Animal (2009), **3:10**, pp 1420–1427 © The Animal Consortium 2009
doi:10.1017/S175173110999036X

animal

Effects of feeding propylene glycol on dry matter intake, lactation performance, energy balance and blood metabolites in early lactation dairy cows

Q. Liu[1†], C. Wang[1], W. Z. Yang[1,2], W. W. Zhang[1], X. M. Yang[3], D. C. He[3], K. H. Dong[1] and Y. X. Huang[1]

[1]*College of Animal Sciences and Veterinary Medicines, Shanxi Agricultural University, Taigu 030801, Shanxi, P. R. China;* [2]*Agriculture and Agri-Food Canada, Research Centre, PO Box 3000, Lethbridge, AB, Canada;* [3]*Institute of Animal Science, Shanxi Academy of Agricultural Science, Taiyuan 030032, Shanxi, P. R. China*

(Received 26 October 2008; Accepted 2 June 2009; First published online 29 June 2009)

The objectives of this study were to evaluate effects of feeding propylene glycol (PG) on feed intake, milk yield and milk composition, blood metabolites and energy balance in Holstein dairy cows from 1 to 63 days in milk. Thirty-two multiparous cows, blocked by lactation number, previous 305-day milk production and expected calving date, were arranged into four groups in a randomized block design. Treatments were: control, low PG, medium PG and high PG with 0, 150, 300 and 450 ml PG per cow per day, respectively. The supplement of food grade PG (0.998 g/g PG) was hand-mixed into the top one-third of the daily ration. Cows were fed ad libitum *a total mixed ration consisting of forage and concentrate (50 : 50, dry matter basis). Feed intake, milk yield and milk components were not affected (*$P > 0.05$*) by PG supplementation. Overall, body weight (BW) loss tended (*$P < 0.08$*) to be linearly reduced, and energy status was linearly improved with increasing PG supplementation. Concentrations of glucose in plasma were higher for cows fed PG relative to control (55.6 v. 58.9 mg/dl) and linearly increased (*$P < 0.01$*) with increasing PG supplementation. Plasma concentrations of non-esterified fatty acids and beta-hydroxybutyrate were linearly increased, but urine acetoacetate concentration was quadratically changed with the highest for control diet and the lowest for 450 ml/day of PG. These results indicated that supplementation of PG in the early lactating cow diets had minimal effects on feed intake and milk production, but may potentially reduce contents of milk fat and milk protein. Supplementation of early lactating dairy cow diets with PG is beneficial in terms of improving energy status and reducing BW loss.*

Keywords: propylene glycol, lactation performance, energy balance, blood metabolites, dairy cows

Implications

Satisfying the nutritional requirements of high-producing dairy cows can be a challenge. Feed intake may decrease as much as 30% during the week before calving (Bertics *et al*., 1992) and cows often continue to be in a negative energy balance during at least the first 5 weeks of lactation (DeFrain *et al*., 2004). Because of our inability to overcome the low dry matter intake during transition period, dairy producers often use oral drenches and pastes to provide glucose precursors to prevent ketosis and other metabolic disorders. Propylene glycol (PG) is a glucogenic precursor that is either rapidly absorbed from the rumen and converted to glucose, or partially metabolized to propionate in the rumen before being absorbed (Nielsen and Ingvartsen, 2004). PG has been used in the treatment of ketosis since the 1950s and is still used today. Most previous studies differ in quantity, timeframe and method of delivery of PG. However, little is known about effects of PG in early lactation period on health, milk production, blood metabolic profiles and energy balance. The aim of this work was to evaluate whether PG can be supplemented in dairy cow diet to improve feed intake, milk yield and composition, and energy balance in early lactation Holstein dairy cows.

Introduction

Satisfying the nutritional requirements of high-producing dairy cows can be a challenge. Feed intake may decrease as much as 30% during the week before calving (Bertics *et al*., 1992) and cows often continue to be in a negative energy balance during at least the first 5 weeks of lactation

† E-mail: liuqiangabc@163.com

(DeFrain *et al*., 2004). The negative energy balance stimulates lipid mobilization from adipose tissues followed by a rise of non-esterified fatty acids (NEFA) concentrations in blood and an increased uptake of NEFA by the liver. Extreme lipid mobilization exceeds the metabolizing capacity of the liver, leading to an increased triglyceride accumulation and formation of ketone bodies (Herdt, 1988). Associations between NEFA concentrations and incidence of ketosis, displaced abomasum and retained fetal membranes have been described (Grummer *et al*., 1994). Because of our inability to overcome the low dry matter (DM) intake during the transition period, dairy producers often use oral drenches and pastes to provide glucose precursors to prevent ketosis and other metabolic disorders (Van Knegsel *et al*., 2007).

