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Original article

Effects of dietary clinoptilolite on reproductive performance, serum progesterone and insulin-like growth factor-1 concentrations in dairy cows during pregnancy and lactation

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Abstract

The objective of this study was to evaluate the effects of dietary zeolite clinoptilolite on reproductive performance, serum progesterone and insulin-like growth factor 1 (IGF-1) concentrations in 78 Holstein Friesian (HF) cows during pregnancy and lactation. The cows were divided into two groups comprising 40 (control group; CON) and 38 (CPL group) cows. To assess reproductive performance of HF cows the following variables were registered: the interval from calving to first insemination (days open to first service, DFS), the interval from calving to pregnancy (days open to pregnancy, DOP), and the number of services per pregnancy (NSP). The average values of progesterone (5.64±0.59 ng/mL *vs.* 5.16±0.64 ng/mL) were not statistically different ($p<0.05$) and IGF-1 levels (400.17±17.72 ng/mL *vs.* 348.36±20.39 ng/mL) were higher in the CON than in the CPL group which received 50 g of clinoptilolite twice a day. However, in the CPL group ovarian cyclity resumed on days 40 and 60 postpartum. In addition, DFS ($p<0.05$) and DOP ($p>0.05$) were shorter in the CPL than in the CON group (115.1±19.9 and 137.5±36.3 days *vs*. 124.2±17.3 and 143.8±33.5 days, respectively). During 305 days of lactation, milk production was higher in the CPL *vs.* CON (8325.5±628.8 kg *vs.* 8050±586.8 kg). The NSP was lower in the CPL than in CON group (1.91 *vs.* 2.14). The dietary clinoptilolite supplement had a positive influence on milk yield, exhibited modulating effects on endocrine status of dairy cows, and improved reproductive performance, with the decreased NSP, and fewer DFS and DOP.

Key words: dietary zeolite, endocrine status, reproduction, cows

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Introduction

Natural zeolites are crystalline, hydrated aluminosilicates and cations clustered to form micro aggregates with three-dimensional structures comprising multiple micropores. They are found mainly in sedimentary rocks of volcanic origin, and are commonly used as commercial adsorbents and catalysts (Bosi et al. 2002, Kralj and Pavelić 2003, Valpotić et al. 2017). Among the 140 types of natural zeolites, clinoptilolite (CPL) is the most widespread scientifically studied substance used in farm animal nutrition, because it positively influences growth in many domestic animal species (Papatsiros et al. 2013). Clinoptilolite is very significant because it increases in feed efficiency (Dschaak et al. 2010) and milk production in dairy cattle (Dyachenko and Lysenko 1988, Ilić et al. 2011, Karatzia et al. 2013, Ural et al. 2013, Khachlouf et al. 2018), besides it may also reduce mastitis problems (Karatzia et al. 2013, Ural 2014, Đuričić et al. 2017). This beneficial outcome may be attributed to the antibacterial, detoxifying, antioxidative and immunostimulating effects of CPL on the metabolism of cows, as shown by a decreased incidence of intramammary infections (Valpotić et al. 2017, Đuričić et al. 2018). Furthermore, CPL is thought to modulate metabolic, endocrine and antioxidative status in dairy cows, thus improving their health and fertility (Valpotić et al. 2017).

There is no doubt that milk production per cow has increased over time, but it is unclear how much this increase can explain the apparent decrease in fertility (Gröhn and Rajala-Schultz 2000, Ghavi Hossein-Zadeh 2013). There is no doubt that production per cow has increased over time, but it is unclear how much of this increase can explain the apparent decrease in fertility (Royal et al. 2000, LeBlanc 2013). Higher producers are more likely to be inseminated and less likely to be culled (Grohn and Rajala-Schultz 2000). Traditional methods to evaluate reproductive performance in lactating cows rely on indirect measures such as time to first insemination, days open, non-return rates, calving interval, and others (LeBlanc 2013). The hypothesis of our study was that dietary zeolite clinoptilolite could improve reproductive performance and milk yield in dairy cows.

The objective of this study was to evaluate the inclusion of dietary zeolite clinoptilolite to the diet of cows on the reproductive performance and serum progesterone (P4) and insulin-like growth factor 1 (IGF-1) concentrations in Holstein Friesian (HF) cows during pregnancy and lactation. To assess the reproductive performance of HF cows, the following variables were detected: the interval from calving to first insemination (days open to first service, DFS), the interval from calving to pregnancy (days open to pregnancy, DOP), and the number of services per pregnancy (NSP).

