ABSTRACT

Young Holstein male calves, fitted with re-entrant duodenal fistulae, were fed 25% fat milk replacers containing either butter oil, lard, or corn oil in a 3 x 3 Latin square experiment. Proteins were supplied by skim milk powder. Patterns of changes with time after feeding were observed for the composition and pH of duodenal digesta, recovery rates of fresh and dry matter, rates of abomasum emptying for fresh and dry matter, gastric proteolysis, and diarrhea.

The composition and pH of duodenal digesta changed markedly with time after feeding, but the only notable differences between milk replacers were for contents of protein nitrogen and total nitrogen. Recovery rates of fresh matter were higher than 100%, presumably due to salivary and gastric secretions. Dry matter was recovered at a rate averaging 100% suggesting that the method used to measure the flow rate and sample duodenal digesta was adequate. The highest flow rates of fresh matter were during the first 3 h after feeding. Differences were marked among the flow rates of constituents of dry matter. Gastric proteolysis was not influenced by the nature of dietary fats. Fecal dry matter, a measure of the severity of diarrhea, was markedly lower for the milk replacer filled with corn oil than for the other two milk replacers.

INTRODUCTION

Highly unsaturated vegetable oils increased the incidence of diarrhea when used as energy sources in the formulation of milk replacers for the young dairy calf (1, 3, 6, 10, 25). The mechanism by which highly unsaturated fats cause diarrhea in the young calf has not been elucidated.

Factors that reduce or prevent the coagulation of milk in the abomasum may increase the incidence of diarrhea (4, 7, 14, 15, 17). Diarrhea in such cases is believed to result from the rapid passage of proteins through the abomasum, leading successively to a reduced gastric proteolysis, an increased concentration of partially digested proteins in the small intestine, massive bacterial activity in this region, and finally diarrhea (4, 24).

The degree of unsaturation of dietary fats influences time for stomach emptying in dog, cat, rat, and children (20, 23). Therefore, the diarrhea in calves fed highly unsaturated oils could be due to an increase rate of passage of proteins through the abomasum which successively would reduce gastric proteolysis, augment the concentration of partially digested proteins in the small intestine, and lead to massive bacterial activity in that region resulting in diarrhea.

The purpose of our study was to compare the rate of passage of material through the abomasum and the degree of gastric proteolysis in young dairy calves fed milk replacers containing either corn oil as an unsaturated vegetable fat or butter oil or lard as saturated fats.

MATERIALS AND METHODS

Animals

Three Holstein male calves, 3 to 4 days old, were purchased from local farmers and housed in individual cages in a room where temperature was thermostatically controlled (19°C). Three to four days later the calves were fitted with re-entrant duodenal fistulae. The surgical procedure was the same as described by Markowicz et al. (9) using T-shaped cannulae prepared from medical-grade Silastic® tubing 9.5 mm ID (inside diameter). The cannulae were inserted in the proximal duodenum below the pyloric valve.
sphincter and above the bile duct. Externally the two cannulae were connected to each other by a U-tube prepared from Tygon® tubing. The calves were allowed 2 wk to recover from surgery before this experiment was started.

Diets

Three milk replacers were formulated to contain 25% fat dry matter. The sources of fat were either butter oil (BO), lard (LA), or corn oil (CO). The composition of the milk replacers is in Table 1. They were prepared in two steps: First, a 40% fat concentrate was prepared by spray-drying a homogenized mixture of warm water (65 C), skim milk powder, a fat source (butter oil, lard, or corn oil), and an emulsifier. Second, the remaining ingredients were added for the desired milk replacer formulation.

Each of the three milk replacers was offered to each of the three animals for 7 days in a 3 x 3 Latin square. During the first 14 days, each feeding consisted of 290 g of milk replacer dissolved in 1800 ml of water at 45 C. During the last 7 days each feeding consisted of 330 g of milk replacer dissolved in 2000 ml of water. The calves were fed by nipple pail twice daily at 0800 and 2000 h.

