

Opportunities for Glycerol Use in Dairy Diets

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Introduction

Glycerol, also referred to as glycerin or glycerine, is a product of the processing of fats for the chemical industry and for biodiesel production. Its main component is glycerol (propane-1,2,3-triol or 1,2,3-propanetriol), with varying amounts of water and other impurities. While pure glycerol has high value as a starting material for a number of industrial applications, crude or unrefined glycerol that arises as a byproduct of biodiesel production has a much lower value. Given the unprecedented recent increase in prices for corn and other cereals, crude glycerol has been considered as an economically attractive feed ingredient for dairy cattle. However, prices for crude glycerol have now increased to the point where it does not appear attractive even when compared with corn over \$6 per bushel. Moreover, crude glycerol has a number of limitations that must be borne in mind by nutritionists and dairy producers. Given that economic evaluations of the potential role of glycerin for dairy cattle may change as conditions change, this review will summarize findings from research conducted on use of glycerol in dairy cattle diets, including information on its metabolism, potential problems, and practical issues in its use in rations.

Glycerol Production

Glycerol is obtained as a by-product from the industrial processing of fats and oils and in the manufacture of "biodiesel" fuels. Large industrial operations that "split" or hydrolyze fats for production of pure fatty acids may also chemically refine and purify glycerol to food grade that can be used in human food, cosmetic, and drug applications. Such purified glycerol is too expensive to be considered as a dairy feed. However, many biodiesel manufacturers lack the size or scope to efficiently refine the crude glycerol, which results in the byproduct being a drain on the profitability of biodiesel production. The low value is a result of the impurities present in crude glycerol. Hence, its low value makes it potentially competitive as an animal feedstock.

Production of biodiesel in the USA has increased by a factor of 1,000 over the period of 1999 through the end of 2007, resulting in co-production of 50 million gallons of crude glycerol annually (Dasari,

2007). Supply of glycerol in the United States and worldwide is projected to grow over the next decade as long as government policies and incentives favor increased processing of plant oils for production of biodiesel fuels and as worldwide production of fats and oils continues to increase. However, the recent run-ups in oilseed prices have greatly diminished the profitability of biodiesel production (Toh and Koh, 2008). Glycerol prices were relatively low throughout 2006 and into 2007, but have climbed recently along with prices of all feeds. Whether glycerol becomes economically attractive again depends on many considerations in the prices of raw commodities, fuels, supply, and demand. In the long term, the incentive certainly exists for manufacturers to invest in capabilities to further refine and purify crude glycerol to higher value products (Toh and Koh, 2008), which will decrease supply to the animal feed market and increase its cost.

Metabolism of Glycerol

Glycerol is a potential glucose precursor via the pathways of gluconeogenesis if it is absorbed into the portal blood via the rumen or small intestine and then taken up by the liver. However, glycerol also is easily fermented in the rumen by bacteria, thus increasing VFA supply but only contributing to glucose production for the proportion of the VFA that is propionate. This is similar to the situation for propylene glycol, which contributes to total glucogenic substrate supply both by being absorbed as propylene glycol and via its fermentation to propionate in the rumen (Neilsen and Ingvarsen, 2004).

Because of its gluconeogenic properties, most early research in which glycerol was given orally (by either feeding or bolus doses) to dairy cows concerned potential benefits of glycerol as a treatment or preventative for ketosis (Johnson, 1953; Fisher et al., 1971; Fisher et al., 1973; Sauer et al., 1973). Glycerol disappears rapidly from the rumen, but the relative amount of absorption as glycerol vs. fermentation has been difficult to determine. Reports suggests that a portion of the glycerol entering the rumen can be absorbed directly (Remond et al., 1993). In studies where 15 to 25% glycerol was added most disappeared within 6 h (Bergner et al., 1995). A German study (Kijora et al., 1998) demonstrated that

twice-daily infusion of 200 g of glycerol into the rumen of steers increased plasma glycerol compared with non-infused steers (0.06 mM vs 0.19 mM). Disappearance of glycerol from the rumen was rapid after its administration, but no glycerol was detected in duodenal digesta. More than 85% of glycerol disappeared within 2 h of administration in the glycerol-adapted steers (Kijora et al., 1998). Maximal rates of glycerol disappearance determined using in vitro fermentors was 0.52 to 0.62 g/h, compared with rumen disappearance rates ranging between 1.2 to 2.4 g/h when 240 g of glycerol were dosed into the rumen (Remond et al., 1993).

