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Ruminal and Plasma Responses in Dairy Cows to Drenching or Feeding Glycerol

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ABSTRACT

Four Holstein dairy cows (137 DIM, 60 kg milk/d) were used in a Latin square with 1-wk periods to evaluate the effect of methods of oral delivery of glycerol on ruminal VFA and plasma concentrations of glucose, ß-hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA), and insulin. All cows were fed only grass hay for ad libitum consumption during 12 h before the experiment. At the start of the experiment, time 0, all cows were fed 5 kg of cracked corn. Treatments administered at time 0 were: 1) control (C), no glycerol; 2) fed glycerol (F), 1 kg of glycerol solution (80% glycerol) added to the corn; 3) drench glycerol (D), 1 kg of glycerol solution in 1 L of water and delivered as oral drench; and 4) tube delivery of glycerol (T), 1 kg of glycerol solution in 9 L of water and delivered into the rumen via an esophageal tube. Blood samples were collected at -1, -0.5, 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, and 24 h after administering glycerol. Rumen samples were collected at 0, 2, 4, and 6 h. After administration of glycerol, concentrations of acetate decreased in rumens of cows while propionate and butyrate were increased by glycerol with peak concentrations at 4 h. Concentrations of glucose were increased in plasma of D and T compared with C, reaching peak concentrations at 1.5 and 3 h for D and T, respectively. Glucose response expressed as area under the curve (AUC) over baseline for 6 h was greater for D and T compared with C. Insulin concentrations in plasma were increased for D and T reaching peak concentrations at 1.4 and 1.1 h for D and T respectively. The 6-h AUC for insulin concentrations were greater for D and T than for F and C. The BHBA was increased in plasma of D, T, and F compared with C, reaching peak concentrations at 2.5, 2.4, and 1.6h for D, T, and F, respectively. These data demonstrate that the ability of glycerol to increase plasma concentrations of glucose and insulin is dependent upon rapid delivery.
Keywords: ß-hydroxybutyrate, dairy cows, glucose, glycerol

INTRODUCTION

Ketosis is an ongoing problem in high producing diary cows. Ketosis usually occurs between the second and seventh week of lactation when metabolic priority is given to the demands of milk production and appetite is often limited (Baird, 1982). Ketosis can be characterized by a drop in blood glucose and the subsequent elevation of blood ketone bodies and NEFA as fat stores are mobilized for energy (Schultz, 1968). One of the most

common methods of ketosis treatment is to administer glucogenic precursors, such as propylene glycol, through a drench. It has been known for some time that glycerol serves as an equal or better treatment for ketosis than common drenches like propylene glycol. (Johnson, 1955) The problem with glycerol has always been that the high cost eliminates it as a viable alternative for most producers.

With the advent of the biodiesel industry, of which glycerol is a byproduct, glycerol may become affordable for the average producer as a means of ketosis prevention/ treatment when delivered as an oral drench (Crandall, 2004). The question still remains as to the best method of delivery. It would be ideal to simply feed the glycerol and thereby avoid the need to provide it as a drench which is both labor intensive and stressful to the animal. The objective of this study was to determine whether feeding glycerol would provide the same positive effects as using it as a drench.

MATERIALS AND METHODS

Cows and Sampling

Four multiparous, lactating, Holstein cows (137 DIM, 60 kg milk/ day) were selected in July 2003 at the South Dakota State University Dairy Research and Training Facility. (Brookings, SD) The cows were used in a Latin square with 1-wk periods. Using protocols approved in accordance with SDSU IACUC policyCows were housed in individual tie-stalls, and fed ad libitum grass hay for 12 h prior to the start of the experiment to try to induce a mild ketosis. At the start of the experiment all cows were fed 5 kg of cracked corn as well as their assigned treatment. The treatments were: 1) control (C), no glycerol; 2) fed glycerol (F; West Central Soy, Ralston, IA), 1 kg of glycerol solution mixed with the cracked corn; 3) drench glycerol (D), 1 kg of glycerol solution mixed with 1 L of water and delivered as an oral drench directly into the oral cavity; 4) tube delivery of glycerol (T), 1 kg of glycerol solution mixed with 9 L of water and delivered directly into the rumen by a Cattle Pump System esophageal pump (The McGrath Company McCook, NE). The composition of the glycerol solution was 80.2% glycerol, 11.5% salt 6.6% water, and 1.3% methanol (DeFrain, 2004).

