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RESEARCH ARTICLE

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Effects of feeding dry glycerol on milk production, nutrients digestibility and blood components in primiparous Holstein dairy cows during the early postpartum period

Farokh Kafilzadeh, Vahid Piri, and Hamed Karami-Shabankareh
Razi University, Faculty of Agriculture, Department of Animal Science. Kermanshah, 6719685416, Iran

Abstract

The aim of this study was to evaluate the glucogenic property of glycerol supplementation in the dairy cow's diet. Sixty primiparous cows (control, n=30, and glycerol supplemented, n=30) were used to measure milk yield and components, blood hormone and metabolite profiles, and body condition score. Feed intake and apparent total-tract digestibility were also measured using 10 primiparous cows (control, n=5, and glycerol supplemented, n=5). Dry glycerol was top dressed at 250 g/day/cow from parturition to 21 days postpartum. Average feed intake, milk yield and components were not affected by glycerol supplementation. Apparent total–tract digestibility of organic matter and neutral detergent fibre were not influenced by dry glycerol supplementation, but lipid digestibility was greater (p=0.01) in cows fed glycerol. The serum concentration of glucose and insulin tended to be higher in dry glycerol-supplemented cows (p=0.1; p=0.06, respectively). While, serum concentrations of nonesterified fatty acids and β-hydroxybutyrate were not affected. Supplemented cows had lower body condition loss during weeks 1 to 5 after calving (p=0.09). The glucogenic effect of glycerol did not affect milk yield during the first 3 weeks of lactation. However, daily milk yield during the 13 weeks recording period was higher in the glycerol-supplemented cows (28.5 *vs*. 30.3 kg, p<0.001). Percentages of cows cycling at the planned breeding date was greater (p=0.01) for cows fed dry glycerol. The results demonstrated that feeding dry glycerol as a glucogenic supply could be useful in saving body reserves and improving energy balance of primiparous Holstein dairy cows during the early postpartum period.

Additional key words: milk production; feed intake; apparent total–tract digestibility; body condition score; blood metabolites.

Abbreviations used: ADF (acid detergent fibre); BCS (body condition score); BHBA (β-hydroxybutyrate); BW (body weight); CP (crude protein); DIM (days in milk); DM (dry matter); DMI (dry matter intake); FCM (fat corrected milk); MUN (milk urea nitrogen); NDF (neutral detergent fibre); NEFA (none esterified fatty acid); OM (organic matter); TMR (total mixed ration).

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Correspondence should be addressed to Farokh Kafilzadeh: kafilzadeh@razi.ac.ir

Introduction

Higher prices of corn and other feedstuffs, and the reduction of the availability of corn for animal feed due to increase in biofuel production have resulted in the search for alternate feed sources for livestock producers (Abo El-Nor *et al*., 2010). At the same time, the rapid growth of biodiesel produduction is expected to increase availability and promote favorable pricing of glycerol which is the main by-product of this industry (Thompson & He, 2006). Glycerol is a carbohydrate molecule ($C_3H_8O_3$) with a net energy concentration of 1.98–2.29 Mcal/kg which is approximately equal to the energy contained in corn starch (Schroder & Südekum, 1999). Glycerol can be converted to glucose to obtain energy for cellular metabolism by the liver (Krebs *et al*., 1966) and kidneys (Krebs & Lund, 1966).

Glycerol was used for treating ketosis in dairy cows for the first time by Johnson (1954). Evaluation of glycerol as a ketosis treatment was further explored in the 1970s (Fisher *et al*., 1971, 1973). Glycerol has also been used as a supplement or feed ingredient (Chung *et al*., 2007; Donkin *et al*., 2009) to improve the energy status of animals. DeFrain *et al*. (2004) and Boyd *et al*. (2013) fed crude glycerol and found a reduction in dry matter intake (DMI) of periparturient and early lactation dairy cows. Using pure source of glycerol resulted in no change in DMI (Chung *et al.*, 2007; Donkin *et al.*, 2009; Lomander *et al.,* 2012). However, Kass *et al.* (2012) reported an increase in intake by increasing crude glycerol levels.

