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Effect of Two New Calcium Limiting Methods on Milk Traits, Hypocalcaemia and Prevention of some Reproductive Disorders in Early Lactating Cows

Research Article

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ABSTRACT

New methods of dietary calcium restriction to control hypocalcaemia and reproductive problems have always been the focus of research. This study was conducted to compare two new methods including two diets containing Zeolite and anionic salt with a conventional method of the low-Ca diet. Sixty periparturient cows were used through a completely randomized statistical design (CRD) with repeated measurements in time. Experimental diets were included: 1. low-Ca (Ca=0.44%), 2. anionic-Kipro (Ca=1%), 3. zeolite (Ca=0.44%). Traits were included: feed and nutrients intake, milk characteristics, plasma calcium concentration, body condition score (BCS), and incidence of reproductive problems. Feed, energy, and nutrient intake of low-Ca were higher than anionic-Kipro and zeolite groups ($P \leq 0.01$). Raw and corrected milk yield of anionic-Kipro was at maximum, but fat% and fat yield of zeolite was higher than other groups ($P \leq 0.01$). Total plasma and ionized calcium concentration at 6 and 12 hours after calving of low-Ca and zeolite diets were higher than the anionic-Kipro group ($P \leq 0.01$). BCS of zeolite and anionic-Kipro diets (2.98 and 2.95) was higher than the low-Ca diet (2.86) significantly ($P \leq 0.01$). The frequency of severe and subclinical hypocalcaemia and reproductive problems in two new proposed methods and younger cows was at least ($P \leq 0.01$). New dietary calcium limiting methods with zeolite and anionic-Kipro supplementation had good results on milk traits with better control on plasma calcium concentration and prevention of reproductive disorders and could be advised.

KEY WORDS early lactating cows, hypocalcaemia, reproductive disorders.

INTRODUCTION

New parturition cows, especially if they have an improper transition period and are exposed to a variety of stresses, such as summer heat stress, will suffer irreparable damage and their milk production will be further declined (Karimi *et al.* 2015). During the transition from calving to early lactation, most refreshing cows will face a variety of problems, including hypocalcaemia, and metabolic and reproductive disorders (Timothy *et al.* 2011; Overton and Rapnicki, 2015). Many reports elegantly demonstrated that adding anion salts to the pre-calving diet of cows to reduce dietary cation-anion difference or DCAD (-100 to -150 mEq/kg), or the use of low-calcium diets improved calcium status and effectively prevented hypocalcaemia (Moore *et al.* 2000; Shahzad *et al.* 2008; Wu *et al.* 2008). Hypocalcaemia is a serious metabolic disorder associated with increased postpartum calcium demand for producing colostrum and milk. Lack of attention to this issue is an increased risk for metabolic diseases such as ketosis, fatty liver, retained placenta, displaced abomasum, and other disorders (Lean *et al.* 2006; Timothy *et al.* 2011).

Induced acidosis by anions increases the sensitivity of bones to parathyroid hormone, which results in increased calcium uptake from bone reserves (Goff and Koszewski, 2018; Jahani-Moghadam *et al.* 2020). Anions are also able to increase the production of hormones 1, 25-hydroxy cholecalciferol by affecting the kidneys. This active form of vitamin D raises blood calcium levels by absorption of calcium from the urinary collecting tubes of the nephrons. As well as through stimulating the production of calcium-binding protein for absorption of calcium from the small intestine (Wilkens *et al.* 2020; Seely *et al.* 2021).

But existing methods also have some disadvantages including the use of low-calcium diets is very difficult in practice. In this method level of calcium should be declined by reducing the feed intake or use of low-quality feed ingredients. In any case, it has many adverse effects on the production and health of newborn cows (Melendez *et al.* 2002). Anionic salts should be consumed up to 3 weeks before parturition until the results. Managing and dividing a low number of cows using a unique diet is also practically difficult and costly. In addition, these salts are non-edible and reduce dry matter intake, which causes a negative energy balance and creates subsequent reproductive problems (Thilsing *et al.* 2002; Wilkens *et al.* 2020). Therefore, it has a special priority due to the necessity of study on the use of new methods that do not have the limitations of common methods. Zeolites, as a natural mineral, are well able to bind divalent cations and absorb fungal toxins, gases, and excess products in their structure. The most important zeolite used in dairy cattle nutrition is clinoptilolite, a group of hydrous sodium aluminosilicate minerals (Kerwin *et al.* 2019; Khachlouf *et al.* 2019). These compounds can bind calcium in the diet or gastrointestinal tract (Grabherr *et al.* 2009). This mechanism results in less absorbable calcium reduces plasma calcium concentrations and ultimately stimulates the release of bone calcium reserves at calving time (Thilsing *et al.* 2006; Kerwin *et al.* 2019).

