Effect of colostrum heat treatment and bacterial population on immunoglobulin G absorption and health of neonatal calves

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ABSTRACT

Improved IgG absorption in calves fed heat-treated colostrum has been attributed to the reduced bacteria content in colostrum after heat treatment. However, at least one study reported that colostrum bacteria content did not affect IgG absorption. The main objective of the current study was a more conclusive test of the combined effects of colostrum bacteria content and heat treatment on IgG absorption. Comparison of conclusions from plasma IgG as measured by radial immunodiffusion and ELISA and comparison of health scores in the first week of life were secondary and tertiary objectives. Colostrum from individual cows was pooled, divided, either heat treated or unheated, and allowed to incubate for bacterial growth or not. The 4 treatments were unheated, low bacteria; unheated, high bacteria; heat-treated, low bacteria; and heat-treated, high bacteria. Plasma samples were collected from bull calves (n = 25–27 per treatment) before and 48 h after colostrum feeding for IgG and total protein analysis. Fecal, respiratory, and general health scores were assigned daily for the first 7 d. Plasma IgG, total protein, apparent efficiency of IgG absorption, and frequency of illness were analyzed using the MIXED and FREQ procedures in SAS (SAS Institute Inc., Cary, NC). Plasma IgG values from ELISA were lower than radial immunodiffusion; however, conclusions were similar. Greater colostrum bacteria content reduced total protein, plasma IgG, and efficiency of IgG absorption. Heat treatment tended to improve 48-h plasma IgG as measured by ELISA. Respiratory scores were not affected by colostrum treatment, but calves fed heat-treated, low-bacteria colostrum tended to experience fewer scour days. These results provide conclusive evidence for the benefits of minimizing bacterial contamination in colostrum for feeding calves.

Key words: colostrum, heat treatment, neonatal calf, health

INTRODUCTION

Calves are born in a state of immunodeficiency caused by a lack of circulating IgG and other Ig molecules that function in antigen recognition and presentation. Calves are able to absorb Ig from colostrum if ingested within 24 h after birth (Stott et al., 1979). Absorption is most efficient immediately after birth; however, even when colostrum is provided in a timely manner, apparent efficiency of IgG absorption (AEA) is rarely >50% and frequently <35% (Quigley and Drewry, 1998; Godden et al., 2009). Heat treatment of colostrum is a method of colostrum management that can improve efficiency of IgG absorption by 20 to 35% (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009a).

The mechanism by which colostrum heat treatment improves IgG absorption is not known, but may be due to reduced competition between IgG and non-Ig proteins or bacteria that are denatured or killed by heat treatment (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009a). Bacteria may also bind IgG within the intestinal lumen, induce sloughing of the absorptive neonatal intestinal cells, or interfere with IgG receptors (Corley et al., 1977; Staley and Bush, 1985). Intestinal exposure to bacteria before receiving colostrum significantly decreases IgG absorption in calves; however, it is not clear how bacteria affect IgG absorption when exposure occurs concurrently (Corley et al., 1977; James et al., 1981).

Elizondo-Salazar and Heinrichs (2009b) attempted to investigate the combined effects of colostrum bacteria content and heat treatment on IgG absorption and reported no effect of bacteria on IgG absorption, whereas colostrum heat treatment improved IgG absorption. These results are contested, however, because the study lacked a sufficient number of calves and did not include a heat-treated, high-bacteria colostrum treatment. In contrast, Godden et al. (2012) reported a negative correlation between colostrum coliform count and IgG absorption, suggesting that bacteria decrease IgG absorption with concurrent exposure.

Two primary methods exist for quantification of plasma IgG: radial immunodiffusion (RID) and ELISA. Our laboratory recently compared these methods...
and found them correlated but not directly comparable (Gelsinger et al., 2015).

The main objective of the current experiment was a more conclusive test of the combined effects of colostrum bacteria content and heat treatment on IgG absorption. Comparison of health scores in the first week of life and comparison of conclusions from plasma IgG as measured by RID and ELISA were secondary and tertiary objectives. Initial expectations were that both heating colostrum and low bacteria content would improve IgG absorption and health. Results from RID and ELISA methods were expected to be similar.

