Effects of high concentrations of dietary crude glycerin on dairy cow productivity and milk quality

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ABSTRACT

An increasing worldwide interest in alternative fuel sources and in a more diversified energy matrix has provided incentives for the biodiesel industry, generating large amounts of the by-product crude glycerin, a potential alternative feed for dairy cows. A replicated 3 × 3 Latin square study was conducted to evaluate the effects of high concentrations of crude glycerin on dry matter intake, milk yield and composition, milk fatty acid profile, and blood metabolites of medium-yield cows. Ruminally cannulated Holstein cows ($n = 6$; 587 ± 39 kg of body weight; 114 ± 29 days in milk; and 20 ± 1.5 kg/d milk yield) were used in the study. The experimental period included 2 wk for adaptation and 1 wk for data collection. Cows were fed diets containing 0 (control), 15, or 30% crude glycerin (83% glycerol). Cows were milked, milk weights were recorded twice daily, and milk samples were collected for milk quality analyses at d 18 and 19 in each experimental period. Feeding cows with crude glycerin linearly decreased dry-matter intake, the 3.5% fat-corrected milk, and the solid-corrected milk yield. Hepatic enzymes were not affected by dietary treatments, except gamma-glutamyl transferase, which was decreased with the 15% crude glycerin diet. Serum glucose and albumin showed quadratic effect with increasing inclusion of crude glycerin. Plasma cholesterol as well as total protein linearly decreased with increasing inclusion of crude glycerin. Milk fat concentration and yield showed a quadratic effect of treatments. Solid yield decreased linearly with increasing inclusion of crude glycerin. Odd-chain fatty acids and conjugated linoleic acid in milk fat linearly increased with addition of crude glycerin in the diets. Together, these results suggest that crude glycerin has potential to replace corn; however, feeding diets in

which corn is replaced with crude glycerin at 30% of dietary dry matter greatly reduces animal performance.

Key words: blood metabolite, crude glycerin, fatty acid, milk production

INTRODUCTION

An increasing worldwide interest in alternative fuel sources and in a more diversified energy matrix has provided incentives for the biodiesel industry, especially in tropical countries with large productive areas. Crude glycerin, composed of water, glycerol, salts, and methanol, represents the main by-product of biodiesel production. The pharmaceutical, cosmetic, and food industries use purified glycerin (>95% glycerol) in several products (Thompson and He, 2006). However, increasing supply has outweighed these industrial demands and the price of glycerin has fallen, opening the possibility for its use as an alternative to conventional food energy sources, such as corn, in the diets of livestock, including dairy cows.

Once ingested and in the animal rumen, glycerol mostly disappears in the first 24 h (Trabue et al., 2007). It may be directly absorbed through the rumen wall, it may be transformed into propionate by ruminal microorganisms, or it may go through the rumen without transformation (Krehbiel, 2008). Glycerol constitutes an excellent substrate for gluconeogenesis and animal energy generation. Glycerol enters the gluconeogenic pathway at the level of dihydroxyacetone phosphate and 3-phosphoglyceraldehyde and can be converted to glucose in the liver of cattle, providing energy for cellular metabolism (Goff and Horst, 2003).

Researchers have supplemented dairy cow diets with 0.25 to 3.6 kg/d of glycerol for almost 60 yr, initially in the control of ketosis (Johnson, 1954) and more recently as an energy ingredient in the purified (Carvalho et al., 2011) or crude (Boyd et al., 2013) form. Addition of up to 15% purified glycerin in the diets of high-yield dairy cows does not significantly affect DMI,

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milk production, or milk composition (Donkin et al., 2009). In fact, a lower concentration of dietary purified glycerin (3.1%) has been shown to increase milk yield and milk protein concentration (Bodarski et al., 2005). When crude glycerin (82.6% glycerol) was added up to 15.6% in diets for medium-yield cows, no changes were observed in milk production and quality (Harzia et al., 2013), evidencing no difference in results of studies using crude or purified glycerin, as reported by Omazic et al. (2013). Altogether, these experiments indicate that purified or crude glycerin can be fed up to 15% of dietary DM to lactating cows without deleterious effects.

Despite this body of work, and in light of the increasing interest in glycerin as alternative energy source for dairy cows, more studies are warranted for the evaluation of higher concentrations of the compound. Thus, we have assessed the effects of up to 30% dietary crude glycerin on DMI, milk yield and composition, milk FA profile, and blood metabolites of medium-yield cows.

