2018

Effect of feeding X-Zelit during transition on blood macro-minerals, productivity and health events in dairy cattle

QUADRANT FARMING SOLUTIONS
OUR KNOWLEDGE YOUR STRENGTH

Contributors:

RuralVet
Animal Health Solutions
Your partners in profitable animal production
www.ruralvet.com.au

COW SIGNALS
AUSTRALIA

QUADRANT FARMING SOLUTIONS PTY LTD

ABBREVIATIONS

ADF	Acid Detergent Fibre
ADICP	Acid detergent insoluble crude protein
AI	Artificial Insemination
AMS	Automated Milking System
BCS	Body Condition Score
BHB	Beta-Hydroxybutyrate
Chol	Cholesterol
Gluc	Glucose
Ca	Calcium
CP	Crude Protein
DCAD	Dietary Cation-Anion Difference
DM	Dry matter
DIM	Days in milk
DMI	Dry matter intake
EE	Ether Extract
GPX	Glutathione peroxidase
ME	Metabolizable Energy
MF	Milk Fever
Mg	Magnesium
NDF	Neutral Detergent Fibre
NDICP	Neutral detergent insoluble crude protein
NFC	Non-Fibre Carbohydrate
NEFA	Non-esterified fatty acid
Phos	Phosphorous
RFM	Retained fetal membrane
RUD	Rumen Undegradable Protein
RDP	Rumen Degradable Protein

Table of Contents

1. BACKGROUND

The incidence of metabolic disease in dairy cattle increases as milk production increases and, in particular, as the rate of increase in milk production increases (milk yield acceleration). In dairy cows, the total disease incidence rapidly increases in the very late peri-parturient period, peaks on the day of parturition and then declines until day 7 day of lactation (Ingvartsen, 2006). This critical 7 day window starting with parturition has a tremendous influence on morbidity, production, reproductive performance and mortality of dairy cows.

The transition period from 3 weeks before calving until 3 weeks post calving is a crucial stage in the production cycle of the dairy cow and no other period can affect subsequent production, health and reproductive performance so greatly. The success of the transition period effectively determines the profitability of the cow during that lactation. Nutritional or management limitations during this time may impede the ability of the cow to reach maximum milk production.

During the transition period, dairy cows undergo large metabolic adaptations in glucose, fatty acid and mineral metabolism. The practical goal of nutritional management during this period is to support these metabolic adaptations. Cows during the transition period also undergo a period of reduced immunologic capacity. This immune dysfunction is broad in scope and affects multiple functions of various cell types and lasts from approximately 3 weeks before calving until 3 weeks post calving. Cows during this period are more susceptible to mastitis.

Parturition is followed by the sudden onset of a profuse lactation, which, if the nutrient reserves have already been seriously depleted, may result in clinical metabolic disease. Most attention has been paid to variations in calcium balance at calving as this mineral has a profound effect on the health, wellbeing and performance of the dairy cow. A depression in the level of ionized calcium in the extracellular space, including plasma, is the basic biochemical disturbance in hypocalcaemia (milk fever). A transient period of subclinical hypocalcaemia (plasma calcium < 2.1 mmol/L) occurs at the onset of lactation caused by an imbalance between calcium influx from gut and bone and output in colostrum and milk. The incidence of hypocalcaemia, with serum calcium concentrations between 1.4 and 2.1 mmol/L in multiparous peri-parturient cows has been estimated at 50% (Constable et al, 2017). Clinical milk fever is estimated to occur in at least 5% of dairy cows in the US. The incidence of milk fever in Australia has been shown to range from 1.6% to 5.4% but in some years the incidence in individual herds may reach as high as 20%. The incidence of subclinical hypocalcaemia can range widely but has shown to affect 40-50% of dairy cows in pasture based systems (Constable et al, 2017).

Current strategies for prevention of clinical and subclinical milk fever is i) increasing the content of anions in the ration (DCAD i.e. a low dietary cation-anion difference ration), ii) low dietary calcium intake (less than 20 g/head/day) or iii) oral administration of calcium before and after calving.

DCAD diets induces a mild metabolic acidosis which causes calcium to be drawn from the cows' skeleton. DCAD rations normally require a 21-day programme to be fully effective. Despite the effectiveness of feeding DCAD rations for prevention of milk fever and other metabolic diseases, this method may not be adequately efficient when using commonly available feeds, especially in pasture based dairy farming systems. Using forages such as pasture with high levels of potassium (usually as high as 4-5%) can easily offset the negative DCAD and reduce its effectiveness. Furthermore, DCAD diets are often unpalatable which can reduce feed intake. Oetzel and Miller (2012) found that more than 50% of dairy cows still have hypocalcaemia (plasma calcium<2.1 mmol/L) despite being fed a DCAD diet.

The approach of feeding diets low in calcium have practical problems as most farms utilizing home-grown forages find it difficult to obtain forages low in calcium. Formulating a dry cow diet (transition diet) that contains less than 20 g of calcium per day and at the same time maintaining adequate intake of forages and concentrates during the pre-calving period can be a challenge. A low dietary calcium intake during the last two weeks prior to calving stimulates the cows' natural homeostatic response which allows cows to mobilise calcium.

X-Zelit (Vilofoss) is a sodium aluminium silicate that binds dietary calcium and thereby induces a negative calcium balance pre-calving which stimulates the mobilisation of calcium. It is vital to bind enough calcium to reach a diet calcium level of less than 20 g/day. X-Zelit or any part thereof (such as aluminium or other ions) does not cross the rumen wall. X-Zelit is a patented product and the Commission of European Communities (CEC) allowed in 2004 its use for reduction of milk fever with no adverse side effects on the cow, its calf, milk as a food and the environment.

A number of studies have demonstrated that feeding X-Zelit during the transition period results in stable blood calcium levels, after calving, above the critical 2.1 mmol/L, and has the potential to prevent Ca deficiency and associated metabolic disorders including milk fever (Thilsing-Hansen and Jørgensen, 2001; Wilson, 2001). X-Zelit is fed to the dry cows during the transition period, 14 days before the expected calving date. Evidence shows that supplementing cows with X-Zelit during the pre-parturition period (14 days) has reduced the risk of clinical hypocalcaemia by 86% (Thilsing et al., 2009) and improved milk yield and somatic cell counts (SCC) in herds experiencing low levels of milk fever (classified as “non-problem herds” with approximately 2-3% of clinical cases of milk fever within the herd; Theilgaard, 2011).

This study was conducted to demonstrate that X-Zelit can be considered as an effective product that can reduce the risk of metabolic disorders in Australian dairy farming systems. Therefore it was decided to compare the effectiveness of X-Zelit with other DCAD products (e.g. BIO-CHLOR) in the market place and to demonstrate that X-Zelit can be efficient and cost-effective. BIO-CHLOR was chosen as the DCAD option due to its high efficacy and superior performance. The study was aimed to provide the dairy industry with locally-generated evidence-based data to show the effectiveness of X-Zelit in improving the metabolic condition of cows during the transition period and reduce the risk of hypocalcaemia and cost of therapeutic interventions.

2. OBJECTIVES

The primary objective of this study is to compare the effect of feeding a dietary calcium binder (X-Zelit®, Vilofoss, Denmark) versus a DCAD ration (BIO-CHLOR®, Arm & Hammer, USA) during the transition period on the incidence of metabolic disease, milk production/composition and other health events in a commercial dairy herd. These include; i) quantifying the incidence of post-partum diseases, such as clinical and subclinical milk fever (MF), and other health events, ii) monitoring milk production and composition (fat & protein) up to 100 DIM and iii) measuring blood macro-minerals (Ca, P, Mg) concentrations.

3. MATERIALS AND METHODS

Daily events and sampling procedures during each period were recorded on pre-designed forms by farm staff and study personnel. Therapeutic interventions during the trial period, if required, were based on the standard operating procedure for treatment of metabolic or infectious diseases.

3.1. Animals and experimental procedures

3.1.1. Study design

The study design was a randomised control trial. The operators involved in the trial were not blinded from knowledge of the contents of formulated diets when administering.

3.1.2. Test product

Composition, formulation, usage and source of test products are presented in Table 1.

Table 1. Composition/Information on ingredients

Test Article	Source	Formulation/ingredients	Storage Use
BIO-CHLOR®	Arm&Hammer (imported by Cows-R-Us)	Dried condensed, extracted glutamic acid fermentation product; dried condensed corn, fermentation solubles; processed grain by-products; magnesium chloride. Crude Protein: 42.31 percent Chloride: 7.9 percent Sulphur: 3.13 percent DCAD: -337.87 meq/100g	25 kg Bags Stored in a cool, dry area
X-Zelit®	Vilofoss (imported by Nutrifoss)	80% Synthetic aluminium silicate 20% Wheat Starch	20 kg Bags Stored in a cool, dry area

3.2. Cows and herd locations

Healthy, lactating cows (total n=160) from a robotic commercial dairy herd (QLD) of parity 1 or greater were selected approximately 30 days before anticipated calving dates and monitored for up to 100 days during the lactation. A total of 72 cattle (dairy cows= 52; heifers = 20) were enrolled in this study. Cattle were assigned randomly and blocked to one of two treatment groups (BIO-CHLOR and X-Zelit) according to the expected calving date, DIM, parity and previous lactation yield (305-day). Cows in BIO-CHLOR group were fed a transition DCAD diet, 21 days before their expected calving dates, and cows in the other group were fed X-Zelit for 14 days before the expected calving date.

3.2.1. Transition diet & feeding

The goal during the transition period was to provide a ration with two different treatments that are claimed to reduce the risk of hypocalcaemia and incidence of metabolic diseases (e.g. clinical milk fever) around calving. Cows in the BIO-CHLOR group were targeted to have a negative DCAD (–15 meq/100 g). Cows in the X-Zelit group were targeted to be on a low calcium diet (less than 20 g/head/day) (Table 2). All cows in both BIO-CHLOR and X-Zelit groups were fed the transition diet 3 and 2 weeks before expected calving date, respectively. The transition diets of both groups were offered to the animals in typical feeding facility available at a commercial dairy farm. Transition cows were housed in a loafing paddock which contained limited/no grass and had access to water at all time. The transition diet was formulated using CPM-Dairy (V3, 2008) to meet or exceed energy and metabolizable protein requirements and meet requirements for trace element and vitamin needs. Both rations (BIO-CHLOR and X-Zelit) were equivalent for nutrients such as energy, protein, starch, sugar, NDF, ADF, fat and minerals and vitamins. The concentrate rations for both groups were mixed as a batch mix and were fed daily at the recommended amounts. Both groups were offered ad libitum access to forage which consisted of sweet corn starch, hay or silage as per requirements.

