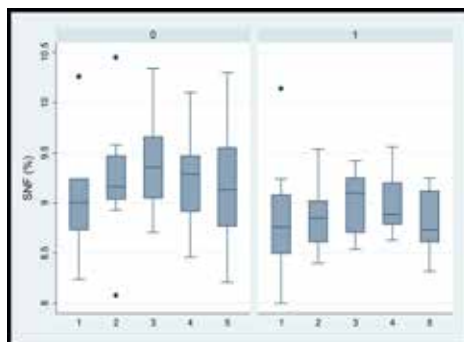


Figure 5. The urea content in milk of the control group (0) and CPL-fed group (1) as determined in five consecutive monthly samplings between the 3rd and 7th months of pregnancy

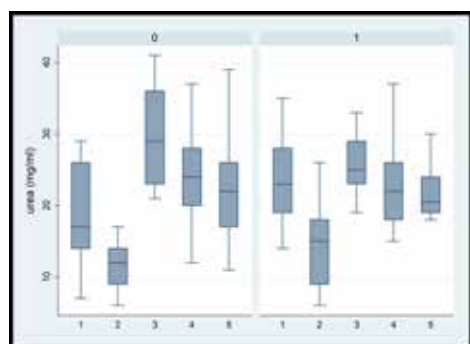


Figure 6. The number of SCC in milk of the control group (0) and CPL-fed group (1) as determined in five consecutive monthly samplings between the 3rd and 7th months of pregnancy, expressed in logarithmic scale

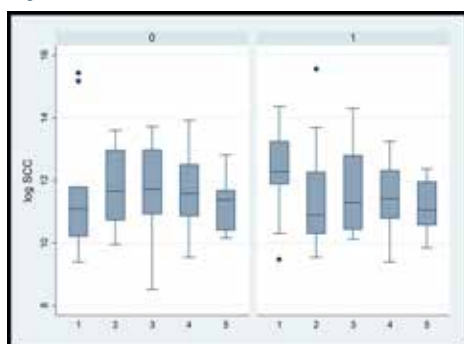


Table 2. The influence of dietary clinoptilolite (CPL) supplementation on somatic cell counts (SCC x 10³) and range in the milk of dairy cows as determined in five consecutive monthly samplings between the 3rd and 7th months of pregnancy following calculation using repeated ANOVA with logarithm transformed values of SCC

Group: control (n=10)/CPL- fed (n=10)	No. of milk samplings:					P values
	1	2	3	4	5	
0*	65.5 (12 - 5050)	116.5 (21 - 806)	131.5 (5 - 911)	107.5 (14 - 1107)	88 (26 - 369)	0.5406
1**	216 (13 - 1732)	55.5 (14 - 5708)	84.5 (25 - 1630)	93.5 (12 - 566)	65 (19 - 236)	0.3273

*0= control cows;

**1= CPL-fed cows

trial, the causative agent of mastitis was isolated from 7 of 10 cows in the control group. In the CPL-fed group, the causative agent of mastitis was isolated from only one cow. The values recorded in the frequency of isolation of the mastitis causative agent differed significantly between groups ($P=0.01$). In the current study, the control group had a 21-fold higher odd of intramammary infection than CPL-fed cows during the observation period (Odds Ratio=21, $P=0.016$).

Discussion

The quality of milk yield is important in the dairy industry, and milk yield is primarily influenced by diet (Ilic et al., 2011). According to Bosi et al. (2002), CPL did not affect milk production, milk fat, milk protein or SCC, while Alic Ural (2014) found that supplementing with 3% CPL in dairy cows increased milk production and decreased SCC. Similarly, Dyachenko and Lysenko (1988) recorded that addition of type A/B zeolite to the diet resulted in increased milk production. In addition, Ilić et al. (2011) described that milk yield was increased by supplementation of 4% (w/v) and 2% (w/v) zeolite, while Ural et al. (2013) suggested that 2% (w/v) CPL supplementation for 120 days significantly ($P<0.01$) improved milk yield. The results obtained by most studies suggest that CPL supplementation may have a positive influence on milk yield. Furthermore, other management practices to CLP supplementation of diets in dairy farms must be applied with maximum care (Alic Ural, 2014).