Measurement and Sampling of Duodenal Flow

Duodenal flow was measured for 12 h from 0800 to 2000 h during the last 3 days of each 7-day feeding by a manual flowmeter (5). The flowmeter also was used to collect samples of the digesta flowing from the abomasum. Digesta samples of approximately 50 ml were collected just before feeding, at 0800 h, and at hourly intervals from 0900 to 2000 h. Immediately after collection, the pH of the samples was determined with a glass electrode pH meter. The samples then were stored at −20 C until analyzed. The amounts of dry matter and of its constituents flowing out of the abomasum during a given 1 h were calculated from the volume of digesta flowing through the flowmeter and the average composition of digesta samples at the beginning and at the end of the same hour.

Measurement of Gastric Proteolysis

The proportion of ingested protein nitrogen converted into nonprotein nitrogen during its passage through the abomasum was a measure of gastric proteolysis. This proportion was calculated:

\[
P_1 = 100(P_2 - P_3)/P_4
\]

TABLE 1. Composition of the milk replacers.

<table>
<thead>
<tr>
<th>Item</th>
<th>Butter oil (BO)</th>
<th>Lard (LA)</th>
<th>Corn oil (CO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skim milk powder</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Butter oil</td>
<td>25</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Lard</td>
<td>...</td>
<td>25</td>
<td>...</td>
</tr>
<tr>
<td>Corn oil</td>
<td>...</td>
<td>...</td>
<td>25</td>
</tr>
<tr>
<td>Additives a</td>
<td>See note</td>
<td>See note</td>
<td>See note</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical analysis (%)</th>
<th>Milk replacers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>97.7</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>4.02</td>
</tr>
<tr>
<td>Protein nitrogen</td>
<td>3.84</td>
</tr>
<tr>
<td>Nonprotein nitrogen</td>
<td>.18</td>
</tr>
<tr>
<td>Fat</td>
<td>25.3</td>
</tr>
<tr>
<td>Reducing substances</td>
<td>40.5</td>
</tr>
<tr>
<td>Ash</td>
<td>6.2</td>
</tr>
</tbody>
</table>

| All three milk replacers were supplemented with the following additives: Vitamin A, 32,500 IU/kg of dry matter; vitamin D₃, 11,000 IU/kg of dry matter; vitamin E, 80 IU/kg of dry matter; terramycin, 1.0 g/kg of dry matter, 55 mg/g of oxytetracycline hydrochloride, Pfizer Ltd., 17,300 Route Transcanadienne, Kirkland, Quebec; and emulsifier, 2.0 g/kg of fat, glycerol monostearate, Fisher Scientific Co., Ltd., 2480 Chemin Sainte-Foy, Sainte-Foy, Quebec.
where: \( P_1 \) = the percentage of ingested protein nitrogen converted into nonprotein nitrogen during its passage through the abomasum; 
\( P_2 \) = nonprotein nitrogen as percent of the total nitrogen which left the abomasum during the 12-h sampling (0800 to 200 h); 
\( P_3 \) = nonprotein nitrogen as percent of the total nitrogen ingested; 
\( P_4 \) = protein nitrogen as percent of the total nitrogen ingested.

Measurement of Diarrhea

During the periods when duodenal flow was measured, feces were collected in plastic bags and stored at \(-20^\circ C\) until analyzed for dry matter content. Fecal dry matter was a measure of the severity of diarrhea (4, 8, 13). Feces with a dry matter content over 20% were normal, under 12% diarrheic, intermediate "loose" (8).

Chemical Analyses

*Duodenal digesta.* Dry matter was determined by drying at 80 \(^\circ\)C for 12 h in a vacuum oven and ash by incineration in a muffle furnace at 550 \(^\circ\)C for 12 h. Total and nonprotein nitrogen (TN and NPN) were measured by Kjeldahl method with a Technicon AutoAnalyzer (18). Nonprotein nitrogen was determined on the supernatant following precipitation of the protein fraction with trichloroacetic acid at 12% final concentration. Protein nitrogen (PN) then was calculated by difference between TN and NPN. Reducing substances (RS) mainly lactose and/or its hydrolysis products were measured on the protein-free supernatant used for NPN determination by a colorimetric method based on the reduction of 3,5-dinitrosalicylic acid. Fat was measured by the Roese-Gottlieb method (2) adapted to use 3-ml aliquots.

