Supplementing fresh bovine colostrum with gut-active carbohydrates reduces passive transfer of immunoglobulin G in Holstein dairy calves

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ABSTRACT

High concentrations of coliform bacteria in maternal colostrum (MC) have been associated with reduced IgG absorption in calves. Mannan-oligosaccharide, a gut-active carbohydrate (GAC) derived from yeast cell wall, has been shown to adsorb pathogens expressing type-1-fimbriae, reducing their ability to colonize the gastrointestinal tract. The objective of this study was to investigate if addition of a GAC to colostrum would result in increased IgG absorption in newborn calves. Newborn Holstein heifer and bull calves were enrolled in summer 2012 at a commercial transition cow facility in western Wisconsin. Each day, 7.6-L pools of fresh, first milking MC were created, split into 3.8-L aliquots, and refrigerated until feeding. Eligible newborn calves were removed from the dam 30 to 60 min after birth, weighed, and randomly assigned to be fed either 3.8 L of the MC pool (control) or 3.8 L of the MC pool with 30 g of GAC mixed in immediately before feeding. Duplicate 10-mL samples of colostrum were collected and frozen at -20°C before feeding (and before addition of GAC) for bacterial culture and IgG determination. A 10-mL venous blood sample was collected from calves before feeding colostrum and 24 h after colostrum feeding, for laboratory determination of serum IgG using radial immunodiffusion analysis. Colostrum and calf characteristics, including colostrum IgG concentration (g/L), colostrum bacteria counts ($\log_{10}$ cfu/mL), calf dystocia scores (1 to 4), birth weights (kg), and age at first feeding (min) were not different between the group fed GAC ($n = 47$) and the control group ($n = 48$). Mixed linear regression analysis showed that calves fed colostrum supplemented with 30 g of GAC had lower mean (standard error) apparent efficiency of absorption of IgG and lower serum IgG concentrations at 24 h [23.9% (1.0); IgG = 24.0 (1.1) g/L] than did control

calves [30.4% (1.0); IgG = 30.8 (1.0) g/L]. Given the negative effect observed in this study, it is not recommended that fresh colostrum be supplemented with 30 g of GAC.

Key words: gut-active carbohydrate, colostrum, passive transfer, calves, immunoglobulin

INTRODUCTION

Achieving early and adequate intake of high-quality colostrum is widely recognized as the single most important management factor in determining health and survival of the neonatal calf (Davis and Drackley, 1998; Weaver et al., 2000; McGuirk and Collins, 2004). In addition to reduced risk for preweaning morbidity and mortality, additional long-term benefits associated with successful passive transfer include reduced mortality in the postweaning period, improved rate of gain and feed efficiency, reduced age at first calving, improved first and second lactation milk production, and reduced tendency for culling during the first lactation (Robison et al., 1988; DeNise et al., 1989; Wells et al., 1996; Faber et al., 2005). But, whereas colostrum is a critical source of nutrients and immune factors for newborn calves, it can also represent one of the earliest potential exposures of dairy calves to several infectious agents including *Mycoplasma* spp., *Mycobacterium paratuberculosis*, fecal coliforms, *Salmonella* spp., and bovine leukemia virus (Streeter et al., 1995; Steele et al., 1997; Walz et al., 1997; McGuirk and Collins, 2004). These pathogens can cause early calthood morbidity or mortality (e.g., enteritis, respiratory disease, septicemia, joint infections) or could contribute to chronic subclinical infections that manifest later in life (e.g., Johne's disease).

Microbial contamination of colostrum is also a concern because increased concentrations of bacteria, and in particular coliform bacteria, in maternal colostrum (MC) have been associated with reduced passive absorption of colostral IgG from the intestine into the circulation, resulting in lower serum IgG concentrations in the calf (James et al., 1981; Poulsen et al., 2002;

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Johnson et al., 2007; Godden et al., 2012). Experts recommend that fresh colostrum fed to calves contain a total plate count (**TPC**) and total coliform count (**TCC**) of fewer than 100,000 and 10,000 cfu/mL, respectively (McGuirk and Collins, 2004). As such, producers are encouraged to adopt management approaches to reduce the negative effects of microbial contamination in colostrum.

