



Complete replacement of corn grain with crude glycerin for dairy cows

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HIGHLIGHTS

- The replacement of corn starch with glycerin reduced lactation performance and feed efficiency.
- The efficiency of utilization of digestible organic matter for milk energy was reduced by glycerin.
- Glycerin reduced acetate and increased butyrate in rumen fluid and had no effect on microbial yield.
- Glycerin increased OM digestibility, but lowered plasma glucose concentration.

ARTICLE INFO

Keywords:

Crude glycerin
Digestibility
Feed efficiency
Glycerol
Rumen fermentation
Dairy cattle

ABSTRACT

The biodiesel process generates crude glycerin as a coproduct, which is primarily glycerol, but also includes varying amounts of other materials that may impact the value of crude glycerin as a feed for livestock. The response of dairy cows to the complete substitution of finely-ground mature corn with crude glycerin in diets containing a high content of non-forage fiber sources was evaluated. The goal was to maximize milk yield per unit of cultivated corn and to have a high replacement rate of corn starch by glycerol in the diet. Eighteen Holsteins (185±58 days in lactation) were housed in sand-bedded tie stalls and individually fed. Cows received a sequence of two treatments in a crossover design with 28-day periods. Treatments were: 1) control diet containing 14.8% ground corn (Control, CTL), or 2) a diet with complete substitution of ground corn with an iso-nitrogenous mixture of crude glycerin and soybean meal (Glycerin, GLY) at 12.3 and 2.7% of the ration dry matter (DM), respectively. Diets had (% of DM) 33.8% forage, 19.4% citrus pulp, and 8.6% whole cottonseeds. Crude glycerin contained 6.3% moisture, 76.2% glycerol, and 8,800 ppm of methanol, on an as-fed basis. No effect on DM intake (DMI, 16.7 kg/d) was observed, whereas GLY decreased milk yield from 23.4 to 21.3 kg/d. The daily secretion of lactose was lower (0.94 vs 1.05 kg/d) and there was a trend for total solids (2.57 vs 2.79 kg/d) to be lower for GLY than CTL. Plasma glucose concentration was decreased for cows fed the GLY diet (51.6 vs 58.3 mg/dL). Glycerin increased butyrate in rumen fluid (19.9 vs 15.6% of total volatile fatty acids) and decreased acetate (58.7 vs 64.6% of total volatile fatty acids) and the acetate to propionate ratio (2.79 vs 3.30). Total tract apparent OM digestibility was increased with GLY (74.1 vs 70.8% of intake). The ratios of milk yield to DMI (1.40 vs 1.30 kg milk/kg DMI) and milk energy secretion to digestible OM intake (DOMI) (1.47 vs 1.30 Mcal/kg) were decreased in GLY. There was no difference between treatments on rumen microbial yield, estimated by the daily excretion of urinary allantoin, and urinary allantoin per unit of DOMI. Eating time per unit of DMI was reduced for cows fed GLY (12.6 vs 14.2 min/kg DMI) and rumination rate did not differ between treatments (18.2 min/kg DMI). Overall, the substitution of ground corn with a crude glycerin and soybean meal mixture increased OM digestibility and decreased rumen acetate to propionate ratio, plasma glucose concentration, milk yield, and efficiencies of feed and energy utilization.

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<https://doi.org/10.1016/j.livsci.2022.104893>

Received 4 January 2022; Received in revised form 25 February 2022; Accepted 2 March 2022

Available online 3 March 2022

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Introduction

The biofuel industry around the world has generated crude glycerin as a residue, containing varying amounts of glycerol, catalyst, soap, water and alcohol (usually methanol) (Thompson and He, 2006). Although pure glycerin is a valuable industrial compound, its purification can be uneconomical and insufficient to consume the potential growth in world glycerin production. Crude glycerin, as a replacement for corn, in diets for cattle may provide an outlet to utilize this biodiesel coproduct without further purification. However, direct characterization of crude glycerol containing impurities needs to be assessed to evaluate the tolerance of cattle to impurities and the impact on productivity and economic efficiency.

The use of glycerol for the prevention and treatment of ketosis in dairy cows has been known for a long time (Johnson et al., 1954; Kupczyński et al., 2020). There is also research suggesting that purified glycerol (Donkin et al., 2009) or crude glycerin (Ezequiel et al., 2015) can replace corn grain in rations for dairy cows up to as much as 15% of diet dry matter (DM), when starch from concentrates is not totally removed from the diet. The replacement of cracked corn grain (36.5 vs 2.8% of diet DM) with crude glycerin at 30% of diet DM reduced milk yield of low yielding Holstein cows on a 45% corn silage diet (Ezequiel et al., 2015). Karlsson et al. (2019) observed a reduction in milk yield, even with increased DM intake (DMI) per day, when refined glycerol replaced food-grade wheat starch at 20% of diet DM in a ration with grass silage at 60.5% of DM, suggesting that a high replacement rate of starch with glycerol may reduce lactation performance. Glycerol is a rapidly fermentable substrate in the rumen (Rémond et al., 1993) and is a gluconeogenic precursor (Lin, 1977). Although, glycerol supplementation in the diet around calving has been shown to decrease blood glucose content in dairy cows (Carvalho et al., 2012). The response of dairy cows to glycerol feeding may vary depending on product and dietary content, stage of lactation, basal diet, or the feeding management adopted (DeFrain et al., 2004).

