Addition of Clinoptilolite In The Diet Reduces Uterine PMN Leukocytes And Open Days In Multiparous Lactating Dairy Cows Managed In A Mountain Tropical Pasture-Based System

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Research Article

Abstract

This study was aimed to assess the effect of adding clinoptilolite in the diet on uterine health and reproductive performance in multiparous lactating dairy cows managed in a tropical pasture-based system above 2500 meters of altitude. Seventy-seven multiparous Holstein crossbred cows were allocated randomly into two groups: clinoptilolite supplemented cows (CLG, n = 42) and non-supplemented cows as control (CG, n = 35). Cows from CLG were supplemented with clinoptilolite from 30 days (50 g/cow/day) before to 60 days after calving (200 g/cow/day). Data were analyzed by general linear model, least squares means and chi-square test of SAS. In CLG cows, percentages of uterine PMN leukocytes (P<0.0001) and proportion of subclinical endometritis (P=0.0187) were lower than in CG. The interval calving to first corpus luteum was shorter (P=0.0759) in CLG than CG, and calving to first service interval was similar between treatments. Cows from CLG became pregnant 35 days earlier than CG cows (P=0.0224). In conclusion, daily addition of clinoptilolite in the diet decreased the proportion of cows with subclinical endometritis and shortened the interval from calving to conception in lactating dairy cows.

Introduction

The transition period extends from 6 to 8 weeks before the end of gestation to the onset of lactation (Roche et al., 2017). In this phase, dairy cattle shift from a physiological stage of gestation at which does no milk is produced to one of non-gestation and intense milk production (Drackley, 1999). During the transition important hormonal and metabolic changes occur. Cows undergo a process of adaptation of the digestive system and high demand for nutrients due to fetal growth and milk synthesis (Zebeli et al., 2015). This physiological stage leads the cows to develop a state of oxidative stress and negative energy balance that significantly reduce voluntary dry matter intake and depresses the immune system (Sordillo, et al., 2009; Zebeli et al., 2015). This physiological, metabolic and nutritional context favors occurrence of numerous disorders such as ketosis, placental retention, abomasal displacement, and bacterial infections such as mastitis and endometritis. These conditions seriously affect productive and reproductive performance (Mulligan and Doherty, 2008), and cause death or culling of around 10% of affected cows (Pinedo et al., 2010).

Subclinical endometritis, an inflammatory disease of the reproductive tract, occurs very frequently in early postpartum in dairy cattle, because of the natural lacerations to superficial layer of the endometrium during calving and concomitant bacterial contamination of the uterus (Pascottini and LeBlanc, 2020). This situation is aggravated because the immune system is depressed during the peripartum period (Sordillo et al, 2009). Likewise, as this reproductive disorder occurs without clinical symptoms, it is not detected or treated, increasing the risk of developing more serious and difficult-to-treat uterine diseases (Bromfield et al., 2015). In addition, subclinical endometritis reduces reproductive efficiency (Quintela et al., 2017), increases the risk of cows being culled due to infertility (LeBlanc et al., 2002), and causes significant economic losses in the herd (Inchaisri et al., 2010).

Considerable attention has been paid to nutritional management of cows during transition (Drackley and Cardoso, 2014; Lacasse et al., 2018). The addition of some supplements in diets to meet the nutritional needs of dairy cows in transition (Little et al., 2017), has contributed to reducing adverse effects during this critical period, and has favored milk production, reproduction and herd health (Osorio et al., 2016; Little et al., 2017).

Zeolites are natural minerals of volcanic origin described as crystalline hydrated aluminosilicates of alkali and alkaline earth cations having an infinite and open three-dimensional structure (Ivkovic et al., 2004). These chemical and physical characteristics confer catalytic, adsorption and ion exchange properties to zeolites (Valpotić et al., 2017). Among natural zeolites, clinoptilolite has been used as a feed supplement in zootechnical and biomedical fields with multiple beneficial effects (Valpotić et al., 2017). Addition of zeolite to a cattle diet modified the molar ratio of volatile fatty acids, decreasing acetate and increasing propionate, which may have a positive effect on energy efficiency (Urías-Estrada et al., 2017). Daily administration of 200 g of clinoptilolite to heifers from day 210 of gestation until the end of first lactation significantly increased body condition, serum concentration of glucose, milk production and reproductive variables (Karatzia et al., 2013). In a previous study, a greater proportion of multiparous Holstein cows supplemented with zeolite during the prepartum and postpartum (180 and 270 g respectively) achieved better body condition at 45 days postpartum, a follicle ≥ 10 mm was detected at 35 days postpartum and had a lower percentage of PMN leukocytes in the uterus compared to non-supplemented group (Garzón et al., 2017).