Propylene glycol (PG) is a glucogenic precursor that is either rapidly absorbed from the rumen and converted to glucose, or partially metabolized to propionate in the rumen before being absorbed (Nielsen and Ingvartsen, 2004). PG has been used in the treatment of ketosis since the 1950s (Johnson, 1954; Maplesden, 1954) and is still used today (McClanahan *et al*., 1998). PG has been shown to alleviate the minus effect of decreased DM intake on negative energy balance (Studer *et al*., 1993; Formigoni *et al*., 1996) and to lower the risk of ketosis and fatty liver syndrome (Studer *et al*., 1993). However, results on effects of PG supplementation are not consistent in the literature. Short-term experiments using non-lactating cattle showed that administration of PG as an oral drench decreased concentrations of NEFA and beta-hydroxybutyrate (BHBA) in plasma (Grummer *et al*., 1994; Christensen *et al*., 1997). Studer *et al*. (1993) determined that 1 l of PG administered during the last 10 days before parturition increased concentrations of glucose and decreased concentrations of NEFA and BHBA in plasma before parturition. Burhans *et al*. (1997) reported no effect of PG on plasma NEFA and BHBA concentrations prior to parturition, whereas after parturition, PG drenches resulted in significantly decreased concentrations of NEFA and BHBA in plasma. Butler *et al*. (2006) reported the improved energy balance and milk lactose content of cows drenched daily with either 500 ml of water or PG from day 10 before parturition until day 25 *postpartum*, but this effect was not observed in other studies (Pickett *et al*., 2003; Moallem *et al*., 2007). Those inconsistencies may be due to differences in quantity, timeframe and method of delivery of PG. Furthermore, the information on the effects of PG supplementation in early lactation period on feed intake, milk production, blood metabolic profiles and energy balance is limited. We hypothesize that supplementation of PG will provide additional glucogenic substrates to dairy cows in early lactation, thus improve energy balance, milk production and blood metabolites. The aim of this work was to evaluate effects of PG supplementation on feed intake, milk yield and milk composition, blood metabolic profiles and energy balance in early lactation Holstein dairy cows from 1 to 63 days in milk (DIM).

Material and methods

Animals and experimental design

Thirty-two multiparous (mean parity: 2.4 ± 0.25) Holstein dairy cows, averaged 617 ± 16.2 of body weight (BW) and 7921 ± 345.6 kg of milk per 305 days from the previous lactation, were used during days 1 to 63 of lactation. Cows were blocked by lactation number, previous 305-day milk production and expected calving date and were arranged into four groups in a randomized block design. Treatments were: control, low PG, medium PG and high PG with 0, 150, 300 and 450 ml PG fed per cow per day, respectively. The supplement of food grade PG (0.998 g/g PG; The Dow Chemical Company, Midland, USA) was purchased commercially and hand-mixed into the top one-third of the daily ration. Cows were housed in a naturally ventilated tie-stall barn after calving and allowed to exercise for 2 h in an open dry lot before each milking. Cows were milked three times daily at 0600, 1400 and 2100 h and were fed after each milking *ad libitum* a total mixed ration (TMR). The diet was formulated based on NRC (2001) recommendations for a 620 kg cow producing 26 kg/day of milk containing 3.4% of milk fat and 3.2% protein (Table 1). The amount of PG fed was determined from the work by Kim *et al*. (2005) who found that PG (2.5 ml/kg $BW^{0.75}$) supplemented to the ration decreased blood NEFA. PG fed in the current study was used as a glucogenic supplement to provide additional glucogenic substrates to dairy cows in early lactation, and no adjustment in net energy (NE_l) concentration was made in the control diet to mimic practical feeding conditions.

Table 1 *Ingredient and chemical composition of the basal diet (g/kg dry matter)*

Ingredients	
Corn stover	210
Corn silage	265
Alfalfa hay	30
Corn grain, ground	224
Wheat bran	60
Soybean meal	85
Cottonseed cake	70
Rapeseed meal	38
Calcium carbonate	5
Salt	4
Dicalcium phosphate	3.5
Mineral and vitamin mixture†	5.5
Chemical composition	
Organic matter	943.2
Crude protein	165.4
Neutral detergent fibre	422.5
Acid detergent fibre	271.6
Calcium	7.4
Phosphorus	4.6
NE_L‡ (MJ/kg)	6.72

NE_L = net energy required for lactation.
†Contained 42 ppm Co, 3500 ppm Cu, 20 000 ppm Fe, 12 000 ppm Mn, 12 000 ppm Zn, 1200 ppm I, 600 ppm Se, 3800 IU/g of vitamin A, 1200 IU/g of vitamin D and 30 IU/g of vitamin E.
‡Estimated based on NRC (2001).

The experimental protocol was approved by the Animal Care and Use Committee of the Shanxi Agriculture University (Taigu County, Jinzhong City, Shanxi Province, China).

