Use of Glycerol in Dairy Rations

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INTRODUCTION

Glycerol is a colorless, odorless, hygroscopic, and sweet-tasting viscous liquid. Synonomous names for glycerol include: glycerin, glycerine, propane-1,2,3-triol, 1,2,3-propanetriol, 1,2,3-trihydroxypropane, glyceritol, and glycyl alcohol. By definition glycerol is a sugar alcohol. Because of its humectant properties, energy content, and high solubility index in water; glycerol is widely utilized in the food, pharmaceutical, and cosmetic industries. With expansion of the biofuels industry, including further processing of soybean oil to produce biodiesel fuel, there is a potential for increased availability of unrefined glycerol, a by-product, as a valuable energy source for livestock. Although glycerol may be an alternative energy source for livestock there are unanswered question regarding the handling, inclusion rates, impact and level of contaminants, and feeding value relative to other energy sources. This review will explore some of the attributes and issues pertinent to glycerol as a feed for lactating dairy cows and highlight results from ongoing research at Purdue University; where the value of glycerol has been examined as a replacement for corn grain.

GLYCEROL FROM BIODIESEL PRODUCTION

The term *bio diesel* is used to describe the primary end-product of the methyl or sometimes ethyl esters of fatty acid that are produced from processing of lipid derived from a variety of plant sources. Every 10 gal of biodiesel that is produced generates about 7.6 lb of crude glycerol. According to the National Biodiesel Board the production of biodiesel in the U.S. over the next decade is expected to grow (http://www.biodiesel.org/). Current annual production is 395 mil gal. Planned expansions in the biodiesel industry are expected to drive that capacity to more than 1.1 bil gal within the next 18 mo, generating more than 800 mil lb of glycerol annually. Corresponding price projections suggest glycerol could be priced competitively with grains as a source of energy for livestock. The value of glycerol in this regard may be further amplified with increasing diversion of corn and other grains to ethanol production.

GLYCEROL PRODUCTION AND QUALITY CONCERNS

Most biodiesel is currently produced by a reaction that utilizes a base catalyzed transesterification of the oil. For soy diesel production, soybean oil is reacted with an equal weight of a short chain alcohol (usually methanol but sometimes ethanol) in the presence of a catalyst (sodium hydroxide; caustic soda or potassium hydroxide; potash) to yield biodiesel and crude glycerol. This process requires low temperature and pressure, yields high conversion (98 %) with minimal side reactions and reaction time, and results in direct conversion of soybean oil to biodiesel with no intermediate compounds. The biodiesel is separated from the glycerol by gravity separation or by centrifugation. Because most commercial biodiesel production utilizes a 6 to 1 molar ratio of alcohol to oil, or excess alcohol to drive the reaction to completion, methanol can partition to the glycerol and biodiesel phases.

Alcohol is removed from biodiesel and glycerol phases by flash evaporation or distillation, to be recovered, and re-used. The resulting glycerol contains unused catalyst and soaps, which are then neutralized by the addition of acid to produce crude glycerin containing 80-88 % glycerol. Further purification of crude glycerin to 99 % or higher purity is needed for use in the cosmetic and pharmaceutical industries. Impurities devalue crude glycerol as high levels of residual catalyst, salts, and methanol may be problematic in using of glycerol as a livestock feed. Recent evaluation of crude glycerol from soy biodiesel production indicates 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 Ca, 6.8 ppm Mg, 53 ppm P, and 1.2 % Na (Thompson and He, 2006).