The stimulating effect of clinoptilolite on the immune system has been also documented in several studies in cattle. For instance, in Holstein cows, addition of 3% clinoptilolite to the basal diet, from 4 weeks prior to expected parturition until 12 weeks after calving, increased milk production and decreased somatic cell count (Ural, 2014). In a recent study, supplementation of Holstein cows with 100 g of clinoptilolite daily from 7 months of pregnancy to day 75 postpartum (Đuričić et al., 2020), decreased the number of bacteria species causing mastitis and the number of affected quarters, as well the risk of intramammary infection compared to control group (Đuričić et al., 2017).

Information is scarce about the effect of zeolites on the reproductive activity in cattle. This study was aimed to evaluate the effect of adding clinoptilolite in the diet, from 30 days before to 60 days after calving, on uterine health and reproductive performance in multiparous lactating dairy cows managed in a pasture-based system above 2500 meters of altitude, in the southern of Ecuadorian Andean highlands.

Materials And Methods

Study location

This study was conducted in two commercial dairy farms located at 15 to 20 kilometers from the city of Cuenca, Azuay province, Ecuador, over 2500 meters above sea level. The area has an average temperature of 12.3°C and a rainfall of 1080 mm/year. These dairy farms had, in general, a similar

management system, based on grazing, mechanical milking (twice daily) and a milk production of approximately 5000 kg/year.

Animals, feeding and treatments

Seventy-seven multiparous (4.3 ± 1.3 calving) Holstein cows with a body condition score (BCS) at calving of around 3 (2.95 ± 0.08) (1−5 scale; Edmonson et al., 1989) were included in this study. All cows grazed a mix of fresh pasture consisting of perennial ryegrass (*Lolium perenne*), kikuyo (*Cenchrus clandestinus*), annual ryegrass (*Lolium multiflorum*), white clover (*Trifolium repens*), red clover (*Trifolium pratense*), which varied in different proportions according to farm and pastures where the cows grazed before and after calving. Cows were supplemented with 2 kg/day of concentrate from 30 days before calving, and with 4−6 kg/day after calving; also, cows had free access to mineral supplementation and water. The chemical composition of the basal diet and the concentrate are shown in Table 1.

Table 1. Chemical composition of the grass and concentrate used in the diet of multiparous lactating dairy cow

	Diet composition (%)				
	DM	EE	CP	CF	NFE
Farm A					
Grass					
dry period	12.3	2.3	19.2	27.9	34.4
lactation	15.1	2.5	23.8	24.5	38.4
Concentrate	89.5	3.4	18.0	18.2	56.8
Farm B					
Grass					
dry period	10.4	2.4	18.2	27.5	33.5
lactation	15.5	2.5	23.6	23.9	38.4
Concentrate	89.1	3.4	17.5	17.6	54.5

DM: dry matter; EE: ethereal extract; CP: crude protein; CF: crude fiber; NFE: nitrogen free extract

Cows were allocated randomly into two groups: clinoptilolite supplemented group (CLG, n = 42) and control group (CG, n = 35). The experiment was conducted from day 30 before the expected date of calving until day 60 postpartum. During prepartum period, CLG cows were supplemented with 50 g/cow/day of clinoptilolite (Captalin®; La Colina Cia. Ltda., Cuenca, Ecuador) (www.lacolina.com.ec) and after calving with 200 g/cow/day (following the manufacturer's recommendation). Clinoptilolite mixed with the concentrate was dispensed once a day in individual feeders. Cows with dystocia at calving, retention of foetal membranes, purulent or mucopurulent vaginal discharge after calving were excluded; hence, only cow clinically healthy were included in this study. According to farm, cows were distributed as follow, farm A (n = 42): CLG: 23 (4.2 ± 1.5 calving; BCS: 3.08 ± 0.35) and CG: 19 (4.3 ± 1.5 calving; BCS:

3.0 ± 0.30) cows; farm B (n = 35): CLG: 19 (4.3 ± 1.5 calving; BCS: 2.86 ± 0.35) and CG: 16 (4.4 ± 2.0 calving; BCS: 2.88 ± 0.45) cows.