The content of glycerol in crude glycerin varies, as well as content of impurities (Hansen et al., 2009). We hypothesized that crude glycerin could completely replace corn grain in a dairy cow diet rich in non-forage fiber sources. The complete replacement of ground corn with crude glycerin, in a low forage diet was adopted to simulate a scenario of high milk yield per unit of land cultivated with corn and also to evaluate the effect of a high replacement rate of starch with glycerol. The objective of this study was to evaluate the response of dairy cows in performance, rumen fermentation, and diet digestibility to the complete substitution of finely ground mature corn with crude glycerin.

Materials and methods

Eighteen Holsteins [185±58 (mean±SD) days in lactation at the beginning of the experiment] were housed in sand-bedded individual tie stalls and fed at 07:00 h and 14:30 h for ad libitum intake and milked three times a day starting at 05:00 h, 13:00 h, and 20:00 h. A total mixed ration was offered in amounts to provide at least 10% of the feed offered as dailyorts. Cows (4 primiparous and 14 multiparous) were paired blocked based on parity and milk yield and randomly assigned to a sequence of treatments in a crossover design, with 28-day periods, with 21 days for treatment adaptation. Treatments were: Control diet with finely ground mature corn (CTL) or diet in which corn was completely replaced by an isonitrogenous mixture of crude glycerin (Almad Agro-indústria Ltda, Diadema, Brazil) and soybean meal (GLY) (Table 1). The CTL diet had 15% finely ground mature corn and GLY had 12% crude glycerin, both at dietary 28% corn silage DM.

On days 22 through 26 of each period, forages, concentrates, and refusals from each cow were sampled daily and frozen. Composite samples were formed, dried at 55 °C for 72 h, ground through a 1 mm mesh diameter screen with a Thomas-Willey mill, and a sub-sample was dried at 100 °C for 24 h for DM determination. Crude protein (CP) was

determined with a micro Kjeldhal steamer distillator (AOAC International, 2002) and ether extract (EE) according to AOAC International (1990). Ash content was determined by incinerating samples at 550 °C for 8 h. Neutral detergent fiber (NDF) was analyzed with a fiber analyzer TE-149 (Tecnal Equipamentos para Laboratórios, Piracicaba, Brazil), using sodium sulfite and amylase. The composition of the crude glycerin (Table 2) was assessed by the ASTM D 6,584-00E01 method for glycerin and by the EN 14,110 method for methanol (ASTM, 2006).

Samples from six consecutive milkings were collected on days 22 and 23 of each treatment period for protein, fat, lactose, total solids and MUN analysis by mid-infrared spectroscopy (Nexgen FTS/FCM. Bentley Instruments Inc., Chaska, USA) at the Paraná State Holstein Association Laboratory (APCBRH, Curitiba, Brazil). Daily milk yield and DMI on days 22 to 25 compared treatments. The daily milk energy secretion (Mcal/d) was calculated (NRC, 2001): [(0.0929 × fat%) + (0.0547 × protein%) + (0.0395 × lactose%)] × kg of milk. Feed efficiencies were calculated as the ratio of milk yield to DMI (Efficiency 1), the ratio of milk energy secretion to DMI (Efficiency 2), and the ratio of milk energy secretion to daily intake of digestible organic matter (DOMI) (Efficiency 3).

Body weight and body condition score were measured on day 28 of each period. Body condition was assessed on a 1 to 5 scale (Wildman et al., 1982), by three independent evaluators and a mean value was generated per cow per period. Body weight was determined immediately after the morning milking with an individual walk-over weigh scale (Eziweigh2. Tru-Test Brasil, Porto Alegre, Brazil).

On day 28, chewing activity was assessed by visual observation of each cow, at five-minute intervals, for 24 h as in Pereira et al. (1999). Chewing activities considered were eating, rumination, and idle. Chewing time, in min/d, was defined as the sum of the eating and rumination times. Chewing, eating, and rumination times per unit of DMI were calculated considering the DMI of the day in which chewing activity was evaluated.

The total tract apparent digestibility of DM, organic matter (OM), NDF, and non-NDF OM were determined by manual collection of feces concurrently to defecation during three 8-h sampling periods, on days 25 to 27. Feces were collected in buckets; for every 2 cows, 1 researcher stood behind them for 8 h and collected any fecal excretion into the bucket. On each day, sampling was shifted ahead by eight hours relative

Table 1

Ingredient and nutrient composition of the Glycerin (GLY) and Control (CTL) treatment diets.

