Absorption of Colostral Immunoglobulin G in the Newborn Dairy Calf

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ABSTRACT

Five groups of eight newborn calves were used to study absorption of colostral immunoglobulin G. One feeding of 2 liters of pooled colostrum was given at one of 6, 12, 24, 36, or 48 h after birth. Concentrations of immunoglobulin G in blood plasma and feces were measured by an immunodiffusion technique. Plasma volume and fecal excretion also were measured. When colostrum was given 6 h after birth, 65.8% of the ingested immunoglobulin G appeared in the plasma. This percentage declined rapidly to reach 46.9%, 11.5%, 6.7%, and 6.0% when colostrum was given at the ages of 12, 24, 36, and 48 h. Total fecal immunoglobulin G increased linearly with age. The quantities not recovered from plasma and feces reached a maximum when colostrum was given at 24 or 36 h after birth. Immunoglobulin G can be "lost" to a great extent via routes other than plasma and feces during this time. Quantities of immunoglobulin G measured in plasma represent apparent rather than true absorption.

INTRODUCTION

The dairy calf is born with insufficient immunity to resist infection in its new environment. Passive immunity is acquired through colostral immunoglobulins. However, the ability to absorb colostral immunoglobulins is restricted to a short time, which is believed to last between 24 (23) and 36 h (5, 10) after birth. The exact time through which the newborn calf can absorb colostral immunoglobulins, however, has not been determined directly and definitely. This question was reviewed by Bush and Staley (2).

The efficiency of immunoglobulin absorption in the newborn calf was evaluated by measuring either concentrations in blood plasma alone or concentrations and volumes of plasma. Husband et al. (7) observed that 44% of the immunoglobulins G (IgG) ingested appeared in the blood stream when colostrum was given 2 to 7 h after birth and volume of plasma was estimated to be 7% of body weight. Bush et al. (3) reported that the efficiency of absorption of colostral IgG was 66% during the first 24 h after birth. When plasma volume and immunoglobulin concentration were measured, Husband et al. (8) recorded an efficiency of absorption of 46%, but McEwan et al. (16) observed that only 25% of the ingested IgG were transferred to the blood circulation when colostrum was offered before the age of 15 h. Kruse (10) proposed an absorption coefficient taking into account both blood volume and immunoglobulin transfer from intravascular to extravascular spaces. Using this coefficient, he observed a linear decrease in efficiency of absorption from 2 to 20 h after birth. When Kruse's coefficient was used in our laboratory to predict the efficiency of absorption of IgG in newborn calves, the quantity in plasma was twice as high as predicted (Matte, unpublished data). In view of this discrepancy, the apparent and true absorption of IgG by newborn calves was measured by our taking into account quantity of IgG ingested, IgG concentration in plasma, plasma volume, fecal IgG concentration, and fecal volume.
MATERIALS AND METHODS

Colostrum Pool

About 100 liters of colostrum from first and second milkings after parturition were collected, immediately frozen, and kept at -20°C. A single pool of colostrum having a fixed IgG content was prepared by thawing and mixing in a single container all batches of colostrum collected. Then the colostrum was transferred in 2-liter plastic containers and stored at -20°C. Concentration of IgG was measured in duplicate by an immunodiffusion technique with quantitative radial immunodiffusion kits. A 5-μl syringe set at 2.5 μl was used to deposit plasma and standards on the plates. Determinations were on the pooled colostrum before storage and during the feeding trial. All determinations showed a uniform concentration of 40 mg/ml.

Colostrum Feeding

Forty newborn Holstein male calves were used. They were purchased from local farmers, and exact time of birth was recorded. Calves were housed in individual wooden cages with slatted floors and placed in a room where temperature was controlled thermostatically at 20 ± 2°C and relative humidity was maintained at 65% by a humidistat installed in the ventilation system. Eight animals were randomly assigned to each of the groups to be given a single dose of 2 liters of colostrum (80 g IgG) at 6, 12, 24, 36, or 48 h after birth. This amount of colostrum was consumed readily by all calves. In addition, each calf was fed 1.8 liters of milk replacer twice a day at 12-h intervals except the day colostrum was fed when only one feeding of milk replacer was offered. Milk replacer (Table 1) was reconstituted with tap water to contain 13% total solids. Colostrum and milk replacer were fed at body temperature in nipple-type pails.

Blood Sampling

Blood samples were drawn from the jugular vein in 10-ml heparinized syringes immediately before and 6 h after colostrum was fed. The blood was transferred into glass tubes and centrifuged at 6,700 × g for 20 min. Then the plasma was withdrawn, transferred into 10-ml Virtis bottles, and stored at -20°C. Concentrations of IgG in plasma were measured in duplicate by the immunodiffusion technique described. A third determination followed a difference between duplicates greater than 15%.

Measuring Plasma Volume

Plasma volume was measured in each calf with Evans Blue dye as an indicator. In general, 3 ml of a 2% sterile saline dye solution was injected intravenously 6 h after colostrum feeding, time at which IgG absorption was expected to have reached its peak (4). Ten minutes after injection of the dye solution, a blood sample was taken and handled as described. In the series of calves fed colostrum at 36 or 48 h, the quantity of Evans Blue was reduced to 2 ml when calves weighed less than 40 kg.

