Effects of supplementing glycerol and soybean oil in drinking water on feed and water intake, energy balance, and production performance of periparturient dairy cows

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ABSTRACT

The objective of this study was to determine the effects of supplementing glycerol and soybean oil in drinking water on feed and water intake, calculated energy balance, and production performance of periparturient dairy cows. Ninety multiparous Holstein dairy cows were randomly assigned to 1 of 3 treatments: 1) no nutrients supplemented in the drinking water (control); 2) 20 g/L of glycerin supplemented in the drinking water (glycerol); and 3) 10 g/L of soybean oil supplemented in the drinking water (SBO). The trial lasted from 7 d prepartum to 7 d postpartum. Cows were offered a close-up and milking cow TMR for ad libitum intake, pre- and postpartum, respectively. The dry matter intake of cows supplemented with glycerol and SBO was lower than for the control cows throughout the experimental period but not different from each other. Water intake for the control cows was greater than the average for the glycerol and SBO cows prepartum, and greater than for SBO cows but similar to that of glycerol cows postpartum. Glycerol cows consumed more water than SBO cows. There were no differences in energy intake and energy balance of the cows pre- and postpartum. Serum triacylglycerol concentration for glycerol cows was lower than for the control and SBO cows prepartum and was lower than for the SBO cows postpartum. There were no differences in the serum nonesterified fatty acids and glucose concentrations throughout the experiment. There were no differences in the serum β -hydroxybutyrate (BHBA) concentrations at parturition, but serum BHBA concentration of the glycerol cows was greater than for control and SBO cows during the prepartum period. However, during the postpartum period, serum BHBA concentrations of the

control cows were greater than for glycerol and SBO cows. There were no differences in calf birth weights or milk yield and composition. Although the glucogenic property of glycerol supplemented in the drinking water at 20 g/L may not have been sufficient to elicit a milk yield response, it did reduce the concentration of BHBA postpartum.

Key words: glycerol, soybean oil, drinking water, transition period

INTRODUCTION

The transition period can be divided into 2 phases: 7 to 0 d prepartum, characterized by a 30% reduction in DMI (Grummer, 1995), and 0 to 21 d postpartum, when intake increases rapidly. The transition period remains a problematic area on many dairy farms, and metabolic disorders continue to occur at economically important rates on commercial dairy farms (Burhans et al., 2003). Furthermore, many of the metabolic disorders afflicting dairy cows during the periparturient period are interrelated and are linked to the diet fed during the prepartum period (Curtis et al., 1985). Several excellent reviews have been written to describe the metabolic adaptations in energy, protein, and mineral metabolism required for dairy cows to transition successfully to lactation (e.g., Grummer, 1993; Drackley et al., 2001; Overton and Waldron, 2004) and the rapid increase in energy demand at parturition has resulted in recommendations for energy dense diets 2 to 3 wk before and after parturition (NRC, 2001).

Water is an important nutrient in dairy production (Haupt, 1984; Murphy, 1992). Osborne et al. (2002) showed that glucose supplementation in the drinking water of transition cows improved the N status of the cow by reducing rumen ammonia levels and blood urea. Thomas et al. (2007) showed that flavoring agents need not be added only to the starter feed for calves, but that flavor can also stimulate DMI and BW gain when added in drinking water. Additionally, Osborne et al. (2008), in an attempt to bypass the rumen with fish oil and increase n-3 polyunsaturated fatty acids in milk, showed that drinking water was a useful vehicle to ad-

Received July 14, 2008.

Accepted September 7, 2008.

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minister nutrients to dairy cows. Furthermore, because dietary fat largely bypasses the portal venous system and is released into circulation via thoracic lymph nodes, the fat can be used for energy by nonhepatic tissues, thus sparing glucose. However, there is no information on the effects of fat supplementation in the drinking water of transition dairy cows on the energy content of colostrum, energy balance of the cows, or the birth weight of the calves. Additionally, the increased production of glycerin (or glycerol) from the biodiesel industry has made various forms of glycerin readily available and an attractive glucogenic substrate for feeding livestock. This is especially important with the reduced availability and higher price of corn because of demand by the ethanol industry. The objective of this study was to determine the effects of supplementing glycerol and soybean oil in drinking water on feed and water intake, energy balance, and production performance of periparturient Holstein dairy cows.

