Clean Colostrum and Ig Absorption

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The successful transfer of passive immunity via colostrum to the newborn dairy calf continues to be a significant challenge. In spite of years of research and implementation of systems for colostrum management, over 30% of calves exhibit failure of passive transfer (FPT) (NAHMS, 1993). A discussion of the challenges of transfer and development of immunity in the calf represented a significant portion of the program of a recent ADSA Discover Conference held in 2008 entitled: “Biology of the Calf”. It is interesting to note that this conference resulted in the generation of more questions than answers concerning the value of colostrum as well as causes of FPT. The objective of this presentation is to discuss microbial risks associated with feeding colostrum.

Nearly all practitioners and their clients are familiar with the three “Q’s” requisite for successful colostrum management:

- Quickness – feed colostrum as soon as possible after birth with a goal of less than 6 hours.
- Quantity – 4 liters of colostrum is commonly recommended to be fed to large breed calves within the first 12 hours of life.
- Quality - Good quality colostrum is commonly indicated as containing >50g of IgG/liter

However, there is growing evidence to support adding another letter to the colostrum protocol list - a “C” for “clean” in reference to the microbial growth in colostrum and the microbial colonization of the calf intestine. A discussion of the mechanisms of IgG absorption by the intestine is useful to understand how early microbial colonization of the intestine might influence IgG absorption. Absorption of the large molecular weight immunoglobulin occurs in the jejunum and proximal ileum via pinocytosis during the first 2 to 24 hours of life. The calf exhibits some selectivity for macromolecular absorption. In such cases some non immune globulin macromolecules may enter the intestinal epithelial cells but do not pass through the basal membrane. It also appears that factors exist in the colostrum serum which are requisite for passage of macromolecules across the basal membrane of the intestinal epithelial cell and into the villus lacteal. The termination of macromolecular absorption in the neonate is termed closure. In the calf closure appears to be a one step process in which uptake of IgG and transport out of the cell cease simultaneously. Various theories exist to explain onset of closure including: development of gastric and enzymatic function in the newborn, reduction in permeability of the villus epithelial cells or replacement of the villus epithelium with a generation of cells incapable of pinocytosis. It appears that the latter two events are probably the primary causes.

Integrity of the intestinal mucosa, villus morphology and cell differentiation play an important role in intestinal absorptive function. Considering that the calf intestine is sterile at birth, early microbial colonization of the intestine may have an influence on Ig absorption. This is evident when comparing intestinal morphology of germfree animals
with normally reared animals. Any factor altering the rate of cell production in the crypts, cell migration up the villus or desquamation of cells from the villus tip may accelerate replacement of cells capable of macromolecular absorption by a population incapable of Ig absorption. Microbes might also act competitively by occupying binding sites on the apical plasma membrane of the epithelial cell. Evidence of interaction of intestinal microflora with the absorptive surface of intestinal epithelial cells was demonstrated by Corley et al. (1977). They observed microbial attachment, exfoliation of microvilli and intracellular penetration of ileal epithelial cells when *E. coli O55* was administered to colostrum-deprived calves. When colostrum was fed prior to *E. coli O55*, attachment and intracellular penetration did not occur as shown in the figures below.

Intestinal epithelial cell from a colostrum-deprived calf administered *E. coli* O55
16,000 X
(Corley et al, 1977)

Intestinal epithelium from that received colostrum prior to *E. coli* O55
14,000 X
(Corley et al, 1977)

A relationship of the early intestinal microflora was also suggested in work by the author (James and Polan, 1978). When an inoculum of live intestinally derived microorganisms was administered with the first colostrum meal, serum gamma globulin levels at 24 hours of age were lower than in calves not receiving the inoculum. However, it was unclear if the apparent impaired absorption was due to the microorganisms or other substances in the carrier. A later study (James et al, 1981) utilizing newborn calves compared the uptake of $^{125}$ labeled gamma globulin in isolated segments of intestinal tissue exposed to an anaerobic mixed culture of intestinal microorganisms ($10^8$/ml), an autoclaved culture of intestine microorganisms, or the sterile culture medium. Intestinal segments were injected with one ml of each treatment followed four hours later with 1.5 h exposure to an $^{125}$ labeled gamma globulin solution. Uptake of gamma globulin was lower in the segments exposed to the live culture as compared to those receiving the autoclaved culture, or sterile culture medium. There was a significant negative correlation between number of bacteria in the gut segments and gamma globulin uptake. It is important to note that our studies evaluated aerobic as well as anaerobic microbial growth in ileal tissue using roll tube techniques. Intestinal segments receiving the autoclaved inoculum or sterile broth culture contained an average of 7.5X10$^7$ organisms / g of intestinal tissue at the end of the trial (24
– 48 h of age). In contrast, segments receiving the live culture contained 5.94X10^8 organisms at the same age. Microbial growth in segments receiving sterile inocula indicates that colonization of the gut of the calf is rapid. In addition, we demonstrated that there is a vigorous microbial population intimately associated with the intestinal epithelium which may be quite different from the more transitory populations existing in the lumen of the intestine. These findings suggest that microbial exposure of the calf during the first hours of life may have a significant impact on acquisition of immunity.