Mannann-oligosaccharide is a gut-active carbohydrate (**GAC**) derived from the cell wall of yeast which has been shown to adsorb pathogens expressing type-1-fimbriae, reducing their ability to colonize the gastrointestinal tract (Spring et al., 2000). In one recent Swiss study, newborn dairy calves treated with GAC in MC at a rate of 0.6 g of GAC/kg experienced a significant increase in serum IgG concentrations for the first 21 d of life as compared with control calves (Lazarevic et al., 2010). Though the mechanism was not studied, the authors hypothesized that GAC may prevent attachment of pathogenic bacteria, such as *Escherichia coli*, to enterocytes by competition for receptor sites (Spring et al., 2000). If this effect is genuine, it could result in healthier calves, both through improved passive transfer of IgG as well as through reduced gut colonization by pathogenic bacteria (Lazarevic et al., 2010). However, and despite the positive results reported, that study had several limitations including a small sample size (12 calves per treatment group), failure to report both colostrum characteristics (e.g., IgG concentrations; bacteria levels) and calf characteristics (e.g., birth weight, which could confound study findings), and failure to report the apparent efficiency of absorption (**AEA**, %) of IgG in study calves.

During the winter of 2011 and 2012, a study was conducted on a commercial transition cow facility in western Wisconsin, whereby newborn calves were randomly assigned to either a GAC treatment group [30 g of GAC was mixed into 1.5 doses (150 g of IgG) of commercial colostrum replacer (**CR**) supplemented with 30 g of GAC ($n = 119$)] or a control group fed 1.5 doses (150 g of IgG) of unsupplemented CR ($n = 122$; Robichaud et al., 2014). Results were that the addition of GAC to a CR had no effect on passive transfer as measured by serum total protein (control = 5.7; GAC = 5.7 g/dL), serum IgG (control = 20.3; GAC = 20.2 mg/mL), and AEA of IgG at 24 h of age (control = 54.3; GAC = 54.3%; $P > 0.05$). Furthermore, no effect of GAC supplementation was noted of the CR on the incidence of diarrhea or pneumonia, survival, or ADG between birth and weaning. However, if GAC interferes with the attachment of pathogenic bacteria including *E. coli* or other pathogens, and if these bacteria directly interfere with IgG absorption, then perhaps their study failed to detect a positive effect of GAC supplementa-

tion because USDA-licensed CR products, such as the one used in that study, contain low levels of bacteria and no pathogenic bacteria including coliforms (Robichaud et al., 2014). Conversely, and although colostrum bacteria levels were not reported, the Lazarevic et al. (2010) study may have been in a position to more easily detect a beneficial effect of GAC by using fresh maternal colostrum that may have contained higher levels of bacterial contamination including coliforms.

Given these disparate study findings, further study of the relationship between GAC supplementation of colostrum and IgG absorption was warranted. The objective of the current study was to investigate the effect of supplementing maternal colostrum with GAC on the absorption of IgG in neonatal dairy calves. We hypothesized that calves fed bovine colostrum supplemented with GAC would experience improved AEA of IgG (%), improved serum IgG concentrations (g/L), and improved serum total protein (**STP**) concentrations at 24 h of age as compared with calves fed unsupplemented colostrum.

MATERIALS AND METHODS

Study Farm

Study activities were approved by the University of Minnesota's Institution of Animal Care and Use Committee. The study was conducted on a large commercial Holstein dairy farm in western Wisconsin from June through August 2012, with all project activities completed by a study technician. This is a 400-cow facility that houses approximately 300 immediate prepartum dry cows and heifers (0 to 28 d precalving), and approximately 100 immediate postpartum lactating cows between 1 and 14 DIM. Freestalls are walked hourly and cows are moved into individual straw-bedded maternity pens when parturition is imminent. Following parturition, the cow is allowed to lick the calf clean for approximately 20 to 30 min. However, the calf is removed before it has an opportunity to suckle. First milking colostrum is routinely harvested within 1 h after parturition and calves are fed 3.8 L of fresh colostrum using an esophageal tube feeder.