MATERIALS AND METHODS

Animals were cared for and handled in accordance with the Canadian Council on Animal Care regulations, and the University of Guelph Animal Care Committee reviewed and approved the experiment and all procedures carried out in the study.

Animals and Experimental Design

Ninety multiparous Holstein dairy cows were randomly assigned to 1 of 3 treatments: 1) no nutrients supplemented in the drinking water (control), and 2) isoenergetic treatments: 2) 20 g/L of glycerin (99.7% glycerol, specific gravity, 1.249; The Kissner Group, Cambridge, Ontario, Canada) supplemented in the drinking water from 7 d prepartum to 7 d postpartum (glycerol); and 3) 10 g/L of soybean oil supplemented in the drinking water from 7-d prepartum to 7-d postpartum (SBO). The level of inclusion of glycerol and SBO was based on previous work, which supplemented glucose at 20 g/L in the drinking water of periparturient cows (Osborne et al., 2002) such that approximately 800 g/d of glycerol and 400 g/d of SBO were delivered prepartum and 1,600 and 800 g/d of glycerol and SBO, respectively, were delivered postpartum (based on a 40- and 80-L/d water consumption pre- and postpartum, respectively). The water supplementation and delivery system has been described elsewhere (Osborne et al., 2008). Cows were housed in individual maternity pens and tie-stalls and offered a close-up and milking cow TMR twice daily at 0800 and 1500 h for ad libitum intake pre- and postpartum, respectively. The ingredients and chemical composition of the close-up and milking cow TMR are presented in Table 1.

Table 1. Ingredients and chemical composition of close-up and lactating cow TMR

Item	TMR	
	Close-up	Lactating cow
Ingredient, %		
Corn silage	61.0	32.5
Alfalfa silage	10.8	21.7
High-moisture corn	5.8	20.5
First-cut mixed hay	7.1	5.3
TMR dairy supplement	15.3 ¹	20.0 ²
Chemical composition		
DM, %	37.9	43.3
CP, % of DM (N × 6.25)	13.8	16.1
ADF, % of DM	23.7	21.9
NDF, % of DM	41.5	39.3
Ca, % of DM	0.81	0.91
P, % of DM	0.36	0.42
K, % of DM	1.26	1.29
Mg, % of DM	0.29	0.29
Na, % of DM	0.15	0.35
NE _L , ³ Mcal/kg	1.51	1.54

¹Contained (g/kg of DM): 48% soybean meal, 673; canola meal, 90; wheat bran, 80; calcium carbonate (limestone), 49.5; dicalcium phosphate, 14.5; fine salt, 16.1; molasses (in pelleted), 20; urea, 14.0; magnesium oxide (56% Mg), 6.9; vitamin E (50 kIU/kg), 14.8; Organic ruminant Micro Premix (Floradale Feed Mill Ltd., Floradale, Ontario, Canada), 7.5; selenium 200, 6.8; sulfur flour 99.5%, 1.8; Yea-Sacc¹⁰²⁶ (Alltech Inc., Nicholasville, KY), 4.8; Rovimix H-2 (DSM Nutritional Products Inc., Parsippany, NJ), 0.3.

²Contained (g/kg of DM): 48% soybean meal, 237; high-protein corn gluten meal, 200; canola meal, 100; roasted soybean (whole), 100; soy hulls (ground), 50.5; fish meal (herring), 50; beet pulp, 50; sodium bicarbonate, 33; calcium carbonate (limestone), 54.6; dicalcium phosphate, 23.6; fine salt, 21.7; golden flake (Floradale Feed Mill Ltd.), 20; molasses (in pelleted), 20; urea, 20; magnesium oxide (56% Mg), 7.2; organic ruminant Micro Premix (Floradale Feed Mill Ltd.), 5.4; Fibrozyme (Alltech Inc.), 2.7; sulfur flour 99.5%, 2.3; Yea-Sacc¹⁰²⁶ (Alltech Inc.), 1.9; Rovimix H-2 (DSM Nutritional Products), 0.1.