All animals were handled according to procedures approved by the Veterinary Medicine Career Committee, Faculty of Agricultural Science, University of Cuenca, Ecuador, and the research was conducted following Chap. 7.8 of the Terrestrial Animal Health Code – 2019© OIE (08/07/2019), regarding to protection of animals used in scientific experiments.

Uterine cytology

Samples for endometrial cytology were collected in duplicate between days 35 and 45 (38.9 ± 3.6 days) after calving as described by Garzón et al. (2017). The cytobrush (CE-Model, ZY-H001, Caricia, Ecuador) was rotated onto a microscope slide, and each smear was air-dried and fixed with ethyl alcohol to preserve cellular morphology. Endometrial samples were stained with Wright® staining (JQWRG-1 K0-00; Quimical, Ecuador), and observed under a microscope (Olympus CX31; Germany) at × 400 magnification. Two hundred cells were counted to determine the proportion of PMN leukocytes in each slice. Cows with ≥10% of polymorphonuclear leukocytes were considered to have subclinical endometritis, as described by Kasimanickam et al. (2004).

Ultrasonography

The ovaries of each cow were examined by ultrasound once a week for three consecutive weeks, starting at day 19.6 ± 3.6 after calving, with a portable ultrasound scanner (Draminski, iScan, Poland) and a 7.5 MHz linear array transductor. The diameters of all detected follicles, day of detection, and size of the first corpus luteum were recorded. Follicles were categorized according to size into three classes: < 5 mm (class A), 5–10 mm (class B), ≥ 10 mm (class C).

Reproductive variables

Following a voluntary waiting period of 60 days after calving, estrous detection was performed by visual observation for 1 hr twice a day (06:00 to 07:00 hr and 17:00 to 18:00 hr). In cows in estrus, artificial insemination was performed according to AM-PM rule, using frozen-thawed commercial bull semen with proven fertility. Pregnancy diagnosis was performed by rectal examination 45 days after artificial insemination in cows with non-return to estrus by an experienced veterinarian. The insemination dates and number of service per conception were recorded, and the following reproductive variables were calculated: intervals from calving to first corpus luteum, to first service and to conception, number of services per conception and first service conception rate.

Statistical analysis

Variables showing a skewed distribution according to the Shapiro-Wilks test were *Log10* transformed. Intervals to first corpus luteum, to first service and to conception, number of service per conception, number and percentage of PMN (number of PMN leukocytes divide by total cell number × 100) were

analyzed by general lineal model of SAS (V 9.3; SAS Institute, Inc., Cary, NC, USA). Effects of treatment, farm, parity and treatment · farm interaction were included in the statistical model. Means were compared using least squares means test of SAS, and data were expressed as means ± SEM. Proportion of cows with subclinical endometritis, first service conception rate and accumulated pregnancy at 45, 60, 75, 90 days after calving were analysed by chi-square test of SAS. Statistical significance was considered as P < 0.05, and P values between 0.051 and 0.1 were considered as tendency.

Results

Body condition decreased as the postpartum days increased, without significant differences between experimental groups during the first 36 days after calving (Table 2). Numbers of follicles in different classes were similar between groups at all three ultrasound evaluations, with the exception of 5–10 mm (class B) follicles, whose number was significantly greater at the second ultrasound assessment in CG cows (P = 0.0077). The CLG group had the largest follicle at the first (P = 0.0325) and third (P = 0.0282) ultrasound examinations (Table 2).