When one considers how colostrum is collected on many dairies and how newborn calves are managed, it becomes apparent that there are significant opportunities for either tremendous microbial growth in colostrum or the “contamination” of the calf from feces, the environment or administration of probiotic preparations prior to or with the first colostrum feeding.

A field study (Swan et al., 2007) involving 12 Minnesota and Wisconsin dairies provides an understanding of the variation in colostrum quality on commercial dairies. Median total plate count was 6.15 X 10^8/ml. However, it varied from 7X10^7 to over 10^9/ml. Over 93% of samples of colostrum fed to calves exceeded the desired upper limit of 100,000 cfu/ml TPC. This study indicates that the quality of maternal colostrum fed to calves on most farms probably rarely remains below the goal of 100,000 cfu/ml TPC. In another field study, Poulsen et al. (2002) found that 82% of colostrum samples contained more than 100,000 cfu/ml.

In an effort to define critical control points for colostrum contamination, Stewart et al. (2005) obtained samples of colostrum from mammary glands from 39 cows, from the milking bucket and from the esophageal feeder tube. They found that bacterial counts were low (log_{10} cfu/ml =1.44) when collected directly from the mammary gland. However, significant bacterial contamination during the harvest process as log_{10} cfu/ml increased to 4.99 in samples obtained from the harvest bucket. No additional contamination appeared to occur between the harvest bucket and the esophageal feeder tube. Only 64% of samples obtained from the harvest bucket within 15 to 20 minutes of harvest contained less than 100,000 cfu/ml. It should be noted that the harvest bucket was thoroughly cleaned and sanitized between milkings by rinsing with warm water, scrubbing with a detergent, acid sanitizing and thoroughly drying prior to the next milking. Effects of refrigeration and treatment with potassium sorbate (0.5% wt/vol) on bacterial growth were also evaluated. Bacterial counts increased to 7.26 cfu/ml (log_{10}) when stored at room temperature for 24 hours. Refrigeration (4°C or 39°F) resulted in lower total plate count of 5.75 cfu/ml (log_{10}). Furthermore, addition of potassium sorbate to samples at ambient temperature resulted in similar TPC as refrigeration. The combination of refrigeration and addition of potassium sorbate was most successful with TPC of 3.60 cfu/ml (log_{10}). In most cases TPC increased rapidly after 24 h of storage and then remained relatively stable. Declines in TPC were attributed to the initiation of fermentation in colostrum samples which may not be desirable. The authors noted that coliform counts remained high in spite of the decreasing pH of the colostrum. Some
workers (Snyder et al., 1974) observed reductions in Ig in fermented as compared to fresh or frozen colostrum. This study demonstrated that significant contamination of colostrum occurred during the harvest process. Sources of contamination likely came from the teat skin, milking equipment or the floor bucket. Improvement in udder preparation or milking equipment sanitation should result in lower TPC. The esophageal feeder was not a source of additional contamination, probably due to the limited time that colostrum was contained in the esophageal feeder.

In addition to the impact of bacterial growth influencing Ig absorption, there is also concern that colostrum might serve as a vector for infecting calves with numerous potential pathogens including *Mycobacterium avium* spp. *paratuberculosis* (MAP), *Mycoplasma spp.*, *Escherichia coli* and *Salmonella spp.*... Fortunately, many well designed pasteurizers have been developed with practical application for the treatment of smaller volumes of milk commonly used on dairy farms. Waste milk is commonly pasteurized at 63°C (145°F) for 30 minutes with batch pasteurizers or 72°C (161°F) for 15 seconds with HTST units. However, research has shown (Elizondo-Salazar et al., 2008, Godden, 2006) that these temperatures result in reduced Ig concentration and increased viscosity. Batch systems are better suited for pasteurization of the smaller quantities of colostrum as they can effectively kill potential pathogens through lower temperature (60°C) for longer times while maintaining liquid consistency and Ig content and activity.