Similar contradictory results have been observed in performance when glycerol has been fed either as a supplement or a feed ingredient. Working with multiparous cows (Chung *et al.*, 2007; Donkin *et al.*, 2009; Wilbert *et al.*, 2013) found no effect on milk yield, while Lomander *et al.* (2012) reported an increase in milk production.

Other than the conflicting results on DMI and performance of cows which could be due to the purity and the level of the glycerol used and/or the severity of negative energy balance in the experimental animals, all the studies with the exception of Kass *et al.* (2012) who used only eight primiparous cannulated cows and fed crude glycerol, have been carried out on multiparous dairy cows. First-lactation cows have lower energy balance because they eat less and have energy requirements for growth in addition to lactation (Lucy *et al.*, 2001).

Therefore, the objective of the present experiment was to analyze the effects of feeding a pure source of dry glycerol as an energy supplement on milk production and components, feed intake, nutrient digestibility, body condition score (BCS), body weight (BW), and serum metabolites and hormone concentrations of primiparous Holstein dairy cows that were calved with high demand to energy during the early postpartum period.

Material and methods

Farm and the animals

This study was carried out at the Kangavar dairy farm, Kermanshah, Iran, housing approximately 3000 black and white Holstein Friesian lactating cows. The research protocol was approved by Razi University Animal Care and Use Committee.

At the beginning of the experiment, 120 Holstein Friesian heifers (in their 1–2 months of parturition) were selected. After parturition 70 cows with the same BCS, same age and no post-partum problems such as retained placenta, metritis and metabolic disorders were selected. From these, 60 cows were randomly divided into two groups (control; n=30 and glycerol supplemented; n=30) and were moved from their maternity pen into a separate free-stall barn from the other cows. The remaining 10 cows, were also randomly divided into two groups (control; n=5 and glycerol supplemented; n=5) and were moved to individual stalls for measurement of DMI and apparent total-tract digestibility. Cows used were similar in age at first parturition (756±28 days).

Experimental design and treatments

This study was conducted as a completely randomized design. Cows were milked three times daily at approximately 06:30, 14:30 and 22:30 hours. From parturition to 21 days in milk (DIM) they received a common total mixed ration (TMR) (Table 1) once daily after the morning milking, which was either supplemented (glycerol treatment) or not supplemented (control treatment) with 250 g/day/cow of a dry glycerol product (Phoderush, Phodé S.A., Albi Terssac, France;

Table 1. Ingredient composition of basal total mixed ration (TMR) offered from parturition through 21 days postpartum

Ingredient % of diet DM	Mean
Corn silage	40.40
Barley grain	13.63
Sugar beet pulp	13.13
Alfalfa hay	12.17
Soybean meal	5.15
Cottonseed hulls	4.04
Wheat bran	2.71
Cottonseed meal	2.27
Corn grain	2.27
Canola meal	2.27
Fish meal	0.66
Calcium carbonate	0.36
Zeolite	0.36
Mineral and vitamin premix[1]	0.33
Salt	0.22
Biotin	0.02
Monensin	0.01
Chemical composition[2]	
DM, %	55.63 (3.02)
CP, % of DM	17.90 (0.77)
ADF, % of DM	24.01 (1.29)
NDF, % of DM	34.27 (2.78)
NEL[3], Mcal/kg	1.64
Ca, % of DM	1.09 (0.07)
P, % of DM	0.38 (0.03)
Mg, % of DM	0.43 (0.04)
K, % of DM	1.48 (0.06)
Na, % of DM	0.29 (0.03)