Table 2. Diet ingredients and estimated average nutrient compositions of the transition diets during the experimental period (CPM-Dairy V3; 2008)

Ingredients	**Transition BIO-CHLOR Group (day -21 to calving)**		**Transition X-Zelit Group (day -14 to calving)**	
	Kg AF/head/day	Kg DM/head/day	Kg AF/head/day	Kg DM/head/day
Canola Meal	0.540	0.487	1.150	1.037
BIO-CHLOR	0.816	0.713	0.000	0.000
X-Zelit	0.000	0.000	0.500	0.498
Magnesium Sulphate	0.113	0.112	0.000	0.000
Micro mineral premix	0.027	0.026	0.027	0.026
Magnesium Oxide	0.045	0.045	0.060	0.060
Urea granular	0.000	0.000	0.080	0.079
Wheat grain (hammer milled)	2.959	2.634	3.183	2.833
Sweet Corn Trash	25.00	4.325	25.00	4.325
Forage sorghum hay (ad lib)	4.00	2.8	4.00	2.80
TOTAL	33.50	11.14	34.00	11.66
Diet composition (% of diet DM)	**Transition BIO-CHLOR Group (day -21 to calving)**		**Transition X-Zelit Group (day -14 to calving)**	
CP (%)	16.36		16.39	
RUP (%CP)	26.27		26.90	
RDP (%CP)	73.73		73.10	
Soluble protein (%CP)	41.21		39.60	
ME (MJ/kg)	9.85		9.38	
ADF (%)	25.51		25.01	
NDF (%)	44.75		42.96	
NFC (%)	32.20		32.34	
Sugar (%)	10.26		9.94	
Starch (%)	18.40		18.69	
EE (fat)	2.38		2.31	
DCAD (meq/100g)	-14.64		8.91	
Calcium (%)	0.32		0.34	
Phosphorus (%)	0.37		0.37	
Magnesium (%)	0.64		0.60	
Potassium (%)	1.56		1.50	
Sulphur (%)	0.46		0.20	

3.2.2. Lactation period

After calving, cows in both groups were offered an identical dairy ration after calving up to 100 DIM. The ration was be formulated using CPM-Dairy with the ingredients available at the farm. The dietary ingredients were adjusted to provide a balanced ration (Table 3) and meet the lactation requirements. The lactating cows were housed in a “compost bedded pack” system which provided a soft bedding area in a shaded facility. The bedding area allocation was 10m^2/cow (1600 m^2 for 160 lactating cattle).

Table 3. Diet ingredients and estimated average nutrient compositions of the lactating diet during the experimental period (CPM-Dairy V3; 2008)

Ingredients	**Lactation (calving to day 100)**	
	Kg AF/head/day	**Kg DM/head/day**
Protein/Fat Pellet (robots)	3.500	3.148
Wheat grain (hammer milled)	7.000	6.230
Sodium Bicarbonate	0.200	0.199
Magnesium Oxide	0.060	0.159
DCP	0.050	0.050
Salt	0.080	0.080
Micro mineral premix	0.025	0.024
BioFix Mycotoxin Binder	0.030	0.027
Lucerne Hay	1.000	0.900
Canola Meal	4.600	4.148
Sweet Corn Trash	47.50	9.500
TOTAL	64.05	24.47
Diet composition (% of diet DM)	**Lactation (calving to day 100)**	
CP (%)	17.93	
RUP (%CP)	34.82	
RDP (%CP)	65.18	
Soluble protein (%CP)	32.49	
ME (MJ/kg)	10.92	
ADF (%)	18.45	
NDF (%)	32.83	
NFC (%)	42.12	
Sugar (%)	11.45	
Starch (%)	26.53	
EE (fat)	3.26	
DCAD (meq/100g)	13.87	
Calcium (%)	0.71	
Phosphorus (%)	0.56	
Magnesium (%)	0.39	
Potassium (%)	1.09	
Sulphur (%)	0.27	

3.2.3. Trial diet ingredients and feed sampling

Diets (transition and lactating) were recorded and feeds were analysed to determine dietary changes occurring during the study period. Additional feed samples were taken, when changes were made in the diets of either transition or lactating feed ingredients. The feed samples were transferred to labelled plastic bags, frozen at -20ºC until analysis at Feedcentral Laboratory (Dairy One Equipment and Calibrations). Each sample was analysed (wet chemistry analysis) for DM, NDF, crude protein (CP), degradable protein, crude fat, ash, acid detergent fibre (ADF), lignin, non-fibrous carbohydrate (NFC), non-structural carbohydrate (NSC), starch, sugar, calcium (Ca), phosphorus (Phos), magnesium (Mg), potassium (K) and sulphur (S) contents.

The feed test analysis of ingredients used in transition and lactation diets and analysis of the batch transition grain mixes (Bio-Chlor and X-Zelit) are presented in table 4.

Table 4. Feed test analysis of ingredients used in transition and lactating diets

Nutrient composition (% of DM)	Sweet Corn Trash	Forage Sorghum Hay	X-Zelit Grain Mix	Bio-Chlor Grain Mix
CP (%)	13.30	15.50	23.00	23.10
ADICP	0.40	0.90	0.20	1.60
NDICP	1.00	4.00	1.80	1.80
Soluble protein (%CP)	47.80	45.20	43.00	38.00
ME (MJ/kg)	12.20	9.20	13.10	13.90
ADF (%)	23.80	32.70	6.80	6.90
NDF (%)	49.80	57.40	10.60	8.30
NFC (%)	25.80	19.20	55.00	59.50
TDN (%)	76.70	57.80	75.60	82.10
Water Soluble Carb (%)	9.80	7.00	Not reported	Not reported
Lignin (%)	1.00	5.70	2.50	0.50
Ash (%)	4.90	9.20	9.40	6.60
Starch (%)	12.90	1.30	32.40	40.80
Fat (%)	6.10	2.70	2.60	2.90
Calcium (%)	0.10	0.47	0.31	0.24
Phosphorus (%)	0.45	0.35	0.54	0.48
Magnesium (%)	0.15	1.14	0.73	1.26
Potassium (%)	1.50	1.22	0.68	0.65
Sulphur (%)	0.16	0.16	0.31	1.38
Chloride (%)	0.32	1.73	0.13	1.36
Sodium (%)	0.03	0.06	0.83	0.25
DCAD (meq/kg DM)	206.17	-251.84	306.21	-967.66

3.3. Sampling, analysis and storage

3.3.1. Blood sampling

Blood samples were taken from a subgroup of animals (cows=14, heifers=6) on the morning of being placed on the transition diet (day -27 to -21 BIO-CHLOR, day -20 to -14 X-Zelit, relative to expected calving day), weekly during the transition period, one sample at calving (up to 24hrs after calving), 48 to 72 hrs post-calving, 7 days post calving, and then batch sampling (14-20 DIM and 21-30 DIM) post-calving. Blood samples were taken by venepuncture of tail vein, using an 18-gauge needle, and collected into 3 plain (red top) tubes. Blood samples were then centrifuged at 3500rpm for 15 minutes and transferred into corresponding 5ml vials and stored at -20°C until analysis for macro-minerals (Ca, P & Mg).

3.4. Management of study animals and experimental procedure

All cows within the herd that were enrolled in the study were run as a single herd and were milked using an automatic milking system (AMS), consisting of 3 milking robots (Lely Holding, Maassluis, The Netherlands). Cows received an allocated amount (3.5 kg) of a custom grain/protein pellet in the robots to encourage them to enter the AMS system and get milked. Cows were then offered a TMR at the feed-pad with ad libitum access to feed at all time, but no access to grass during the experimental period. Frequency of milking ranged from 1 or 6 times per day, and milk yield and compositions (fat and protein %) were recorded at every milking for each cow. The amount of milk production during each milking were measured and pooled to provide daily milk yield. Milk fat and protein contents (%) were measured at each milking. Duration and frequency of rumination were also measured using a Qwes-HR tag (Lely Holding, Maassluis, The Netherlands), which included an acceleration sensor, specially tuned rumination microphone, a microprocessor and memory. The tag records a general activity index and analyses the vocal signals to calculate rumination time. The tag is attached to the upper part of the cow's neck with a strap and weight in a way that prevents false movements and protects the tag from mechanical damage. Changes in rumination are the earliest signs that can provide warning about potential health problems.

Cows were monitored daily by the farm staff and all metabolic and health events were recorded. The definitions of these disorders were determined by farm managers in consultation with study investigator. The incidence of metabolic and health diseases was based on agreed definitions of these disorders at the onset of the study.

3.5. Statistical analysis

A total of 72 cows were initially enrolled in this study. Four cows were excluded from a subgroup of cows (n=24) that have been selected for blood sampling, due to incomplete macro-mineral profile. Blood profile from Day -21 to day 21 were used for statistical analysis. Cows and heifers (n=12) with transition period of less than 12 days or more than 40 days were excluded from the statistical analysis. Animals (n=4) with an unforeseen health event prior to enrolment were excluded from statistical analysis. List of excluded animals and reason for exclusion are provided in Appendix A. Production data from calving to day 82 were included in the statistical analysis. Descriptive statistics (mean ± SD) of blood Ca, Mg and Phosphorous, production data (milk production, milk fat & protein (% and yield) and total milk solids) and rumination time were estimated.

Production and blood macro-mineral data were analysed using a mixed effects linear regression with repeated measures (with restricted maximum likelihood estimators (REML) approach), using the R program (The R Foundation for Statistical Computing Platform, 2014) program and nlme package (Pinheiro et al., 2014). Dependent variables were macro-mineral blood profile, production parameters and duration of rumination, and independent variables included treatment groups (BIO-CHLOR and X-Zelit), sampling intervals (weeks or days) and other covariates. The interactions between time and groups were also explored. Coefficients (± se), probability of significance (P-value) and least square means (LSM) were estimated to determine the differences in blood concentrations of Ca, Mg and Phosphorous and productivity measures between the treatment groups. The included covariates in the model were remained in the model if these were significant or changed the magnitude of coefficients for the treatment groups.

4. RESULTS

4.1. Macro-minerals

4.1.1. Descriptive analysis

Descriptive statistics of parity, DIM, transition periods and serum concentration of Ca, Mg and Phosphorous for cows and heifers at enrolment, before feeding the treatment diets, are presented in Table 5. There was no significant difference in parity, DIM, transition period and serum concentrations of macro-minerals between BIO-CHLOR and X-Zelit groups (P> 0.05, Figure 1).