More recent information indicates that net absorption of glycerol in cows is limited, even when large doses are administered. Kristensen and Raun (2007) measured glycerol absorption and liver metabolism of glycerol in cows given 925 g/d of 85% glycerol once daily through a ruminal cannula. Only about 10% of the glycerol administered was recovered as glycerol in the portal vein, but nearly all of that absorbed was taken up by the liver and most likely converted to glucose. The remainder of glycerol that was not recovered in the portal vein presumably was fermented in the rumen.

Ruminal fermentation of glycerol could contribute to its role as a glucogenic substrate to the extent that it is converted to propionate. However, data on the ruminal fate of glycerol are conflicting. Some early reports of glycerol fermentation indicated that it was fermented to large proportions of propionate (Johns et al., 1953; Garton et al., 1961), while other studies reported increased acetic and propionic acids (Wright, 1969) or increased propionic and butyric acids (Czerkawski and Breckenridge, 1972). Using rumen fluid inoculum from cows previously adapted to glycerol feeding resulted in greater production of propionate and butyrate at the expense of acetate when glycerol was added to in vitro fermentations (Remond et al., 1993). Bergner et al. (1995) showed that ^{14}C -labeled glycerol was mostly converted to propionate. Kijora et al. (1998) found that intraruminal administration of glycerol decreased ruminal pH (from 6.3 to 5.4) and decreased the acetate to propionate ratio (from 3.5 to 2.1).

Linke et al. (2004) administered 1 kg of crude glycerine (80% purity) by feeding or oral administration via drench or stomach tube. Their results (Table 1) showed that glycerol decreased molar percentages of acetate and increased molar percentages of propionate and butyrate at 4 h after either feeding or oral administration although butyrate increased to a greater degree than did propionate. Kristensen and Raun (2007) also found that glycerol administration into the rumen decreased the molar percentage of acetate and increased butyrate, but did not affect the molar percentage of propionate. Trabue et al. (2007) compared ruminal

fermentation characteristics when either propylene glycol or glycerol was provided as the single substrate to in vitro fermentations. They found that propylene glycol addition resulted in much larger increases in propionate concentration over time than did addition of glycerol. Furthermore, addition of glycerol also increased concentrations of butyrate, valerate, and caproate compared to the control or addition of propylene glycol. Schröder and Südekum (1999) noted increased concentrations of butyrate when diets were supplemented with glycerol. DeFraen et al. (2004) found greater proportions of butyrate in ruminal fluid from cows administered glycerol as a drench. Together, these data indicate that providing glycerol into the rumen via feeding or oral bolus would contribute to ruminal fermentation as a microbial energy source; however, it appears that glycerol fermentation likely will not provide as much propionate for glucose production as would propylene glycol.

Table 1. Ruminal fermentation characteristics at 4 h postadministration for cows administered 1 kg of glycerine (80% glycerol). From Linke et al. (2004).

VFA	Treatment				P value
	Control	Fed	Drench	Tube	
Acetate, mol %	53.3	44.9	44.6	43.0	0.05
Propionate, mol %	26.4	28.7	30.4	30.4	0.05
Butyrate, mol %	14.1	20.0	20.3	21.5	0.05

Glycerol in the diet also might affect other aspects of ruminal fermentation. Kijora et al. (1998) found that incorporation of ^{15}N -labeled urea into microbial protein was decreased when 200 g of glycerol was administered into the rumen twice daily. Roger et al. (1992) examined the impact of glycerol addition to in vitro microbial cultures on growth, adhesion, and cellulolytic activity of two major cellulolytic bacterial species (*Ruminococcus flavefaciens* and *Fibrobacter succinogenes*). They found that low concentrations (0.1 to 1% of volume) did not affect any of the aspects studied; however, addition of glycerol at 5% of volume had strong negative effects on growth and cellulolytic activity of these organisms. Ruminal liquid volume of lactating dairy cows typically ranges from 50 to 60 L, which indicates that glycerol inclusion at low rates (~250 g or less at one time) is unlikely to result in negative effects; however, greater amounts (~1.25 kg at one time) could result in some negative effects on ruminal cellulolytic activity (Overton, 2007). Recent research at Purdue University showed that glycerol at up to 15% of the dietary substrate maintained DM fermentation rates, whereas more than 5% molasses depressed DM digestibility (Donkin and Doane, 2007).