Measurements and collection of samples

Feed offered and refusals were measured for each cow and recorded daily through the experimental period to calculate daily DM intake. Milk yield were recorded daily from parturition through 63 DIM. Samples of TMR were collected weekly to determine DM content and then composited for later chemical analysis. BWs were recorded on two consecutive days at parturition and at 21, 42 and 63 DIM after the 1400 h milking. Milk samples from each cow were collected weekly from each milking on three consecutive milkings within a day. Milk samples were preserved with 2-bromo-2-nitropropane-1,3-diol and pooled by day (weighed to milk yield of each milking) for each cow before chemical analysis. Urine samples were collected using urine collection aprons (Hobbs *et al*., 1950; Liu *et al*., 2008) from 3 days during 7, 14 and 21 DIM. Concentrations of urine acetoacetate were measured from a midstream urine sample using Ketostix reagent strips (Ketostix; Bayer Corporation Diagnostics Division, Elkhart, USA) (Chung *et al*., 2007). Blood samples were obtained from three consecutive days on days 6, 7, 8; 13, 14, 15 and 20, 21, 22 of parturition, 2 h after the morning feeding, from the coccygeal vein into 7 ml vacuum tubes (Vacutainer®; Becton Dickinson, Rutherford, NJ, USA) containing potassium oxalate and 4 g/100 ml sodium fluoride for glucose analysis; 10 ml tubes containing sodium heparin for analyses of BHBA and NEFA; and 13 ml tubes (serum separator tubes) containing clot activator were used to collect serum for analysis of insulin concentration. Blood tubes were immediately placed on ice and transported to the laboratory. Plasma was separated from whole blood by centrifuging at $2000 \times \mathbf{g}$ for 15 min in a refrigerated centrifuge at 4°C. Blood tubes for serum collection were allowed to sit for approximately 0.5 h and centrifuged at room temperature at $1000 \times \mathbf{g}$ for 15 min to separate serum. All plasma and serum samples were stored frozen at −20°C until further analyses.

Chemical analyses

Samples of TMR and refusals were dried in an oven at 55°C for 48 h, and ground to pass a 1-mm screen with a mill (FZ102; Shanghai Hong Ji instrument Co., Ltd, Shanghai, China) for chemical analysis. Analytical DM content of oven-dried samples was determined by drying at 135°C for 3 h (Association of Official Analytical Chemists, 1990; method 930.15). The neutral detergent fiber (aNDF) and acid detergent fiber (ADF) contents were determined using the methods described by Van Soest *et al*. (1991) with heat stable alpha amylase and sodium sulfite used in the aNDF procedure, with both aNDF and ADF expressed inclusive of residual ash. The content of N in the samples was determined by a Kjeldahl method (AOAC, 1990; method 976.05). Milk samples were analyzed for fat, true protein and lactose using infrared spectrophotometry (Foss 120 Milko-Scan; Foss Electric, Hillerød, Denmark) according to AOAC (1997, method 975.16) procedures.

Concentrations of glucose in plasma were determined using glucose oxidase (Sigma kit #315; Sigma Diagnostics, St. Louis, MO, USA) based on the method of Trinder (1969). Concentrations of BHBA were determined (Sigma kit 310-A; Sigma Diagnostics) following the method of Williamson *et al*. (1962). Concentrations of NEFA were determined using a colorimetric assay (NEFA C Assay Kit; Nanjing Jian Cheng Institute of Bio-Engineering, Nanjing, China) with the modifications of Johnson and Peters (1993). Serum samples were analyzed for concentrations of insulin by using a radioimmunoassay (Insulin Assay Kit; Jian Cheng Institute of Bio-Engineering, Nanjing, China).

Calculations and statistical analyses

Net energy intake (MJ/day) was calculated by multiplying the DM intake by the NE density of the diet from the ingredient (Feng *et al*., 2000). The energy for lactation (NE_L) of PG, assumed to be 16.73 MJ/kg (Miyoshi *et al*., 2001), was included in NE intake. The NE required for maintenance (NE_M; MJ/day) was calculated as $BW^{0.75} \times 0.08 \times 4.184$ (NRC, 2001). Net energy required for lactation (NE_L; MJ/day) was calculated as milk yield (kg) $\times$ ((0.00929 $\times$ fat) + (0.00563 $\times$ protein) + (0.00395 $\times$ lactose)) (g/kg) $\times$ 4.184 (NRC, 2001). Energy balance was calculated as $NE_I - (NE_M + NE_L)$ and expressed in MJ/day.

Milk yield data collected on the first 3 days of lactation was not included to avoid sampling colostrum, and corresponding DM intake were also excluded. Data were summarized by period for BW and blood metabolites, and for the overall experiment. The data were then analyzed using the mixed model procedure of SAS (2000) as a completely randomized block experiment with treatment as a fixed effect, cows within treatment and block as random effects. Data for DM intake, milk yield and milk composition were averaged weekly and analyzed using the same model, but with week considered as a repeated measure using compound symmetry as the variance–covariance error structure. Specific pre-planned contrasts were used to assess the effects of PG (0 *v.* all levels of PG), and to determine the linear or quadratic response to increasing PG dose. Treatment effects were declared significant at $P < 0.05$, and trends were discussed at $0.05 < P < 0.10$, unless otherwise stated.