MATERIALS AND METHODS

Colostrum Treatments

First milking colostrum was collected from individual cows at the Pennsylvania State University and frozen in 1.89-L containers. Colostrum was later thawed at 4°C, pooled, and thoroughly mixed to create a single batch. The total weight of colostrum used was approximately 427 kg, which was divided equally and processed as follows to create the 4 colostrum treatments. Half of the batch remained unheated and was immediately re-bottled in new, clean containers. Half of this unheated colostrum was refrozen at −20°C until needed for feeding calves (unheated, low bacteria). The other half of the unheated colostrum was incubated at 20°C for 60 h and subsequently stored at −20°C until needed for feeding calves (unheated, high bacteria).

The second half of the initial batch of colostrum was heat treated in stainless steel containers (28 L each) placed in a commercial steam vat pasteurizer (Girton Manufacturing Co., Millville, PA; Elizondo-Salazar and Heinrichs, 2009a). All containers were fitted with agitators to ensure even heating of the colostrum. Both water and colostrum temperatures were monitored continuously during the heat treatment process. Colostrum was heated to 60°C, maintained at that temperature for 30 min, then rapidly cooled and bottled in new, sterile containers. This method of heat treatment has been used in previous studies by our laboratory group and has been shown to reduce bacteria counts by 1 log cfu/mL with minimal effect on IgG concentration (Elizondo-Salazar et al., 2010). Other groups have also done studies by heating colostrum to 60°C for 60 min (Johnson et al., 2007; Godden et al., 2012).

Half of the heat-treated colostrum was immediately frozen at −20°C until needed for feeding calves (heat-treated, low bacteria). The other half of the heat-treated colostrum was inoculated with 20 mL of unheated, low-bacteria colostrum, stored at 20°C for 72 h to allow bacteria to grow to a concentration similar to that of the unheated, high-bacteria colostrum, and subsequently stored at −20°C until needed for feeding calves (heat-treated, high bacteria). Samples were collected before freezing from each colostrum treatment for IgG and bacteria analysis.

Calf Enrollment and Sampling

All procedures were approved by the Pennsylvania State University institutional animal care and use committee (Institutional Animal Care and Use Committee #41739). All bull calves born in the Pennsylvania State University dairy herd from January through October 2013 were removed from their dam upon discovery, weighed, and randomly assigned to a colostrum treatment. Calving areas were monitored throughout the day with the last check at 2300 h and the first check at 0500 h. Colostrum treatments were provided to all bull calves by staff members at the research herd. Colostrum was thawed in a warm water bath and fed in 2 feedings of 1.89 L each. Any colostrum that was not consumed via nipple bottle (Nasco, Fort Atkinson, WI) was supplied via esophageal feeder (Nasco) so each calf received 3.78 L of colostrum from a single treatment within 12 h after removal from dam. Blood samples were collected into evacuated tubes containing sodium heparin (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) via jugular venipuncture before and 48 h after feeding colostrum. Hematocrit was measured and whole blood samples were centrifuged at 1,500 × g for 15 min to separate plasma, which was tested for total protein concentration using an optical refractometer (SPR-T2, Atago Co. Ltd., Tokyo, Japan; calibrated before each use) and stored at −20°C until analyzed for IgG concentration. Calves received 3 L of whole waste milk twice/d starting approximately 36 h of age and starter grain was made available ad libitum starting 2 d after birth.

The main objective of our experiment was to compare IgG absorption rather than overall health. However, some calves in the current study received a large dose of bacteria in their colostrum feeding, and Corley et al. (1977) have shown that the neonatal gut is capable of absorbing whole bacteria cells during the same time that IgG absorption is occurring. Whereas quantification of absorbed bacteria was beyond the scope of our study, health scores were conducted for 7 d to assess the early effects of absorbed bacteria between treatments. Fecal, respiratory, and general health scores were assigned daily on d 1 to 7 at the time of morning feeding. The CalfTrack system described by Lesmeister and Heinrichs (2004) was used with scores from 1 to 5 (1 = healthy; 5 = severely ill). The same person scored calves for the duration of the experiment and was not
blinded to treatment; however, no indication as to treatment was apparent on the calf or the pen.

