Glycerol as a partial replacement for lactose in milk replacer for young dairy calves

R. A. Ebert-Allen,1* G. M. Willis,2† and J. K. Drackley1‡

Graphical Abstract
Treatments were milk replacers

Control: All milk protein, 28% CP, 15% fat, 46% lactose
Glycerol: 15% replacing lactose in controls

12 calves 12 calves

Results:
- No differences in body weight, stature measurements, or gain:feed
- No health issues

Conclusion:
Glycerol can replace 15% of the lactose in an all-milk milk replacer without loss of performance or health

Summary
Two groups of 12 calves were used to determine whether glycerol could be included at 15% of the milk replacer formula replacing lactose. Liquid glycerol was added to a base milk replacer at each feeding. Calves were fed the diets from d 3 of age to d 49. There were no differences between treatments in body weight, stature measurements, gain:feed, or health issues. Glycerol can replace 37.5% of the lactose (15% of the formula) in milk replacer with no loss of performance.

Highlights
- Liquid glycerol was added to a base milk replacer at each feeding.
- Glycerol replaced lactose (15% of the formula, replaced 37.5% of the lactose).
- Calves were fed diets from d 3 through 49.
- No differences were detected between diets for growth, feed efficiency, or health.
Glycerol as a partial replacement for lactose in milk replacer for young dairy calves

R. A. Ebert-Allen,1* G. M. Willis,2† and J. K. Drackley1‡

Abstract: Glycerol (glycerin) is increasingly available from biodiesel manufacture and edible oil refining and it has been used successfully in diets for chickens, pigs, and adult cattle; however, less information is available on its nutritional value in young calves. Our objective was to determine the effects on calf growth and health when glycerol replaced a portion of lactose in milk replacer. Holstein calves (12 male, 12 female) born at the University of Illinois dairy unit were assigned alternately to 1 of 2 treatments (24 calves total): control milk replacer or milk replacer supplemented with 15% glycerol in replacement of lactose. 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 that of the control, except that glycerol replaced some lactose. Calves were housed in individual hutches bedded with straw, and water was freely available. Starter was offered beginning on d 36. The amount of milk replacer offered was reduced by half on d 43, and calves were weaned at d 49. 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, and the 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 0.25 Mcal/kg of metabolic body weight (BW) (about 1.5% of BW daily as powder, or approximately 675 g/d) and from wk 2 to 6 at 0.30 Mcal/kg of metabolic BW (about 2% of BW daily, or approximately 900 to 1,200 g/d). Measurements of BW and stature were made weekly through d 56. Calf BW and average daily gain through d 35 (0.66 vs. 0.65 kg/d for controls and glycerol, respectively) did not differ significantly between treatments. Stature measurements (withers height, body length, heart girth) and measures of health (fecal scores, medical treatments) did not differ between treatments. Under the conditions of this experiment, glycerol was an acceptable replacement for at least 37.5% of the total lactose in milk replacer (15% of the formula) if economically favorable.

Lactose is the primary ingredient in milk replacers for young calves, constituting 40 to 50% of the DM in the formula. The high and variable cost of dairy ingredients creates an economic incentive to replace ingredients such as lactose with nondairy energy sources. One possible ingredient is glycerol, which is widely available from biodiesel manufacture and edible oil refining. Glycerol is rapidly absorbed from the small intestine (Höber and Höber, 1937) by passive diffusion and by Na+-dependent carriers (Kato et al., 2005). Glycerol can be directly oxidized or converted into glucose by the liver in the process of gluconeogenesis. Glycerol has been successfully used in feeding poultry (Lima et al., 2013), swine (Hernández et al., 2016), and adult cattle (Kholif, 2019). However, less is known about its potential use in milk replacer for young dairy calves.

Our hypothesis was that growth would be less when calves were fed a milk replacer containing 15% glycerol. Our objective was to determine whether glycerol could replace a portion of the lactose in an “all-milk” milk replacer formula without compromising growth and health of young calves.