GLYCEOL FOR TRANSITION COWS AT LOW INCLUSION LEVELS

The use of glycerol in the treatment of ketosis was reported as early as 1954 (Johnson et al., 1954) and evaluation of glycerol, as well as propylene glycol, as a ketosis treatment was further explored in the 1970's (Fisher et al., 1971, 1973). More recently the value of glycerol has been examined as a preventative aid for metabolic problems associated with transition cows. Goff and Horst (2001) used up to 3 L in ketosis treatment and prevention and DeFrain et al. (2004) fed 0.86 kg/d to transition dairy cattle. Recent studies evaluating 162.5 g/d of glycerol in a dry product containing 65 % food grade glycerol for transition cows did not alter feed intake, milk yield and components, blood metabolites, and serum insulin concentrations during the first 3 wk of lactation; but tended to increase milk production 3 wk after cessation of feeding (Chung et al., 2007). While these studies demonstrate the potential value of glycerol in treating ketosis; there is a lack of data to examine the value of glycerol as a primary ration ingredient for post-transition dairy cattle. Feeding rates for transition cows range from 5 to 8 % of the diet dry matter (**DM**).

FEEDING STUDIES USING HIGHER INCLUSION LEVELS OF GLYCEROL

Although there is supporting evidence for use of glycerol for transition cows there is little information that examines the use of glycerol as a macro-ingredient in rations for lactating dairy cows. Feeding studies have typically been lower from 150 to 472 g/d (Fisher et al., 1971, 1973; Kalili et al., 1997). There are only a handful of studies with glycerol feeding rates that approach 5 % or more of the ration on a DM basis.

Schröder and Südekum (1999) fed 10 % glycerol to dairy cattle, 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 3.6 % glycerol to mid-lactation dairy cows was without effect on intake, milk production, or gross milk composition; but slightly altered the profile of fatty acid in milk and increased rumen propionate and butyrate at the expense of reduced acetate (Khalil et al., 1997). Feeding 0.86 g of glycerol to +21 d relative to calving (5.4 % of ration DM) did not have any effects on milk production or feed intake (DeFrain et al., 2004). Feeding 500 ml of glycerol, or approximately 3.1 % of DM, from 3 wk prior to calving through to 70 d in milk caused an increase milk yield and milk protein content in milk (Bodarski et al., 2005). Taken together these experiments indicate that glycerol may be added to diets for lactating cows to a level of at least 10 % of DM without deleterious effects and in some cases beneficial effects on milk production and composition.

ENERGY VALUE FOR GLYCEROL

Because glycerol has not been used as a macro ingredient, the estimates of net energy of lactation (**NE$_l$**) are not available for typical feeding scenarios. Schröder and Südekum (1999) reported estimates from 0.90 to 1.03 Mcal/lb with energy values decreasing for higher starch diets, and recently DeFrain et al. (2004) reported 0.87 Mcal/lb when feeding glycerol in early lactation. Part of the uncertainty for assigning an energy value for glycerol is the impact or the potential impact of the levels of glycerol that have been fed to ruminants and unknown interactions with other ration components. The energy value of glycerol is approximately equal to the energy contained in corn starch. However the experimental estimates for the energy value of glycerol, when it replaced corn in the ration is reduced if the diet already has a high proportion of corn and a high starch content (55 % of ration DM; Schröder and Südekum, 1999). The energy estimates for glycerol are greater when it is fed in a low starch ration (40 % of DM); therefore, based on the typical starch levels in rations fed to dairy cattle, an energy value for glycerol equal to the energy value of corn starch is an appropriate starting point until more information is available.

RUMEN METABOLISM OF GLYCEROL

Glycerol is fermented to volatile fatty acid (**VFA**) in the rumen. Early reports of glycerol fermentation indicated that glycerol was almost entirely fermented to propionate (Johns et al., 1953; Garton et al., 1961). Other reports indicate an increase in acetic and propionic acid (Wright, 1969) or increased propionic and butyric acid (Czerkawski and Breckenridge, 1972). *In vitro* glycerol fermentation, using rumen fluid inoculum from cows adapted to glycerol feeding, indicates increased production of propionate and butyrate at the expense of acetate (Remond et al., 1993). Studies using 14C labeled glycerol indicate that most of the glycerol was found in propionate (Bergner et al., 1995). Rumen microbes adapt to glycerol feeding, as the rates of glycerol disappearance from rumen fluid are more rapid after 7 d of glycerol feeding to donor animals used as a source of rumen-fluid (Kijora et al., 1998). In studies where 15 to 25 % glycerol was added, most of the glycerol disappeared within 6 h (Bergner et al., 1995).