[1]Premix contained 0.273% of sulfur; 4.3 ppm of cobalt; 19.8 ppm of vitamin A; 420 ppb vitamin D; and 852.36 ppm vitamin E. [2]Mean analysis for composite samples (n = 2) and associated standard deviations (in parentheses). [3]NEL: net energy, lactation; estimation based on NRC (2001). DM: dry matter, CP: crude protein, ADF: acid detergent fibre, NDF: neutral detergent fibre

net energy for lactation (NEL = 2.6 Mcal/kg of DM). The product contained 33% ash, <2% glycerol fatty acid esters, <2% water, negligible amounts of salt and methanol, and flavoring substances other than glycerol and was stabilized on a dry mineral carrier providing 65% food grade glycerol (corresponding to 162.5 g of food grade glycerol/day/cow). After top dressing, the cows were locked for a short period of time to consume their allotment at once. The TMR was formulated based on the NRC (2001) guidelines for milking primiparous Holstein dairy cows at 500 kg of BW and producing 30 kg of milk/day with 3.7% of milk fat. Dry glycerol was used as a glucogenic supplement to provide additional glucogenic substrates in the early postpartum period; therefore, no adjustment in the energy concentration was made in the diet of the control group to represent the feeding condition in the field.

Sample collection and measurements

Calf birth weight was recorded, and calving difficulties were evaluated based on a 5-point scale, 1 = no assistance and 5 = caesarean (National Association of Animal Breeders, NAAB; Columbia, MO, USA). The amount of feed offered was adjusted each day to achieve 7 to 10% refusals. To measure feed intake in cows housed in individual stalls the amount of feed offered and refused was recorded daily. DMIs were calculated based on daily intake of TMR and the dry matter (DM) content of the feed. Samples of the TMR were collected weekly during the experimental period. Fecal samples were collected from cows kept in individual stalls after a 2-weeks acclimation period to the stall environment and ration. Fecal samples were collected once per day during the last 4 days of the feeding period and sampling was shifted 6 hours in time during the 4-days collection schedule. Samples of diet were collected for 4 days before initiation of fecal collection. Fecal and TMR samples were placed in plastic bags, duly identified, and stored in a freezer (−8°C).

Milk from individual cows was sampled once per week from three consecutive milkings for the first 3 weeks of lactation. Samples were placed in tubes, refrigerated, and submitted to the laboratory. Individual milk yield was automatically recorded daily from parturition up to 13 weeks postpartum.

BCS was evaluated based on a 5-point scale (Wildman *et al*., 1982) from parturition to 35 days postpartum. All the weekly BCS evaluations were made by the same technicians. BW was recorded 3 times on days 1, 11, and 21 during the experimental period. Estrous was detected by observation of estrous behavior for five 30-min periods daily by a veterinary technician.

The blood samples were collected approximately 2 hours before the morning feeding on 1, 7, 14, and 21 day after parturition. Blood samples were collected via vacuum tube (10 mL) from the coccygeal vein and were transported immediately on ice to the laboratory after sampling. For serum collection, the tubes were centrifuged at room temperature at 1000 × g for 15 min to separate serum. Serum samples were stored frozen at -20°C until further analyses.

Laboratory analysis

Chemical analyses were performed at the Animal Nutrition Laboratory and the biochemical analyses in the Veterinary Clinical Analyses Laboratory. At the end of the collection period fecal samples were thawed and an aliquot of each sample in the collection shift was taken and homogenized to obtain a sample pooled by animal and treatment. The DM content of the samples was determined by drying in a forced-air oven at 55°C for 48 h. Fecal and TMR samples were ground to pass through a 1-mm screen. Weekly samples of TMR were analyzed for DM, crude protein (CP), acid detergent fiber (ADF), and minerals (Ca, P, Mg, K and Na) by wet chemistry following AOAC (2000) procedures and for neutral detergent fiber (NDF) following the method of Goering & Van Soest (1970). Nutrient composition of the basal TMR is reported in Table 1.

Daily TMR samples as well as daily fecal samples taken during digestibility trial from each cow were pooled and were analyzed for acid detergent insoluble ash as described by Van Keulen & Young (1977). These samples were also analyzed for organic matter (OM), nitrogen (N) and lipids by wet chemistry following AOAC (2000) procedures, and also for NDF by the method of Goering & Van Soest (1970).

Milk samples were analyzed for fat, true protein, lactose, solid not fat (SNF), somatic cells, and milk urea nitrogen (MUN) by using infrared spectrophotometry (Fossomatic 4000 Milko-Scan, Foss Electric, Hillerød, Denmark) according to AOAC (1997) procedures.