	GLY	CTL
	% of dry matter	
Ingredient		
Corn silage	27.6	27.7
Tifton hay	6.1	6.2
Finely ground mature corn	–	14.8
Crude glycerin	12.3	–
Soybean meal	21.3	18.6
Citrus pulp	19.4	19.4
Whole cottonseeds	8.6	8.6
Premix ¹	4.7	4.7
Chemical composition		
Crude protein	17.5	17.7
Neutral detergent fiber	32.4	34.4
Neutral detergent fiber from forages	18.7	19.0
Ash	9.7	9.3
Ether extract	5.8	6.1
Non-fiber carbohydrates ²	34.5	32.5
Dry matter,% of as-fed	49.9	49.6

¹ Premix: 25% sodium bicarbonate, 25% Megalac, 15% limestone, 13% soybean meal, 8% NaCl, 6% magnesium oxide, 8% minerals and vitamins (200 g/kg Ca; 156 g/kg P; 30 g/kg Mg; 35 g/kg S; 150 mg/kg Co; 2,000 mg/kg Cu; 5,000 mg/kg Mn; 11,900 mg/kg Zn; 82 mg/kg Se; 200 mg/kg I; 1,000 KUI/kg vit A; 220 KUI/kg vit D; 6.2 KUI/kg vit E).

² Non-fiber carbohydrates = 100 - (CP + NDF + EE + Ash).

Table 2
Composition of the crude glycerin on an as-fed basis.

Glycerol, %	76.2
Moisture, %	6.3
Crude protein, %	0.3
Ether extract, %	1.3
Ash, %	2.9
Phosphorus, %	0.61
Sodium, %	0.81
Methanol, ppm	8,800
Acidity index, mg KOH/g	1.74
Gross energy, kcal/g	4.008

to the previous sampling period to obtain a 24 h profile of fecal output. Fecal samples were frozen and composites were dehydrated and the NDF and ash contents determined as previously described. The daily DOMI was calculated by multiplying OM intake on days 22 through 24 by OM digestibility determined on days 25 through 27.

Total urine excreted was collected in buckets at the same time as total fecal collection. The daily excretion of allantoin was determined in urine and used as a relative measure of rumen microbial yield (Chizzotti et al., 2008). Urine was immediately acidified upon collection by the addition of 10% sulfuric acid solution (in a 1 to 9 vol to vol ratio) and the sample was stored at 4 °C. A composite sample was diluted with distilled water (1 to 3 ratio) and frozen at -20 °C. Allantoin was analyzed by a colorimetric procedure (Chen and Gomes, 1992). The ratio of daily excretion of allantoin to DOMI estimated the efficiency of rumen microbial synthesis (Pereira et al., 2021).

On day 27, samples of ruminal fluid were obtained with a flexible oro-gastric tube, with the goal of having minimum saliva contamination. Samples were obtained at 12.6 ± 0.4 h after the morning feeding. Rumen pH was measured immediately with a portable pH meter probe (Phmetro Digimed DM 20. Digicrom Analítica, São Paulo, Brazil). Samples of ruminal fluid were frozen in liquid nitrogen and stored at -20 °C, and then centrifuged at 4 °C at 8,855 × g for 15 min. The supernatant was analyzed for volatile fatty acids (VFA) by gas-liquid chromatography (CP 3800 Gas Chromatography Varian. Varian Chromatography Systems, Palo Alto, USA), with a capillary column [CP-Wax 58 (FFAP) CB. Varian Analytical Instruments, Palo Alto, USA].

On day 28, blood samples were obtained from the coccygeal vessels to determine plasma glucose concentration. Samples were obtained 12.2 ± 0.1 h after the morning feeding. Blood was collected in tubes containing 100 µl potassium fluoride, were centrifuged at 1,000 × g for 15 min, and the plasma was frozen at -20 °C. Glucose was analyzed with a laboratory kit (Cat. 27. Labtest Diagnóstica S.A., Lagoa Santa, Brazil).

Data were analyzed with Proc Mixed of SAS statistical software (version 9.3, 2011; SAS Institute Inc., Cary, USA) with a model containing the random effects of block (1 to 9) and cow within block (1 to 18) and the fixed effects of period (1, 2) and treatment (CTL, GLY). Variables with repeated measures across days were averaged and digestibility evaluated during 8 h on three consecutive days generated 1 value per cow per treatment per period. Differences between least squares means with probability values below 0.05 were considered significant and between 0.05 and 0.10 as trends.

Results and discussion

Analysis of the crude glycerin used in this experiment revealed a gross energy content of 4.28 kcal/g of DM (Table 2), which is typical of carbohydrates and matches the energy value of 4.3 kcal/g of pure glycerol (Maynard et al., 1979). The non-glycerol, non-fiber carbohydrate content of crude glycerin was 13% of DM, estimated by subtracting the water, glycerol, ash, CP, and EE from the fresh sample weight. The estimated content of rapidly degradable carbohydrates in the sample was around 94% of DM. Hansen et al. (2009) observed that the concentration of glycerol in 11 samples of crude glycerin in Australia was

72.4%, ranging from 38.4 to 96.5%. Samples of crude glycerin with glycerol purity exceeding 80% are frequently reported in the literature (DeFrain et al., 2004; Kass et al., 2012; Shin et al., 2012; Boyd et al., 2013; Ezequiel et al., 2015), demonstrating that crude glycerin is a potential energy source for dairy cows, in spite of its variability in composition.