Results

Intake, milk yield and milk components

Dry matter intake was not affected by PG supplementation, but intake of NE linearly increased ($P = 0.04$) with increasing PG supplementation (Table 2). Milk yield (actual and 4% fat-corrected milk) and yields of milk components were similar among the treatments. Whereas milk fat content tended ($P = 0.09$) to be lower with PG supplementation (3.27%; average of three PG dosages) than with control diet (3.38%). Milk protein content tended ($P = 0.09$) to linearly decrease

Table 2 *Effects of propylene glycol supplementation on dry matter intake, milk yield and milk components in early lactating dairy cows*

	Supplementation of PG (ml/day)					Contrast[†] (*P*)	
Item	0	150	300	450	s.e.	0 *v.* PG	Linear
Intake							
DM (kg/day)	16.4	16.4	16.3	16.3	0.68	0.31	0.14
NE (MJ/day)	111.3	113.2	115.2	117.2	0.89	0.08	0.02
Milk production[‡] (kg/day)							
Actual	26.0	26.1	26.4	26.4	1.35	0.24	0.13
4% FCM	23.6	23.5	23.5	23.3	1.24	0.41	0.32
Milk fat	0.88	0.87	0.86	0.85	0.04	0.14	0.16
Milk protein	0.84	0.84	0.84	0.84	0.03	0.38	0.35
Milk lactose	1.19	1.20	1.22	1.23	0.04	0.21	0.14
Milk composition (%)							
Milk fat	3.38	3.33	3.26	3.22	0.12	0.09	0.15
Milk protein	3.22	3.21	3.20	3.20	0.05	0.12	0.09
Milk lactose	4.58	4.61	4.64	4.65	0.05	0.24	0.16
Feed efficiency							
Milk/DM intake	1.59	1.59	1.62	1.62	0.10	0.11	0.09
FCM/DM intake	1.44	1.43	1.44	1.43	0.09	0.15	0.17

PG = propylene glycol; DM = dry matter; NE = net energy; FCM = fat-corrected milk.
[†]Quadratic effect was not significant ($P > 0.10$).
[‡]As milk yield from 0 to 3 days in milk (DIM) was excluded to avoid sampling of colostrum, DM intake from 0 to 3 DIM were not included.

Table 3 *Effects of propylene glycol supplementation on body weight changes and energy balance in early lactating dairy cows*

	Supplementation of PG (ml/day)					Contrast[†] (*P*)	
Item	0	150	300	450	s.e.	0 *v.* PG	Linear
BW (kg)							
Parturition	617	618	617	616	14.3	0.84	0.58
21 DIM	582	584	588	588	12.5	0.51	0.25
42 DIM	585	587	591	592	13.1	0.23	0.08
63 DIM	589	592	599	602	13.4	0.09	0.10
BW change[‡] (kg)							
DIM 1 to 21	−35.8	−34.1	−29.7	−27.9	5.23	0.08	0.10
DIM 22 to 42	2.9	3.1	3.6	3.9	0.38	0.16	0.09
DIM 43 to 63	4.3	5.1	7.6	10.3	1.12	0.06	0.07
Overall	−9.53	−8.63	−6.17	−4.57	1.24	0.09	0.08
Energy balance[§] (MJ/day)							
DIM 1 to 21	−13.9	−11.9	−9.7	−7.6	1.73	0.03	<0.01
DIM 22 to 42	−5.9	−3.8	−2.6	−0.2	1.25	0.09	0.04
DIM 43 to 63	0.1	1.6	2.9	4.8	0.67	0.09	0.02
Overall	−6.6	−4.7	−3.1	−0.9	1.29	0.08	0.03

PG = propylene glycol; DIM = days in milk.
[†]Interaction between treatments and time was not significant ($P > 0.05$) for overall variable. Quadratic effect was not significant ($P > 0.10$).
[‡]BW change was calculated as the difference during 3-week period.
[§]Energy balance was expressed as differences between energy input NE_I (net energy intake) and output $NE_M + NE_L$ (net energy required for maintenance and lactation).

with increasing PG supplementation with no differences in milk lactose content. Milk efficiency (kg of milk/kg DM intake) tended ($P < 0.09$) to be linearly improved with increasing PG supplementation.

Body weights, changes in body weights and energy balance
Cows had approximately 5% to 6% BW loss during the 21 DIM, and then slightly gained at 42 and 63 DIM, respectively, compared with the previous weighing day (Table 3). Consequently, BW changes were considerably negative during the period of 21 DIM, but greatly improved overall due to positive BW changes during period of 22 to 42 DIM or 43 to 63 DIM. BW was similar at the parturition and at 21 DIM among the treatments, whereas it tended to linearly increase at the 42 ($P = 0.08$) and the 63 DIM ($P = 0.10$) with increasing PG supplementation. In consequence, the BW changes were consistently improved ($P < 0.10$) overall and each period with PG supplementation.