Table 2
Body condition and follicular characteristics (mean ± SEM) in early postpartum in multiparous lactating dairy cows supplemented with clinoptilolite

Variables	Treatment		P-value		
	Clinoptilolite (n = 42)	Control (n = 35)	T	F	T × F
Body condition at calving	2.92 ± 0.08	2.99 ± 0.08	0.4827	0.1027	0.3778
First ultrasound (days) [1]	20.0 ± 3.8	19.3 ± 3.4	-	-	-
body condition	2.84 ± 0.06	2.88 ± 0.06	0.5707	0.3791	0.7702
class A follicles (n)	7.7 ± 2.0	10.2 ± 1.8	0.2440	0.0254	0.6993
class B follicles (n)	1.32 ± 0.44	1.40 ± 0.40	0.7754	0.0034	0.9684
class C follicles (n)	1.20 ± 0.21	1.15 ± 0.19	0.9886	0.3714	0.8793
largest follicle (mm)	14.2 ± 1.3	11.3 ± 1.2	0.0325	0.7896	0.3150
Second ultrasound (days) [1]	28.1 ± 3.8	27.6 ± 3.7	-	-	-
body condition	2.82 ± 0.07	2.81 ± 0.07	0.9097	0.6799	0.6847
class A follicles (n)	7.8 ± 1.3	7.3 ± 1.4	0.8661	0.4538	0.8275
class B follicles (n)	0.98 ± 0.24	1.90 ± 0.26	0.0077	0.2506	0.3551
class C follicles (n)	1.17 ± 0.16	1.05 ± 0.16	0.9001	0.1813	0.9172
largest follicle (mm)	12.5 ± 1.2	11.4 ± 1.3	0.4897	0.7153	0.9248
Third ultrasound (days) [1]	34.1 ± 3.0	35.6 ± 3.7	-	-	-
body condition	2.80 ± 0.07	2.68 ± 0.08	0.1874	0.3881	0.5306
class A follicles (n)	8.0 ± 1.9	8.0 ± 1.8	0.8416	0.5881	0.7829
class B follicles (n)	1.03 ± 0.33	0.98 ± 0.32	0.5767	0.0357	0.1259
class C follicles (n)	0.97 ± 0.17	0.73 ± 0.16	0.2865	0.4060	0.1947
largest follicle (mm)	14.7 ± 1.5	10.2 ± 1.5	0.0282	0.2489	0.6884

T: treatment; F: farm; T × F: treatment × farm. [1] Mean ± SD.

Follicle categories: class A: ≤5 mm; class B: >5 < 10 mm; class C: >10 mm.

Number and percentage of uterine PMN leukocytes were lower (P < 0.0001) in CLG than in CG (Fig. 1). For number and percentage of uterine PMN leukocytes there was no effect of farm, treatment · farm or parity.

The interval from calving to first corpus luteum was shorter (P = 0.0759) in CLG than CG, while calving to first service interval was similar between treatments. Likewise, cows from CLG became pregnant in 35 days earlier than CG cows (P = 0.0224). Number of services per conception, first service conception rate,

diameter of the corpus luteum, and proportion of cows with a corpus luteum at day 30 postpartum did not differ between groups (Table 3). Proportion of cows with subclinical endometritis was significantly lower (P = 0.0187) in CLG than in CG. For days open there was a treatment · farm interaction. On farm A, calving to conception interval was 18.1 days longer in CLG than in CG (123.3 ± 14.6 v 105.2 ± 15.7 days, respectively; P = 0.3750), whereas in farm B this interval was 86.2 days shorter in CLG than in CG (96.5 ± 16.1 v 182.7 ± 17.5 days, respectively; P = 0.0002).

Table 3. Postpartum intervals, services per conception, corpus luteum diameter (mean ± SEM), first service conception and proportion of multiparous lactating dairy cows with a corpus luteum at day 30 postpartum, and with subclinical endometritis according to whether or not they were supplemented with clinoptilolite.

Variables	Treatment		P-value		
	Clinoptilolite (n=42)	Control (n=35)	T	F	T × F
Postpartum intervals to (days)					
1st corpus luteum	23.5 ± 1.6	27.0 ± 1.6	0.0759	0.6227	0.0743
first service	76.6 ± 6.3	75.4 ± 6.2	0.8620	0.3032	0.9937
conception	109.9 ± 12.0	144.0 ± 12.4	0.0224	0.0878	0.0010
Number of services per conception	1.48 ± 0.19	1.78 ± 0.18	0.1882	0.9177	0.0831
Corpus luteum diameter (mm)	25.9 ± 1.5	24.0 ± 1.6	0.3258	0.5963	0.8708
Cows with a CL at day 30 (%) *	54.7	42.8	0.2982	–	–
First service conception (%)	58.8	52.0	0.6019	–	–
Subclinical endometritis (%)	4.7	22.8	0.0001	–	–

T: treatment; F: farm; T ´ F: treatment ´ farm. * proportion of cows with a corpus luteum at day 30 postpartum

Although not significant, cumulative pregnancy rates in the first 90 days postpartum were between 4.7 and 15.7 percentage points greater in CLG than in CG (Fig. 2).

