Journal of Applied Animal Research

ISSN: 0971-2119 (Print) 0974-1844 (Online) Journal homepage: https://www.tandfonline.com/loi/taar20

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To cite this article: Khouloud Khachlouf, Houda Hamed, Radhouane Gdoura & Ahmed Gargouri (2019) Effects of dietary Zeolite supplementation on milk yield and composition and blood minerals status in lactating dairy cows, Journal of Applied Animal Research, 47:1, 54-62, DOI: 10.1080/09712119.2018.1563548

To link to this article: https://doi.org/10.1080/09712119.2018.1563548

Published online: 01 Jan 2019.

JOURNAL OF APPLIED ANIMAL RESEARCH
2019, VOL. 47, NO. 1, 54–62
https://doi.org/10.1080/09712119.2018.1563548

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Effects of dietary Zeolite supplementation on milk yield and composition and blood minerals status in lactating dairy cows

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ABSTRACT

The purpose of this study was to determine effects of inclusion of Zeolite in lactating dairy cows' rations on blood Ca, P, and Mg status during periparturient period, as well as on milk yield and composition during early lactation. Forty-two pregnant dry Holstein cows were randomly assigned as Zeolite treated cows (EG) or untreated cows (CG) comprising 21 cows each. The EG group received the same diet as the CG group, but with addition of 200 g/cow/day of Zeolite. There was no treatment effect on milk total solids, milk fat, milk protein, lactose, milk ash, milk Ca, milk P, milk Mg, plasma P, and plasma Mg for CG and EG. Conversely, milk yield, fat corrected milk (FCM), fat yield, protein yield, lactose yield, and plasma Ca were significantly increased by Zeolite addition. These results indicate that Zeolite can be effectively used in the rations of dry and lactating cows with positive effects on milk production and components yields and no deleterious effects on milk composition or blood parameters. Blood Ca was enhanced around calving and at the beginning of lactation. It is suggested that prepartum Zeolite supplementation may alleviate the negative Ca balance and therefore reduce the incidence of subclinical hypocalcaemia during the periparturient period.

ARTICLE HISTORY
Received 27 September 2018
Accepted 19 December 2018

KEYWORDS
Dairy cows; blood parameters; productive parameters; Zeolite

1. Introduction

A major challenge for dairy producers, managers, and veterinarians is to maintain a dairy cow's health during the periparturient period (the dry period and the first 3–4 weeks after calving). This transition period is characterized by the appearance of periparturient disorders, such as hypocalcaemia or milk fever. It is a metabolic disease caused by a low blood Ca level close to calving. It occurs when cows are unable to compensate for the dramatic increase in Ca needed for colostrum and milk production at calving (Smith and Risco 2005; Charbonneau et al. 2006). The most dramatic clinical milk fever is made when Ca blood concentration levels are lower than 60 mg/L (Allen and Sansom 1993). The diagnosis of subclinical milk fever, a milder form of hypocalcaemia, using blood Ca concentration values is more difficult, but measured values in the range of 62–75 mg/L have been suggested (Risco et al. 1984; Shearer and van Horn 1992).

Milk fever or parturient paresis (between 3% and 10% of cows) generally increases the risk for dystocia, retained foetal placenta, ketosis, and mastitis, and tends to increase calving intervals and slightly reduce milk yield (Stevenson and Call 1988; Goff 2006). Occurrence of milk fever increases when high-calcium feeds are offered during the dry period. Older cows and all cows with high milk production are at greater risk for milk fever. One potential way of preventing parturient hypocalcaemia involves feeding a prepartal diet that is Ca deficient (<20 g/d or 0.5% Ca diet). However, this method of prevention has been almost abandoned because it is difficult to formulate rations sufficiently low in calcium when using commonly available feeds (Thilsing-Hansen et al. 2002, 2007).

Recent studies have shown, however, that it is possible to prevent milk fever, as well as subclinical hypocalcaemia, by supplementing the dry cow ration with substance that has the capacity to bind Ca in the intestinal tract such as sodium aluminium silicate (Zeolite) and thereby making it unavailable for absorption (Thilsing-Hansen and Jørgensen 2001; Wilson 2001; Thilsing-Hansen et al. 2002, 2003, 2007), resulting in an activation of the calcium homeostatic mechanisms before calving.

Natural (Clinoptilolite) or synthetic Zeolites (Zeolite A) are crystals formed from a microporous aluminosilicate skeleton of alkali and alkaline earth cations which are encountered worldwide and having an infinite, open, three-dimensional structure. These materials have unique chemical and physical properties and are characterized by their ability to lose and gain water reversibly, to absorb substances with a suitable cross-sectional diameter (adsorption property) and to switch their cations with cations from their environment such as Ca^{2+}, Mg^{2+}, K^{+}, and NH_4^{+}, without major structural change (cation exchange capacity-CEC $\approx$ 220 mEq/100 g) (Filippidis et al. 1996; Bosi et al. 2002).