Table 5. Descriptive statistics of parity, DIM, transition periods and serum concentration of Ca, Mg and Phosphorous for cows & heifers at enrolment (before feeding the treatment diets)

	BIO-CHLOR	**X-Zelit**	
	N (Mean ± SD)	**N (Mean ± SD)**	**P value**
No of cows/heifers (N)	N = 9 (7 cows/2 heifers)	N = 11 (7 cows/4 heifers)	NA
Parity (N)	N=7 3.29 ± 0.50	N=7 3.29 ± 0.50	P =1.00
Days in milk previous lactation (N)	N=7 270.86 ± 10.53	N=7 282.0 ± 33.83	P = 0.743
Transition period (days)	N=9 30.47 ± 9.17	N=11 27.64 ± 16.28	P = 0.630
Serum Ca Con. (mmol/L)	N=9 2.35 ± 0.10	N=9 2.25 ± 0.19	P = 0.146
Serum Mg Con. (mmol/L)	N=9 0.99 ± 0.08	N=9 1.04 ± 0.11	P = 0.366
Serum Phos Con. (mmol/L)	N=9 2.08 ± 0.26	N=9 1.81 ± 0.41	P = 0.092

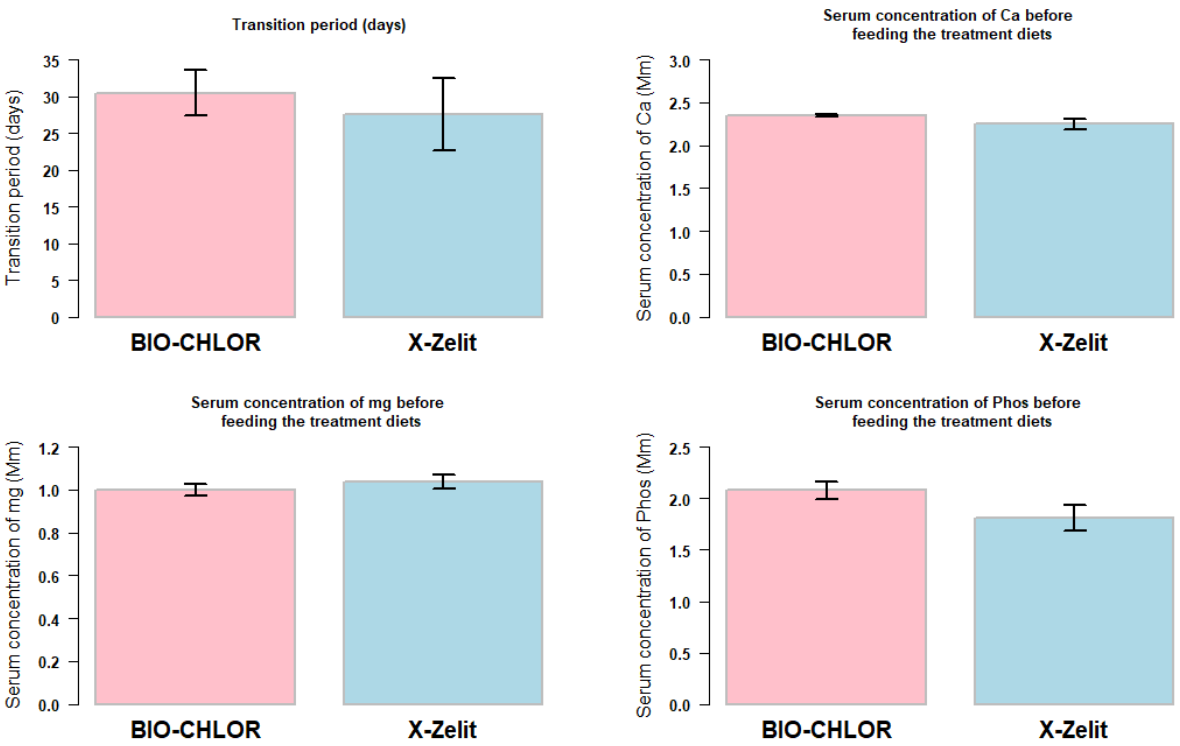

Figure 1: Mean (± SD) of parity, DIM, transition periods and serum concentration of Ca, Mg and Phosphorous for cows & heifers at enrolment (before feeding the treatment diets)

Descriptive statistics (mean ± SD) of serum concentrations of Ca, Mg and Phosphorous during pre-calving, at calving and post-calving periods are presented in Table 6.

Table 6. Average (±SD) of serum concentration of Ca, Mg and Phosphorous during pre-calving period (-2 week to calving), calving (0-24 hr) and post calving (0 to +3 week)

	Serum concentrations (mmol/L)					
	Pre-calving (days -14 to calving)		**At calving (0 to 24hr)**		**Post calving (calving to day 21)**	
	BIO-CHLOR	**X-Zelit**	**BIO-CHLOR**	**X-Zelit**	**BIO-CHLOR**	**X-Zelit**
	No of observations Mean ± SD	**No of observations Mean ± SD**	**No of observations Mean ± SD**	**No of observations Mean ± SD**	**No of observations Mean ± SD**	**No of observations Mean ± SD**
Ca	N= 18 2.30 ± 0.09	N= 21 2.43 ± 0.17	N= 9 2.16 ± 0.13	N= 11 2.40 ± 0.31	N= 27 2.31 ± 0.20	N= 33 2.33 ± 0.18
Mg	N= 18 0.96 ± 0.10	N= 21 0.94 ± 0.14	N= 9 1.01 ± 0.09	N= 11 1.14 ± 0.20	N= 27 0.96 ± 0.09	N= 33 1.10 ± 0.22
Phos	N= 18 2.08 ± 0.18	N= 21 1.32 ± 0.69	N= 11 1.92 ± 0.21	N= 11 1.39 ± 0.34	N= 27 1.90 ± 0.30	N= 33 1.77 ± 0.37

4.1.2. Multivariable analysis

Estimated coefficients and least square means of serum concentration of Ca, Mg and Phosphorous during pre-calving (day -14 to calving), at calving (day 0) and post-calving (calving to day 21) are presented in Tables 7-9. Serum concentrations were adjusted for transition period (days) and for pre-feeding concentrations.

Serum Ca: Serum concentrations of Ca were significantly higher in X-Zelit group than BIO-CHLOR group during the pre-calving period (P=0.003). The concentrations of Ca were not impacted by serum Ca levels before feeding the treatment (Table 7, Figure 2).

Table 7. Estimated coefficients (±Se) and least square means of serum concentration of Ca from day -14 to day 21

	Serum Ca concentrations (mmol/L)					
	Day -14 to calving (pre-calving)		**Day 0 (calving)**		**Calving to day 21 (post-calving)**	
	Coefficient ±Se	**P value**	**Coefficient ±Se**	**P value**	**Coefficient ±Se**	**P value**
Treatment Group	0.208 ± 0.060	0.003	0.193 ± 0.118	0.123	0.145 ± 0.10	0.164
Week	0.010 ± 0.027	0.715	NA	NA	0.110 ± 0.027	0.0002
Days in Transition	0.113 ± 0.057	0.067	0.001 ± 0.004	0.782	0.003 ± 0.003	0.283
Pre-feeding concentration	0.263 ± 0.176	0.154	-0.488 ± 0.377	0.215	0.389 ± 0.205	0.075
	LSM & 95%CI		**LSM & 95%CI**		**LSM & 95%CI**	
BIO-CHLOR	2.258 (2.169, 2.346)		2.189 (2.008, 2.370)		2.283 (2.177, 2.388)	
X-Zelit	2.466 (2.385, 2.546)		2.382 (2.220, 2.544)		2.348 (2.257, 2.439)	

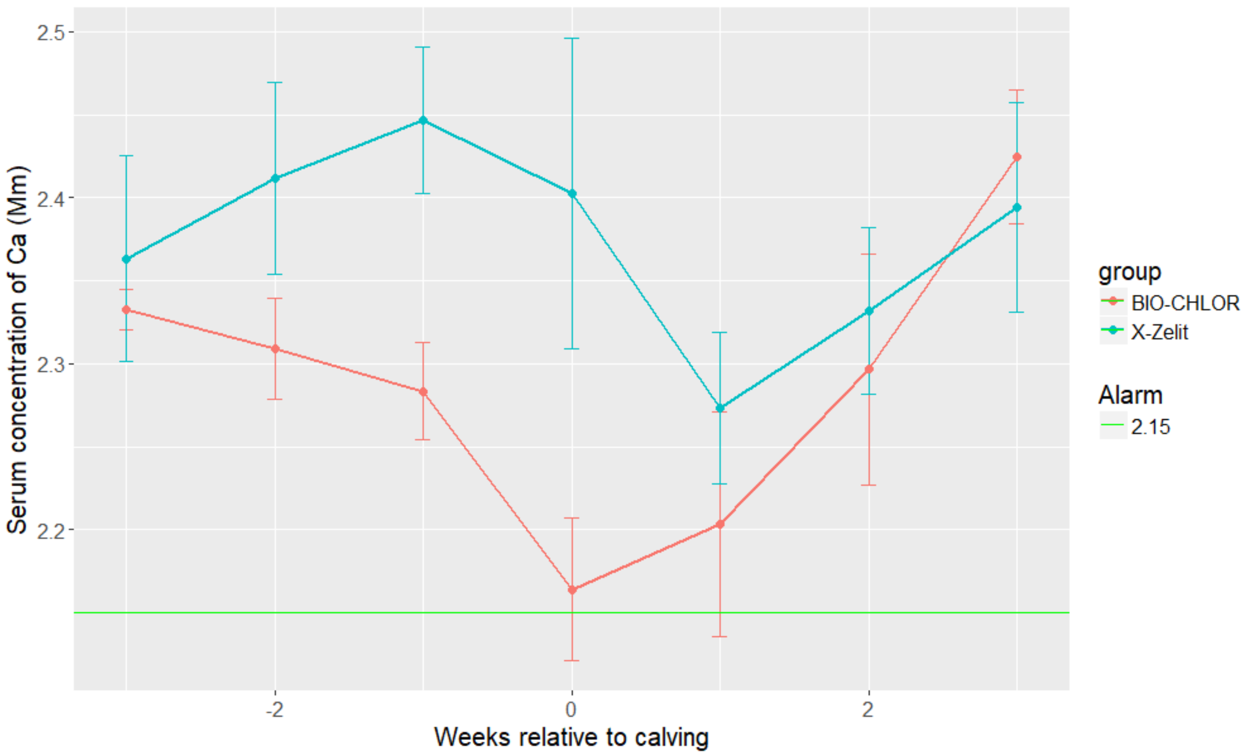

Figure 2. Serum concentrations of Ca from (mean ±Se) from week -3 to week +3 after calving, and alarm level

Serum Mg: Concentration of Mg during the experimental period (day -21 to day 21) didn't differ between the treatment groups. The level of Mg before feeding the treatment diets didn't influence the concentrations of Mg during transition period and lactation (Table 8, Figure 3).

Table 8. Estimated coefficients (±Se) and least square means of serum concentration of Mg from week -2 to week +3

	Serum Mg concentrations (mmol/L)					
	Day -14 to calving (pre-calving)		**Day 0 (calving)**		**Calving to day 21 (post-calving)**	
	Coefficient ±Se	**P value**	**Coefficient ±Se**	**P value**	**Coefficient ±Se**	**P value**
Treatment Group	-0.166 ± 0.098	0.110	0.083 ± 0.076	0.293	0.107± 0.074	0.168
Week	0.042 ± 0.040	0.307			0.011 ± 0.015	0.473
Days in Transition	-0.0004 ± 0.002	0.804	-0.001 ± 0.003	0.684	0.0003 ± 0.003	0.903
Pre-feeding concentration	-0.319 ± 0.159	0.063	-0.444 ± 0.244	0.0870	-0.304 ± 0.227	0.198
	LSM & 95%CI		**LSM & 95%CI**		**LSM & 95%CI**	
BIO-CHLOR	0.945 (0.899, 1.051)		1.036 (0.919, 1.153)		0.963 (0.852, 1.075)	
X-Zelit	0.921 (0.852, 0.990)		1.119 (1.014, 1.224)		1.092 (0.994, 1.190)	