Table 4 *Effects of propylene glycol supplementation on plasma metabolites and urine ketone in dairy cows*

	Supplementation of PG (ml/day)					Contrast† (*P*)		
Item	0	150	300	450	s.e.	0 *v.* PG	Linear	Quadratic
Glucose (mg/dl)								
7 DIM	50.8	53.5	55.1	55.7	1.35	<0.01	<0.01	0.01
14 DIM	55.9	57.8	60.7	62.4	2.12	<0.01	<0.01	0.64
21 DIM	60.1	62.4	63.9	64.5	2.43	<0.01	<0.01	0.02
Overall	55.6	57.9	59.9	60.9	2.07	<0.01	<0.01	0.28
Insulin (μIU/ml)								
7 DIM	5.29	5.55	5.67	5.72	0.44	<0.01	<0.01	0.26
14 DIM	6.76	6.77	6.88	6.94	0.53	<0.01	<0.01	0.37
21 DIM	6.84	7.09	7.20	7.21	0.78	<0.01	<0.01	0.43
Overall	6.30	6.47	6.58	6.62	0.56	<0.01	<0.01	0.35
NEFA (μEq/l)								
7 DIM	455.9	443.7	434.8	431.8	24.12	<0.01	<0.01	0.19
14 DIM	348.8	343.9	337.9	331.4	21.35	<0.01	<0.01	0.65
21 DIM	257.9	250.0	246.2	241.9	18.54	<0.01	<0.01	0.31
Overall	354.2	345.9	339.6	335.0	20.67	<0.01	<0.01	0.26
BHBA (μmol/l)								
7 DIM	894.0	887.9	869.2	852.8	64.35	<0.01	<0.01	0.35
14 DIM	824.9	806.2	787.9	765.7	59.23	<0.01	<0.01	0.29
21 DIM	737.8	722.8	713.9	706.8	61.41	<0.01	<0.01	0.46
Overall	818.9	805.7	790.3	775.1	62.57	<0.01	<0.01	0.52
Urine acetoacetate (mg/dl)								
7 DIM	16.1	14.4	12.9	12.0	1.22	<0.01	<0.01	0.01
14 DIM	15.3	12.8	10.9	9.0	1.53	<0.01	<0.01	0.01
21 DIM	14.5	11.1	7.8	5.9	2.16	<0.01	<0.01	0.12
Overall	15.3	12.7	10.5	8.9	1.62	<0.01	<0.01	0.01

PG = propylene glycol; DIM = days in milk; NEFA = non-esterified fatty acids; BHBA = beta-hydroxybutyrate.
†Interaction between treatments and time was not significant ($P > 0.05$) for overall variable.

Energy balance was negative during the first 42 DIM and overall experimental period (Table 3). Increasing PG supplementation linearly improved energy balance from calving till 63 DIM.

Blood metabolic profiles

Concentrations of glucose in plasma and insulin in serum linearly ($P < 0.01$) increased with increasing PG supplementation on day 7, 14 and 21 *postpartum* (Table 4). Cows supplemented with PG (average of three PG levels) had overall 6% higher blood glucose relative to control cows. In contrast, blood concentrations of NEFA and BHBA linearly ($P < 0.01$) decreased with increasing PG supplementation on day 7, 14 and 21. Overall, supplementation of PG in the dairy cow diets reduced blood NEFA by 3.9% (average of the three PG groups) and BHBA by 3.4% (average of the three PG groups) compared with control. Similarly, concentrations of urine acetoacetate linearly decreased ($P < 0.01$) with increasing PG supplementation. Supplementation of PG reduced urine acetoacetate (average of the three PG groups) by 17%, 31% and 42% at day 7, 14 and 21, respectively.

Discussion

Increasing supplementation of PG in the dairy cow diets did not affect DM intake and milk production, whereas linearly increased intake of NE due to addition of PG, and numerically reduced the contents of milk fat and protein with increasing PG supplementation. Feeding PG particularly reduced BW loss and improved energy balance during the first 21 DIM. In addition, supplementation of PG linearly increased concentrations of glucose and decreased concentrations of NEFA and BHBA in plasma for the first 3 weeks of lactation.