Few findings have been reported on the efficacy of clinoptilolites during the transition period of cows. The most important functions of clinoptilolites can be introduced as follows: Unique properties by ability to lose and gain water reversibly, along with ion-exchange property. Also, they are used as feed additives mainly to improve production traits. Although, further investigations showed that clinoptilolites can be used for the prevention of metabolic diseases (Katsoulos *et al.* 2006; Kerwin *et al.* 2019; Khachlouf *et al.* 2019).

The results indicated that the dietary administration of clinoptilolite (2.5% concentrate mixture) during the last month of the dry period is effective in preventing milk fever and ketosis after calving. The further study proved that the dietary daily intake of 200 g clinoptilolite enhances the immune response of calves vaccinated against *E. coli* and also prevents the reduction of ruminal pH when they consume a high concentrate diet (Katsoulos *et al.* 2006).

Other research papers addressed the hypothesis that oral supplementation with clinoptilolites will improve colostrum quality in primiparous dairy cows without adverse effects on the cows' health. Clinoptilolite in the colostrum of newborn calves increases the absorption of immunoglobulins in the intestine and reduces the incidence of diarrhea (Sadeghi and Shawrang, 2008; Khachlouf *et al.* 2018).

In the latest study, synthetic zeolites were used to control the availability of dietary minerals (e.g., Ca, Mg, and P) in transition dairy cows. So, feeding zeolite at pre-calving time has few effects on immune functions. The lower gene expression of neutrophil inflammatory mediators may be due to the altered availability of dietary minerals prepartum and indicates that zeolite-A may control inflammation during the transition period (Crookenden *et al.* 2020).

According to the hypothesis of the current study research, due to the urgent need of cows to high-quality feeds during the prepartum period, enrichment of zeolite and anionic salts with associated compounds to make them high quality with more palatability can greatly reduce their negative effects on feed intake and further performances. In this regard, two special supplements included enriched zeolite and anionic-Kipro were used to compare their impacts on milk production, prevention of hypocalcemia, and some reproductive disorders in early lactating cows under summer heat stress. This research seems to be necessary due to the lack of sufficient information.

MATERIALS AND METHODS

All procedures used on the cows in this study were approved by the Islamic Azad University, Institutional Animal Care and Use Committee.

Cows, treatments, and management

A number of 60 periparturient Holstein cows in 3 different parity (1 year 30%, 2 and 3 years 30%, >3 years 40%) were used by a completely randomized statistical design (CRD) with repeated measurements. The experiment was performed on a dairy farm of Pakdasht Fashafoyeh agriculture and Industry Company near Tehran in the summer of 2020. Experimental diets included: 1. LC: low-Ca (Ca=0.44%), 2. AK: anionic-Kipro (Ca=1%), 3. Z: zeolite (Ca=0.44%). The dietary cation-anion difference (DCAD) of diets were +100, -100, and +100 mEq/kg of dry matter (DM), respectively. The mean body condition score (BCS) of the cows at the beginning of the experiment was 3.50±0.25 and the average milk yield was 40 ± 2.20 kg. The experiment was performed under summer heat stress (Thermal Humidity Index was between 71 to 83) on a commercial dairy farm. Adaptation period was 15 days and the experimental duration was 45 days from -15 to +30 days after parturition time. The experimental feed samples were sent to the laboratory to determine the nutrient values based on acceptable standards according to AOAC (2010). The enriched AK and Z supplements were used at 5.75% (800 g/cow/day) and 1.50% (200 g/cow/day) of DM. Zeolite supplement (type A) made by the Iranian chemical industry (general formula: $nAl_2O_3.mSiO_2.\times H_2O$, two-layer and 8-sided type clinoptilolite with zeolite/Ca binding ratio of 6:2 under approved code:2004-11-03 IZA). Anionic-Kipro supplement contained a pure mix of anionic salts (ammonium chloride, calcium chloride, calcium sulphate, magnesium sulphate provided at 28, 56, 24, and 85 g/kg DM) made by an Iranian chemical company (Sana™ Chemical Co. Under approved code: A1-2053) with a special formulation (DM=90%, CP=40-41%, Ca=2-3%, P=0.60%, Mg=3-4% and dietary cation-anion difference (DCAD)=-3400 mEq/kg). Both of Z and AK supplements enriched with essential ingredients included protein (coated soy protein 48%), vitamins (1000000 IU vitamin A, 200000 IU vitamin D, 10000 mg vitamin E), and minerals (3700 mg Mn, 4300 mg Zn, 1100 mg Cu, 50 mg Co, 60 mg I, 25 mg Se, 1000 mg anti-oxidant). All pregnant cows were moved to calving site 3 weeks before parturition. Common feed was used to prepare the total mixed ration (TMR) diets and nutrient requirements of the cows were determined according to standard tables of NRC (2001). Measured traits were included milk yield (raw and corrected based on fat 4%), milk composition (fat and protein), total and ionized plasma Ca concentration and frequency of some after-parturition reproductive abnormalities (hypocalcaemia, reproduction disorders, and fertility and culling rate).