The serum concentration of glucose was determined using glucose oxidase (Pars Azmoon, Tehran, Iran). Serum β-hydroxybutyrate (BHBA) and nonesterified fatty acid (NEFA) concentrations were measured using D-3-hydroxybutyrate and NEFA kits (Mabna Laboratories, Tehran, Iran). The minimum detectable concentration of D-3-hydroxybutyrate and NEFA was determined as 0.1 mmol/L and the accuracy of each assay was verified using a commercial reference. The serum samples were analyzed for concentration of insulin by ELISA (Lise-Meltner Strallo Kit; DRG Instruments, Marburg, Germany); the intra- and inter assay coefficients of variation were 2 and 4%, respectively. The detection limit of the assay was 0.89 μIU/mL.

Calculations and statistical analysis

Change in BW was calculated as the difference between the averages BW of first day in milk DIM to the average BW of 21 DIM for each group. Change in BCS was calculated as the difference between the average BCS at week 1 to the average BCS at week 5 of lactation for each group. Milk yield for the first 3 days of lactation was excluded to avoid sampling of colostrums. Fat-corrected milk (FCM) was estimated according to Gaines (1928):

$$\text{FCM (kg/day)} = [0.4 \times \text{milk production (kg/day)}] + [15.0 \times (\text{fat yield (kg/day)})].$$

Data of intake of nutrients, apparent total-tract digestibility, and body weight after calving, BCS at calving, calf birth weights, and calving difficulties were analyzed using t-test procedure (SAS, 2003). Before data analysis, daily measurements for milk and DMI were condensed to weekly averages. Average weekly milk production, DMI, and milk composition, BCS, BW, concentration of blood metabolites and hormones were analyzed as repeated measure using PROC MIXED (version 9.1; SAS, 2003). The general linear mixed model was:

$$y_{ijk} = \mu + \alpha_i + b_j + (\alpha b)_{ij} + \gamma(\alpha)_{ik} + \beta_1(x_f - \overline{x}_f) + \beta_2(x_g - \overline{x}_g) + \varepsilon_{ijk}$$

where y_{ijk} is the observation on cow *k* at sampling time *j* given treatment *i* ; μ is the overall mean; α_i is the fixed effect of treatment *i* (control or glycerin); b_j is the fixed effect of sampling week *j*; $(\alpha b)_{ij}$ is the 2-way interaction of treatment *i* by sampling time *j*; $\gamma(\alpha)_{ik}$ is the random effect of cow *k* nested within treatment *i*; $\beta_1(x_f - \overline{x}_f)$ is the covariate of calf weight, and $\beta_2(x_g - \overline{x}_g)$ is the covariate of body weight after calving. The PDIFF option was used for multiple comparison tests. Statistical significance was declared at $p \leq 0.05$ and a tendency to significance was declared at $0.05 < p \leq 0.1$. The data were presented as means ± standard deviations or least squares means ± standard errors of the means.

Results

Allocation of cows, body weight after calving, BCS at calving, calf birth weight, and calving difficulties are reported in Table 2. Cows age at first parturition, body weight, BCS at calving, calf birth weight, and calving difficulties data were similar (p>0.05) in the two groups. Milk yield, milk components, and DMI are reported in Table 3. Production of milk, 4% FCM, percentage and yield of milk components were not significantly affected when dry glycerol was fed. DMI was not significantly different between two groups. The average daily milk yield (Fig. 1) during the 13 weeks recording period was 28.5 *vs*. 30.3 kg, for the control and glycerol fed groups, respectively.

Intake of OM, NDF, N, and lipids were not significantly affected by feeding dry glycerol (Table 4). Supplementation of dry glycerol had no effects on apparent total-tract digestibility of OM, NDF, and N, with the exception of lipid digestibility which was higher in dry glycerol fed cows (p = 0.01).

BW, BCS, changes in BW and BCS, serum concentrations of metabolites and serum insulin are reported in Table 5. BW and BCS decreased as lactation progressed, reflecting the mobilization of body reserves. BW was not affected with glycerol supplementation. While BCS in cows received no glycerol tended to be lower (p=0.09) compared with cows fed dry glycerol (mean ± SE: 2.65 ± 0.015 and 2.71 ± 0.018, respectively).

