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The effect of a product with three gluconeogenic precursors during the transition period on blood metabolites and milk yield in Chilean Holstein cattle

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ABSTRACT

The objective of this study was to determine the effect of a product containing calcium propionate 70 g/kg, propylene glycol 95 g/kg and glycerol 330 g/kg on blood β-hydroxy butyrate, non-esterified fatty acids and milk yield in Chilean Holstein cows. Cows were housed in a free-stall with head-locks, milked 3× and fed a total mixed ration. At 21 days before expected parturition 40 cows were randomly assigned to a control group ($n = 20$) and a treated group ($n = 20$). Controls received the farm diet. Treated cows received 300 g of the supplement, top dressed on the same diet, during the morning until 15 cows per group accomplished 30 days pp. A blood sample was obtained at calving for non-esterified fatty acids determination and at 7, 14 and 21 days pp for β-hydroxy butyrate determination. Milk yield up to 60 days pp was higher (2 kg/day) in the treated than control group. Non-esterified fatty acids were higher in the control than treated group. The control group at 14 days pp had a lower concentration of β-hydroxy butyrate than the treated group. In conclusion, a product with three gluconeogenic precursors improved milk yield and maintained a moderated energy status during the transition period of Chilean dairy cattle.

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KEYWORDS
Transition dairy cow; ketone bodies; NEFA; milk yield

1. Introduction

The transition period of dairy cows is defined as the last 3 weeks of gestation and the first 3 weeks postpartum (Grummer 1995). This period is characterized by tremendous metabolic, endocrine and physiological changes; consequently, the cow must adjust in order to succeed during the ensuing production cycle (Goff and Horst 1997; Drackley et al. 2001). Unfortunately some cows are unable to adjust, resulting in metabolic diseases such as hypocalcaemia, hypomagnesaemia, ketosis, left displacement of abomasum and fatty liver (Goff and Horst 1997). These periparturient disorders determine significant economic losses for the dairy industry worldwide; therefore efficient management and prevention play key roles to optimize profitability of dairy operations (Melendez and Risco 2005).

During the transition period, feed intake decreases at a time when energy requirements increase. Glucose demands in Holstein cows start to increase during the last trimester of gestation due to growth of the conceptus and after calving due to milk yield. When blood glucose concentration decreases, as occurs around parturition, non-esterified fatty acids (NEFA) mobilization from adipose tissue is stimulated and fatty acid uptake by the liver is increased (Drackley et al. 2001); however, when blood glucose levels are sufficient, insulin is secreted and oxidation of fatty acids is inhibited and triglycerides synthesis is stimulated. When blood glucose concentrations are low, a lack of insulin and increased glucagon secretion favour the transport of NEFA into the mitochondria, with a consequent increase in ketone body formation (Herdt 2000). Because of this characteristic lipolysis, NEFA concentrations are typically highest at parturition (0.9–1.2 mEq/L) with a slow decrease after 3 days postpartum (Melendez et al. 2002). Extreme rates of lipid mobilization lead to excessive uptake of NEFA by the liver and increased triglyceride accumulation with the consequent development of hepatic lipidosis (Drackley et al. 2001). In the last years, a state of insulin resistance during the transition period has been described in dairy cattle, affecting the entrance of glucose to the hepatocyte (De Koster and Opsomer 2013). In this sense, the supply of 3 carbon gluconeogenic precursors such as propionate, propylene glycol (1, 2 propanediol) and glycerol (1, 2, 3 propanetriol), which are not insulin dependent to enter the liver cells, either separately or in combination may be useful.

Glycerol is an efficient gluconeogenic precursor depending upon its absorption rather than ruminal fermentation to propionate and butyrate (Remond et al. 1993). Propylene glycol is another gluconeogenic product in which its activity will occur whether it is fermented to propionate in the rumen or absorbed and metabolized by the liver (Studer et al. 1993). Finally, propionate is the primary glucogenic substrate in the dairy cow (Drackley et al. 2001). Conversion of propionate to glucose depends upon conversion to succinyl co-A before entering the Krebs cycle (Herdt 2000). As a result, each of these compounds has a different route of conversion to glucose. Therefore, mixing products may be best suitable by taking advantage of the different pathways to synthetize glucose in the liver. Consequently, the objective of this study was to

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determine the effect of a product containing calcium propionate, propylene glycol and glycerol on blood β-hydroxy butyrate (BHB), NEFA and milk yield in Holstein cows managed under Chilean conditions.

