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Effects of calcium propionate by different numbers of applications in first week postpartum of dairy cows on hypocalcemia, milk production and reproductive disorders

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ABSTRACT

This study was conducted to evaluate effects of calcium propionate on hypocalcemia, dry matter intake, body condition score, milk production and reproductive disorders in dairy cows. Twenty four multiparous Holstein cows were sorted by parity, body condition score (BCS) in close-up period and season of calving and assigned to one of the three treatments. The cows in treatment 1 (T1) received two drenches at calving and 24h after calving. The cows in treatment 2 (T2) received three drenches at calving, 24h after calving and 7 days after calving. The cows in treatment 3 (T3) were the control. Each drench contained 143g of calcium as calcium propionate (0.68kg). Parameters studied were serum calcium, glucose and nonesterified fatty acid (NEFA) concentrations, dry matter intake (DMI), BCS, milk production (MP), incidence of retained placenta (RP) and metritis. Milk fever developed in 5 of 8 cows, in 3 of 8 cows and in 3 of 8 cows in T1, T2 and T3, respectively, at calving. There was no cow with milk fever in T1 and T2 at 4h after second drench (about 28h after calving) but 3 of 8 cows in T3 had still milk fever at this time. The cows receiving two drenches recovered from milk fever in a shorter term as compared to the cows in T3. There were no differences among treatments for DMI, BCS, MP, RP, serum glucose and NEFA concentrations during the experimental period. There was no difference for metritis between T1 and T3 but incidence of metritis in T2 was significantly lower as compared to T3 ($P<0.05$). Two drenches of calcium propionate were beneficial in treating milk fever and three drenches of calcium propionate were considered to have had a preventive effect for metritis.

Key words: Calcium propionate, Milk fever, Subclinical hypocalcemia, Milk production, Reproductive disorder.

RIASSUNTO

EFFETTI DI DIFFERENTI SOMMINISTRAZIONI DI PROPIONATO DI CALCIO SU IPOCALCEMIA, PRODUZIONE LATTEA E DISORDINI RIPRODUTTIVI IN VACCHE DA LATTE NELLA PRIMA SETTIMANA DEL POST-PARTUM

Il presente studio è stato condotto al fine di valutare gli effetti del propionato di calcio su ipocalcemia, sostanza secca ingerita, body condition score, produzione di latte e stato riproduttivo in vacche da latte. Ventiquattro bovine Holstein pluripare sono state suddivise in gruppi omogenei per numero di parti, body condition score (BCS) rilevato in fase di asciutta e giorni di lattazione; ogni gruppo è stato poi assegnato a uno dei tre trattamenti previsti. Gli animali del primo trattamento (T1) hanno ricevuto due somministrazioni: al momento del parto e 24 ore dopo. Le vacche del secondo trattamento hanno ricevuto tre somministrazioni: al momento del parto, 24 ore dopo e a sette giorni dal parto. Gli animali del terzo trattamento rappresentavano il gruppo di controllo. Ogni somministrazione conteneva 143 grammi di calcio preparato come propionato di calcio (0,68Kg). I parametri studiati sono stati la concentrazione ematica di calcio, di glucosio e di acidi grassi non esterificati (NEFA), la sostanza secca ingerita (DMI), il BCS, la produzione lattea (MP), l'incidenza di ritenzioni placentari (RP) e di metriti.

I casi di febbre lattea si sono verificati in 5 animali su 8 nel primo gruppo (T1) e in tre animali su 8 nel secondo (T2) e nel terzo gruppo (T3). Non si sono registrati casi di vacche colpite da paresi da parto 4 ore dopo la seconda somministrazione (a 28 ore circa dal parto) sia nel primo (T1) che nel secondo gruppo (T2), mentre i casi clinici interessati nel gruppo di controllo (T3), 3 su 8, non erano ancora risolti in tale periodo.

I tempi di prognosi si sono ridotti negli animali che avevano ricevuto due somministrazioni rispetto a quelli del gruppo T3. Non si sono rilevate differenze significative tra i diversi trattamenti per quanto concerne produzione di latte, BCS, sostanza secca ingerita, ritenzioni placentari, NEFA e glicemia. Non si sono registrate differenze tra i gruppi T1 e T3 sul numero di metriti diagnosticate, ma l'incidenza nel gruppo T2 è stata significativamente più bassa rispetto al gruppo di controllo (T3) ($P < 0,05$).