The facility of these supplements to liberate progressively ions in the rumen has created in the past 20 years an interest to use them as feed additives for ruminants, mainly in order to improve performance traits and for the prevention of

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certain metabolic diseases in dairy cattle. Recently, Zeolite has been approved as an additive in farm animals feeds by the European Committee at the highest inclusion rate of 2% of dry matter (European Commission Regulation 2005). The effectiveness of Clinoptilolite against intoxication by mycotoxins, as well as the increased interest for the use of feed additives that do not have residuals on the animal products, are expected to increase the use of Clinoptilolite as a feed additive (Valpotić et al. 2017; Benić et al. 2018).

Most of the studies concerning the use of Zeolite in the dairy cows ration were conducted during the lactation period and focused on the productive parameters (milk yield and composition) and ruminal environment. Among these studies we can mention, for example, those of Bosi et al. (2002), Dschaak et al. (2010), Sulzberger et al. (2016) and Đuričić et al. (2017). For more details, an interesting review on the effects of Zeolite supplementation on dairy cow production and ruminal parameters was recently published by Khachlouf et al. (2018).

On the other hand, there is a paucity of published information concerning the use of Zeolite during the critical periparturient period. Therefore, the current study was undertaken to evaluate the effect of introducing the calcium binding substance Zeolite in the dairy cow rations before and after calving on blood Ca, P, and Mg levels, as well as on milk production and composition during early lactation. It is also tested the hypothesis that Zeolite supplementation will benefit periparturient Ca status.

2. Materials and methods

2.1. Animals and diets

This experiment was conducted in a state farm in the region of Sfax (Tunisia). This farm adopts an aboveground farming system (Zero grazing) and applies the loose-housing systems. The trial took place from the 40 d before the expected date of calving until the 60 d of lactation. The cows were evenly allocated into two homogeneous groups: control group (CG) and experimental group (EG) kept in separate pens. Each treatment group consisted of 21 Holstein cows that calved between February and August 2016. The groups were balanced with respect to lactation number (20 primiparous + 22 multiparous), body weight (≈ 550 ± 25 kg), expected date of calving and milk production during the previous lactation (22.40 ± 6.32 kg/d for multiparous). The basic control diet for CG and EG consisted of oat hay, and alfalfa pellets during the dry period and oat hay, oat silage, and alfalfa pellets after calving. This daily ration was formulated to meet the energy (Forage Unit for Lactation: UFL), and the protein (Digestible Protein in the small Intestine: PDI) requirements of the pregnant and lactating cows according to INRA (2007). The offered quantities were calculated to allow 10% orts (feed refused from a daily offering). The diets were supplemented with concentrates at a level of 6 kg per cow/d in dry period, and 12 kg per cow/d in early lactation. During these two periods, the total dry matter intake (DMI) was estimated 11.5, and 19.0 kg/cow per d, respectively. Additionally, cows in the dry period and lactation were supplemented with a commercial mineral balancer to meet the requirements of Ca, P, and Mg.

Cows were allowed ad libitum access to the basal ration and water, individually fed concentrate (06:00 and 16:00 h), and milked twice daily during lactation period (06:00 and 16:00 h).

The experimental diet (EG) was the same control ration, but additionally supplemented with 200 g/cow/d of Zeolite (sodium alumino silicate; Zeoline, B-4480 Engis, Belgium) thoroughly mixed into the morning meal concentrate of each cow. For this group, the adaptation period to the Zeolite diet started one week before launching the experiment. Cows remained in their treatment groups for the entire experiment. The different feed distributed to cows and their quantities are listed in Table 1. The chemical composition and nutritive values of the concentrate and basal diet used in this study are also presented in Tables 2 and 3.

2.2. Milk production and composition

After calving, individual milk yield was recorded at days 7, 20, 40, and 60 post-calving during the morning and the evening milking. The controls coincided with those carried out by the Livestock and Grazing Office. Simultaneously, milk samples were collected from each cow at each milking, using aseptic pots of 100 ml capacity. Samples were held at 4°C in a cooler box to prevent milk spoilage during the transport to the laboratory. Representative sub-samples for mineral analysis (20 mL)

Table 1. Quantities of food (kg/cow/day) distributed to control and experimental groups before and after calving.

	Before calving		After calving	
Feed	Control	Experimental	Control	Experimental
N (cows)	21	21	21	21
Concentrate (kg/cow/d)	6	6	12	12
Zeolite[a] (g/cow/d)	0	200	0	200
Oat hay (kg/cow/d)	6	6	4	4
Oat silage (kg/cow/d)	0	0	15	15
Alfalfa pellets (kg/cow/d)	1	1	1	1

[a]The Zeolite was mixed with the concentrate.

Table 2. Ingredients, chemical composition and nutrient values of concentrate of control and experimental diets used in this study.

