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COMMUNICATION

Supplementing transition cows with calcium propionate-propylene glycol drenching or organic trace minerals: implications on reproductive and lactation performances[#]

Suplementación de vacas en periodo de transición con propionato-propilen glicol o minerales traza orgánicos: implicancias para la eficiencia reproductiva y la lactancia

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RESUMEN

El objetivo del presente estudio fue el de evaluar los efectos de una suplementación de propionato de calcio-propilen glicol y una formulación de minerales traza orgánicos (4-Plex) en la eficiencia reproductiva y productiva de vacas durante el periodo postparto. En el ensayo 1 se suplementó aleatoriamente a vacas en lactancia (n = 37) con una mezcla de propionato de calcio-propilen glicol (DR) o con un control (CODR), al parto (día 0) y al pico de lactancia (día 30). En el ensayo 2 se trató a las vacas (n = 33) con una fórmula de minerales trazas (TM) o un placebo (COTM) durante el postparto. Los niveles de calcio, fósforo y ácidos grasos no esterificados (NEFA) fueron detectados en suero. Se evaluó la composición de grasa, proteína, células somáticas y cuerpos cetónicos en la leche. Los estros fueron detectados utilizando un sistema HeatWatch y las ovulaciones fueron estimadas mediante detección de progesterona en la leche. La suplementación de DR resultó en mayores ($P < 0,05$) concentraciones de calcio en suero comparado con CODR. No se detectaron diferencias en la composición de la leche entre los grupos tratados. La suplementación de TM resultó en un menor ($P < 0,0001$) número de servicios por concepción comparado con COTM. Por lo tanto, la suplementación de DR fue efectiva en aumentar los niveles de calcio en suero; sin embargo, no fue suficiente para inducir otras respuestas metabólicas, reproductivas o productivas. La suplementación diaria de minerales redujo el número de servicios requeridos para la concepción; sin embargo, no mostró otros efectos reproductivos o productivos.

Key words: calcium propionate, propylene glycol, trace minerals, fertility.
Palabras clave: propionato de calcio, propilen glicol, minerales traza, fertilidad.

INTRODUCTION

Lactating dairy cows undergo important changes in energy and mineral metabolism during the transition period corresponding from the last three weeks of gestation until the first three weeks post-partum (Grummer 1993). These changes are consequence of the increased nutritional demand associated with the later stages in fetal development followed by the onset in milk production. Furthermore, the gradual decrease in dry matter intake results in considerable mobilization of body reserves and deterioration of body condition (Butler and Smith 1989). Nutritional status of high producing dairy cattle during transition period has important implications for fertility performance and productive state (Butler 2000).

Calcium and energy precursors such as calcium propionate and propylene glycol have been used for several years for prevention of metabolic disorders in transition cows (Johnson 1954). Feeding calcium propionate at calving and 12 h after calving has been shown to reduce the number of cows suffering from subclinical hypocalcemia (Goff *et al* 1996). Propionate plays a major glucogenic effect as a volatile fatty acid in the rumen and can decrease beta-hydroxy butyrate (BHB) and non-esterified fatty acids (NEFA) during the first 2 days post-partum (Goff *et al* 1996). Similarly, propylene glycol as an oral drench can reduce concentrations of NEFA and BHB (Christensen *et al* 1997) and increase levels of glucose and insulin before parturition (Studer *et al* 1993). Nevertheless, the effect of energy precursor has also been tested in fertility performance during post-partum in dairy cows. Oral supplementation of calcium propionate plus propylene glycol showed no effect on fertility performance in cows fed anionic diets (Melendez *et al* 2003). However, propylene glycol administration during post-partum improved ovarian activity as showed by earlier first ovulation and longer first luteal phase after calving (Miyoshi *et al* 2001). We hypothesized that supplementation of a calcium propionate and propylene glycol drenching at parturition and peak of lactation may have a positive metabolic effect and improve reproductive and productive performance in lactating dairy cows. Supplementation at parturition may help in reducing NEB, while administration at Day 30 post-partum may impact over the physiology of first

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ovulation and the start of luteal activity (Beam and Butler 1998, Vries and Veerkamp 2000).

