Use of glycerol and propylene glycol as additives in heat-treated goat colostrum

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ABSTRACT

This experiment aimed to evaluate the suitability of glycerol and propylene glycol to reduce microbial count and preserve immune properties in heat-treated goat colostrum. Colostrum samples from 11 goats were each divided into 9 aliquots. Different concentrations (2, 6, 10, and 14%; vol/vol) of either glycerol or propylene glycol were added to the aliquots. Phosphate buffer solution was added to one aliquot, which was set as the control (CG). After the respective additions, all colostrum samples were heat treated at 56°C for 1 h. After cooling, aerobic mesophilic bacteria were cultured. The samples were frozen until free fatty acid, IgG, IgA, and IgM concentrations and chitotriosidase activity were measured. No differences were found in aerobic mesophilic bacteria counts between either 10 or 14% glycerol and propylene glycol additives. These additions reduced bacterial count to a greater extent than CG, and 2 or 6% additions. Colostrum IgG concentration was not affected by either of the additives or their concentrations. The propylene glycol additive reduced IgA and IgM concentrations and chitotriosidase activity, compared with CG. Conversely, glycerol did not affect any of the studied immune variables. In conclusion, glycerol addition to goat colostrum before heat treatment is suitable to enhance bacterial reduction, whereas colostrum immune properties were not affected.

Key words: sanitization, passive immune transfer, pasteurization, dairy goat

INTRODUCTION

Early colostrum intake is critical for newborn ruminant survival (Castro et al., 2009; Hernández-Castellano et al., 2015a). Colostrum contains a complex mixture of lipids, proteins, carbohydrates, and minerals, which is the first source of nutrition for newborn mammals (Boschetti et al., 2019). Colostrum has numerous bioactive components (Borad and Singh, 2018). Besides lactose, several carbohydrates have been identified within colostrum (Aquino et al., 2017; Fischer et al., 2018). Similarly, a wide lipids range of such as free fatty acids (FFA), triacylglycerides, cholesterol, tocopherol, and phospholipids have been reported in colostrum (Contarini et al., 2014; Marziali et al., 2018). Most of the bioactive components in colostrum are proteins that actively participate in protecting the neonate against pathogens and other postpartum environmental challenges (Hernández-Castellano et al., 2018). The most abundant immunoglobulins in goat colostrum are IgG, IgM, and IgA (Rodríguez et al., 2009; Rudovsky et al., 2008; Hernández-Castellano et al., 2015b), and they play an essential role in passive immune transfer from the dam to the newborn (Castro et al., 2018). Chitotriosidase (**ChT**) is another immune protein present in goat colostrum (Argüello et al., 2008; Castro et al., 2011; Sánchez-Macías et al., 2014). The activity of this enzyme is strongly related to the host defense against fungi and nematodes (Barone et al., 1999) in humans (van Eijk et al., 2005), goat kids (Argüello et al., 2008; Moreno-Indias et al., 2012a), and newborn lambs (Hernández-Castellano et al., 2015b,c).

Besides the beneficial effect of colostrum intake in newborn ruminants, colostrum feeding may be a potential transmission route for different pathogenic bacteria such as *Mycobacterium avium* ssp. *paratuberculosis*, *Sal-*

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monella spp., *Listeria monocytogenes*, and *Escherichia coli* (Steele et al., 1997), and viruses such as bovine leukosis (Perrin and Polack, 1988) or caprine arthritis encephalitis (Guerrault, 1990). Sanitization methods are of specific interest in regions where these diseases are present. Thus, heat treatment is one of the most commonly used sanitization methods. For instance, Adams et al. (1983) demonstrated the inactivation of caprine arthritis encephalitis virus in colostrum by heat treatment at 56°C for 1 h. Godden et al. (2006) observed no growth of *Mycobacterium avium* after 1 h at 60°C. Also, it has been demonstrated that heat-treated colostrum can increase intestinal absorption of IgG, through bacterial count reduction (Gelsinger et al., 2015; Saldana et al., 2019), and increase prebiotic activity (Fischer et al., 2018).

