Influence of Bovine Antiserum (Bo-Bac 2X) Injection on Colostral Immunoglobulin G Absorption in Neonatal Dairy Calves

R. E. Pedersen, C. O. Paulrud, and W. B. Tucker
Department of Animal and Dairy Sciences
Mississippi State University, MS 39762

ABSTRACT

On the background of positive survival data from farms in Mississippi, treating calves with antiserum injection in addition to normal colostrum administration, the objective of the present study was to evaluate the influence of a single subcutaneously administered bovine antiserum injection (0.031 g of IgG/kg of body weight) and pooled colostrum administration on efficiency of Ig absorption and on 24-h plasma IgG concentration in neonatal bull calves. Twenty-nine male dairy calves (21 Holsteins and 8 Jerseys) were assigned randomly at parturition to receive one of four treatments: 1) colostrum (n = 9), 2) colostrum and bovine antiserum injection (n = 7), 3) milk replacer (n = 5), or 4) milk replacer and bovine antiserum injection (n = 8). At birth, calves either did or did not receive an injection of bovine antiserum and were fed pooled colostrum or milk replacer (Holsteins, 3.8 L; Jerseys, 1.9 L) via an esophageal feeder. Blood was collected immediately before administration of the colostrum or milk replacer, then again at 24 and 48 h postpartum. Immunoglobulin G concentrations of colostrum, milk replacer, antiserum, and plasma were monitored by single radial immunodiffusion. Colostrum administration and injection of bovine antiserum each increased plasma Ig concentration at 24 h posttreatment. In addition, antiserum injection increased the apparent efficiency of absorption of colostral Ig by 42% over that for calves fed colostrum alone. The increase in plasma IgG for antiserum-treated calves exceeded the total amount of IgG administered in the antiserum injection; hence, this increase appeared to be the result of an increase in total absorption of colostral IgG, or possibly antiserum injection somehow triggered active synthesis of IgG. Injection of antiserum might possibly serve as a beneficial adjunct to a colostrum management program by enhancing the acquisition of passive immunity from colostral sources.

Key words: calves, antiserum, immunoglobulin, colostrum

INTRODUCTION

Bovines have a thick epitheliochorial placentation. As a result, placental transfer of Ig is minimal, and bovine neonates are born essentially agammaglobulinemic (Brambell, 1970; Kruse, 1970). Calves are therefore highly dependent on the passive transfer of Ig from the colostrum of the dam. The Ig in colostrum are derived largely from plasma proteins and are transported selectively through mammary secretory cells into the colostrum. In addition, mammary gland lymphocytes produce a minimal amount of colostral Ig (Larson et al., 1980).

In the neonate, Ig bind to receptors in the microvillous border of the small intestine and are absorbed by nonspecific endocytosis into the specialized epithelial cells of the jejunum and ileum. This absorption is independent of the molecular weight of the absorbed molecules. Upon absorption into the cell, a vacuole containing the Ig is formed and transported to the cell membrane, where it expels its contents via exocytosis into the lamina propria. From there, the Ig pass into the systemic circulation via the lymphatics and venous capillaries (Bush and Staley, 1980; Staley and Bush, 1985). The transfer of macromolecules ceases at approximately 24 h postpartum (Husband et al., 1972, Staley and Bush, 1985). The mechanisms involved in the closure of the intestine to absorption of macromolecules are still not fully understood.

Colostral antibody deficiencies, ingestion failure, or failure of absorption are all factors that can compromise passive acquisition of immunity by neonatal calves. Each of these factors has been associated with increased morbidity and mortality (Center of Animal Health Monitoring, 1993; Gay, 1983; McGuire et al., 1976, Nocek, et al., 1984; Penhale et al., 1970; Smith, 1962; Smith and Little, 1922). Penhale et al. (1970) reported that IgG is the single most important factor in resistance to infections. Rea et al. (1996) observed the greatest risks of mortality in calves with serum protein concentrations

Received March 21, 2000.
Accepted August 22, 2000.
Corresponding author: W. B. Tucker, e-mail: btucker@ads.mss-state.edu.
1Journal article no. J-9665 of the Mississippi Agricultural and Forestry Experiment Station.
<45 g/L, serum IgG concentrations <5 g/L, and sodium sulfite test scores <1+. The Center for Animal Health Monitoring (1993) reported preweaning mortality rates of 8.4% for dairy heifer calves. This survey also indicated that over 40% of calves failed to achieve serum concentrations of 10 g of IgG/L, and that more than 55% of the total mortality risk among calves with serum concentrations below 10 g of IgG/L was associated with this failure of passive transfer. The losses associated with this mortality include not only the cost of the calf and investment in labor, feed, housing, and health care but also the potential value of the lost genetics. Hence, it is critical to develop management practices that will optimize the absorption of Ig and assure adequate immunity in neonatal calves.