2. Material and methods

2.1. Dairy farm and management

The study was conducted in a commercial dairy farm located in the central area of Chile (33°19′S, 71°24′W) characterized by a Mediterranean climate with an average rainfall of 300 mm per year and a temperature-humidity index of 57–70 (Arias and Mader 2010).

The farm consisted of 800 milking cows producing 11,000 kg of milk per lactation, housed in a free-stall system with sand bedding, milked 3 times a day and fed a total mixed ration (TMR) based on alfalfa hay, corn silage and concentrate (Tables 1 and 2).

Cows were dried off two months before expected parturition (BEP), housed in an early dry pen and fed oat silage *ad libitum*. At approximately 30 days BEP cows were moved weekly to a transition *pre-partum* pen. They were fed a TMR with anionic salts to meet or exceed the requirements of the National Research Council (2001) and a dietary cation–anion difference of −85 mEq/kg of dry matter (DM).

Cows calved in a maternity pen and were evaluated for reproductive tract status and the presence of retained foetal membranes after 12 hours post parturition. After that, cows were moved to a *post-partum* pen and subjected to a health monitoring programme evaluating on a daily basis the presence of fever, metritis and/or displacement of abomasum until 13 days *postpartum*. Subsequently cows were monitored daily for milk yield until 30 days *postpartum*. If the cow experienced reduction in milk yield the animal was separated and evaluated clinically for any disease and treated accordingly. Both *pre-partum* and *post-partum* transition pens had a system of head-locks to restrain the animals for examination, artificial insemination or treatment purposes.

Table 1. Ingredients of experimental diets.[a]

Ingredient (dry matter kg/d)	Pre-partum diet	Post-partum diet
Ground dry corn	1.98	5.59
Canola meal	0.18	0.27
Feather meal	0.49	0.42
Roasted Soybean	0.10	1.80
Soybean meal 47%	0.26	2.64
Wheat brand	0.45	2.0
Wet brewers	2.16	3.0
Minerals	0.24	0.15
Vitamin ADE	0.03	0.02
Calcium carbonate	0.10	0.20
Micotoxin binder	0.04	0.05
Salt	0.02	0.02
Sodium bicarbonate	0.05	0.25
Alfalfa hay	0.88	2.64
Corn silage	6.65	7.70
Oat silage	0.74	1.11
Total	14.37	27.86

[a]Treatment diet in both pre-partum and post-partum periods was the same as the control group plus 300 g of a commercial product containing 21 g of calcium propionate, 28.5 g of propylene glycol and 99 g of glycerol. The product was top dressed on the diet every morning feeding.

Table 2. Nutrient composition of diets.

Item	Prepartum	Postpartum
Nutrient		
Dry matter (%)[a]	40.3	48.8
Forage (%)	58.1	40.0
Crude protein (%)[a]	14.2	17.5
NEL (Mcal/kg)[d]	1.59	1.75
ADF (%)[a,e]	22.2	19.1
NDF (%)[a,f]	39.4	31.9
Forage NDF (%)	27.6	19.5
Lignin (%)[a]	2.18	2.08
NFC (%)[b,g]	37.6	41.0
Sugars (%)[a]	2.35	4.58
Starch (%)[a]	19.9	27.5
Soluble fibre (%)[b]	3.9	5.76
Fat (%)[a]	4.0	4.95
Ash (%)[a]	8.36	7.78
Minerals[a]		
Calcium (%)	0.94	0.77
Phosphorus (%)	0.34	0.40
Magnesium (%)	0.32	0.26
Potassium (%)	1.08	1.37
Sulfur (%)	0.30	0.27
Sodium (%)	0.15	0.38
Chloride (%)	0.84	0.31
Iron (ppm)	212.8	180.7
Zinc (ppm)	78.9	78.4
Copper (ppm)	18.9	16.2
Manganese (ppm)	54.1	45.9
Selenium (ppm)	0.39	0.37
Cobalt (ppm)	0.15	0.35
Iodine (ppm)	0.68	0.78
DCAD (mEq/kg)[c,h]	−84.3	229