In addition, colostrum preservation and storage are essential in dairy farms. The most common methods to preserve colostrum are freezing (Morand-Fehr, 1989; Holloway et al., 2001; Argüello et al., 2003), refrigeration (Valenta, 1982; Argüello et al., 2003), lyophilizing (Klobasa et al., 1998; Castro et al., 2005), and addition of either acidifying (Muller and Syhre, 1975) or buffering substances (Jenny et al., 1984). Chemical preservation methods are recommended for storage at room temperature in cases where freezing or cooling facilities are not available (Foley and Otterby, 1978). Chemical additives can contribute to preserve colostrum, and some also may reduce microbial counts. Sodium dodecyl sulfate has been used as a sanitation component in raw goat colostrum (Morales-de la Nuez et al., 2011) without a negative effect on colostrum IgG. A 1% concentration of SDS decreased the total bacterial count in colostrum, similar to those observed in low heat-treated colostrum (56°C for 1 h). No literature is available about the use of additives combined with heat treatment to improve sanitation methods. Glycerol and propylene glycol are used as co-solvents in the pharmaceutical and cosmetic industry, and as antimicrobial and antifungal agents (Kinnunen and Koskela, 1991; Darwish and Bloomfield, 1997).

This experiment aimed to evaluate the suitability of glycerol and propylene glycol to reduce microbial count and preserve immune properties in heat-treated goat colostrum.

MATERIALS AND METHODS

Colostrum Samples and Treatments

Eleven pregnant Majorera dairy goats were used in this study. At parturition each goat was milked with a bucket milking machine. After milking, a composite

colostrum sample (774 mL) was collected and stored at -20°C. After thawing overnight at 4°C, each of the 11 colostrum samples was divided into 9 aliquots (86 mL). Each colostrum aliquot was treated with one of the following additions: **G2** (12 mL of PBS + 2 mL of glycerol), **G6** (8 mL of PBS + 6 mL of glycerol), **G10** (4 mL of PBS + 10 mL of glycerol), **G14** (14 mL of glycerol), **P2** (12 mL of PBS + 2 mL of propylene glycol), **P6** (8 mL of PBS + 6 mL of propylene glycol), **P10** (4 mL of PBS + 10 mL of propylene glycol), and **P14** (14 mL of propylene glycol). For the control aliquots (**CG**), 14 mL of PBS was added.

Heat treatment was performed in a water bath at 56°C for 1 h following recommendations by Argüello et al. (2003). Immediately after that, samples were transferred into ice water (4°C), and microbiological analyses were carried out. Five aliquots (1.5 mL) of each treatment were stored at -20°C, until immune variables and FFA concentration were determined.

Colostrum Analyses

Aerobic mesophilic bacteria count (**AMC**) was performed following standard procedures on plate count agar (Trujillo et al., 2007). The FFA concentration was measured according to Shipe et al. (1980). Briefly, 0.1 mL of HCl (0.7 N) was added to 0.5 mL of colostrum in a test tube, vortex mixed, and then 2 mL of copper reagent and 6 mL of solvent were added. The mixture was shaken for 30 min at 240 rpm and then centrifuged for 10 min at $1,940 \times g$ at room temperature. Then, 3.5 mL of the solvent layer was transferred to a tube containing 0.1 mL of the color reagent. Finally, the color was measured with a spectrophotometer at 440 nm within the first hour. The concentration of the different immunoglobulins in colostrum, specifically IgG, IgA, and IgM, was quantified using commercial ELISA kits (Bethyl Laboratories, Montgomery, TX). Chitotriosidase activity was analyzed following the procedure described by Argüello et al. (2008). The ChT activity was expressed as micromoles of substrate hydrolyzed per milliliter per hour. Each analysis (AMC, FFA, IgG, IgA, IgM, and ChT activity) was performed in duplicate. If the values from duplicates differed more than 10%, the sample was reanalyzed.