Diets and chemical analysis

Feed ingredients of the diets before and after parturition are presented in Table 1. The chemical composition of the diets is also presented in Table 2.

Samples and laboratory analysis

Crude protein (micro-kjeldal method), net energy (regression equations), calcium, phosphorus, and other minerals (spectrophotometry), dry matter (moisture evaporation method), ether extract (Soxhlet method), neutral detergent fiber (NDF) (total cellulose, hemicellulose, and lignin), and acid detergent fiber (ADF) (cellulose and lignin) were measured using AOAC (2010). Milk samples were tested for fat and protein percentage using a MilkoScan-605 device of Foss Denmark (Bondoc, 2007). Total plasma calcium concentration was measured by photometric method (kits of Pars-Azmoon chemical company, TS.M.91.13.4) using Analyzer device of Roche Hitachi-911 Chemistry Analyzer Company. Plasma ionized calcium concentration was measured by Ion Selective Electrode method with an Electrolyte Analyzer of AC-9800 Audicom company.

Statistical analysis

Data were analyzed by SAS (2004) software in a CRD statistical design. Data of continuous traits were analyzed by one-way variance analysis using a mixed procedure with repetitive observations in time. For discontinuous data or numerical traits, nonparametric analysis methods were employed. Also, the Shapiro-Wilk test was used to ensure normal distribution of data. The mean differences of the treatments were compared at a level of $P \leq 0.01$ using Duncan's Multiple Range Test. The statistical model was as follows:

$$Y_{ijk} = \mu + T_i + D_j + (TD)_{ij} + \varepsilon_{ijk}$$

Where:
Y_{ijk}: indicated as an observation of a treatment.
μ: overall mean of a trait.
T_i: treatment effect.
D_j: time effect.
TD_{ij}: interaction effect of treatment × time.
ε_{ijk}: residual effects.

RESULTS AND DISCUSSION

Dry matter intake (DMI) of low-Ca (LC) diet with 12.80 kg/day was the highest (P≤0.01) compared to anionic-Kipro (AK) and zeolite (Z) diets with 12.45 and 12.35 kg/day, respectively (Table 3). Consumption of energy of the diets had also a similar trend to DMI, so the mean energy intake of LC diet was higher than AK and Z diets (P≤0.01). At 30 days of postpartum, AK and LC diets had a higher amount of raw and corrected milk yield than Z diet (P≤0.01). Inversely, the fat% of Z diet was higher than LC and AK diets (P≤0.01), but protein% and feed efficiency had no significant difference between the diets.

The effect of different diets on the plasma calcium concentration of cows at parturition time and 24 hours after that is presented in Table 4.

At parturition time, the total plasma calcium concentration means of Z diet were almost similar to LC and AK diets, but the ionized calcium concentration means of AK diet was maximum (P≤0.01). Generally, at 6, 12 and 24 hours after parturition total and ionized plasma calcium concentration means of Z and then AK diets were also higher than LC diet (P≤0.01). Therefore, the positive effect of Z and AK diets on the plasma calcium concentration of experimental cows was confirmed.