*Milk replacers.* Dry matter was determined by drying 3-g samples at 80 \(^\circ\)C for 12 h in a vacuum oven. The dried samples then were incinerated in a muffle furnace at 550 \(^\circ\)C for 12 h. The TN, PN, NPN, RS, and fat in the reconstituted milks (10 g made up to 100 ml) were determined by the same methods as for duodenal digesta.

*Feces.* To determine dry matter content, the feces were first freeze-dried to remove most of the water and then placed in a vacuum oven at 80 \(^\circ\)C for 24 h.

*Statistical analyses.* All results were submitted to analysis of variance, and when applicable, Duncan's new multiple range test was used to compare treatment means (16).

**RESULTS AND DISCUSSION**

Flow of Fresh Matter from the Abomasum

The hourly and cumulative flow rates of fresh matter over 12 h are in Fig. 1. The highest flow rates of fresh matter were during the first 3 h after feeding when 43.5% ± 8.8% fresh matter passed through the flowmeter. No difference between milk replacers was significant (\(P<.05\)), suggesting that the rate of abomasum emptying in these calves was not influenced by the degree of unsaturation of the dietary fats. This was contrary to observations in the cat, rat, and child where the rate of stomach emptying decreased as the degree of unsaturation of the dietary fats increased (20).
Over 12 h, the recovery rate of the fresh matter ingested averaged 192 ± 24% (Table 2). No difference between milk replacers was significant (P<.05). The high recovery rate of fresh matter was presumably due to salivary and gastric secretions. On the average, the volume of these secretions over 12 h, calculated as the difference between the ingested and the recovered fresh matter volumes, was 1949 ± 523 ml, which is comparable to values in calves fed whole milk (11, 19, 22).

**Composition of Duodenal Digesta**

The composition and pH of duodenal digesta changed markedly with time after feeding (Fig. 2 and 3). Dry matter content remained near 8% for 7 h after feeding, then progressively decreased to 5% until the next meal (Fig. 2A). The TN (Fig. 2B), PN (Fig. 2C), and fat (Fig. 3F) contents decreased during the 1st h after feeding, then increased to reach a peak at about 6 to 7 h after feeding and decreased again until the next meal. The peaks in PN and fat contents 6 to 7 h after feeding were presumably due to the lysis of the coagulum. The RS content (Fig. 3E) increased sharply during the 1st h after feeding, then decreased gradually to its initial concentration. The variations in NPN (Fig. 2D) and ash (Fig. 3G) contents throughout the 12 h were much less pronounced than for the other constituents. In the case of NPN, the concentrations are always lower for BO than for the other two sources of fat, but at no time interval were the differences significant (P<.05).

The only notable differences between milk replacers were for PN content (Fig. 2C) and, consequently, for TN content (Fig. 2B); although the patterns of changes in PN and TN contents with time after feeding were about the same for all three milk replacers, variations were more pronounced with milk replacer BO than with milk replacers LA and CO.

The pH of duodenal digesta (Fig. 3H) increased sharply during the 1st h after feeding, then decreased gradually to its initial value. No difference between milk replacers was significant (P<.05), suggesting that gastric secretion of HCl in these calves was not influenced by the degree of unsaturation of the dietary fats. This was contrary to observations in rat, cat, and man where gastric secretion of HCl decreased as the degree of unsaturation of