Colostrum Preparation and Sampling

First milking MC was collected from cows within 1 h postparturition and refrigerated. When sufficient volume was collected colostrum from 1 or more dams was pooled to create a 7.6-L batch. Because a typical cow on this dairy produces approximately 4 L of colostrum, then each batch usually included colostrum from 2 cows. However, the occasional batch might include

colostrum from 3 cows if individual cows produced less than 4 L. The 7.6-L batch was thoroughly mixed, then separated into 2 equal 3.8-L aliquots, and stored in clean, sanitized bottles in the refrigerator until assigned for feeding to a newborn calf. Colostrum was stored for no longer than 48 h before feeding to a calf. For each batch, 1 of the two 3.8-L aliquots was designated to be fed either as supplemented with GAC (treated; bottle A) or unsupplemented with GAC (control; bottle B), respectively. Over the course of the study, 60 unique 7.6-L batches were created (enough to feed 120 calves). When a newborn calf was born and enrolled, the randomly assigned bottle was removed from the refrigerator and warmed in a hot water bath (49–51°C) for approximately 20 min and until it reached feeding temperature (35–39°C). Immediately before feeding the colostrum was thoroughly mixed and two 10-mL colostrum samples were collected into sterile sample vials and frozen at –20°C for later IgG determination and microbial culture.

Calf Enrollment and Sampling

To be eligible for inclusion in our study, newborn female or male calves had to be from a singleton observed birth, ≥ 31.8 kg of birth weight, with a dystocia score of 1 to 4 on a scale of 1 to 5 (where 1 = unassisted; 2 = easy pull; 3 = moderate pull; 4 = hard pull; 5 = caesarian section), and without obvious congenital defects. Calves were removed from the maternity pen within 1 h of birth and before suckling. The calf was weighed using a calibrated electronic scale, a unique ear tag applied, and the navel dipped with 7% tincture of iodine solution. A 0-h 10-mL venous blood sample was collected from the jugular vein immediately before colostrum feeding. The calf was then randomly assigned to 1 of 2 treatment groups: GAC group ($n = 52$), which received 3.8 L of colostrum supplemented with 30 g of a commercial GAC product mixed in immediately before feeding (Bio-Mos, Alltech Inc., Nicholasville, KY; bottle A from each batch); or control group ($n = 51$), which received 3.8 L of unsupplemented colostrum (bottle B from each batch).

The warmed colostrum was fed in a single feeding using an esophageal tube feeder within 1 to 2 h of birth. Information recorded for each calf included dam identification, calf identification, birth date, birth time, birth weight, sex, dystocia score, dam parity, colostrum feeding time, batch number of colostrum fed, and treatment group assigned (GAC or control). After colostrum feeding the calf was placed in a clean, well-bedded pen for the next 24 h. While in this pen, calves were offered 1.9 L of a milk protein-based commercial milk replacer via nipple bottle twice daily until the calf was 24 h old

(Amplifier Max Calf Growth Formula; 22% CP, 20% crude fat; Land O' Lakes Animal Milk Products, Shoreview, MN). This milk replacer formula did not contain a GAC and should not have affected study results. A second 10-mL venous blood sample was collected at 24 h of age (± 1 h), after which calves exited the study and were managed and housed according to the farm's routine protocols.

Sample Analysis

Colostrum Analysis. One of each pair of frozen colostrum samples was submitted, on ice, to the Udder Health Laboratory, University of Minnesota (St. Paul, MN). The sample was thawed at room temperature and then cultured to determine TPC and TCC (cfu/mL). The second tube of each pair of colostrum samples was shipped on ice to the Quality Assurance Laboratory of the Saskatoon Colostrum Company (Saskatoon, SK, Canada) for determination of colostrum IgG (g/L) levels by radial immunodiffusion analysis essentially as described by Chelack et al. (1993).

Serum Analysis. All prefeeding (0–1 h) and postfeeding (24 h) blood samples were refrigerated overnight, allowing a clot to form. The next day the samples were centrifuged at $12,000 \times g$ for 10 min at 21°C. One milliliter of serum from each sample was then transferred into duplicate storage cuvettes and frozen at –20°C. Serum samples were transported on ice to the Quality Assurance Laboratory of the Saskatoon Colostrum Company (Saskatoon, SK, Canada) to determine serum IgG (g/L) and STP (g/dL) concentrations by radial immunodiffusion analysis, essentially as described by Chelack et al. (1993).