[a]Laboratory determination.
[b]Calculation by formula.
[c]DCAD equation: (Na + K) – (Cl + S).
[d]Net energy of lactation.
[e]Acid detergent fibre.
[f]Neutral detergent fibre.
[g]Non-fibre carbohydrates.
[h]Dietary cation–anion difference.

2.2. Experimental design

This study was approved by the Institutional Animal Care and Use Committee of the University Santo Tomas, Chile. In order to find a difference in daily milk yield of 1.5 kg (SD = 1.5) from 4 to 60 days *post-partum* between a control and a treated group, with a 95% confidence and 80% of power, a sample size of 15 cows per group was calculated. For dairy management reasons, only multiparous cows (2 lactations or more) were considered for the study. When cows were moved to the *pre-partum* pen they were randomly assigned to both groups until 20 animals per group were reached. This number of cows was considered assuming that 5 cows per group would be unable to finish the protocol or ineligible for the following reasons: twin births, severe dystocia or trauma. Both treated and control cows resided in the same pen and were managed in the same way. During the morning feeding cows were head-locked. At this time, treated cows were supplemented with the experimental product. The protocol consisted of top-dressing over the TMR a powder product (300 g per cow) containing 70 g of calcium propionate, 95 g of 1, 2 propanediol (propylene glycol) and 330 g of 1, 2, 3 propanetriol (glycerol) per kilogram of product (glukosa®, Centrovet, Santiago, Chile). The protocol was continued during the *post-partum* period until 15 cows per group accomplished 30 days of milk production. After that, cows were followed up to 60

days post calving recording the daily milk yield using a computerized milking system (Afimilk® S.A.E., Afikim, Kibbutz Afikim, Israel).

2.3. Blood sampling and metabolite analysis

A blood sample was obtained from tail vessels at calving, 7, 14 and 21 days *postpartum*, because most of periparturient disorders occur within the first 3 weeks of lactation (Goff and Horst 1997; Drackley et al. 2001). Samples were centrifuged at 3000 g for 10 minutes. Immediately after collection serum was separated, stored and frozen at – 20°C until analysis. Samples were assayed at the biochemistry laboratory of campus Catemito, University Santo Tomas, Chile. Non-esterified fatty acids were determined at calving by an enzymatic-colorimetric method (Johnson and Peters 1993), using a commercial kit (NEFA-C kit WAKO, Osaka, Japan). β-hydroxy butyrate was determined at 7, 14 and 21 days *postpartum* by an enzymatic-colorimetric method (Williamson and Mellanby 1974), using a commercial kit (Pointe Scientific, Inc. BHA Set., Lincoln Park, MI, USA).

2.4. Statistical analysis

Non-esterified fatty acids at calving were analysed by ANOVA developing a mixed model considering the cow as a random effect. The model also considered the effect of treatment, parity number and body condition score at calving (scale 1 to 5, ¼ point increment) (Ferguson et al. 1994).

Milk yield and the concentrations of BHB at 7, 14 and 21 days *postpartum* were analysed by ANOVA for repeated measures. The experimental data were analysed proposing the following mixed model:

$$Y_{ijklm} = \mu + T_i + \text{time}_j + \text{Cow}_k(T_i) + P_l + \text{BCS}_m + (T \times \text{time})_{ij} + e_{ijklm},$$

where Y_{ijklm} is the dependent variable (milk yield or BHB), μ is the overall mean, T_i is the fixed effect of treatment (treatment, control), time_j is the fixed effect of time (day or week), Cow_k (T_i) is the random effect of the cow nested within treatment, P_l is the fixed effect of parity number (2, ≥3), BCS_m is the random effect of body condition score at calving (1 to 5 in a ¼ point scale), $(T \times \text{time})_{ij}$ is the fixed effect of the interaction of treatment by time which is the most important effect of the model because it compares the parallelism of the curves between groups and e_{ijklm} is the experimental error.