**IgG and Bacteria Analysis**

Colostrum samples were thawed at 20°C for analysis of bacteria content and IgG concentration. Bacteria analysis used methods described by Jayarao et al. (2004). Samples were tested for SPC, coliform and noncoliform gram-negative bacteria, environmental and contagious streptococci, and CNS. Colostrum was incubated at 32°C (SPC) or 37°C (all others), and bacterial colonies were counted after 48 h.

Colostrum IgG concentration was determined using RID. Colostrum samples were thawed, diluted 1:10 in 0.85% saline solution, and tested using a kit according to manufacturer’s instructions (#728411; Triple J Farms, Bellingham, WA). Plasma samples were not diluted for RID, but 48-h samples were diluted 1:500,000 in Tris buffer saline solution containing Tween 20 (50 mM Tris, 0.14 M sodium chloride, 0.05%M Tween 20, pH 8.0) for ELISA. Plasma collected from calves at birth was tested by RID only. All ELISA antibodies were purchased from Bethyl Laboratories (Montgomery, TX). The protocol was adapted from that described by Vetter et al. (2013). A detailed description of RID and ELISA methods has been reported by Gelsinger et al. (2015). All samples were duplicated for each assay and coefficient of variation were <10.5% for RID and <15% for ELISA. Samples that yielded greater coefficient of variation were retested until an acceptable value was obtained. Plasma IgG values measured by RID and birth weight were used to calculate AEA (Quigley and Drewry, 1998).

**Statistical Analysis**

The study used a completely randomized design with 2 × 2 factorial arrangement of treatments. Descriptive statistics were generated to describe colostrum treatments and characteristics of calves that received each treatment. Calf characteristics were compared using Proc MIXED in SAS (Version 9.3, SAS Institute, Cary, NC) to confirm that birth weight, age at colostrum feedings, and blood parameters at birth were similar between treatments. Least squares means were determined for plasma total protein, IgG, and AEA and compared between treatments using Proc MIXED in SAS. Colostrum heat treatment (unheated vs. heat-treated), relative bacteria content (low vs. high), and their interaction were included as fixed effects in the model. Hematocrit at 48 h, plasma IgG at birth, birth weight, and age at first and second colostrum feeding were offered as covariates in the model. Insignificant covariates were removed. Akaike information criterion was used to choose the best fitting final model. The final model for predicting IgG as measured by RID and ELISA as well as total protein was:

\[ Y_{ijkl} = \mu + \alpha_i + \beta_j + \alpha\beta_j + \gamma_l + \varepsilon_{ijkl} \]

where \( Y_{ijkl} \) represents the mean response, \( \alpha \) represents the fixed effect of heat treatment (\( i = 1, 2 \); heat-treated or unheated), \( \beta \) represents the fixed effect of relative bacteria concentration within day (\( j = 1, 2 \); high or low), \( \gamma \) represents the covariate of hematocrit at 48 h, and \( \varepsilon \) represents the residual error. Hematocrit was not included in the model to predict AEA as plasma volume was included in that calculation. Multiple comparisons with Tukey-adjusted \( P \)-values were used to compare least squares means and assign superscripts for individual treatments.

Health scores were analyzed as frequency data using Proc FREQ in SAS. A Chi-squared test was used to determine whether colostrum treatment affected the number of calf days with scores >2 for fecal, respiratory, or general score. When treatment significantly affected health score, data were analyzed by logistic regression (low = 1, 2; high = >2) in Proc GENMOD using colostrum treatment as a fixed effect and odds ratios were calculated. Final \( P \)-values were adjusted by Tukey’s method for multiple comparisons. In all analyses, significance was declared at \( P < 0.05 \) and a tendency when 0.05 < \( P < 0.10 \).