Trace minerals such as zinc, magnesium, copper and cobalt have important roles in protein synthesis, vitamin metabolism and immune function (Spears and Weiss 2008). The final impact of mineral supplementation to dairy cows; however, may depend on the presence of a dietary mineral imbalance or deficiency (Vanegas *et al* 2004). Particularly, copper supplementation at the dry period has showed to improve fertility during post-partum (Black and French 2004). The role of copper in the immune function may have a beneficial effect in the resumption of the reproductive performance after calving (Torre *et al* 1996). Trace minerals has also been reported to improve lactation performance by increasing milk production and reducing somatic cells count (Nocek *et al* 2006). Thus, our second hypothesis for this study postulates that daily TM supplementation during two months of the post-partum period may improve reproductive and productive performances. The aim of this study was to evaluate the metabolic, reproductive and productive impacts of two different dietary supplements 1) calcium propionate-propylene glycol drenching, and 2) commercial organic trace mineral formulation during the post-partum period.

MATERIAL AND METHODS

COWS AND TREATMENT PROTOCOLS

This study was conducted at the Virginia Tech Dairy Center using 70 Holstein cows fed twice daily a TMR of corn silage, alfalfa hay, and cotton seed (table 1). No anionic salts were included in the diet. Mean milk yield for the herd was 9500 kg/year with 380 kg of fat and 280 kg of protein using twice daily milking and freestall barn housing. Cows were randomly assigned to each dietary supplement group. In trial 1, the drenched group (DR; n = 18) received 3 liters of a mixture of calcium propionate, propylene glycol, water, and minerals (table 1), and the control group was administered 3 liters of a saline solution (NaCl 0.9%) (CODR; n = 19). Both groups were dosed 12 h post-partum and at 30 days in milk (DIM) using a Cattle Pump System (Springer Magrath Company, Mc Cook, Nebraska). In trial 2, cows were supplemented daily with one gel cap bolus containing 14 g of a trace mineral (TM; n = 17) formula (4-Plex, Zinpro Corporation, Eden Praire, MN) or with an empty bolus as a control (COTM, n = 16) from 12 hrs post-partum until 60 DIM (table 1). Boluses were administered orally using a plastic balling

Table 1. Nutrient composition of basal diet[1] and propionate-propylene glycol (DR) and trace minerals (TM) supplements for lactating cows.

Composición nutricional de la dieta basal[1] y suplementos de propionato-propilen glicol (DR) y minerales trazas (TM) para vacas en lactancia.

Basal Diet			Supplements		
Components (DM basis)	Lactating cow diet	NRC[1] requirements	Components	DR	TM
Crude protein (%)	16	14.1	Calcium propionate[2] (g)	375	
NE_L (Mcal/kg)	0.32	0.28	Propylene glycol (g)	400	
Fiber (ADF) (%)	25	17 to 21	NaCl (g)	50	
Ca (%)	1.2	0.62	Ca[3] (g)	97.8	
P (%)	0.38	0.32	P[4] (g)	26.5	
Mg (%)	0.32	0.2	Mg[5] (g)	15	
K (%)	1.78	1.0	Mn (mg/kg)		> 14,300
S (%)	0.24	0.3	Zn (mg/kg)		> 25,800
Na (%)	0.4	0.22	Cu (mg/kg)		> 9,000
Zn (mg/kg)	60	43	Co (mg/kg)		> 1,800
Mn (mg/kg)	71	13	Methionine (mg/kg)		> 82,100
Cu (mg/kg)	8	11	Lysine (mg/kg)		> 38,000
Co (mg/kg)	1	0.11			

1 NRC requirements for a Holstein cow, 680 kg BW, 25 kg/d of milk production. Dry matter intake of 20 kg/d.

2 98% purity and 80.6 g of Ca.

3 in 100 g of Monocalcium Phosphate and 375 g of Calcium propionate.

4 in 100 g of Monocalcium Phosphate.

5 in 25 g of Magnesium oxide.

gun. All animal experimentation was performed according to the Institutional Animal Care and Use Committee at Virginia Tech.