Data Analysis

A priori sample size calculations were determined based on the primary outcome of serum IgG at 24 h of age. It was estimated that 50 calves per treatment group would provide in excess of 95% confidence and 80% power to detect a predicted difference in serum IgG of 3 g/L (GAC = 20 g/L, control = 17 g/L; SD = 5 g/L; 2-tailed test).

Calf serum IgG at 24 h (g/L) and calf birth weight (kg) measures were used to calculate the AEA of IgG (%), which is an estimate of the proportion of the total IgG mass fed absorbed into the calf's circulation. This was calculated using a formula previously described by Quigley et al. (2002), assuming a plasma volume of 9.9% of birth weight.

All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC). Descriptive statistics (mean, SD, range) were first calculated to

describe, by treatment group, the following calf enrollment and colostrum characteristics: birth weight (kg), dystocia score (1–4), serum IgG (g/L) and STP (g/dL) before colostrum feeding, age at time of colostrum feeding (min), IgG concentration in the colostrum fed (g/L), and TPC and TCC counts in the colostrum fed (cfu/mL). Descriptive statistics (mean, SD, range) were also calculated to describe, by treatment group, the following dependent variables of interest at 24 h of age: serum IgG (g/L), AEA IgG (%), and STP (g/dL). All variables were examined for outliers and for normality. Because the TPC and TCC data were right-skewed, these values were transformed (Log_{10}) before inclusion in the analysis.

Mixed linear regression (MIXED procedure) was used to describe the relationship between colostrum treatment group (explanatory variable, forced) and each of the 3 dependent variables measured at 24 h of age: serum IgG, AEA IgG, and STP. Additional covariates offered as main effects into each of these 3 models, either to control for potential confounding or to improve model efficiency, included dystocia score (1 to 4), sex (male/female), IgG concentration in the colostrum fed (g/L), and either colostrum TPC (log_{10} , cfu/mL) or colostrum TCC (log_{10} , cfu/mL). Batch number was included as a random effect in all models to control for the clustering of 2 calves (1 GAC; 1 control) within each batch of colostrum fed. All covariates were first examined in a univariable model, with variables significant at $P < 0.20$ carried forward to offer into a multivariable model. A manual backward, stepwise elimination process was then used to remove nonsignificant covariates (removed if $P > 0.05$). Interaction terms were then investigated between the treatment variable (treatment vs. control) and any remaining significant covariates. Model fit was

evaluated by examining the residual log-likelihood statistic. Final significance was declared at $P < 0.05$.

RESULTS

A total of 103 calves (51 control; 52 GAC) were originally enrolled into the study. However, 8 calves were omitted from the final analysis for the following reasons: 1 GAC calf was omitted because a colostrum sample was unavailable for testing; 1 GAC calf died after enrollment but before the 24-h blood sample was collected (i.e., a stillbirth. Berglund et al., 2003); and 6 calves (3 GAC, 3 control) were omitted because the 0-h serum IgG concentration exceeded 1.0 g/L. Reasons for prefeeding IgG values exceeding 1.0 mg/mL could include unobserved suckling of the dam before enrollment, in utero exposure to an antigen and subsequent titer production, or analytic variation. Although it is highly unlikely the calves nursed, given that all births were observed, these 6 calves were omitted from the final analysis to remove any doubt. It should be noted that when a separate analysis was conducted including these 6 calves, the results and statistical inferences did not change (results not shown). The final data set analyzed included 95 calves: 48 control calves and 47 GAC calves. There was no difference between treatment groups for any of the variables describing the colostrum fed or calf enrollment characteristics ($P > 0.05$; Table 1).

The final model describing serum IgG at 24 h showed a negative effect of treatment, with the adjusted mean (SE) for serum IgG concentration 6.74 (1.26) g/L higher for control calves [30.75 (1.04) g/L] as compared with treated calves [24.02 (1.05) g/L; $P < 0.0001$; Table 2]. This final model also retained covariates describing

Table 1. Crude descriptive statistics describing colostrum characteristics and calf enrollment characteristics by colostrum treatment group [reported (mean; SD)]