**RESULTS AND DISCUSSION**

A description of colostrum treatments is given in Table 1. Colostrum IgG concentration was similar to colostrum treatments used by Elizondo-Salazar and Heinrichs (2009b) and other studies investigating heat treatment (Johnson et al., 2007). The IgG concentration was similar among treatments in agreement with previous results that colostrum heat treatment does not affect IgG concentration (McMartin et al., 2006; Elizondo-Salazar and Heinrichs, 2009a).

Mean (±SD) bacteria counts for SPC, coliform and noncoliform gram-negative bacteria, environmental and contagious streptococci, and CNS counts are also given in Table 1. High-bacteria treatments were similar and contained approximately 3 log cfu/mL more bacteria than low-bacteria treatments. For all bacteria types except environmental streptococci, greater difference was noted between high and low bacteria compared with the treatments used by Elizondo-Salazar and Heinrichs (2009b).

A total of 104 calves were enrolled in the experiment. Based on plasma IgG concentration at birth
EFFECT OF COLOSTRUM HEAT TREATMENT AND BACTERIA

(18.5 mg/mL), 1 calf was assumed to have suckled the dam and data from this calf was removed from the analysis. This calf received the heated, low-bacteria colostrum treatment. The final sample size given in Table 2 was assumed adequate to compare IgG absorption between treatments based on previous studies (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009a). Mean weight, age at first and second colostrum feeding, plasma total protein and IgG concentrations, and hematocrit are reported in Table 2 and were not different between colostrum treatments. Total protein values reported here are higher than many other manuscripts (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009a). One possible explanation is that the numbers reported here are plasma total protein and would therefore include clotting factors that are not included in serum total protein measurements.

Least squares means from ELISA and RID measurement of plasma IgG values are given in Table 3. A thorough comparison of these tests is made in a separate technical note (Gelsinger et al., 2015). Briefly, values from the ELISA and RID are correlated, but ELISA values are consistently lower than RID values. The secondary objective of our study was to compare IgG values between treatments as measured by each test to determine whether the same conclusions would be reached regardless of laboratory method. Most studies investigating IgG concentrations in colostrum and plasma have used RID. Our laboratory has shown that RID and ELISA values cannot be directly compared; therefore, RID results may be more easily interpreted and compared with the results of other laboratories. The RID is a more consistent test requiring fewer repeated analyses per sample to obtain values meeting the predetermined acceptance criteria (CV ≤10.5%; Gelsinger et al., 2015). The ELISA is a more precise method, but more data are needed from multiple laboratories to compare and interpret values.

Table 1. Mean (±SD) IgG concentration and bacterial populations in each colostrum treatment

<table>
<thead>
<tr>
<th>Item</th>
<th>Unheated colostrum</th>
<th>Heat-treated colostrum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low bacteria</td>
<td>High bacteria</td>
</tr>
<tr>
<td>IgG (mg/mL)</td>
<td>67.3 ± 2.9</td>
<td>71.8 ± 6.1</td>
</tr>
<tr>
<td>SPC (log cfu/mL)</td>
<td>4.59 ± 0.02</td>
<td>8.63 ± 0.21</td>
</tr>
<tr>
<td>CC (log cfu/mL)</td>
<td>3.78 ± 0.04</td>
<td>8.91 ± 0.01</td>
</tr>
<tr>
<td>NC (log cfu/mL)</td>
<td>3.55 ± 0.37</td>
<td>5.50 ± 0.28</td>
</tr>
<tr>
<td>ES (log cfu/mL)</td>
<td>3.80 ± 0.28</td>
<td>7.51 ± 0.08</td>
</tr>
<tr>
<td>CS (log cfu/mL)</td>
<td>1.65 ± 2.33</td>
<td>0.00</td>
</tr>
<tr>
<td>CNS (log cfu/mL)</td>
<td>3.84 ± 0.09</td>
<td>7.73 ± 0.20</td>
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</table>

1CC = coliform count; NC = noncoliform, gram-negative bacteria; ES = environmental streptococci; CS = contagious streptococci.