Materials and Methods

An ethical approval for the study was obtained from the Ethical Committee of the Faculty of Veterinary Medicine, University of Zagreb, Croatia. The research protocol and animal management were in compliance with the Directive 2010/63/EU of the European Parliament (2010) on the protection of animals used for scientific purposes.

Animals and housing

Seventy-eight HF-cows, aged 3 to 5 years and kept on a commercial dairy farm near Đurđevac, Croatia were used in the study. They were randomly assigned into two groups: a clinoptilolite group (CPL) of cows (*n*=38), which received 50 g of natural clinoptilolite (CPL) twice a day (morning and afternoon), and a control group (CON) (*n*=40). The cows were fed a ration composed of haylage, corn silage, hay and a complete feed mixture for dry cows and lactating cows with 19% crude protein. Cows were housed in a free stall barn with straw beding, fed a 50:50 forage-to-concentrate ration and milked twice a day at 6 hours *a.m.* and 4 hours *p.m*. The supplemented cows received natural CPL modified by vibroactivation and micronization (Vibrosorb®, Viridisfarm, Podpićan, Croatia) from the third month of pregnancy (day 180 prior to parturition) to the end of observation.

Blood sampling and examinations

Blood samples were taken after the morning milking by 'Vacutainer' system from the tail vein (*v. coccygea*) into tubes without anticoagulant but with clot activator. After clotting at room temperature for 1 h, blood samples were centrifuged at 1500 g for 15 min. The sera were separated and stored at -70°C until analyses. The samples were taken on days 90, 180, 210 and 240 of pregnancy, on day 10 before and day 0 after parturition, and on days 5, 12, 19, 26, 40 and 60 of lactation. Serum concentrations of P4 were measured in duplicate by VIDAS-PRG-ELFA (Biomerieux, France) and IGF-1 were determined by the quantitative ELISA method with the use of commercial Bovine insulin-like growth factor 1 (IGF-1) test kit (Novatein Biosciences, Cambridge, MA, USA) by the Microplate Reader DV 990BV4; (Lab services, Italy). All the cows were examined, including vaginal and transrectal uterine palpation 12-36 hours after partu-

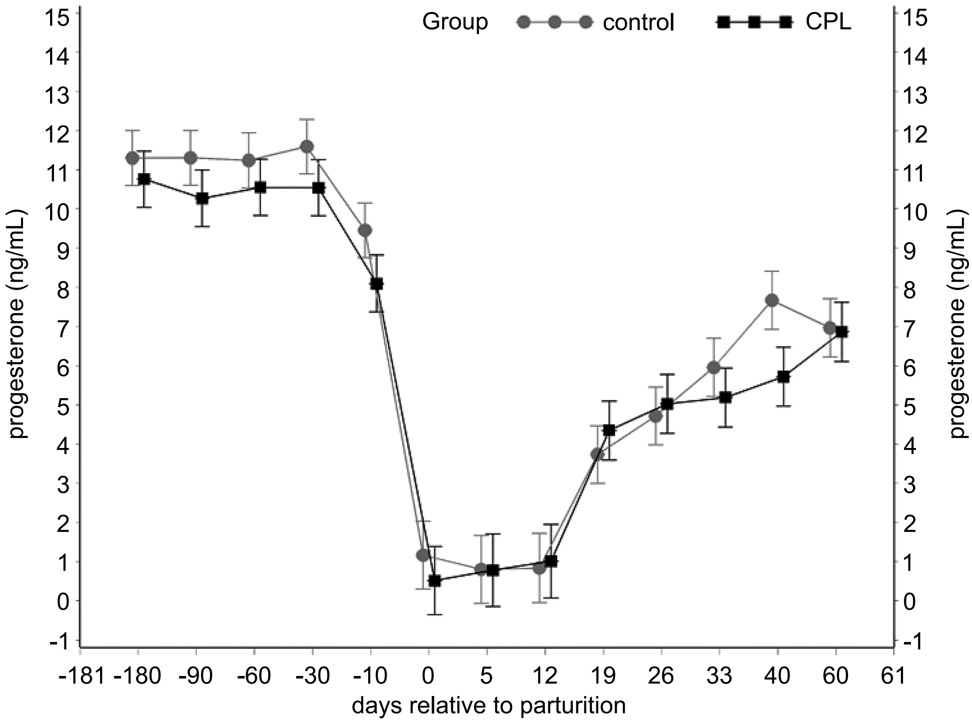

Fig. 1. Serum progesterone concentrations in the cows supplemented with clinoptilolite (CPL) and the control group during pregnancy and lactation.

rition. They were examined again 19-26 days post-partum or earlier, and 45 days after insemination with transrectal ultrasonography with a 5 MHz linear-array transducer (SonoVet 2000, SonoVet, New Braunfels, Texas, USA) until confirmation of pregnancy. After the examination, the cows with reproductive diseases (endometritis, ovarian cyst diseases, ovarian atrophy) were excluded from the trial and managed in line with usual herd management (intrauterine or hormonal therapy, etc.) The cows were artificially inseminated with frozen-thawed semen by an experienced inseminator. The reproductive performance and culling data of individual animals were observed for 10 months following the last blood samples being taken. The milk yield for each cow was recorded monthly and the 305-day milk yield calculated. Animal recording was carried out according to International Committee for Animal Recording (ICAR) rules. The data included lactation, parity, DFS, DOP, and NSP.