Discussion

This study aimed to assess the effect of supplementing clinoptilolite to multiparous dairy cows managed in a mountain tropical pasture-based system on reproductive performance and uterine health. The results showed that addition of clinoptilolite to the diet significantly reduced the interval calving to conception, the percentage of uterine PMN leukocytes, and the proportion of cows with subclinical endometritis. Clinoptilolite may have improved uterine health and reduced the interval to conception through two mechanisms: 1) modifying ruminal physiology (Urías-Estrada et al., 2017) and as a consequence

improving the metabolic status (Karatzia et al., 2013), and 2) strengthening the immune system of cows (Ural, 2014; Đuričić et al., 2017; Đuričić et al., 2020).

In the current study, body condition did not differ between groups along the first 35 days postpartum, indicating that addition of clinoptilolite in the diet did not improve the energetic balance of cows in the early postpartum. In a previous study, supplementation of a greater dose of clinoptilolite (180 and 270 g/cow/day during 60 days before and 45 days after calving, respectively) to the basal diet improved body condition score in multiparous lactating dairy cows managed under similar conditions to the current study (Garzón et al., 2017). In another study, clinoptilolite supplementation (200 g daily) increased body condition score and blood serum concentration of glucose and acetoacetate, and decreased blood serum concentration of ketone bodies, without affecting milk production (Karatzia et al., 2013). However, both studies were conducted in animals (cows and heifers respectively) in which supplementation with clinoptilolite began between 210 to 225 days of gestation, and exhibited a considerably better body condition at calving.

Although the follicular categories were, in general, similar between treatments, ultrasonographic monitoring determined larger follicles in CLG cows. That finding is consistent with numerical trends in later reproductive variables, such as interval to the first corpus luteum, first service conception rate, proportion of cows with a corpus luteum at day 30 postpartum (these variables did not differ statistically), and the significant difference in open days.

Rapid resumption of cyclic ovarian activity after calving is essential to achieve maximum productivity in the dairy herd (Giordano et al., 2012). In this study, resumption of ovarian cyclicity determined by detection of a corpus luteum by ultrasound in the first 35 days after calving was slightly faster in CLG than CG, although diameter of luteal tissue did not vary between groups. As a reference, proportion of cows with a corpus luteum at day 30 after calving was around 12 percentage points greater in CLG than in CG (P = 0.2982). No published data were found on the effect of the addition of clinoptilolite in the basal diet on resumption of ovarian cyclicity, as determined in the present study. However, supplementation of dairy heifers with 200 g of clinoptilolite reduced the interval to the first postpartum estrous by 6 days (Karatzia et al., 2013). Based on the detection of a follicle $\geq$ 10 mm as indicative of resumption of ovarian activity, Garzón et al. (2017) found that supplementation of multiparous dairy cows with clinoptilolite (180 and 270 g/cow/day during the dry period and early postpartum respectively) doubled the proportion of cows that returned to ovarian activity within a period of time 45 days after calving.

In the aforementioned studies, the supplementation of greater amounts of clinoptilolite began at least 60 days before the expected date of calving, whereas in our study it began 30 days before parturition with 50 g/cow/day of clinoptilolite. The addition of clinoptilolite prior to parturition was intended to promote the adaptation of this additive in the rumen. The considerably lower amount added to the diet during this period in the current study may explain the lack of effect on body condition and on resumption of reproductive cyclicity and calving to first service interval. It is reasonable that adding a larger amount of clinoptilolite over a longer period before parturition may have an effect on specific aspects of the body

physiology leading to an energy balance favorable to reproductive function, as demonstrated by Karatzia et al. (2013) and Garzón et al. (2017). It is well known that a positive energy balance and a suitable postpartum body condition influence reproductive performance (Spicer et al., 1990; Butler and Smith, 1989). Specific traits included increased circulating concentrations of progesterone, estradiol and LH, enhanced number and quality of oocytes retrieved by ultrasound-guided transvaginal aspiration (Kendrick et al., 2010), reduced intervals to first estrus and increased conception rate (Beam and Butler, 1999; Butler, 2003).