Table 1 Feed ingredients of the before and after parturition experimental diets (% of DM unless otherwise noted)

Ingredients/diets	Before parturition			After parturition
	LC	AK	Z	Normal
Alfalfa hay	16.11	15.78	15.85	17.20
Corn silage	31.11	31.57	30.64	28.70
Wheat straw	6.37	6.24	6.28	2.20
Wheat bran	3.94	3.20	3.88	-
Ground barley	11.82	11.10	11.65	13.50
Ground corn	11.96	11.51	11.78	15.80
Soybean meal 44%	10.94	8.50	10.77	11.93
Canola meal	3.57	2.42	3.52	3.95
Corn gluten meal	1.88	0.80	1.85	1.95
Propylene glycol	0.98	0.97	0.97	1.10
Vitamin-mineral premix[1]	0.98	0.97	0.97	1.20
Zeolite[2]	-	-	1.50	-
Kipro[3]	-	5.75	-	-
Calcium carbonate	-	0.85	-	0.6
Magnesium oxide	0.34	0.34	0.34	0.3
Dicalcium Phosphate	-	-	-	0.22
Sodium Bicarbonate	-	-	-	1.20
Sodium Chloride	-	-	-	0.15
Vitamin A (IU)	8500	8500	8500	5500
Vitamin D (IU)	3200	3200	3200	1500
Vitamin E (mg)	150	150	150	75
Total	100	100	100	100

[1] Each kg of vitamin-mineral premix in experimental diets contained: vitamin A: 5 million IU; vitamin D_3: 1 million IU; vitamin E: 30 mg; Mg: 19 g; Fe: 12 g; Mn: 10 g; Zn: 13 g; Cu: 300 mg; Co: 100 mg; Se: 30 mg and I: 100 mg.
[2] Zeolite manufactured by Iranian Chemical Industry (general formula: $nAl_2O_3.mSiO_2.xH_2O$) two-layer and 8-sided, class of Clinoptilolite with Zeolite/Ca binding ratio of 6: 2. DM= 90%; DCAD= -3400 mEq/kg of DM.
[3] Kipro manufactured by Sana[TM] Chemical Co. contained anionic salts plus coated soy protein, essential vitamins, and trace minerals.
LC: low-Ca; AK: anionic-Kipro and Z: zeolit.

Table 2 Feed composition of the before and after parturition experimental diets (% of DM unless otherwise noted)

Nutrients/diets[1]	Before parturition			After parturition
	LC	AK	Z	Normal
Crude protein	16.24	16.24	16.24	16.54
NE (Mcal/kg)	1.58	1.58	1.58	1.61
NDF	35.50	33.20	35.50	31.74
ADF	21.00	20.20	21.00	19.22
NFC	38.70	38.00	38.70	40.57
EE	2.65	2.50	2.70	2.65
Ca	0.44	1.00	0.44	0.44
P	0.36	0.31	0.36	0.36
Mg	0.44	0.44	0.44	0.44
Cl	0.16	0.25	0.16	0.16
K	1.26	1.22	1.26	1.26
Na	0.05	0.05	0.05	0.05
S	0.22	0.28	0.21	0.22
DCAD (mEq/kg DM)	+100	-100	+100	+300

LC: low-Ca; AK: anionic-Kipro and Z: zeolit.
NE: net energy; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: non-fiber carbohydrate; EE: ether extract and DCAD: dietary cation-anion difference.

The effect of different diets on the frequency of severe and subclinical hypocalcaemia as well as postpartum reproductive disorders is presented in Table 5.

The corrected percentage of the incidence of severe hypocalcaemia in LC, AK and Z diets was 37.9, 52.3 and 29.65%, respectively. The incidence of subclinical hypocalcaemia also was 40.45, 36.27, and 33.21%, respectively. The frequencies were more common in cows with parity 2 or more than 2. Therefore, to control severe hypocalcaemia, Z diet was better than AK or LC diets, and also to control of subclinical hypocalcaemia, AK and Z diets were better than LC diet, respectively.

Table 3 Comparison of feed and nutrient intake and milk production traits of different diets at day 30 after parturition (kg/day unless otherwise noted)