Statistical analysis

Statistical analyses were performed by the use of SAS 9.4 software (2002-2012 SAS Institute Inc., Cary, NC, USA). A mixed model (PROC MIXED) was used to analyse serum IGF-1 and progesterone concentration. The statistical model included the fixed effects of group and time, and their interactions. The effects on cows of repeated measures over time were included in the model by RANDOM statement. The Tukey-Kramer multiple comparison test method was performed with a SLICE option to compare each group level within each time level. To assess the reproductive performance of the cows DFS, DOP and NSP were measured. Non-parametric log-rank and Wilcoxon tests (PROC LIFETEST) were conducted, and the shape of the Kaplan-Meier curves verified and survival estimates used to calculate the median days open. The values were censored when observations were terminated for reasons beyond the investigator's control. For a continuous variable (305 days of milk yield), a semi-parametric model, the Cox proportional hazard regression (PROC PHREG), was used. To test their influence on treatment groups, parity and milk yield were included in the model with a PHREG procedure and remained in the final model if $p<0.05$. The hazard ratio was interpreted as the relative daily probability of conception or relative pregnancy rate. Graphs were prepared using the GPLOT procedure, and one was made with two plot statements, showing the Kaplan-Meier survival curves and adjusted survival curves where the mean of the significant covariate for each group adjusted to be equal.

Results

The average values of serum P4 (5.64±0.59 ng/mL *vs.* 5.16±0.64 ng/mL) were without statistically significant differences ($p>0.05$) and IGF-1 levels

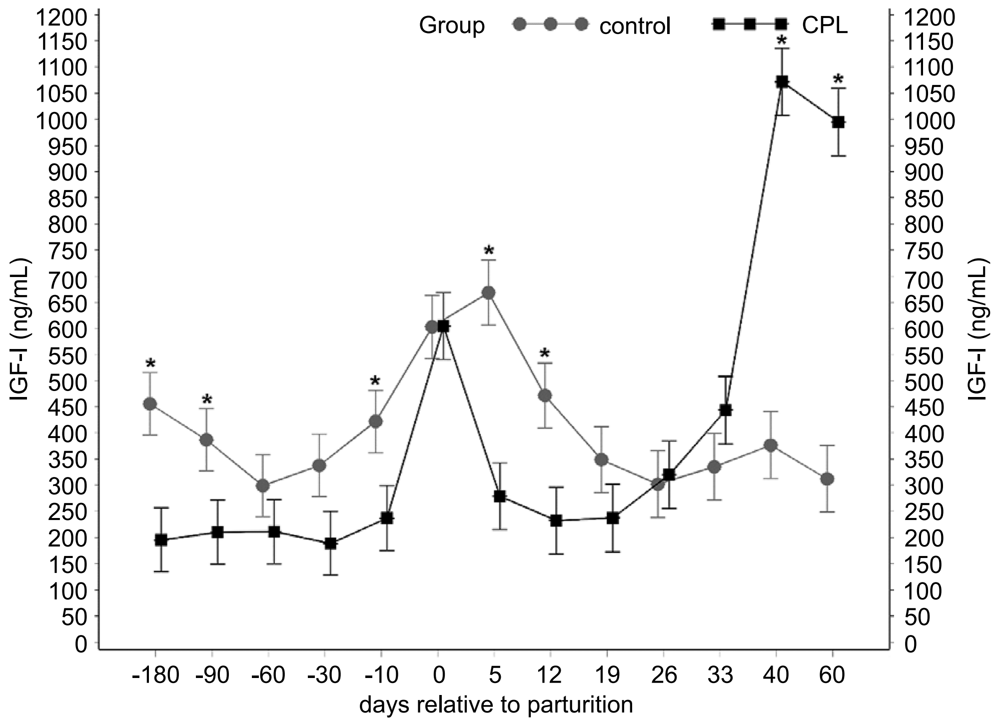

Fig. 2. Serum insulin-like growth factor-1 (IGF-1) concentrations in the cows supplemented with clinoptilolite (CPL) and the control group during pregnancy and lactation (an asterisk means a statistically significant difference between the groups).