Nutritional Value of Glycerol

In assessing the nutritional value of glycerol, the purity must be taken into account. Pure glycerol would be almost entirely available as an energy source. As mentioned earlier, however, pure sources of glycerol are unlikely to be used in dairy cattle feeding because of their high cost. Crude glycerol from the biodiesel industry may range from >70% to more than 90% glycerol (Dasari, 2007). The water content of glycerol used in various scientific studies has ranged from a low of 1% (for purified glycerol sources) to as much as 26.8%. German researchers determined that the net energy (NEL) value of glycerol (1.03 to 1.05 megacalories per pound dry matter) in sheep, steers, and dairy cows was equal to or greater than that of corn grain (Schroder and Sukekum, 1999). Similar conclusions have been reached for glycerol use in poultry (Dozier et al., 2008) and swine (Lammers et al., 2008) diets. Net energy values were approximately 13% lower in high-starch diets (about 0.90 megacalories per pound) than in lower-starch diets, which was attributable to decreased cell wall (NDF) digestibility caused by addition of glycerol to the higher-starch diets. Therefore, the economic value of energy from glycerol can be compared directly with that of corn grain after correcting for the glycerol content (analogous to the “dry matter” content of the glycerol) of the material. This contention is supported by recent research at Purdue (Donkin et al., 2007; Donkin and Doane, 2007).

It must be remembered that glycerol, unlike corn, supplies essentially no protein and few nutritionally important minerals, which would need to be compensated if glycerol was used as an energy source. The South Dakota study reported that glycerol contained 11.5% “salt”, but other sources have reported low sodium contents. Potassium content in the German research ranged from 2.2 to 2.3% of the dry matter and phosphorus from 1.05 to 2.36%. Contributions to mineral intake by glycerol might be a factor in dry matter intake and need to be accounted for in ration formulation. A recent evaluation of crude glycerol from soy biodiesel production indicated a glycerol content of 76.2% and as much as 7.98% fat, 0.05% protein, and 2.73% ash. The latter was composed of 11 ppm calcium, 6.8 ppm magnesium, 53 ppm phosphorus, and 1.2% sodium (Thompson and He, 2006).

An important consideration for crude glycerol use is the impurities that may be present, including methanol, spent catalysts, and salts after neutralization. For example, a low-purity source of glycerol tested by German researchers contained 26.7% methanol on a dry matter basis (Schröder and Südekum, 1999) and the glycerol used in the South Dakota study contained 1.3% methanol (DeFrain et al.). While methanol may be detoxified to some

degree in the rumen, methanol consumption from larger amounts of less pure glycerol sources may be excessive. Methanol would be even more detrimental for preruminant calves and other nonruminants. Methanol content of crude glycerol should be less than 0.5%. A recent regulatory letter issued by FDA indicates that methanol levels higher than 150 ppm (interpreted as in the total diet) could be considered unsafe in animal feeds.

Use of Glycerol in Diets for Dairy Cows during the Transition Period

Glycerol has been proposed as a preventative for metabolic problems in transition cows. Goff and Horst (2001) drenched up to 3 L in ketosis treatment and prevention. Administration of 1, 2 or 3 L of glycerine (80% glycerol) by esophageal pump increased plasma glucose by 16, 20 and 25%, respectively, over pretreatment values. DeFrain et al. (2004) evaluated glycerol supplementation in the diet of transition cows. Glycerol (0, 0.43, or 0.86 kg/d) was topdressed on the TMR and fed to transition cows (n = 10 per treatment) from 14 d prepartum to 21 d postpartum. Glycerol did not affect prepartum concentrations of glucose, insulin, NEFA or BHBA; however, postpartum concentrations of plasma glucose tended to be higher for the cows fed the control diet compared to those fed glycerol (65.8 vs. 63.0 and 60.1 mg/dL). Cows fed either 0.43 or 0.86 kg/d of glycerol had decreased prepartum DMI compared with the control cows, but postpartum DMI was not affected by treatment. Energy-corrected milk yield tended to be decreased in cows fed glycerol compared with the control. Feeding 500 ml of glycerol, or approximately 3.1% dietary DM, from 3 wk before calving through 70 days in milk increased milk yield and milk protein content in milk (Bodarski et al., 2005).