Addition of clinoptilolite significantly decreased the number and percentage of uterine PMN leukocytes. In a similar study in the southern Ecuadorian Andes (Garzón et al., 2017), cows supplemented with clinoptilolite had a significantly lower percentage (3.4%) of PMN leukocytes than the control group (22.3%), which agrees with findings in this study. Moreover, clinoptilolite significantly reduced the proportion of cows with subclinical endometritis, a finding not previously reported in cows. In a different experimental context, addition of clinoptilolite to lactating dairy cows decreased somatic cell count (Ural, 2014), and numbers of bacterial causing mastitis, as well as the risk of intramammary infection compared to control group (Đuričić et al., 2017; Đuričić et al., 2020).

Bacteria colonizing the uterus during and immediately after calving, many of them pathogenic (Williams et al., 2007), adhere to endometrium, and even penetrate deeper layers, causing varying degrees of infertility (Lazzari et al., 2011). Likewise, pathogenic bacteria produce endotoxins that affect reproductive performance of cows in various ways (Suzuki et al., 2001; Williams et al., 2001; Williams et al., 2008; Lavon et al., 2008), several of them related to the production of inflammatory mediators (Williams et al., 2007).

Clinoptilolite has been shown to have antibacterial, antioxidant and anti-inflammatory and immunomodulatory effects in various species of mammals (review by Valpotić et al., 2017; Pavelić et al., 2018; Mastinu et al., 2019). For instance, in mice, adding clinoptilolite in the food ration for 5 months reduced cell death induced by reactive oxygen species (ROS) and production of ROS in mitochondria (Montinaro et al., 2013). In weaned pigs, supplementing clinoptilolite in the diet reduced total bacteria in jejunum and diarrhea severity, and increased recruitment of CD54RA^{+} cells (Valpotić et al., 2017). Addition of 200 g of clinoptilolite for 70 days in cows (E. coli-vaccinated on day 210–240 of gestation) increased specific antibody titers against E. coli in their serum and colostrum and in the serum of their calves (Karitzia et al., 2010). Feeding newborn calves with 3 liters of maternal colostrum containing 5 g/liter of clinoptilolite caused 50% greater IgG levels at 24 and 48 h after birth than control calves (Fratric et al., 2005). In poultry, addition of 2 or 3% of clinoptilolite in the diet (for 40 days) to broiler chicks increased percentages of T lymphocytes expressing CD4 + CD25+, and B lymphocytes expressing BU-1 + and MHC Class II + molecules, and in the CD4:CD8 ratio (Jarosz et al., 2017). Although the precise mechanism by means clinoptilolite increases immune activity in animals is unknown yet, this body of evidence clearly demonstrates the immune-stimulating property of this compound.

In light of the experimental evidence cited above, and results of the current study, it is likely that cows on CG diet had a weaker immune system, and as a consequence, had a greater number and percentage of uterine PMN leukocytes. Therefore, more of them had percentages of uterine PMN leukocytes indicative of subclinical endometritis.

According to evidence discussed above, it is plausible that the better reproductive performance achieved by CLG cows occurred due to the antioxidant and immune-stimulating properties of clinoptilolite. As a consequence, a healthier uterine environment favored clinoptilolite-supplemented cows to became pregnant earlier, with a first service conception rate 7 percentage points greater and 0.3 fewer inseminations than CG group. Moreover, CLG cows reached an accumulated pregnancy at 90 days postpartum 15.7 percentage points greater than CG. Ninety days postpartum is a critical time by which the reproductive goal of one calf and one lactation per year is attained (Perea et al., 2001). Each additional day to achieve pregnancy has a significant economic cost for dairy farms (De Vries, 2006). Thus, addition of clinoptilolite to the feed ration from prepartum and during postpartum may be implemented as a routine strategy to improve uterine health and reproductive efficiency in dairy farms.