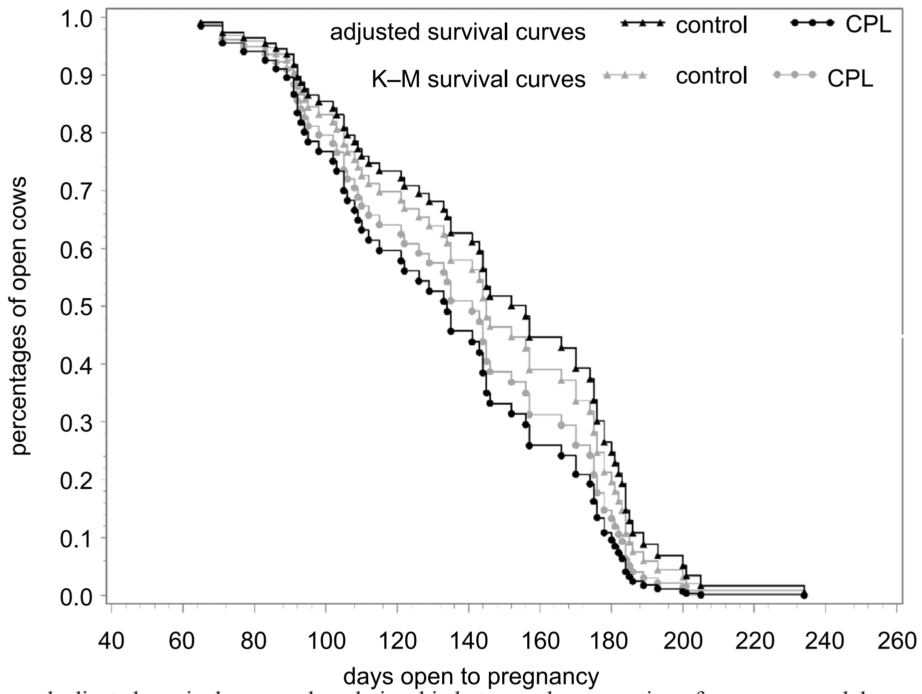

Fig. 3. Kaplan-Meier and adjusted survival curves: the relationship between the proportion of open cows and days open to pregnancy for the cows supplemented with clinoptilolite (CPL) and the control group.

(400.17±17.72 ng/mL *vs.* 348.36±20.39 ng/mL) were higher ($p<0.05$) in the CON than in the CPL group (Figs. 1 and 2). During pregnancy, the highest P4 concentration was recorded in the treatment group on day 90 before parturition (11.25±1.69 ng/mL) and in the control group on day 30 before parturition ($p>0.05$) (11.84±1.78 ng/mL). In the CPL group, ovarian cyclicity resumed on days 40 and 60 postpartum (PP), when P4 concentration was above 1 ng/mL at first time after parturition, and when the IGF-1 level was highest during the PP period, which was also higher ($p<0.05$) than in the control group (1072.20±64.73 and 995.04±64.73 ng/mL *vs.* 376.85±63.80 and 312.32±63.80 ng/mL, respectively). In addition, a con-

Table 1. Hazard ratio for dairy cows supplemented or non-supplemented with clinoptilolite (CPL) with days open to first service (DFS) and days open to pregnancy (DOP), and differences in reproductive performance.

	Parameter	GROUP		p-value	
		Control (n=37)	CPL (n=36)		
mean±SE	305 days milk yield (kg)	8003±133	8368±131	p=0.06	
	parity	2.61±0.18	2.60±0.18	p=0.94	
	number of services per pregnancy	2.14±0.17	1.91±0.17	p=0.34	
				Log-Rank	Wilcoxon
median days (95% CI)	DFS	122 (116-130)	112 (105-123)	p=0.07	p=0.04
	DOP	152 (122-176)	143 (108-157)	p=0.20	p=0.18
		CPL group relative to control group		Chi-Square	
hazard ratio (95% CI)	DFS	0.52 (0.31-0.88)		p=0.01	
	DOP	0.59 (0.35-1.00)		p=0.05	

secutive increase in serum P4 concentrations was recorded from days 26 to 60 PP in both groups.