Item	
Ingredients (%)	
Barley grain	19
Corn grain	49
Soybean meal	27
Premix (mineral/vitamin)	5
Chemical composition (% CM)[a]	
DM	87.5
OM	79.7
NDF	12.0
ADF	4.3
CP	17.6
EE	2.6
Ca	2.1
P	1.4
Nutritional value[b]	
UFL (/kg CM)	0.98
PDIN (g/kg CM)	129
PDIE (g/kg CM)	118

[a]Crude matter.
[b]Estimated from INRA Tables (2007).

Table 3. Chemical composition and nutrient values of forages used in cow diet.

	Oat hay[a]	Oat silage[a]	Alfalfa pellets[a]
Composition (% of DM)			
DM[b]	90.5	26.4	90.3
OM[c]	91.5	88.8	89.0
NDF[d]	58.0	53.5	42.6
ADF[e]	36.4	34.2	31.9
CP[f]	9.1	8.2	17.3
EE[g]	2.2	4.0	2.5
Ca	0.34	0.62	1.86
P	0.22	0.25	0.28
Nutritional value			
UFL[h] (/kg DM)	0.55	0.72	0.72
PDIN[i] (g/kg DM)	30	63	115
PDIE[j] (g/kg DM)	50	56	95

[a]Estimated from INRA Tables (2007), Nefzaoui and Chermiti (1989) and Nutrient Requirements of Dairy Cattle 7th revised edition (2001).
[b]Dry matter.
[c]Organic matter.
[d]Neutral detergent fibre.
[e]Acid detergent fibre.
[f]Crude protein.
[g]Ether extract.
[h]Forage unit for lactation.
[i]Digestible protein in the small intestine supplied by microbial protein from rumen-degradable protein (INRA 2007).
[j]Digestible protein in the small intestine supplied by microbial protein from rumen-fermented OM (INRA 2007).

were stored at −20°C and thawed at 4°C overnight before analysis.

Milk samples were analysed by an external collection centre in duplicate for total solids, fat, protein, and lactose using a near-infrared milk analyser (Milkoscan 6000; FOSS, Denmark) according to method 972.16 of AOAC (1990).

Additionally, the flame atomic absorption spectrometric method was used for the determination of milk minerals (Ca, P, and Mg) after mineralization and dilution of the samples with nitric acid (AFNOR NF ISO 8070 2007).

Finally, 3.5% fat corrected milk (FCM) was calculated by equation: (0.432 × milk yield) + (16.23 × milk fat yield) according to NRC (2001).

2.3. Blood sampling

Throughout the experimental period, individual blood samples (7–8 ml) were collected in the morning before the morning feeding from the jugular vein into Vacutainer tubes, 40 and 20 days before the expected date of calving, and at approximately 72 h and 20 days postpartum. At the laboratory, blood plasma was separated by centrifugation (1500 × *g* for 15 min at 4°C) and stored at −18°C until the analyses were performed.

The plasma Ca was performed by the colorimetric 5-nitro-5′-methyl-(1,2-bis(o-aminophenoxy) ethan-N,N,N′,N′-tetraacetic acid (NM-BAPTA) method developed by Roche on c701/Cobas Integra 7000® analyser (NM-BAPTA, Roche Diagnostics, Mannheim, Germany). Briefly, the calcium NM-BAPTA is based on the NM-BAPTA molecule which reacts with calcium ions under alkaline conditions to form a complex, modifying the colour of the mixture. This complex reacts in a second step with EDTA and the reaction equilibrium shifts towards the calcium–EDTA complex and free NM-BAPTA. The change in absorbance, proportional to the calcium concentration is measured at 340 nm (Bourguignon et al. 2014).

The plasma inorganic P was also assessed on c701/Cobas Integra 7000® analyser. This method utilizes ammonium molybdate as the colour-forming reagent. Measurement of the final product occurs at 340 nm. Inorganic phosphate forms an ammonium phosphomolybdate complex having the formula $(NH_4)_3[PO_4\ (MoO_3)_{12}]$ with ammonium molybdate in the presence of sulphuric acid. The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration.

The Mg level was measured by the Chlorophosphonazo III colorimetric assay on Roche Cobas Integra 7000® automate. In this method magnesium reacts with Chlorophosphonazo III (CPZ III) and causes an increase in absorbance. EDTA is added to inhibit calcium interference. Addition of EDTA to the reaction allows for accurate sample blanking. The reaction is a two-point, end-point method that is measured photometrically at 660 nm.

2.4. Statistical analysis

All data were analysed by repeated measures ANOVA using the general linear model (GLM) procedure of SPSS version 18. The main effects included in the initial model were treatment diet (CG, EG), period, and treatment × period interactions. These data were compared at the significance level of $P < .05$.

3. Results and discussion

Descriptions and summary statistics of relevant measured variables for control (CG) and experiment (EG) groups are provided in Table 4. In addition to the treatment effect, period, and the interaction of treatment and period were also studied for all parameters.

3.1. Milk production and composition

The milk yield was significantly affected by treatment, lactation period and their interaction (Table 4). The dietary Zeolite addition resulted in a significant increase in milk production

Table 4. Milk and blood parameters from cows on each treatment.