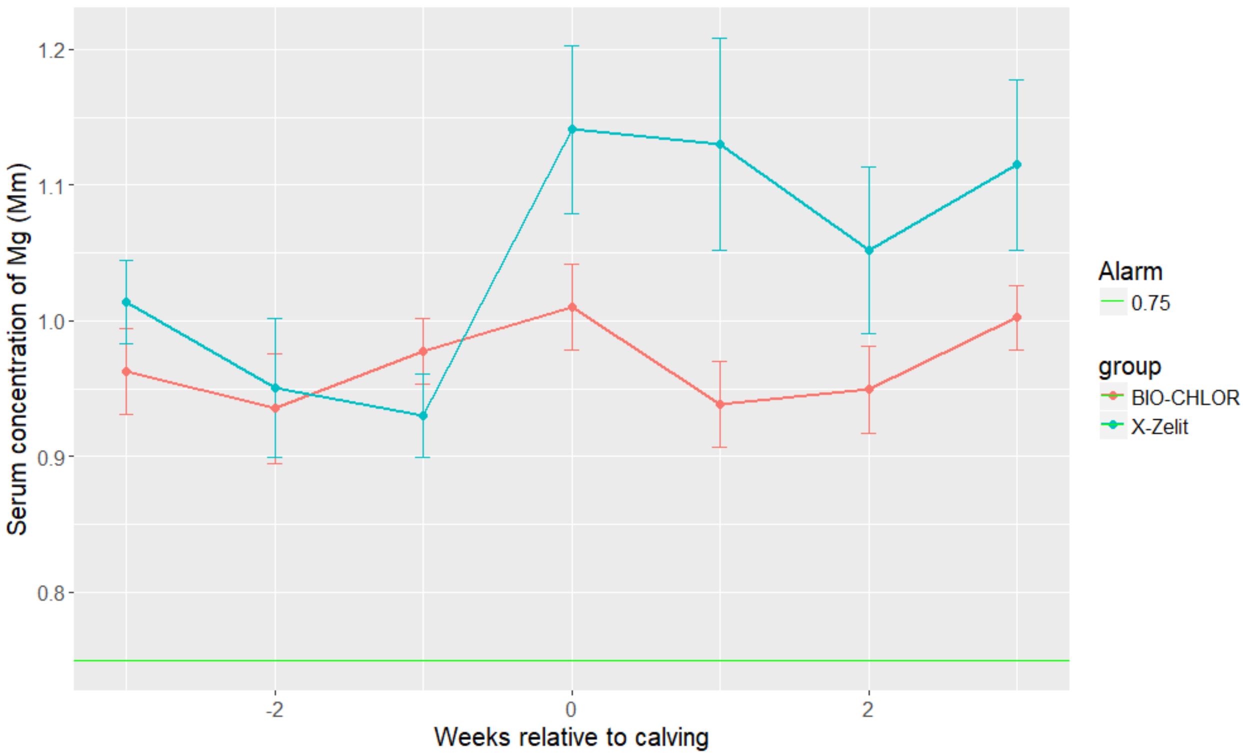

Figure 3. Serum concentrations of Mg from (mean ±Se) from week -3 to week +3 after calving, and alarm level

Serum Phosphorous: Serum concentration of Phosphorous was significantly higher in BIO-CHLOR group than those in the X-Zelit group during pre-calving and calving periods. The concentration of Phosphorous before feeding the treatment diets negatively impacted the Phosphorous concentration in the X-Zelit group during the pre-calving and calving periods (Table 9, Figure 4).

Table 9. Estimated coefficients (±Se) and least square means of serum concentration of Phosphorous from week -2 to week +3

	Serum P concentrations (mmol/L)					
	Day -14 to calving (pre-calving)		**Day 0 (calving)**		**Calving to day 21 (post-calving)**	
	Coefficient ±Se	**P value**	**Coefficient ±Se**	**P value**	**Coefficient ±Se**	**P value**
Treatment Group	-0.818 ± 0.236	0.003	-0.503 ±0.142	0.003	-0.359 ± 0.223	0.126
Week	-0.0873 ± 0.116	0.463	0.004 ± 0.005	0.451	-0.084 ± 0.075	0.271
Days in Transition	-0.002 ± 0.009	0.855	-0.373 ± 0.757	0.629	0.006 ± 0.003	0.105
Pre-feeding concentration	-0.428 ± 0.749	0.576	-0.503 ± 0.142	0.003	0.645 ± 0.299	0.045
	LSM & 95%CI		**LSM & 95%CI**		**LSM & 95%CI**	
BIO-CHLOR	2.112 (1.753, 2.470)		1.906 (1.687, 2.126)		1.858 (1.713, 2.000)	
X-Zelit	1.294 (0.968, 1.620)		1.403 (1.205, 1.601)		1.807 (1.679, 1.936)	

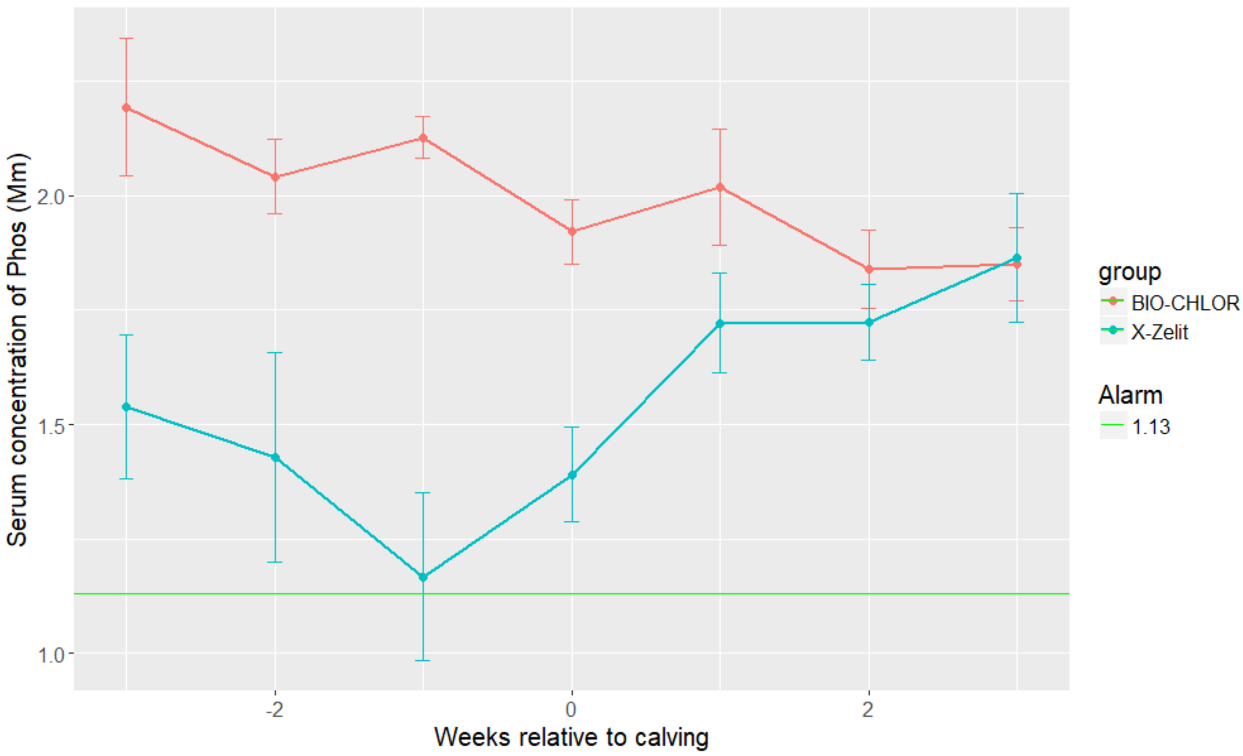

Figure 4. Serum concentrations of Phosphorous from (mean ±Se) from week -3 to week +3 after calving, and alarm level

4.2. Milk production & milk compositions

4.2.1. Descriptive analysis

Number of animals (cows and heifers) and average parity, transition period and previous lactation yield of cows enrolled in this study are presented in Table 10.

Table 10. Descriptive statistics (mean ± SD) of cows enrolled in the study

	BIO-CHLOR		**X-Zelit**		
	N (Mean ± SD)		**N (Mean ± SD)**		Range
No of animals enrolled (N)	36		36		
No of animals included in statistical analysis	24		32		
Cows	18		24		
Heifers	6		8		
Average parity (range 1 to ≥ 6)	N=24 3.08 ± 1.67		N=32 2.78 ± 1.54		BIO-CHLOR= (1– ≥ 6) X-Zelit = (1– ≥ 6)
Transition period (days)	N=24 25.6 ± 6.15		N=32 20.8 ± 4.72		BIO-CHLOR= (14 – 37) X-Zelit = (12 – 36)
No of animals 10-20 days in transition	Cows N = 7	Heifers N = 0	Cows N = 14	Heifers N = 4	
No of animals 21-30 days in transition	Cows N = 6	Heifers N = 5	Cows N = 10	Heifers N = 3	
No of animals 31-40 days in transition	Cows N = 5	Heifers N = 1	Cows N = 0	Heifers N = 1	
305-day milk yield (L) (previous lactation)	N=18 10,424 ± 1,443		N=24 10,894 ± 2,569		BIO-CHLOR = (8301 – 12853) X-Zelit = (6223 – 16629)

Due to the disproportionate distribution of the number of animals in days in transition (table 10), it was not appropriate to include days in transition in the multivariable model. Interactions between treatment and time (days in lactation) were included in the model if these were significant.

Table 11 shows the descriptive statistics of herd production parameters and rumination time for all animals, and cows and heifers separately.

Table 11. Descriptive statistics (mean ± SD) of production parameters and rumination for cows & heifers from calving to day 82 in lactation

	No of observations (Mean ± SD)					
	All animals		**Heifers**		**Cows**	
	BIO-CHLOR (N=1988)	**X-Zelit** (N=2652)	**BIO-CHLOR** (N=497)	**X-Zelit** (N=662)	**BIO-CHLOR** (N=1491)	**X-Zelit** (N=1990)
Milk yield (L)	35.6 ± 9.84	39.6 ± 12.37	29.8 ± 7.88	29.7 ± 8.49	37.5 ± 9.69	42.9 ± 11.68
Milk fat (%)	3.62 ± 0.843	3.43 ± 0.717	3.36 ± 0.520	3.36 ± 0.603	3.71 ± 0.910	3.45 ± 0.750
Milk fat yield (kg)	1.29 ± 0.475	1.34 ± 0.425	0.987± 0.243	0.967± 0.221	1.39 ± 0.489	1.46 ± 0.405
Milk protein (%)	3.45 ± 0.480	3.36 ± 0.443	3.38 ± 0.388	3.39 ± 0.388	3.47 ± 0.505	3.35 ± 0.460
Milk protein yield (kg)	1.20 ± 0.300	1.30 ± 0.365	0.987± 0.231	0.986± 0.255	1.27 ± 0.286	1.40 ± 0.336
Milk solids (kg)	2.49 ± 0.716	2.63 ± 0.739	1.97 ± 0.449	1.95 ± 0.444	2.66 ± 0.704	2.86 ± 0.676
Rumination time (min/day)	428 ± 102	470 ± 102	453 ± 91.2	442 ± 93.8	420 ± 104	480 ± 103
Number of milking's (no/day)	2.79 ± 0.824	2.95 ± 0.869	2.90 ± 0.915	2.59 ± 0.859	2.75 ± 0.789	3.07 ± 0.839

4.2.2. Multivariable analysis

Milk yield: Average daily milk yield was higher in the X-Zelit group, for both cows and heifers (Table 12, Figure 5). Figure 5 shows that the milk production level was consistently greater in the X-Zelit group during the experiment.