Although the ash content of the crude glycerin in this trial was 2.9%, which is low when compared to reported values (Hansen et al., 2009), the methanol content (Table 2) was above the minimum threshold considered to be safe for animal feed usage based on European standards. Glycerol used in this experiment contained 8,800 ppm of methanol, while the maximum security limit has been set at 5,000 ppm in Europe (European Commission, 2013). Given an intake of crude glycerin DM of approximately 2.0 kg/d in this experiment, the corresponding consumption of methanol was 19,272 mg/d or 31.2 mg/kg of body weight. Based on animal observations, this level of methanol intake for 28 days did not induce any clinical symptoms of methanol toxicity (Eells et al., 1996; Sebe et al., 2006). The available information suggests that methanol is highly metabolized in the rumen to methane (Czerkawski and Breckenridge, 1972; Pol and Demeyer, 1988), and as a consequence, capable of generating a measure of energetic inefficiency (digestible OM in methanol being transformed to methane gas).

The replacement of starch from ground corn by glycerol from crude glycerin depressed ($P = 0.02$) the daily milk yield by about 10%, with no detectable effect ($P = 0.85$) on DMI (Table 3). The daily production of lactose was depressed ($P = 0.01$) and there was a tendency ($P = 0.06$) for decreased total solids production. The ratio of milk yield to DMI (Efficiency 1) was 7% lower ($P = 0.05$) in GLY than in CTL. Boyd et al. (2013) also observed that feeding dairy cows a diet supplemented with 400 g of crude glycerin reduced milk yield from 37.2 to 35.8 kg/d. Donkin et al. (2009) replaced ground corn at 20% of diet DM with purified glycerol at 5, 10 or 15% of the diet DM and observed no negative impact on milk yield for cows averaging 37 kg/d. The replacement of purified wheat starch with refined glycerol, both at 20% of diet DM, reduced milk yield and lactose concentration despite greater DMI (Karlsson et al., 2019). Feed efficiency (4% fat corrected milk/DMI) was reduced when crude glycerin was included at 5 and 10% of DM to a low roughage diet formulated with cottonseed hulls and grass hay, but was increased when glycerin was added to a diet based on corn and alfalfa silages (Shin et al.,

Table 3

Intakes of dry matter (DMI), organic matter (OMI), and digestible OM (DOMI), lactation performance, body weight (BW), body condition score (BCS), and feed efficiency of dairy cows on treatments Glycerin (GLY) and Control (CTL).

	GLY	CTL	SEM ¹	P
	kg/d			
DMI	16.7	16.8	0.37	0.85
OMI	15.1	15.2	0.34	0.79
DOMI	11.2	10.7	0.31	0.29
Milk	21.3	23.4	0.58	0.02
Fat	0.750	0.797	0.027	0.24
Protein	0.680	0.736	0.024	0.13
Lactose	0.942	1.050	0.028	0.01
Solids	2.571	2.792	0.079	0.06
	% of milk			
Fat	3.53	3.43	0.097	0.48
Protein	3.24	3.15	0.063	0.32
Lactose	4.42	4.46	0.070	0.63
Solids	12.13	11.92	0.219	0.50
BW, kg	617	619	4.6	0.76
BCS, 1 to 5	3.60	3.63	0.050	0.71
Milk energy, Mcal/d	14.41	15.58	0.435	0.07
Ef 1 ²	1.30	1.40	0.037	0.05
Ef 2 ² Mcal/kg	0.87	0.93	0.030	0.14
Ef 3 ² Mcal/kg	1.30	1.47	0.043	0.01

¹ SEM = Standard error of the means.

² Efficiency 1 = Milk yield/DMI. Efficiency 2 = Milk energy/DMI. Efficiency 3 = Milk energy/DOMI.

2012). The interaction of crude glycerin supplementation with the type of forage, stage of lactation, the replacement rate of starch by glycerol, the starch content of the basal diet, or the level of contaminants in crude glycerin may contribute to the variability in the response to glycerin feeding.