For all models, the best goodness of fit was specified according to the best covariance structure, based on Schwarz's Bayesian Criterion (Littell et al. 1998).

Least squares means (LSM) ± Standard Error of the Mean (SEM) were reported. Significant effects were considered when p was ≤0.05. A tendency was considered with a p value between .05 and .1. Statistical analysis was conducted using the software SAS 9.0 (2003).

3. Results and discussion

In Figure 1, milk yield for the treated and control groups until 60 days postpartum is shown. The interaction effect of treatment by day was significant ($p \leq .05$); consequently curves were not parallel and milk yield was significantly higher in treated than control cows. Correcting for parity number and body condition score at calving, LSM for the entire period were 42.5 kg/day for the treated group and 40.5 kg/day for the control group. Consequently, the treated cows produced on average 2 kg per day more milk than the control cows.

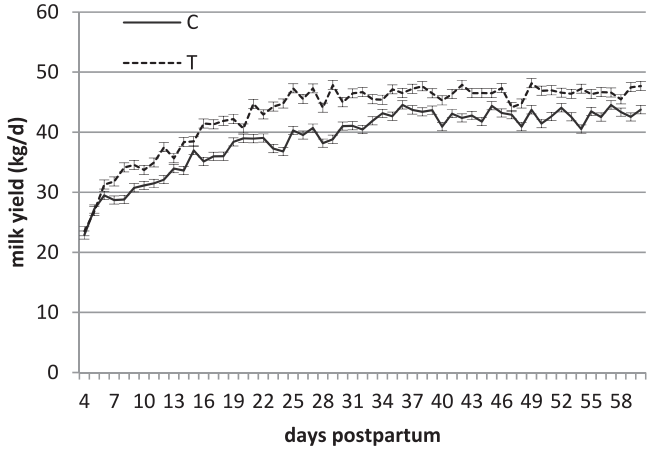

Figure 1. Daily milk yield, kg/d (LSM ± SEM) from 4 to 60 days postpartum in control and treated groups. C: control group ($n = 15$); *T*: treated group ($n = 15$). Interaction group by day ($p \leq .05$).

One of the strengths of this study was that gluconeogenic precursors were fed to cows during daily head-lock restraint, enabling the individual cow to serve as the experimental unit. This study design allowed cows from both treatment and control groups to be commingled in the same lot and managed in the same way, eliminating pen effect, thereby avoiding a crossover experimental design. In addition, only healthy animals that remained in lactation for the first 30 days in milk were evaluated. This permitted to assess the pure influence of metabolic homeostasis with no other concurrent diseases. Cows with twin births, severe dystocia or trauma at calving were excluded from the analysis. Furthermore, the statistical model was corrected for parity number and body condition score at calving. Consequently, with this study design variability was intended to be reduced in its minimal expression. Nevertheless, one of the weaknesses of this study was the lack of quantifying individual DM intake. The study was conducted in a commercial dairy farm; consequently it was unfeasible to evaluate DM intake.

Several studies have evaluated products containing either calcium propionate, propylene glycol or glycerol; however few studies have assessed products containing a mix of the three gluconeogenic precursors. The combination of these 3 gluconeogenic precursors was able to improve milk yield in Chilean dairy cattle. This may be related to specific effects of each compound individually, although a negative or positive interaction among the ingredients may be possible. In the present study, the experimental design did not allow the evaluation of these potential interactions. Each of these gluconeogenic precursors has been demonstrated to impact milk yield in dairy cattle. However, doses used for each of these compounds tested independently have been larger than the combined doses considered in the current investigation. Indeed, in the present