At 14 h prior to treatments, jugular catheters (Angiocath, Becton Dickinson and Co., Franklin Lakes, NJ) were inserted to assist in the collection of serial blood samples. Blood samples were collected into evacuated tubes containing K-EDTA (Becton Dickinson and Co., Franklin Lakes, NJ) at –1, -.5, 0, .25, .5, .75, 1, 1.5, 2, 4, 6, 8, 12, and 24 h relative to time of administration of glycerol, and immediately placed on ice. Plasma was separated by centrifugation (American Scientific Products, McGraw Park, Ill.) at 5,800 x g for 10 minutes and stored at -20°C for later analysis. Rumen fluid was collected at 0, 2, 4, and 6 h relative to time of administration of glycerol by applying vacuum pressure to an esophageal tube fitted with a suction strainer. (DeFrain, 2004) One milliliter of 0.5 M sulfuric acid was mixed with a 10 ml sample of rumen fluid and the sample was frozen at -20°C for later analysis.

Laboratory Analysis

Plasma samples were thawed and concentrations of glucose were determined using glucose oxidase (Sigma Kit #315, Sigma Diagnostics, St. Louis, MO) according to the procedures of Trinder (1969). Concentrations of ß-hydroxybutyrate (BHBA) were determined (Sigma Kit 310-A, Sigma Diagnostics, St. Louis, MO) following the methods of Williamson et al. (1962), and concentrations of nonesterified fatty acids (NEFA) were determined using a colorimetric assay (NEFA-C Kit, Wako Chemicals, Richmond, VA), following modifications by Johnson and Peters (1993). Insulin was quantified by solid-phase radioimmunoassay. (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA)

For determination of VFA, rumen fluid samples were thawed and centrifuged at 32,000 x g for 20 min. (Jouan Inc. Winchester, VA). The concentrations of individual VFA were determined by gas chromatography (model 6890. Hewlett-Packard) using 2-ethylbutyrate as an internal standard in a 2-m glass column packed with GP 15% SP-1220/1% H3PO4 on Chromosorb w AW (Supelco Inc., Bellefonte, PA). Helium was used as the carrier as at a flow rate of 20 ml/min, and the temperature was isothermal at 200°C for injector, column and detector.

Statistical Analyses

Plasma data were analyzed as repeated measures using PROC MIXED of SAS software, version 8.1 (1999). For each variable, cow, period, and treatment were subjected to 5 covariance structures: variance components, autoregressive order one, compound symmetry, toeplitz, and unstructured. The structure yielding the Akaike's information criteria closest to 0 was used. For all metabolites, the model included treatment, period, time, and the treatment x time interaction. Plasma response data for glucose, BHBA, and insulin, included time to peak, peak height, and area under curve (AUC) and were calculated by integration of the first 6 h of data using Microcal Origin version 6.0. For calculation of response data, the mean values of the three samples collected prior to and including time 0 were used to determine baseline. Concentrations during the first 6 h that were greater than baseline were used to calculate AUC. Response data was analyzed with treatment and period as main effects.

Rumen VFA data, including acetate, propionate, butyrate, acetate:propionate, butyrate:propionate, and acetate+butyrate:propionate, were analyzed as repeated measures using PROC MIXED of SAS software, version 8.1 (1999). The model for rumen VFA included treatment, period, time, and the treatment x time interaction. Preplanned contrasts for comparison of all data included: 1. glycerol vs. control, 2. feed vs. forced, and 3. drench vs. tube.

Results and Discussion

DeFrain, et al. (2004) established the current knowledge available on feeding glycerol to transition cows. This research did not demonstrate the increases in blood glucose described by earlier researchers who delivered glycerol via esophageal drench (Johnson, 1955; Goff and Horst, 2001). The current experiment was able to directly compare methods of delivery as a possible explanation for inconsistencies.