There was no detectable difference ($P = 0.29$) in energy consumption, measured as DOMI for CTL and GLY treatments (Table 3). However, apparent digestibility of OM was increased by 3.3%-units ($P = 0.02$) when glycerol replaced starch (Table 4), similar to the observation of Donkin et al. (2009). However, a smaller proportion of DOMI was secreted as milk energy for the GLY treatment, as determined by the ratio of milk energy secretion to DOMI (Efficiency 3). This measure was lower ($P = 0.01$) for the GLY diet than for CTL (Table 3), denoting that glycerin feeding reduced the efficiency of energy usage for lactation in dairy cows. A plausible explanation for this may be the energy loss from methane for crude glycerin. However, a greater energy loss in the form of methane was not supported by a change in rumen VFA profiles (Table 4), given that the substitution of corn with glycerin increased ($P = 0.01$) the molar proportion of butyrate (19.9 vs 15.6% of VFA) and decreased acetate (58.7 vs 64.6% of VFA), without affecting ($P = 0.18$) rumen propionate content (20.7% of VFA). The decrease in the molar proportion of acetate in GLY (Table 4) was apparently not the result of depressed fiber digestion induced by glycerol feeding, since total tract NDF digestibility was similar (Table 4). Although classical studies suggest that the ruminal metabolism of glycerol results in propionate production (Garton et al., 1961), some researchers have shown that the ruminal fermentation of glycerol is also able to increase the proportion of butyrate and reduce the proportion of acetate (Rémond et al., 1993; Carvalho et al., 2011; Boyd et al., 2013; Avila-Stagno et al., 2014). The constancy in propionate proportion in our experiment may have been the result of an already high propiogenesis in the CTL diet with corn starch and low forage content. A decrease in the acetate to propionate ratio, even with increased molar proportion of butyrate, should reduce the net production of reduced equivalents in this treatment, therefore reducing the energy loss in the form of methane. However, it has been suggested that the production of butyrate and propionate from glycerol can increase methane relative to propiogenesis from glucose, since there is no net electron incorporation in the conversion of glycerol to propionate (Avila-Stagno et al., 2014; Benedetti et al., 2018). The replacement of starch with glycerol has not reduced methane production in vitro (Avila et al., 2011; Avila-Stagno et al., 2014; Benedetti et al., 2018) and in vivo (Karlsson et al., 2019), in spite of the reduced acetate to propionate ratio.

Another explanation for the lower efficiency (Milk energy/DOMI) on GLY would be differences in the post-absorptive metabolism of glycerin-derived metabolites. Donkin et al. (2009) observed an increased partial efficiency of DMI for BW gain with increasing level of glycerol in the diet. Karlsson et al. (2019) observed a reduction in pre-formed long chain fatty acids (C18:0) in milk fat when glycerol replaced starch in the diet of mid-lactation cows, suggesting a possible change in post-absorptive metabolism in favor of body tissue gain. Glycerol has been demonstrated to increase palmitate uptake and decrease β -oxidation in myocytes (Gambert et al., 2005). When purified wheat starch was replaced with glycerol at 20% of diet DM there was a reduction in the respiratory quotient (CO_2/O_2) and in the ratio of energy corrected milk to net energy intake, at similar methane excretion per unit of DMI (Karlsson et al., 2019). Therefore, the replacement of starch with glycerol can reduce the energetic efficiency of milk secretion by ruminal and post-absorptive mediated mechanisms.

Glycerin decreased ($P \leq 0.03$) plasma glucose concentration by 12% (Table 4) and the daily secretion of milk lactose by 10% (Table 3). The effect of glycerin supplementation on plasma glucose has been variable in the literature, although the use of glycerol as a source of gluconeogenic carbon has justified its use as a drench for the prevention and treatment of ketosis (Goff and Horst, 2003). The absence of positive response in plasma glucose to the supplementation with glycerol has been observed elsewhere (Kass et al., 2012; Boyd et al., 2013). Alternatively, lower blood glucose has been observed in early lactation cows supplemented with crude glycerin (DeFrain et al., 2004) or glycerol (Carvalho et al., 2011). Ezequiel et al. (2015) observed that when ground corn, at 36% of diet DM, was reduced to 19% in a diet with 15% crude glycerin, plasma glucose was increased, but when corn was further reduced to 2.8% of DM in a diet with 30% crude glycerin, plasma glucose returned to the basal level, and the increase in crude glycerin in the diet induced a linear reduction in the daily yields of milk lactose and fat. However, Wang et al. (2008), supplementing 0, 100, 200 or 300 g of purified glycerin and Donkin et al. (2009), also evaluating diets with 0, 5, 10 or 15% of purified glycerin, both observed linear increases in plasma glucose in response to the supplementation. It is not clear whether the difference in response results from the manner and amount of glycerin supplemented, the metabolic profile of animals used, the composition of the basal diet or the purity of the glycerin. In cases where glycerol feeding is accompanied by greater ruminal butyrate, the reduction in blood glucose may be a consequence of decreased liver gluconeogenesis from propionate in the presence of butyrate (Aiello and Armentano, 1987). Carvalho et al. (2011) observed that the reduction in

Table 4

Total tract apparent digestibility (D) of dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), and non-NDF OM (Non-NDF OM), rumen fermentation profile, plasma glucose, urine volume, and excretion of allantoin in urine (Alla) of dairy cows on treatments Glycerin (GLY) and Control (CTL).

	GLY	CTL	SEM ¹	P
	% of intake			
D DM	73.8	70.1	0.47	0.10
D OM	74.1	70.8	1.01	0.02
D NDF	39.7	36.7	2.25	0.36
D Non-NDF OM	93.8	92.4	0.69	0.18
	molar% of volatile fatty acids			
Acetate	58.7	64.6	1.28	0.01
Propionate	21.5	19.8	0.88	0.18
Butyrate	19.9	15.6	0.76	0.01
Acetate/Propionate	2.79	3.30	0.170	0.04
Rumen pH	6.53	6.41	0.074	0.29
Glucose, mg/dL	51.6	58.3	1.98	0.03
Urine, L/d	26.6	22.6	2.16	0.20
Alla, mmol/d	68.6	66.9	7.32	0.87
Alla/DOMI, ² mmol/kg	6.33	5.95	0.58	0.64

¹ SEM = Standard error of the means.