Dye concentrations were measured in fresh plasma by a Bausch and Lomb spectrophotometer set at 624 μm. Whenever the optical density was above .80, the plasma was diluted with saline. Plasma volume for each calf was calculated according to the respective Evans Blue concentrations in the plasma and the amount injected. Total IgG in plasma was calculated on plasma concentration and volume.

Collecting Feces

Feces were collected quantitatively for each calf during 48 h following colostrum feeding.

TABLE 1. Composition of the milk replacer.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High fat pre-mix (32.8% tallow)</td>
<td>60.98</td>
</tr>
<tr>
<td>Skim milk powder</td>
<td>27.35</td>
</tr>
<tr>
<td>Corn starch</td>
<td>5.00</td>
</tr>
<tr>
<td>Whey powder</td>
<td>7.70</td>
</tr>
<tr>
<td>Dextrose</td>
<td>.67</td>
</tr>
<tr>
<td>Vitamin mixture1</td>
<td>.11</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>.20</td>
</tr>
</tbody>
</table>

1 Vitamin-mix No. 10376, Pfizer Co. Ltd., Montréal, Québec.

2 Quantitative kits for Bovine IgG Determination, Miles Laboratories, Inc., Elkhart, IN.

3 Spectronic 20.
They were collected by a simple device of two plastic bags fitted to a plastic collar. One of the bags cut open at both ends was glued around the anus; the second bag connected to the first one by means of an elastic band and a hard plastic collar served to collect feces.

Feces then were stored at −20°C. Prior to immunoglobulin analysis, feces were allowed to thaw overnight, pooled for each calf, and homogenized in a 4-liter Waring Blender. When feces were too hard for proper homogenization, a known quantity of phosphate buffered saline solution (PBS) was added. Two 40-ml aliquots were centrifuged at 4°C for 30 min at 27,000 \( \times \) g, and the supernatant was measured accurately to determine the total volume of fecal liquid. The concentration of IgG in the fecal liquid was ascertained by the immunodiffusion technique described above. Total immunoglobulin excretion in feces was calculated on IgG concentration and total fecal liquid volume.

Statistical Analysis

All data were submitted to the test of Bartlett (20) for homogeneity of variance. Square roots of the data were used when the variance was not homogeneous. Regression analyses were according to Snedecor and Cochran (20).

**RESULTS AND DISCUSSION**

**Plasma Volume**

Plasma volume and plasma IgG are in Table 2. Plasma volume, which was near 6,000 ml (14.2% of body weight) for calves receiving colostrum at 6 or 12 h of age, decreased to approximately 3,000 and 3,500 ml (8.5% of body weight) in calves fed the colostrum at 36 or 48 h. The group fed colostrum at 24 h of age had intermediate amounts. A plasma volume of 8.5% of body weight would be intermediate between volumes obtained by McEwan et al. (15) and Husband et al. (8), but volumes equal to 14.2% of live weight would be markedly higher than those previously reported. In our study, plasma volume was measured 6 h after colostrum feeding instead of 12 or 72 h as was the case in experiments reported by Husband et al. (8) and McEwan et al. (15). This may partly explain the greater plasma volumes in our work.
Colostrum was the only source of water ingested by the calves fed at 36 or 48 h after birth. For these calves, feeding milk replacer before feeding colostrum had to be discontinued, because this procedure induced severe diarrhea and high mortality. Therefore, they received no liquid until colostrum was fed. This may explain, at least in part, why blood volume in these two groups of calves was lower than in the other groups, which were fed colostrum at a younger age. It is possible that fasting during 36 or 48 h induced tissue dehydration and consequently lowered plasma volume.

According to Shannon and Lascelles (19), the increase in plasma volume following colostrum feeding is influenced, in part, by the quantity of immunoglobulins absorbed because of an osmotic phenomenon. If absorption of IgG is reduced considerably in calves older than 24 h, as we observed, it could be expected that feeding colostrum then would increase plasma volume less than at a younger age on account of a smaller osmotic pressure induced.

**Plasma IgG**

Plasma volumes and IgG concentrations were bases for calculating quantities of IgG appearing in plasma. Plasma IgG’s are in Table 2, and the relationships with age are illustrated in Figure 1. When colostrum was given at the age of 6 h, 65% of the ingested IgG appeared in the plasma, but this percentage decreased rapidly to plateau near 6% when colostrum was given at 36 or 48 h after birth. The regression equation was \( Y = 9.469 - .374X + .004X^2 \), where \( Y \) = the square root of total plasma IgG and \( X \) = age at the time of colostrum feeding. This equation would explain 98% of the variation at \( P < .05 \) (\( r = .992 \)).

Figure 1 and its regression equation show that calves still were absorbing some IgG when colostrum was fed at 48 h of age. This is not in complete agreement with Devery et al. (5), who concluded that calves do not absorb labeled IgG after the age of 36 h. In our work, the amount of IgG absorbed was obtained by difference between quantities of circulating IgG immediately before and 6 h after colostrum feeding. Therefore, the increase of IgG in plasma must have resulted from absorption of fed colostral IgG. It is possible then that non-denatured IgG in colostrum were absorbed more effectively than labeled IgG (5).