Dry matter intake

Inconsistency of NE intake with DM intake due to PG supplementation was resulted from adding PG in the estimation of dietary NE. Thus, energy intake would be similar if the PG portion was removed from the calculation. Lack of the effect of PG supplementation on DM intake in this study is consistent with some results (Pickett *et al.*, 2003; Rukkwamsuk *et al.*, 2005; Moallem *et al.*, 2007), but contrasts to other report (Miyoshi *et al.*, 2001). The last researchers reported decreased feed intake after 2 days of top-dressing 518 g PG per day and suggested that the decrease of DM intake was due to the low palatability of PG. We suggest that the similar DM intake among the treatments in the present study could be due to an opposite effect that, on one hand, high energy of PG has potential to increase DM intake, and on the other hand, PG is an unpalatable additive. Furthermore, Nielsen and Ingvartsen (2004) explained in a

review article that lack of PG stimulation on DM intake of early lactating cows fed up to 518 g of PG per day was due to the dosage of PG used that did not increase the energy density of the feed sufficiently to induce an increase in DM intake. In addition, feed intake in early lactating cows can be influenced by metabolic factors such as the increase in insulin triggered by PG may stimulate negative feedback signals that reduce DM intake (Ingvartsen and Andersen, 2000). However, this effect was not observed in the present study even though blood insulin concentration linearly increased with increasing PG supplementation, indicating that DM intake could be affected by multifactors.

Milk yield and milk components

Even though intake of energy linearly increased with increasing supplementation of PG, there was no effect of PG supplementation on milk yield. The results are consistent with the similar DM intake among treatments in this study. Increasing energy supply may not be necessary to increase milk production but reduce BW loss (Table 3). The mechanism by which the energy from PG supplementation contributed to the BW deposit rather than milk production is not clear. Nevertheless, fail to increase milk production with increasing PG supplementation is in agreement with previous studies (Formigoni *et al*., 1996; Miyoshi *et al*., 2001; Pickett *et al*., 2003; Rukkwamsuk *et al*., 2005). In contrast, Miettinen (1995) reported higher milk yield on the second test day in the treated group than in the control group. Stokes and Goff (2001) reported that milk yield of cows on a commercial dairy farm was increased by administration of PG on the first 2 days *postpartum*. Nevertheless, these studies only monitored milk production for a very short period in lactation (ranged from 2 to 25 DIM).

Numerical reduction of milk fat content with PG supplementation is in agreement with the most published work as reviewed by Nielsen and Ingvartsen (2004). Those authors suggested that reduced milk fat content could be due to, first, the decrease in plasma NEFA since lowered NEFA concentrations lead to decreased NEFA-uptake by the mammary gland (Emery and Herdt, 1991; Nielsen and Riis, 1993); and second, PG could lower proportion of acetate in the rumen (Grummer *et al*., 1994; Shingfield *et al*., 2002) which may reduce the amount of acetate available for *de novo* fatty acid synthesis in the mammary gland. The linear reduction of plasma NEFA with increasing PG supplementation is consistent with numerical ($P < 0.15$) linear decrease of milk fat content in the present study. The trends towards linearly decreased milk protein content with increasing PG supplementation, although the decrease was biologically subtle, is not expected, due to the assumption that supplementation of PG would decrease amino acid requirements for gluconeogenesis, and thus there would not be a shortage of the spared amino acids for the milk protein synthesis. Furthermore, an increase in energy content of the feed by adding PG would stimulate an increase in milk protein percentage (Sutton, 1989). However, this assumption is not supported by the present results as well as the published results as reviewed by Nielsen and Ingvartsen (2004). It suggests that the relatively small doses of PG have not been able to increase the energy content of the diet sufficiently and/or there has not been a shortage of the spared amino acids for the milk protein synthesis. Thus, it has been argued that reductions in the catabolism of glutamine, glutamate and aspartate will not directly increase the supply of limiting amino acids (Reynolds *et al*., 1997).

Body weight changes and energy balance

Cows fed PG tended to increase BW at a higher rate relative to those fed the control diet. This response is similar to Formigoni *et al*. (1996) who reported that cows in early lactation supplied with PG tended to have less body condition lose than control cows. However, it contrasts to other reports (Studer *et al*., 1993; Pickett *et al*., 2003; Moallem *et al*., 2007) that no effect of PG supplementation on BW or body condition score was found. The reduced BW loss during the first 21 DIM was paralleled by lower concentrations of plasma NEFA for cows supplemented with PG.

The improvement of energy balance due to PG supplementation in this study is consistent with the report of Miyoshi *et al*. (2001) and Butler *et al*. (2006). Miyoshi *et al*. (2001) found a tendency towards a higher energy balance in multiparous cows drenched with 518 g PG daily from 7 to 42 DIM compared to control cows. Butler *et al*. (2006) reported that energy balance was improved with adding 500 ml of PG (2 Mcal) to the dairy cow diet. However, Moallem *et al*. (2007) reported no differences in energy balance due to PG supplementation. The difference between the present study and that of Moallem *et al*. (2007) might be due to the quality or dose of the PG product used, or the ratio of concentrate to forage and the quality of forage in the diets. Perhaps the glucogenic potential of PG may be most effective during early lactation. Linearly improved energy balance and reduced BW loss with increasing PG supplementation suggest that the PG supplemented in dairy cow diet could be efficiently used for improving energy balance and minimizing body mobilization in early lactating cows without reducing milk production under the current feeding conditions. Furthermore, failure to observe the quadratic effect on BW change and energy balance indicates that there may be potential to improve energy balance with higher dosage of PG supplementation than that used in the current study.