Due somministrazioni di propionato di calcio hanno avuto azione benefica nel trattamento della ipocalcemia da parto, mentre tre somministrazioni hanno esercitato azione preventiva sull'insorgenza di metriti.

Parole chiave: Propionato di calcio, Milk fever, Ipocalcemia sub-clinica, Produzione di latte, Disordini riproduttivi.

Introduction

The transition from late gestation to early lactation is regarded as one of the most challenging elements of the production cycle (DeFrain *et al.*, 2005). It is characterized by tremendous metabolic and endocrine adjustments that the cows must experience from late gestation to early lactation (Drackley *et al.*, 2001; DeFrain *et al.*, 2005). Perhaps the most important physiological change occurring during this period is the decrease in dry matter intake around parturition and the sudden increase in nutrients that cows need for milk production (Drackley, 1999; Ingvarsen and Andersen, 2000). As a result of these remarkable changes, most of the infectious diseases and meta-

bolic disorders occur during this time (Goff and Horst, 1997; Drackley, 1999).

Milk fever is a hypocalcemic disorder associated with the onset of lactation in dairy cows, leading to decreased DMI after calving, increased risk of secondary diseases, decreased milk production and decreased fertility. The hypocalcemia occurs because Ca leaves the extracellular fluid pool to enter the mammary gland faster than it can be replaced by intestinal Ca absorption or bone Ca resorption (Goings *et al.*, 1974; Jorgensen, 1974; Goff and Horst, 1997). Low blood calcium can be explained by increasing cortisone and estrogen levels in blood and decreasing receptor number of D vitamin in intestine during parturition (Horst *et al.*, 1989; Tveit *et al.*, 1991).

Hypocalcemia may be clinical or subclinical. Clinical signs of milk fever (clinical hypocalcemia) often are not seen until calcium is about 4mg/dl. (Goff and Horst, 1997). Subclinical hypocalcemia is defined as <7.5mg/dl plasma calcium (Goff *et al.*, 1996; Oetzel, 1996). Subclinical hypocalcemia affects about 50% of all adult dairy cattle and approximately 75% of all cases of milk fever occur within 24 hours of calving (Goff and Horst 1997; Oetzel and Goff, 1999).

Hypocalcemia has been related to dystocia and retained placenta (Grunert, 1986; Eiler, 1997; Houe *et al.*, 2001). The cows that developed milk fever were 3.0 to 4.2 times more likely to experience retained placenta (RP) than normal cows (Curtis *et al.*, 1985; Erb *et al.*, 1985; Houe *et al.*, 2001).

The clear links between milk fever, dystocia and RP, together with the link between milk fever and periparturient immunosuppression, provide a strong basis for the association between milk fever and metritis (Kimura *et al.*, 2006).

Milk fever can be prevented by measures that increase the rate of entry of Ca into the extracellular fluid compartment from intestine, bone, or both. Oral administration of Ca salts prior to and at parturition has been suggested (Jonsson and Pehrson, 1970; Goff and Horst, 1993) to prevent milk fever in dairy cows. The use of a source of calcium that would have no adverse effects on the forestomach is preferable, provided that the calcium is equally available for absorption. Large amounts of propionic acid are produced in the rumen as a result of carbohydrate metabolism, and no adverse effects are obvious. Therefore, calcium propionate might be expected to be a satisfactory source of calcium (Pehrson *et al.*, 1998).

Calcium propionate was administered orally to dairy cows as two doses at calving and again 12h after calving (Goff *et*

al., 1996) or at calving and again 24h after calving (Stokes and Goff, 2001) for treating milk fever and subclinical hypocalcemia. However, three doses of calcium propionate were not administered orally. The objectives of this study were to evaluate the effects of applications of calcium propionate as two oral drenches (at calving and 24h after calving) or three oral drenches (at calving, 24h after calving and seven days after calving) on hypocalcemia, dry matter intake, body condition score, milk production and reproductive disorders in dairy cows.