MATERIALS AND METHODS

Cows, Dietary Treatments, and Experimental Design

The São Paulo State University Institutional Animal Care and Use Committee approved all experimental protocols of the present study (approval number: 1892108). The experiment was conducted in the Animal Unit of Digestive and Metabolic Studies and Laboratory of Ingredients and Pollutant Gases, at the Animal Science Department of São Paulo State University, Campus of Jaboticabal, Brazil.

Six ruminally cannulated, multiparous Holstein cows (587 ± 39 kg, 114 ± 29 DIM, and 20 ± 1.5 kg/d milk production) were distributed in a replicated 3×3 Latin square design. Cows were paired according to initial weight, parity, and milk production. After lactation peak, each pair of matched cows was assigned randomly across squares to 1 of 3 dietary treatments with cows being housed in individual tie stalls.

The animals were fed diets with similar amount of protein and energy, meeting NRC (2001) guidelines for 550-kg Holstein dairy cows producing 25 kg of milk/d with 3.5% of milk fat. All diets were formulated with 14.1% CP and with roughage:concentrate ratio of 45:55 (DM basis). The base ration contained corn silage, cracked corn grain, sunflower meal, corn gluten meal, urea, vitamins, and minerals (Table 1). Cows were fed twice daily (0800 and 1800 h) for ad libitum intake (10 to 15% weighbacks) for 21 d in each experimental period (2 wk for adaptation and 1 wk for data collection). The dietary treatments were **G0** (control treatment, containing no crude glycerin), **G15** (containing 15%

crude glycerin as a percentage of ration DM), and **G30** (containing 30% crude glycerin as a percentage of ration DM). The crude glycerin used in this trial was in liquid form, soybean-based, and composed of 83% glycerol, 89% DM, 6% NaCl, and less than 0.01% methanol. Corn silage was top-dressed with crude glycerin and then mixed with concentrate at the time of feeding.

Table 1. Ingredient, chemical, and FA composition of experimental diets

Item	Treatment ¹		
	G0	G15	G30
Ingredient, % DM			
Corn silage	45.0	45.0	45.0
Cracked corn grain	36.5	19.3	2.8
Sunflower meal	10.7	11.4	11.3
Corn gluten meal	5.1	6.6	8.1
Urea	0.5	0.5	0.6
Crude glycerin ²	0.0	15.0	30.0
Mineral–vitamin premix ³	1.7	1.7	1.7
Limestone	0.6	0.6	0.6
Chemical composition, %			
CP	14.4	14.3	14.1
RDP ⁴	9.8	9.7	9.5
RUP ⁴	4.6	4.6	4.6
NDF	34.8	33.3	31.5
ADF	19.0	18.9	18.6
NFC ⁵	42.0	43.3	45.0
Ether extract	3.2	2.6	2.1
Ash	5.6	6.5	7.3
Na	0.12	0.66	1.21
Cl	0.32	0.66	1.01
ME, ⁴ Mcal/kg	2.8	2.8	2.8
NE _L , ⁴ Mcal/kg	1.6	1.6	1.6
FA profile, % FAME			
C12:0	0.02	0.02	0.04
C14:0	0.08	0.11	0.15
C15:0	0.03	0.04	0.05
C16:0	12.76	13.37	13.78
C16:1	0.16	0.17	0.21
C17:0	0.10	0.11	0.14
C 17:1	0.04	0.05	0.06
C18:0	2.52	2.62	2.75
C18:1n-9	33.58	32.58	31.47
C18:1n-7	1.41	1.48	1.22
C18:2n-6	46.62	46.33	46.55
C18:3n-6	0.08	0.12	0.17
C18:3n-3	1.25	1.51	2.04
C 20:0	0.56	0.56	0.51
C20:1n-9	0.23	0.25	0.23
C22:0	0.25	0.32	0.30
C23:0	0.05	0.05	0.04
C24:0	0.26	0.31	0.29

¹G0 = control, no crude glycerin added; G15 = 15% crude glycerin; G30 = 30% crude glycerin.

²Crude glycerin: 83% glycerol, 89% DM, 6% NaCl, <0.01% methanol.