Plasma glucose concentrations are illustrated in Figure 1. Peak glucose concentrations for all treatments occurred between 0.75 and 3h post feeding. Peak glucose concentrations were 77.4, 91.6, 79.0, and 90.0 mg/dl ($P < 0.01$) for C, D, F, and T respectively. The significant increase in plasma glucose for drenching and tubing is in agreement with Goff and Horst (2001). Contrasts for glycerol vs. none and fed vs. forced glycerol were significant ($P < 0.01$).

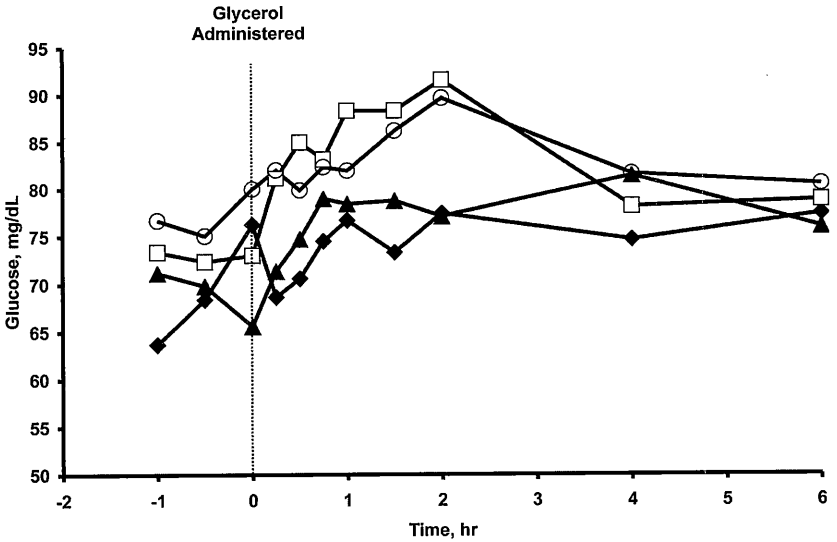


Figure 1. Concentrations of glucose (pooled SEM = 4.21) in plasma of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

The 6-h response curves for plasma glucose are in Table 1. Peak height was increased significantly by glycerol supplementation, and again by forcing glycerol vs. feeding it ($P = 0.04$ and $P = 0.03$ for contrasts 1 and 2 respectively). Area under the response curve was increased ($P = 0.05$) by glycerol supplementation, and there was a tendency for further increase ($P = 0.08$) by delivering glycerol as either a drench or by espohageal tube. The slight increase in plasma glucose associated with feeding glycerol is in contrast with DeFrain, et al. (2004). This could possibly be explained by feeding glycerol in a concentrate vs. feeding glycerol as part of a total mixed ration.

	Treatment					Contrast		
	Control	Drench	Feed	Tube	SEM	Glycerol	Feed v. Forced	Drench v. Tube
						P<		
Glucose								
TP[1], h	2.9	1.5	1.6	3.0	0.8	0.38	0.44	0.24
PH[2], mg/dl	5.8	21.0	8.0	18.5	3.9	0.04	0.03	0.62
AUC[3],(mg x h)/dl	9.5	54.6	23.7	58.2	13.8	0.05	0.08	0.85
Insulin								
TP, h	2.2	1.5	1.0	1.1	0.8	0.29	0.80	0.83
PH, mg/dl	27.6	96.7	29.3	114.8	18.8	0.03	< 0.01	0.44
AUC,(mg x h)/dl	80.2	244.5	67.1	270.1	47.6	0.05	< 0.01	0.66
BHBA								
TP, h	1.2	2.5	1.6	2.4	0.5	0.12	0.2	0.86
PH, mg/dl	1.3	1.3	2.2	3.0	0.4	0.16	0.94	0.03
AUC,(mg x h)/dl	4.0	7.8	5.5	8.4	2.8	0.34	0.48	0.87

1 TP = Time to Peak; 2 PH = Peak Height; 3 AUC = Area Under Curve

Table 1. Plasma metabolite responses in cows treated with glycerol by adding to diet, drenching, or by esphogeal tube.

