Effect of heat treatments of goat colostrum on bacterial counts, viscosity, and immunoglobulin G concentration

Harish Kumar, Naveen Kumar, Raman Seth, Arun Goyal and Chand Ram

Received: August 2014 / Accepted: January 2015

Abstract The objective of present study was to determine the effect of different time temperature treatment on microbial count, IgG concentration and viscosity of goat colostrum. Aliquots were prepared from pooled goat colostrum samples collected during first three days and were subjected to three different heat (60, 63 & 65°C) treatments for 60 min. Samples were analyzed for viscosity, IgG concentration, standard plate, coliform, *Staphylococcus aureus* and *Salmonella spp.* counts. Reduced bacterial counts were observed in all the heat treated samples as compared to untreated colostrum samples. Except for standard plate count, the above mentioned pathogens were undetectable in all the samples subjected to heat treatment. Colostrum samples heated at 60°C for 60 min had significantly (P<0.01) higher levels of IgG than the other two temperature treatments. Viscosity was unaffected after heating colostrum at 60°C for 60 min whereas significant (P<0.01) increase was observed at 63 and 65°C. In this study, heat treatment of goat colostrum at 60°C for 60 min significantly (p<0.001) reduced bacterial count, slightly reduced IgG concentration, and did not affect viscosity.

Keywords: Goat, colostrum, IgG, heat treatment, bacterial count

Introduction

Colostrum, a nutrient-rich, first secretion produced by mammals after parturition (Linzell and Peaker, 1974), is necessary for goat kids as they are agammaglobulinemic at birth and therefore need to consume colostrum during the first few hours after birth. The goat neonate is born agammaglobulinemic and depends on colostrum IgG intake to obtain adequate passive immunity (Besser and Gay, 1994; Weaver et al., 2000). Thus, early ingestion of colostrum by the newborn is critical for its survival. Failure of passive transfer of colostrum IgG is associated with increased morbidity and mortality due to neonatal diseases (Salazar et al., 2010; Gelsinger et al., 2014). Immunoglobulin is the most prevalent of the colostrum antibodies and is commonly measured as an indicator of successful passive transfer of immunity (Butler, 1969). However, dairy animals are infected with diseases through feeding of infected colostrum. Pathogens such as *Mycobacterium avium ssp. paratuberculosis*, *Salmonella spp.*, *Listeria monocytogenes* and *Escherichia coli* are transmitted to newborn, either by direct sucking of teats or due to unhygienic milking and storage (Steele et al. 1997; Salazar et al., 2010; Doyle et al., 1987). James et al. (1981) and Poulsen et al. (2002) reported that bacteria in bovine colostrum was associated with decreased IgG absorption and suggested that heat treatment might be an ideal approach to decrease microbial contamination in colostrum. Godden et al. (2006) observed that heat treatment at 60°C for 60 min reduced SPC and coliform counts without significantly affecting IgG concentration. Slight reduction in IgG concentration was observed when the bovine colostrum samples were subjected to heat treatment at 60°C for 60 min (Weave et al., 2000) whereas viscosity was unaltered after heat treatment at 60°C for 120 min (Elizondo-Salazar and Heinrichs, 2009; Donahue et al., 2012; Gelsinger et al., 2014, Johnson et al., 2007). Numerous studies had been conducted on temperature treatments of bovine colostrum and its subsequent effect on pathogens, viscosity and IgG concentration whereas the effect of heat treatment on goat colostrum is rather scarce. With this perspective, the present study was conducted with an objective to identify the time temperature treatment of colostrum that would result in significant reduction of bacterial count while having minimal effects on IgG concentration and viscosity of goat colostrum.
Materials and Methods

Collection of goat colostrum samples

The study was conducted on fifteen Sannen x Beetal and fifteen Alpine x Beetal crossbred dairy goats at National Dairy Research Institute, Karnal, India. All the goats were healthy and were provided with similar housing system and diet regime. Colostrum samples were collected at an interval of 12 h from 0 to 72 h after kidding in both the breeds. After collection, the colostrum of both breeds were pooled and immediately stored at -20°C in aliquots until further used. Before heat treatment the colostrum samples were thawed at 4°C and then thoroughly mixed for 10 min.