³Composition of mineral–vitamin premix (g/1,000 g) = vitamin A, 200 kIU; vitamin D, 60 kIU; vitamin E, 60 IU; Ca, 190 g; P, 73 g; Na, 62 g; Mg, 44 g; Cl, 90 g; S, 30 g; Zn, 1,350 mg; Mn, 940 mg; Co, 3 mg; Cu, 340 mg; I, 16 mg; Se, 16 mg; Fe, 1,064 mg; F, 730 mg.

⁴Estimated based on NRC (2001).

⁵NFC = $100 - (\% \text{ CP} + \% \text{ NDF} + \% \text{ ash} + \% \text{ ether extract})$.

Sampling, Laboratory Procedures, and Calculations

Cows were milked twice daily (0700 and 1700 h). Dry-matter intake and milk weights were recorded during the 7 d of data collections in each period. Milk samples for chemical analyses were obtained at d 18 and 19, at 4 consecutive milkings of each period and analyzed for protein, determined as total nitrogen (AOAC, 1990; method 33.2.11; 991.20) multiplied by 6.38 (Barbano et al., 1990), fat, lactose, and total solids (B-2000 Infrared Analyzer; Bentley Instruments Inc., Chaska, MN) and MUN (MUN spectrophotometer; Bentley Instruments Inc.). The nonfat solids were obtained by the difference between total solids and fat percentage. The 3.5% corrected milk production (3.5% FCM) was calculated using the equation $3.5\% \text{ FCM} = 0.4324 \times \text{kg of milk} + 16.216 \times \text{kg of fat}$, according to Piepenbrink et al. (1996). The milk production corrected for total solid (SCM) was obtained using the equation $\text{SCM} = (12.3 \times \text{g of fat}) + (6.56 \times \text{g of nonfat solids}) - (0.0752 \times \text{kg of milk})$, according to Tyrrell and Reid (1965).

Milk NPN and noncasein nitrogen (NCN) were determined using methodologies proposed by IDF (1964) and IDF (1993), respectively. The concentration of CN nitrogen, true nitrogen (TN), and serum nitrogen (SN) were calculated by difference, where CN nitrogen = TN – NCN; TN = total nitrogen – NPN; and SN = TN – CN nitrogen. Efficiency of milk production was calculated by dividing milk production by DMI. Milk energy secretion (MES) was calculated according to the NRC (2001) equation: $\text{MES (Mcal/kg)} = (0.029 \times \% \text{ fat}) + (0.0547 \times \% \text{ CP}) + (0.0395 \times \% \text{ lactose})$, and the milk energy (Mcal/d) was obtained by multiplying the milk energy concentration of daily milk by daily milk production.

After milk removal, milk was homogenized, and samples of 250 mL were collected from individual milk buckets and reserved at 4°C. Samples from 4 consecutive milkings were composited and centrifuged at $1,000 \times g$ for 10 min at 4°C to separate milk fat. The supernatant fat was kept in a freezer (–20°C) overnight, and the transesterification of triacylglycerol was performed according to the method of the International Organization for Standardization (ISO, 1978). The methyl esters were prepared according to method 2301 (IUPAC, 1987) and analyzed by gas chromatograph model 14-B (Shimadzu, Kyoto, Japan), along with fused silica capillary column, type Omewax250 (30 m $\times$ 0.25 mm $\times$ 0.25 μm) Cat. No. 24136-Supelco, with the following analytical conditions and programing: 100°C for 2 min; heating 4°C/min up to 220°C and maintaining this temperature for 25 min; injector temperature of 250°C; detector temperature of 280°C; carrier gas velocity (H_2)

of 1 mL/min; SPLIT 1/100; injection volume of 1 μL ; using flame ionization detector. The FA profile of TMR was determined using the same gas chromatograph after total lipid extraction following procedures of Bligh and Dyer (1959).

Blood samples were collected 3 h after morning milking (2 h after morning feeding) on d 21 in each experimental period, through coccygeal vessels puncture, using evacuated tubes and centrifuged at $3,000 \times g$ for 15 min at 4°C to separate the plasma. Tubes containing potassium oxalate and 4% sodium fluoride were used for glucose analysis and tubes containing sodium heparin for analysis of the other variables. Once collected, samples were placed on ice and transported to the Clinical Pathology Laboratory of Veterinary Hospital at Faculdade de Ciências Agrárias e Veterinárias (FCAV)-Unesp, where they were immediately processed and analyzed for the concentrations of liver enzymes alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, and alkaline phosphatase using kinetic method (Labtest Diagnóstica, Lagoa Santa, MG, Brazil) to evaluate liver activity after addition of elevated concentrations of a gluconeogenic substrate, and possibly hepatocytes damages caused by crude glycerin impurities. Plasma glucose and serum triglycerides, cholesterol, amylase, total protein, urea, albumin, and creatinine were also investigated using commercial kits (Labtest Diagnóstica).