Other methods to transfer Ig to neonatal calves have been suggested. Injection of Ig derived from blood from an abattoir was ineffective in preventing scours and reducing mortality in neonatal calves (1996). However, several antiserum products are available commercially that contain concentrated IgG that have been harvested from animals hyperimmunized against pathogens that cause diseases in young calves. These products are used routinely to treat sick calves. In addition, their use is recommended when colostrum is unavailable for neonatal calves. These products might be used as a supplement to colostrum to boost the availability of IgG that are specific for pathogens. Calf survival on farms that feed colostrum plus a subcutaneous injection of antiserum (Bo-bac-2X, Anchor Products Co., Addison, IL, at 0.728 ml/kg of BW) was improved (personal observation). However, the influence of these products on colostral Ig absorption has not been scientifically evaluated. Hence, the objective of our study was to determine the effects of a single subcutaneous injection of antiserum at birth on plasma IgG concentration and the efficiency of IgG absorption from colostrum fed to neonatal calves.

MATERIALS AND METHODS

Experimental Design

Twenty-nine Holstein (n = 21) and Jersey (n = 8) naturally born bull calves at the Mississippi State University Dairy Research Center were removed from the calving pens within 15 min after birth and assigned randomly, to receive one of four treatments immediately after removal: 1) pooled colostrum, 2) pooled colostrum plus a subcutaneous injection of antiserum (Bobac-2X, Anchor Products Co., Addison, IL, at 0.728 ml/kg of BW), 3) milk replacer (Instant Maxi Care NT, Land O’ Lakes) or 4) milk replacer plus antiserum (0.728 ml/kg of BW). The antiserum preparation was divided into two equal volumes and injected subcutaneously on both sides of the neck approximately 10 min before either colostrum or milk replacer was fed. Pooled colostrum was collected from the first milking postpartum and predominantly from multiparous cows within the herd. Colostrum was stored in plastic containers at 8 °C. When refrigerated colostrum was unavailable, frozen colostrum was utilized. Colostrum and milk replacer were warmed to 37°C and administered via esophageal feeder (Holsteins, 3.8 L; Jerseys, 1.9 L) in a single feeding at 1 to 2 h postpartum. Calves were then placed in individual hutches (2 m²) bedded with straw. The calves were offered water and commercial calf starter ad libitum from birth and were fed milk replacer once daily. Only calves from observed deliveries were included in the trial.

Sampling and Analysis

Calves were weighed before treatment and again 7 d later, before feeding. Blood samples were collected from the jugular vein in 10-ml syringes and transferred to 7-ml heparinized, evacuated tubes (Vacutainer) at 0, 24, and 48 h after the removal from the dam. Plasma was separated by centrifugation (3000 × g), collected and stored (−20°C) pending analysis of IgG by single radial immunodiffusion (SRID; VMRD Inc., Pullman, WA). Before colostrum was administered to each calf, and an aliquot of colostrum was collected and frozen at −20°C for subsequent analysis of IgG content. Colostrum was diluted 1:4 with physiological saline before analysis. Concentrations of IgG in milk replacer and antiserum also were analyzed via SRID. Rectal temperatures were measured and the consistency of the feces was scored (Larson et al., 1977) before the initial treatment at 0 h and daily immediately before feeding, for 7 d postpartum.

Statistical Analysis

Plasma IgG concentration at 24 and 48 h, BW increase, days of scouring (fetal consistency score > 2), and mean body temperature were analyzed according to the GLM procedure of SAS (SAS, 1985). Main effects included antiserum injection and the effects of colostrum supplementation. Interactions between the main effects were also evaluated. The apparent efficiency of absorption (AEA) of colostral antibodies at 24 h was calculated as: 

\[ \text{AEA} = \left( \frac{\text{plasma IgG at 24h (g/L)} - \text{plasma IgG at 0h (g/L)}}{\text{plasma volume × IgG consumed (g)}} \right) \times 100 \]  

