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Short communication: Regulation of hepatic gluconeogenic enzymes by dietary glycerol in transition dairy cows

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ABSTRACT

Nutritional status and glucose precursors are known regulators of gluconeogenic gene expression. Glycerol can replace corn in diets fed to dairy cows and use of glycerol is linked to increased rumen propionate production. The effect of dietary glycerol on the regulation of gluconeogenic enzymes is unknown. The objective of this study was to examine the effect of glycerol on expression of pyruvate carboxylase (PC), cytosolic and mitochondrial phosphoenolpyruvate carboxykinase (PEPCK-C and PEPCK-M), and glucose-6-phosphatase. Twenty-six multiparous Holstein cows were fed either a control diet or a diet where high-moisture corn was replaced by glycerol from −28 through +56 d relative to calving (DRTC). Liver tissue was collected via percutaneous liver biopsy at −28, −14, +1, +14, +28, and +56 DRTC for RNA analysis. Expression of PC mRNA increased 6-fold at +1 and 4-fold at +14 DRTC relative to precalving levels. Dietary glycerol did not alter expression of PC mRNA expression. Expression of PEPCK-C increased 2.5-fold at +14 and 3-fold at +28 DRTC compared with +1 DRTC. Overall, dietary glycerol increased PEPCK-C expression compared with that of cows fed control diets. The ratio of PC to PEPCK-C was increased 6.3-fold at +1 DRTC compared with precalving and tended to be decreased in cows fed glycerol. We detected no effect of diet or DRTC on PEPCK-M or glucose-6-phosphatase mRNA, and there were no interactions of dietary treatment and DRTC for any transcript measured. Substituting corn with glycerol increased the expression of PEPCK-C mRNA during transition to lactation and suggests that dietary energy source alters hepatic expression. The observed increase in PEPCK-C expression with glycerol feeding may indicate regulation of hepatic gene expression by changes in rumen propionate production.
Key words: glycerol, gluconeogenesis, transition cow

Short Communication

Glycerol is a byproduct of biodiesel production, which is increasing with the current emphasis on alternatives to petroleum, thus reducing some of the historical cost constraints for glycerol as a feed ingredient. Previous research has supported use of oral administration of glycerol to alleviate ketosis (Johnson, 1954; Goff and Horst, 2003) and as an energy substrate and glucose precursor that increases feed intake and improves energy balance (Fisher et al., 1971, 1973). Previous feeding studies have indicated that glycerol can be incorporated into rations of mid-lactation cows to 15% of ration DM (Chung et al., 2007; Donkin et al., 2009) without adverse effects on cow health, milk production, or milk composition. Incorporation of glycerol to 11.5% of DM in rations for transition cows also did not negatively affect feed intake or milk production (Carvalho et al., 2011). Feeding glycerol reduced feed sorting in transition cows and increased the proportion of propionate and butyrate in the rumen, at the expense of acetate (Carvalho et al., 2011).

Propionate produced in the rumen is the primary gluconeogenic precursor in ruminant animals (Wiltrout and Satter, 1972; Lomax and Baird, 1983; Annison and Bryden, 1999) and conversion of propionate carbon to glucose is controlled by the abundance of cytosolic phosphoenolpyruvate (**PEPCK-C**), a key enzyme for gluconeogenesis in liver (Aschenbach et al., 2010). Increased expression of PEPCK-C in transition cows is linked to increased ruminal propionate production, including increased feed intake (Greenfield et al., 2000) and monensin feeding (Karcher et al., 2007) and suggests feed-forward control of propionate metabolism. Given the shift in VFA produced in the rumen with dietary glycerol inclusion (Carvalho et al., 2011), we hypothesized that inclusion of glycerol into transition cow diets would alter gluconeogenic enzymes, specifi-

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Table 1. Ingredient and nutrient composition of the pre- and postpartum experimental diets

Item	Prepartum		Postpartum	
	Control	Glycerol	Control	Glycerol
Ingredient, % of DM				
Corn silage	35.4	35.4	39.0	39.0
Alfalfa haylage	8.0	8.0	15.5	15.5
Grass hay	13.0	13.0	3.5	3.5
Wheat straw	—	—	1.5	1.5
Cotton seed hulls	6.0	6.0	—	—
Soybean hulls	7.8	7.8	2.0	2.0
High moisture corn	14.0	—	12.5	—
Glycerol	—	11.5	—	10.8
Soybean meal	—	2.5	10.0	11.0
Megalac R[1]	—	—	0.7	0.7
Protein blend[2]	—	—	5.3	6.0
Supplement[3,4]	15.8	15.8	10.0	10.0
Chemical composition[5]				
DM, %	50.9	49.4	46.8	46.0
CP, % of DM	16.6 (1.00)	16.6 (1.35)	18.2 (0.83)	18.7 (1.00)
ADF, % of DM	22.9 (1.75)	25.5 (1.79)	19.5 (1.77)	20.8 (2.32)
NDF, % of DM	38.0 (1.18)	42.2 (1.35)	31.4 (2.71)	34.2 (1.67)
Starch, % of DM	22.6 (2.64)	15.0 (1.22)	26.7 (1.73)	19.2 (1.12)
NE_L, Mcal/kg of DM	1.58 (0.02)	1.61 (0.05)	1.65 (0.02)	1.61 (0.02)
Ca, % of DM	1.09 (0.17)	1.02 (0.12)	1.11 (0.07)	1.11 (0.20)
P, % of DM	0.36 (0.02)	0.34 (0.02)	0.43 (0.02)	0.40 (0.04)
Mg, % of DM	0.39 (0.04)	0.36 (0.02)	0.36 (0.04)	0.35 (0.02)
K, % of DM	1.22 (0.05)	1.29 (0.09)	1.47 (0.11)	1.44 (0.03)
Na, % of DM	0.15 (0.01)	0.15 (0.01)	0.32 (0.01)	0.32 (0.02)