Table 2. Allocation, initial body weight (BW), body condition score (BCS) at calving, calf birth weight, and calving difficulty for primiparous Holstein dairy cows not supplemented or supplemented with 250 g/day/cow dry glycerol from parturition to 21 days postpartum.

	Treatment		SEM	*p*-value
	Control	Glycerol		
Cows, n	30	30	–	–
BW[1], kg	496.3	503.5	6.3	0.59
BCS[2,3]	3.04	3.00	0.020	0.43
Calf BW, kg	39.0	38.9	0.47	0.91
Calving difficulties[4]	1.6	1.8	0.12	0.49

[1] Collected after parturition. [2] Collected at parturition. [3] Wildman *et al*. (1982). [4] Five-point scale: 1= no assistance; 2 = slight problem; 3 = needed assistance; 4 = considerable force; and 5 = cesarean.

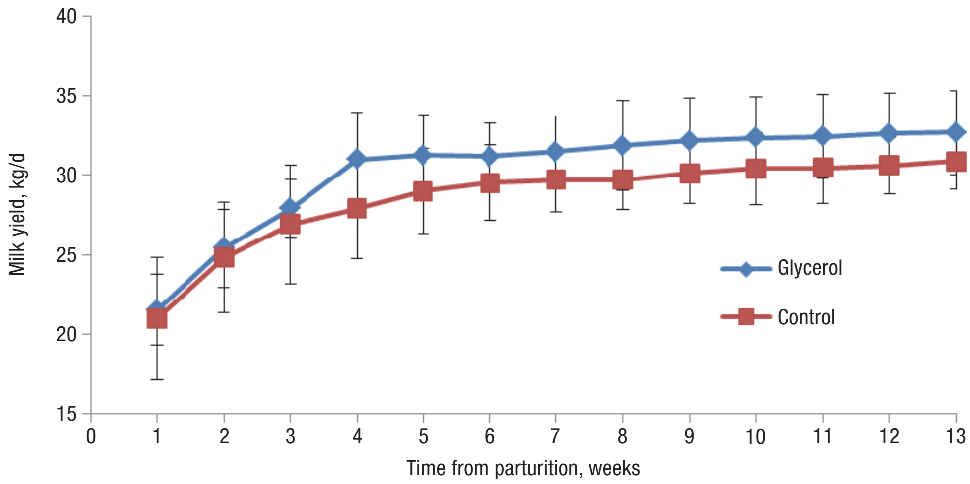

Figure 1. Milk yield during the first 13 weeks postpartum in primiparous Holstein dairy cows not supplemented (control) or supplemented with 250 g/day/cow dry glycerol (glycerol) from parturition to 21 days postpartum.

Table 3. Yield of milk and 4% FCM, and milk components for primiparous Holstein dairy cows not supplemented or supplemented with 250 g/day/cow dry glycerol from parturition to 21 days postpartum.

	Treatment		SEM	*p*-value		
	Control	Glycerol		Treatment	Week	Treatment×Week
Milk yield, kg/d	24.27	24.97	0.461	0.3	<0.001	0.8
DMI, kg/d	13.74	14.51	0.424	0.27	<0.001	0.44
4% FCM, kg/d	24	24.06	0.530	0.93	<0.001	0.99
Fat, %	3.91	3.76	0.065	0.18	0.57	0.94
Fat yield, kg/d	0.95	0.93	0.025	0.7	<0.001	0.97
Protein, %	3.33	3.38	0.049	0.48	0.21	0.93
Protein yield, kg/d	0.81	0.84	0.019	0.24	<0.001	0.98
Lactose, %	5.23	5.26	0.031	0.54	0.03	0.11
Lactose yield, kg/d	1.27	1.31	0.026	0.27	<0.001	0.53
SNF %	9.57	9.56	0.045	0.83	0.02	0.22
SNF yield, kg/d	2.32	2.38	0.047	0.39	<0.001	0.88
SCC, 1,000 cells/mL	277	357	56.8	0.32	0.03	0.08
MUN, mg/dL	14.5	14.4	0.24	0.8	0.91	0.48

DMI: dry matter intake, SNF: solid not fat, SCC: somatic cell count, MUN: milk urea nitrogen.