Parameter	Control group ¹	GAC group ²
Colostrum fed		
Number of unique batches fed	48	47
Colostrum IgG (g/L)	105.6 (26.7; 51.5 to 164.6)	105.5 (26.5; 47.2 to 172.0)
Colostrum total plate count (Log_{10} , cfu/mL)	5.49 (1.33; 3.56 to 8.97)	5.43 (1.33; 3.72 to 8.91)
Colostrum total coliform count (Log_{10} , cfu/mL)	4.64 (1.58; 2.23 to 8.18)	4.69 (1.56; 2.19 to 8.75)
Calf enrollment characteristics		
Number of calves	48	47
Dystocia score (1 to 4)	1.5 (1.0; 1 to 3)	1.4 (0.9; 0 to 4)
Birth weight (kg)	39.7 (4.6; 31.8 to 50.3)	38.5 (3.4; 31.8 to 45.4)
Age at feeding (min)	85.6 (27.4; 30 to 145)	87.6 (28.0; 40 to 140)
Serum TP, 0 h (g/dL)	4.42 (0.29; 3.9 to 5.2)	4.39 (0.29; 3.6 to 4.9)
Serum IgG, 0 h (g/L)	0.32 (0.13; 0.2 to 0.8)	0.30 (0.15; 0.2 to 1.0)

¹3.8 L of maternal colostrum.

²3.8 L of maternal colostrum supplemented with 30 g of gut-active carbohydrates (GAC).

Table 2. Final multivariable models describing the effect of supplementing fresh bovine colostrum with 30 g of gut-active carbohydrates (GAC) on serum IgG, serum total protein, and apparent efficiency of absorption of IgG at 24 h of age¹

Model outcome	Parameter	Treatment group	Adjusted mean (SE)	Estimate (SE)	P-value
Serum IgG (g/L)	Treatment group	GAC	24.02 (1.05)	−6.74 (1.26)	<0.0001
		Control	30.75 (1.04)	Referent	
STP ² (g/dL)	Birth weight (kg)			−0.56 (0.18)	0.0033
	Colostrum IgG (g/L)			0.13 (0.03)	0.0004
	Treatment group	GAC	5.81 (0.08)	−0.45 (0.10)	<0.0001
		Control	6.26 (0.08)	Referent	
AEA IgG ³ (%)	Colostrum IgG (g/L)			0.0095 (0.0026)	0.0009
	Treatment group	GAC	23.9 (0.97)%	−6.52 (1.15)	<0.0001
		Control	30.4 (0.96)%	Referent	
	Colostrum TCC ⁴			−1.03 (0.50)	0.0447
	Colostrum IgG (g/L)			−0.14 (0.03)	<0.0001

¹All models control for batch as a random effect.

²STP = serum total protein (g/dL).

³AEA IgG = apparent efficiency of absorption of IgG (%).

⁴TCC = total coliform count (Log₁₀, cfu/mL).

the birth weight of the calf and the quality of colostrum fed. A negative association was observed between calf birth weight (kg) and serum IgG at 24 h (g/L; estimate = −0.56 (0.18); $P = 0.0033$). A positive association between colostrum IgG concentration (g/L) and calf serum IgG was noted at 24 h [g/L; estimate = 0.13 (0.033); $P = 0.0004$; Table 2]. The variables describing birth weight and colostrum IgG did not interact with or otherwise confound the relationship between colostrum treatment group and serum IgG at 24 h.

The final model describing STP at 24 h also showed a negative effect of treatment, with the adjusted mean (SE) STP concentration 0.45 (0.10) g/dL higher for control calves [6.26 (0.08) g/dL] as compared with treated calves [5.81 (0.08) g/dL; $P < 0.0001$; Table 2]. This final model also retained the covariate describing the IgG concentration (g/L) in the colostrum fed. There was a positive association between colostrum IgG concentration (g/L) and calf STP at 24 h [estimate = 0.0095 (0.0026); $P = 0.0009$; Table 2]. The variable describing colostrum IgG did not interact with, or otherwise confound, the relationship between colostrum treatment group and STP at 24 h.