study, animals received 21 g of calcium propionate, 28.5 g of propylene glycol and 99 g of glycerol per head daily. As a result, it may be suggested that most of the positive effect of the mixed product might be related to glycerol, because of its proportional greater participation. Indeed, calcium propionate is a compound poorly fermented by the rumen microorganisms. A single dose of 510 g (25 times of the dose tested in the present study) drenched at parturition increased slightly milk yield during the first 2 weeks after calving (Higgins et al. 1996). A high proportion of propylene glycol escapes degradation of the rumen and it is absorbed in the small intestine; the rest is metabolized to propionate. Ruminal molar percentage of acetate decreases and acetate to propionate ratio also decreases as propylene glycol dose is increased, indicating ruminal conversion of propylene glycol to propionate (Grummer et al. 1994; Christensen et al. 1997). In the liver, propylene glycol is converted to glucose, primarily via the lactaldehyde pathway and subsequent oxidation to lactate (Kristensen and Raun 2007). In most studies, administration of propylene glycol *prepartum* and/or *postpartum* has not been shown to significantly affect milk yields in lactating dairy cows (Fisher et al. 1973; Studer et al. 1993; Formigoni et al. 1996). However, Laranja da Fonseca et al. (1998) observed positive effects of propylene glycol on milk yield (300 ml per head daily) during the early *post-partum* period (2.5 kg of milk/day).

The concentration of NEFA at calving was significantly higher in the control group (0.75 ± 0.1 mEq/L) than the treated cows (0.55 ± 0.1 mEq/L) ($p \leq .05$). The interaction of time by treatment for BHB was different between groups ($p \leq .05$) (Figure 2). LSM for control group at 14 days *postpartum* had a lower concentration of serum BHB (0.60 ± 0.11 mmol/L) than the treated group (0.98 ± 0.11 mmol/L) ($p \leq .05$). At 7 and 21 days *postpartum* the concentrations of BHB were similar between groups ($p > .5$).

The concentration of NEFA was assessed only at calving because this metabolite has been demonstrated to be a health predictor when it is measured either precalving or at calving (Ospina et al. 2010). The concentration of NEFA at calving was significantly higher in the control group than the treated cows. This was expected because glucose precursors

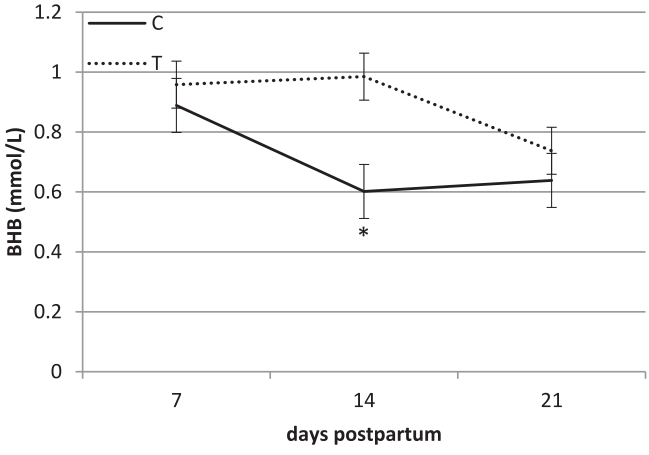

Figure 2. Serum concentration of BHB (mmol/L) at 7, 14 and 21 days postpartum in control and treated groups. (LSM ± SEM). C: control group ($n = 15$); *T*: treated group ($n = 15$). Interaction group by week was significant ($p \leq .05$). *LSM at day 14: $p \leq .05$.