Material and methods

This study was carried out at a commercial dairy farm in Bursa, Turkey. Data collection extended from July 10, 2007 to March 01, 2008.

Cows, management and diet

Twenty four Holstein cows, with calvings between July 10 and October 16, 2007, which were entering their second or greater lactation, were sorted by parity, BCS in close-up and season of calving and assigned to one of the three treatments in the study. Each treatment group consisted of eight cows.

All cows were fed the dry diet described in Table 1 during dry period. After calving, cows were moved into the lactation pen and fed the lactation diet described in Table 1. Diets were formulated for a 600kg cow consuming 10.5kg and 19.3kg of dry matter per day for dry and lactation period, respectively, and producing 29kg of milk per day containing 3.5% fat according to recommendations by NRC (2001). Rations were delivered as total mixed ration.

Experimental design

Experimental treatments consisted of a control and two application protocols.

Treatment 1 (T1): The cows received two drenches of calcium propionate; the first

Table 1. Ingredients and chemical composition of dry and lactation diets.

| Dry Diet | |
|---|-------|
| Ingredients (% DM): | |
| Wheat straw | 58.02 |
| Commercial concentrate mixture ¹ | 41.98 |
| Chemical composition (% DM): | |
| Neutral detergent fibre | 60.20 |
| Acid detergent fibre | 35.30 |
| Crude protein | 11.61 |
| Ether extract | 3.05 |
| Ash | 7.80 |
| Non-fibre carbohydrates | 17.34 |
| Calcium | 0.64 |
| Phosphorus | 0.38 |
| Lactation Diet | |
| Ingredients (% DM): | |
| Wheat straw | 2.44 |
| Oats hay | 24.38 |
| Alfalfa hay | 15.70 |
| Commercial concentrate mixture ² | 56.70 |
| Sodium bicarbonate | 0.78 |
| Chemical composition (% DM): | |
| Neutral detergent fibre | 43.60 |
| Acid detergent fibre | 24.41 |
| Crude protein | 16.08 |
| Ether extract | 4.81 |
| Ash | 8.00 |
| Non-fibre carbohydrates | 27.51 |
| Calcium | 0.90 |
| Phosphorus | 0.65 |

¹ProYem, Dry Period Concentrate Mixture, Matli Feed Industry, Karacabey/TURKEY

²ProYem, Lactation Period Concentrate Mixture, Matli Feed Industry, Karacabey/TURKEY

drench at calving and the second at 24 h after calving.

Treatment 2 (T2): The cows received three drenches of calcium propionate; the first drench at calving, the second at 24h after calving and the third at 7 days after calving.

Treatment 3 (T3-control): The cows did not receive calcium propionate.

Calcium propionate that we used in our study contained 21% calcium. Each drench contained 143g of calcium as calcium propionate (0.68kg) (Lunapik®, Luna Chemical Materials Firm, İstanbul/TURKEY). Application time and number of drenches are presented in Table 2. All drenches were delivered into the esophagus via an esophageal feeder tube.

Sampling and chemical analyses

Blood samples were collected from the jugular vein in 10ml volume serum separator tubes at calving (prior to first drench), 4h after first drench, 4h after second drench, 4h after third drench, on day 10 postpartum and week 4 postpartum. Blood samples were centrifuged at ambient temperature for 10 min at 3000 x g (NF 615, Nüve Industrial Materials Firm, Ankara/TURKEY). Serum was divided into two equal sub-samples and was placed in eppendorf tubes. The first sub-sample was analyzed for calcium and glucose within 24h of collection in Department of Biochemistry, Uludag University Veterinary Faculty. Serum calcium and glucose concentrations were determined colorimetrically (Calcium-Oc, Glucose-TR SPINREACT, S.A.–Ctra. Santa Coloma, 7-E-17176 Sant Esteve de Bas-(Girona) SPAIN). The second sub-sample was stored in a freezer at -20°C for non-esterified fatty acids (NEFA) analysis. The second sub-sample was also analyzed in Department of Biochemistry, Uludag University Veterinary Faculty. Serum NEFA concentrations were also determined colorimetrically (NEFA C KIT, WAKO Pure