The final model describing apparent efficiency of absorption of IgG (AEA IgG, %) showed a negative effect of treatment, with the adjusted mean (SE) AEA IgG 6.52% (1.15) higher for control calves [30.4% (0.96)] as compared with treated calves [23.9% (0.97); $P < 0.0001$; Table 2]. This final model also retained the covariates describing TCC and IgG concentration in the colostrum fed. A negative association was seen between colostrum TCC and AEA IgG [estimate = −1.03% (0.50); $P = 0.0447$]. There was also a negative association between colostrum IgG and AEA % [estimate = −0.14% (0.03); $P < 0.0001$; Table 2]. The variables

describing colostrum TCC and colostrum IgG did not interact with, or otherwise confound, the relationship between colostrum treatment group and AEA IgG (%).

DISCUSSION

This is the first randomized controlled clinical trial conducted on a North American commercial dairy farm to investigate the effects of supplementing maternal colostrum with GAC on passive transfer of IgG in newborn Holstein calves. The results indicate that the addition of 30 g of GAC to colostrum reduced, rather than enhanced, absorption of IgG. These findings contradict those of a smaller earlier study of 24 newborn calves that reported a positive effect on calf serum IgG when 22.5 g of GAC was added to maternal colostrum (Lazarevic et al., 2010), and a more recent North American study of 241 Holstein dairy calves that reported no effect on IgG absorption when 30 g of GAC was added to a reconstituted colostrum replacer (Robichaud et al., 2014). Lazarevic et al. (2010) reported higher serum IgG concentrations in both groups of calves (GAC = 58.1 g/L; control = 43.0 g/L; $P < 0.0001$) as compared with the current study (GAC = 24.0 g/L; control = 30.8 g/L; $P < 0.0001$); however, the mean serum IgG concentrations were excellent in both studies, far exceeding the goal of >10 g/L IgG for acceptable passive transfer (McGuirk and Collins, 2004).

The source of the contradictory findings between the Lazarevic et al. (2010) study and the current study are uncertain given the many known or possible differences in study methodologies. Unfortunately, the Lazarevic et al. (2010) study failed to describe many of the study methods used, as well as many potentially confounding factors including colostrum and calf char-

acteristics. The dose of GAC used and the colostrum-feeding schedule also differed between studies. The 12 GAC-treated calves in the Lazarevic et al. (2010) study reportedly received 22.5 g of GAC mixed into 4.5 L of colostrum, equating to approximately 0.6 g of GAC/kg of BW. By comparison, the 47 GAC-treated calves in the current study received 30 g of GAC mixed into 3.8 L of colostrum, equating to approximately 0.77 g of GAC/kg of BW.

Another possible difference in study methodologies was the schedule and method of colostrum feeding; the current study used an esophageal tube feeder and provided a single 3.8-L feeding within 2 h of birth. By comparison, the Lazarevic et al., (2010) study provided 4.5 L of colostrum over multiple colostrum feedings (2, 12, and 24 h after birth). And, whereas the method of feeding was not reported in the Lazarevic et al. (2010) study, it is assumed that the calves were fed by nipple bottle. Regardless, we do not consider the potential difference in method of colostrum feeding to be important, as earlier experiments have reported no effect of feeding method on IgG absorption at the volumes fed (Godden et al., 2009; Chigerwe et al., 2012).

Other factors that may have differed between these 2 studies that are known to affect AEA of IgG (%) and final serum IgG (g/L) include colostrum quality (IgG concentration) and colostrum bacteria counts (TCC). For example, the IgG mass (concentration $\times$ volume) in colostrum is positively associated with final serum IgG concentrations because more grams of IgG are presented to the gut, even though there is often a negative association between colostrum IgG concentration and AEA percentage, presumed to be attributable to saturation of intestinal capacity for macromolecular absorption (Besser and Osborn, 1993; Quigley et al., 1998; Weaver et al., 2000). Both these relationships were observed in the current study. Similarly, a negative association between colostrum TCC and serum IgG concentration, which has also been previously described (Godden et al., 2012), was observed in the current study. Unfortunately, the Lazarevic et al. (2010) study did not report the quality of colostrum fed (IgG, g/L) nor colostrum bacteria counts (cfu/mL). And because colostrum quality was not tested, the Lazarevic et al. (2010) study could not report the total dose of IgG fed (g) nor calculate the AEA (%) for study calves. Because of the many differences in study methodologies, many of which may be confounding factors, it is impossible to provide an exact explanation for differences in study findings.