³Estimated according to the NRC (2001).

Measurements

Water and feed intake were monitored daily. The amount of TMR offered and refused for each cow was measured daily. Samples of TMR and orts were obtained daily and dried at 60°C for 48 h for DM determination. The TMR samples were pooled twice weekly and sent to a commercial laboratory (Agri-Food Laboratories, Guelph, Ontario, Canada) for chemical composition analysis.

All cows were weighed and body condition scored before morning feeding at 7 d before parturition and at parturition. At 7 d postpartum, the cows were weighed and body condition scored after the morning milking. Body condition score was assessed by the same individual using a 5-point scale (1 = thin to 5 = fat) in 0.25-unit increments (Edmonson et al., 1989). Calf weights at birth were recorded before the first colostrum was offered.

Cows were milked twice daily at 0630 and 1630 h, and milk production was recorded at each milking. Composite morning and evening milk samples were obtained on the first 6 d of lactation. Blood samples were obtained daily at 0700 h into evacuated 10-mL test tubes (Vacutainer, Becton Dickinson, Rutherford, NJ) by venipuncture of the coccygeal vessels. Samples were placed on ice and centrifuged at $2,000 \times g$ for 15 min in a refrigerated centrifuge (Model TJ-6, Beckman, Palo Alto, CA) at 4°C within 30 min. Serum was separated and transferred to 7-mL plastic scintillation vials and stored at -70°C until analysis.

Chemical Analysis

Analytical DM content of the TMR was determined by oven drying at 135°C for 2 h (method 3.002; AOAC, 1990). The CP contents were determined using Leco FP 428 N analyzer (Leco Corporation, St. Joseph, MI; method 4.2.08; AOAC, 1990). The TMR samples were also analyzed for ADF (method 973.18c; AOAC, 1990) and NDF (Van Soest et al., 1991) using α -amylase (A3306; Sigma Chemical Co., St. Louis, MO), and sodium sulfite corrected for ash concentration adapted for an Ankom 200 fiber analyzer (Ankom Technology, Fairport, NY). Calcium, phosphorus, potassium, magnesium, and sodium were analyzed by inductively coupled plasma spectroscopy (method 945.46; AOAC, 1990).

Milk samples were analyzed for true protein, fat, and lactose using infrared spectroscopy (MilkoScan FT 120, Foss Analytical A/S, Hillerød, Denmark). The concentration of BHBA in serum was determined using the Sigma Diagnostics enzymatic method (kit 310-A, Sigma) following the methods of Williamson et al. (1962). Nonesterified fatty acid concentrations were determined using a colorimetric assay (NEFA-C kit, Wako Chemicals, Richmond, VA), following modifications by Johnson and Peters (1993), and glucose concentrations were determined using a colorimetric assay (kit GAGO20, Sigma) following the methods of Raabo and Terkildsen (1960). Triacylglycerol concentrations were determined using a colorimetric assay (kit TRO100, Sigma) following the methods of McGowan et al. (1983). Glycerol and soybean oil gross energy values were determined using a C5000 bomb calorimeter (IKA Works Inc., Wilmington, NC).

Energy Balance Calculations

Total net energy intake (TNE_I) was determined from DMI and glycerol or SBO intakes in water. The NE_L content of the TMR was calculated according to NRC (2001) and NE_L contents of glycerol and SBO were esti-

mated at 59 and 64%, respectively, of the gross energy values (NRC, 2001) to be 2.5 and 6.0 Mcal/kg of DM, respectively. Net energy for maintenance (Mcal/d) was computed as $BW^{0.75} \times 0.08$ (NRC, 2001). Pregnancy energy expenditures (NE_P) were calculated as $[(2 \times 0.00159 \times \text{days pregnant} - 0.0353) \times \text{calf BW}/45]/0.14 \times 0.64$ (NRC, 2001). Milk energy was calculated using the equation NE_L (Mcal/d) = $MP \times [(0.0929 \times F) + (0.0563 \times P) + (0.0395 \times L)]$, where MP is milk production (kg/d), and F, P, and L are fat, protein, and lactose percentages in the milk, respectively (NRC, 2001). Energy balance (Mcal/d) was estimated as $TNE_I - (NE_M + NE_P + NE_L)$.