The maximal rates of glycerol disappearance in the rumen determined using *in vitro* fermentors is

0.52 to 0.62 g/h (Remond et al., 1993). There is lack of agreement for *in vivo* disappearance from the rumen as microbial metabolism. Estimates from disappearance of a 200 g dose of glycerol indicate that more than 85 % of glycerol in the rumen disappears within 2 h in cattle acclimated to glycerol feeding (Kijora et al., 1998). Other data using a dose of 240 g of glycerol, indicates rumen disappearance rates ranging between 1.2 to 2.4 g/h (Remond et al., 1993). Likewise there have been reports suggesting that a portion of the glycerol entering the rumen can be absorbed directly (Remond et al., 1993). The fate of any absorbed glycerol is metabolism in the liver and requires glycerol kinase (Lin, 1977), an enzyme responsible for channeling glycerol into the triose phosphate step of glycolysis/gluconeogenesis. When glucose demands are high, such as the case for lactating cows, the fates of absorbed glycerol or propionate produced by rumen fermentation are likely to be identical.

FEEDING VALUE OF GLYCEROL AS A REPLACEMENT FOR CORN GRAIN IN RATIONS FED TO LACTATING DAIRY COWS

The objective of our feeding trial was to evaluate the value of glycerol as a replacement for corn grain in diets of lactating dairy cattle. For this experiment 60 lactating Holstein cows were housed in individual tie stalls at the Purdue Dairy Research and Education Center and adjusted to a basal diet for 2 wk period. Cows were then assigned to diets containing 0, 5, 10, or 15 % glycerol as a % of ration DM. For these experiments we used 99.5 % USP/FCC, Kosher grade glycerol in order to avoid any impact of potential contaminants found in crude glycerol. The basal (0 glycerol) ration was balanced to meet or exceed NRC requirements and contained corn silage, alfalfa haylage, hay, dry-rolled corn, vitamins, and minerals (Table 1). Corn was replaced by an equivalent amount of food grade glycerol and corn gluten feed. The addition of corn gluten adjusted for the protein removed with corn grain. Diets were offered once daily for ad libitum intake (5 to 10 % weighbacks), feed refusals were measured daily, and feed intake determined by difference. Cows were milked twice daily and milk samples were obtained weekly at 2 consecutive milkings and analyzed for fat, protein, lactose, total solid, milk urea N, and somatic cells.

Glycerol was well-tolerated by the cows and there were no differences in DM intake or milk production when the entire 8 wk experimental period was considered (Table 2). Feed intake was reduced by inclusion of 15 % glycerol during the first 7 d of the trial. Negative effects on intake were only evident during the first week of the test and differences were not detected for the subsequent 7 wk. Recovery of intake within 7 d suggests that achieving a feeding rate of 15 % glycerol might be best accomplished with a protocol that gradually introduces glycerol into the ration.

Milk production and milk composition were not altered in response to glycerol feeding with the exception of decreased milk urea nitrogen in response to glycerol. These changes were observed at all levels of glycerol feeding. Reduced MUN suggests improved use of dietary protein by rumen bacteria and reduced losses as ammonia. Cows fed the highest amount of glycerol gained the most weight during the 8 wk feeding period. Cows fed 10 and 15 % glycerol gained more weight than cows fed 5 % glycerol or the control diet. Weight gain for the control cows and 5 % glycerol did not differ.