Table 2. Application time and number of drenches.

| Drench | Time | T1 | T2 | T3 |
|---------------|---------------------|------------|----|------------|
| First drench | calving | + | + | Only water |
| Second drench | 24 h after calving | + | + | Only water |
| Third drench | day 7 after calving | Only water | + | Only water |

Chemical, Code no. 994–75406). Shimadzu UV–1601 spectrophotometer was used for reading serum Ca, glucose and NEFA concentrations, with 570nm, 505nm and 550nm wave length, respectively.

A cow was considered to have milk fever if she was recumbent and if her blood calcium concentration was ≤ 5.5 mg/dl and sub-clinical hypocalcemia was defined as a blood calcium concentration ≤ 7.5 mg/dl (Goff *et al.*, 1996; Oetzel, 1996).

Dry matter intake was measured individually twice a week in close-up period and week 1, 3, 5, 10 of lactation.

Cows were scored for body condition in close-up period and week 1, 5, 10 of lactation. Scoring was based on a five-point scale with 0.25 unit intervals, where 1=emaciated, and 5=obese (Wildman *et al.*, 1982).

Cows were milked twice a daily and milk production was measured individually three times a week in week 2, 4, 6, 8 and 10 of lactation.

Reproductive disorders were assessed as retained placentas and metritis. Incidences of metritis and retained placentas were recorded during the experiment.

The chemical analyses (crude protein, ether extract, ash, calcium, phosphorus) of diets were performed according to AOAC (1990), NDF and ADF analyses were performed according to Van Soest *et al.* (1991).

Statistical analysis

Dry matter intake, body condition score, milk production and serum calcium, NEFA and glucose concentrations were analyzed

by analysis of variance using the ANOVA procedure and the significance controls of the differences among the treatments were determined by TUKEY test. As for proportional values (retained placenta and metritis), they were analyzed by Chi-square test. SPSS 13 (2004) computer program package was used for the statistical analyses. Significance was accepted at $P < 0.05$.

Results and discussion

Concentrations of serum calcium were indicated in Table 3. Serum calcium concentrations at calving (prior to first drench) were similar for both treated and control cows. There were no statistical differences for serum calcium concentrations at calving, 4h after first drench, day 10 and week 4 postpartum among treatments (Table 3). Goff *et al.* (1996) also observed that serum calcium concentrations were similar at day 10 after calving between treatments. Serum calcium concentrations at 4h after second drench and 4h after third drench (7 days after calving) were similar for T1 and T3 but T2 had a higher concentration of serum calcium than other treatments at the same times ($P < 0.01$) (Table 3). There were no statistical differences for serum calcium concentrations between at calving (prior to first drench) and 4h after first drench inside each treatment. (Table 3). We did not observe acute effect of calcium propionate on serum calcium concentration for 4h after first drench. However, Goff and Horst (1994) observed that calcium propionate treatment acutely increased blood calci-

Table 3. Comparison of serum calcium concentrations (mg/dl) among and inside treatments.

| | T1 ¹ (2 drenches) $\bar{x} \pm S\bar{x}$ | T2 ² (3 drenches) $\bar{x} \pm S\bar{x}$ | T3 (Control) $\bar{x} \pm S\bar{x}$ | p |
|---------------------------------|---|---|---|------|
| Calving (prior to first drench) | 5.21 ^{ce} ± 0.20 | 5.83 ^b ± 0.32 | 5.57 ^c ± 0.23 | ns |
| 4 h after first drench | 5.90 ^{be} ± 0.21 | 6.28 ^b ± 0.27 | 5.70 ^c ± 0.21 | ns |
| 4 h after second drench | 6.57 ^{bd} ± 0.23 | 7.94 ^{*a} ± 0.62 | 5.81 ^c ± 0.27 | 0.01 |
| 4 h after third drench | 6.75 ^{bd} ± 0.17 | 8.21 ^{*a} ± 0.44 | 6.67 ^{bc} ± 0.28 | 0.01 |
| Day 10 postpartum | 7.25 ^{ad} ± 0.29 | 7.97 ^a ± 0.33 | 7.45 ^{ab} ± 0.43 | ns |
| Week 4 postpartum | 8.21 ^a ± 0.28 | 8.23 ^a ± 0.16 | 8.11 ^a ± 0.33 | ns |

¹Drenches were administered at calving and 24h after calving.