Plasma insulin also significantly increased ($P < 0.01$) with glycerol supplementation (Figure 2). Plasma insulin concentrations reached peak levels for all treatments between 1 and 2 h post feeding. Peak plasma insulin levels were 73.4, 144.9, 78.0, and 136.5 pg/ml for C, D, F, T respectively. These results, unlike the plasma glucose concentrations, agree with DeFrain, et al. (2004) for feeding glycerol. Peak height (Table 1) was increased significantly by glycerol supplementation, and again by forcing glycerol vs. feeding it ($P = 0.03$ and < 0.01 for contrasts 1 and 2 respectively). Area under the response curve was also increased by glycerol supplementation ($P = 0.05$) and again by either drenching or tubing glycerol ($P < 0.01$).

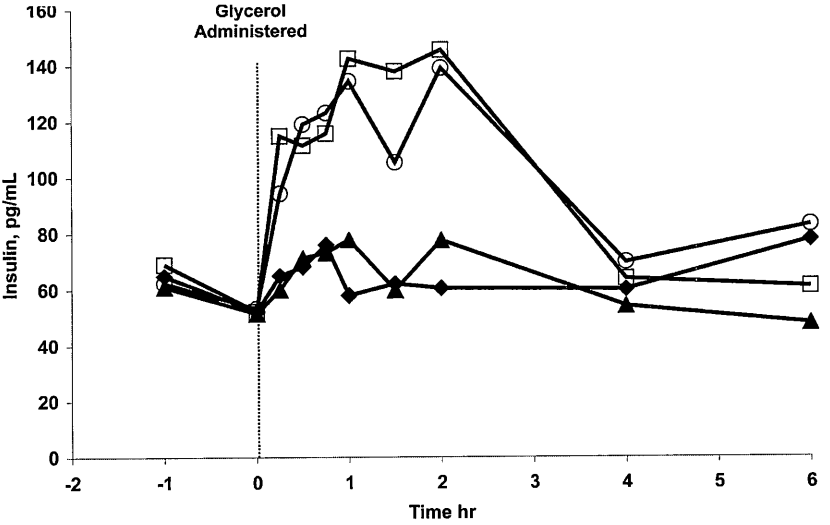
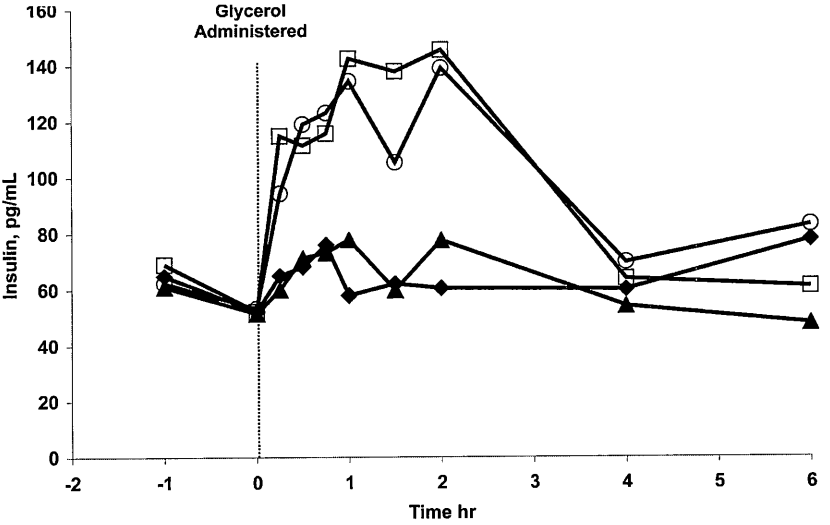


Figure 2. Concentrations of insulin (pooled SEM = 17.02) in plasma of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

Plasma BHBA (Figure 3) was also significantly increased by glycerol supplementation. ($P < 0.01$). Peak plasma BHBA levels were 4.81, 6.98, 6.54, 7.04 mg/dl for C, D, F, and T, respectively. The greater increase of plasma BHBA for feeding glycerol vs. drenching or tubing glycerol as compared with plasma glucose or insulin is in agreement with DeFrain et al. (2004), and could possibly be explained by the significant increase of butyrate in the rumen (Figure 7). Peak height of BHBA (Table 1) over baseline values was greatest for tubing glycerol ($P = 0.03$ for contrast 3).