Item	Diets			SEM	P-value		
	LC	AK	Z		Diet	Time	Diet × Time
DMI (kg/day)	12.80^a	12.45^b	12.35^b	0.163	< 0.001	< 0.011	< 0.010
NE_L (Mcal/day)	20.22^a	19.67^b	19.51^b	0.216	0.811	0.445	0.560
CP (kg/day)	2.08	2.02	2.00	0.236	0.518	0.368	0.685
NDF (kg/day)	4.29	4.13	4.38	0.223	< 0.016	0.633	< 0.018
ADF (kg/day)	2.69	2.52	2.59	0.158	< 0.022	0.755	< 0.025
Row milk yield (kg/day)	42.17^a	42.72^a	40.19^b	0.933	0.001	0.001	< 0.011
Corrected milk yield (kg/day)*	35.78^{ab}	36.31^a	35.25^b	0.402	0.001	0.001	< 0.012
Fat %	2.99^b	3.02^b	3.18^a	0.046	< 0.021	0.000	< 0.002
Protein %	2.96	2.97	2.96	< 0.018	0.322	0.562	0.652
Feed efficiency	2.79	2.99	2.85	0.139	0.326	0.449	0.857

* Milk yield based on standard fat 4% calculated from formula: FCM 4%= 0.4 (kg milk) + 15 (kg milk×fat%). Feed efficiency calculated as: corrected milk yield (kg/day) / DMI (kg/day).
LC: low-Ca; AK: anionic-Kipro and Z: zeolit.
DMI: dry matter intake; NE_L: net energy for lactation; CP: crude protein; NDF: neutral detergent fiber and ADF: acid detergent fiber.
The means within the same row with at least one common letter, do not have significant difference (P>0.01).
SEM: standard error of the means.

Table 4 Effect of different diets on plasma calcium concentration at parturition time and 24 hours after that (mg/dL)

Item	Diets			SEM	P-value		
	LC	AK	Z		Diet	Time	Diet × Time
7 days before parturition							
Ca	9.27	9.42	9.38	0.131	0.533	0.478	0.655
Ca^{++}	4.31	4.46	4.44	0.052	0.412	0.128	0.112
Ca^{++}/Ca	0.46	0.47	0.47	0.010	0.415	0.354	0.215
Parturition time							
Ca	7.43^a	7.43^a	7.32^{ab}	0.125	0.000	< 0.012	< 0.022
Ca^{++}	3.49^b	3.52^a	3.44^b	0.052	< 0.018	< 0.033	< 0.025
Ca^{++}/Ca	0.47	0.47	0.47	0.010	0.321	0.245	0.255
Hour 6 after parturition							
Ca	7.89^{ab}	7.82^{ab}	7.95^a	0.136	0.000	< 0.011	< 0.011
Ca^{++}	3.63^b	3.73^{ab}	3.82^a	0.074	0.000	< 0.045	< 0.044
Ca^{++}/Ca	0.46	0.48	0.48	0.009	0.451	0.633	0.555
Hour 12 after parturition							
Ca	8.38^{ab}	8.35^{ab}	8.53^a	0.134	0.000	< 0.041	< 0.035
Ca^{++}	4.02^{ab}	4.16^a	4.10^a	0.055	0.000	0.005	< 0.017
Ca^{++}/Ca	0.48^b	0.50^a	0.48^b	0.007	0.000	0.285	0.911
Hour 24 after parturition							
Ca	8.71^{ab}	9.06^a	8.89^a	0.131	0.000	< 0.050	< 0.045
Ca^{++}	4.18^b	4.45^a	4.27^b	0.055	0.000	< 0.022	< 0.037
Ca^{++}/Ca	0.48^b	0.49^a	0.48^b	0.008	0.000	0.501	0.713
BCS	2.86^{ab}	2.95^a	2.98^a	0.046	0.000	0.005	0.008

LC: low-Ca; AK: anionic-Kipro and Z: zeolit.
The means within the same row with at least one common letter, do not have significant difference (P>0.01).
SEM: standard error of the means.

AK and Z diets had a significantly lower frequency of reproductive disorders, conversely, significant higher frequency of successful pregnancy than LC diet (P≤0.01).

Due to the lack of palatability of anionic salts decrease in DMI was predictable (Goff and Koszewski, 2018). As a rule, nutrient intake in diet included energy, protein, NDF and ADF had a similar trend due to their positive correlation with DMI (Jahani-Moghadam *et al.* 2020). There was no significant difference in the percentage and yield of protein through the diets. Due to the natural effect of anionic diets on DCAD, which maintains the rumen fermentation pattern and balanced production of fatty acids (acetate and butyrate) and also on calcium homeostasis, milk production, as well as prevention of metabolic disorders, was improved (Moore *et al.* 2000; Lean *et al.* 2006).

In this experiment, an attempt was made to reduce the negative effect of AK and Z diets by enriching them with protein, minerals, and vitamins. The results indicate the positive effect of this type of supplementation on nutrient intake and milk traits. It should be noted that the nature of LC diets is that they are more palatable than anionic diets and have a higher DMI.