Cornell University researchers studied the use of glycerol either as a dietary supplement or as a short-term postcalving drench (Ogborn et al., 2004; Ogborn, 2006). In the dietary study, 48 Holstein cows entering second or greater lactation were assigned to two prepartum treatments beginning at 21 d before calving and subsequently to four postpartum treatments after calving. Prepartum treatments consisted of a control or dietary addition of crude glycerine (80.6% glycerol) at 5% of diet DM. Postpartum treatments consisted of a control, dietary addition of glycerine (3.3% of diet DM), oral glycerine drench (500 ml; 625 g) once daily for the first 5 d postcalving, or a combination of diet addition and oral drench of glycerine. Postpartum dietary addition of glycerine continued through 21 d postcalving; thereafter, all cows were fed the same diet. Cows fed glycerol during the prepartum period consumed on average 0.59 kg/d of glycerol (0.74 kg/d of crude glycerine). In contrast to the DeFrain

et al. (2004) study, prepartum glycerol feeding increased prepartum DMI (14.8 vs. 13.2 kg/d); however, this increase in prepartum DMI did not translate into any effects on postpartum DMI or milk yield and composition. Furthermore, prepartum dietary glycerol did not affect plasma metabolites (glucose, NEFA, BHBA) and liver composition (triglycerides and glycogen) during either the prepartum or postpartum periods.

Cows fed glycerol during the postpartum period consumed on average 0.50 kg/d of glycerol (0.63 kg/d of crude glycerine). Glycerol feeding tended to decrease postpartum DMI by about 1 kg/d and drenching glycerol markedly decreased postpartum DMI by about 1.5 kg/d. Feeding glycerol postpartum did not affect milk yield or composition, BW and BCS change, or plasma metabolites and liver composition during the postpartum period. Drenching glycerol did not affect milk yield or milk composition but did result in greater BW and BCS loss during early lactation. Overall concentrations of plasma metabolites and liver composition were not affected by drenching glycerol. Short-term responses of plasma metabolites on d 5 postcalving indicated that glycerol drench tended to increase plasma glucose and decrease plasma NEFA concentrations during the first 6 h after administration (Ogborn, 2006). This glucogenic effect of glycerol is weaker than that typically expected when propylene glycol is drenched (Pickett et al., 2003; Neilsen and Ingvarsen, 2004).

Chung et al. (2007) topdressed 250 g/d of a blended "dry" glycerin source (65% glycerol) from calving through 21 d postpartum. Postpartum DMI and metabolic responses during the first 21 d postcalving were not affected by treatment. Milk yield and composition was largely not affected by treatment, although there was a trend for increased milk yield of cows fed glycerin by wk 6 of lactation, suggesting some residual effect of the non-significant alterations in metabolism during the transition period.

Use of Glycerol in Diets for Dairy Cows in Established Lactation

Fewer studies have examined glycerol supplementation as an energy source in diets for dairy cows. Glycerol at 3.6% of the diet fed to mid-lactation cows did not affect DM intake, milk production, or milk composition but increased rumen propionate and butyrate at the expense of acetate (Khalili et al., 1997).

Donkin et al. (2007) evaluated pure glycerol (99.5% glycerol) as an ingredient in dairy rations by replacing corn grain with a combination of glycerol and corn gluten feed (6.25:1). They fed Holstein cows (n = 60) diets containing 0, 5, 10, and 15% glycerol on a DM basis. Some results from this experiment are

shown in Table 2. Dietary glycerol at up to 15% of total diet DM did not affect overall DMI or milk yield, although the authors reported that cows fed the 15% glycerol diet had decreased DMI for the first 7 d of the experiment. Glycerol did not affect milk fat or protein content, but decreased milk urea N content, suggesting that the glycerol was more fermentable in the rumen than ground shelled corn. Cows fed glycerol had larger increases in BW during the experiment.

Table 2. Effects of feeding glycerol in diets for lactating cows (Donkin et al., 2007; Donkin and Doane, 2007; as summarized by Overton, 2007).