Table 4. Effect of feeding dry glycerol on nutrient intake and apparent total- tract digestibility of primiparous Holstein dairy cows not supplemented or supplemented with 250 g/day/cow dry glycerol from parturition to 21 days postpartum.

	Treatment		SEM	*p*-value
	Control	Glycerol		
Intake				
Organic matter, kg	12.67	12.76	0.031	0.18
Neutral detergent fiber, g	4.71	4.67	0.041	0.61
Nitrogen, g	406.95	409.68	1.113	0.25
Lipid, g	563.68	565.45	5.824	0.76
Digestibility, apparent total-tract				
Organic matter, %	78.26	78.35	0.171	0.82
Neutral detergent fiber, %	61.16	60.50	0.242	0.20
Nitrogen, %	71.62	72.09	0.254	0.38
Lipid, %	76.34[b]	79.90[a]	0.792	0.01

[a,b] Row means with different superscripts differ significantly at $p < 0.05$.

Table 5. Body weight (BW), BW change, body condition score (BCS), BCS change, concentration of serum insulin and some metabolites for primiparous Holstein dairy cows not supplemented or supplemented with 250 g/day of dry glycerol from parturition to 21 days postpartum.

	Treatment		SEM	*p*-value		
	Control	Glycerol		Treatment	Week	Treatment×Week
BW (week 1 to 3), kg	458.92	489.47	2.035	0.22	<0.001	0.23
BW change[1], kg	-28.7	-21.4	2.550	0.81	NA	NA
BCS (week 1 to 5)	2.65	2.71	0.023	0.09	<0.001	0.12
BCS change[3]	-0.41	-0.34	0.017	0.39	NA	NA
Glucose, mg/dL	58.41	59.78	0.440	0.10	0.08	0.38
Insulin, µIU/mL	6.99	8.38	0.393	0.06	0.41	0.54
NEFA, mmol/L	0.45	0.43	0.013	0.24	0.67	0.49
BHBA, mmol/L	0.51	0.46	0.017	0.13	0.68	0.38

[1] Body weight change was calculated as the difference between the averages BW on day 1 of experiment to 21 DIM. [2]Calculated as the difference between the average BCS of weeks 1 to 5 of lactation. NA: not applicable. NEFA: non esterified fatty acids, BHBA: β-hydroxybutyrate

Overall, serum concentration of NEFA and BHBA were not affected by glycerol feeding. Concentrations of glucose and insulin tended to be higher (p=0.1 and p=0.06, respectively) in glycerol supplemented cows.

Because supplemented cows ovulated earlier than the control cows (Fig. 2), the percentages of cows cycling at the planned start of mating (13 weeks) for each group (83.3 and 69.69, respectively) also differed (p=0.01) for cows fed dry glycerol compared with those in the control treatment.

Discussion

Milk yield and components were not significantly affected when dry glycerol was fed, which is in agreement with previous studies in which glycerol was fed to transition (DeFrain *et al*., 2004), early postpartum (Fisher *et al*., 1973; Chung *et al*., 2007), and mid lactation cows (Khalili *et al*., 1997; Donkin *et al*., 2009; Kass *et al*., 2012). In contrast, top dressing of rations fed to dairy cattle from 2 weeks before calving through 10 weeks of lactation with 300 and 500 mL glycerol