Table 12. Estimated coefficients (± se) and least square means (LSM) using a mixed model linear regression (repeated measures) for milk yield for cows & heifers from calving to day 82

	Coefficients ± se					
	All animals		**Heifers**		**Cows**	
	Coefficients ± se	***P* value**	**Coefficients ± se**	***P* value**	**Coefficients ± se**	***P* value**
Treatment	4.219 ± 1.991	0.039	0.983 ± 2.167	0.658	4.088 ± 2.224	0.074
Days in lactation	0.157 ± 0.004	<0.0001	0.150 ± 0.006	<0.0001	0.157 ± 0.005	<0.0001
Parity	2.171 ± 0.626	0.001	NA		1.053 ± 1.106	0.347
No of milking's	2.927 ± 0.126	<0.0001	3.662 ± 0.179	<0.0001	2.609 ± 0.161	<0.0001
Previous lactation yield (305-day)	NA		NA		0.001 ± 0.001	0.042
	LSM & 95% CI		**LSM & 95% CI**		**LSM & 95% CI**	
BIO-CHLOR	35.465 (32.45, 38.48)		28.160 (24.43, 31.89)		38.239 (34.85, 41.63)	
X-Zelit	39.683 (37.08, 42.29)		30.985 (27.82, 34.15)		42.327 (39.39, 45.26)	

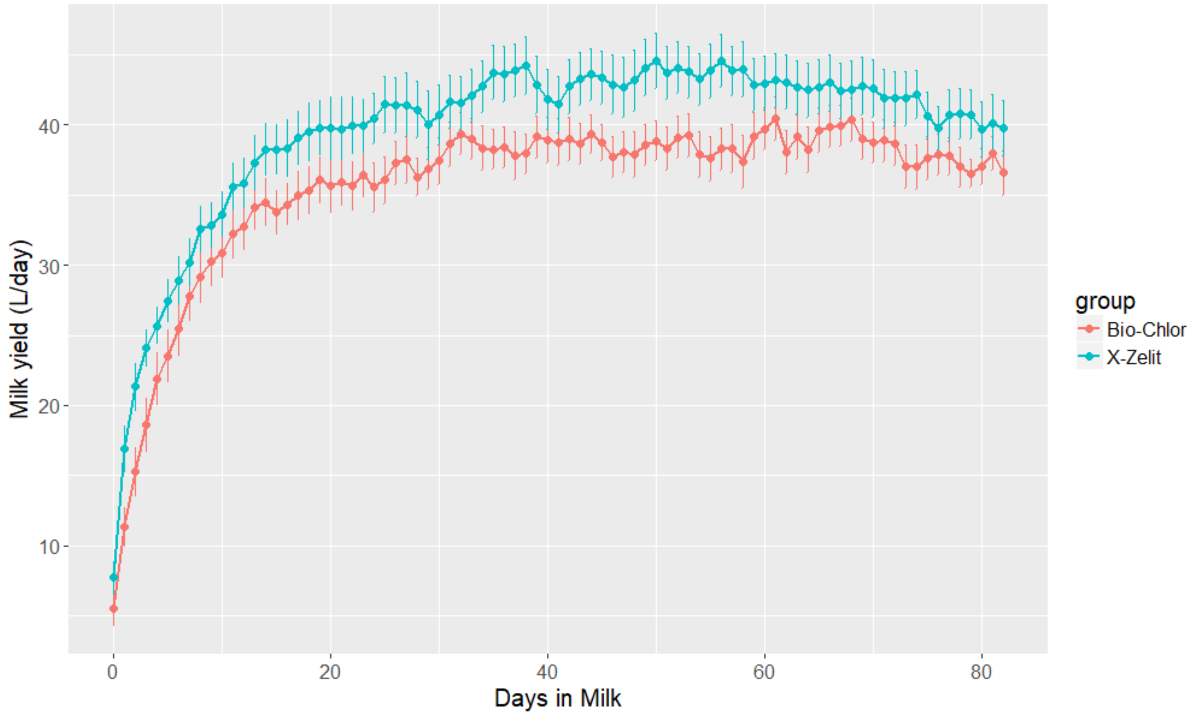

Figure 5. Daily milk yield (L/day) from calving to day 82 in cows and heifers fed BIO-CHLOR or X-Zelit during transition period

Milk fat (%): Average milk fat content (%) was, non-significantly, higher in cows and heifers fed BIO-CHLOR than those in X-Zelit group (Table 13, Figure 6). The milk fat percentage was higher in BIO-CHLOR group than X-Zelit cows throughout the lactation period (Figure 6).

Table 13. Estimated coefficients (± se) and least square means (LSM) using a mixed model linear regression (repeated measures) for milk fat content (%) for cows & heifers from calving to day 82

	Coefficients ± se					
	All animals		**Heifers**		**Cows**	
	Coefficients ± se	***P* value**	**Coefficients ± se**	***P* value**	**Coefficients ± se**	***P* value**
Treatment	-0.015 ± 0.139	0.914	-0.013 ± 0.170	0.941	0.105 ± 0.162	0.520
Days in lactation	-0.006 ± 0.001	<0.0001	-0.006 ± 0.001	<0.0001	-0.003 ± 0.001	<0.0001
Parity	0.115 ± 0.043	0.010	NA		0.304 ± 0.073	0.0002
No of milking's	0.033 ± 0.011	0.003	-0.041 ± 0.017	0.017	0.067 ± 0.014	<0.0001
Previous lactation yield (305-day)	NA		NA		-0.0002 ± 0.00004	0.001
Treatment x Days	-0.004 ± 0.001	<0.0001	-	ns	-0.005 ± 0.001	<0.0001
	LSM & 95% CI		**LSM & 95% CI**		**LSM & 95% CI**	
BIO-CHLOR	3.604 (3.40, 3.81)		3.370 (3.09, 3.65)		3.607 (3.37, 3.84)	
X-Zelit	3.441 (3.26, 3.62)		3.357 (3.11, 3.60)		3.525 (3.32, 3.73)	

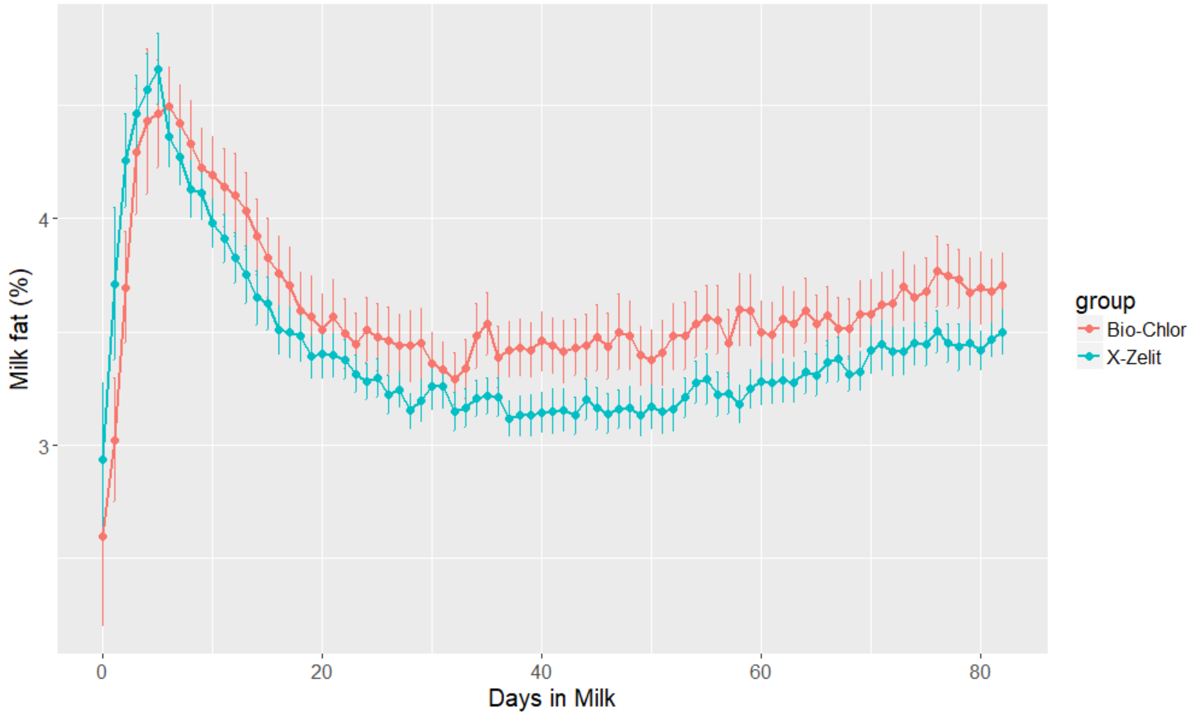

Figure 6. Milk fat content (%) from calving to day 82 in cows and heifers fed BIO-CHLOR or X-Zelit during transition period

Milk fat yield (kg): The milk fat yield didn't significantly differ between BIO-CHLOR and X-Zelit groups, for both cows and heifers (Table 14, Figure 7).

Table 14. Estimated coefficients (± se) and least square means (LSM) using a mixed model linear regression (repeated measures) for milk fat yield (kg) for cows & heifers from calving to day 82

	Coefficients ± se					
	All animals		**Heifers**		**Cows**	
	Coefficients ± se	***P* value**	**Coefficients ± se**	***P* value**	**Coefficients ± se**	***P* value**
Treatment	0.127 ± 0.086	0.147	0.016 ± 0.056	0.777	0.108 ± 0.115	0.353
Days in lactation	0.004 ± 0.0002	<0.0001	0.004 ± 0.0001	<0.0001	0.004 ± 0.0002	<0.0001
Parity	0.126 ± 0.027	<0.0001	NA		0.094 ± 0.056	0.098
No of milking's	0.111 ± 0.004	<0.0001	0.120 ± 0.005	<0.0001	0.105 ± 0.005	0.006
Previous lactation yield (305-day)	NA		NA		-0.00001 ± 0.00003	0.743
Treatment x Days	-0.002 ± 0.0003	<0.0001	-	ns	-0.002 ± 0.0003	<0.0001
	LSM & 95% CI		**LSM & 95% CI**		**LSM & 95% CI**	
BIO-CHLOR	1.282 (1.15, 1.41)		0.966 (0.87, 1.06)		1.414 (1.24, 1.59)	
X-Zelit	1.343 (1.23, 1.45)		0.99 (0.90, 1.06)		1.443 (1.29, 1.59)	