(Quigley and Drewry, 1998). Plasma volume was estimated to be 10% of birth weight (Quigley and Drewry, 1998). The AEA at 24 h and plasma IgG concentration at 24 h for colostrum-fed calves versus those that received colostrum and antiserum were compared with Student’s t test (SAS, 1985). Significance was declared at \( P < 0.05 \) unless otherwise noted.
Table 1. Effects of antiserum injection and oral colostrum administration on BW, plasma IgG, and health of neonatal calves.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C</th>
<th>C+AS</th>
<th>SEM</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>5</td>
<td>8</td>
<td>9</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW, kg 0 h</td>
<td>37.3</td>
<td>36.1</td>
<td>33.2</td>
<td>38.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>35.2</td>
<td>35.3</td>
<td>34.2</td>
<td>40.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW change, %3</td>
<td>−5.6</td>
<td>−2.3</td>
<td>3.2</td>
<td>4.2</td>
<td>1.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Plasma IgG, g/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.512</td>
<td>0.424</td>
</tr>
<tr>
<td>At 24 h</td>
<td>0.73</td>
<td>1.93</td>
<td>17.58</td>
<td>22.98</td>
<td>1.17</td>
<td>0.0001</td>
</tr>
<tr>
<td>At 48 h</td>
<td>0.72</td>
<td>1.90</td>
<td>16.14</td>
<td>20.95</td>
<td>1.34</td>
<td>0.0001</td>
</tr>
<tr>
<td>Days of scouring4</td>
<td>1.60</td>
<td>1.83</td>
<td>2.89</td>
<td>1.71</td>
<td>0.29</td>
<td>0.310</td>
</tr>
<tr>
<td>Mean rectal temp, °C</td>
<td>38.6</td>
<td>38.6</td>
<td>38.4</td>
<td>38.6</td>
<td>0.1</td>
<td>0.667</td>
</tr>
</tbody>
</table>

1MR = Calves fed milk replacer at birth; MR+AS = calves fed milk replacer and injected with antiserum at birth; C = calves fed colostrum at birth; C+AS = calves fed colostrum and injected with antiserum at birth.
2Contrasts: 1 = Effect of feeding colostrum or milk replacer at birth; 2 = effect of injecting or not injecting antiserum at birth; 3 = interaction of the main effects.
3Represents the increase in BW from birth to d 7 postpartum.
4Days with fecal consistency score >2.

RESULTS AND DISCUSSION

BW and Fecal Scores

One calf with plasma IgG >2.5 g/L at 0 h was excluded from the study; 62% of the calves (n = 29) had undetectable concentrations of IgG in plasma at 0 h, and the remaining 38% had levels far below the range of the method used and was therefore set to 0. Mean colostral IgG concentration was 91 g/L (SEM = 10.6). The change in BW from birth to 7 d postpartum (Table 1) was affected (P < 0.01) by the administration of colostrum at birth. Calves receiving colostrum at birth gained weight from birth to d 7, whereas those receiving milk replacer at birth lost weight during that period. The positive influence of colostrum on BW gain might have resulted from the high nutrient density in the colostrum, or could have been related to enhanced immune function from the passive transfer of Ig from the colostrum. However, fecal consistency score (Table 1) did not appear to be affected by colostrum administration. In contrast to the effects of colostrum, the injection of antiserum (Table 1) did not affect BW change from birth to d 7.

Plasma IgG

Compared with milk replacer, oral administration of colostrum (Table 1) yielded a higher (P < 0.001) plasma IgG content at 24 and at 48 h. Analyses of the IgG content of the commercial antiserum preparation revealed an immunoglobulin content of 43 g of IgG/L. At the dosage utilized in this study, this equals a maximum theoretical transfer of <0.5 g of IgG/L of plasma in the calf. Calves injected with antiserum (Table 1) at birth exhibited a larger (P = 0.058) increase in plasma IgG content from 0 to 24 h and a tendency for a larger increase (P = 0.105) from 0 to 48 h, than did those calves not receiving the antiserum.

In colostrum-fed calves (Table 2), the plasma IgG content at 24 h was 30% higher (P = 0.084) for calves injected with antiserum than for those that were not injected. The boost in plasma IgG for calves injected with antiserum was 5.1 g/L above that for calves that did not receive antiserum. This represents fully one-half of the 10 g/L of plasma IgG concentration required for minimal protection against disease. Because the antiserum contained such small quantities of IgG, the increase in plasma IgG associated with antiserum injection is suggested to be related to enhanced colostral IgG absorption by the calf or possibly a triggering of the IgG synthesis. Although the mechanism behind this response is not clear, the practical benefits of boosting colostral IgG absorption in the neonatal calf could be

Table 2. Mean increase in plasma IgG and apparent efficiency of absorption (AEA) for calves fed colostrum at birth or fed colostrum and injected with antiserum at birth.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Colostrum</th>
<th>Colostrum + antiserum</th>
<th>SEM</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>5</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in Plasma IgG from 0 to 24 h, (g/L)</td>
<td>17.58</td>
<td>22.98</td>
<td>1.48</td>
<td>0.084</td>
</tr>
<tr>
<td>AEA1, %</td>
<td>20.2</td>
<td>28.6</td>
<td>2.05</td>
<td>0.037</td>
</tr>
</tbody>
</table>

1AEA = [(plasma IgG at 24h (g/L) – plasma IgG at 0h (g/L)) × plasma volume ÷ IgG consumed (g)] ÷ 100.
substantial. To our knowledge, this response has not been reported previously.