Statistical Analyses

Statistical analyses were performed using SAS, version 9.00 (SAS Institute Inc., Cary, NC). The SAS PROC MIXED procedure for repeated measures evaluated the effect of colostrum treatments (CG, G2, G6, G10, G14, P2, P6, P10, and P14) on AMC, FFA, IgG,

IgA, and IgM concentrations, and ChT activity. Means were analyzed using Tukey's test (Ott and Longnecker, 2001).

RESULTS

Microbial Reduction in Colostrum and Free Fatty Acid

The effect of glycerol and propylene glycol additions on the AMC reduction in heat-treated colostrum is shown in Figure 1. The AMC reduction was greater in all treatment groups (40 to 84%) compared with CG. The G10, G14, P10, and P14 treatments caused the greatest bacterial reductions (74–84%). The G6 and P6 treatments reduced AMC by 60%, whereas G2 and P2 decreased AMC by 40%. It is noteworthy that no significant differences were observed in AMC reduction between glycerol and propylene glycol at the same concentrations. In addition, no significant differences in FFA concentrations (Figure 2) were found between treatments ($P = 0.969$).

Colostrum Immune Quality

The effects of glycerol and propylene glycol additions to heat-treated colostrum on the immune variables, IgG, IgM, IgA, and ChT activity can be observed in

Figure 1. Percentage of aerobic mesophilic bacteria count reduction in heat-treated colostrum with different concentrations of glycerol (G, ■) or propylene glycol (P, □). The aerobic mesophilic bacteria count reduction in the control colostrum (heat treatment without additives) is 0%. G2 = 2% glycerol; G6 = 6% glycerol; G10 = 10% glycerol; G14 = 14% glycerol; P2 = 2% propylene glycol; P6 = 6% propylene glycol; P10 = 10% propylene glycol; P14 = 14% propylene glycol. Error bars represent SE of each aerobic mesophile reduction mean on different treatments. Means with different letters (a–c) are significantly different ($P < 0.05$).

Figure 2. Free fatty acid level in heat-treated colostrum with different concentrations of glycerol (G, ■) or propylene glycol (P, □), and control colostrum (CG, ●). G2 = 2% glycerol; G6 = 6% glycerol; G10 = 10% glycerol; G14 = 14% glycerol; P2 = 2% propylene glycol; P6 = 6% propylene glycol; P10 = 10% propylene glycol; P14 = 14% propylene glycol. Error bars represent SE of absorbance (Abs) at 440 nm means on different treatments.

Table 1. No significant differences in IgG concentrations were found between treatments ($P = 0.310$). No differences in IgA concentrations were observed between CG and glycerol addition at any studied concentration ($P = 0.466$). However, IgA was negatively affected when propylene glycol addition was increased, P14 being the treatment that caused the greatest reduction in IgA ($P = 0.040$). Similar IgM concentrations were observed at the different concentrations of glycerol addition ($P = 0.552$). In case of propylene glycol, the P14 treatment caused the greatest reduction in IgM concentration ($P = 0.043$). Chitotriosidase activity was not affected by glycerol addition, but increased amounts of propylene glycol decreased activity of this enzyme ($P = 0.009$).

DISCUSSION

Heat treatment reduces microorganism counts in cow (Johnson et al., 2007; Donahue et al., 2012; Saldana et al., 2019) and goat colostrum (Argüello et al., 2003; Trujillo et al., 2007; Morales-de la Nuez et al., 2011). In the present study, the bactericidal effect of heat treatment was enhanced by the use of 2 different additives. Glycerol and propylene glycol are commonly used in hygiene and cosmetic products, and have antimicrobial properties (Nalawade et al., 2015). Both additives could also be combined with FFA to produce mono-, di-, and triglycerides. For instance, Kelsey et al. (2006) found that glycerol monolaurate negatively affects *Staphylococcus aureus* growth, as well as bacterial protein se-

Table 1. Immunoglobulin concentration (IgG, IgM, and IgA) and chitotriosidase (ChT) activity in heat-treated colostrum with different concentrations of glycerol or propylene glycol and control colostrum