Fifteen aliquots (10 ml each) were prepared from the thoroughly mixed colostrum samples. The samples were heated at 60, 63 and 65°C for 60 min in water bath. Temperatures were continuously measured by 2 thermometers, one in water bath and second in sample. After heat treatment, samples were placed in an ice bath until they cooled to 30°C.

Colostrum analysis

Heat-treated and untreated samples were examined for standard plate count (SPC), coliform (CC), Staphylococcus aureus, and Salmonella spp. counts as per the method described by Houghtby et al. (1993). Colostrum samples were serially diluted in a ratio of 1:10 with 0.85% NaCl for 5 times. Each dilution was plated on plate count agar for total plate count (TPC), MacConkey agar for total coliform count (TCC), Baird Parker agar for Staphylococcus aureus count, SS agar for Salmonella spp. and Shigella and Potato Dextrose agar for yeast mold counts. All plates were incubated at 37°C for 48 h except for the yeast mold which was incubated at 27°C for 5 days and the numbers of colonies were recorded (cfu/mL). Viscosity was measured with a digital viscometer using parallel plate geometry.

IgG determination

The IgG in colostrum samples were determined by a goat specific IgG ELISA kit (Koma Biotech Inc., Seoul, Korea) following the manufacturer's instructions. As described in these instructions, samples were diluted to IgG concentrations between 1.23- 100 ug/mL which is the quantification range of the test kit. For each sample, two wells were prepared and quantified on a microtitre plate. Briefly, washing solution 200ul was added to each well. Aspirate the wells to remove liquid and was washed the plate 4 times using 300ul of washing solution per well. After the last wash, plate was inverted to remove residual solution and blot on paper towel. The goat IgG standard provided with kit was diluted with assay diluent in range of 7.8 to 500 ng/ml. Add 100ul of standard and samples to each well in duplicate. Cover with the plate sealer provided and the plate was incubated at room temperature (25°C) for at least 1 h. Then the wells were aspirated to remove the liquid and washed five times. Add 100ul of diluted detection antibody (1:25000) in each well and incubated for 1 hour. After incubation wells were washed five times and add 100ul of color development solution for color development. To stop the color reaction, 100ul of stop solution (H2SO4) was added to each well. Absorbance for each well was determined using a plate reader at 450 nm.

Statistical Analysis

The differences in IgG concentration, viscosity and SPC between the samples subjected to different heat treatments were statistically determined by using one way ANOVA (Tukey's multiple comparison tests). Graph pad Prism version 5 software was used to generate figures and for statistical analysis.

Results and Discussion

Microbial count of goat colostrum after various time and temperature treatments is presented in Table 1. Coliform (CC), Staphylococcus aureus, Salmonella spp. and Yeast mold were undetected at all the three temperature treatments. Standard plate count of 4.29 ± 0.15 cfu/ml was observed in unheated colostrum which was significantly (P<0.001) higher as compared to the samples subjected to heat treatments. Significant decrease in SPC was observed in samples which were heated to 60°C for 60 min whereas the differences in SPC between 60 and 63°C & 60 and 65°C were non-significant (P>0.05).

These observations suggest that the heat treatment of colostrum at 60°C for 60 min is sufficient to decrease the bacterial counts to a significant level. Similar results were obtained in the previous experiments conducted on bovine colostrum by Salazar et al. (2010) and McMartin et al. (2010), who concluded that the heat treatment of bovine colostrum to 60°C for 60 min significantly reduced the bacterial count.

Effect of heat treatment on viscosity and IgG concentration of colostrum

IgG concentrations and viscosity of colostrum samples subjected to different heat treatments are given in Table 2. IgG concentration of colostrum samples significantly (P<0.01) declined as the treatment temperature increased (Fig.1). Significant decrease (P<0.001) in IgG concentration was observed in all the three different temperature treated colostrum as compared to the samples at ambient temperature. IgG concentration of colostrum subjected to 60°C for 60 min was...
Table 1  Least squares means for bacterial load of goat colostrum after heat treatment at 3 different temperatures for 60 min

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>Bacterial count (log10 cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SPC</td>
</tr>
<tr>
<td>Ambient</td>
<td>0</td>
<td>4.29 ± 0.15</td>
</tr>
<tr>
<td>60</td>
<td>60</td>
<td>3.07 ± 0.18</td>
</tr>
<tr>
<td>63</td>
<td>60</td>
<td>2.78 ± 0.12</td>
</tr>
<tr>
<td>65</td>
<td>60</td>
<td>2.65 ± 0.15</td>
</tr>
</tbody>
</table>

SPC = Standard plate count; CC = Colifom count; SA = Staphylococcus aureus aureus; SM= Salmonella spp.