Table 5 Effect of different diets on the prevalence of hypocalcaemia and reproduction disorders after parturition

Item (% and ratio)[1]	Diets			SEM	P-value
	LC	AK	Z		
Severe hypocalcaemia	40.45 (8/19)	36.27 (7/20)	33.21 (6/20)	4.330	0.0001
Subclinical hypocalcaemia	34.19 (6/19)	22.74 (3/20)	26.56 (4/20)	2.720	0.0001
Calving difficulty	25.24 (2/11)	17.55 (1/11)	16.78 (1/12)	0.159	0.0001
Retained placenta	31.10 (4/15)	26.56 (3/15)	26.56 (3/15)	0.247	< 0.0002
Endometritis	33.21 (6/20)	26.56 (4/20)	30.00 (5/20)	2.536	< 0.0025
Abomasal displacement	17.55 (1/11)	0.00 (0/10)	0.00 (0/10)	1.746	0.0001
Culled cows	12.92 (1/20)	0.00 (0/20)	0.00 (0/20)	2.356	0.2124
Successful pregnancy	53.72 (13/20)	63.43 (16/20)	67.21 (17/20)	4.857	0.0001

[1] Frequency noted based on corrected percentage and number of animals that showed the sign of abnormality.
LC: low-Ca; AK: anionic-Kipro and Z: zeolit.
SEM: standard error of the means.

But it is so difficult to prepare such diets and also to adjust a diet with low-calcium levels, lower quality feed should be used, thus the supply of sufficient nutrients to produce enough milk will not be done adequately (Lean *et al*. 2006; Shahzad *et al*. 2008; Wu *et al*. 2008).

There are several reports on the positive effects of zeolite on feed intake, energy consumption, milk production, and composition (Grabherr *et al*. 2009; Khachlouf *et al*. 2019). Zeolite has a different mechanism of action due to the binding of calcium in the gastrointestinal tract and reduced access to it will activate the physiological process mentioned earlier, which will result in regulating plasma calcium levels during parturition (Thilsing *et al*. 2002; Thilsing *et al*. 2006). However, according to the hypothesis of this study, it is necessary to provide sufficient essential nutrients with zeolite to reduce the negative effect of zeolite on the binding of divalent cations and other essential nutrients. The results of the present study confirmed such effects and no negative effects were observed in this field.

The critical threshold of plasma calcium concentration for severe hypocalcemia symptoms in this study was considered ≤7.30 mg/dL (1.82 mmol/L) and for subclinical hypocalcaemia ≤8.50 mg/dL (2.12 mmol/L). So at parturition time, several cows showed degrees of severe hypocalcaemia. Severe hypocalcaemia lasted for about 6 hours after parturition. From 6 to 12 hours after parturition conditions changed to onset of subclinical hypocalcemia in several cows. From hour 12 onwards the situation improved and at hour 24 only a small number of cows showed signs of diseases. From 6 to 24 hours after parturition, the calcium concentration of zeolite diet was higher than two other diets (Table 3). The cut-off border of plasma calcium concentration as a criterion for the diagnosis of subclinical hypocalcaemia in lactating cows is a challenging and controversial issue and has been the subject of numerous studies (Wilkens *et al*. 2020). In a report, this level was stated less than 7.20 mg/dL (1.80 mmol/L) (Goff, 2000), and in another report less than 8.80 mg/dL (2.20 mmol/L) (Chapinal *et al*. 2011). Oetzel (2013) showed that the critical threshold was less than 8.50 mg/dL. Martinez *et al*. (2012) reported being less than 8.59 mg/dL. The important point based on the available findings is that if the critical threshold is less than 8.5 mg/dl, anionic diets are no longer able to well prevent subclinical hypocalcaemia and this is a relatively complex issue in controlling the complication (Chapinal *et al*. 2011; Wilkens *et al*. 2020). Based on the results of tables 3 and 4 AK and Z diets had better control on hypocalcaemia than LC diet. The BCS values in table 3 also confirm the superiority of AK and Z diets over LC diet. BCS index is a useful and economical tool for monitoring energy changes and the risk of metabolic diseases in transition cows due to reduction of energy and DM intake (Leand and DeGaris, 2010; Overton and Rapnicki, 2015).