Statistical Analysis

The ANOVA for DMI, BW, DMI as a percentage of BW, BW change, BCS, BCS change, water and energy intake, energy balance, and milk production and composition was conducted separately by period (i.e., d -7 to d -1, at parturition, and d 1 to d 7) using PROC MIXED of SAS (v. 9.1; SAS Inst. Inc., Cary, NC). The model was: $Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \epsilon_{ij}$, where Y_{ij} is the dependent variable, μ is the overall mean, α_i is the effect of treatment ($i = 1, 2, 3$), β_j is the effect of time in days ($j = -7, -6, -5, -4, -3, -2, -1$ or $1, 2, 3, 4, 5, 6, 7$), $\alpha\beta_{ij}$ is the effect of a treatment $\times$ time interaction ($ij = 1, 2, \dots, 21$), and ϵ_{ij} is the random residual error. The repeated measurement option in time was used with cow nested within treatment as the repeat subject for variables with repeated measurements. The first-order autoregressive covariance model was fitted to the data. The correlation between energy intake, energy balance and blood metabolites was conducted using PROC CORR in SAS. Differences among treatment means were tested for significance using Tukey's honestly significant difference, and effects were considered significant at $P < 0.05$. Data are expressed as least squares means $\pm$ SEM, which represents the pooled SE for the model.

RESULTS AND DISCUSSION

Of the 90 cows assigned to treatments, only 70 completed the trial (20 on the control treatment, 24 on glycerol, and 26 on soybean oil). The remaining cows calved early and were not included in the statistical analysis.

DMI and Water Intake

During the prepartum period, cows supplemented with glycerol and SBO had lower ($P = 0.03$) DMI than the control cows (Table 2). However, DMI of glycerol

Table 2. Effects of supplementing glycerol and soybean oil (SBO) in drinking water of dairy cows from d 7 before calving to 7 d postpartum on DMI, BW, BCS, water intake, and energy balance

Item	Treatment			SEM	<i>P</i> -value
	Control	Glycerol	SBO		
Prepartum ¹					
DMI, kg/d	12.4 ^a	10.9 ^b	11.3 ^b	0.42	0.03
DMI, % of BW	1.82	1.57	1.62	0.134	0.37
BW, ² kg	731	756	747	14.3	0.47
BW change ³	14.9 ^a	34.0 ^b	18.5 ^a	2.58	<0.01
BCS ⁴	3.74	3.69	3.58	0.116	0.62
BCS change ⁴	0.14	0.15	0.21	0.047	0.51
Water intake, L/d	34.9 ^a	33.6 ^{ab}	29.4 ^b	1.64	0.05
NE intake, ⁵ Mcal/d	18.8	18.7	18.7	0.67	0.99
NE balance, ⁵ Mcal/d	3.36	3.17	3.16	0.774	0.98
Postpartum ⁶					
DMI, kg/d	15.1 ^a	12.6 ^b	13.3 ^b	0.48	<0.01
DMI, % of BW	2.22	1.87	2.00	0.082	0.01
BW, ⁷ kg	682	701	689	16.8	0.74
BW change ⁸	-27.2 ^a	-30.3 ^{ab}	-33.7 ^b	1.38	<0.01
BCS ⁹	3.57	3.53	3.45	0.106	0.72
BCS change ⁹	-0.16	-0.18	-0.16	0.017	0.85
Water intake, L/d	68.7 ^a	66.8 ^a	58.3 ^b	2.66	0.02
NE intake, ⁵ Mcal/d	23.3	24.0	23.8	0.79	0.83
NE balance, ⁵ Mcal/d	-14.6	-14.2	-14.4	1.28	0.98

^{a,b}Means within a row with different superscript letters differ ($P < 0.05$).

¹Last 7 d of gestation.

²Mean BW from 7 d precalving to calving.

³Difference between precalving and calving data.