Metabolites

The linear increase in plasma glucose due to increasing PG supplementation demonstrates the glucogenic capacity of this compound as reported previously (Studer *et al*., 1993; Grummer *et al*., 1994). The present results are consistent with previous studies (Grummer *et al*., 1994; Butler *et al*., 2006; Kristensen and Raun, 2007), but contrast to other findings. Moallem *et al*. (2007) reported no differences in plasma glucose, and insulin concentrations between the control and PG groups. Concentrations of NEFA and BHBA

in plasma concur with the relatively minimal mobilization of body fat, as indicated by less reduction in BW by PG supplementation in the present study. Canfield and Butler (1991) reported that plasma NEFA correlate negatively with energy balance in early lactating cows and NEFA can be used as an indicator of energy balance and body mobilization. This finding is confirmed with the present results of linearly reduced plasma NEFA and linearly improved energy balance as well as increasing BW gain with increasing PG. The linear decrease in plasma concentrations of NEFA or BHBA with increasing PG is consistent with linear reduction of BW loss and linear improvement of energy balance. These results are also in agreement with other studies (Grummer *et al*., 1994; Butler *et al*., 2006; Kristensen and Raun, 2007). Butler *et al*. (2006) observed that NEFA was decreased in response to a PG drench *postpartum*. Kristensen and Raun (2007) reported that infusion of 650 g PG into rumen decreased the plasma concentrations of BHBA. However, Moallem *et al*. (2007) found no differences in plasma NEFA, and BHBA concentrations between the control and PG groups. Those researchers suggested that the lack of effect of PG on blood metabolites might have been caused by the long interval between PG administration and blood sampling (15 h). Nielsen and Ingvartsen (2004) reported that the effect of PG on plasma glucose and insulin occurs shortly after consumption, and the time of blood sampling is a crucial factor in determining the effects of PG. Linearly reduced urine acetoacetate might be due to linearly decreased concentration of plasma BHBA and that of plasma NEFA with increasing PG supplementation. The linear positive effects but with lack of quadratic effect of increasing PG on blood metabolites indicates the potential benefits to further increase the dosage of PG in the present experimental conditions.

Conclusion

Supplementation of multiparous Holstein dairy cow diets with up to daily 450 ml of PG did not affect DM intake and milk production with only a subtle decrease of milk fat and milk protein contents during the first 63 days of lactation. However, increasing supplementation of PG reduced BW loss and improved energy status of early lactating cows. The present results indicate that the PG can be used as an effective glucogenic precursor to improve the metabolic status of transition dairy cows. The PG supplementation in dairy cow diet was beneficial to improve the energy status and blood metabolites, and the potential benefits to further increase the dosage of PG over 450 ml per day in the present experimental conditions were suggested.

Acknowledgements

This work was supported by a grant from 11th Five-Year key projects of National Scientific Research Foundation of China (2007BAD56B04). The authors thank the staff of Shanxi Agriculture University dairy cow unit for care of the animals.

References

Association of Official Analytical Chemists 1990. Official methods of analysis, 14th edition. Association of Official Analytical Chemists, Int., Arlington, VA, USA.

Association of Official Analytical Chemists 1997. Official methods of analysis, 16th edition. Association of Official Analytical Chemists, Int., Gaithersburg, MD, USA.

Bertics SJ, Grummer RR, Cadorniga-Valino C and Stoddard EE 1992. Effect of prepartum dry matter intake on liver triglyceride concentration in early lactation. Journal of Dairy Science 75, 1914–1922.

Burhans WS, Briggs EA, Rathmacher JA and Bell AW 1997. Glucogenic supplementation does not reduce body tissue protein degradation in periparturient dairy cows. Journal of Dairy Science 80 (suppl. 1), 167(abstract).

Butler ST, Pelton SH and Butler WR 2006. Energy balance, metabolic status, and the first postpartum ovarian follicle wave in cows administered propylene glycol. Journal of Dairy Science 89, 2938–2951.

Canfield RW and Butler WR 1991. Energy balance, first ovulation and the effects of naloxone on LH secretion in early postpartum dairy cows. Journal of Animal Science 69, 740–746.

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

Chung YH, Rico DE, Martinez CM, Cassidy TW, Noirot V, Ames A and Varga GA 2007. Effects of feeding dry glycerin to early postpartum Holstein dairy cows on lactational performance and metabolic profiles. Journal of Dairy Science 90, 5682–5691.

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

Emery RS and Herdt TH 1991. Lipid nutrition. The Veterinary Clinics of North America. Food Animal Practice 7, 341–352.