	Group			Statistic (*P*-value)		
Item	CG	EG	SEM	Group	Period	Group × Period
Milk parameters						
Milk yield (kg/d)	21.54	23.72	0.34	**.001**	**.000**	**.005**
FCM[a]	19.30	21.35	0.31	**.001**	**.000**	**.007**
Milk total solids (%)	11.88	12.01	0.54	.456	**.000**	.990
Milk fat (g/kg)	29.09	29.47	1.10	.273	**.000**	.988
Milk fat (kg/d)	0.62	0.68	0.01	**.001**	**.000**	**.018**
Milk protein (g/kg)	28.31	28.46	0.99	.638	**.000**	.995
Milk protein (kg/d)	0.61	0.68	0.01	**.001**	**.000**	**.004**
Lactose (g/kg)	43.07	43.09	0.15	.963	.624	.999
Lactose (kg/d)	0.93	1.02	0.10	**.002**	**.000**	**.006**
Milk ash (g/kg)	7.99	8.13	1.06	.706	**.076**	.998
Milk Ca (mg/kg)	1166.2	1168.6	12.0	.923	.400	.990
Milk P (mg/kg)	946.6	951.5	13.7	.861	.427	.988
Milk Mg (mg/kg)	119.8	118.2	1.0	.428	.988	.479
Blood parameters						
Plasma Ca (mg/L)	91.20	94.59	3.77	**.002**	**.000**	**.001**
Plasma P (mg/L)	66.01	68.72	9.38	.356	**.087**	.840
Plasma Mg (mg/L)	20.50	20.78	1.80	.622	**.000**	.763

Note: CG = Control group; EG = Experimental group; SEM = Standard error of mean.
[a]Calculated as (0.432 × milk yield) + (16.23 × milk fat yield).

during the first two months of lactation (+2.18 kg/cow/d; $P < .001$; Table 4). Owing to the interactive effect of treatment and period ($P = .005$; Table 4; Figure 1), the difference between both groups was more pronounced at the end of the experimental period. Many researchers have proved that the dietary inclusion of Zeolite improves average of yield of dairy cows (Katsoulos et al. 2006; Ilić et al. 2011; Ural et al. 2013; Cruywagen et al. 2015). Generally, an increase in milk production can result from increased ruminal concentrations of propionate, increased postruminal digestion of starch, increased microbial protein synthesis, increased by-pass protein, or from a combination of these factors (Garcia-Lopez et al. 1992; Katsoulos et al. 2006).

Others authors suggested that cows supplemented with Zeolite were able to convert feed into milk more efficiently (Cassida et al. 1988; Ural 2014; Sulzberger et al. 2016). The results obtained from the present study and aforementioned researchers indicated that the administration of Zeolite improves the energy status of the animals and may have positive influence on milk yield (Katsoulos et al. 2006; Karatzia et al. 2013).

Another explanation for increases in milk production might be the potential effect of Zeolite on the DCAD (dietary cation-anion difference) of diets fed to the cows. Previous experiments indicate that buffers increased DCAD and that DCAD and milk yield are closely related (Hu et al. 2007). It appears that the DCAD of the diet affects the acid-base balance regulation, which in turn increased DMI of dairy cows. This might explain a part of the increase in milk production.

In certain studies, the milk yield was not affected by Zeolite inclusion in the diet (Thilsing-Hansen et al. 2002; Katsoulos et al. 2006; Migliorati et al. 2007; Grabherr et al. 2009) although the cows showed a reduced feed intake and hypophosphataemia. It appears that at low level of Zeolite inclusion (1–1.4% on a DM basis), milk yield was not affected.

The discrepancies in results indicate that several factors influence milk production when incorporating Zeolite in the rations and that there may be limitations that need to be considered.

In our study, milk composition (total solids, fat, protein, lactose, and ash) were unaffected by Zeolite inclusion (Table 4, Figures 2 and 3). These data are in agreement with results of other experiments using different sources of buffers (Cassida et al. 1988; Solorzano et al. 1989; Migliorati et al. 2007; Clark et al. 2009; Đuričić et al. 2017).

In contrast, other researchers reported that milk fat content increased or tended to increase when 1–2% of sodium sesquicarbonate or Zeolite was added to the diet (Cruywagen et al. 2015; Sulzberger et al. 2016). Ruminal buffers have been shown to prevent milk fat depression with rations based on corn silage or low-fibre diets (Harrison et al. 1989; Kennelly et al. 1999) by stabilizing rumen pH and thus offering a more suitable environment for microbial growth. Additionally, the buffer may increase ruminal outflow, increasing the acetate:propionate ratio and thus improving milk fat tests (Snyder et al. 1983; Cruywagen et al. 2015).

In general, it has been accepted that dietary buffers do not consistently alter protein percentage of milk (Cassida et al. 1988; Ghorbani et al. 1989; Katsoulos et al. 2006; Đuričić et al. 2017) during early or midlactation. This trend was confirmed in our study (Table 4; Figure 3), suggesting that protein metabolism was unaffected by the addition of Zeolite.