²Drenches were administered at calving, 24h after calving and day 7 after calving.

*indicates significant differences in the same row among treatments ($P < 0.01$).

ns: not significant.

Different letters in the same column indicate significant differences inside treatments ($P < 0.05$).

um concentration for 4 to 6h after treatment. Perhaps calcium propionate treatment could increase serum calcium concentration not within the first 4h after first drench but for 5 to 6h after first drench. After the cows in T1 and T2 received two drenches at calving and 24h after calving, their serum calcium concentrations significantly increased for 4h after second drench (approximately 28h after calving) in comparison to calving ($P < 0.05$) (Table 3). Serum calcium concentration was higher for T2 at 4h after second drench, because T2 had a higher serum calcium concentration at calving as compared to T1 (Table 3). In T1 and T2, we observed differences for serum calcium concentrations between at calving (prior to first drench) and 4h after third drench (7 days after calving) ($P < 0.05$) (Table 3). At 4h after third drench, T2 had a higher serum calcium concentration as compared to T1, because the cows in T2 received three drenches. In T3 receiving no supplemental calcium as calcium propionate, serum calcium concentration did not significantly increase between at calving and 4 h after third drench (Table 3).

Milk fever developed in 5 of 8 cows, 3 of 8 cows and 3 of 8 cows in T1, T2 and T3, respectively, at calving (Table 4). At 4h after first drench, the cows with milk fever decreased in T1 and T2. There was no cow with milk fever in T1 and T2 at 4 h after second drench (approximately 28h after calving) but 3 of 8 cows in T3 had still milk fever at this time (Table 4). Treating the cows with two drenches of calcium propionate, both T1 and T2, removed milk fever. However, there were the cows with subclinical hypocalcemia in T1 and T2 at 4h after second drench. Subclinical hypocalcemia was observed in all treatments throughout the first 4 weeks of lactation. When the treatments were combined 6 of 24 cows remained subclinically hypocalcemic (Table 4). Goff *et al.* (1996) suggested that treatment with calcium propionate could significantly reduce the incidence of milk fever when a problem with milk fever existed in a dairy herd. In the study of plasma calcium concentrations of periparturient Holstein cows, Goff *et al.* (1996) observed that 10 to 50% of cows remained subclinically hypocalcemic (serum calcium $< 7.5\text{mg}/100\text{ml}$) up to 10d after calving,

Table 4. Milk fever and subclinical hypocalcemia occurrences.

| | T1 | | T2 | | T3 | |
|---------------------------------|----|----|----|----|----|----|
| | MF | SH | MF | SH | MF | SH |
| Calving (prior to first drench) | 5 | 3 | 3 | 5 | 3 | 5 |
| 4h after first drench | 2 | 6 | 1 | 7 | 3 | 5 |
| 4h after second drench | - | 7 | - | 4 | 3 | 5 |
| 4h after third drench | - | 7 | - | 3 | - | 6 |
| Day 10 postpartum | - | 5 | - | 4 | - | 5 |
| Week 4 postpartum | - | 3 | - | 1 | - | 2 |

MF: Milk fever; SH: Subclinical hypocalcemia.

depending on management efforts to combat milk fever. Ramberg (1974) also observed that cows were at greatest risk of negative Ca balance during the first 10d of lactation, because of which we administered to the cows in T2 third drench at 7 days after calving. However, we did not observe effect of the third drench of calcium propionate in subclinically hypocalcemic cows. In T2, despite the fact that subclinically hypocalcemic cows at 4h after third drench was less than those at 4h after second drench, subclinically hypocalcemic cows increased again at day 10 postpartum (Table 4).