² Alla/Digestible organic matter intake.

Table 5

Chewing activity of dairy cows on treatments Glycerin (GLY) and Control (CTL).

	GLY	CTL	SEM ¹	P
Eating, min/d	228	254	8.98	0.05
Rumination, min/d	318	328	12.56	0.57
Chewing, ² min/d	546	582	16.3	0.13
Eating, min/DMI	12.6	14.2	0.49	0.03
Rumination, min/DMI	17.6	18.7	0.82	0.36
Chewing, min/DMI	30.2	32.9	1.07	0.09

¹ SEM = Standard error of the means.² Chewing = Eating + Rumination.

blood glucose around calving in response to the supplementation of food grade glycerol was associated with increased butyrate in rumen fluid and also with increased plasma β -OH-butyrate (BHB). Increased production of ruminal butyrate can increase the production of BHB in the rumen epithelium. Increased BHB concentration has been observed for transition cows fed glycerol (DeFrain et al., 2004; Chung et al., 2007).

There was no evidence ($P \geq 0.64$) for the effect of treatments on the relative rumen microbial yield, estimated by the daily excretion of allantoin in urine, or the efficiency of microbial synthesis, estimated by the ratio of urinary allantoin to DOMI (Table 4). Similarly, Donkin et al. (2009) did not observe an effect of the replacement of corn with glycerol on the purine derivative to creatinine ratio in urine of dairy cows.

The substitution of corn by glycerin increased ($P = 0.03$) feeding rate, without affecting ($P = 0.57$) rumination time (Table 5). This suggests that palatability was not a problem for the GLY diet, a pertinent finding due to the viscosity fluid nature and the presence of contaminants in crude glycerin. The increase in dietary density and agglutination of feed particles by liquid glycerin is a plausible explanation for the increased intake rate on treatment GLY, potentially due to less sorting (Greter and DeVries, 2011). This is consistent with the reduction in sorting behavior of dairy cows when pure liquid glycerol was added to the diet of transition dairy cows (Carvalho et al., 2012). The lower energetic efficiency of the GLY diet cannot be explained by possible differences on the physical work of eating and rumination behaviors between treatments.

Conclusions

The complete substitution of ground corn by crude glycerin at 12% of diet DM in a low forage, high non-forage fiber sources diet increased OM digestibility and decreased rumen acetate to propionate ratio, plasma glucose concentration, milk yield, and efficiencies of feed and energy utilization.

Financial support

Brazilian National Council for Scientific and Technological Development (CNPq).

Minas Gerais State Agency for Research and Development (FAPEMIG).

CRediT authorship contribution statement

Ozana F. Zacaroni: Investigation, Formal analysis, Data curation, Writing – original draft, Visualization. **Naina M. Lopes:** Investigation. **Gilson S. Dias Júnior:** Investigation. **Trevor J. DeVries:** Writing – review & editing. **Renata A.N. Pereira:** Resources, Supervision, Project administration, Funding acquisition. **Shawn S. Donkin:** Formal analysis, Writing – review & editing. **Marcos N. Pereira:** Conceptualization, Methodology, Formal analysis, Resources, Writing – original draft, Visualization, Supervision, Funding acquisition.

Declarations of Competing Interest

None.

Acknowledgments

The authors are grateful to Brazilian National Council for Scientific and Technological Development (CNPq), Minas Gerais State Agency for Research and Development (FAPEMIG), and Grupo do Leite of University of Lavras. M.N. Pereira received a CAPES/PRINT grant “Senior Visiting Professor” at the University of Guelph (88887.582823/2020–00).