However, Tucker et al. (1994) and Dschaak et al. (2010) reported that milk protein percentage was increased with Zeolite supplementation during the complete lactation. This difference did not appear until midlactation and was most apparent during late lactation. Solorzano et al. (1989) noted that sodium sesquicarbonate increased digestibilities of CP (crude protein) and NDF (neutral detergent fibre). Addition of buffer may have increased AA (amino acid) supply to the mammary gland by promoting digestion of dietary proteins.

Finally, the FCM and the yields of fat, protein, and lactose were significantly affected by feeding system, lactation period, and the interaction of feeding system and lactation period, as a consequence of increasing milk yield in the Zeolite group (Table 4).

3.2. Milk minerals

Minerals such as Ca, P, and Mg play an important role in the structure and stability of casein micelles, and the susceptibility of the protein to aggregation during dairy processing (Holt and Jenness 1984; Gaucheron 2005). Especially, the calcium and phosphate contents are higher in milks rich in proteins. Also, Ca and P have a major influence on processing characteristics,

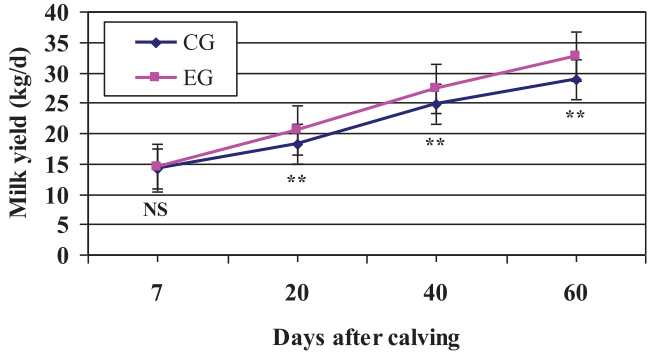

Figure 1. Mean milk yield in Zeolite-treated cows (EG) and untreated control cows (CG).

Note: NS, nonsignificant; $^{**}P < .01$.

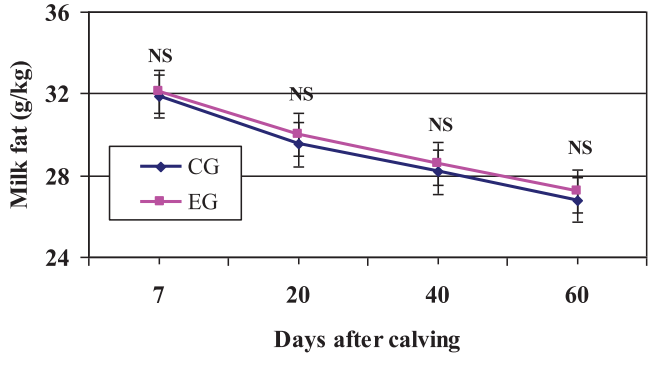

Figure 2. Mean milk fat content in Zeolite-treated cows (EG) and untreated control cows (CG).

Note: NS, nonsignificant.

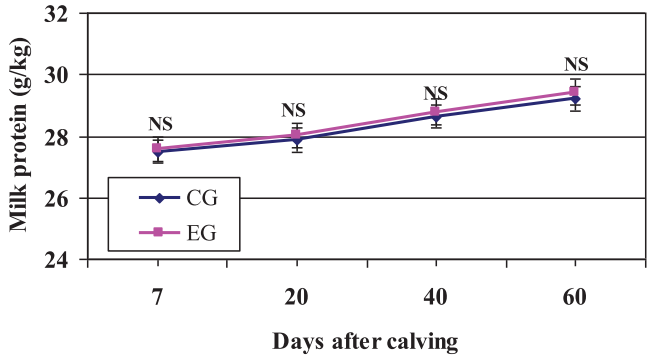

Figure 3. Mean milk protein content in Zeolite-treated cows (EG) and untreated control cows (CG).

Note: NS, nonsignificant.

such as rennet coagulation, heat stability, and ethanol stability (Tsioulpas et al. 2007; Sandra et al. 2012; Horne 2016). Apart from their effects on protein aggregation, variation in mineral content can also alter the nutritional value of milk and milk products (Cashman 2011; Pirilä et al. 2011).

Indeed, cow's milk and dairy products, widely consumed by children, are the best sources of Ca in human diet, helping to achieve adequate dietary Ca intake (Gao et al. 2005; Pirilä et al. 2011). Consequently, milk with higher Ca, P and Mg concentrations, is thus desirable.