Several authors have reported a reduction in the motility of the rumen and abomasum (Daniel, 1983; Jorgensen *et al.*, 1998) in subclinically and clinically hypocalcemic cows. This reduction in ruminal and abomasal motility likely causes a reduction in dry matter intake. In our study, we observed no significant increase in dry matter intakes of the cows receiving calcium propionate as a source of calcium (Table 5).

There were no statistical differences for body condition scores among treatments. Cows lost 0.63, 0.50 and 0.81 body score unit between close-up period and week 10 of lactation for T1, T2 and T3, respectively. Body condition variation was the lowest for T2. However, the differences for body condition variations were numerical (non-significant) (Table 5), which would suggest that fat mobi-

lization was similar among treatments. Thus, Serum NEFA concentrations were also similar for all the treatments (Figure 1).

Cows should calve with a BCS of 3.5-3.75 (Beede, 1997; Studer, 1998). In our study, average body condition scores for each treatment, during close-up period, were lower than those recommended. Cows should not lose more than 1 BCS during early lactation (Melendez, 2006). In our study, body condition variation was the highest for T3 (-0.81) (Table 5).

Goff *et al.* (1996) observed no significant increase in milk production in a commercial Holstein herd (multiparous cows only) receiving two calcium propionate tubes (74g of Ca) at calving and again at 12h after calving (four tubes totaling 148g of Ca). In our study, despite the higher dose and more application, we also observed no significant increase in milk production (Table 5). There were no statistical differences for milk production among treatments. Stokes and Goff (2001) also reported that two drenches of calcium propionate (680g per drench) did not affect milk production. Mean daily milk production of cows were 25.39, 25.87 and 24.70kg for T1, T2 and T3, respectively, in this study. Milk production, averaged across all weeks, were 1.17kg and 0.69kg greater for the cows in T2 and T1, respectively, as compared to the cows in T3. However, these differences in milk production were not significant (Table 5).

Table 5. Comparison of dry matter intakes, body condition scores and milk production among treatments.

| | T1 $\bar{x} \pm S\bar{x}$ | T2 $\bar{x} \pm S\bar{x}$ | T3 $\bar{x} \pm S\bar{x}$ | P |
|------------------------------|------------------------------|------------------------------|------------------------------|----|
| Dry Matter Intakes (kg/day): | | | | |
| Close-up | 9.24 ± 0.24 | 9.09 ± 0.22 | 9.35 ± 0.23 | ns |
| 1 st Week | 14.51 ± 0.22 | 14.19 ± 0.37 | 14.24 ± 0.19 | ns |
| 3 rd Week | 15.70 ± 0.23 | 15.61 ± 0.58 | 15.30 ± 0.18 | ns |
| 5 th Week | 17.09 ± 0.32 | 17.12 ± 0.44 | 16.53 ± 0.59 | ns |
| 10 th Week | 18.47 ± 0.13 | 18.71 ± 0.26 | 17.78 ± 0.34 | ns |
| Body Condition Scores: | | | | |
| Close-up | 3.19 ± 0.18 | 3.19 ± 0.18 | 3.31 ± 0.15 | ns |
| 1 st Week | 2.88 ± 0.13 | 2.97 ± 0.14 | 3.03 ± 0.13 | ns |
| 5 th Week | 2.66 ± 0.09 | 2.78 ± 0.14 | 2.66 ± 0.10 | ns |
| 10 th Week | 2.56 ± 0.06 | 2.69 ± 0.13 | 2.50 ± 0.07 | ns |
| Variation | -0.63 ± 0.14 | -0.50 ± 0.11 | -0.81 ± 0.10 | ns |
| Milk Production (kg/day): | | | | |
| 2 nd Week | 21.90 ± 2.02 | 22.60 ± 1.70 | 23.84 ± 1.25 | ns |
| 4 th Week | 24.14 ± 1.25 | 24.66 ± 1.66 | 24.07 ± 0.85 | ns |
| 6 th Week | 25.92 ± 1.70 | 25.47 ± 1.40 | 24.77 ± 0.75 | ns |
| 8 th Week | 26.67 ± 1.53 | 27.50 ± 1.60 | 25.04 ± 1.02 | ns |
| 10 th Week | 28.32 ± 1.35 | 29.13 ± 1.06 | 25.77 ± 1.16 | ns |
| Mean | 25.39 ± 0.76 | 25.87 ± 0.73 | 24.70 ± 0.45 | ns |

ns: not significant.