In conclusion, this study showed that the daily addition of clinoptilolite in the diet prior to calving (50 g daily) and up to 60 days postpartum (200 g daily) had beneficial effects on reproduction by reducing the prevalence of subclinical endometritis and open days in multiparous lactating dairy cows managed in a mountain tropical pasture-based system.

Declarations

Author contribution

JG, AG and GL conceived and designed research. FS and JZ conducted experiment and wrote original draft. GC and FQ contributed new reagents or analytical tools. FP: analyzed data, reviewed and edited the manuscript.

Conflict of interest

The authors declare to have no conflict of interest.

Data availability

Data will be made available on reasonable request.

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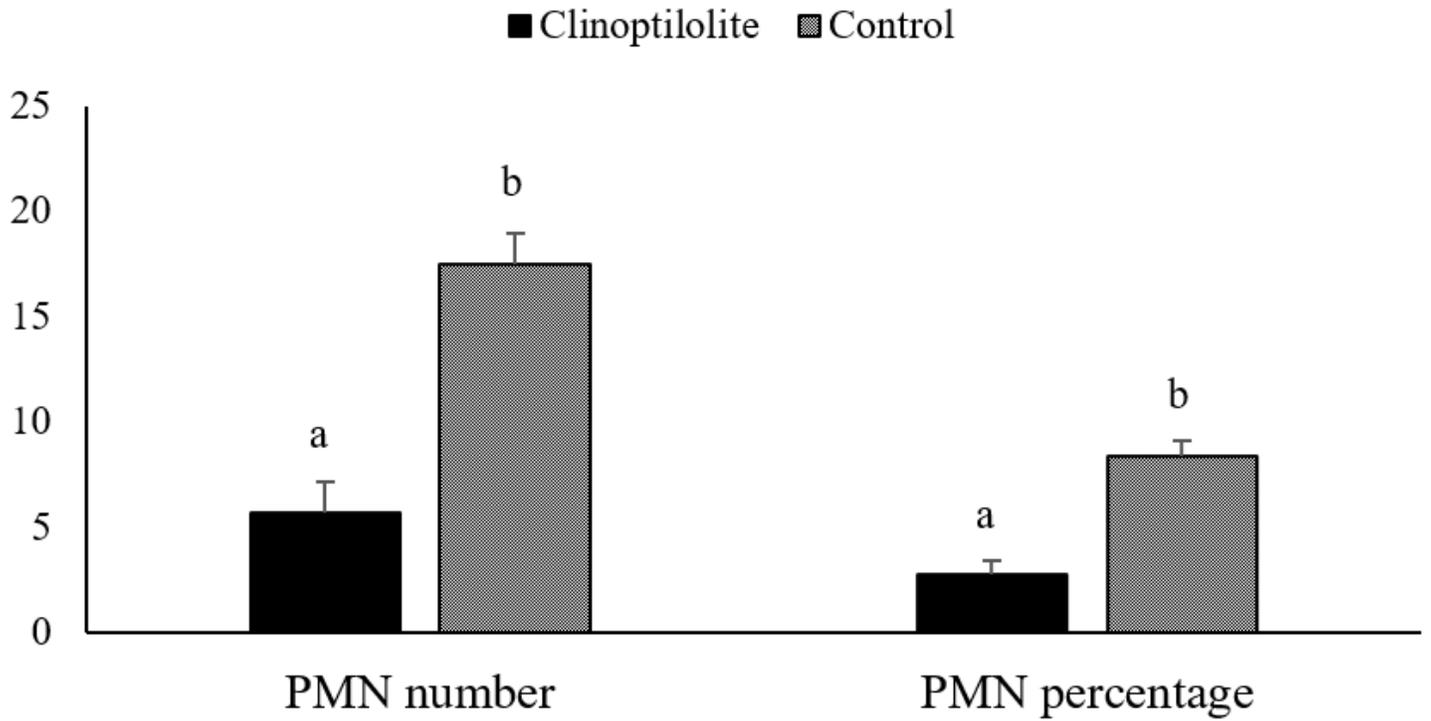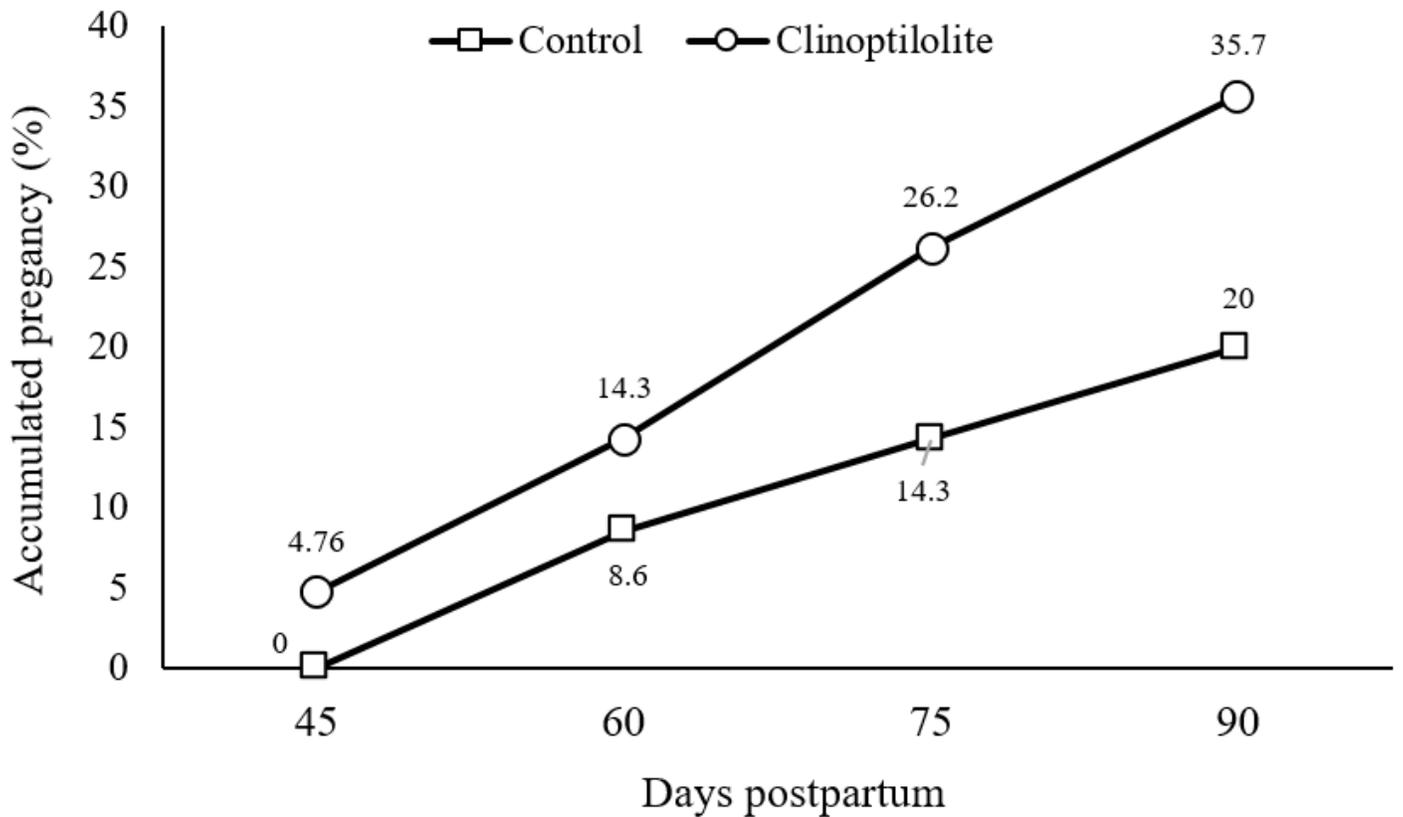
Figures

Figure 1

Number and percentages of uterine PMN leukocytes in the early postpartum in multiparous lactating cows according to whether or not they were supplemented with clinoptilolite. a-b P<0.0001.

Figure 2

Accumulated pregnancy at 45, 60, 75 and 90 days postpartum in multiparous lactating dairy cows according to whether or not they were supplemented with clinoptilolite.