Statistical Analysis

Data were analyzed as a replicated 3×3 Latin square design using PROC MIXED of SAS version 9.1 (SAS Institute Inc., Cary, NC). The fixed effect consisted of treatment, and random effects consisted of cow and period. The statistical model for the trial was as follows:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_{k(j)} + \lambda_{l(j)} + \alpha\beta_{(ij)} + \varepsilon_{ijkl}$$

where Y_{ijkl} = value observed in the plot for treatment i , Latin square j , period k , and cow l ; μ = overall mean; α_i = effect of treatment i ; β_j = effect of Latin square j ; $\gamma_{k(j)}$ = effect of period k within Latin square j ; $\lambda_{l(j)}$ = effect of cow l within Latin square j ; $\alpha\beta_{(ij)}$ = effect of the interaction treatment i and Latin square j ; and ε_{ijkl} = residual error.

Treatment effects were tested using the following contrasts: (1) linear effect of crude glycerin concentration and (2) quadratic effect of crude glycerin level. Values are reported as least squares means and associated standard errors, and significance was defined as $P < 0.05$ and trends as $0.05 \leq P \leq 0.10$.

Table 2. Feed intake and performance of lactating Holstein cows fed diets of 0, 15 or 30% crude glycerin

Item	Treatment ¹			SE	Contrast, <i>P</i> -value	
	G0	G15	G30		Linear	Quadratic
DMI, kg/d	17.8	17.2	15.0	1.48	0.04	0.32
Milk yield, kg/d	17.9	16.9	16.0	1.59	0.13	0.98
3.5% FCM, kg/d	17.0	15.1	14.9	1.81	0.04	0.33
SCM, kg/d	16.9	14.7	14.7	2.73	0.02	0.19
Efficiency, milk/DMI	0.95	0.95	1.11	0.15	0.01	0.16

¹G0 = control, no crude glycerin added; G15 = 15% crude glycerin; G30 = 30% crude glycerin.

RESULTS

Increasing inclusion of crude glycerin in the diets linearly decreased DMI ($P = 0.04$, Table 2). Cows fed G30 had DMI 15.7% lower than G0 and 13.1% lower than G15. However, animals fed crude glycerin had greater milk production per unit of DM consumed (linear, $P = 0.01$). Milk yield did not change between control and glycerin diets ($P > 0.05$), but when milk production was corrected to fat (3.5%) or solids concentrations, linear decreases were observed ($P = 0.04$, $P = 0.02$, respectively). Milk fat concentration showed a quadratic effect ($P = 0.01$, Table 3), with lower values observed for G15, whereas fat yield linearly decreased with glycerin inclusion ($P = 0.03$). Milk protein concentration, milk protein yield, and milk CN did not differ among treatments ($P > 0.05$). Milk lactose concentration and milk lactose yield tended to decrease linearly with increasing inclusion of crude glycerin ($P = 0.07$ and $P = 0.06$, respectively), whereas milk solids concentration was

not altered by the different diets; however, milk solids yield was linearly decreased in glycerin treatments ($P = 0.03$). Milk SNF concentration and yield were not altered with increasing inclusion of crude glycerin in the diets. None of the milk nitrogen fractions (MUN, total nitrogen, true nitrogen, CN nitrogen, serum nitrogen, and NPN) differed among treatments ($P > 0.05$). Treatments tended to quadratically affect net energy ($P = 0.06$) but did not change milk total daily net energy.