3.2.1. Calcium

In our study, the range of Ca concentration for all milk samples collected over the experimental period (900–1290 mg/kg, data not shown) was lower in magnitude to that (1110–1470 mg/kg) reported by O'Brien et al. (1999), Auldist et al. (1999) and Gulati et al. (2018). Variations in milk Ca content have been clearly related to the breed, season, stage of lactation, parity, and feed strategy (Nogalska et al. 2017; Gaignon et al. 2018). In addition, it is well established that the secretion of Ca into milk was regulated by mammary gland secretion of parathyroid hormone-related protein (PTHrP), bone accretion and resorption dynamics, the digestive tract, and kidneys (Boudon et al. 2016; Gaignon et al. 2018).

The lower milk Ca content in Tunisian dairy heard was most probably related to the intensive production system based on conserved grass (such as silage or hay), and high quantities of concentrates (Nogalska et al. 2017; Gaignon et al. 2018).

Zeolite supplementation had no significant effect on the amount of Ca exported in milk ($P > .05$; Table 4 and Figure 4). In spite of the fact that blood Ca concentration in experimental cows was enhanced by Zeolite (see mineral blood section and Figure 7), it seems that the availability of blood Ca is not a determinant for Ca uptake by the mammary gland, at least under our experimental conditions.

As expected, during the early weeks of lactation, most cows remain under the negative Ca balance (Gabryszczuk et al. 2010). Milk Ca content slightly decreased (not significantly) during the first days of lactation for both groups (Figure 4) as a consequence of highest milk production and lowest milk casein content in that period (Litwińczuk et al. 2004; Nogalska et al. 2017; Gaignon et al. 2018). According to Bijl et al. (2013) and Gulati et al. (2018), there is a significant positive correlation between milk Ca and protein content (or casein content: about 66% of Ca is intimately associated with casein). In this study, as it was mentioned before, milk protein was not affected by Zeolite addition (see Figure 3). This can explain the non-notable improvement in the milk Ca content observed here.

On the other hand, Boudon et al. (2016) signalled that milk Ca content was highly correlated with DCAD, which was enhanced by Zeolite supplementation (Hu et al. 2007). Thus, the slight improvement in the milk Ca content in EG (Figure 4) was probably associated with increased DCAD and with decreased in the amount of Ca excreted in urine.

3.2.2. Phosphorus

The range of P content in all milk samples collected over the experimental period (750–1200 mg/kg, data not shown) was comparable in magnitude to that reported by Sola-Larrañaga and Navarro-Blasco (2009) and Gulati et al. (2018) (550–1120 mg/kg).

The concentration of P was not affected by treatment, period, and their interaction ($P > .05$; Table 4 and Figure 5). Thus, the Zeolite supplementation was shown to have no significant effect on the milk P concentration over the experimental period. This result was expected because milk protein content was not enhanced in the current study (about 50% of

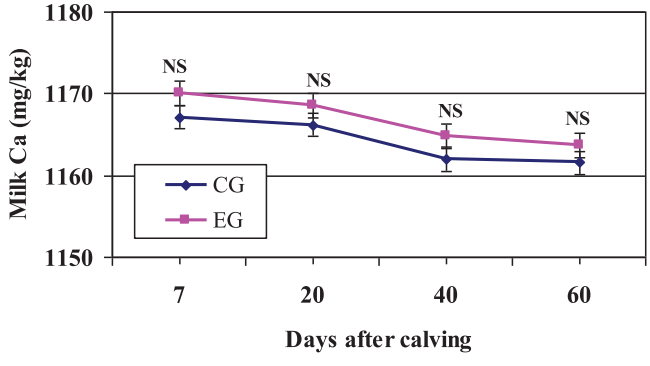

Figure 4. Mean milk Ca content in Zeolite-treated cows (EG) and untreated control cows (CG).

Note: NS, nonsignificant.

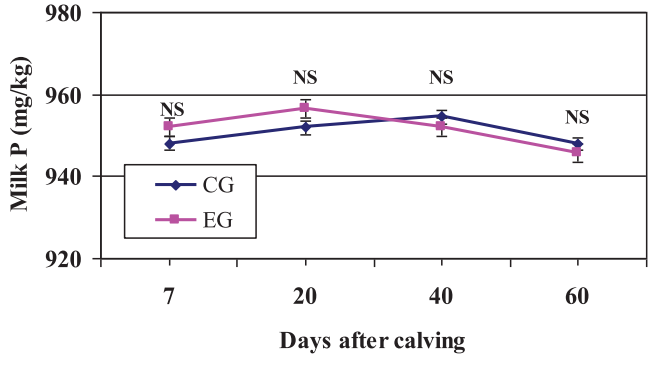

Figure 5. Mean milk P content in Zeolite-treated cows (EG) and untreated control cows (CG).

Note: NS, nonsignificant.

P is intimately associated with casein according to Gaucheron 2005 and Petrera et al. 2016).

3.2.3. Magnesium

The range of Mg across all milks (98–132 mg/kg, data not shown) was of similar magnitude to that previously reported in the literature (Rodríguez-Rodríguez et al. 2001; Gaucheron 2005; Gulati et al. 2018) and stayed within the reference interval (100–150 mg/kg) suggested by Litwińczuk et al. (2004). The mean Mg content was unaffected neither by treatment system nor by period (Table 4), and it remained stable along the experimental period (Figure 6). The absence of effect of Zeolite on milk Mg content might be related to the non-change in milk protein content in the current study (Bijl et al. 2013; Gulati et al. 2018). These authors found that the content of this mineral had a significant positive correlation with protein content which was largely due to its association in the casein micelle.