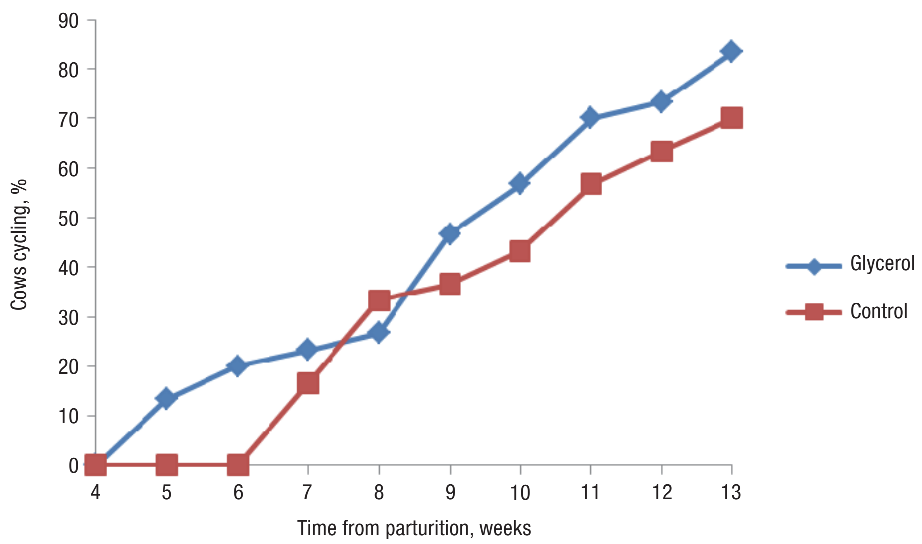

Figure 2. Percentage of cows cycling during the first 13 weeks after calving in primiparous Holstein dairy cows, not supplemented (Control) or supplemented with 250 g/day/cow dry glycerol (Glycerol).

resulted in greater milk production (Bodarski *et al*., 2005). Similarly, Lomander *et al*. (2012) reported that cows supplemented with 450 g/day glycerol had a higher milk yield during the first 90 days in milk. The glucogenic property of dry glycerol via feeding at the level used in present study (162.5 g of glycerol/day) may not have been adequate to elicit a milk yield response.

DeFrain *et al*. (2004) reported tendencies for a lower milk fat yield and MUN when 430 and 860 g/day glycerol was fed. Donkin *et al*. (2009) also reported that MUN decreased when diets containing 5, 10, and 15% refined glycerol were fed and suggested that N efficiency was better with glycerol feeding probably due to the changes in postruminal or post absorptive N metabolism. This effect of glycerol on milk fat yield and MUN, however, was not observed in the current study or in some other studies (Fisher *et al*., 1973; Ogborn, 2006; Chung *et al*., 2007).

Dry glycerol used as a top dressing in the present study had no effect on feed intake. Similar results have been reported by Khalili *et al*. (1997); Chung *et al*. (2007) and Donkin *et al*. (2009), who fed dry glycerol to multiparous Holstein dairy cows during the postpartum period. In contrast, prepartum DMI decreased but postpartum DMI was unaffected when glycerol was administered as a top dress to transition dairy cows (DeFrain *et al*., 2004).

Boyd *et al*. (2013) fed 200 g and 400 g glycerol/day and reported that DMI decreased as the amount of glycerol fed increased. However, Kass *et al*. (2012) found that intake increased when barley meal was replaced with 1 kg, 2 kg or 3 kg crude glycerol per day as an energy source in the ration of mid-lactation primiparous Holstein dairy cows. They noted that the increased feed intake could be related to several properties of glycerol that might increase its palatability, such as that it is a sweet-tasting and viscous liquid. The inconsistent effects of glycerol on feed intake may be due to the quality of the glycerol product used. Substances such as salts and methanol, which are coproduced during the synthesis of glycerol, may influence the palatability of the final glycerol product (Schroder & Südekum, 1999).

There was no difference in apparent digestibility of OM, NDF and N, between the two groups. These results are in agreement with Khalili *et al*. (1997), who found that digestibility of DM, OM and NDF were not different in glycerol fed cows. Also, Wilbert *et al*. (2013) and Boyd *et al*. (2013) reported no effects of glycerol inclusion in the diet on nutrients intake and nutrients digestibility. In contrast, Donkin *et al*. (2009) reported that digestibility of DM, OM and NDF were influenced by glycerol feeding. This difference may be due to the greater levels of glycerol fed. In addition, Wang *et al*. (2009) reported an increase in OM and CP digestibility with incremental increase in glycerol feeding as a supplement up to medium level (200 g/day). However, a slight decrease in digestibility was observed when high level (300 g/day) of glycerol was supplemented. Glycerol in excess of 1% in bacterial culture media inhibited growth and cellulolytic activity of *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* in vitro, although bacterial adherence to cellulose was not affected (Roger *et al*., 1992). Apparent digestibility of lipids was influenced by supplementation of glycerol. Khalili *et al*. (1997) reported that digestibility of lipids was not altered when 3.6% glycerol was fed. They indicated that the supply of glycerol along with plants that have a high content of fatty acids (lipids) increases the digestibility of lipids. They noted that the interaction of glycerol and fatty acids could result in an increase in the digestibility of lipids. However, one should bear in mind that addition of any high digestible ingredient in diet could increase apparent digestibility.