SAMPLING AND ANALYSES

Blood samples for calcium and phosphorus concentrations were obtained at 0, 6 and 12 h post-treatment administration corresponding to 12, 18, and 24 h post-partum, respectively. Analysis for calcium and phosphorus was performed using a colorimetric enzymatic kit (Olympus diagnostica GnbH Irish Branch, Lismeehan, O'Callaghan's Mills, Co. Clare, Ireland). Serum samples for NEFA analysis were taken by venipuncture of the median coccygeal vein or artery using vaccutainer tubes (Becton Dickinson, Franklin Lakes, NJ) at 7 days before partum, at calving, at treatment administration, and weekly during 9 weeks post-partum. Samples were allowed to clot for 4 hrs and spun at 900 x g for 15 min. Serum was decanted and frozen at -20 ºC. NEFA was measured using a colorimetric enzymatic kit (Wako NEFA C kit, Wako chemicals Inc., Osaka, Japan). Milk samples were taken 3 times weekly until 60 DIM for determination of acetoacetate using a qualitative test for detection of ketone bodies (Ketocheck test, Great States, Animal Health, St. Joseph, MO). Liver biopsies were taken 30 days before and 30 days after parturition for determination of trace mineral content. Liver samples were obtained trans-cutaneously between the 11^{th} and 12^{th} ribs on the right side of the cow using a reverse cutting custom made biopsy instrument. Element analysis was conducted by inductively coupled plasma-atomic emission spectrometry (ICP-AES). Progesterone concentration was measured in milk 3 times weekly from 7 days post-partum until 60 DIM for estimation of the time of ovulation using a non extraction progesterone assay kit (Coat-A-Coat, Diagnostic products Corp., Los Angeles, CA). An increase in milk progesterone concentrations of > 1 ng mL^{-1} for a period of 2 to 3 days was considered as an indication of ovulation 3 to 4 days before. A HeatWatch transmitter (DDx Inc., Denver, CO) was installed by 10 DIM to determine time of onset of estrus and number of standing events. Cows were artificially inseminated (AI) after voluntary waiting period (60 DIM) and pregnancies were diagnosed by palpation *per rectum*. Daily milk yield and weekly somatic cells, fat and protein content were estimated until 70 DIM.

DATA AND STATISTICAL ANALYSIS

All statistical analyses were performed using SAS software package (SAS version 9.1.3., SAS Institute, Inc., Cary, NC). Serum concentrations of calcium, phosphorous, NEFA, copper and zinc content, and somatic cell score were compared between treatments using a linear mixed model appropriate for repeated measurements per subject (animal) with the compound symmetry for modeling the error covariance structure. The fixed effects part of the model included the effects of treatment, day of sampling, parity and interactions between variables. Intervals of days from calving to first ovulation and first detected estrus were compared between groups using the Lifetest analysis. The survival data curves were compared using a log-rank test. The Phreg procedure was used for calculation of the equality of the survivor function and risk ratios for ovulation and estrus occurrence considering treatment, parity, and service number effects. Control cows were considered to have a risk ratio of 1. Milk yield, fat, and protein accumulated until 60 DIM were compared between groups using the GLM procedure including treatment, parity, and interactions between variables. Presence of acetoacetate was compared between groups using a logistic regression analysis. The level of significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

We first analyzed metabolic response of lactating cows supplemented with DR at parturition. Calcium levels increased ($P < 0.05$) at 6 h and remained higher for 12 h after DR supplementation (Figure 1A). Other studies have shown a rise in calcium concentration within 30 and 60 min of calcium propionate administration and then sustained levels within the next 6 to 8 h (Goff and Horst 1994). Sustained levels of calcium in serum for the initial 6 h may be explained by the slow gastrointestinal absorbance of the calcium propionate constituent of the DR formula (Goff and Horst 1994). Goff *et al* (1996) reported higher levels of calcium 12 h after treatment in cows supplemented with two doses of calcium propionate at calving and 12 h later. Our data showed that a single dose of calcium propionate 12 h after calving was sufficient to increase calcium concentrations for at least 12 h. Although, there was not a clear effect after treatment administration, lower values of phosphorous in cows supplemented with DR (Figure 1C) may be a homeostatic response after increased concentrations of calcium. Supplementation of this glycogenic formula at 12 h post-partum was not effective in reducing NEFA concentrations (Figure 2A). The acute increase of NEFA values at calving are associated with fat mobilization consequence of decreased DMI and increased NEB before parturition (Grummer 1993, Gerloff and Herdt 1999). Previous studies have supplemented propylene glycol for more than10 days during pre-partum in order to increase glycemia and subsequently reduce NEFA levels during transition period (Studer *et al* 1993, Grummer *et al* 1994, Goff *et al* 1996, Stockes and Goff 2001). However, this data indicate that a single day supplementation is insufficient to reduce fat mobilization and consequent NEFA levels in cows under NEB. Moreover, concentrations of NEFA in our herd were within normal values during post-partum indicating that an energetically balanced diet may have minimized the effect of