References

- AOAC International, 1990. Official Methods of Analysis, 15th ed. AOAC International, Gaithersburg.
- AOAC International, 2002. Official Methods of Analysis, 16th ed. AOAC International, Gaithersburg.
- ASTM, 2006. American Society for Testing and Materials. Petroleum products and lubricants. D6557-latest, West Conshohocken.
- Avila, J.S., Chaves, A.V., Hernandez-Calva, M., Beauchemin, K.A., McGinn, S.M., Wang, Y., Harstad, O.M., McAllister, T.A., 2011. Effects of replacing barley grain in feedlot diets with increasing levels of glycerol on in vitro fermentation and methane production. Anim. Feed Sci. Technol. 166–167, 265–268. <https://doi.org/10.1016/j.anifeeds.2011.04.016>.
- Avila-Stagno, J., Chaves, A.V., Ribeiro-Jr, G.O., Ungerfeld, E.M., McAllister, T.A., 2010. Inclusion of glycerol in forage diets increases methane production in a rumen simulation technique system. Br. J. Nutr. 111, 829–835. <https://doi.org/10.1017/S0007114513003206>.
- Benedetti, P.D.B., Fonseca, M.A., Shenkoru, T., Marcondes, M.I., de Paula, E.M., da Silva, L.G., Faciola, A.P., 2018. Does partial replacement of corn with glycerine in beef cattle diets affect in vitro ruminal fermentation, gas production kinetic, and enteric greenhouse gas emissions? PLoS ONE 13, e0199577. <https://doi.org/10.1371/journal.pone.0199577>.
- Boyd, J., Bernard, J.K., West, J.W., 2013. Effects of feeding different amounts of supplemental glycerol on ruminal environment and digestibility of lactating dairy cows. J. Dairy Sci. 96, 470–476. <https://doi.org/10.3168/jds.2012-5760>.
- Carvalho, E.R., Schmelz-Roberts, N.S., White, H.M., Doane, P.H., Donkin, S.S., 2011. Replacing corn with glycerol in diets for transition dairy cows. J. Dairy Sci. 94, 908–916. <https://doi.org/10.3168/jds.2010-3581>.
- Carvalho, E.R., Schmelz-Roberts, N.S., White, H.M., Wilcox, C.S., Eicher, S.D., Donkin, S.S., 2012. Feeding behaviors of transition dairy cows fed glycerol as a replacement for corn. J. Dairy Sci. 12, 7214–7224. <https://doi.org/10.3168/jds.2010-3584>.
- Chen, X.B., Gomes, M.J., 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: an overview of technical details. In: Int. Feed Res. Unit. Occasional Publ. Rowett Research Institute, Aberdeen, UK.
- Chizzotti, M.L., Valadares Filho, S.C., Valadares, R.F.D., Chizzotti, F.H.M., Tedeschi, L.O., 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. Livest. Sci. 113, 218–225. <https://doi.org/10.1016/j.livsci.2007.03.013>.
- Chung, Y.H., Rico, D.E., Martinez, C.M., Cassidy, T.W., Noirot, V., Ames, A., Varga, G.A., 2007. Effects of feeding dry glycerol to early postpartum Holstein dairy cows on lactational performance and metabolic profiles. J. Dairy Sci. 90, 5682–5691. <https://doi.org/10.3168/jds.2007-0426>.
- Czerkawski, J.W., Breckenridge, G., 1972. Fermentation of various glycolytic intermediates and other compounds by rumen microorganisms with particulate reference to methane production. Br. J. Nutr. 27, 131–146. <https://doi.org/10.1079/bjn19720077>.
- DeFrain, J.M., Hippen, A.R., Kalscheur, K.F., Jardon, P.W., 2004. Feeding glycerol to transition dairy cows: effects on blood metabolites and lactation performance. J. Dairy Sci. 87, 4195–4206. [https://doi.org/10.3168/jds.S0022-0302\(04\)73564-X](https://doi.org/10.3168/jds.S0022-0302(04)73564-X).
- Donkin, S.S., Koser, S.L., White, H.M., Doane, P.H., Cecava, M.J., 2009. Feeding value of glycerol as a replacement for corn grain in rations fed to lactating dairy cow. J. Dairy Sci. 92, 5111–5119. <https://doi.org/10.3168/jds.2009-2201>.
- Eells, J.T., Salzman, M.M., Lewandowski, M.F., Murray, T.G., 1996. Formate-induced alterations in retinal function in methanol-intoxicated rats. Toxicol. Appl. Pharmacol. 140, 58–69. <https://doi.org/10.1006/taap.1996.0197>.
- European Commission, 2013. Commission Regulation (EU) No 68/2013 of 16 January 2013 on the catalogue of feed materials. Off. J. Eur. Union 29, 1–64 <https://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:029:0001:0064:EN:PDF>.
- Ezequiel, J.M.B., Sancanari, J.B.D., Machado Neto, O.R., Silva, Z.F., Almeida, M.T.C., Silva, D.A.V., van Cleef, F.O.S., van Cleef, E.H.C.B., 2015. Effects of high concentrations of dietary crude glycerol on dairy cow productivity and milk quality. J. Dairy Sci. 98, 8009–8017. <https://doi.org/10.3168/jds.2015-9448>.
- Gambert, S., Héliers-Toussaint, C., Grynberg, A., 2005. Regulation of intermediary metabolism in rat cardiac myocyte by extracellular glycerol. Biochim. Biophys. Acta 1736, 152–162. <https://doi.org/10.1016/j.bbali.2005.08.004>.
- Garton, G.A., Lough, A.K., Vioque, E., 1961. Glyceride hydrolysis and glycerol fermentation by sheep rumen contents. J. Gen. Microbiol. 25, 215–225. <https://doi.org/10.1099/00221287-25-2-215>.