Table 2  Least squares means of IgG1 and IgG2 concentrations and viscosity of goat colostrum after heat treatment at 3 different temperatures for 60 min

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>IgG (mg/ml)</th>
<th>Viscosity [log10(Pa-s)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient</td>
<td>0</td>
<td>22.4 ± 0.28</td>
<td>1.88 ± 0.02</td>
</tr>
<tr>
<td>60</td>
<td>60</td>
<td>19.5 ± 0.23</td>
<td>2.09 ± 0.01</td>
</tr>
<tr>
<td>63</td>
<td>60</td>
<td>17.5 ± 0.28</td>
<td>3.80 ± 0.09</td>
</tr>
<tr>
<td>65</td>
<td>60</td>
<td>15.8 ± 0.20</td>
<td>4.20 ± 0.03</td>
</tr>
</tbody>
</table>

SPC = Standard plate count; CC = Colifom count; SA = Staphylococcus aureus aureus; SM= Salmonella spp.

Figure 1. Changes in total IgG concentration in goat colostrum samples after heat treatment at various time and temperature combinations (top of the bars represent SEM).

The reduction observed in the IgG concentration after heat treatments was similar to that observed in bovine colostrum by Meylan et al. (1996) and McMartin et al. (2006) at 63°C for 60 min (34% IgG reduction). Previous researchers (Salazar et al., 2010) also reported that 60°C for 60 min slightly reduced IgG concentration, and did not affect viscosity. The results of this research suggest that goat colostrum can be successfully heated to 60°C for up to 60 min without affecting viscosity or reducing IgG concentration.
Heat treatment of colostrum significantly reduced the bacterial load and could serve as an effective method for reducing pathogen exposure to newborn. Goat colostrum heated at 60°C for 60 min contained slightly lowered IgG concentration. Viscosity was not affected when the temperature was held at 60°C for 60 min. The findings of this study suggest that heat treatment of goat colostrum at 60°C for 60 min may be used as an optimal temperature and timing, at which heat treatment would produce no significant changes in viscosity, a small reduction in IgG concentration, and a significant reduction in bacterial count.

**Conclusions**

Heat treatment of colostrum significantly reduced bacterial load in goat colostrum samples, indicating that heat treatment of colostrum could serve as an effective method for reducing pathogen exposure to newborn calves. In conclusion, the results of this study indicate that heat treatments of goat colostrum at 60°C for 60 min could help to obtain hygienic goat colostrum of good microbiological quality while preserving the IgG concentration. Viscosity was not affected when the temperature was held at 60°C for 60 min. The findings of this study suggest that heat treatment of goat colostrum at 60°C for 60 min may be used as an optimal temperature and timing, at which heat treatment would produce no significant changes in viscosity, a small reduction in measured IgG concentration, and a significant reduction in bacterial count.

**Acknowledgments**

Financial support provided by Ministry of Agriculture, India and National Dairy Research Institute are greatly acknowledged. The authors thank Dr. Bharath Kumar B.S. for thorough scrutiny of the paper.

**References**


Elizondo-Salazar JA, Jayarao BM, Heinrich AM (2010) Effect of heat treatment of bovine colostrum on bacterial counts, viscosity, and
Linzell JL, Peaker M (1974) Changes in colostrum composition and in the
permeability of the mammary epithelium at about the time of parturition
in the goat. J Physi 24:129-151
McMartin S, Godden S, Metzger L, Feirtag J, Bey R, Stabel J, Goyal S,
Colostrum::Effects of Temperature on Viscosity and Immunoglobulin G
Poulsen KP, Hartmann FA, McGuirk SM (2002) Bacteria in colostrum:
Steele M (1997) Survey of Ontario bulk tank raw milk for food-borne
pathogens. J Food Protec 60:1341-1346
Passive transfer of colostral immunoglobulins in calves. J Vet Intern
Med 14:569-577.