The inhibitory effect of zeolite and Anionic supplementation in preventing of hypocalcaemia and reproductive disorders was confirmed through several reports (Goff and Koszewski, 2018; Kerwin *et al*. 2019; Khachlouf *et al*. 2019). Reason for the high frequency of hypocalcaemia in cows of the parity 2 and > 2 is related to physiological characteristics, nutrition and calcium stores in the body, which is an expected phenomenon (Thilsing *et al*. 2002; Lean and DeGaris, 2010).

A notable point is that, except considering an adequate number of samples, it is not appropriate to consider a short time immediately after parturition to assess the short-term effects of the diet and there should be a sufficient time between samples (Oetzel, 2013; Seely *et al.* 2021).

Despite the advantages of LC diet in preventing hypocalcaemia and reproductive disorders due to some problems supporting the calcium intake (<20 g/day) is less useful and the frequency of reproductive disorders in this diet is often high, this was also confirmed in this experiment (Thilsing *et al.* 2002; Timothy *et al.* 2011). Most reproductive problems after parturition related to calcium deficiency and their abundance are good benchmark for evaluation of reproduction in the farm (Underwood and Loor, 2005; Wu *et al.* 2008; Wilkens *et al.* 2020). Hypocalcaemia causes a loss of muscle contraction in the uterus and gastrointestinal tract which increase the risk of placental abruption and abomasum displacement and older cows are more at risk (Drackley *et al.* 2005; Lean and DeGaris, 2010). Cows with retained placenta are at higher risk for acute mastitis and ketosis, and also cows with ketosis are about 12 times more likely to develop abomasum displacement. A healthy cow must defecate placenta by 12 hours after parturition otherwise the remaining placenta will cause infection and subsequent problems (Melendez *et al.* 2004; Timothy *et al.* 2011). Predisposing factors included a history of abnormal calving, uterine infection and placental abruption, environmental and nutritional factors, decreased immune system function and mineral deficiency (Emtenan *et al.* 2011; Seely *et al.* 2021). In comparison to reported average placental retention in dairy herds (4-16%), our averages were significantly higher. This figure may be much higher in poorly managed herds (Emtenan *et al.* 2011).

The frequency of abomasal displacement in our study was low and only 1 case was observed in LC diet. AK and Z diets had no case of abomasum displacement compared to LC diet. This abnormality is directly related to a decrease in plasma calcium concentration, so better calcium control through these supplements appears to have a direct effect on gastrointestinal muscle contraction and prevention of abomasum displacement. Nutritional factors and dietary fiber content also have a direct impact on it (Lean and DeGaris, 2010; Emtenan *et al.* 2011).

Inflammation of the uterus, which is most often associated with infection through 21-28 days after parturition, is called endometritis (Chapianl *et al.* 2011). This may affect up to 60% of the herd and prevent the cow from moving and feeding normally and cause them susceptible to postpartum diseases. The frequency of endometritis relatively in this experiment was high (26 to 33%), but Z diet had the lowest incidence of endometritis. Some factors such as infection and inadequate nutrition (reduced DMI) will increase the risk of developing endometritis (Chapinal *et al.* 2011; Emtenan *et al.* 2011). More than 75% of reproductive disorders in dairy cows occur in the first month after parturition due to energy metabolism deficiencies, mineral metabolism disorders, and immune system problems (Lean *et al.* 2006; Wilkens *et al.* 2020). In fact, these problems are interrelated and each of them is a precursor to the other. Therefore, to better control reproductive problems, a comprehensive approach should be taken in all fields.

CONCLUSION

Results of this study indicated that new dietary calcium limiting methods as supplementation of zeolite and anionic-Kipro in the preparturition diets of the cows could be advised due to good results on milk traits with better control of plasma calcium concentration and prevention of reproductive disorders. According to our findings dry matter, energy and nutrient intake of the cows consumed the zeolite and anionic-Kipro diets were improved. Raw and corrected milk yield of the mentioned diets were higher than low-Ca diet. The percentage and amount of fat in the zeolite diet was higher than others. Total plasma and ionized calcium concentration means of zeolite and anionic-Kipro diets were improved and the frequency of severe and subclinical hypocalcaemia as well as the incidence of reproductive disorders was significantly reduced when compared to the low-Ca diet. In conclusion, new limiting methods of calcium proposed by the current study with supplementation of essential nutrients had good results on milk traits with better control on plasma calcium concentration and reproductive disorders and so could be introduced as alternatives to the conventional methods.

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