Feng YL, Mo F and Lu ZN 2000. Nutrient requirements of dairy cattle (in Chinese), pp. 14–15. Chinese Agriculture University Press, Beijing, China.

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

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

Herdt TH 1988. Fatty liver in dairy cows. The Veterinary Clinics of North America. Food Animal Practice 4, 269–287.

Hobbs CS, Hansard SL and Barrick ER 1950. Simplified methods and equipment used in separation of urine from feces eliminated by heifers and by steers. Journal of Animal Science 9, 565–570.

Ingvartsen KL and Andersen JB 2000. Integration of metabolism and intake regulation: a review focusing on periparturient animals. Journal of Dairy Science 83, 1573–1597.

Johnson RB 1954. The treatment of ketosis with glycerol and propylene glycol. The Cornell Veterinarian 44, 6–21.

Johnson MM and Peters JP 1993. Technical note: an improved method to quantify nonesterified fatty acids in bovine plasma. Journal of Animal Science 71, 753–756.

Kim YK, Choi H and Myung KH 2005. Effects of propylene glycol on carcass traits and its related gene expression in Korean native steers. Journal of Animal Science 83, 344–349.

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

Liu Q, Wang C, Huang YX, Dong KH, Yang WZ and Wang H 2008. Effects of lanthanum on rumen fermentation, urinary excretion of purine derivatives and digestibility in steers. Animal Feed Science and Technology 142, 121–132.

Maplesden DC 1954. Propylene glycol in the treatment of ketosis. Canadian Journal of Comparative Medicine and Veterinary Science 18, 287–293.

McClanahan S, Hunter J, Murphy M and Valberg S 1998. Propylene glycol toxicosis in a mare. Veterinary and Human Toxicology 40, 294–296.

Miettinen PVA 1995. Prevention of bovine ketosis with glucogenic substances and its effects on fertility in Finnish dairy cows. Berliner und Munchener Tierarztliche Wochenschrift 108, 14–19.

Miyoshi S, Pate JL and Palmquist DL 2001. Effects of propylene glycol drenching on energy balance, plasma glucose, plasma insulin, ovarian function and conception in dairy cows. Animal Reproduction Science 68, 29–43.

Moallem U, Katz M, Lehrer H, Livshitz L and Yakoby S 2007. Role of peripartum dietary propylene glycol or protected fats on metabolism and early postpartum ovarian follicles. Journal of Dairy Science 90, 1243–1254.

Nielsen NI and Ingvartsen KL 2004. Propylene glycol for dairy cows: a review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis. Animal Feed Science and Technology 115, 191–213.

Nielsen MO and Riis PM 1993. Somatotropin. Acta Veterinaria Scandinavica 89 (suppl.), 47–54.

NRC 2001. Nutrient requirements of dairy cattle, 7th revised edition. National Academy of Sciences, Washington, DC, USA.

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

Reynolds CK, Sutton JD and Beever DE 1997. Effects of feeding starch to dairy cattle on nutrient availability and production. In Recent advances in animal nutrition (ed. PC Garnsworthy and J Wiseman), pp. 104–134. Nottingham University Press, Nottingham, UK.

Rukkwamsuk T, Rungruangb S, Choothesaa A and Wensing T 2005. Effect of propylene glycol on fatty liver development and hepatic fructose 1,6 bisphosphatase activity in periparturient dairy cows. Livestock Production Science 95, 95–102.

SAS 2000. SAS User's Guide: Statistics, Version. 8.1 Edition. SAS Institute, Inc., Cary, NC, USA.

Shingfield KJ, Jaakkola S and Huhtanen P 2002. Effect of forage conservation method, concentrate level and propylene glycol on diet digestibility, rumen fermentation, blood metabolite concentrations and nutrient utilization of dairy cows. Animal Feed Science and Technology 97, 1–21.

Stokes SR and Goff JP 2001. Case study: evaluation of calcium propionate and propylene glycol administered into the esophagus at calving. The Professional Animal Scientist 17, 115–122.

Studer VA, Grummer RR and Bertics SJ 1993. Effect of prepartum propylene glycol administration on periparturient fatty liver in dairy cows. Journal of Dairy Science 76, 2931–2939.

Sutton JD 1989. Altering milk composition by feeding. Journal of Dairy Science 72, 2801–2814.

Trinder P 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry 6, 24–27.

Van Knegsel ATM, Van Den Brand H, Dijkstra J, Van Straalen WM, Jorritsma R, Tamminga S and Kemp B 2007. Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. Journal of Dairy Science 90, 3397–3409.

Van Soest PJ, Robertson JB and Lewis BA 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583–3597.

Williamson DH, Mellanby J and Krebs HA 1962. Enzymic determination of D(–)-β-hydroxybutyric acid and acetoacetic acid in blood. The Biochemical Journal 82, 90–96.