Complicating this discussion further are the results of a different randomized clinical trial of 241 newborn Holstein calves that reported no difference in AEA percentage or serum IgG at 24 h when calves were

randomly assigned to a control group and fed 1.5 doses (150 g IgG) of a lacteal derived commercial CR (Land O'Lakes Colostrum Replacer; Land O'Lakes Inc., St. Paul, MN) or a treated group fed 30 g of GAC mixed into 1.5 doses of the same CR product (Robichaud et al., 2014). The Robichaud et al. (2014) study was conducted on the same Wisconsin Holstein farm as the current study, but during the winter and spring of 2011 and 2012. Differences between the CR-GAC study and the current study include season (winter vs. summer), dose of IgG fed (CR = 150 g vs. MC = an average of 405 g), source of IgG (CR vs. fresh MC), and volume of colostrum fed (approximately 3 vs. 3.8 L). A pertinent difference might be the level of bacterial load in the colostrum treatments, given that TPC are lower and coliforms should be absent in the Center for Veterinary Biologics (CVB; USDA, Animal and Plant Health Inspection Service, Veterinary Service, Ames, IA)-licensed CR product used by Robichaud et al. (2014). Conversely, TPC and TCC levels were present, and sometimes in very high concentrations, in the current study feeding pooled maternal colostrum. Whereas bacterial cultures of the reconstituted CR was not conducted for the Robichaud et al. (2014) study, purity testing is routinely conducted by the manufacturers for each serial and these are monitored by a USDA laboratory to verify that all lots of CVB-licensed CR products are free of coliforms, *Salmonella* spp., and fungi (USDA 2012a,b). As such, the only time a CVB-licensed CR might become contaminated would be on the farm at the time of reconstitution and feeding (e.g., from contaminated mixing or feeding equipment).

Ultimately, to learn why 3 different and conflicting results were found among these studies (current study, Lazarevic et al., 2010; Robichaud et al., 2014), it will be necessary to develop a better understanding of the mechanism(s) of GAC action in the gut. Mannan-oligosaccharide is a GAC derived from the cell wall of yeast, which has been shown to adsorb pathogens expressing type-1-fimbriae, reducing their ability to colonize the gastrointestinal tract (Spring et al., 2000). Lazarevic et al. (2010) hypothesized that the increased serum IgG observed in GAC-treated calves might be attributed to the fact that GAC may prevent attachment of pathogenic bacteria, such as *Escherichia coli*, by competition for receptor sites (Spring et al., 2000). In theory, this should result in healthier calves, both through passive transfer status as well as through reduced gut colonization by pathogenic bacteria. Lazarevic et al. (2010) did not measure calf weights and did not test colostrum IgG concentrations, and therefore were unable to calculate and report the AEA of IgG (%) values for the treated and control group of calves. Therefore, the authors cannot rule out that the enhanced serum IgG results they

observed in GAC-treated calves was not attributable to other unmeasured differences (i.e., confounding factors) between the 2 treatment groups.

Despite this limitation in study design or reporting, the hypothesis proposed by Lazarevic et al., (2010) still has merit. James et al. (1981) reported that the presence of coliform bacteria in colostrum may interfere directly with passive absorption of colostral antibodies across the intestine and into the circulation, reducing passive transfer of immunity in the calf. This finding is supported by a recent study of 924 calves fed heat-treated ($n = 490$) or fresh ($n = 434$) colostrum, wherein high TCC concentrations in colostrum were negatively associated with serum IgG concentrations in calves (Godden et al., 2012). As such, and assuming that GAC prevents attachment of pathogenic bacteria by competition for receptor sites, it was assumed that adding 30 g of GAC to colostrum in the current study would result in enhanced absorption of IgG. Why, in the current study, did we observe a negative effect of GAC-supplementation of colostrum on passive transfer of IgG? One possibility is that the 30-g dose of GAC used in the current study prevented colonization of the gut by pathogenic bacteria, but is, itself, blocking intestinal sites for nonselective transfer of IgG molecules. However, this would not explain the absence of negative effect observed the GAC supplementation study using CR (Robichaud et al., 2014). Another possibility is that the formation of very large aggregates of bacteria, GAC, and IgG might result in diminished absorption of the IgG, or perhaps this aggregate is absorbed but is immediately phagocytized and was not apparent in the serum. Might a lower dose of GAC result in a positive (or at least not negative) effect on IgG absorption? Future studies will be needed to investigate this hypothesis. Overall, the conflicting results among the 3 studies suggests that there is poor understanding of how GAC interacts with bacteria or IgG molecules in the gut of the newborn calf and that further research is needed to investigate the use of these molecules.

