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Addition of glycerol to lactating cow diets stimulates dry matter intake and milk protein yield to a greater extent than addition of corn grain

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ABSTRACT

The objective of this study was to determine if the addition of glycerol to the diet of dairy cows would stimulate milk protein yield in the same manner as the addition of corn grain. Twelve multiparous lactating dairy cows at 81 ± 5 d in milk were subjected to 3 dietary treatments in a replicated 3 × 3 Latin square design for 28-d periods. The diets were a 70% forage diet considered the basal diet, the basal diet with 19% ground and high-moisture corn replacing forages, and the basal diet with 15% refined glycerol and 4% added protein supplements to be isocaloric and isonitrogenous with the corn diet. Cows were milked twice a day and samples were collected on the last 7 d of each period for compositional analysis. Within each period, blood samples were collected on d 26 and 27, and mammary tissue was collected by biopsy on d 28 for Western blot analysis. Dry matter intake increased from 23.7 kg/d on the basal diet to 25.8 kg/d on the corn diet and 27.2 kg/d on the glycerol diet. Dry matter intake tended to be higher with glycerol than corn. Milk production increased from 39.2 kg/d on the basal diet to 43.8 kg/d on the corn diet and 44.2 kg/d on the glycerol diet. However, milk yield did not differ between corn and glycerol diets. Milk lactose yields were higher on the corn and glycerol diets than the basal diet. Milk fat yield significantly decreased on the glycerol diet compared with the basal diet and tended to decrease in comparison with the corn diet. Mean milk fat globule size was reduced by glycerol feeding. Milk protein yield increased 197 g/d with addition of corn to the basal diet and 263 g/d with addition of glycerol, and the glycerol effect was larger than the corn effect. The dietary treatments had no effects on plasma glucose concentration, but plasma acetate levels decreased 27% on the glycerol diet. Amino acid concentrations were not affected by dietary treatments, except for branched-chain amino acids, which decreased 22% on the glycerol diet compared with the corn diet. The decreases in plasma acetate and branched-chain amino acid concentrations with glycerol and the larger effects of glycerol than corn on milk protein and fat yields suggest that glycerol is more glucogenic for cows than corn grain.
Key words: glycerol, milk synthesis, mammary, milk fat globule

INTRODUCTION

Milk protein yield in dairy cattle is stimulated by an elevated postruminal supply of glucose or propionate (Rulquin et al., 2004; Raggio et al., 2006), as well as higher inclusion levels of dietary grain (Macleod et al., 1983). The mechanism responsible for this stimulation of milk protein yield is unclear. The effect of grain feeding may be explained in part by an increase in microbial protein outflow from the rumen (Rode et al., 1985), leading to greater absorption of EAA, but microbial outflow is not always affected (Klusmeyer et al., 1991; Cameron et al., 1991). Insulin, which is released during glucose infusion and grain feeding (Macleod et al., 1983; Rulquin et al., 2004), stimulates milk protein yield (Mackle et al., 2000; Bequette et al., 2001), presumably through the insulin signaling cascade inside mammary epithelial cells. Essential amino acids and insulin are both activators of the mechanistic target of rapamycin complex 1 (**mTORC1**) in mammary epithelial cells (Burgos et al., 2010; Appuhamy et al., 2011) that may be responsible for nutritional stimulation of milk protein synthesis. Glucose, glucose precursors, and grains also depress milk fat production (Balch et al., 1955; Sutton, 1989; Lemosquet et al., 1997; Hurtaud et al., 1998; Rigout et al., 2002), partly through conjugated linoleic acid formation in the rumen (Loor and Herbein, 1998; Bauman et al., 2008) and partly through insulin-

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mediated inhibition of mammary lipoprotein lipase activity (Mackle et al., 2000; Cant et al., 2002).