**Apparent Efficiency of Absorption**

The AEA for calves fed colostrum in the present study was consistent with other trials (Besser et al., 1985; Morin et al., 1997), although notably higher AEA was reported by Besser and Osborn (1993). In our study, absorption may have been influenced by the relatively large volume of colostrum fed, and by the high immunoglobulin concentration in the colostrum. Stott and Feller (1983) reported that the ability to absorb IgG from colostrum decreased as volume fed and immunoglobulin concentration of the colostrum increased. In our study, the AEA of colostral antibodies (Table 2) was 28.6% for calves that received an antiserum injection at birth, versus 20.2% for those that did not. The reason for enhanced AEA by calves receiving antiserum is not clear, but might be related to increased immunoglobulin half-life, increased rate of immunoglobulin transfer, an increased interval postpartum in which enterocytes allow macromolecule transport, or perhaps a combination of these factors. One might argue that an additional possibility for the enhanced AEA could be that components of the antiserum injection are, in an unknown fashion, able to alter the inhibitory effect of maternal derived Ig on the active synthesis of immunoglobulin. Then, however, one can argue that an equivalent synthesis of Ig should also take place in the milk replacer plus antiserum treated calves, but that did not occur. A last possibility, involving active synthesis, would be that the subcutaneous antiserum treatment somehow triggers immunoglobulin synthesis from passively transferred B-cells.

Besser et al. (1985) reported that normogammaglobulinemia can be established in almost all normal calves by using an esophageal feeder to administer good quality colostrum. However, the esophageal feeder does not induce closure of the esophageal groove; hence, colostrum can settle in the reticulorumen, thereby delaying its entry into the lower digestive tract by approximately 3 h (Lee et al., 1983). This delay might lessen the absorption of colostral Ig compared with colostrum administered by suckling.

An intracellular micropinocytotic transport of IgG is dominant throughout the entire small intestine, increasing from duodenum to ileum (Jochims et al., 1994). The presence of specialized transport vesicles, called coated vesicles, at the microvillous membrane of duodenal and jejunal enterocytes suggests a selective, receptor-mediated IgG transport (Jochims et al., 1994). Jochims et al. (1994) defined intestinal closure as a multifactorial event initiated by a replacement of fetal intestinal epithelial cells by a more mature cell population, cessation of transport at the basal and lateral cell membrane of the enterocytes, and increased proteolytic activity by lysosomes. In the present research, this increased proteolytic activity theoretically could have been inhibited by possible protease inhibitors in serum in the antiserum treatment.

Future research in this area should focus on the availability of specific Ig to the neonate, the enhancement of intestinal absorption of Ig, and alternative methods for administering Ig. Even if IgG is normally chosen as the reference immunoglobulin because IgG is the major immunoglobulin in colostrum, the importance of the other immunoglobulin classes and bioactive substances in colostrum should not be neglected (Schanbacher et al., 1997). Researchers (Caldow et al., 1988) have reported that neonates can be healthy and productive, even when serum Ig concentrations are low. The development of infectious disease is determined not only by the immune defense but also by the infectious challenge. The influence and importance of Colostral cellular and nonspecific immune factors is poorly understood and may be more important than earlier recognized.

**CONCLUSIONS**

The increase in plasma IgG content for antiserum-treated calves quantitatively exceeded the amount of IgG administered in the antiserum injection. This could indicate that antiserum administration enhanced the absorption of IgG from colostrum. Hence, antiserum might be utilized as an adjunct to a colostrum management program to enhance the benefits of colostrum administration. Other possible reasons for calves that received both antiserum and colostrum to have achieved higher IgG concentration than those calves receiving only colostrum could be that potential protease inhibitors in the antiserum preparation inhibited lysosome proteolytic activity and thereby theoretically induced a prolonged time of IgG absorption or the possibility that the subcutaneous antiserum treatment somehow triggered IgG synthesis from passively transferred B-cells. The true mechanism behind the enhanced IgG concentration among the colostrum and antiserum treated calves is unclear, but nevertheless of great interest.

**ACKNOWLEDGMENTS**

The authors thank Herdsman Mike Scott and his staff at Mississippi State University Dairy Research Center for their professional support and great patience.
REFERENCES