Regarding the milk FA profile, the proportion of C4:0 was quadratically affected by treatments ($P = 0.04$; Table 4). Feeding crude glycerin did not change concentrations of C6:0, C8:0, and C10:0 but promoted linear increase in milk concentrations of C7:0. Milk concentrations of C9:0, C11:0, and C13:0 were linearly increased with crude glycerin ($P = 0.02$). Lauric acid (C12:0) increased linearly with crude glycerin addition ($P = 0.02$). Fatty acids C14:0, C14:1, and C16:0 were not altered by diets. Milk C15:0 concentration increased

Table 3. Milk composition of lactating Holstein cows fed diets of 0, 15 or 30% crude glycerin

Item	Treatment ¹			SE	Contrast, <i>P</i> -value	
	G0	G15	G30		Linear	Quadratic
Fat, %	3.17	2.71	3.01	0.47	0.25	0.01
Fat yield, kg/d	0.57	0.48	0.48	0.08	0.03	0.14
Protein, %	3.03	3.05	3.24	0.15	0.26	0.54
Protein yield, kg/d	0.54	0.52	0.52	0.04	0.45	0.65
Lactose, %	4.66	4.66	4.41	0.10	0.07	0.21
Lactose yield, kg/d	0.82	0.78	0.71	0.08	0.06	0.68
CN yield, kg/d	0.36	0.37	0.33	0.03	0.27	0.34
Solids, %	11.68	11.31	11.59	0.44	0.72	0.14
Solids yield, kg/d	2.09	1.89	1.84	0.19	0.03	0.30
Nonfat solids, %	8.70	8.62	8.41	0.18	0.19	0.34
Nonfat solids yield, kg/d	1.51	1.40	1.39	0.11	0.17	0.38
MUN, mg/dL	12.46	12.39	11.86	2.40	0.65	0.83
Total nitrogen, %	0.48	0.49	0.49	0.02	0.33	0.90
True nitrogen, %	0.44	0.45	0.45	0.02	0.39	0.78
CN nitrogen, %	0.32	0.34	0.33	0.02	0.83	0.28
Serum nitrogen, %	0.12	0.11	0.13	0.01	0.33	0.18
NPN, %	0.04	0.04	0.04	0.004	0.99	0.43
Net energy, Mcal/kg	0.64	0.60	0.63	0.04	0.69	0.06
Total net energy, Mcal/d	11.49	10.40	10.20	1.11	0.14	0.36

¹G0 = control, no crude glycerin added; G15 = 15% crude glycerin; G30 = 30% crude glycerin.

linearly with dietary crude glycerin ($P = 0.03$), showing an increase of 129% from G0 to G30. Milk concentrations of C17:0 and C17:1 were linearly increased from G0 to G30 ($P = 0.006$ and $P = 0.003$, respectively). Milk concentrations of C18:0, C18:1n-9, C18:1n-7, C18:2n-6, C18:3n-6, and C18:3n-3 did not differ among treatments. Regarding *cis-9,trans-11* C18:2 (CLA), milk concentrations increased linearly with increasing dietary glycerin ($P = 0.007$), showing an increase of approximately 100% from G0 to G15 and of 200% from G0 to G30. Feeding crude glycerin did not change concentrations of C20:0, C20:1n-9, C20:2, C20:3n-6, C20:5n-3, and C22:0. Milk concentrations of C20:4n-6 and C22:4n-6 increased linearly with dietary glycerin ($P < 0.0001$ and $P = 0.008$, respectively). Total SFA, total unsaturated FA, total MUFA, and unsaturated FA:SFA ratio were not influenced by dietary treatments. Total PUFA increased linearly with increasing inclusion of crude glycerin ($P = 0.03$).

Blood concentrations of hepatic enzymes and minerals as well as energy and protein metabolism parameters are displayed in Table 5. Dietary glycerin did not affect alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase ($P > 0.05$). The serum gamma-glutamyl transferase was quadratically affected by treatments ($P = 0.01$), showing the lowest value on G15; on the other hand, serum glucose concentrations were quadratically affected by increasing inclusion of crude glycerin ($P = 0.04$), showing the greater value on G15. Plasma cholesterol was reduced linearly ($P = 0.002$) from 93.9 (G0) to 59.5 mg/dL (G30), and triglycerides were not affected by dietary treatments ($P > 0.05$). Plasma amylase concentrations were not affected by crude glycerin addition ($P = 0.848$), but this by-product reduced linearly the total protein ($P = 0.005$) and had a quadratic effect on albumin ($P = 0.04$) concentrations. The other indicators evaluated, including urea, creatinine, and plasma minerals (Ca,

Table 4. Milk FA profile in lactating Holstein cows fed diets of 0, 15 or 30% crude glycerin