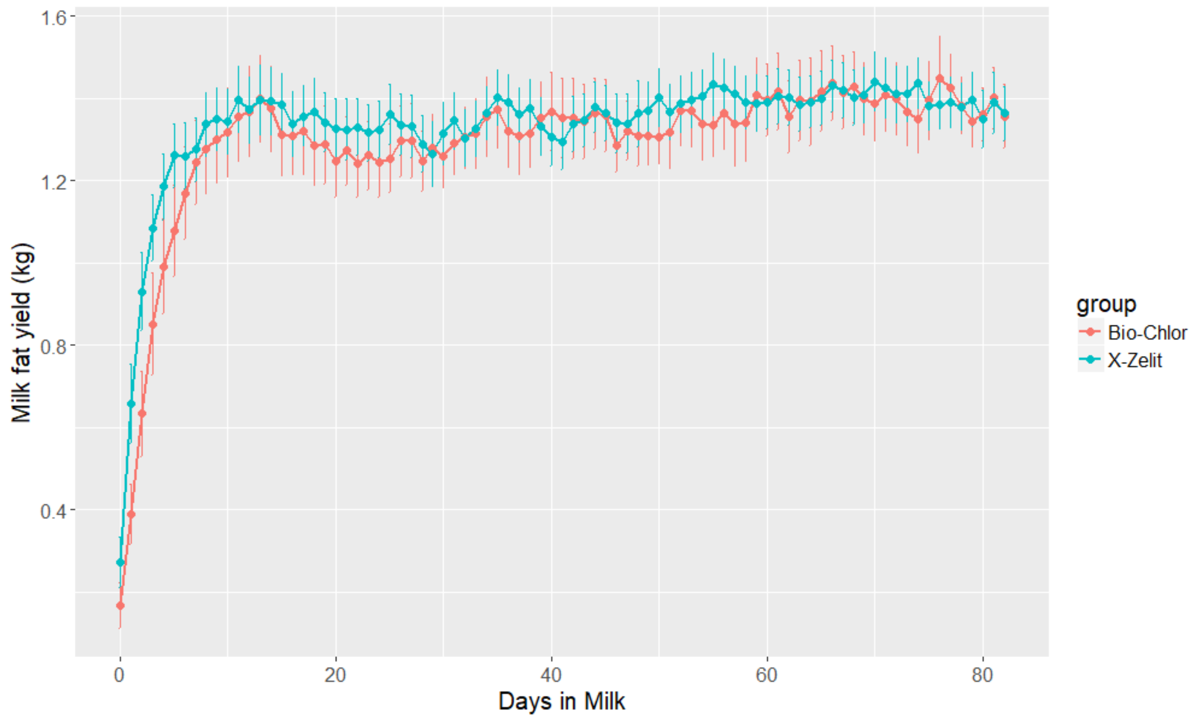

Figure 7. Milk fat yield (kg/day) from calving to day 82 in cows and heifers fed BIO-CHLOR or X-Zelit during transition period

Milk protein content (%): Milk protein content (%) was significantly and consistently higher in BIO-CHLOR than X-Zelit cows during the lactation period (Table 15, Figure 8).

Table 15. Estimated coefficients (± se) and least square means (LSM) using a mixed model linear regression (repeated measures) for milk protein content (%) for cows & heifers from calving to day 82

	Coefficients ± se					
	All animals		**Heifers**		**Cows**	
	Coefficients ± se	***P* value**	**Coefficients ± se**	***P* value**	**Coefficients ± se**	***P* value**
Treatment	-0.167 ± 0.050	0.0016	-0.022 ± 0.052	0.680	-0.210 ± 0.064	0.002
Days in lactation	-0.012 ± 0.0003	<0.0001	-0.012 ± 0.0003	<0.0001	-0.012 ± 0.0004	<0.0001
Parity	-0.008 ± 0.015	0.571	NA		-0.037± 0.030	0.235
No of milking's	-0.058 ± 0.006	<0.0001	-0.094 ± 0.008	<0.0001	-0.040 ± 0.008	<0.0001
Previous lactation yield (305-day)	NA		NA		0.00005 ± 0.00002	0.7674
Treatment x Days	0.002 ± 0.0004	<0.0001	-	ns	0.002 ± 0.001	<0.0001
	LSM & 95% CI		**LSM & 95% CI**		**LSM & 95% CI**	
BIO-CHLOR	3.445 (3.37, 3.52)		3.397 (3.31, 3.48)		3.466 (3.37, 3.56)	
X-Zelit	3.360 (3.30, 3.42)		3.375 (3.30, 3.45)		3.353 (3.27, 3.43)	

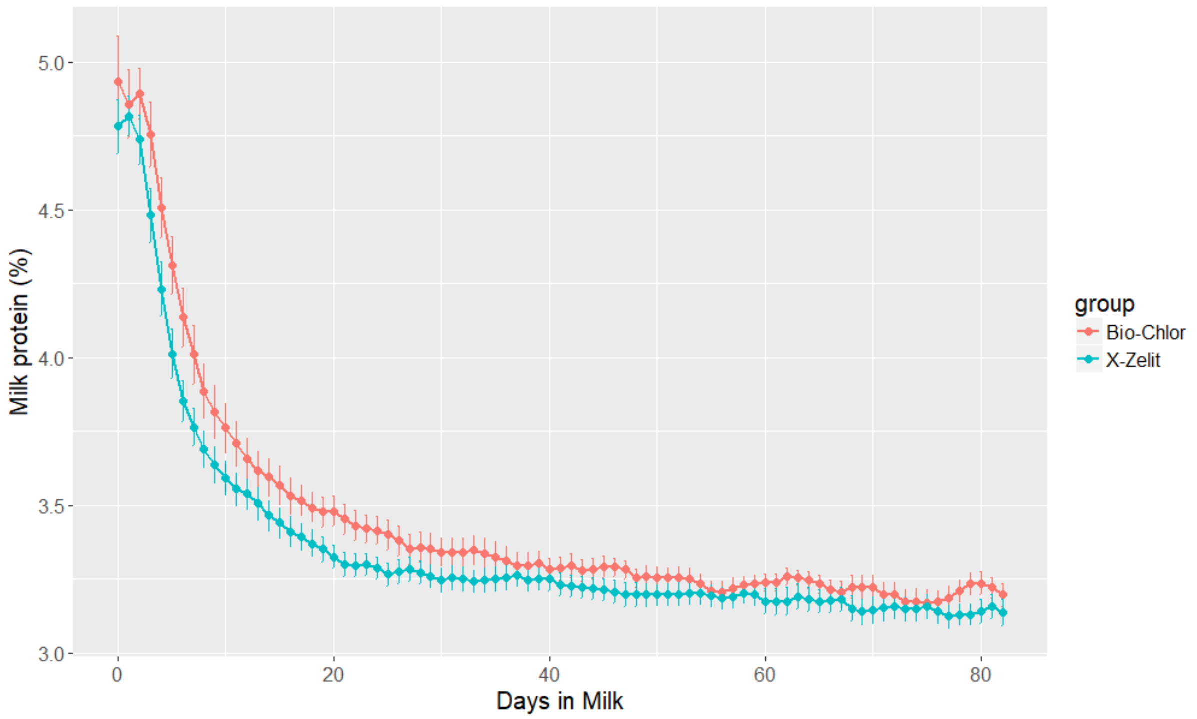

Figure 8. Milk protein content (%) from calving to day 82 in cows and heifers fed BIO-CHLOR or X-Zelit during transition period

Milk protein yield (kg): Estimated average milk protein yield was, non-significantly, greater in X-Zelit group than BIO-CHLOR cows during the lactation (Table 16, Figure 9).

Table 16. Estimated coefficients (± se) and least square means (LSM) using a mixed model linear regression (repeated measures) for milk protein yield (kg) for cows & heifers from calving to day 82

	Coefficients ± se					
	All animals		**Heifers**		**Cows**	
	Coefficients ± se	***P* value**	**Coefficients ± se**	***P* value**	**Coefficients ± se**	***P* value**
Treatment	0.105 ± 0.061	0.089	0.035 ± 0.069	0.622	0.092 ± 0.064	0.159
Days in lactation	0.002 ± 0.0001	<0.0001	0.002 ± 0.0002	<0.0001	0.002 ± 0.0002	<0.0001
Parity	0.067 ± 0.019	0.001	NA		-0.050 ± 0.031	0.115
No of milking's	0.093 ± 0.004	<0.0001	0.117 ± 0.006	<0.0001	0.083 ± 0.005	<0.0001
Previous lactation yield (305-day)	NA		NA		0.00003 ± 0.00001	0.033
	LSM & 95% CI		**LSM & 95% CI**		**LSM & 95% CI**	
BIO-CHLOR	1.196 (1.10, 1.29)		0.966 (0.85, 1.08)		1.293 (1.20, 1.39)	
X-Zelit	1.301 (1.22, 1.38)		1.001 (0.90, 1.10)		1.385 (1.30, 1.47)	

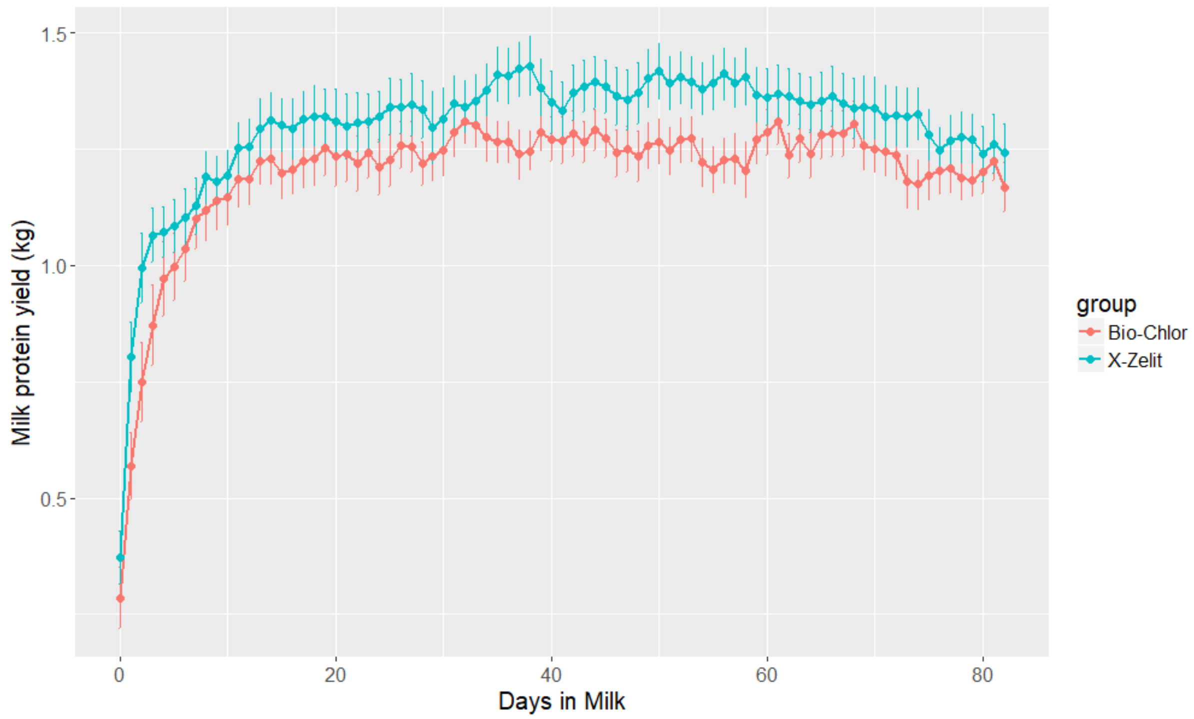

Figure 9. Milk protein yield (kg/day) from calving to day 82 in cows and heifers fed BIO-CHLOR or X-Zelit during transition period

Milk solid (kg): The amount of total daily milk solid (fat + protein yields) were greater in X-Zelit group than those in the BIO-CHLOR group, but this difference was not statistically significant (Table 17, Figure 10).