⁴BCS: 1 = thin, 5 = fat; mean BCS at 7 d precalving.

⁵Calculated according to the NRC (2001).

⁶First 7 d of the lactation period.

⁷Mean BW from calving to 7 d postcalving.

⁸Difference between calving data and data at 7 d postcalving.

⁹Mean BCS at 7 d postcalving.

and SBO cows were not ($P > 0.05$) different. DeFrain et al. (2004) evaluated the antiketogenic effects of glycerol top-dressed on the TMR of dairy cows during the transition period and observed that prepartum, DMI was greater for control cows compared with those fed glycerol in agreement with the results of the current study (Table 2).

During the postpartum period, cows supplemented with glycerol and SBO had lower ($P < 0.01$) DMI than the control cows. However, DMI of glycerol and SBO cows were not ($P > 0.05$) different (Table 2). Previous studies have shown that feeding unsaturated oil depressed DMI (Lock and Shingfield, 2004) because of impaired rumen function (Jenkins, 1993). Fisher et al. (1971) found that glycerol acted as an appetite stimulant when fed at 472 g/d within the concentrate mix of a component-based diet compared with cows fed propylene glycol, whereas Fisher et al. (1973) found that feeding glycerol at 174 or 347 g/d did not improve feed intake, milk yield, and energy balance compared with unsupplemented cows or cows supplemented with propylene glycol. Chung et al. (2007) fed a dry glycerol product (food grade, 65% glycerol) to supply 163 g of

glycerol per day to 39 multiparous Holstein dairy cows from calving to 21 DIM and found no effect on feed intake. However, in a recent study, Donkin and Doane (2007) evaluated the dose response of feeding 0, 50, 100, and 150 g/kg of glycerol (99.5% pure glycerol) of diet DM (1,222, 2,454, and 3,600 g/d of glycerol for 50, 100, and 150 g/kg of glycerol, respectively) to lactating dairy cows and found that feed intake was decreased at the 150 g/kg level of glycerol during the first 7 d of the experiment. In the current study, supplementing glycerol and SBO in the drinking water lowered DMI during the first 7 d of the experiment. The effects of glycerol both pre- and postpartum in the current study are consistent with previous studies although our study is different from those that have added glycerol or SBO to the TMR because we provided these energy sources via drinking water instead of top-dressing on the basal diet. Because animals eat primarily to meet their energy requirements (Church and Pond, 1988), if an animal is in positive energy balance providing additional metabolizable energy would theoretically decrease feed intake and thus improve efficiency. Ogborn (2006) drenched multiparous Holstein cows with 500 mL/d of glycerol

Figure 1. Water intake for the control cows (■) and cows supplemented with glycerol (●) or soybean oil (▲) in drinking water from d 7 before calving to 7 d postpartum.

for the first 5 DIM and observed decreased DMI. Feeding glycerol during the prepartum period in the Ogborn (2006) study increased DMI, but feeding glycerol during the postpartum period tended to decrease DMI.

During the prepartum period, there were no differences ($P > 0.05$) in the water intake of glycerol cows and either the control cows or those supplemented with SBO. However, during the postpartum period, water intake of the control cows was greater ($P = 0.02$) than that of cows supplemented with SBO but similar to that of cows supplemented with glycerol. This is in contrast to Schröder and Südekum (1999) who found that feeding glycerol in the diet stimulated water intake. Water intake of the lactating cows supplemented with glycerol in the current study was greater ($P = 0.02$) than that of cows supplemented with SBO (Table 2, Figure 1).

Energy Balance, BW, and BCS

All cows were in positive energy balance before calving and in negative energy balance after calving (Figure 2). There were no differences ($P > 0.05$) in energy intake and energy balance of the cows throughout the experimental period (Table 2, Figure 2). During the prepartum period, cows supplemented with glycerol showed an almost 2-fold increase in BW gain compared with the control or cows supplemented with SBO. However,

no conclusion(s) can be drawn from weight changes in such a short experimental period. Furthermore, there are great limitations to measuring BCS change over such a short duration to avoid ending up with artifacts. There were no differences in the change in BW between cows supplemented with SBO and the control cows. There were no differences ($P > 0.05$) in BW, BCS, or BCS changes.