All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (IACUC protocol number 06132). Holstein calves (24 in total; 6 male and 6 female calves per treatment) born at the University of Illinois dairy unit from May through June 2007 were housed in individual hutches bedded with straw; water was freely available at all times. The number of calves chosen was sufficient to detect a 0.15 kg/d difference in ADG between treatments at the $P = 0.05$ level with 80% power. Calves were managed at birth and for the first 2 d according to standard protocols at the dairy unit. Calves received a minimum of 3 L of colostrum as soon as possible after birth and were then fed colostrum and transition milk (2 L per feeding) through d 2 of life.

Calves were assigned alternately to each of 2 milk replacer treatments (24 calves total) beginning on d 3 of life. Treatments were an all-milk protein-based milk replacer (Excelerate, Milk Specialties Global) containing 28% CP, 2.6% lysine, and 15% fat, with all carbohydrate as lactose; or an all-milk protein-based milk replacer with similar CP, lysine, and fat contents as the control but supplemented with 15% glycerol (Sigma Chemical Co.) replacing a portion of the lactose. The base milk replacer for the glycerol diet (Table 1) contained greater amounts of protein, fat, minerals, and vitamins than the control diet so that when glycerol was added, the final composition would be the same as that of the control, except that glycerol replaced 37.5% of the lactose. The control milk replacer contained 40% lactose; the glycerol milk replacer, as fed, contained 25% lactose. The substitution of glycerol for lactose would be expected to increase the osmolality of a 15% reconstituted milk replacer solution by 179 mOsm/kg. Diets were formulated to meet or exceed established requirements for all other nutrients (NRC, 2001). Both milk replacers were reconstituted to 15% solids and fed twice daily. Glycerol (liquid) was added to reconstituted base milk replacer at each feeding.

---

1Department of Animal Sciences, University of Illinois Urbana-Champaign, Urbana 61801. 2Milk Specialties Global, Eden Prairie, MN 55344. *Present address: Jump Simulation, 1306 N. Berkeley Ave., Peoria, IL 61603. †Present address: 1463 Valley Dr., Robertsville, MO 63072. ‡Corresponding author: drackley@illinois.edu. © 2022, The Authors. Published by Elsevier Inc. and Fass Inc. on behalf of the American Dairy Science Association®. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/). Received October 25, 2021. Accepted December 14, 2021.
Table 1. Formulated composition of milk replacers (% as received unless otherwise noted)

| Component   | Control | Glycerol |
|-------------|---------|----------|
| CF          | 28.5    | 33.5     |
| Crude fat   | 15.0    | 17.6     |
| Ash         | 7.0     | 6.7      |
| Lactose     | 40.1    | 32.9     |
| Gross energy (kcal/kg) | 4.635 | 4.882 |
| Calcium     | 0.90    | 0.98     |
| Phosphorus  | 0.67    | 0.67     |
| Lysine      | 2.79    | 3.31     |
| Methionine  | 0.58    | 0.68     |
| Cysteine    | 0.87    | 1.05     |
| Histidine   | 0.46    | 0.54     |
| Isoleucine  | 1.76    | 2.07     |
| Leucine     | 2.98    | 3.52     |
| Valine      | 1.66    | 1.97     |
| Arginine    | 0.76    | 0.88     |
| Phenylalanine | 0.92  | 1.09     |
| Tyrosine    | 0.82    | 0.97     |
| Threonine   | 1.95    | 2.32     |
| Tryptophan  | 0.44    | 0.51     |

Milk replacers were fed at a rate of 0.25 Mcal of gross energy/kg of BW0.75 (where BW0.75 represents metabolic BW) during wk 1, and at a rate of 0.30 Mcal/kg of BW0.75 from wk 2 through 6. These feeding rates equated to approximately 1.5% and 2% of BW (as powder) daily, respectively. Amounts fed were adjusted weekly as calves grew. No starter feed was offered until the beginning of wk 6 to avoid the variability introduced by dry feed consumption. During wk 6 to 8, starter (18% CP, 14% NDF) was offered for ad libitum intake to all calves. At the start of wk 7, the amount of milk replacer was increased according to that week’s BW and then the amount fed was reduced by half (i.e., the afternoon feeding was eliminated). Calves were weaned at the end of wk 7 and remained in the hutches through wk 8. Measurements and feed amount adjustments were made on the same day each week. The calf’s actual age at each experimental week was recorded and used as a covariate in statistical analysis where appropriate.