Item, ¹ %	Treatment ²				Contrast, <i>P</i> -value	
	G0	G15	G30	SE	Linear	Quadratic
C4:0	0.98	0.66	0.85	0.14	0.29	0.04
C6:0	1.03	0.99	1.04	0.07	0.96	0.38
C7:0	0.05	0.05	0.12	0.02	0.01	0.11
C8:0	0.91	0.91	0.98	0.06	0.31	0.44
C9:0	0.05	0.15	0.22	0.06	0.02	0.74
C10:0	2.48	2.58	2.53	0.19	0.59	0.80
C11:0	0.11	0.31	0.46	0.11	0.02	0.78
C12:0	3.54	3.61	4.03	0.17	0.02	0.29
C13:0	0.19	0.42	0.58	0.15	0.02	0.75
C14:0	11.14	10.30	10.34	0.34	0.11	0.31
C14:1	1.02	0.99	0.92	0.15	0.35	0.81
C15:0	1.61	2.81	3.69	0.68	0.01	0.78
C16:0	33.99	33.59	32.94	1.71	0.65	0.95
C17:0	0.65	0.99	1.22	0.16	0.006	0.56
C17:1	0.26	0.42	0.50	0.06	0.003	0.48
C18:0	10.45	8.80	8.81	0.77	0.20	0.45
C18:1n-9	23.42	23.26	21.61	1.41	0.41	0.70
C18:1n-7	1.18	1.32	1.28	0.21	0.58	0.61
C18:2n-6	4.11	3.98	4.03	0.18	0.92	0.55
C18:3n-6	0.09	0.08	0.09	0.009	0.41	0.58
C18:3n-3	0.16	0.17	0.18	0.02	0.53	0.54
<i>cis-9,trans-11</i> C18:2	0.31	0.63	0.93	0.09	0.007	0.89
C20:0	0.14	0.16	0.16	0.02	0.14	0.32
C20:1n-9	0.04	0.05	0.06	0.005	0.11	0.59
C20:2	0.06	0.07	0.06	0.009	0.93	0.38
C20:3n-6	0.12	0.13	0.12	0.01	0.93	0.13
C20:4n-6	0.23	0.27	0.31	0.01	<0.0001	0.24
C20:5n-3	0.05	0.04	0.05	0.006	0.86	0.30
C22:0	0.05	0.04	0.04	0.005	0.11	0.33
C22:4n-6	0.04	0.06	0.07	0.006	0.008	0.43
SFA	67.56	66.46	68.06	1.73	0.79	0.54
UFA ¹	32.60	33.26	31.77	1.62	0.71	0.60
MUFA	27.45	27.74	25.88	1.53	0.49	0.61
PUFA	5.21	5.46	5.89	0.22	0.03	0.73
UFA/SFA	0.49	0.51	0.47	0.03	0.71	0.54

¹UFA = unsaturated FA.

²G0 = control, no crude glycerin added; G15 = 15% crude glycerin; G30 = 30% crude glycerin.

P, Mg, Na, K, ionized Ca), were not altered by dietary treatments.

DISCUSSION

Increasing grain prices worldwide have led to an increasing demand for alternative gluconeogenic sources for livestock. On the other hand, a booming biodiesel industry has resulted in greater availability of glycerin, a by-product with potential to replace starch-based ingredients. The current study evaluated the effects of feeding medium-yield cows with high concentrations of dietary glycerin on blood metabolites, milk yield, milk composition, and FA profile.

A linear reduction in DMI was detected when cows were fed up to 30% crude glycerin. This result is in contrast with previous findings showing no changes to DMI or milk production of high-yield dairy cows fed up to 15% purified glycerin (Donkin et al., 2009). The reduced intake observed with G15 or G30 may result from changes to the physical form of diets, because glycerin makes the TMR become more viscous; decreasing diet palatability because of its impurities, such as methanol and catalysts; or from increasing concentration of salt, mainly NaCl, which increased 500% from G0 to G30. According to ARC (1980), increased dietary intake of salt could be detrimental to animal health, leading to

reduced consumption or poor utilization of feed, and damage the kidneys. The NRC (2001) reported that maximum concentration of dietary NaCl for dairy cattle is approximately 4% on a DM basis, corresponding to 1.6% Na. The recommendation of Na for cows in the condition of the present study is 0.32% Na, but diets containing 15 or 30% crude glycerin had, respectively, 0.66 and 1.2% Na. Donkin et al. (2009) and Shin et al. (2012) reported reduced digestibility of fiber when they fed cattle purified or crude glycerin, respectively, and this fact could also be an explanation to the reduced DMI observed in current study.