Table 17. Estimated coefficients (± se) and least square means (LSM) using a mixed model linear regression (repeated measures) for milk solid (kg) for cows & heifers from calving to day 82

	Coefficients ± se					
	All animals		**Heifers**		**Cows**	
	Coefficients ± se	***P* value**	**Coefficients ± se**	***P* value**	**Coefficients ± se**	***P* value**
Treatment	0.245 ± 0.134	0.075	0.051 ± 0.111	0.652	0.226 ± 0.166	0.182
Days in lactation	0.007 ± 0.0003	<0.0001	0.006 ± 0.0004	<0.0001	0.007 ± 0.0004	<0.0001
Parity	0.193 ± 0.042	<0.0001	NA		0.045 ± 0.082	0.589
No of milking's	0.204 ± 0.008	<0.0001	0.237 ± 0.011	<0.0001	0.188 ± 0.010	<0.0001
Treatment X days	-0.002 ± 0.0005	<0.0001	-		-0.002 ± 0.001	0.0002
	LSM & 95% CI		**LSM & 95% CI**		**LSM & 95% CI**	
BIO-CHLOR	2.478 (2.27, 2.68)		1.932 (1.75, 2.11)		2.700 (2.45, 2.95)	
X-Zelit	2.645 (2.47, 2.82)		1.983 (1.83, 2.14)		2.836 (2.62, 3.05)	

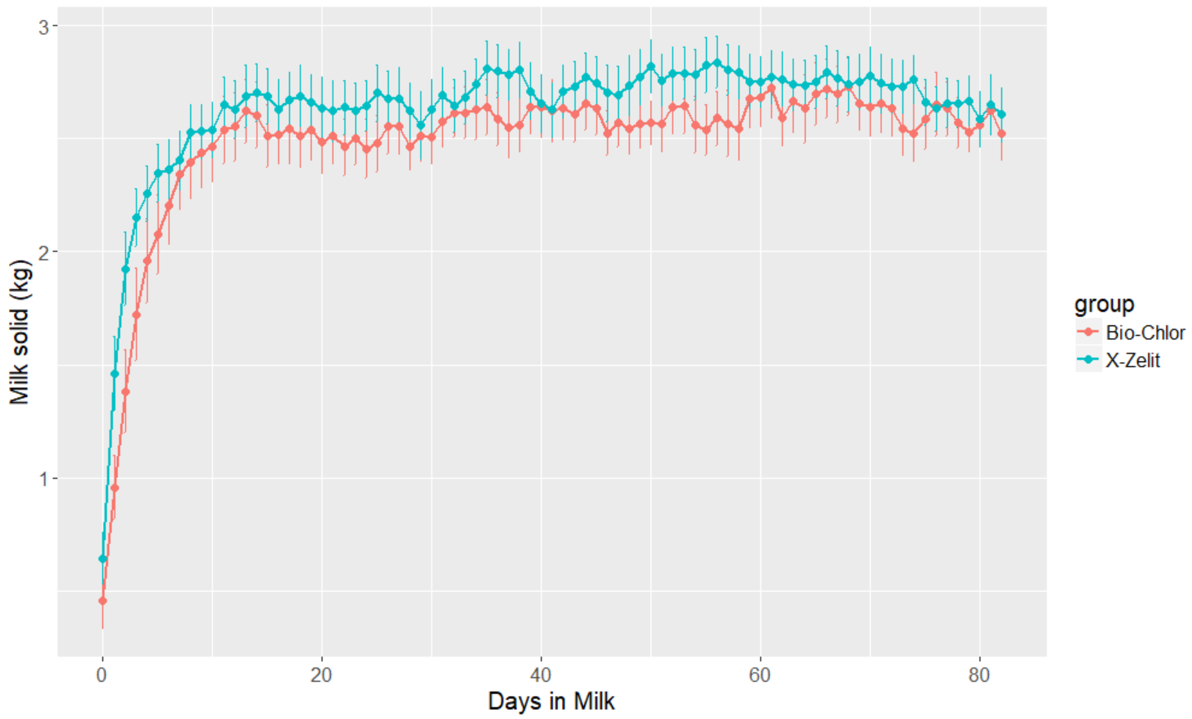

Figure 10. Milk solid (kg/day) from calving to day 82 in cows and heifers fed BIO-CHLOR or X-Zelit during transition period

4.3. Rumination time

The average duration of rumination (min/day) during lactation was significantly higher in cows fed X-Zelit than those in BIO-CHLOR group (Table 18, Figure 11).

Table 18. Estimated coefficients (± se) and least square means (LSM) using a mixed model linear regression (repeated measures) for rumination (minutes/day) for cows & heifers from calving to day 82

	Coefficients ± se					
	All animals		**Heifers**		**Cows**	
	Coefficients ± se	***P* value**	**Coefficients ± se**	***P* value**	**Coefficients ± se**	***P* value**
Treatment	39.308 ± 15.030	0.012	-3.089 ± 28.311	0.915	55.820 ± 17.505	0.003
Days in lactation	0.918 ± 0.050	<0.0001	0.625 ± 0.092	<0.0001	0.986 ± 0.059	<0.0001
No of milking's	14.931 ± 1.603	<0.0001	25.606 ± 2.646	<0.0001	10.948 ± 1.983	<0.0001
	LSM & 95% CI		**LSM & 95% CI**		**LSM & 95% CI**	
BIO-CHLOR	429.893 (407.13,452.66)		448.254 (402.03,494.48)		422.416 (395.70,449.13)	
X-Zelit	469.201 (449.48,488.93)		445.165 (404.79,485.54)		478.235 (455.08,501.39)	

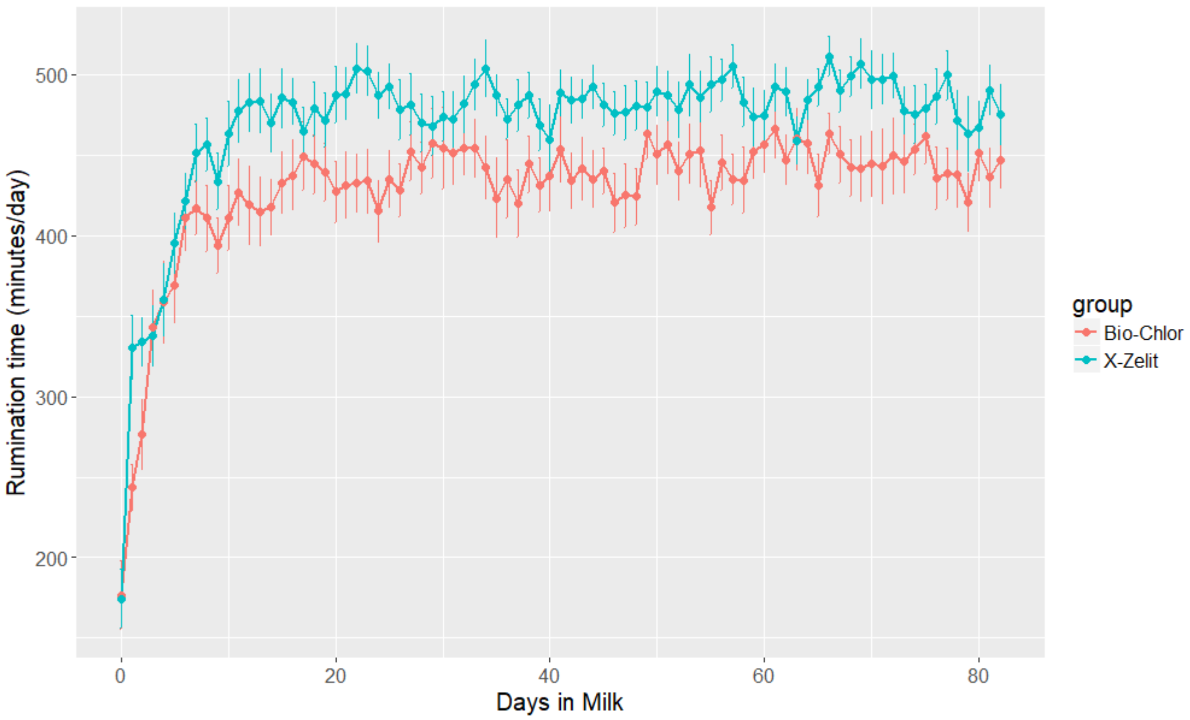

Figure 11. Rumination time (mins/day) from calving to day 82 in cows and heifers fed BIO-CHLOR or X-Zelit during transition period

4.4. Reproduction

Due to the study being terminated at 82 days in lactation and that some animals left the farm, there were only a portion of animals that had accurate pregnancy data. All animals were pregnancy tested at completion of the study and results from animals that had been served in the 30 days previously were excluded. The total number of animals suited for pregnancy analysis were 37. Table 19 shows the first service conception rate and days to conception for the BIO-CHLOR and X-Zelit group.

Table 19. First service conception rate and days to conception for BIO-CHLOR and X-Zelit Groups

	Number of Animals	**No of animals pregnant and % pregnant to 1st Service**		**Days to conception**
Bio-Chlor	16 (5 heifers, 11 cows)	9	56.25%	69
X-Zelit	21 (8 heifers, 13 cows)	12	57.14%	71

Figure 12 illustrates the proportion of cows non-pregnant from calving until the last pregnancy test for the BIO-CHLOR and X-Zelit group.

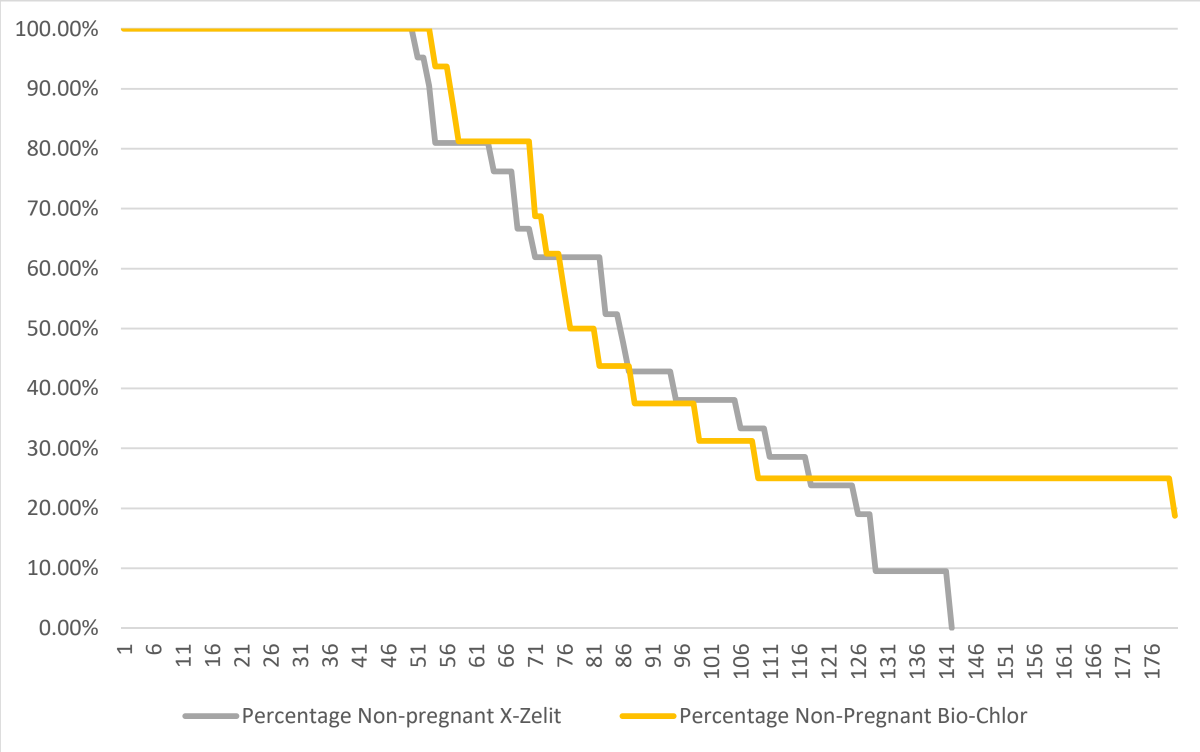

Figure 12. Proportion of cows non-pregnant in BIO-CHLOR and X-Zelit Groups by days in lactation

4.5. Metabolic and health events

Table 20 shows the metabolic and health events that were recorded for the first 21 days in lactation for X-Zelit and BIO-CHLOR groups.