Intakes of milk replacer and starter were recorded daily. Feeds were sampled weekly and then formed into monthly composites.

Table 2. Health data for calves (n = 12 per diet) fed only a control milk replacer or a similar milk replacer with glycerol replacing a portion of the lactose during wk 1 to 5, with starter offered during wk 6 to 8

| Item                        | Diet       |
|-----------------------------|------------|
|                             | Control    | Glycerol | SEM | P <  |
| Average fecal score1        |            |          |     |      |
| Wk 1 to 5                   | 2.72       | 2.73     | 0.037 | 0.75 |
| Wk 1 to 8                   | 2.62       | 2.58     | 0.047 | 0.54 |
| Average days with fecal score > 31 |            |          |     |      |
| Wk 1 to 5                   | 1.33       | 1.75     | 0.53  | 0.59 |
| Wk 1 to 8                   | 1.58       | 1.75     | 0.58  | 0.84 |
| Respiratory disease2        |            |          |     |      |
| Wk 1 to 5                   | 1          | 1        | —     | —    |
| Wk 1 to 8                   | 2          | 8        | —     | —    |
| Ear infection3              |            |          |     |      |
| Wk 1 to 5                   | 0          | 1        | —     | —    |
| Wk 1 to 8                   | 1          | 0        | —     | —    |

1 On a 1 (firm) to 5 (watery) visual scale.
2 Number of calves with at least one incidence.

Data were analyzed statistically using the Mixed procedure of SAS (v. 9.1; SAS Institute Inc.). Separate statistical analyses were conducted for the period from birth through wk 5 (when only milk replacer was fed), from wk 6 through 8 (when starter was fed), and from birth through the end of the trial at wk 8. Variables measured at the end of wk 5 (total milk replacer intake, BW, ADG, stature measurements, and gain:feed) and wk 8 (starter intake, total DMI, BW, ADG, gain:feed, stature measurements) were analyzed using an initial model that contained birth BW and age at measurement as continuous variables (covariates), diet, sex, and diet × sex. Effects of birth BW, age, sex, and diet × sex were not retained in the final model if P > 0.15. Weekly growth measurements were analyzed as repeated measures with an initial model that contained the effects of birth BW as a continuous variable (covariate), diet, sex, diet × sex, week, diet × week, sex × week, and diet × sex × week. Calf was the experimental unit, and the effects of week and its interactions were tested with the model residual error. Several covariance structures were examined, and the one that yielded the lowest Akaike’s information criterion (first-order autoregressive with heterogeneous variance) was used in the model. Health data were evaluated by logistic regression using a binomial distribution in the GLIMMIX procedure in SAS. Least squares means were calculated and are presented throughout. Because animal numbers were limited, results were considered statistically significant when P < 0.10.

Diets mixed easily and were stable in solution. Calves readily consumed both diets with no apparent palatability or acceptability problems. Mean fecal scores (Table 2) did not differ during the milk replacer feeding period (mean 2.7 on a 5-point scale) or for the entire trial (mean 2.6). Incidences of other health events are tabulated in Table 2. Of note is the greater incidence of navel infections for calves fed the glycerol-supplemented milk replacer. In all...
but one calf, the first diagnosis of navel infection was made within 3 d after birth, when the experimental treatments began. For the other calf, the navel infection occurred subsequent to respiratory illness and heat stress. Consequently, it is highly unlikely that the greater number of navel infections was related to the glycerol supplementation. Nevertheless, the high incidence of navel infections could have increased maintenance needs for energy and protein, thus decreasing growth in the affected calves.