Estimates of NE_l for the diets were calculated from intake, production data, and body weight (**BW**) changes. The energy content of each ration was calculated for each cow over the experimental period using total energy expenditure (milk, maintenance, BW gain) with DM intake. An estimate for NE_l (Mcal/lb) for each diet was determined from NE_l used (Mcal) divided by DM consumed for the corresponding interval. Estimated energy values for the diets were 0.70, 0.70, 0.71 and 0.72 ± 0.02 Mcal/lb and were not different (P = 0.90). The lack of differences in this regard suggests that glycerol can be substituted for corn without adjustments for the energy content. However the feed energy value of crude glycerol is likely to be less than that of pure glycerol and must be adjusted for the levels and energy content of the impurities. It should be noted that the energy values of the TMR, determined by chemical analysis, are slightly higher than the estimates determined by difference of milk produced and BW change. These differences may reflect the effects of increasing intake, and therefore passage rate, to reduce the NE value of a ration.

EFFECT OF GLYCEROL ON FEED PARTICLE DISTRIBUTION

Glycerol is typically added to either a TMR or added in the grain portion of the feed. Addition of a 50:50 soybean oil/glycerol blend to either 85:15 or 70:30 blend of HM ground corn and spray-dried whey in swine diets increased flowability as determined by the angle of repose of the mixture (Groesbeck et al.,

Table 1. Diet composition of lactating dairy cow rations fed to evaluate glycerol at various levels as a replacement for corn grain.

	% Glycerol			
Ingredient, % DM	**0**	**5**	**10**	**15**
Corn silage	31.94	31.94	31.94	31.88
Alfalfa haylage	10.00	10.00	10.00	9.98
Alfalfa hay	12.16	12.16	12.16	12.14
Soy hulls	7.66	7.66	7.66	7.64
48% Soybean meal	6.62	6.62	6.62	6.61
Roasted soybeans	5.40	5.40	5.40	5.39
Fish meal	0.66	0.66	0.66	0.66
Urea	0.30	0.30	0.30	0.30
Megalac R	0.98	0.98	0.98	0.98
Corn, ground	20.00	14.20	8.40	2.79
Glycerol	-	5.00	10.00	14.97
Corn gluten meal	-	0.80	1.60	2.40
Mineral/vitamin	4.28	4.28	4.28	4.27
Chemical Analysis, % DM				
Crude Protein	18.1	17.5	17.9	18.1
ADF	19.1	19.2	19.4	19.3
NDF	30.9	32.4	29.7	31.0
NE_l, Mcal/lb	0.77	0.76	0.77	0.77
Ca	1.03	1.01	1.06	1.05
P	0.41	0.39	0.41	0.41
Mg	0.34	0.31	0.32	0.33
K	1.88	1.85	1.88	1.88
Na	0.25	0.24	0.28	0.27

2007). Glycerol added to pelleted feed containing wheat, soybean meal, rapeseed meal, beet pulp, wheat bran, corn, and vitamin-mineral premix suppressed fungal growth (Schröder and Südekum, 1999). Glycerol added to a TMR containing corn silage, haylage, chopped hay, and concentrates at the time of mixing resulted in a disproportional increase in weight of feed retained on the top 2 screens of the Penn State particle separator, suggesting that glycerol adheres to particles based on their surface areas (Figure 1). Data for digestibility of fiber (described below) indicates a lack of a detrimental impact in this regard. If liquid glycerol is added to a TMR, then the use of tools, such as the Penn State particle separator to assess effectiveness of fiber in the ration, will need to be modified to account for the change in particle due to adherence of glycerol to long particles.

IN VITRO FERMENTATION OF GLYCEROL OR MOLASSES

The effects of pure glycerol on *in vitro* DM and fiber digestion of the nonglycerol components of the ration were assessed *in vitro* and compared with molasses as an alternative energy source. The fermenter diets were the same as those fed to the cows in the experiment described above. Diets were placed in bags inside Ankom Daisy Fermenters that contained either 0, 5, 10, or 15 % of either pure

Table 2. Effect of glycerol on feed intake, milk production, body weight change, and body condition score change.