No reduction in milk yield was observed, thus feed efficiency increased with dietary glycerin. The lack of response on milk production could be partially explained by the high SEM observed for this parameter (1.59). The improvement in feed efficiency in 21-d periods may be maintained by NEFA and will likely not be continued in a longer feeding period because of lower DMI in animals fed especially G30. Santana Junior et al. (2013), evaluating changes in body condition and DMI, hypothesized that feed efficiency decreases with increasing dietary glycerin. This apparent discrepancy may result from the different indicators evaluated in the 2 studies: in the current study changes in milk yield were observed but not in BCS (average 3.13, in a 1-to-5 scale).

Table 5. Blood metabolites of dairy cows fed diets of 0, 15, or 30% crude glycerin

Item	Treatment ¹			SE	Contrast, <i>P</i> -value	
	G0	G15	G30		Linear	Quadratic
Hepatic enzymes ²						
ALT, U/mL	32.62	35.88	33.73	2.07	0.65	0.21
AST, U/mL	50.52	60.41	57.80	7.33	0.26	0.19
GGT, U/mL	38.97	32.81	46.75	7.29	0.07	0.01
ALP, U/mL	43.21	43.45	43.22	5.48	0.42	0.23
Energy metabolism						
Glucose, mg/dL	63.87	74.87	66.33	4.11	0.59	0.04
Cholesterol, mg/dL	93.90	68.27	59.50	6.21	0.002	0.28
Triglycerides, mg/dL	15.08	10.94	10.10	1.93	0.12	0.30
Amylase, U/mL	198.25	207.33	213.17	18.74	0.56	0.94
Protein metabolism						
Total protein, g/dL	8.35	7.99	7.80	0.28	0.005	0.40
Urea, mg/dL	26.51	25.40	24.55	3.17	0.55	0.96
Albumin, g/dL	2.57	1.99	2.16	0.34	0.05	0.04
Creatinine, mg/dL	1.04	1.24	1.27	0.13	0.08	0.35
Minerals						
Ca, mg/dL	7.96	8.03	8.18	0.44	0.48	0.86
P, mg/dL	6.59	7.03	6.05	0.45	0.42	0.23
Mg, mg/dL	2.26	2.23	2.15	0.17	0.41	0.82
Na, mmol/L	130.69	130.47	130.50	2.36	0.88	0.90
K, mmol/L	3.20	3.46	2.93	0.55	0.47	0.31
Ionized Ca, mg/dL	0.98	0.75	0.79	0.14	0.61	0.31

¹G0 = control, no crude glycerin added; G15 = 15% crude glycerin; G30 = 30% crude glycerin.

²ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; ALP = alkaline phosphatase.

Dietary glycerin did not affect milk yield but linearly reduced the FCM yield. Previous studies using lower glycerin concentrations also showed a reduction in milk fat (Khalili et al., 1997; Chung et al., 2007; Santana Junior et al., 2013). The increases in ruminal propionate:acetate ratio previously reported for animals on glycerol diets (Carvalho et al., 2011) may explain these changes, because acetate represents the main precursor to milk fat. Alternatively, other authors suggest that a reduction in NEFA plasma concentrations of animals fed glycerol may indicate lower lipid mobilization and lipolysis, thus explaining the reduction in milk fat (Kass et al., 2012; Kupczynski et al., 2012). Nevertheless, it was observed that milk fat content showed a quadratic effect, increasing back with 30% dietary glycerin. The speculation is that this U-shaped dose-response curve may result from a threshold effect. For example, to maintain an isocaloric intake across diets, the 15 and 30% diets have 47 and 92% less cracked corn grain, respectively. It might be that below a certain level of dietary starch, animal metabolism switches back to a higher lipid mobilization profile. This potential explanation, however, should be evaluated in further studies.

Regarding milk protein, previous studies have controversial results. Authors have reported decreases in milk protein concentration with up to 3 kg/d of dietary glycerol (Harzia et al., 2013), whereas others have shown increases in milk protein (Kass et al., 2012; Wilbert et al., 2013). In the current study the milk protein concentration and the daily production of milk protein remained constant regardless of dietary treatment.