The percentage of censored values was 8.1% and 2.8% for the CON and CPL groups, respectively. Table 1 shows the reproductive performance of cows and the hazard ratio of the CPL group relative to the CON group. During 305 days of lactation, milk production tended (p=0.06) to be higher in the CPL than in the CON group (8368±131 kg *vs*. 8003±133 kg). The NSC was lower in the CPL group than in the CON group (1.91±0.17 *vs.* 2.14±0.17), but not significantly different, as parity was. The median DFS was significantly different early in the time period of the survival curves (Wilcoxon test; p=0.04). Open to first service was shorter (112; 95% CI: 105-123) in the CPL than in the CON group (122; 95% CI: 116-130). The estimate of the hazard ratio for the zeolite-supplemented cows compared to controls for DFS was 0.52 (95% CI: 0.31-0.88; p=0.01), implying that the cows in the control group were not pregnant until the first insemination, *i.e.* the relative rate of first service in the control group decreased by 48% in comparison to that found in the CPL group. The median days open to pregnancy (DOP) was not significantly different between the groups, but the estimate of the hazard ratio for the CPL group relative to the control group was 0.59 (95% CI: 0.35-1.00) and fairly significant (p=0.05) after including the variable with a significant influence on DOP. The variable that had an influence on DFS and DOP was the 305 days milk yield in kg (HR=0.99; 95% CI: 0.99-1.00, p<0.01). Fig. 3 shows the relationship between the proportion of open cows and DOP for the CPL and CON groups of cows with Kaplan-Meier survival curves and adjusted survival curves, where the mean of the significant covariate (305 days of milk yield) for each group was adjusted to be equal, *i.e*. the covariate patterns differed only in their group values.

Discussion

In intensive systems, milk production per cow has increased over time in most of the world, on average 1-2% per year (Royal et al. 2000). However, higher milk production has reduced reproductive efficiency (Maizon et al. 2004). Washburn et al. (2002) found an increase in DFS from 84 days (in 1985-1987) to 100 days (ten years later) in Holstein herds, and an average increase in days to conception from 121 days (in 1980--1982) to 158 days (in 1997-2000). In US dairy farms, days open increased from 112 to 166 partly because of on-purpose delayed pregnancy in dairy systems (Groenendaal et al. 2004). Moussavi (2008) found an extension of the period from calving to conception to an average of 155 days. On the other hand, in our study, after the voluntary waiting period, cows were inseminated as soon as possible to shorten the time to conception in line with the management on the farm and low price of milk in the country. According to Cilek (2009), the interval from calving for Holstein cows in Turkey were 111.6±3.7, somewhat less than those registered for the CPL cows in our study, but the DOP in the Turkish Holstein cows were higher at 149.6±4.4 than in our CPL group and lower than in the CON group. Popov et al. (2016) found that DOP in first lactation was 141.6±13.3 and shorter in the third lactation at 126.7±15, similar to the median days open (123 days) in Iranian Holstein cows (Bahonar et al. 2009), while in South Africa the average DOP in HF cows was 133.9 (Muller et al. 2014).

The increased NSP often indicates problems with the cow reproductive system, which has a negative impact on farm profitability (LeBlanc 2007). An increase in the NSP at the previous calving results in an increase in age at the next calving in later lactations, which lowers the number of lactations. The maximal NSP was in the third and fourth lactation in Latvian Holstein cows, with 2.36 and 2.28 services (Cielava et al. 2017). The average NSC in South African Holstein cows was 2.55 ± 1.79 (Muller et al. 2014). Previous results for NSC were higher than those in our study for both groups of cows, but in the CON group there were more services per pregnancy than in the CPL group.

It is well known that an analysis of hormonal profiles represents a reliable method of monitoring ovarian cyclic activity. After parturition, serum P4 concentration is low until the first ovulation. Elevated concentrations of progesterone during the first and second diestral periods in cows after parturition increases the concentration of IGF-I in cows with a positive energy balance (Spicer et al. 1990). In the CPL group of cows, ovarian cyclity resumed on days 40 and 60 postpartum, when the IGF-1 level was highest during the PP period, which was also higher than in the CON group of cows.

Clinoptilolite supplementation may have a positive influence on milk yield (Ural et al. 2013, Ural 2014), as also found in our study where the CPL group tended to have a higher milk yield at 305 day of lactation. Karatzia et al. (2013) concluded that clinoptilolite increased milk production and improved reproductive variables. Other authors administrated 200 g daily, twice the dose used in the present study. In spite of this, our results also indicate that the dietary administration of clinoptilolite improved the reproductive performance of dairy cows. Perhaps the physical properties of the clinoptilolite (natural CPL modified by vibroactivation and micronization to reduce particles to 4.28 µm in size) used in our study were critical for the similar efficacy.

It could be concluded that the dietary clinoptilolite supplementation had a positive influence tended to improved reproductive performance in terms of a decreased NSP, and fewer DFS and DOP, exhibited modulating effects on the endocrine status of dairy cows, and improved milk yield.

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