[1]Church & Dwight Co., Princeton, NJ.

[2]Contained 44% Aminoplus (Ag Processing Inc., Omaha, NE), 3% menhaden fish meal, 53% ProvAAL STD 5000 (Perdue Agribusiness, Salisbury, MD).

[3]Prepartum: contained 38.29% soybean meal, 25.65% Bio-Chlor (Church & Dwight Co.), 5.4% $CaCO_3$, 2.16% dicalcium phosphate, 1.08% MgO, 1.08% NaCl, 1.65% mineral-vitamin premix (16.11% Ca, 2.11% S, 31,505 mg/kg Zn, 8,036 mg/kg Cu, 26,020 mg/kg Mn, 140 mg/kg Se, 473 mg/kg Co, 284 mg/kg I, 1,440 kIU/kg vitamin A, 416 kIU/kg vitamin D, 6,647 IU/kg vitamin E), 2.16% $MgSO_4$, 5.08% Megalac R (Church & Dwight Co.), 0.49% Niacinamide (99.5% niacin), 2.62% yeast culture (Diamond V Mills, Cedar Rapids, IA), 1.8% vitamin E 20,000, 0.08% Rumensin 80 (Elanco, Greenfield, IN), 2.62% Omnigen-AF (Prince-Agri Products, Quincy, IL), 1.08% urea, 4.38% blood meal, 3.81% Aminoplus, 0.57% menhaden fish meal.

[4]Postpartum: contained 25% dried molasses, 42.75% finely ground corn, 7.5% $CaCO_3$, 5% dicalcium phosphate, 6.2% $NaHCO_3$, 2% MgO, 2% DCAD plus, 0.5% potassium magnesium sulfate, 2.5% NaCl, 2.025% mineral/vitamin premix (16.11% Ca, 2.11% S, 31,505 mg/kg Zn, 8,036 mg/kg Cu, 26,020 mg/kg Mn, 140 mg/kg Se, 473 mg/kg Co, 284 mg/kg I, 1,440 kIU/kg vitamin A, 416 kIU/kg vitamin D, 6,647 IU/kg vitamin E), 0.25% Niacinamide (99.5% niacin), 2% yeast culture (Diamond V Mills), 0.213% vitamin E 20,000, 0.062% Rumensin 80, 2% Omnigen-AF (Prince-Agri Products).

[5]Mean (SD) analysis for composite samples (n = 5).

cally PEPCK-C. The objective of this experiment was to determine the effects of replacing high-moisture corn with glycerol on expression of hepatic gluconeogenic enzymes during the transition to lactation period.

Twenty-six multiparous Holstein cows were paired by expected calving date and randomly assigned to receive either a diet containing corn silage, alfalfa haylage, hay, high-moisture corn, cottonseed hulls, soybean hulls, vitamins, and minerals (control) or a diet in which high-moisture ear corn was replaced with a mixture of glycerol and soybean meal (glycerol). Refined glycerol (99.5% USP grade glycerin; Pt Sumi Ashi Oleochemicals Industry, Jakarta, Indonesia) was included at 11.5 and 10.8% of the ration DM for the pre- and postpartum diets, respectively (Table 1). Soybean meal was added to the glycerol diets to adjust for the protein removed from the diet with the removal of high-moisture corn.

Cows were housed in individual tie stalls at the Purdue Dairy Research and Education Center (W. Lafayette, IN) and fed diets formulated to meet or exceed the NRC (2001) guidelines for 600-kg dairy cattle. Diets were fed as a TMR once daily between 0630 to 0730 h in amounts that ensured ad libitum consumption and approximately 10 to 15% feed refusals. Cows were milked twice daily at approximately 0700 and 1830 h. Animal use and handling protocols were approved by the Purdue Animal Care and Use Committee.

Liver biopsy samples were collected on −28, −14, +1, +14, +28, and +56 d relative to calving (**DRTC**), as described previously (Greenfield et al., 2000; Velez