3.3. Blood minerals

In this study, we also investigated whether the chosen calcium binding substance had any effect on the blood constituents normally linked to calcium homeostasis (Ca, P, and Mg) during the dry period and early lactation.

3.3.1. Calcium

Statistical analysis revealed a significant ($P < .01$) difference in plasma Ca level between the two groups before and after calving. It is also interesting to report that there was a significant interaction between treatment and period on blood Ca content (Table 4 and Figure 7). Blood analysis showed a linear decrease ($P < .05$) in concentrations of plasma Ca before and after calving. But, the drop in the plasma Ca level was more pronounced in CG than that in the EG. This is usually due to the feed, especially the dietary Ca, being insufficient to meet the heavy demand due to the rapidly growing foetus in dry period or milk production in early lactation.

From looking at Figure 7, it is obvious that Zeolite supplementation had a stabilizing effect on blood Ca around calving. The increase in plasma Ca level by Zeolite supplementation around calving was in agreement with Thilsing-Hansen and Jørgensen (2001) and Thilsing-Hansen et al. (2002, 2003).

In this herd, the mean plasma Ca level in both groups did, however, stay above the hypocalcaemia limit of 85.0 mg/L suggested by DesCoteaux et al. (1997) and thus no cases of milk fever were recorded, and no cows were treated with calcium solutions intravenously.

The increased plasma calcium level closing to calving in the Zeolite-treated cows compared to untreated group is thought to result from an activation of the Ca homeostatic mechanisms before calving, rendering the cow ready for the sudden and massive draw on blood calcium around the time of calving (Thilsing-Hansen et al. 2002). Briefly, Zeolite supplementation was expected to decrease the availability of ration Ca below the requirements. This places the cow in negative Ca balance before calving. A drop in blood Ca stimulates parathyroid hormone (PTH) secretion from the parathyroid glands, which in turn increases renal reabsorption of Ca, enhances bone resorption and promotes renal vitamin D metabolism towards production of 1,25-dihydroxyvitamin D (1,25$(OH)_2$D). This last also increases the intestinal absorption of Ca (Goff et al. 1991; Horst et al. 1997).

The activation of the calcium homeostatic mechanisms in Zeolite group before calving could also be related to the increase in the dietary cation-anion difference (DCAD) by Zeolite supplementation. It is now well known that blood Ca concentration is negatively associated with DCAD (Charbonneau et al. 2006; Boudon et al. 2016; Goff and Koszewski 2018).

Combined, our results are a confirmation of prior research that reports the effectiveness of including Zeolite in the rations of dry dairy cows to activate the calcium homeostatic mechanisms before calving and thus to avoid postpartum hypocalacemia.

3.3.2. Phosphorus

In our study, the plasma P content did not differ between treatments before calving as well as post calving (Table 4 and Figure 8). In both groups, the mean plasma inorganic phosphate was above the lower limit of the reference interval (55–65 mg/L) given by Kaneko et al. (1997).

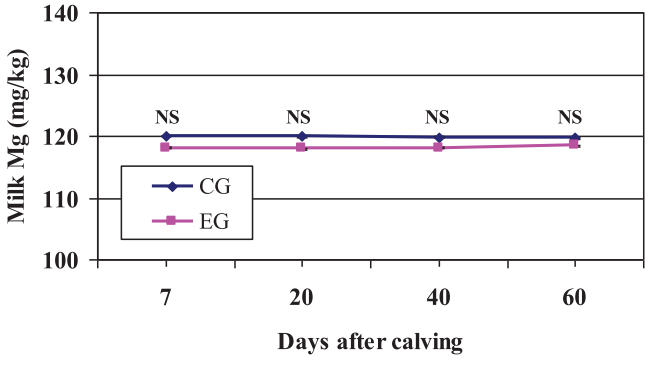

Figure 6. Mean milk Mg content in Zeolite-treated cows (EG) and untreated control cows (CG).

NS, nonsignificant.

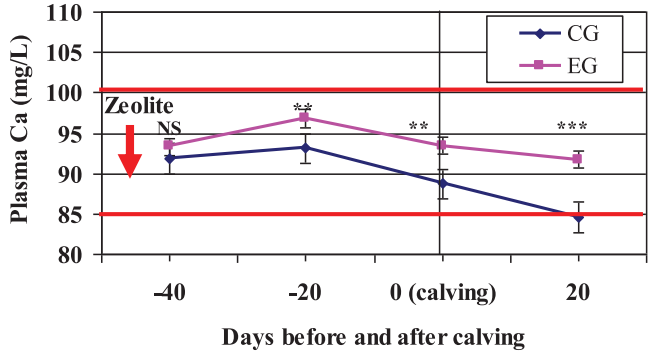

Figure 7. Mean plasma Ca in Zeolite-treated cows (EG) and untreated control cows (CG).