Variable	Dietary glycerol (% of DM)				SEM	P
	0	5	10	15		
DMI, lb/d	52.8	53.9	54.1	53.0	1.1	0.82
Milk, lb/d	81.4	81.2	82.1	80.1	1.3	0.71
Milk fat, %	3.70	3.52	3.58	3.58	0.11	0.69
Milk protein, %	2.79	2.84	2.86	2.89	0.06	0.62
Milk urea N (MUN), mg/dl	12.5 ^a	10.9 ^b	10.7 ^b	10.2 ^b	0.4	0.05
BW change, lb	69.3 ^a	89.3 ^{ab}	109.1 ^b	113.3 ^b	10.1	0.05

^{ab}Means within a row with different superscripts differ, $P < 0.05$

Taken together these experiments indicate that glycerol may be added to diets for lactating cows to a level of at least 10% of dry matter without deleterious effects and in some cases beneficial effects on milk production and composition.

Glycerol in Diets for Calves and Growing Cattle

We recently examined whether glycerol could be used as a partial replacement for lactose in milk replacers (Drackley et al., 2008). Holstein calves (6 male, 6 female) born at the University of Illinois dairy unit were assigned alternately to each of two treatments (24 calves total): control milk replacer or milk replacer supplemented with 15% glycerol. The experimental base milk replacer contained greater protein, fat, minerals, and vitamins so that when glycerol was added the composition would be the same as control, except that glycerol replaced some lactose. Calves were housed in individual hutches bedded with straw and had water freely available; no starter was offered until d 36. Calves were fed milk replacers twice daily from d 3 of life. Milk replacers contained 28% protein (all from whey proteins), 2.6% lysine, and 15% fat. Control milk replacer contained 40% lactose; glycerol milk replacer contained 25% lactose. Both replacers were reconstituted to 15% solids. Glycerol (liquid) was added to reconstituted base milk replacer at each feeding. During wk 1 milk replacers were fed at a rate of 1.5% of BW daily as powder and from wk 2 through 6 at 2% of BW daily. Starter was offered beginning on d 36. Milk replacer

offered was reduced by half on d 43 and calves were weaned at d 49. Measurements of BW and stature were made weekly through d 56. Calf BW through d 35 did not differ significantly between treatments (1.50 vs. 1.41 kg/d for controls and glycerol, respectively). Stature measurements (withers height, body length, heart girth) and measures of health (fecal scores, medical treatments) also did not differ between treatments. Our results indicate that glycerol could be an acceptable replacement for at least 37.5% of the total lactose in milk replacer if economically favorable. At this point glycerol cannot be incorporated into spray-dried protein-fat mixtures or in dry milk replacers. Liquid glycerol could be added into on-farm mixes of liquid milk replacer such as are often used for feeding veal calves.

In growing cattle, Schröder and Südekum (1999) fed 10% glycerol to dairy steers, effectively replacing over one-half of the starch in the diet, without negatively affecting intake, ruminal digestibility, rumen microbial synthesis or total tract nutrient digestibility in steers. Feeding glycerol increased water content of rumen digesta, and has been reported to stimulate water intake in other species.

Glycerol in Manufactured Feeds

Large farms may be able to handle glycerol as a bulk liquid and incorporate it into total mixed rations. However, glycerol may work well in pelleted concentrates as well. An interesting set of evaluations of glycerol addition to pelleted feeds was made by German researchers (Schröder and Südekum, 1999). Glycerol was added in increasing amounts to a concentrate containing wheat, soybean meal, rapeseed meal, beet pulp, wheat bran, corn, and vitamin-mineral premix. The mixture then was pelleted and stored under different conditions for different amounts of time. As little as 5% glycerol added to the mixture was effective in preserving higher-moisture pellets as indicated by suppression of fungal growth. Other measures of pellet quality and integrity were unchanged or improved by glycerol addition. Pelleting mixtures with less-pure glycerol resulted in little methanol detected in the pellets, probably because the heat generated in the pelleting process caused the methanol to volatilize. Consequently, the prospects for addition of glycerol to pelleted feed mixtures for dairy cattle seem promising.

Conclusions

Based on the body of research conducted in recent years, it is evident that glycerol could be used at up to 15% of the dietary DM without negative effects on cow performance. Glycerol could be an effective replacement for corn grain on an energy basis, with approximately the same efficiency of use as corn grain. Economics will dictate whether that replacement is feasible.

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