may increase insulin in blood; therefore hormone-sensitive lipase is inhibited with less release of NEFA from the adipose tissue, even though an insulin resistance state during the transition period of dairy cows has been reported (De Koster and Opsomer 2013). Indeed, Higgins et al. (1996) indicated that calcium propionate increased blood glucose 24 hours after administration, and reduced BHB and NEFA during the first two days *postpartum*. In a previous study, the administration of propylene glycol before parturition decreased hepatic fat accumulation and ketone body formation. Plasma NEFA concentration was 403 and 234 μM, for control and treated cows, respectively, from 1 to 7 days *prepartum*. However, milk production and composition through 21 days *postpartum* were not different between treated and control groups (Studer et al. 1993). In addition, Christensen et al. (1997) demonstrated that administration of propylene glycol as an oral drench or mixed with concentrate resulted in higher serum insulin and lower plasma NEFA concentrations than did feeding propylene glycol as part of a TMR system. In the same line, cows receiving either 0.43 or 0.86 kg of glycerol during the *pre-partum* and *post-partum* periods, respectively, did not have changed *pre-partum* plasma glucose, insulin and BHB; however, 860 g of glycerol reduced NEFA at 7 days *post-partum* compared to the control group (DeFrain et al. 2004). In a large-scale Swedish study, 673 fresh cows from 12 commercial herds were randomly subjected to daily supplementation with 450 g of glycerol, 300 g of propylene glycol or nothing (control). Supplements were fed twice daily from 0 to 21 days in milk as a top dressed on concentrates. Blood metabolites were tested at approximately 2, 5 and 8 weeks postpartum. No differences in plasma concentrations of glucose, BHB, NEFA or IGF-1 were found between the control group and any of the treatment groups. However, cows supplemented with glycerol had a higher milk yield during the first 90 days in milk (Lomander et al. 2012). In general, the effectiveness of propylene glycol to increase plasma glucose concentrations depends on the dosage and the mode of administration, with stronger increases occurring in cows given higher dosages and when administered by oral drenches; however, this is less practical for on-farm use than its administration through the diet (Pickett et al. 2003; Bobe et al. 2004; Chung et al. 2009). Consequently, Bobe et al. (2004) proposed that a combination of propionate with glycerol or propylene glycol may be more effective than alone. An old study with a combination of 75 g of sodium propionate, 125 g of glycerol and 100 g of propylene glycol proved to be effective for decreasing ketone bodies in dairy cows (Pehrson 1972).

The results of BHB suggest that treated cows had a more markedly negative energy balance during the first 14 days of lactation perhaps because treated cows experienced higher milk yield than control cows. Thus, although treated cows were receiving gluconeogenic precursors they used more glucose for lactose synthesis; consequently it might be suggested that glucose produced in the liver was directed to the mammary gland, less oxaloacetate was available for the Krebs cycle and more ketone bodies were formed. Nevertheless, although BHB was higher at 14 days *postpartum* in treated than control cows, the levels reported in the present study were below 1.0–1.4 mmol/L, values considered as cut-off for subclinical ketosis (Duffield 2000; Ospina et al. 2010).

4. Conclusions

The supplementation of a product combining 3 gluconeogenic precursors during the *pre-* and *post-partum* periods improved milk yield during the first 60 days of lactation and maintained a moderated metabolic energy status during the first 21 days *postpartum* in Chilean dairy cattle.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

Arias RA, Mader TL. 2010. Determination of potential risk of heat stress of cattle in four locations of Central and Southern Chile. Arch Med Vet. 42:33–39.

Bobe G, Young JW, Beitz DC. 2004. Invited review: pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J Dairy Sci. 87:3105–3124.

Christensen JO, Grummer RR, Rasmussen FE, Bertics SJ. 1997. Effect of method of delivery of propylene glycol on plasma metabolites of feed-restricted cattle. J Dairy Sci. 80:563–568.

Chung IH, Martinez CM, Brown NE, Cassidy TW, Varga GA. 2009. Ruminal and blood responses to propylene glycol during frequent feeding. J Dairy Sci. 92:4555–4564.

De Koster JD, Opsomer G. 2013. Insulin resistance in dairy cows. Vet Clin Food Anim. 29:299–322.

DeFrain JM, Hippen AR, Kalscheur KF, Jardon PW. 2004. Feeding glycerol to transition dairy cows: effects on blood metabolites and lactation performance. J Dairy Sci. 87:4195–4206.

Drackley JK, Overton TR, Douglas GN. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. J Dairy Sci. 84(E. Suppl.):E100–E112.

Duffield TF. 2000. Subclinical ketosis in lactating dairy cows. Vet Clin North Am Food Anim Pract. 16:231–253.

Ferguson JM, Galligan DT, Thomsen N. 1994. Principal descriptors of body condition score in Holstein cows. J Dairy Sci. 77:2695–2703.