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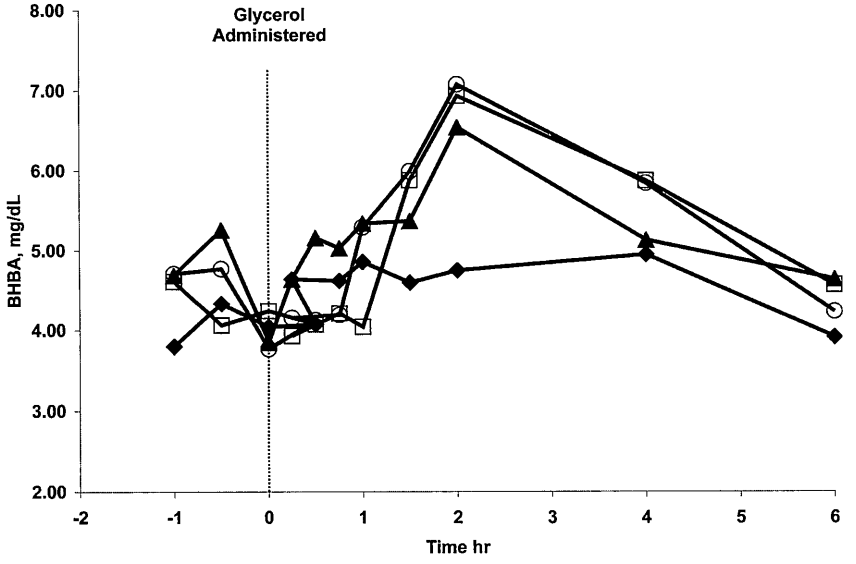

Figure 3. Concentrations of BHBA (pooled SEM = 0.86) in plasma of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

Concentration of NEFA in plasma (Figure 4) was significantly decreased by glycerol supplementation ($P < 0.01$). Plasma NEFA levels at nadir were 530, 260, 380, and 230 μEq/L for C, D, F, and D, respectively. This could be attributable to a greater availability of plasma glycerol for the esterification FA. Plasma glycerol was not quantified.

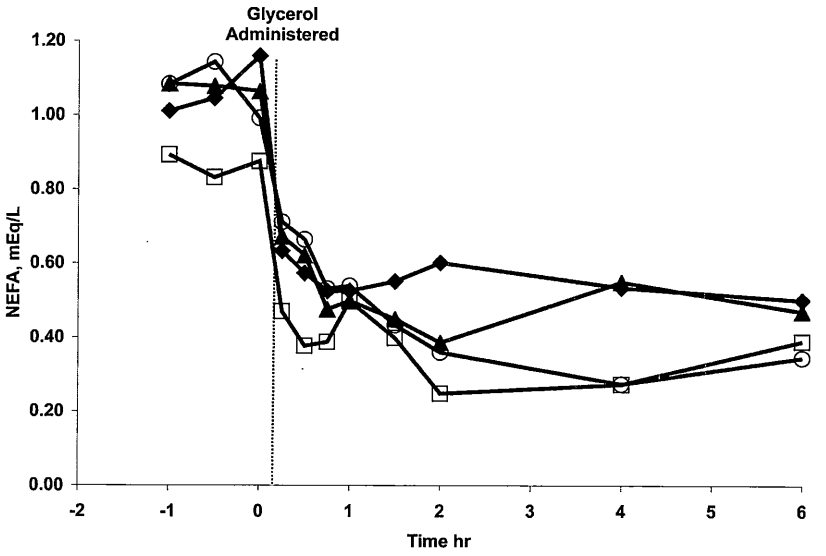

Figure 4. Concentrations of NEFA (pooled SEM = 0.14) in plasma of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