In the current study cows which received no glycerol tended to lose more BCS compared with cows fed dry glycerol. No difference in the BCS was reported by Lomander *et al*. (2012) in cows fed glycerol compared with the control cows. Energy status can be assessed by profiling concentrations of key blood metabolites and insulin (Chung *et al*., 2007). Small reductions of BCS indicate positive energy status, which was supported by trend to higher glucose and insulin levels in the glycerol fed cows. Donkin & Doane (2007) reported that cows with diet supplemented with 10 and 15% glycerol gained more weight after 8 weeks than cows not supplemented. However, in the current study there was no difference in the change in BW between two groups. One unit of BCS represents a change of 82 kg of live weight for Holstein dairy cows (NRC, 2001). On average, the cows produced 24 kg milk/day and lost only 25 kg BW and 0.37 points (5-point scale) BCS during the first 3 weeks of lactation. This observation was opposed to approximately 60 kg BW and 0.5 points BCS reduction, reported by DeFrain *et al*. (2004).

Serum concentrations of NEFA and BHBA were not significantly affected by dry glycerol feeding, which are in agreement with Lomander *et al*. (2012) who fed glycerol to early lactation dairy cows. In contrast, Chung *et al*. (2007) who fed 250 g/day glycerol as a top dress to transition cows, or Osborne *et al*. (2009) who supplied 20 g/L glycerol in water, reported lower concentration of plasma BHBA in glycerol fed cows at the third week of lactation. This reduction of BHBA may be due to a shift to reduce fatty acid oxidation to ketones or to increase utilization of ketones by extrahepatic tissues when glycerol is fed (Donkin *et al*., 2009). Defrain *et al*. (2004) reported lower concentrations of NEFA in the postpartum period in cows fed glycerol compared with controls. In this manner, in our study the concentration of NEFA was lower in supplemented cows compared with not supplemented cows, but this difference was not significant. In the present study the trend to higher insulin level could indicate a more positive energy status which was supported by the trend to higher concentrations of plasma glucose. This is in agreement with Chung *et al*. (2007), who reported higher concentration of glucose in plasma at 14 DIM when multiparous Holstein dairy cows were supplemented with dry glycerol.

When the results of various studies on the effects of glycerol are examined, several factors, such as method of administration, time of sampling in relation to time of administration or time of calving, dose, and physiologic status of the animal need to be considered (Nielsen & Ingvartsen, 2004).

In a previous study (Karami-Shabankareh *et al*., 2013) we found that follicular growth and reproductive efficiency was improved in the glycerol-fed cows; glucogenic effects of dry glycerol and more positive energy status may be led to the resumption of cyclicity in supplemented cows compared with control cows. These results demonstrated that feeding dry glycerol as an energy supplement may improve energy availability to primiparous Holstein dairy cows. However, it should not be forgotten that the primiparous Holstein dairy cows that were used in this study might be less tolerant of negative energy balance after parturition compared with cows that were used in some other studies in this field.-

In conclusion, supplementation of primiparous Holstein dairy cows diet with 250 g/day dry glycerol as a top dressing from parturition to 21 days postpartum tended to improved energy availability (higher blood glucose, numerically) of cows during the first 3 weeks of lactation. This glucogenic effect of dry glycerol did not affect DMI or milk yield during the first 3 weeks of lactation. However, milk yield was significantly improved during 13 weeks DIM. These results demonstrated that feeding dry glycerol as a glucogenic supply may be useful to improve negative energy balance in young cows that calve with a high demand to energy.

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