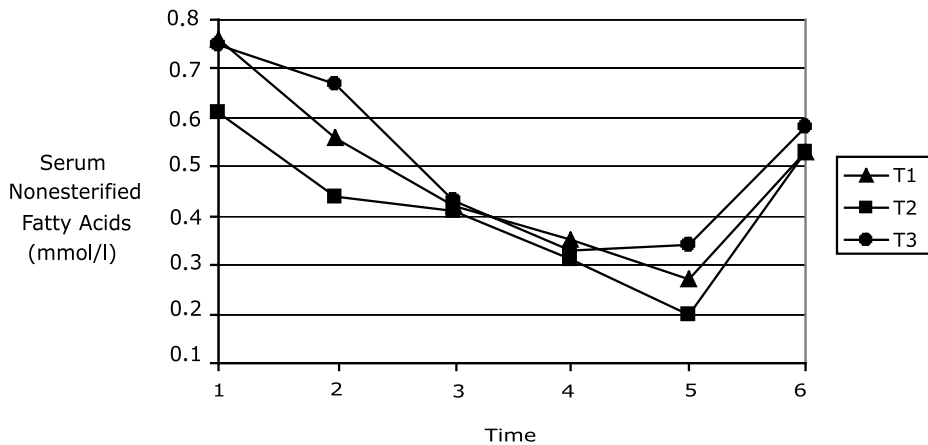
Calcium propionate could serve as a gluconeogenic precursor at a time when the cow is in negative energy balance (Goff *et al.*, 1996). At parturition, calcium propionate increases blood glucose 24 hours after its administration, and reduces NEFA during the first two days postpartum (Higgins *et al.*, 1996). In our study, serum NEFA did not respond to oral drenches (Figure 1). Goff *et al.* (1996), supplying calcium propionate at calving and after 12 hours, observed a smaller concentration of NEFA 24 hours after calving in Jersey cows, but not in Holstein cows.

Normal values for NEFA should be between

0.7 to 0.9mmol/l at parturition; values greater than this indicate excessive fat mobilization (Studer *et al.*, 1993; Kaneene *et al.*, 1997). In our study, none of the cows had a high average milk production and excessive fat mobilization, which explains why we did not observe high concentrations of NEFA (Figure 1).

Glucose has an important impact on milk production because lactose is the major osmoregulator for mammary uptake of water (Mandebvu *et al.*, 2003). There were no statistical differences for serum glucose concentrations among treatments (Figure 2). In our study, drenching with additional energy,

Figure 1. Changing of serum nonesterified fatty acid (NEFA) concentrations (mmol/l).



Time=1: at calving (prior to first drench), 2: 4h after first drench, 3: 4h after second drench, 4: 4h after third drench, 5: day 10 postpartum, 6: week 4 postpartum.

There were no statistically significant differences for serum NEFA concentrations among treatments.

as calcium propionate, had no effect on serum glucose concentration, which may explain that there was no significant increase in milk production in T1 and T2. Stokes and Goff (2001) also observed that calcium propionate treatment did not affect blood glucose level. We observed higher serum glucose concentrations at calving (Figure 2), which may be the result of the combination of glucocorticoid release at calving and failure to secrete insulin when blood calcium is reduced (Goff and Horst, 1997). As lactation began, serum glucose levels declined.