Godden and coworkers (2006) inoculated 30 L batches of first milking colostrum with *Mycoplasma bovis*, *Listeria monocytogenes*, *E. coli* 0157:H7, *Salmonella enteritidis* and MAP. Colostrum was pasteurized using a commercial batch pasteurizer for 120 min at 60°C. Samples were obtained at 15 min intervals throughout the pasteurization treatment for culture, measurement of Ig concentration and antibody activity. There was no effect of heating moderate to high quality colostrum (>50mg IgG/ml) on IgG concentration (60.5 mg/ml pre vs. 59.1mg/ml post pasteurization) or antibody activity. Viable counts of all organisms could not be detected after 30 minutes of treatment. MAP was not detected after 60 min of treatment at 60°C. This work is supported by Penn State researchers (Elizondo-Salazar et al., 2008) who found that heat treatment above 60°C resulted in denaturation of colostral IgG and increased viscosity. Although their work found that 30 min of treatment at 60°C reduced TPC, coliforms, gram negative bacteria, *Streptococcus agalactiae* and *Staphylococcus aureus*, it is probably advisable to extend pasteurization temperature to 60 min to provide greater confidence in the destruction of MAP.

Benefits from pasteurization of colostrum are demonstrated by Johnson et al. (2007) who compared feeding raw and pasteurized colostrum (60°C for 60 min). They found that calves fed heat-treated colostrum had significantly higher serum total protein and IgG concentrations at 24 h of age and higher apparent efficiency of absorption of IgG as shown in the table below.
<table>
<thead>
<tr>
<th>Colostrum</th>
<th>Serum protein</th>
<th>IgG concentration</th>
<th>Apparent efficiency of absorption</th>
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</thead>
<tbody>
<tr>
<td>Raw</td>
<td>6.3 mg/dl</td>
<td>22.3 mg/ml</td>
<td>35.6%</td>
</tr>
<tr>
<td>Pasteurized</td>
<td>5.9 mg/dl</td>
<td>18.1 mg/ml</td>
<td>26.1%</td>
</tr>
</tbody>
</table>

There was no difference in serum concentrations of IgA, IgM, and vitamin A or E, beta carotene or serum bovine viral diarrhea virus Type 1 serum neutralization titers.

**Practical recommendations for management of colostrum feeding programs.**

- Avoid introduction of pathogens from infected cows. Don’t let calves suckle cows.
- Mammary glands of fresh cows should be thoroughly cleaned and sanitized prior to milking.
- Do not pool colostrum.
- Fresh cows should be milked first in the parlor. Where they are milked in a separate facility, the sanitation of equipment and facilities should be equal to that in the primary milking parlor.
- If colostrum will not be fed within 20 – 30 minutes, it should be rapidly cooled. Cooling colostrum from 95°F to 60°F extends coliform generation times from 20 to 150 minutes. This can be achieved by several methods described by Dr. Sam Leadley in the June 2008 issue of Calving Ease. Colostrum can be rapidly cooled by submerging two- or four-quart containers of colostrum into an ice bath. An alternate method is to freeze one-quart containers of water and place them into a gallon of colostrum. Take precautions that the ice containers are thoroughly sanitized on the outside. The practice of placing two-quart bottles into a freezer results in rather slow cooling and provides an opportunity for bacterial growth before temperature has dropped sufficiently.
- Clean feeding buckets and bottles and esophageal feeders by rinsing in lukewarm water, scrubbing with a detergent solution, acid rinsing and air drying before being reused.
- Keep nipple bottles and esophageal feeders well maintained.
  - Inspect nipples for enlarged holes which can facilitate aspiration of fluid into the lungs of calves.
  - Inspect esophageal feeders for milk deposits and scratches on the ball at the end of the feeding tube.
- Pasteurize colostrum if sufficient calvings occur to justify expense of equipment and labor. Pasteurizers should be more strongly considered when the herd has a history of culling for Johne’s or a high incidence of disease. If colostrum is pasteurized, it should be fed as soon as it has cooled to 40°C (110°F). If not fed within 30 minutes, refrigerate or freeze it.
- Consider the use of colostrum replacers when supplies of good quality colostrum are insufficient to meet the herd’s needs. Replacers should be tested and provide at least 150 to 200 g of IgG.
- Avoid using probiotic preparations until after closure has occurred which is usually 24 hours of age.
Colostrum should be processed with the same care afforded to grade A milk sold from the farm. Before practicing the three Q’s of colostrum feeding management (quickness, quantity and quality), the primary emphasis should be placed on “clean” to achieve the goal of less than 100,000 cfu/ml TPC.

References.