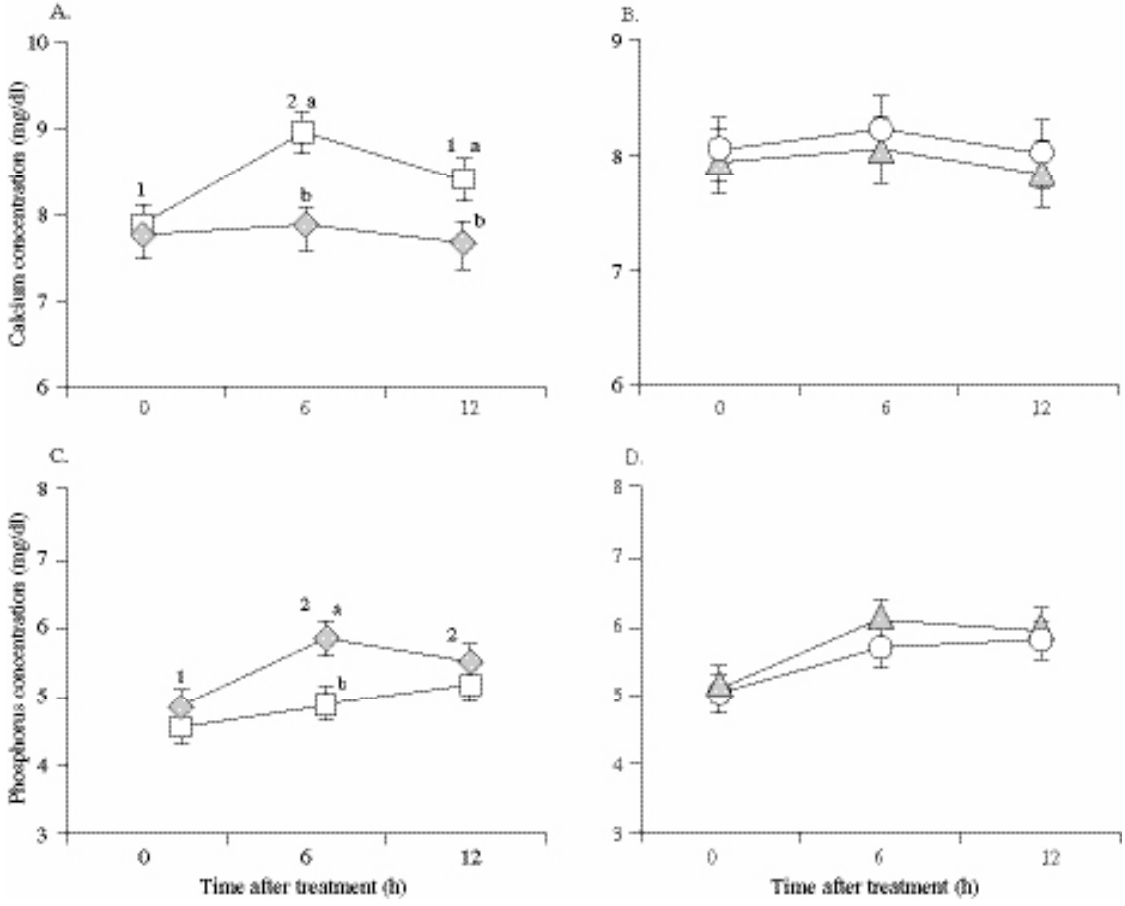

Figure 1. Serum calcium (a,b) and phosphorous (c,d) concentrations at 0, 6, and 12 h post-treatment (12, 18, and 24 h post-partum, respectively) for cows supplemented with drench (DR, □) or trace minerals (TM, △). Control cows (CODR, ◇; COTM, ○) for each respective treatment. Different superscripts indicate significant ($P < 0.05$) difference between hours (1, 2) and treatments (a, b).

Concentraciones de calcio (a,b) y fósforo (c,d) a las 0, 6 y 12 h post-tratamiento (12, 18 y 24 h post-tratamiento, respectivamente para vacas suplementadas con propionato de calcio-propilen glicol (DR, □) y minerales trazas (TM, △). Vacas control (CODR, ◇; COTM, ○) para cada tratamiento respectivo. Superíndices diferentes indican diferencias significativas ($P < 0,05$) entre horas (1,2) y tratamientos (a,b).