Odd-chain FA in milk necessarily originate with microbial production because animals only synthesize even-chain FA. Indeed, Keeney et al. (1962) reported that rumen bacteria are the main sources of odd-chain FA. A linear increase in C7:0, C9:0, C11:0, C13:0, C15:0, C17:0, and C17:1 concentrations with the addition of dietary glycerin was observed in the current study. This alteration indicates improvement in ruminal microbial synthesis, because these FA are only found in ruminal microorganisms. Additionally, odd-chain FA are formed when the propionyl-CoA, rather than acetyl-CoA, is used as primer (Fulco, 1983; Kaneda, 1991). Thus, the increase in rumen propionate seen in animals fed glycerin (Carvalho et al., 2011; Shin et al., 2012; Boyd et al., 2013) also increases the available primers for odd-chain FA production.

Cow milk fat is one of the most important sources of odd-chain FA for humans, and it plays an important role as indicator of dairy product intake by them (Vlaeminck et al., 2006). These FA, specifically C15:0, inhibit the growth of cancer cells in vitro and in vivo

(Yang et al., 2000). Furthermore, C15:0 may have a significant beneficial effect against breast cancer (Wongtangtintharn et al., 2004). These findings allow us to speculate about potential benefits of dietary glycerin for ruminants and the observed increase in odd-chain FA in milk.

Dietary glycerin did not affect the total concentrations in milk of saturated, monounsaturated, and polyunsaturated FA. However, *cis*-9,*trans*-11 C18:2 (CLA) linearly increased in the milk of animals fed crude glycerin, possibly as a result of a slight decrease in ruminal pH, previously reported by Wang et al. (2009), as a consequence of replacement of corn with glycerin, which is fermented more rapidly in the rumen. This particular FA is of great interest to human health because it possesses anticarcinogenic properties (Ha et al., 1987). Nevertheless, previous studies showed a negative effect of dietary glycerin on the growth of *Butyrivibrio fibrisolvens*, a bacteria that hydrogenates *cis*-9,*cis*-12 C18:2 (Abo El-Nor et al., 2010). These distinct results may again stem from the range of concentrations used. Further studies involving the identification and quantification of ruminal microorganisms in animals fed crude glycerin are required.

Dietary lipids directly affect ruminant blood cholesterol concentration (Nunes et al., 2010). Glycerin has a lower lipid concentration than corn and lower availability of FA, resulting in the reduced synthesis of triglycerides and cholesterol (Nelson and Cox, 2005). This fact could explain the lower plasma concentrations of triglycerides and cholesterol that were observed in animals fed glycerin-containing diets.

In ruminants, propionic acid followed by AA and triglycerides represent the main sources of glucose (Van Soest, 1994). Animals fed a 15% glycerin diet had elevated blood glucose possibly derived from ruminal propionate or from the glycerol directly absorbed through the rumen wall. When fed 30% glycerin, however, animal blood glucose returned to control levels. This effect may result from changes to the complex glucose-regulating endocrine system or from the putative threshold effect previously mentioned.

The lower blood concentrations of total protein and albumin may be due to increased ruminal proteolytic activity. The lack of effect of glycerin on BUN was also reported by Boyd et al. (2013) and Chung et al. (2007), whose results also disagree with Shin et al. (2012), which observed linear decrease in BUN concentration and attributed this effect to an improved use of dietary N by ruminal microorganisms.

Dietary crude glycerin did not affect plasma minerals despite its high NaCl (6%) concentration. An increase in blood gamma-glutamyl transferase enzyme in ani-

mals fed 30% crude glycerin was observed, but no other liver enzymes suffered alterations. From this data liver damage cannot be inferred, because no changes were observed in activity of liver enzymes alanine aminotransferase and aspartate aminotransferase; however, long-term effects of high-glycerin-content diets should be further evaluated.

CONCLUSIONS

High concentrations of dietary glycerin (30%) for medium-yield cows reduced DMI, improved feed efficiency, but reduced 3.5% FCM and SCM. Additionally, dietary glycerin increased the concentration of odd-chain FA and CLA in milk, alterations with potential benefits to human health. Together, these results suggest that crude glycerin has potential to replace corn; however, feeding diets in which corn is replaced with crude glycerin at 30% of dietary DM greatly reduces animal performance.

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