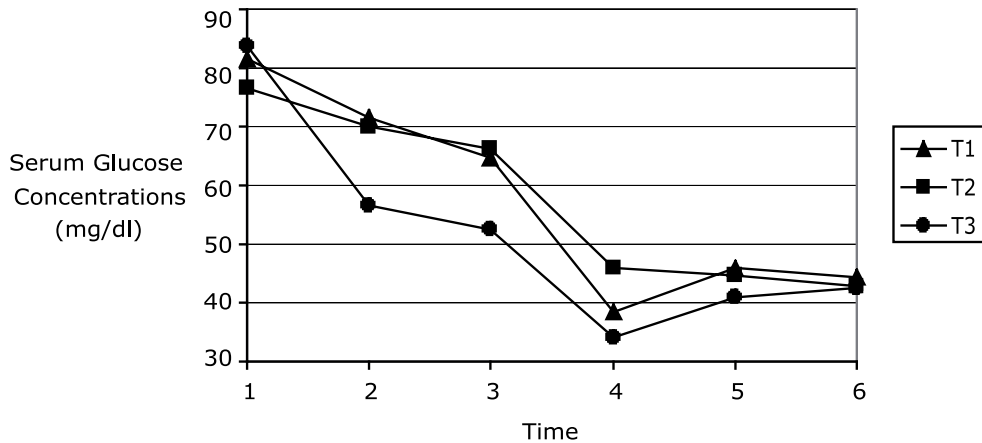
Retained placenta and metritis were diagnosed in 8 of 24 cows and 11 of 24 cows, respectively. The majority of metritis and retained placenta occurred in T3. There were no statistical differences for retained placenta among treatments. The difference for metritis was significant between T2 and T3 ($P < 0.05$) (Table 6).

There are a direct effect and a large indirect effect of milk fever on retained placenta. The direct effect of milk fever is to cause loss

of muscle tone in the uterus (Stokes and Goff, 2001). The indirect effect is due to the fact that milk fever is a risk factor for dystocia, and dystocia is a risk factor for retained placenta (Erb *et al.*, 1985; Correa *et al.*, 1993). None of the cows experienced dystocia in our study but we observed retained placenta. Even if two drenches of calcium propionate removed milk fever in T1 and T2, the addition of calcium and energy at calving did not reduce incidence of retained placenta, because the first drench of calcium propionate had no acute effect on serum calcium concentration in our study. Goff *et al.* (1996) also reported that the incidence of retained placenta was not affected by calcium propionate treatment.

Whiteford and Sheldon (2005) observed a significantly higher incidence rate of metritis in UK cows that suffered clinical hypocalcemia (milk fever) in comparison to normocalcemic cows. In addition, cows with retained placenta were more likely to have metritis than cows without retained placenta (Erb *et al.*, 1985). The incidences of milk fever, retained placenta

Figure 2. Changing of serum glucose concentrations (mg/dl).



Time=1: at calving (prior to first drench), 2: 4h after first drench, 3: 4h after second drench, 4: 4h after third drench, 5: day 10 postpartum, 6: week 4 postpartum.

There were no statistically significant differences for serum glucose concentrations among treatments.

Table 6. Retained placenta and metritis occurrences.

| | n | Retained placenta | | Metritis | |
|----|---|-------------------|------|----------|---------------------|
| T1 | 8 | 2 | % 25 | 3 | %37.5 ^{ab} |
| T2 | 8 | 2 | % 25 | 2 | %25 ^b |
| T3 | 8 | 4 | % 50 | 6 | %75 ^a |

Different letters in the same column indicate significant differences ($P<0.05$).

and metritis were high in our study (Table 4 and 6). This finding was in agreement with the relationship among milk fever, retained placenta and metritis as reported by Whiteford and Sheldon (2005) and Erb *et al.* (1985). In our study, the average DIM at which metritis was diagnosed was day 10. Treating cows with three drenches of calcium propionate reduced incidence of metritis (Table 6). The addition of calcium and energy may have improved smooth muscle contraction and hastened uterine involution. Stokes and Goff (2001) reported that two drenches of calcium propionate decreased the incidence of metritis. The result of our study was not in agreement with Stokes

and Goff (2001). Since there were the higher incidences of milk fever and retained placenta in our study in comparison to Stokes and Goff (2001), two drenches of calcium propionate were not sufficient for treatment of metritis.

Conclusions

According the results of our study, the cows receiving two drenches of calcium propionate recovered from milk fever in a shorter term (about 28 h after calving) as compared to control cows. In addition, three drenches of calcium propionate significantly decreased the incidence of metritis and the-

re were positive effects of calcium propionate treatment on all the parameters that we studied. More studies must be conducted to evaluate effects of three drenches of calcium propionate on blood metabolites, health events and milk production.

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