- Greter, A.M., Devries, T.J., 2011. Effect of feeding amount on the feeding and sorting behaviour of lactating dairy cattle. *Can. J. Anim. Sci.* 91, 47–54. <https://doi.org/10.4141/CJAS10067>.
- Goff, J.P., Horst, R.L., 2003. Oral glycerol as a gluconeogenic precursor in the treatment of ketosis and fatty liver. *Acta Vet. Scand.* 44, 40–41. <https://doi.org/10.1186/1751-0147-44-S1-P40>.
- Hansen, C.F., Hernandez, A., Mullan, B.P., Moore, K., Trezona-Murray, M., King, R.H., Pluske, J.R., 2009. A chemical analysis of samples of crude glycerol from the production of biodiesel in Australia, and the effects of feeding crude glycerol to growing-finishing pigs on performance, plasma metabolites and meat quality at slaughter. *Anim. Prod. Sci.* 49, 154–161. <https://doi.org/10.1071/EA08210>.
- Johnson, R.B., 1954. The treatment of ketosis with glycerol and propylene glycol. *Cornell Vet* 44, 6–21.
- Karlsson, J., Ramin, M., Kass, M., Lindberg, M., Holtenius, K., 2019. Effects of replacing wheat starch with glycerol on methane emissions, milk production, and feed efficiency in dairy cows fed grass silage-based diets. *J. Dairy Sci.* 102, 7927–7935. <https://doi.org/10.3168/jds.2018-15629>.
- Kass, M., Ariko, T., Kaart, T., Rihma, E., Ots, M., Arney, D., Kärt, O., 2012. Effect of replacement of barley meal with crude glycerol on lactation performance of primiparous dairy cows fed a grass silage-based diet. *Livest. Sci.* 150, 240–247. <https://doi.org/10.1016/j.livsci.2012.09.007>.
- Kupczyński, R., Szumny, A., Wujcikowska, K., Pachura, N., 2020. Metabolism, ketosis treatment and milk production after using glycerol in dairy cows: A review. *Animals (Basel)* 10, 1379. <https://doi.org/10.3390/ani10081379>.
- Lin, E.C.C., 1977. Glycerol utilization and its regulation in mammals. *Annu. Rev. Biochem.* 46, 765–766. <https://doi.org/10.1146/annurev.bi.46.070177.004001>.
- Maynard, L.A., Loosli, J.K., Hintz, H.F., Warner, R.G., 1979. *Animal Nutrition*. McGraw-Hill, New York seventh ed.
- NRC, 2001. *Nutrient Requirements of Dairy Cattle*. National Academic Press, Washington seventh ed.
- Pereira, M.N., Garrett, E.F., Oetzel, G.R., Armentano, L.E., 1999. Partial replacement of forage with nonforage fiber sources in lactating cow diets. I. Performance and health. *J. Dairy Sci.* 82, 2716–2730. [https://doi.org/10.3168/jds.S0022-0302\(99\)75528-1](https://doi.org/10.3168/jds.S0022-0302(99)75528-1).
- Pereira, M.N., Morais Júnior, N.N., Caputo Oliveira, R., Salvati, G.G.S., Pereira, R.A.N., 2021. Methionine precursor effects on lactation performance of dairy cows fed raw or heated soybeans. *J. Dairy Sci.* 104, 2996–3007. <https://doi.org/10.3168/jds.2020-18696>.
- Pol, A., Demeyer, D., 1988. Fermentation of methanol in the sheep rumen. *Appl. Environ. Microbiol.* 54, 832–834. <https://doi.org/10.1128/aem.54.3.832-834.1988>.
- Rémond, B., Souday, E., Jouany, J.P., 1993. In vitro and in vivo fermentation of glycerol by rumen microbes. *Anim. Feed Sci. Technol.* 41, 121–132. [https://doi.org/10.1016/0377-8401\(93\)90118-4](https://doi.org/10.1016/0377-8401(93)90118-4).
- Sebe, A., Satar, S., Uzun, B., Topal, M., Yesilgac, H., Avci, A., 2006. Intracranial hemorrhage associated with methanol intoxication. *Mt. Sinai J. Med.* 73, 1120–1122.
- Shin, J.H., Kim, S.C., Wang, D., Adesogan, A.T., Staples, C.R., 2012. Effects of feeding crude glycerol on performance and ruminal kinetics of lactating Holstein cows fed corn silage- or cottonseed hull-based, low-fiber diets. *J. Dairy Sci.* 95, 4006–4016. <https://doi.org/10.3168/jds.2011-5121>.
- Thompson, J.C., He, B.B., 2006. Characterization of crude glycerol from biodiesel production from multiple feedstock. *Appl. Eng. Agric.* 22, 261–265. <https://doi.org/10.13031/2013.20272>.
- Wang, C., Liu, Q., Yang, W.Z., Huo, W.J., Dong, K.H., Huang, Y.X., Yang, X.M., He, D.C., 2008. Effects of glycerol on lactation performance, energy balance and metabolites in early lactation Holstein dairy cows. *Anim. Feed Sci. Technol.* 151, 12–20. <https://doi.org/10.1016/j.anifeedsci.2008.10.009>.
- Wildman, E.E., Jones, G.M., Wagner, P.E., Boman, R.L., Troutt Junior, H.F., Lesch, T.N., 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.* 65, 495–501. [https://doi.org/10.3168/jds.S0022-0302\(82\)82223-6](https://doi.org/10.3168/jds.S0022-0302(82)82223-6).