In the present study, the chemical composition of milk was found to be more stable in CPL-fed cows, since the content of milk fat was statistically different in the third and fourth samplings compared to the first sampling in the control group, whereas in the CPL-fed cows, such a difference was observed only between the first and the third samplings. In addition,

Garcia Lopez et al. (1988) found an improvement in milk fat, with the same dietary addition. Hornig et al. (1999) also observed a significantly increase in milk fat, protein and lactose contents, with 2% CPL supplementation, which was contrary to the findings of Bosi et al. (2002).

There are many factors that affect urea levels in milk, such as temperature, diet, quality of feed, water, season, stage of lactation, etc. (Jonker et al., 1998). Milk urea levels can be increased by CPL inclusion in the diet (Bosi et al., 2002), and they are highly variable, as in this study.

The beneficial effects of CPL in the current study could be ascribed to the dose, which was higher than the doses applied in other studies. Also, the modification of natural CPL by vibroactivation, a novel technology using frequencies that activate and micronize the natural zeolite mineral to particles of 4.28 μm in size (Đuričić and Samardžija, 2016) could be the reason for such a beneficial influence of the agent on the incidence of intramammary infections in dairy cows and more stable chemical composition of milk.

Alic Ural (2014) reported statistically significant lower SCC for the control group than for CPL-fed cows. On the contrary, the present study found no significant differences between the groups of cows were established for SCC, as also reported by Bosi et al. (2002). However, significant differences in SCC were not recorded in the current study, regardless of the feeding regime. Differences in the frequency of isolation of mastitis causative agents from single udder quarters were established between observed group of cows.

Conclusions

This study evaluated the effects of dietary CPL in lactating dairy cows on milk composition, SCC and the incidence

of mastitis from the third to seventh month of gestation. Observed differences in the content of particular milk components did not differ significantly between the groups of cows at any of sampling points. However, the chemical composition of milk was found to be more stable in CPL-fed cows. A statistically significant difference between single samplings in CPL-fed cows was found in milk fat and urea contents. Moreover, significant differences were found between single values of all measured milk components, with exception of non-fatty dry matter in the control group. The SCC in milk did not differ significantly between the groups. However, control cows had a 21-fold higher odd of intramammary infections than CPL-fed cows. This beneficial outcome of the study may be attributed to the antibacterial, detoxifying, antioxidative and immunostimulating effects of CPL on the metabolism of cows, as exhibited by a decreased incidence of intramammary infections during the dry period, parturition and early lactation. Such an outcome might be explained by the moderation of stressful events accompanying such periods, when cows are the most sensitive to metabolic imbalance and environmental detrimental effects, resulting in more pronounced immunosuppression and susceptibility to intramammary infections.

Summary

The aim of the current study was to establish the influence of dietary zeolite clinoptilolite (CPL) on the chemical composition of milk, somatic cell counts and udder health in dairy cows. Twenty cows of the Holstein-Frisian breed, aged between 3 and 5 years, which were pregnant for 3 months and kept in a small family farm in vicinity of Đurđevac, Croatia, were included in the trial. Cows were randomly assigned into two groups, control (n=10) and CPL-fed group (n=10). The CPL group received 100 g zeolite in the ratio for dairy cows on a daily basis. The first milk sampling was taken prior to adding