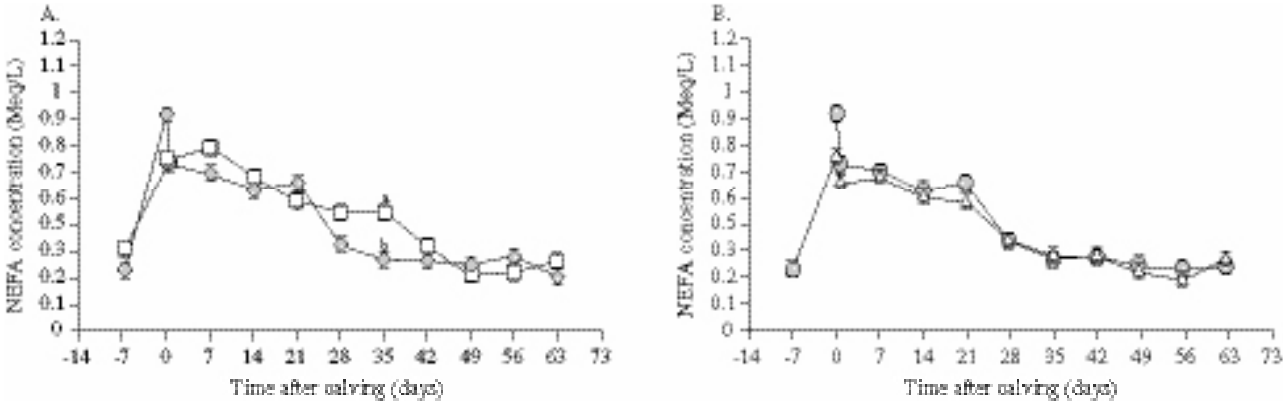

Figure 2. Serum NEFA concentrations detected at pre- and post-partum for cows treated with (a) drench (DR, □) or (b) trace minerals (TM, △). Control cows (CODR, ◇; COTM, ○) for each treatment. Different superscripts indicate significant ($P < 0.05$) difference treatments (a, b).

Concentraciones de NEFA en suero detectadas durante el pre- y post-parto para vacas tratadas con (a) mezcla propionato de calcio-propilen glicol (DR, □) o (b) minerales trazas (TM, △). Vacas control (CODR, ◇; COTM, ○) para cada tratamiento. Superíndices diferentes indican diferencias significativas ($P < 0,05$) entre horas (1,2) y tratamientos (a,b).

energy supplements. We do not have a clear explanation for the higher NEFA concentrations at day 35 in cows supplemented with DR. The metabolic response of TM supplementation in lactating cows during post-partum showed no differences for calcium, phosphorus and NEFA concentrations (Figures 1B-D and Figures 2B). Analyses of trace mineral in liver showed no differences in zinc and copper content between treatments groups; however, cows supplemented with TM showed an increase ($P < 0.05$) in copper levels from 220 ppm at the dry period to 319 ppm at lactation period compared with 268 and 280 ppm in the dry and lactation periods in the COTM group. Prevalence of ketosis was not significantly different between groups (DR = 4.67, CODR = 3.54%; TM = 5.50%; COTM = 3.08%).

We analyzed the return of ovarian activity using a HeatWatch electronic estrus detection system and progesterone measurements in milk. Supplementation of DR at parturition and at peak of lactation had no significant impact over reproductive variables (table 2). Similarly, Melendez *et al* (2003), using 161 cows found no effect of a single supplementation of a similar drench on several reproductive variables including conception rates, calving to conception interval, and services per conception. As described above, the lack of a significant effect of DR supplementation on reproduction performance may be associated to the limited metabolic impact of the administration of a single dose of this supplement separated by a 30-day interval during post-partum. Depending on the concentration present on the diet, supplementation of trace minerals has showed a positive effect on reproductive performance in dairy cows (Campbell *et al* 1999, Vanegas *et al* 2004). In our study, cows supplemented with TM displayed lower ($P < 0.0001$) services per conception (1.14) compared to COTM (2.10) cows (table 2). Previous trials using different supplements containing copper during the dry period have showed significant improvements in fertility (Black and French 2004). The physiological effect of copper on the reproductive physiology of dairy cows is not completely understood; however, copper mediation of the immune function may have a beneficial effect on cellular processes occurring during post-partum including uterine involution, ovarian activity and embryonic development (Torre *et al* 1996). There were no significant differences in milk yield, fat, protein and somatic cell count at 60 DIM between treatments groups (table 3). Previous reports have showed inconsistent results of a similar glycogenic drench administration on productive variables with studies

Table 2. Reproductive parameters for cows supplemented with drench (DR), trace minerals (TM), and respective controls (CODR and COTM) during early lactation.

Parámetros reproductivos para vacas suplementadas con mezcla de propionato de calcio-propilen glicol (DR), minerales trazas (TM) y controles respectivos (CODR y COTM) durante la lactancia temprana.