During the postpartum period, there were no differences ($P > 0.05$) in the BCS change of cows supplemented with glycerol compared with control cows or cows supplemented with SBO. In the Donkin and Doane (2007) study, cows supplemented with 150 g/kg glycerol gained more weight after 8 wk on the diet than did cows fed other treatments. However, in the current study, the experiment was only from d 7 prepartum to d 7 postpartum, and the observed changes in BCS and BW were typical of BCS and BW changes in the herd. This lack of effect was not surprising because BCS is primarily an assessment of subcutaneous tissue change and measuring BCS over a relatively short period of time gives no clear indication of whether a cow was gaining or losing weight. Furthermore, when a cow begins to replenish mobilized tissue, abdominal and intermuscular fat, which contribute significantly to the total energy available for mobilization (Butler-Hogg et al., 1985), are replenished before subcutaneous fat.

Figure 2. Energy balance for the control cows (■) and cows supplemented with glycerol (●) or soybean oil (▲) in drinking water from d 7 before calving to 7 d postpartum.

Triacylglycerol

Soybean oil was supplemented in the drinking water as a source of triacylglycerol for ATP production and milk fat synthesis. The concentration of triacylglycerol in the serum of SBO-supplemented cows was greater ($P < 0.01$) than in glycerol cows during the prepartum period, was greater ($P = 0.03$) than in control cows at parturition, and was greater ($P < 0.01$) than in control and glycerol cows postpartum (Figure 3A). The concentrations of triacylglycerol averaged 189.0 , 174.6 , and 205.3 ± 7.25 $\mu\text{mol/L}$ for the control, glycerol and SBO treatments, respectively, during the prepartum period; 112.7 , 122.8 , and 139.0 ± 8.85 $\mu\text{mol/L}$, respectively, at parturition; and 99.5 , 97.4 , and 120.0 ± 3.43 $\mu\text{mol/L}$, respectively, postpartum. Glycerol caused a decrease in serum triacylglycerol concentrations prepartum, which may have been a consequence of depressed DMI, although the effect did not persist into lactation.

NEFA

The concentration of NEFA in serum of all cows remained relatively constant from d -7 to -3 and then began to increase at 2 d before parturition (Figure 3B). Grummer (1993) suggested that this rapid increase in the concentration of serum NEFA immediately before calving may be hormonally regulated. It has been sug-

gested that the spike at calving is associated with the sharp reduction in intake and with the elevation in plasma lipolytic hormones that occurs in conjunction with the onset of calving (Vázquez-Añón et al., 1994). There were no differences ($P = 0.20$) in the concentration of NEFA in serum during the prepartum period, which averaged 0.21 , 0.30 , and 0.26 ± 0.037 mmol/L for the control, glycerol, and SBO treatments, respectively. The concentration of serum NEFA prepartum was negatively correlated to the concentration of triacylglycerol ($r = -0.62$; $P < 0.01$). Similar to the prepartum period, there were no differences ($P = 0.53$) in the concentration of NEFA in serum at parturition, which averaged 0.57 , 0.62 , and 0.52 ± 0.066 mmol/L for the control, glycerol, and SBO treatments, respectively. These results are in agreement with those of Ogborn (2006), who orally drenched transition cows with crude glycerol (625 g of glycerol/d) during the first 5 d postpartum and observed a reduction in DMI but no changes in plasma glucose and NEFA concentrations. There were also no differences ($P = 0.22$) in the serum concentrations of NEFA postpartum, which averaged 0.63 , 0.50 , and 0.58 ± 0.050 mmol/L for the control, glycerol, and SBO treatments, respectively, in contrast to DeFrain et al. (2004), who observed decreased concentrations of NEFA in serum of glycerol-supplemented cows.