Glycerol supplementation significantly decreased ($P < 0.01$) ruminal acetate production (Figure 5) and tended to decrease acetate further by drenching and tubing ($P = 0.07$). Ruminal propionate production was significantly increased ($P < 0.01$) by glycerol supplementation (Figure 6) and drenching and tubing exhibited greater response than did feeding ($P = 0.02$). Ruminal butyrate production was also significantly increased ($P < 0.01$) by glycerol supplementation (Figure 7) and response was greater by drenching and tubing compared with feeding ($P = 0.05$). These data are in agreement with other research (Fischer et al., 1971; Rémond et al., 1993; Khalili et al., 1997; Schröder and Südekum, 1999) which described an increase in ruminal fermentation to butyrate associated with glycerol supplementation. This increase in fermentation to butyrate may also explain the increase in plasma BHBA in the presence of an increase in plasma glucose, as butyrate is extensively metabolized to BHBA by the rumen epithelium before delivery to circulation (Weigand, 1975).

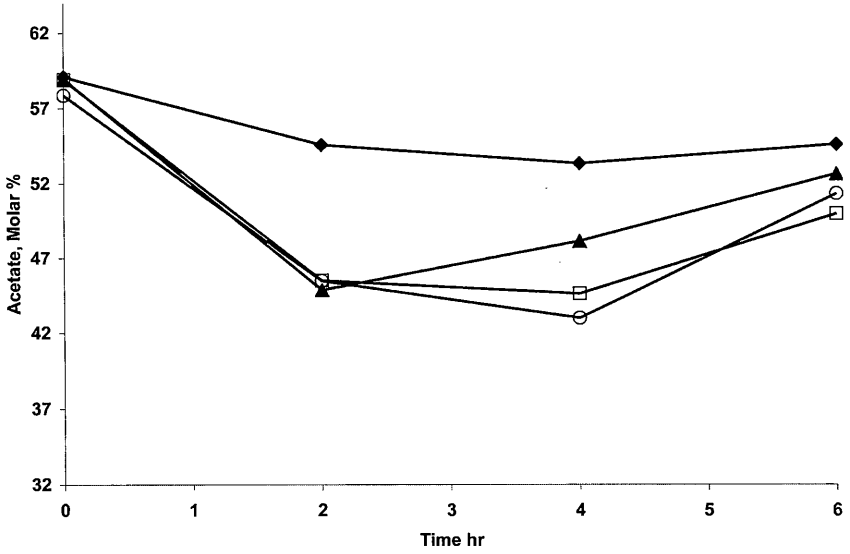

Figure 5. Molar percentages of acetate (pooled SEM = 1.54) in rumen fluid of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

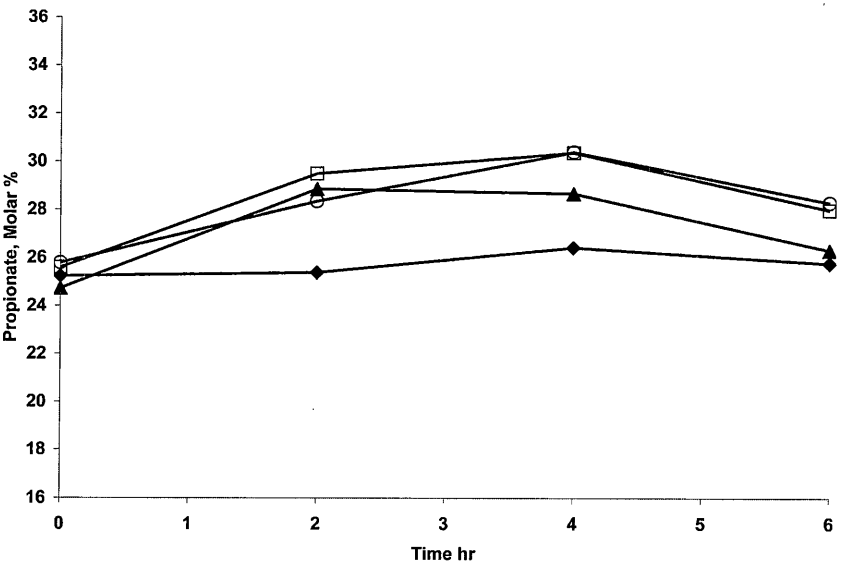

Figure 6. Molar percentages of propionate (pooled SEM = 0.86) in rumen fluid of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