Variable	DR	CODR	TM	COTM	SEM[1]
Conception rate	41.9	44.1	45.8	47.6	
Days to first ovulation					
Mean	32.8	31.7	29.7	30.5	2.73
Median	29	22.5	22	21.7	
Risk ratio	0.8	1	0.87	1	
Days to first estrus					
Mean	45.7	46.4	42.7	45.3	6.96
Median	46	66	42	68	
Risk ratio	0.60	1	0.57	1	
Standing events at first estrus (no)	2.3	3.1	4.3	3.3	0.50
First to second estrus interval (d)	46.2	35.6	38.6	36	7.35
Standing events at second estrus (no)	3.5	4.4	4.8	4.2	0.10
Days to first service					
Mean	98	108	90	103	7.04
Median	94	105	92	98	
Risk ratio	0.68	1	0.71	1	
Services per conception	2.21	2.06	1.14[a]	2.10[b]	0.26

Different superscripts (a,b) indicate significant ($P < 0.05$) difference.

[1] Standard error of the mean.

Superíndices diferentes (a,b) indican diferencias significativas ($P < 0,05$).

[1] Error estándar de la media.

Table 3. Milk yield, fat and protein composition, and somatic cell score for cows supplemented with drench (DR), trace minerals (TM) and respective controls (CODR and COTM) cows during early lactation.

Producción de leche y composición de grasa, proteína e índice de células somáticas en vacas suplementadas con mezcla de propionato de calcio-propilen glicol (DR), minerales trazas (TM) y controles respectivos (CODR y COTM) durante la lactancia temprana.

Variable	DR	CODR	TM	COTM	SEM[1]
Milk yield[2] (kg)	2.181	2.270	2.204	2.189	122.9
Fat[2] (kg)	87.1	94.1	89.4	88.4	2.99
Protein[2] (kg)	60.1	66.12	60.64	58.3	4.29
Somatic cells (Score)	2.5	1.73	2.28	2.23	1.35

Different superscripts (a, b) indicate significant ($P < 0.05$) difference.

1 Standard error of the mean.

2 Accumulated until 70 days in milk.

indicating a positive effect (Higgins *et al* 1996, Stockes and Goff 2001) or no effect (Melendez *et al* 2003). Similarly, supplementation of TM has showed a positive effect on milk yield and somatic cell count (Kellog and Lane 1996) or no effect on milk production and composition (Kellog *et al* 1996, Campbell *et al* 1999).

In conclusion, supplementation of a calcium propionate-propylene glycol drenching at parturition and at peak of lactation was effective in increasing calcium in serum shortly after treatment; however, was not sufficient in inducing other metabolic, reproductive or productive responses. Administration of TM during 60 days after calving resulted in lower number of services required per conception; however, this supplementation showed no effect on other reproductive or productive variables. The impact of DR and TM supplementation on lactation cows during transition period might have been minimized by the proper diet management of the herd used in this study.

SUMMARY

The aim of this study was to estimate the effect of the supplementation of a calcium propionate-propylene glycol drenching and a commercial organic trace mineral formulation (4-Plex) on reproductive and lactation performances in cows during the post-partum period. In trial 1, lactating dairy cows (n = 37) were randomly assigned either to a calcium propionate-propylene glycol drenching (DR) or to a control (CODR). Both groups were treated at 12 h post-partum and at 30 DIM. In trial 2, (n = 33) cows were treated with either a trace mineral (TM) formula or a placebo (COTM) daily from 12 h post-partum until 60 DIM. Blood samples were collected to evaluate calcium, phosphorus and non-esterified fatty acids serum levels. Milk samples were obtained for fat, protein, somatic cell, and ketone bodies composition. Liver biopsies were taken for zinc and copper content. Estruses were detected using a HeatWatch system and ovulations were estimated by detecting progesterone concentrations in milk samples. Supplementation with DR resulted in higher ($P < 0.05$) concentrations of calcium compared to the control group. There were no differences in NEFA, ketone bodies, milk yield, protein, fat and somatic cell count between treatment groups. Supplementation of TM resulted in less ($P < 0.0001$) services per conception compared to COTM. Thus, DR supplementation during post-partum was effective in increasing calcium in serum shortly after treatment; however, was not sufficient to induce other metabolic, reproductive or productive responses. Daily trace mineral supplementation resulted in lower services required per conception; however, this supplementation showed no effect on other reproductive or productive variables.

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