Percentages of absorbed IgG when colostrum was given 6 or 12 h after birth were considerably higher than percents published by Kruse (10). However, when colostrum was given 24 h after birth, our results agreed with those reported. Discrepancies between our results and those of Kruse (10) could be explained by changes in plasma volume that would occur in calves following birth but were not measured directly in the experiment reported by Kruse (10). The rate of IgG absorption based solely on concentration in plasma indicated a plateau at 24 h (Table 2). However, when plasma
IMMUNOGLOBULIN ABSORPTION IN CALVES

Figure 3. Relationship between the quantity of IgG not accounted for and age following the administration of a single dose of 80 g of IgG as colostrum. IgG not accounted for = Ingested IgG - (plasma IgG + fecal IgG).

volume was taken into account, the plateau appeared at 36 h (Figure 1). Measuring plasma volume appears essential in this type of studies.

Immunoglobulin G Balance

The relationship between fecal IgG and age at colostrum feeding is illustrated in Figure 2. According to Kruse (11), rennin acts on IgG to yield one fragment F(ab)2 whereas trypsin yields two Fab and one Fc fragments. Since F(ab)2 and Fab would precipitate in the presence of anti-lgG (11), it is logical to assume that the technique used in our work was adequate to determine total IgG excreted. The balance between total IgG ingested and plasma + fecal IgG is illustrated in Figure 3. The quantities of IgG not accounted for increased rapidly between ages of 6 to 24 h, appeared to have reached a maximum between 24 and 36 h, and rapidly decreased thereafter. The best mathematical model to predict the amount of unrecovered IgG was $Y = 8.978 + 2.680X - 0.043X^2$, where $Y$ = total amount of IgG not accounted for and $X$ = age at the time of colostrum feeding. This equation explained 99.6% of the variation at $P < .01$ ($r = .998$).

A phenomenon of this nature could be explained by a gradual change in the rate of catabolism of IgG in the newborn calf was 6% per day during the first 14 days of life, which would indicate a half-life of approximately 12 days. This half-life is identical to that reported by Sasaki et al. (18) using labeled IgG. Because plasma volumes and IgG concentrations were measured 6 h after colostrum was fed and for only 48 h after birth, the half-life of IgG cannot explain the quantities of IgG not accounted for in our experiment.

Immunoglobulins are absorbed via lymphatic circulation (1, 4, 6). Therefore, a certain amount of IgG would be expected to be in the lymphatic pool 6 h after colostrum feeding (6). El Nageh (6) found that the concentration of γ-globulins in lymph was three times as high as in serum 5 h after intake of labeled γ-globulins. It is not possible, however, to assess the quantity of IgG remaining in the lymphatic circulation at that time because lymph volume per se is unknown. Nevertheless, the quantity of IgG remaining in lymph 6 h after feeding likely would be proportional to the quantity absorbed and would contribute to lower the quantity of IgG not accounted for instead of increasing it with age (Table 2).

Stone and Deyoe (22) observed that 4 to 8 h after colostrum feeding, IgG concentration in synovial fluid of newborn calves reached the concentrations in the adult animal. This partly could explain why a certain amount of IgG could not be recovered in plasma 6 h after colostrum feeding; however, there is no indication that the size of that extravascular pool would change markedly when colostrum was fed at 6, 12, 24, 36, or 48 h after birth and, therefore, could not explain the shape of the curve in Figure 3.

In this experiment, collection of feces was for 48 h following colostrum feeding. It is possible that a certain quantity of unabsorbed IgG was not recovered completely at the end of the collection period. A period of 48 h was chosen for fecal collection to minimize the effect of possible excretion of endogenous IgG in feces (12). However, based on the work of Steck (21) and Logan et al. (13), it is logical to assume that most of the unabsorbed IgG was excreted 48 h after feeding. Incomplete recovery of the unabsorbed IgG in feces would increase the percentage of IgG not accounted for but would not affect the shape of the curve.
curve in Figure 3 because all calves were treated the same way.

The newborn calf exhibits transient proteinuria following colostrum feeding. Protein excretion during that period may be 15 to 20 times greater than before colostrum feeding. During this period, colostral-immunoglobulin-breakdown products would account for most of the urinary protein (9). According to the same authors (9), immunoglobulinuria would increase gradually to reach a maximum at the age of 30 h and sharply decrease thereafter. Furthermore, Pierce (17) showed that proteinuria was independent of the immunoglobulin concentration in serum. Proteinuria, then, would seem to be age-dependent and, therefore, could explain the shape of the curve in Figure 3. If this were the case, it would become clear that quantities of IgG measured in plasma would represent only apparent rather than true IgG absorption. It would be logical then to assume that the true absorption of IgG decreased linearly from 6 to 48 h after birth rather than curvilinearly as in Figure 1. This assumption would be supported by linear increase in excretion of fecal IgG during that time (Figure 2).

However, apparent absorption would be more meaningful for practical purposes than true absorption.

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