Fisher LJ, Erfle JD, Lodge GA, Sauer FD. 1973. Effects of propylene glycol or glycerol supplementation of the diet of dairy cows on feed intake, milk yield and composition, and incidence of ketosis. Can J Anim Sci. 53:289–296.

Formigoni A, Cornil MC, Prandi A, Mordenti A, Rossi A, Portetelle D, Renaville R. 1996. Effect of propylene glycol supplementation around parturition on milk yield, reproduction performance and some hormonal and metabolic characteristics in dairy cows. J Dairy Res. 63:11–24.

Goff JP, Horst RL. 1997. Physiological changes at parturition and their relationship to metabolic disorders. J Dairy Sci. 80:1260–1268.

Grummer RR. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cows. J Anim Sci. 73:2820–2833.

Grummer RR, Winkler JC, Bertics SJ, Studer VA. 1994. Effect of propylene glycol dosage during feed restriction on metabolites in blood of prepartum Holstein heifers. J Dairy Sci. 77:3618–3623.

Herdt TH. 2000. Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. Vet Clin North Am Food Anim Pract. 16:215–230.

Higgins JJ, Sanchez WK, Guy MA, Anderson ML. 1996. An oral gel of calcium propionate plus propylene glycol is effective in elevating calcium and glucose levels in periparturient dairy cows. J Dairy Sci. 79(Suppl. 1):130.

Johnson MM, Peters JP. 1993. Technical note: an improved method to quantify nonesterified fatty acids in bovine plasma. J Anim Sci. 71:753–756.

Kristensen NB, Raun BML. 2007. Ruminal and intermediary metabolism of propylene glycol in lactating Holstein cows. J Dairy Sci. 90:4707–4717.

Laranja da Fonseca LF, Lucci CS, Rodriguez PHM, Santos MV, Lima AP. 1998. Supplementation of propylene glycol to dairy cows in periparturient period: effects on plasma concentration of BHBA, NEFA, and glucose. J Dairy Sci. 81(Suppl. 1):320.

Littell RC, Henry PR, Ammerman CB. 1998. Statistical analysis of repeated measures data using SAS procedures. J Anim Sci. 76:1216–1231.

Lomander H, Frössling J, Ingvartsen KL, Gustafsson H, Svensson C. 2012. Supplemental feeding with glycerol or propylene glycol of dairy cows in early lactation. Effects on metabolic status, body condition, and milk yield. J Dairy Sci. 95:2397–2408.

Melendez P, Donovan A, Risco CA, Hall MB, Littell R, Goff J. 2002. Metabolic responses of transition Holstein cows fed anionic salts and supplemented at calving with calcium and energy. J Dairy Sci. 85:1085–1092.

Melendez P, Risco CA. 2005. Management of transition cows to optimize reproductive efficiency in dairy herds. Vet Clin North Am Food Anim Pract 21:485–501.

National Research Council. 2001. Nutrient requirements of dairy cattle. 7th rev ed. Washington (DC): National Academies Press.

Ospina PA, Nydam DV, Stokol T, Overton TR. 2010. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: critical thresholds for prediction of clinical diseases. J Dairy Sci. 93:546–554.

Pehrson B. 1972. The effect of orally administered glycogenic substance to dairy cows. Nordic Vet Med. 24:409–426.

Pickett MM, Piepenbrink MS, Overton TR. 2003. Effects of propylene glycol or fat drench on plasma metabolites, liver composition, and production of dairy cows during the periparturient period. J Dairy Sci. 86:2113–2121.

Remond B, Souday E, Jouany JP. 1993. In vitro and in vivo fermentation of glycerol by rumen microbes. Anim Feed Sci Tech. 41:121–132.

SAS. 2003. SAS/STAT software: change and enhancements through release 9.1 for windows. Cary (NC): SAS Inst Inc.

Studer VA, Grummer RR, Bertics SJ, Reynolds CK. 1993. Effect of prepartum propylene glycol administration on periparturient fatty liver in dairy cows. J Dairy Sci. 76:2931–2939.

Williamson DH, Mellanby J. 1974. D-3-hydroxybutyrate. In: Bergmeyer HU, editor. Methods of enzymatic analysis. London: Academic Press; p. 1836.