Elevated concentration of serum NEFA during the periparturient period is a common feature in dairy cows

Figure 3. Serum metabolite concentrations of dairy cows on the control treatment (no supplementation; ■) and those supplemented with glycerol (●) and soybean oil (▲) in drinking water from d 7 before calving to 7 d postpartum: a) triacylglycerol; b) NEFA; c) glucose; and d) BHBA.

and reflects increased reliance on adipose reserves to support energy requirements and milk fat synthesis (Pullen et al., 1989). The magnitude of the increase in NEFA after parturition was inversely related to DMI before parturition (Holtenius et al., 2003). High serum NEFA concentration leads to triacylglycerol accumulation in muscle (Roberts et al., 1981) and liver (Roberts et al., 1981; Grummer, 1993) and has been associated with depressed DMI and metabolic disorders (Grummer, 1993).

Glucose

There were no differences in the concentrations of glucose in serum prepartum ($P = 0.82$), at parturition ($P = 0.98$), and postpartum ($P = 0.92$; Figure 3C). The concentration of glucose in serum averaged 2.90 , 2.75 , and 2.83 ± 0.165 mmol/L for the control, glycerol, and SBO treatments, respectively, during the prepartum period; 3.25 , 3.17 , and 3.25 ± 0.313 mmol/L, respectively, at parturition; and 2.24 , 2.15 , and 2.22 ± 0.161 mmol/L,

respectively, postpartum. Although Rukkwamsuk et al. (1999) reported that high-energy diets increased the concentration of plasma glucose during the last week of gestation, our results are in agreement with those of DeFrain et al. (2004) who top-dressed either 430 or 860 g/d of glycerol onto the daily TMR allocation of dairy cows during the transition period from d -14 to d 21 postpartum and found that prepartum plasma glucose and insulin concentrations were not affected by treatments. Postpartum, supplementing 430 g/d of glycerol lowered blood glucose concentrations at d 7 and BHBA concentrations at d 14 and d 21, whereas supplementing 860 g/d of glycerol led to a decrease in glucose concentration from d 14 to 21 and elevated BHBA concentrations from d 7 (DeFrain et al., 2004). DeFrain et al. (2004) suggested that dietary glycerol during the transition period may only provide modest support to increase postpartum serum glucose concentrations and decrease NEFA. The decreased glucose concentrations in all treatments postpartum were probably related to the low DMI and the concomitant reduction in propi-

onate absorption, along with an increased glucose use in milk synthesis (Doepel et al., 2002).

BHBA

The concentration of BHBA in the serum of the glycerol cows was greater ($P = 0.05$) than for control and SBO cows during the prepartum period, whereas during the postpartum period the concentration of BHBA of the control cows was greater ($P = 0.02$). There were no differences in the concentration of BHBA at parturition (Figure 3D). The concentration of BHBA in serum of the control cows was greater ($P < 0.01$) than that for glycerol and SBO cows postpartum. There was no difference ($P > 0.05$) in concentration of BHBA in serum of glycerol and SBO cows. The concentration of BHBA in serum averaged 0.41, 0.52, and 0.41 ± 0.036 $\mu\text{mol/L}$ for the control, glycerol, and SBO treatments, respectively, during the prepartum period; 0.47, 0.49, and 0.45 ± 0.084 $\mu\text{mol/L}$, respectively, at parturition; and 0.79, 0.64, and 0.59 ± 0.053 $\mu\text{mol/L}$, respectively, postpartum. Chung et al. (2007) showed that glycerin-supplemented cows exhibited a more-positive energy status during the second week of lactation as evidenced by greater concentrations of plasma glucose, lower concentrations of plasma BHBA, and lower concentrations of urine ketones. In the current study, there were no differences in the concentration of glucose during the first 7 DIM.

There was a tendency for the concentration of serum BHBA during the prepartum period to be negatively correlated to the concentration of triacylglycerol ($r = -0.41$; $P = 0.06$). The concentration of serum BHBA postpartum was negatively correlated to the concentration of glucose ($r = -0.46$; $P = 0.04$) and tended to be positively correlated to the concentration of NEFA ($r = 0.42$; $P = 0.06$). The increased serum concentrations of NEFA and BHBA postpartum suggest mobilization of body fat as indicated by the reductions in BW and BCS. Although water supplementation had no effect on NE balance or serum glucose and NEFA concentrations, BHBA concentrations in serum were decreased. The decrease may have been unrelated to fat mobilization and due to the reduced DMI on glycerol and SBO treatments, leading to lower production and absorption of butyrate from rumen fermentation.