Table 20. Health events (farmer records) for animals 0-21 days in lactation

Animal	Parity	Group	Day in lactation at event	Event	Treatment
1214	5	Bio-Chlor	0	Milk Fever	4 in 1 Drip
1283	4	Bio-Chlor	13	Died Mastitis	Antibiotics
1517	2	Bio-Chlor	0	Assist, Twins, RFM	Prostaglandin
1646	1	Bio-Chlor	0	Assist	
1650	1	Bio-Chlor	0	Assist	
1651	1	Bio-Chlor	0	Premature calf	
1661	1	Bio-Chlor	0	Assist	
1215	5	X-Zelit	0	RFM	Prostaglandin
1325	4	X-Zelit	0	Assist (head back). Suspected mild MF	4 in 1 Drip
1346	3	X-Zelit	0	Assist, Breach	
1346	3	X-Zelit	5	Suspected Ketosis?	Propylene Glycol
1388	3	X-Zelit	0	RFM, Twins	Prostaglandin
1520	2	X-Zelit	0	Assist	
1669	1	X-Zelit	0	Assist	
1672	1	X-Zelit	0	Assist (easy pull)	
1680	1	X-Zelit	0	Assist	

Table 21 shows a summary of the health events (by percentage of the animals calved) in the first 21 days in lactation for the BIO-CHLOR and X-Zelit groups.

Table 21. Summary of health events and percentage of total animals calved for BIO-CHLOR and X-Zelit groups

	BIO-CHLOR		**X-Zelit**	
	No of animals	% of total calved (N=24)	No of animals	% of total calved (N=32)
Assisted calving	4	16.67%	6	18.75%
RFM	1	4.17%	2	6.25%
Milk Fever	1	4.17%	1	3.13%
Mastitis	1	4.17%	0	-
Death	1	4.17%	0	-
Ketosis	0	-	1	3.13%

5. DISCUSSION & CONCLUSION

Serum concentration of calcium was significantly higher in the X-Zelit group than the BIO-CHLOR group during the pre-calving period and was consistently higher at calving and the first 14 days into lactation. Both groups had serum calcium levels during pre-calving, at calving and post calving periods higher than the alarm level of 2.15 mmol/L. Serum concentration of magnesium during the experimental period (day -21 to day 21 in lactation) didn't differ between the treatment groups; however the concentrations of magnesium were higher for the X-Zelit group. The level of magnesium before feeding the treatment diets didn't influence the concentrations of magnesium during the transition period and in early lactation. Serum concentration of phosphorus was significantly higher in the BIO-CHLOR group than those in the X-Zelit group during the pre-calving and calving periods. The concentration of phosphorus prior to enrolment was lower in the X-Zelit group; however this difference was not significant.

Average daily milk yield was significantly higher in the X-Zelit group. Daily milk yield was consistently greater in the X-Zelit group during the experiment. Average milk fat and protein content (%) was, non-significantly, higher in cows and heifers fed BIO-CHLOR than those in X-Zelit group throughout the lactation period. However, fat and protein yield (kg) and total milk solid yield (kg) was consistently higher in the X-Zelit group.

The cows showed the biggest difference between groups in milk yield and milk solid production whereas there was little difference between the heifer groups.

The average duration of rumination (minutes/day) during lactation was significantly higher in the animals fed X-Zelit. The variation in rumination time was mainly seen in the cows whereas there was little difference between the heifer treatment groups.

There were a limited amount of animals that could be included into the analysis of reproductive performance but both groups showed a very similar fist service conception rate and days to conception. The animals in the X-Zelit group were all in calf by 142 days whereas 18.75% of the animals in the BIO-CHLOR group were not pregnant by 180 days. However, due to the small study number, this would need further exploration.

The animal health events were similar in the BIO-CHLOR and X-Zelit groups. The number of assisted calving's were high in both groups (16.67% and 18.75% respectively). The farm manager did not live on farm and his standard operating procedure was to assist any calving animal before leaving the property even though the animal may not have required assistance.

The results show that X-Zelit is effective in maintaining a perfect calcium balance at calving and in this study the animals fed X-Zelit had better blood serum calcium levels at calving than the animals is the DCAD group. The study also showed that magnesium levels are maintained when using X-Zelit. The blood mineral profiling showed that X-Zelit seems to have a marginal binding effect of phosphorus as serum P levels dropped from enrolment until calving but bounced again at calving and into lactation. This is similar to the results found in the Danish trials. There were some animals in the X-Zelit group that went under the "alarm level" of 1.13 mmol/L; however no adverse health events were observed. In the event of a particular farm experiencing phosphorus deficiencies in plants or animals, it may be beneficial to supplement with additional phosphorus when using X-Zelit. However, it is important to note that high phosphorus concentrations have been associated with increased risk of milk fever (Barton et al 1987; Lean et al 2003; Lean et al 2005). Therefore recommendations are to minimise dietary phosphorus concentrations to less than 0.4% of DM before calving.

In summary, management of both clinical and subclinical hypocalcaemia in post calving cows is important for health, milk yield and reproduction. As an alternative to DCAD management, actualising a low calcium diet approach through the use of X-Zelit substantially improved blood calcium post-calving and milk yield in this study. Trends towards positive effects on reproductive performance, immunity and rumination time warrants further study. Also, further studies into the use of X-Zelit in pasture based systems, where DCAD management is often challenging due to high potassium levels in forages, would be beneficial.

In this study, the farm manager noted that the group of animals fed X-Zelit consumed their grain mix immediately after daily feed-out whereas the animals in the DCAD group picked at their feed over the duration of the day. This could result in feed wastage under adverse weather conditions such as rain events. It is well recognized that DCAD diets can be unpalatable and that feed intake can be depressed with using anionic salts. However, using commercial supplements such as BIO-CHLOR has far less palatability issues than standard anionic salts. X-Zelit has a neutral taste which can be beneficial, especially if the transition diet is fed via the dairy (common practise in Tasmania) or if it is fed as a grain mix in troughs. In a TMR system the reduced palatability of DCAD diets will be less problematic.

6. ACKNOWLEDGEMENTS

We would like to thank Dairy Farm Operations Manager Mr Steven Duncan for his assistance with care and management of the animals, feeding management of the experimental diets, blood sample collection and data collection. Dr Ahmad Rabiee for study design, protocol and statistical analysis. Mrs Kamilla Breinhild of Scibus for dietary information and nutrient specifications of study rations. Dr David Paynter of Regional Laboratory Services for blood sample analysis.

7. REFERENCES

Barton, B.A., N.A. Jorgensen and H.F. DeLuca (1987). Impact of prepartum dietary phosphorus intake on calcium homeostasis at parturition. *J. Dairy Sci*. 70:1186-1191

Constable PD, Hinchcliff KW, Done SH and Grunberg W (2017). Metabolic and Endocrine Diseases. In: Veterinary Medicine. A textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. Elsevier, Edition 11, Chapter 17, Volume 2: 1662.

Ingvartsen KL (2006.) Feeding- and management-related diseases in the transition cow: physiological adaptations around calving and strategies to reduce feeding-related diseases. Animal Feed Science and Technology 126:175-213.

Lean, I.J., P.J. DeGaris, D.M. McNeil and E. Block (2005). Hypocalcemia in dairy cows: meta-analysis and dietary cation anion difference theory revisited. *J. Dairy Sci*. 89:669-684.

Lean, I.J., P.J. DeGaris, L.K. Wade and Z.K. Rajczyk (2003). Transition Management of Dairy Cows: 2003. In: *Australian & New Zealand combined Dairy Veterinarians' Conference*. Foundation for Continuing Education of the N.Z. Veterinary Association, Taupo, New Zealand, pp 221-248.

Oetzel GR and Miller BE (2012). Effect of oral calcium bolus supplementation on early-lactation health and milk yield in commercial dairy herds. Journal of Dairy Science 95:7051-7065.

Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team, 2017, nlme: Linear and Non-linear Mixed Effects Models.
R package version 3.1-131, <URL: https://CRAN.R-project.org/package=nlme>

R Core Team, 2017, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <URL https://www.R-project.org/>

Theilgaard P (2011). Effects of X-Zelit supplementation on milk yield, somatic cell counts and diseases/disorders related to hypocalcaemia. Cattle Practice volume 19 Part 3. BCVA conference 2011.

Thilsing-Hansen T and Jørgensen RJ (2001). Prevention of parturient paresis and subclinical hypocalcemia in dairy cows by zeolite A administration in the dry period. J. Dairy Sci. 84:691–693.

Thilsing T, Joergensen RJ, Eiland F and Hansen T (2009). Testing of a zeolite product for the reduction of the risk of milk fever in 22 private herds. Enclosure 3 complete translation (translation of a project report in Danish) Project J nr. 3412-05-01140 under the Danish Innovation Law.

Wilson GF (2001). A novel nutritional strategy to prevent milk fever and stimulate milk production in dairy cows. N.Z. Vet. J. 49:78–80.

8. APPENDIX A

Table 22. List of animals excluded from analysis in blood profiling and milk production performance

Cow ID	Group	Reason for exclusion
1175	Bio-Chlor	Died before study start
1214	Bio-Chlor	Had milk fever at calving. No blood collected. Died of mastitis during study
1253	Bio-Chlor	Calved to an earlier AI date
1283	Bio-Chlor	Calved early and died
1335	Bio-Chlor	Not pregnant
1339	Bio-Chlor	Calved early
1348	Bio-Chlor	Died before study start
1515	Bio-Chlor	Died of mastitis at 45 days in milk
1637	Bio-Chlor	Transition period 5 days (protocol 12 – 40 days)
1651	Bio-Chlor	Transition period 5 days (protocol 12 – 40 days)
1631	Bio-Chlor	Transition period 68 days (protocol 12 – 40 days)
1661	Bio-Chlor	Transition period 52 days (protocol 12 – 40 days)
1231	X-Zelit	Aborted
1346	X-Zelit	Transition period 7 days (protocol 12 – 40 days)
1670	X-Zelit	Transition period 68 days (protocol 12 – 40 days)
1658	X-Zelit	Transition period 48 days (protocol 12 – 40 days)