Notes: The solid horizontal lines indicate the upper and lower limit of the reference interval. NS, nonsignificant; $^{**}P < .01$; $^{***}P < .001$.

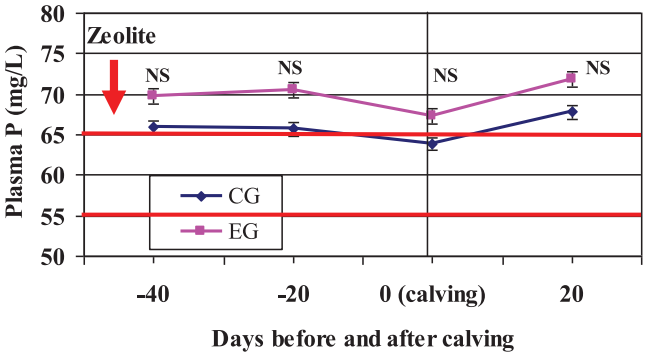

Figure 8. Mean plasma P in Zeolite treated cows (EG) and untreated control cows (CG).

Notes: The solid horizontal lines indicate the upper and lower limit of the reference interval. NS, nonsignificant.

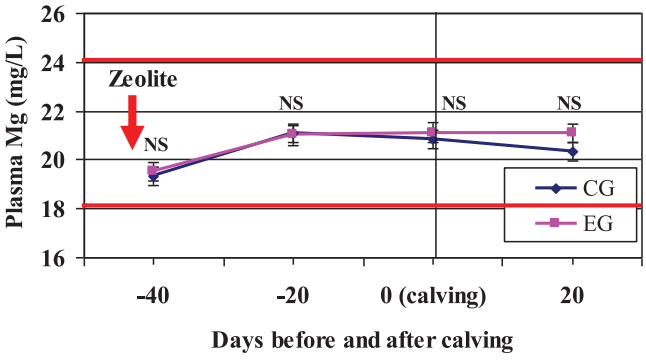

Figure 9. Mean plasma Mg in Zeolite-treated cows (EG) and untreated control cows (CG).

Notes: The solid horizontal lines indicate the upper and lower limit of the reference interval. NS, nonsignificant.

As expected, plasma phosphate in both groups was decreased close to calving ($P = .087$). The hypophosphatemia normally seen in parturient cows can be explained by a higher level of circulating PTH at this point and thereby an increased salivary and renal excretion of phosphate (Kaneko et al. 1997). In the present study, the drop in plasma phosphate in Zeolite-treated cows and the untreated control cows was only transient, since the phosphate level had normalized within 3 weeks after calving (Figure 8).

Unlike our results, Thilsing-Hansen et al. (2002, 2003) saw a lower mean postpartum plasma P concentration in cows fed Zeolite and explained their results by a decreased bioavailability of dietary P by the Zeolite due to formation of insoluble aluminium phosphate complexes in the intestinal lumen.

3.3.3. Magnesium

From Table 4 and Figure 9, plasma Mg concentration was not different between treatments during the entire experimental period. The mean plasma Mg level in both groups stayed within the reference interval (18–24 mg/L) suggested by Kaneko et al. (1997). Because blood magnesium concentration usually reflects dietary magnesium intake (Goff 2008), it could be concluded that normal plasma magnesium in this study was attributed to the correct feed intake.

Somewhat surprisingly, the plasma Mg content was significantly higher around calving than that of the beginning of the dry period (period effect). According to Kaneko et al. (1997) and Thilsing-Hansen et al. (2002, 2003), the plasma Mg concentration is less well controlled than that of Ca and primarily is a result of balance between ruminal and intestinal absorption and renal excretion. The hypermagnesemia seen around calving may be connected to the concurrent drop in blood Ca observed in this study. The mechanism of hypermagnesemia in cows with hypocalcaemia is steel unknown, but increased renal reabsorption of Mg by the renal tubules induced by PTH (secreted in response to hypocalcaemia) could play a role (Riond et al. 1995; Goff and Koszewski 2018).

4. Conclusion

These results indicate that 200 g/d of Zeolite can be effectively included in the rations of lactating dairy cows without any adverse effects on milk composition or milk minerals. Our findings, however, indicate that the rations formulated with Zeolite increased milk yield for cows and consequently enhanced FCM, milk fat, milk protein, and lactose yields. Additionally, prepartum Zeolite supplementation significantly increased the plasma Ca level before and after calving. Combined, our results are a confirmation of prior research that reports the effectiveness of including Zeolite in the rations of dry dairy cows to activate the calcium homeostatic mechanisms before calving and thus to prevent against parturient hypocalcaemia. These findings indicate promising prospects for the utilization of Zeolite, which is often more economical than traditional buffer sources.

Disclosure statement

No potential conflict of interest was reported by the authors.

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