Table 3. Growth measurements for calves (n = 12 per diet) fed a control milk replacer or a similar milk replacer with glycerol replacing a portion of the lactose

| Variable                      | Control  | Glycerol | SEM  | P-value |
|-------------------------------|----------|----------|------|---------|
| Birth BW1 (kg)                | 43.3     | 43.6     | 1.79 | 0.93    |
| Actual age at wk 5 (d)        | 35.5     | 34.4     | 0.57 | 0.19    |
| BW, wk 5 (kg)                 | 66.8     | 66.4     | 0.96 | 0.78    |
| ADG, wk 1 to 5 (kg)           | 0.66     | 0.65     | 0.028| 0.77    |
| Milk replacer intake, wk 1 to 5 (kg/d) | 1.03  | 1.02     | 0.026| 0.67    |
| Gainfeed, wk 1 to 5           | 0.64     | 0.64     | 0.015| 0.75    |
| Withers height, wk 5 (cm)     | 85.3     | 86.2     | 0.66 | 0.39    |
| Heart girth, wk 5 (cm)        | 90.8     | 90.8     | 0.62 | 0.95    |
| Body length, wk 5 (cm)        | 69.5     | 69.0     | 0.47 | 0.49    |
| Actual age at wk 8 (d)        | 56.5     | 55.4     | 0.57 | 0.19    |
| BW, wk 8 (kg)                 | 76.0     | 75.0     | 1.82 | 0.71    |
| ADG, wk 6 to 8 (kg)           | 0.50     | 0.46     | 0.031| 0.34    |
| ADG, wk 1 to 8 (kg)           | 0.58     | 0.56     | 0.032| 0.71    |
| Starter intake, wk 6 to 8 (kg/d) | 0.88  | 0.82     | 0.072| 0.52    |
| Total DMI, wk 1 to 8 (kg/d)   | 0.98     | 0.94     | 0.040| 0.55    |
| Gainfeed, wk 1 to 8           | 0.62     | 0.62     | 0.013| 0.97    |
| Withers height, wk 8 (cm)     | 89.2     | 89.2     | 0.76 | 0.99    |
| Heart girth, wk 8 (cm)        | 95.9     | 95.0     | 1.04 | 0.54    |
| Body length, wk 8 (cm)        | 74.2     | 73.9     | 0.60 | 0.77    |

1Sex effect, P = 0.16 (male = 45.3 kg, female = 41.6 kg).
2Diet by sex interaction, P < 0.05 (male control = 0.63, male glycerol = 0.68, female control = 0.66, female glycerol = 0.60).
3Sex effect, P < 0.03 (male = 84.6 cm, female = 86.9 cm).
4Diet by sex interaction, P < 0.05 (male control = 0.63, male glycerol = 0.67, female control = 0.61, female glycerol = 0.57).
5Sex effect, P = 0.15 (male = 88.4 cm, female = 90.1 cm).
6Sex effect, P = 0.07 (male = 73.2 cm, female = 74.9 cm).
and metabolic and feed intake regulating hormones. Animal 10:919–926. 
https://doi.org/10.1017/S175173111500275X.

Höber, R., and J. Höber. 1937. Experiments on the absorption of organic solutes 
in the small intestine of rats. J. Cell. Comp. Physiol. 10:401–422. https://
doi.org/10.1002/jcp.1030100402.

Kato, T., Y. Hayashi, K. Inoue, and H. Yuasa. 2005. Glycerol absorption by 
Na+-dependent carrier-mediated transport in the closed loop of the rat small 
intestine. Biol. Pharm. Bull. 28:553–555. https://doi.org/10.1248/bpb.28 .553.

Kholif, A. E. 2019. Glycerol use in dairy diets: A systemic review. Anim. Nutr. 
5:209–216. https://doi.org/10.1016/j.janimu.2019.06.002.

Lima, E. M. C., P. B. Rodrigues, R. R. Alvarenga, V. M. P. Bernardino, L. 
Makiyama, R. R. Lima, V. S. Cantarelli, and M. G. Zangeronimo. 2013. 
The energy value of biodiesel glycerine products fed to broilers at different 
ages. J. Anim. Physiol. Anim. Nutr. (Berl.) 97:896–903.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. 
Press.

Vermeire, D. A. 2008. Milk replacer composition and product and method for 
producing the same. US Patent 20080260895A1.

Notes

J. K. Drackley ◂ https://orcid.org/0000-0002-4560-5594

Funding for this study was provided by Milk Specialties Global (Eden Prairie, 
MN).

The authors have not stated any conflicts of interest.