Glycerol is a glucogenic precursor (Cori and Shine, 1935) that may become available as a feed ingredient for cattle as a co-product of biodiesel manufacture. As a glucogenic feedstuff, glycerol has several features in common with corn grain. Degradation of glycerol in the rumen increases the proportions of propionate and butyrate at the expense of acetate (Rémond et al., 1993; Wang et al., 2009a). Ruminal degradation of corn grain also increases propionate and butyrate at the expense of acetate (Hess et al., 1996; Valadares et al., 1999). In addition to VFA production, 30 to 50% of the starch in corn grain is digested to glucose in the small intestine (Rémond et al., 2004; Taylor and Allen, 2005), a portion of which may enter the bloodstream. In a similar fashion, approximately half of the glycerol consumed is absorbed across the rumen wall into blood, where it becomes available for hepatic gluconeogenesis (Rémond et al., 1993). Because of these similarities in fermentation pattern and glucogenicity, glycerol may be comparable to glucose or corn grain in its effect on milk component yields.

Whether addition of glycerol to the diet stimulates milk protein yield the way addition of corn grain does has not been evaluated to our knowledge. In several experiments, isocaloric inclusions of refined glycerol up to 15% of diet DM have not affected intake, rumen microbial protein yield, or milk protein yield (Khalili et al., 1997; Carvalho et al., 2011; Kass et al., 2012). Isocaloric substitution of 8% crude glycerol for corn grain increased milk protein yield in an experiment by Wilbert et al. (2013). Hypercaloric additions of glycerol to the diet, instead of isocaloric substitutions, have been made at much smaller doses and in the context of preventing metabolic disorders associated with the transition into lactation (DeFrain et al., 2004; Chung et al., 2007; Wang et al., 2009b). These additions have not resulted in changes in DM intakes or milk component yields. When glycerol was included at 20 g/L in the drinking water of transition cows, for a glycerol intake equal to 1.3 kg/d or 10% of DMI, ad libitum consumption of the diet decreased so that NE_L intake was not affected and milk component yields remained unchanged (Osborne et al., 2009). Isocaloric replacement of concentrate with glycerol depressed milk fat yield in one experiment (Vallance and McClymont, 1959) but not in others (Khalili et al., 1997; Carvalho et al., 2011; Kass et al., 2012). Effects on milk yield and composition may be difficult to detect with statistical significance when glycerol replaces grain isocalorically, but they may be easier to detect when glycerol is added hypercalorically to a diet. For use as a major feed ingredient in milking cow rations throughout lactation, it would be useful to know if addition of glycerol can stimulate daily milk yield, and if it stimulates milk protein and depresses milk fat production in the manner of a corn grain addition. Our hypothesis was that the fermentability and glucogenicity of glycerol would lead to a milk protein stimulation and milk fat depression when added to increase the energy density of lactating cow diets. Our objective was to compare the hypercaloric effects of corn and glycerol on milk production and composition and mammary mTORC1 signaling.

MATERIALS AND METHODS

Animals, Housing, and Diets

The Animal Care Committee at the University of Guelph approved all experimental procedures in this study. Twelve multiparous, lactating Holstein cows (674 ± 66 kg BW; 81 ± 5 DIM) were housed in a tie-stall barn and given free access to feed and water throughout the study. Cows were assigned to 3 dietary treatments (Table 1) in a replicated 3 × 3 Latin square design of three 28-d periods. The 3 diets were a 70% forage basal TMR (**BAS**), the basal TMR with 19% of the forages substituted for corn grain (**CG**), and the basal TMR with 19% of the forages substituted for refined glycerol from a soybean oil source (99.7% glycerol; Palmera G997U, KLK Oleo, Selangor, Malaysia) plus protein supplements (**GLYC**). The CG and GLYC diets were formulated to be isocaloric and isonitrogenous and to contain 30% NDF, compared with 38% NDF for the BAS diet.

Sample Collection and Analysis

The amounts of feed offered and refused were recorded daily for each individual cow to estimate feed intakes. Orts were sampled from each cow and pooled on the last week of each period. Samples of each diet were collected daily, pooled by period, and submitted for nutrient composition analyses by wet chemistry at a commercial laboratory (Agri-Food Labs, Guelph, ON, Canada). Dry matter contents of feed and orts samples were determined using a forced-air oven at 60°C for 24 h.

Cows were milked at 0500 and 1530 h daily, and milk yields were recorded. Milk samples were collected from each milking during the last 7 d of each period and submitted for compositional analysis by infrared spectroscopy (Laboratory Services Division, University of Guelph, ON, Canada). Fresh milk samples from d 23, 25, and 27 of each period were also subjected to analysis of fat globule size distribution by integrated light scattering (Mastersizer S, Malvern Instruments, South-