CPL to feed. The four consecutive samplings were performed on a monthly basis up to 7th month of pregnancy, *i.e.*, the dry period. The milk samples were analysed for chemical composition (milk fat, proteins, lactose, non-fatty dry matter and urea), somatic cell counts and by bacteriological examination. Observed differences in the content of particular milk components tested did not differ significantly between groups in any of sampling points. However, the chemical composition of milk was found to be more stable in CPL-fed cows. Statistically significant differences were found for milk fat and urea contents between single samplings in CPL-fed cows. Moreover, in the control group, significant differences were found between single samplings for all milk components, except non-fatty dry matter. The number of SCC in milk between the groups did not differ significantly. However, the control cows had a 21-fold higher odd of intramammary infections than CPL-fed cows. This beneficial outcome of the study may be attributed to the antibacterial, detoxifying, antioxidative and immunostimulating effects of CPL on the metabolism of cows, as exhibited by a decreased incidence of intramammary infections during the dry period, parturition and early lactation. Such an outcome might be explained by the moderation of stressful events accompanying such periods, when cows are the most sensitive to metabolic imbalance and environmental detrimental effects, resulting in more pronounced immunosuppression and susceptibility to intramammary infections.

Key words: cows, milk composition, somatic cells, zeolite clinoptilolite

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Dodavanje zeolita klinoptilolita u obrok mliječnih krava utječe na kemijski sastav mlijeka i zdravlje mliječne žlijezde

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Cilj ovog istraživanja bio je ustanoviti utjecaj zeolita klinoptilolita (KPL) dodavanog u obroke mliječnih krava na kemijski sastav mlijeka, broj somatskih stanica (BSS) i zdravlje vimena. Dvadeset krava Holštajn-frizijske pasmine u dobi od 3 do 5 godina, gravidnih tri mjeseca i držanih u maloj obiteljskoj farmi u okolici Đurđevca, Hrvatska, bile su uključene u ovo istraživanje. Krave su bile nasumično razvrstane u dvije skupine, kontrolnu (n=10) i skupinu hranjenu s dodatkom KPL (n=10), i to svakodnevno sa 100 g zeolita u obroku za mliječne krave. Prvo uzorkovanje mlijeka načinjeno je prije dodavanja KPL u hranu. Još su četiri uzorkovanja provedena kroz četiri mjeseca, do sedmog mjeseca gravidnosti, odnosno suhostaja. Uzorci mlijeka bili su analizirani s obzirom na kemijski sastav mlijeka (mliječna mast, bjelanjčevine, laktoza, nemasna suha tvar i ureja), BSS i bakteriološkom pretragom. Uočene razlike u sadržaju pojedinog pretraživanog sastojka mlijeka nisu se značajno razlikovale, ni za pojedinačno uzorkovanje niti između pokusnih skupina krava. Međutim, kemijski sastav mlijeka bio je mnogo stabilniji u krava kojima je dodavan KPL u hranu. Naime, statistički značajna

razlika između pojedinačnih uzorkovanja u krava kojima je dodavan KPL bila je utvrđena za sadržaj mliječne masti i ureje. Štoviše, u mlijeku krava iz kontrolne skupine utvrđene su značajne razlike između pojedinačnih uzorkovanja za sve pretražene sastojke mlijeka, s izuzetkom nemasne suhe tvari. Broj BSS u mlijeku krava iz obiju skupina nije se značajno razlikovao. Međutim, krave iz kontrolne skupine imale su 21 puta veći rizik od pojave intramamarnih infekcija nego krave kojima je KPL dodavan u hranu. Ovakav povoljni ishod naših istraživanja može se pripisati antibakterijskom, detoksikacijskom, antioksidativnom i imunostimulacijskom učinku KPL na metabolizam krava što se očitovalo padom pojavnosti intramamarnih infekcija tijekom suhostaja, porođaja i rane laktacije. Ovaj ishod vjerojatno bi se mogao objasniti ublažavanjem stresnih stanja tijekom spomenutih razdoblja kada su krave najosjetljivije na metaboličke poremećaje i nepovoljne okolišne uvjete koji rezultiraju u izraženijoj imunosupresiji i prijemljivosti za intramamarne infekcije.

Ključne riječi: krave, sastav mlijeka, somatske stanice, zeolit klinoptilolit