	% Glycerol					
Item	**0**	**5**	**10**	**15**	**SEM**	**P[1]**
Milk production, lb/d	81.4	81.2	82.1	80.0	1.3	0.71
Feed intake, lb/d	52.8	53.9	54.1	53.0	1.2	0.82
Efficiency, milk:feed	1.56	1.52	1.52	1.53	0.04	0.85
Milk fat, lb/d	2.93	2.81	2.92	2.80	0.14	0.88
Milk protein, lb/d	2.19	2.28	2.33	2.28	0.09	0.78
Milk lactose, lb/d	3.66	3.71	3.88	3.68	0.18	0.84
Milk solid, lb/d	9.50	9.53	9.85	9.47	0.43	0.91
SCC, 1000 cells/ml	275	490	137	144	111	0.10
Milk urea N, mg/dl	12.5[a]	10.9[b]	10.7[b]	10.2[b]	0.4	<0.05
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 lactose, %	4.64	4.62	4.70	4.66	0.07	0.89
Milk solid, %	12.05	11.89	12.03	12.04	0.19	0.91
BCS change	0.1	0.1	0.1	0.1	0.1	0.91
BW change, lbs	69.4 [a]	89.6 [ab]	109.3 [b]	113.5 [b]	10.2	<0.05

[1] Probability that treatment means are equal.
[a,b] Means with different superscripts differ ($P < 0.05$).

Table 3. Effect of glycerol or molasses on 24 h *in vitro* DM and NDF digestion and VFA accumulation. Diets used were as listed in Table 1.

	% Glycerol				% Molasses				
Measure	**0**	**5**	**10**	**15**	**0**	**5**	**10**	**15**	**SE**
DM digestion, %	62.7[a]	59.8[b]	61.3[ab]	63.1[a]	62.6[a]	62.3[a]	56.9[c]	56.3[c]	0.4
NDF digestion, %	34.9[ab]	30.8[bc]	32.4[abc]	35.2[a]	36.1[a]	35.9[a]	29.9[cd]	26.2[d]	0.8
Acetate, mM	37.1	37.6	37.7	38.7	38.5	40.4	38.4	36.3	0.7
Propionate, mM	18.1[ab]	20.2[ab]	20.1[ab]	21.6[a]	17.0[b]	20.1[ab]	18.4[ab]	16.8[b]	0.8
Butyrate, mM	13.0	13.8	13.3	14.5	13.6	14.7	13.8	12.3	0.5
Valerate, mM	5.7[bc]	9.1[a]	6.9[abc]	8.2[abc]	5.2[c]	6.4[abc]	8.5[ab]	5.9[bc]	0.6

[a,b,c,d] Means within the same row and section with different superscripts differ ($P < 0.05$).

