Comparison of Freezing and Lyophilizing for Preservation of Colostrum as a Source of Immunoglobulins for Calves 1

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ABSTRACT: Lyophilizing was compared to freezing as a method of colostrum storage. Eight lots of colostrum from the first milking were divided into two equal parts; one was frozen, and the other was lyophilized. Twenty-two newborn calves were divided into two groups and fed either 2 L of frozen and thawed colostrum or 2 L of reconstituted lyophilized colostrum. The calves were bled at 12, 18, 24, and 72 h after feeding, and levels of the immunoglobulins IgG1, IgG2, IgM, and IgA were determined with a radial immunodiffusion assay, in colostrum and sera. The mean concentration of individual immunoglobulin isotypes in the sera of calves fed either frozen or lyophilized colostrum did not differ significantly. Calves fed from the same lots of colostrum had similar immunoglobulin concentrations in their sera, irrespective of the method of storage. All immunoglobulin isotypes were absorbed with equal efficiency from frozen and lyophilized colostrum as determined by calculation of the absorption coefficient.

Key Words: Colostrum, Cattle, Immunoglobulins, Absorption, Storage


Introduction

Colostrum is important for the transfer of passive immunity from cow to newborn calf via intestinal absorption of immunoglobulins (Ig) and also for local protective action in the intestine. It is useful to create a colostrum bank for newborn calves that cannot be fed by their own mother immediately after birth.

Freezing of colostrum has been reported to provide the maximum retention of Ig and nutrients (Foley and Otterby, 1978), but the unavailability of equipment to freeze and store colostrum would be a limiting factor in developing countries.

Some studies found lyophilized colostrum to be stable, easy to handle, and suitable for passive immunization (Larson et al., 1974; Meyer et al., 1982; Husu et al., 1993). As a result, two lyophilized products have been commercialized. Calves fed with these products had significantly lower IgG levels but did not have increased susceptibility to disease compared to calves fed fresh colostrum (von Pickel et al., 1991; Zaremba et al., 1993). The objective of the present study was to compare the concentration of bovine Ig-isotypes in blood serum of calves fed either lyophilized or frozen colostrum.

Materials and Methods

Twenty-two calves (BW = 42.9 ± 5.0 kg) born between March and June to Holstein-Friesian cows at the Institute of Animal Husbandry and Animal Ethology were separated from their dams at birth, weighed, kept in separate pens, and randomly assigned to either the frozen or lyophilized colostrum group (L or F). Colostrum from the first postpartum milking was collected from Holstein-Friesian cows in the institute’s herd. Eight lots of colostrum were collected from 14 cows over a period of 1 mo, each lot being either from a single cow or a pool from two cows depending, on calving on a single day. Each lot of colostrum collected on a single day was divided into two equal parts; one part was stored in a 2-L plastic container and the other part was separated into aliquots in 50-mL petri dishes. Both were stored in a freezer at −20°C. The colostrum in petri dishes was lyophilized using a lyophiler (Type Beta 1, Martin Christ, Germany). After lyophilization, colostrum was stored in plastic containers at room temperature until it was used. Before feeding, the frozen colostrum was thawed and warmed to 37°C,

1The authors wish to thank Vivian Hensel and Martin Nietz for their excellent technical assistance, and Deutschen Akademischen Austauschdienst for financial support.

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Received January 13, 1997.
Accepted November 29, 1997.
and lyophilized colostrum was rehydrated in lukewarm water.

After thawing (F) or rehydration (L), the fat content was determined with the sulfuric acid method of Gerber (Methodenkommission, 1963). The lactose content was determined with the method of Betrand (Lenkeit and Becker, 1949). For estimation of casein, the method of Hansson (1956) was used.

Calves were not allowed to suckle immediately after birth, but were fed 2 L of the assigned colostrum from a bucket as soon as possible. From then on, the calves were fed 3 L of whole milk twice a day.

Each calf was bled at 12, 18, 24, and 72 h after feeding of colostrum. Sera were prepared from these samples and stored at –20°C.

The concentrations of immunoglobulins, lactoferrin, and albumin in colostral whey and Ig in sera were determined in four repetitions per sample with the single radial immunodiffusion method of Mancini et al. (1965) as technically modified. Antigen standards and monospecific sera were prepared as described earlier (Klobasa, 1987).

To determine the effect of lyophilization on efficiency of Ig absorption, the coefficient of absorption (C) of each Ig and total Ig was calculated with the following formula:

\[
C = \frac{IgB \times BV \times SPB}{IgW \times CV \times WPC} \times 100
\]

where \(C\) = coefficient of absorption, \(IgB = \) Ig concentration in serum after 18 h of colostrum feeding, \(BV = \) blood volume (5.8 % of body weight; Löffler, 1974), \(SPB = \) serum portion in blood (50%; Pschyrembel, 1986), \(IgW = \) Ig concentration in whey, \(CV = \) volume of colostrum fed, and \(WPC = \) whey portion in colostrum \(\{100 - (\% \text{ fat} + \% \text{ casein})\}/100\).