Table 2. Mean (±SD) birth weight, age at colostrum feedings, and blood parameters at birth for calves in each treatment

<table>
<thead>
<tr>
<th>Item</th>
<th>Unheated colostrum</th>
<th>Heat-treated colostrum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low bacteria</td>
<td>High bacteria</td>
</tr>
<tr>
<td>Calves (no.)</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>41.1 ± 1.1</td>
<td>43.0 ± 1.1</td>
</tr>
<tr>
<td>Age at first colostrum feeding (min)</td>
<td>109 ± 12.8</td>
<td>95 ± 12.1</td>
</tr>
<tr>
<td>Age at second colostrum feeding (min)</td>
<td>662 ± 59.9</td>
<td>653 ± 59.9</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>6.4 ± 0.1</td>
<td>6.5 ± 0.1</td>
</tr>
<tr>
<td>IgG (mg/mL)</td>
<td>0.3 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>35.5 ± 1.1</td>
<td>33.4 ± 1.1</td>
</tr>
</tbody>
</table>

1Measured by radial immunodiffusion.
cally, mean plasma IgG from analogous treatments in the current study were in the same order from greatest to least. Had Elizondo-Salazar and Heinrichs (2009b) included a heat-treated, high-bacteria colostrum treatment, their results may have been similar to those reported here. In addition, the differences in colostrum bacteria levels between treatments were greater here than those studied by Elizondo-Salazar and Heinrichs (2009b), which also may have led to their lack of treatment differences.

The mechanism by which bacteria hamper IgG absorption is yet to be deduced. Descriptions of epithelial cell absorption of bacteria and absorption of IgG were similar, implying that bacteria may directly compete with IgG for absorption (Corley et al., 1977; Baintner, 2007). Bacteria may also bind to IgG within the intestinal lumen, making the IgG unavailable for absorption (Staley and Bush, 1985). Further studies investigating interactions between bacteria, neonatal epithelia, and IgG are needed to more clearly describe these relationships.

Colostrum heat treatment did not affect any of the parameters considered. Other studies have reported plasma IgG concentrations 3.0 to 5.8 mg/mL greater in calves that received heat-treated compared with unheated colostrum (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009a; Godden et al., 2012; Gelsinger et al., 2014). The SPC of unheated, low-bacteria and heat-treated, low-bacteria colostrum treatments are similar to counts reported by other studies before and after heat treatment (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009a; Donahue et al., 2012; Gelsinger et al., 2014). The SPC of unheated, low-bacteria and heat-treated, low-bacteria colostrum treatments are similar to counts reported by other studies before and after heat treatment (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009a; Donahue et al., 2012; Godden et al., 2012). Standard plate count was nearly 2 log cfu/mL greater before heat treatment. Our analysis grouped both treatments together as low bacteria, potentially confounding other effects of heat treatment with the difference in bacteria count. A regression analysis may be more appropriate, however, as the low number of SPC values (1 from each treatment; n = 4) in our study did not allow meaningful regression analysis.

Variation in the age of calves at colostrum feeding and other differences between individual calf handlers may be another explanation for why heat treatment did not significantly improve IgG absorption in our study. The current study relied on members of the dairy farm staff to feed and ensure full consumption of the colostrum treatment whereas, in other studies, a small group of researchers was responsible for feeding colostrum. Godden et al. (2012) also relied on farm staff to provide colostrum treatments; however, sample size in their study was much greater than the current study. Smaller sample size may have limited the power of the current study to observe differences due to heat treatment. Time elapsed from discovery to first colostrum feeding ranged from 0.25 to 5.75 h. Seventy five percent of calves received their first colostrum feeding within 2 h of discovery.