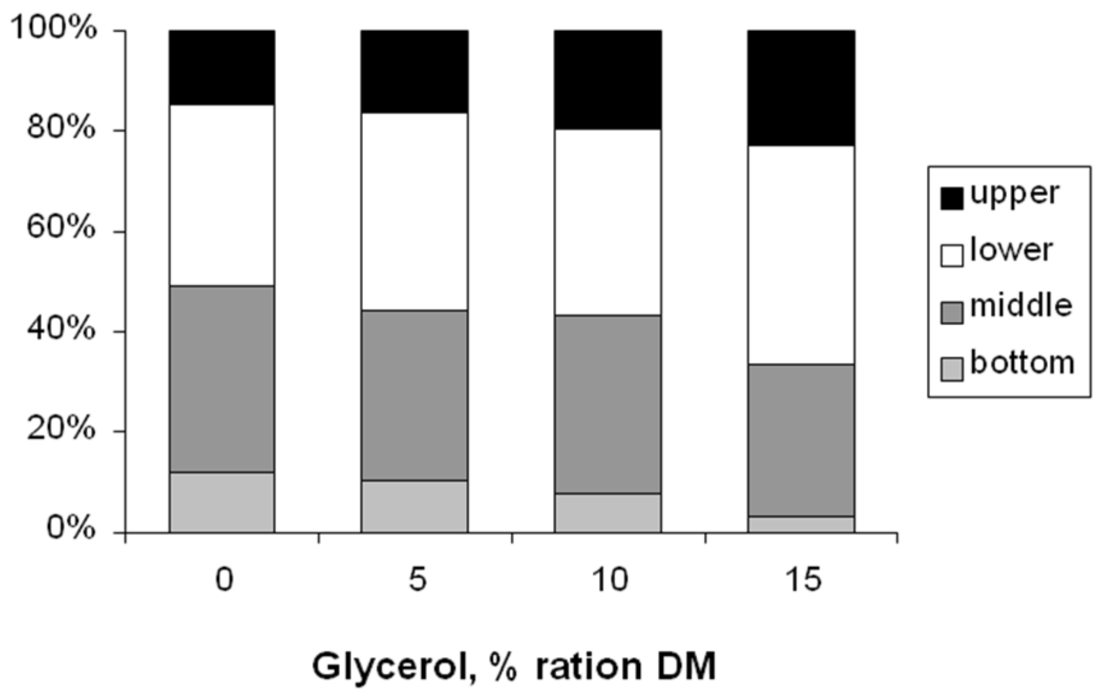
glycerol or cane molasses. Diets were fermented for 24 h. The initial feed and fermentation residue was analyzed for organic matter and NDF content. The VFA profile of the incubation was determined.

Glycerol addition at 10 and 15 % of the ration maintained *in vitro* DM digestion during a 48 h incubation; whereas molasses added above 5 % reduced DM digestion by approximately 10 %. The digestion of NDF was unchanged by the addition of 15 % glycerol but was decreased by approximately 30 % by the addition of 15 % molasses. The effects of glycerol on VFA profile of the incubation were moderate with few significant differences. Although there was no clear trend with inclusion level, glycerol increased the average propionate concentration. There were no significant differences between molasses and glycerol in total VFA production or profile of acetate, butyrate, or valerate.

The data suggest that glycerol improved DM digestion, relative to molasses, largely because of lack of the negative effect of molasses on fiber digestion and the lack of effect of glycerol when added at 15 % of the diet. The decrease in fiber digestion at the highest inclusion level of molasses may suggest the limitations of feeding high levels of sugar. The lack of negative effects of glycerol on fiber digestion suggests differences in glycerol and sugar fermentation. Glycerol appears to be a good dietary source of energy with a larger range for acceptable use compared with cane molasses.

Results from this study clearly indicate that glycerol is a valuable feed ingredient for lactating dairy cows. Glycerol can be included as a macro ingredient in diets for lactating dairy cows without any deleterious effects. *In vitro* data suggest glycerol fermentation to propionate at the expense of acetate and butyrate, as well as no negative impacts on digestibility of DM and NDF with increasing glycerol in the diet. Therefore feeding glycerol in place of corn is an alternative strategy for formulating diets for lactating cows when corn is not priced favorability.

Figure 1. Distribution of TMR particle size with removal of corn grain and addition of glycerol to the ration.



These data point to the feeding value of glycerol when fed in pure form; however depending on the level and composition of impurities the feeding value of crude glycerol cannot be implied directly from these results.

SUMMARY

Previously published research and recent work completed at Purdue University indicate the value of glycerol as a feed for lactating dairy cattle. Increased production of biodiesel and resulting glycerol, when combined with an increased demand for corn in ethanol production, may warrant use of glycerol as livestock feed. Although issues exist relative to the composition of crude glycerol there does not appear to be any detrimental impact of feeding glycerol up to at least 15 % of the total ration DM. Caution should be used however, when introducing glycerol to the diet as approximately 7 d is required to adapt the rumen to glycerol feeding.

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