Item	CG ¹	Glycerol				Propylene glycol				SEM
		2%	6%	10%	14%	2%	6%	10%	14%	
IgG (mg/mL)	23.38	24.65	23.96	22.21	23.45	24.80	23.77	23.42	25.29	2.819
IgA (mg/mL)	0.49 ^a	0.46 ^a	0.43 ^{ab}	0.43 ^{ab}	0.45 ^{ab}	0.46 ^a	0.46 ^a	0.41 ^{ab}	0.37 ^b	0.070
IgM (mg/mL)	0.68 ^a	0.56 ^{ab}	0.44 ^{abc}	0.45 ^{abc}	0.52 ^{abc}	0.64 ^a	0.53 ^{ab}	0.26 ^{bc}	0.17 ^c	0.152
ChT activity (μmol/mL per h)	11.38 ^{ab}	11.60 ^{ab}	11.57 ^{ab}	12.10 ^a	11.45 ^{ab}	11.54 ^{ab}	11.60 ^{ab}	10.82 ^{bc}	10.29 ^c	0.667

^{a-c}Means within a row with different superscripts are significantly different ($P < 0.05$).

¹CG = heat-treated colostrum with 14% PBS.

cretion. Because FFA concentrations were not affected by the addition of either glycerol or propylene glycol, the above combination was not considered. In the present study, aerobic mesophilic bacteria reduction was directly influenced by the antimicrobial activity of the experimental additives. Isopropyl alcohol is widely used for disinfection. Alcohols act by denaturing proteins and dissolving lipids, consequently changing the lipid membrane structure (Tortora et al., 2007). Isopropyl alcohol is structurally similar to glycerol and propylene glycol, although it has higher activity. Antimicrobial action of the studied additives can be improved when combined with heat treatment. Further studies on their effect and action mechanisms are necessary.

The effect of heat treatment on IgG concentration in colostrum has been widely studied. Heating colostrum above 66°C reduces IgG concentrations (Chen and Chang, 1998; Indyk et al., 2008), although the effect of lower temperatures (56–63°C) on colostrum IgG concentration is still controversial. Some authors reported no effect on IgG concentrations in colostrum heated at 60°C (Johnson et al., 2007; Gelsinger et al., 2015) and 56–63°C (Trujillo et al., 2007). However, other authors observed substantial decreases when similar temperatures were used (Argüello et al., 2003; Loste et al., 2008; Morales-delaNuez et al., 2011). Therefore, further studies on the effect of colostrum heat treatment around 60°C are also necessary. In the present experiment, heat-treated colostrum IgG concentration was similar to those described by Argüello et al. (2003) and Trujillo et al. (2007) in the same goat breed, sampling time, and heat treatment. Immunoglobulin A and IgM have not been sufficiently studied in heat-treated milk or colostrum. According to Mainer et al. (1997), heat treatment differently affects immunoglobulins (i.e., IgG, IgA, IgM) in cow milk. Based on these results, IgM is denaturalized to a higher degree than IgA, whereas IgG shows the lowest denaturalization. This finding may explain the reduced IgM and IgA concentrations compared with IgG concentrations described in the present study. Thus, the P14 treatment did not affect IgG concentration, but it caused a 26% reduc-

tion in IgA concentration and a 75% reduction in IgM concentration.

No literature is available about the effects of heat treatment on ChT activity. In the same goat breed, Argüello et al. (2008), Moreno-Indias et al. (2012b), and Hernández-Castellano et al. (2019) reported values of ChT activity in colostrum obtained immediately postpartum of 3.91 μmol/mL per h (4-yr-old goats), 9.43 μmol/mL per h, and 9.74 μmol/mL per h (both using goats of different ages). The results of our study are similar to those observed by Moreno-Indias et al. (2012b) and Hernández-Castellano et al. (2019), since we also used different-aged goats. Further studies are also necessary to test the effect of both heat treatment and animal age on goat colostrum ChT activity.

CONCLUSIONS

The addition of glycerol to goat colostrum before heat treatment would be an appropriate method to improve the bacterial reduction without affecting colostrum immune properties. In contrast, propylene glycol addition decreases several immune variables in colostrum, and is therefore not suitable for colostrum sanitation.

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