Although plasma IgG and AEA were not improved, colostrum heat treatment did successfully reduce bacteria concentration in colostrum, which is clearly capable of improving IgG absorption. A previous study by Elizondo-Salazar and Heinrichs (2009a) showed that IgG comprised a greater proportion of plasma total protein in calves when fed heat-treated compared with unheated colostrum. It is possible that heat treatment could reduce absorption of some non-IgG protein or prevent some effect of colostrum on protein synthesis in calves. Hernández-Castellano et al. (2014) showed increased concentrations of apolipoprotein A-IV, plasminogen, serum amyloid A, and fibrinogen gamma chain in lambs after consuming colostrum. These are low-abundance proteins with multiple roles, including some immunologic function. Heat treatment of bovine colostrum to 60°C for 60 min has been shown to decrease colostrum concentrations of IGF-1 and lactoferrin (El-Fattah et al., 2014); however, the effect of heat treatment on other non-Ig proteins in unknown. The

Table 3. Plasma IgG concentration measured by radial immunodiffusion (RID) and ELISA and efficiency of absorption in calves fed heat-treated or unheated colostrum of high or low bacteria content

<table>
<thead>
<tr>
<th>Item</th>
<th>Unheated¹</th>
<th>Heat-treated¹</th>
<th>P-value²</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
<td>SEM</td>
</tr>
<tr>
<td>IgG (mg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RID (48 h)</td>
<td>20.19a</td>
<td>10.72b</td>
<td>1.6</td>
</tr>
<tr>
<td>ELISA</td>
<td>12.13a</td>
<td>6.78b</td>
<td>1.8</td>
</tr>
<tr>
<td>AEA (%)</td>
<td>33.77a</td>
<td>14.74b</td>
<td>2.3</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>8.24a</td>
<td>7.38b</td>
<td>0.1</td>
</tr>
</tbody>
</table>

¹Relative bacteria concentration in colostrum.
²T = effect of colostrum heat treatment, B = effect of bacteria content.
³AEA = apparent efficiency of absorption calculated from radial immunodiffusion values.
shorter treatment time (30 min) used in our study may explain the lack of an effect of heat treatment on IgG and total protein concentrations. Other studies utilizing a more rigorous method of heat treatment may be necessary to determine an effect beyond the clear benefit of bacterial reduction.

A total of 23 scour days (fecal score >2) were observed in 19 calves, and 6 calves experienced respiratory illness (respiratory score >2) in the first 7 d of life. Colostrum treatment did not affect frequency of respiratory disease, but tended (P = 0.06) to influence frequency of scour. Logistic regression of the probability of a fecal score ≤2 on colostrum treatment revealed no significant differences between treatments. However, calves that received heated, low-bacteria colostrum nearly tended to receive a fecal score ≤2 more often than calves that received unheated, low-bacteria colostrum (odds ratio = 9.9; P = 0.14). Comparison of calf health between treatments was a secondary objective of our experiment and sample size was not adequate to draw conclusions from this data. Post hoc power analysis based on least squares means and standard deviation results from logistic regression indicated a sample size between 32 and 160 calves per treatment might provide adequate power to determine differences between treatments. No calves experienced a general score >2 for the duration of the trial. Johnson et al. (2007) and Elizondo-Salazar and Heinrichs (2009a) reported no differences in fecal, respiratory, or general health scores or in leukocyte counts between preweaned calves that received heat-treated or unheated colostrum at birth; however, in a large field trial including data from 1,051 calves, those fed heat-treated colostrum were less likely to experience scour and healthier when all illnesses were considered (Godden et al., 2012). The present results seem to agree with Godden et al. (2012), but our study lacked sufficient power to draw conclusions about effects on calf health.

CONCLUSIONS

High bacteria content in colostrum negatively affected plasma IgG concentration as measured by RID or ELISA as well as total protein and AEA. Plasma IgG was lower when measured by ELISA compared with RID, but conclusions were similar from both laboratory methods. Feeding heat-treated, low-bacteria colostrum may reduce scour days in calves in the first week of life.

REFERENCES