For these calculations the change in blood volume due to colostrum feeding (Kruse, 1970) and distribution of Ig in extravascular/intravascular spaces (Lavoie et al., 1989) were not taken into consideration.

Data were analyzed with SAS (1989) analysis of variance procedures (Gogolok et al., 1992). The statistical model included the effects of treatment, sex, and colostrum pool. Weight at birth was considered a covariate. All first-order interactions were tested, and they were not significant (\(P > .05\)).

**Results**

The different lots of fresh colostrum varied in their composition (Table 1), the variation being more marked in the Ig concentrations. The total Ig in colostral whey ranged from 13.15 to 91.70 mg/mL. There was no change in the level of either individual or total Ig after freezing of colostrum. Lyophilization did not result in a significant decrease in concentration of immunoglobulins. Our earlier investigations (unpublished data) showed that neither freezing nor lyophilization alters the Ig concentration in whey.

The mean concentration of individual Ig isotypes in the sera of calves fed with either frozen or lyophilized colostrum at different intervals after feeding is presented in Figure 1. There was no difference (\(P > .05\)) between mean level of Ig in sera of the two groups of calves at any of the time intervals. The values for 18 h have been used to compare the mean effect of feeding different lots of colostrum. Table 2 shows the mean concentration of individual and total Ig in the sera of calves fed with different lots of colostrum. There was no apparent relationship between concentration in the neonatal calf sera and that of Ig in the colostrum lots they were fed, except in one lot in which low Ig concentration (13.15 mg/mL) in the colostrum resulted in significantly low Ig level in sera (3.95 mg/mL).

Table 1. Mean composition of colostrum lots (n = 8) used in the study

<table>
<thead>
<tr>
<th>Item</th>
<th>Mean</th>
<th>SEM*</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat, %</td>
<td>4.40</td>
<td>.83</td>
<td>2.10</td>
<td>8.90</td>
</tr>
<tr>
<td>Casein %</td>
<td>5.80</td>
<td>.53</td>
<td>3.90</td>
<td>7.70</td>
</tr>
<tr>
<td>Lactose %</td>
<td>3.26</td>
<td>.23</td>
<td>2.24</td>
<td>4.82</td>
</tr>
<tr>
<td>Lactoferrin, mg/mL whey</td>
<td>.50</td>
<td>.07</td>
<td>.28</td>
<td>.88</td>
</tr>
<tr>
<td>Albumin, mg/mL whey</td>
<td>2.67</td>
<td>.43</td>
<td>.99</td>
<td>4.72</td>
</tr>
<tr>
<td>IgG1, mg/mL whey</td>
<td>41.28</td>
<td>7.78</td>
<td>10.98</td>
<td>77.16</td>
</tr>
<tr>
<td>IgG2, mg/mL whey</td>
<td>2.62</td>
<td>.32</td>
<td>.85</td>
<td>3.83</td>
</tr>
<tr>
<td>IgM, mg/mL whey</td>
<td>4.88</td>
<td>.75</td>
<td>1.14</td>
<td>8.18</td>
</tr>
<tr>
<td>IgA, mg/mL whey</td>
<td>1.41</td>
<td>.24</td>
<td>.18</td>
<td>2.53</td>
</tr>
<tr>
<td>Total Ig, mg/mL whey</td>
<td>50.20</td>
<td>8.79</td>
<td>13.10</td>
<td>91.70</td>
</tr>
</tbody>
</table>

*SEM = standard error of the mean.

Table 2. Concentration of immunoglobulins (mg/mL) in serum of calves 18 hours after feeding with frozen (F) or lyophylized (L) colostrum

<table>
<thead>
<tr>
<th>Storage method</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgM</th>
<th>IgA</th>
<th>Total Ig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM*</td>
<td>Mean</td>
<td>SEM*</td>
<td>Mean</td>
</tr>
<tr>
<td>F</td>
<td>9.80</td>
<td>1.03</td>
<td>.70</td>
<td>.09</td>
<td>1.00</td>
</tr>
<tr>
<td>L</td>
<td>10.70</td>
<td>1.08</td>
<td>.70</td>
<td>.07</td>
<td>1.10</td>
</tr>
</tbody>
</table>

*SEM = standard error of the mean.
Table 3. Absorption coefficient (%) of immunoglobulins after 18 hours of feeding calves with frozen (F) or lyophilized (L) colostrum

<table>
<thead>
<tr>
<th>Storage method</th>
<th>IgG1 Mean</th>
<th>SEMa</th>
<th>IgG2 Mean</th>
<th>SEMa</th>
<th>IgM Mean</th>
<th>SEMa</th>
<th>IgA Mean</th>
<th>SEMa</th>
<th>Total Ig Mean</th>
<th>SEMa</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>20</td>
<td>3.0</td>
<td>21</td>
<td>3.0</td>
<td>16</td>
<td>1.8</td>
<td>19</td>
<td>3.0</td>
<td>19</td>
<td>2.4</td>
</tr>
<tr>
<td>L</td>
<td>20</td>
<td>2.1</td>
<td>20</td>
<td>2.1</td>
<td>16</td>
<td>1.8</td>
<td>19</td>
<td>1.5</td>
<td>19</td>
<td>1.5</td>
</tr>
</tbody>
</table>