Milk Yield and Composition

There were no effects ($P > 0.05$) of glycerol or SBO on calf birth weight or milk yield and composition (Table 3). Khalili et al. (1997) fed 36 g of glycerol/kg of diet DM to mid-lactation dairy cows and reported no effects on feed intake, milk production, and composi-

tion, although the profile of fatty acids in milk was slightly altered. Donkin and Doane (2007) found that milk production and composition were not affected by glycerol addition up to 150 g/kg of dietary DM. Similarly, Chung et al. (2007) fed 163 g of glycerol per day from calving to 21 DIM and reported no difference in milk yield and components. Earlier glycerol feeding studies (Fisher et al., 1971, 1973) also found no effect of glycerol on milk yield. However, Bodarski et al. (2005) observed that feeding 500 mL of glycerol or approximately 31 g/kg of ration DM from 3 wk prepartum through 70 DIM increased milk yield and milk protein content during the second week of lactation but there was no treatment effect over the course of the study.

Milk yield was not affected by feeding or drenching glycerol during the first 5 d of lactation although increasing dietary energy density prepartum has been shown to have a positive effect on milk yield and milk constituents in some studies (Keady et al., 2001; McNamara et al., 2003). There are no studies that have looked at the addition of SBO in the drinking water of dairy cows on milk yield and composition, although addition of SBO to the TMR (0, 17, and 34 g/kg of DM) has been shown to linearly reduce milk fat yield and protein percentage and linearly increase milk yield and milk protein yield (AlZahal et al., 2008). Although milk yield was not affected by addition of SBO in the current study, DMI was depressed. Osborne et al. (2008), in an attempt to bypass the rumen with fish oil and increase n-3 polyunsaturated fatty acids in milk, found that supplementing fish oil in the drinking water of dairy cows had no effect on DMI, water intake, or milk yield compared with top-dressing the fish oil on the TMR.

In conclusion, although the supplementation of energy sources in drinking water was not sufficient to elicit a milk yield response or change the net energy balance of cows during the first week postpartum, it did lower the concentrations of BHBA in serum postpartum. Glycerol and SBO supplementation lowered DMI but had no effect on NE intake. Soybean oil lowered water intake. However, neither treatment had any effect on calf weight at birth, milk production and composition, and serum NEFA and glucose concentration. These results suggest that glycerol supplemented in the drinking water at 20 g/L might not be a viable glucogenic feed additive for transition dairy cows from 7 d prepartum to 7 d postpartum.

ACKNOWLEDGMENTS

The authors thank the Dairy Farmers of Ontario and Ontario Ministry of Agriculture and Food for financial support, the staff at the Elora Dairy Research Centre,

Table 3. Effects of supplementing glycerol and soybean oil (SBO) in drinking water of dairy cows from d 7 before calving to 7 d postpartum on calf weight, milk yield and composition

Item	Treatment			SEM	P-value
	Control	Glycerol	SBO		
Calf weight, kg	47.2	46.3	47.8	1.06	0.62
Milk yield, ¹ kg/d	27.9	26.6	26.5	0.96	0.52
Fat, %	5.51	5.93	5.44	0.217	0.24
Fat yield, kg/d	1.55	1.52	1.44	0.088	0.67
Protein, %	6.08	6.67	6.87	0.439	0.42
Protein yield, kg/d	1.56	1.62	1.73	0.113	0.55
Lactose, %	3.69	3.58	3.60	0.068	0.51
Lactose yield, kg/d	1.04	0.96	0.97	0.040	0.30
SNF, %	10.5	11.0	11.2	0.38	0.42
SNF yield, kg/d	2.79	2.76	2.89	0.132	0.77

¹First 7 d of the lactation period.

and Mollie Campbell, Carolyn Borsy, Carmen Chui, and Norm Purdie (Department of Animal and Poultry Science, University of Guelph) for their technical assistance.

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