<table>
<thead>
<tr>
<th>Feed components</th>
<th>Butter oil</th>
<th>Milk replacers</th>
<th>Corn oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh matter</td>
<td>191.1</td>
<td>192</td>
<td>24.1</td>
</tr>
<tr>
<td>Dry matter</td>
<td>19.9</td>
<td>10.0</td>
<td>11.1</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>1.9</td>
<td>11.3</td>
<td>14.1</td>
</tr>
<tr>
<td>Protein nitrogen</td>
<td>0.9</td>
<td>20.0</td>
<td>12.5</td>
</tr>
<tr>
<td>Neutral nitrogen</td>
<td>8.7</td>
<td>12.0</td>
<td>7.3</td>
</tr>
<tr>
<td>Reducing substances</td>
<td>6.3</td>
<td>12.7</td>
<td>4.2</td>
</tr>
<tr>
<td>Fat</td>
<td>8.0</td>
<td>7.4</td>
<td>8.7</td>
</tr>
<tr>
<td>Ash</td>
<td>1.5</td>
<td>1.6</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*Values are average of nine measurements.*
the dietary fats increased (12, 20).

Flow of Dry Matter and Its Constituents from the Abomasum

The hourly and cumulative flow rates of dry matter and its constituents over 12 h are in Fig. 4 and 5. Differences were marked among the flow rates of the various constituents of dry matter. For example, 90 ± 7% of the total flow of RS occurred in the first 6 h after feeding whereas corresponding values for PN and fat were 61 ± 12% and 57 ± 13%. This agrees with observations in calves fed whole milk (11, 21). Such differences were presumably due to the fact that RS, being soluble, were not retained in the coagulum, unlike PN and fat. No significant (P<.05) difference was between milk replacers, suggesting that the nature of the dietary fats not only had no influence on the rate of abomasum emptying of fresh matter but also did not influence the rate of passage of the constituents of dry matter through the abomasum.

The recovery rates of dry matter and its constituents over 12 h are in Table 2. Dry matter was recovered at a rate averaging 100 ± 11%; this suggests that the method to measure the flow rate and take samples of duodenal digesta was adequate. Recovery rates for different constituents of dry matter varied considerably. For TN and ash, the recovery rates averaged 116 ± 18% and 124 ± 15%; these high values were presumably due to salivary and gastric secretions. For PN and NPN, the recovery rates averaged 90 ± 18% and 694 ± 148%; this could be expected since PN was hydrolyzed to NPN during its passage through the abomasum. Fat and RS were recovered at the respective rates of 86 ± 18% and 85 ± 11%. Fermentation in the stomach was probably
Gastric Proteolysis

The extent of gastric proteolysis was measured by the proportion of ingested PN hydrolyzed into NPN during its passage through the abomasum (see Materials and Methods). These proportions reached 24.8 ± 4.5%, 29.0 ± 6.5%, and 26.0 ± 6.5% for milk replacers BO, LA, and CO. These values were not significantly (P<.05) different, thus suggesting that gastric proteolysis was not influenced by the nature of the dietary fats. This could be expected since the rate of passage of PN through the abomasum and gastric secretion of HCl, two factors which could have influenced gastric proteolysis, were not influenced by the nature of the dietary fats.

Diarrhea

Fecal dry matter, a measure of the severity of diarrhea, averaged 20.0 ± .8%, 22.3 ± 3.5%, 20.0 ± .8%, and 18.0 ± 2.0% for milk replacers BO, LA, and CO. These values were not significantly (P<.05) different. BO = butter oil; LA = lard; CO = corn oil.
FIG. 5. Influence of the nature of dietary fat on the hourly and cumulative flow rates of reducing substances (RS), fat and ash. Data are expressed as percentages of total flow over 12 h. At a given hour, the values were not significantly (P > .05) different. BO = butter oil; LA = lard; CO = corn oil.

and 14.5 ± 6.0% for milk replacers BO, LA, and CO. Fecal dry matter was thus markedly lower for milk replacer CO than for the other two milk replacers; this agrees with observations in the young calf that corn oil and other unsaturated oils have laxative properties, contrary to butter oil, lard, and other saturated fats (1, 3, 6, 10, 25). The differences in fecal dry matter between milk replacers CO and milk replacers BO and LA, though marked, were not statistically significant (P < .05). Nevertheless, the results suggest that corn oil had a laxative effect as shown by the fecal dry matter content of milk replacer CO.

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