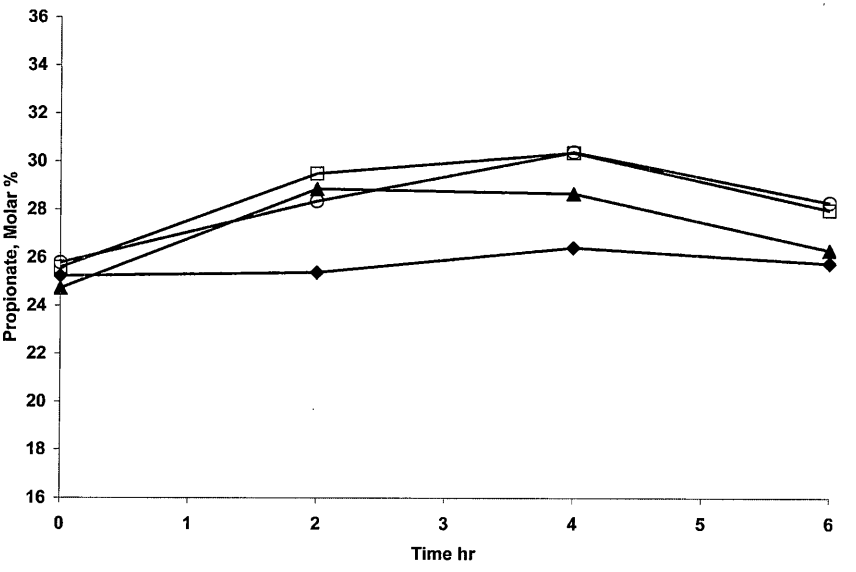

Figure 6. Molar percentages of propionate (pooled SEM = 0.86) in rumen fluid of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

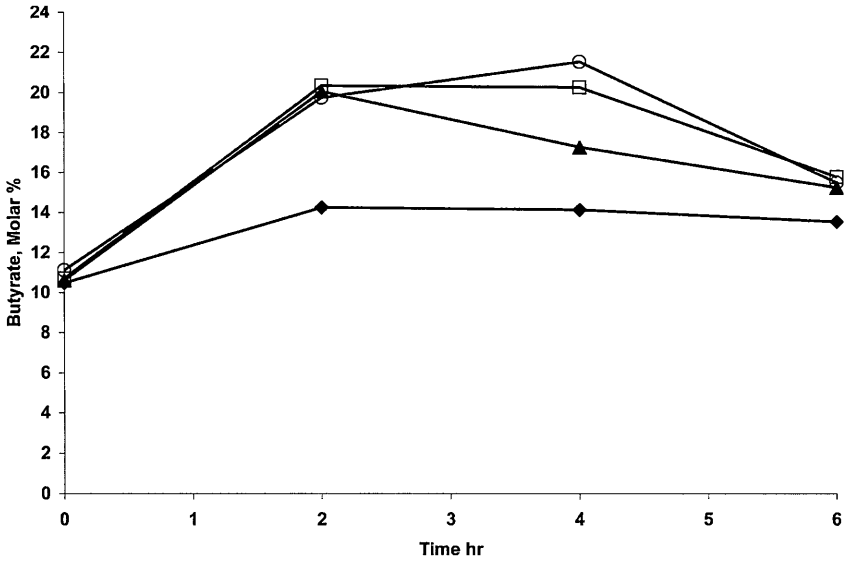

Figure 7. Molar percentages of butyrate (pooled SEM = 0.96) in rumen fluid of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

CONCLUSIONS

Providing the cow with glycerol by drenching or tubing increases plasma glucose, insulin, and BHBA. Feeding glycerol increases plasma BHBA without increasing plasma glucose or insulin. Feeding glycerol also leads to increased ruminal fermentation to propionate and butyrate, and decreased fermentation to acetate. Increased fermentation to butyrate can lead to increased blood ketone bodies, BHBA, without a concurrent increase in blood glucose concentrations, which could lead to an increased likelihood of ketosis in dairy cows. It is the conclusion of these researchers that inclusion of glycerol in the feed may not be the best treatment or preventative for ketosis; however, the benefits of feeding glycerol to the lactating animal in a positive energy balance have not been adequately defined.

ACKNOWLEDGMENTS

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