\(^a\text{SEM = standard error of the mean.}\)

In general, immunoglobulins were absorbed with equal efficiency from the frozen and lyophilized colostrum (Table 3). There was no evidence for competition or saturation of the Ig uptake because variations in the levels of ingested Ig were not reflected in the coefficients of absorption. However, IgM was absorbed less efficiently than the other Ig isotypes. There was some variation in the quality of colostrum obtained from different cows. Colostrum lots with minimum Ig concentration resulted in low serum Ig levels in the F and the L groups receiving this lot. There was one case in which it seemed that a calf had an impaired ability to adsorb Ig. In this case, colostrum had Ig concentrations close to mean values, but the F group calf absorbed only 50%, with low absorption coefficients for all Ig classes. The matched L group calf absorbed 70% of all Ig (except IgM) from the lyophilized colostrum and had normal absorption coefficients. Even though it was not possible to determine conclusively that the poor absorption was due to the calf or to the sample of frozen colostrum it received, it is presumed that the freezing technique itself is consistent and that the calf itself had an impaired ability to take up Ig.

Discussion

The eight lots of colostrum used in the present study showed a wide range of immunoglobulin concentrations. Such variation in the concentration of Ig in the colostrum of individual cows has been reported previously (Kruse, 1970; Logan, 1977; Petrie, 1984). If the colostrum used has insufficient Ig concentrations, the sera Ig concentration will be lower. Mean total Ig concentration of all the eight lots of colostrum used in this experiment was about 50 mg/mL. Thus, 2 L of colostrum from a pool of all the lots would have provided about 100 g of Ig to each calf, which is within the recommended range of 80 to 100 g (Meyer and Steinbach, 1965; Kruse, 1970; Logan, 1977). When colostrum must be preserved for providing passive immunity to calves, it should be pooled from a number of cows to prevent the use of colostrum with low Ig values.

Maximum concentration of absorbed Ig is achieved when 2 L of colostrum (Stott et al., 1979) is given to a calf soon after birth with assisted feeding from the pail (Sundrum et al., 1988). Feeding calves frozen or lyophilized colostrum using this protocol raised mean serum concentration of IgG1, IgG2, IgM, and IgA to approximately 10.0, 7.0, 1.0, and 4.0 mg/mL, respectively, after 12 h. These values were not significantly different at 18 and 24 h, although slightly higher values were recorded at 18 h. No comparable data are available from earlier studies with lyophilized colostrum because workers estimated either only IgG levels (von Pickel et al., 1991; Zaremba et al., 1993) or antibody levels (Husu et al., 1993). Even though these values are lower than those reported in calves fed with fresh colostrum (McGuire et al., 1976; Stott et al., 1979; Sundrum et al., 1988), they are above the

![Figure 1. Mean concentration (mg/mL) of immunoglobulins (G1 = IgG1, G2 = IgG2, M = IgM, A = IgA) in calf sera after the administration of 2 L of colostrum preserved by freezing (F) or lyophilizing (L).](image-url)
IgG1, 0.8 mg/mL IgM, and 0.2 mg/mL IgA.

Using 18-h postfeeding values, there was no significant difference in the concentration of IgG1, IgG2, IgM, and IgA in the sera of calves fed with different lots of frozen or lyophilized colostrum. Similarly, all the four Ig isotypes were absorbed with equal efficiency from frozen and lyophilized colostrums. These results indicate that lyophilization preserves all the Ig isotypes in colostrum and such colostrum can be an adequate source IgG, as has been reported earlier (Meyer et al., 1982; von Pickel et al., 1991; Zaremba et al., 1993), and IgM and IgA. It has already been shown that IgM alone can prevent colisepticemia in calves (Logan and Penhale, 1971a,b; Penhale et al., 1973), but all three Ig isotypes were required for prevention of enterotoxemia. Seemingly, passively acquired Ig, in particular IgG1 and IgA to some extent, influence the occurrence of a number of enteric diseases in calves. Even though transfer of passively acquired IgG1 to the gastrointestinal tract is well established (Newby and Bourne, 1976; Besser et al., 1987), evidence has also been provided for active transfer of IgA from serum to bile in calves (Butler, 1981). Thus, colostrum well preserved by lyophilizing or freezing can provide protection against enteric and systemic infection.

Implications

Freezing or lyophilizing surplus colostrum is a suitable means of creating a colostrum bank with intact nutritional and immunological qualities. Lyophilized colostrum powder is easy to transport, requires no special conditions for prolonged storage, and could be used to provide immunoglobulins to newborn calves.

Literature Cited