CONCLUSIONS

The addition of 30 g of GAC to 3.8 L of bovine colostrum resulted in a reduction in absorption of IgG (AEA %) and lower final serum IgG concentrations in newborn Holstein calves compared with control calves fed colostrum not supplemented with GAC. Until the scientific community acquires a better understanding of the relationship between GAC and passive transfer of immunoglobulins, it is not recommended that colostrum be supplemented with GAC.

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REFERENCES

- Besser, T. E., and D. Osborn. 1993. Effect of bovine serum albumin on passive transfer of immunoglobulin G1 to newborn calves. *Vet. Immunol. Immunopathol.* 37:321–327.
- Berglund, B., L. Steinbock, and M. Elvander. 2003. Causes of stillbirth and time of death in Swedish Holstein calves examined post mortem. *Acta Vet Scand.* 44:111–120.
- Chelack, B. J., P. S. Morley, and D. M. Haines. 1993. Evaluation of methods for dehydration of bovine colostrum for total replacement of normal colostrums in calves. *Can. Vet. J.* 34:407–412.
- Chigerwe, M., D. M. Coons, and J. V. Hagey. 2012. Comparison of colostrum feeding by nipple bottle versus oroesophageal tubing in Holstein dairy bull calves. *J. Am. Vet. Med. Assoc.* 241:104–109.
- Davis, C. L., and J. K. Drackley. 1998. Colostrum. Pages 179–206 in *The Development, Nutrition, and Management of the Young Calf*. 1st Ed. Iowa State University Press, Ames.
- DeNise, S. K., J. D. Robison, G. H. Stott, and D. V. Armstrong. 1989. Effects of passive immunity on subsequent production in dairy heifers. *J. Dairy Sci.* 72:552–554.
- Faber, S. N., N. E. Faber, T. C. McCauley, and R. L. Axe. 2005. Effects of colostrum ingestion on lactational performance. *Prof. Anim. Sci.* 21:420–425.
- Godden, S. M., D. M. Haines, K. Konkol, and J. Peterson. 2009. Improving passive transfer of immunoglobulins in calves II: Interaction between feeding method and volume of colostrum fed. *J. Dairy Sci.* 92:1758–1764.
- Godden, S. M., D. J. Smolenski, M. Donahue, J. M. Oakes, R. Bey, S. Wells, S. Sreevatsan, J. Stabel, and J. Fetrow. 2012. Heat-treated colostrum and reduced morbidity in preweaned dairy calves: Results of a randomized trial and examination of mechanisms of effectiveness. *J. Dairy Sci.* 95:4029–4040.
- James, R. E., C. E. Polan, and K. A. Cummins. 1981. Influence of administered indigenous microorganisms on uptake of [iodine-125] gamma-globulin in vivo by intestinal segments of neonatal calves. *J. Dairy Sci.* 64:52–61.
- Johnson, J. L., S. Godden, T. Molitor, T. Ames, and D. Hagman. 2007. Effects of feeding heat-treated colostrum on passive transfer of immune and nutritional parameters in neonatal dairy calves. *J. Dairy Sci.* 90:5189–5198.
- Lazarevic, M., P. Spring, M. Shabanovic, V. Tokic, and L. A. Tucker. 2010. Effect of gut active carbohydrates on plasma IgG concentrations in piglets and calves. *Animal* 4:938–943.
- McGuirk, S. M., and M. Collins. 2004. Managing the production, storage and delivery of colostrum. *Vet. Clin. North Am. Food Anim. Pract.* 20:593–603.
- Poulsen, K.P., F.A. Hartmann, and S.M. McGuirk. 2002. Bacteria in colostrum: Impact on calf health. Abstr. 52 in *Proc. 20th American College of Internal Veterinary Medicine*. Dallas, TX. American College of Internal Veterinary Medicine, Lakewood, CO.
- Quigley, J. D., D. L. Fike, M. N. Egerton, J. J. Drewry, and J. D. Arthington. 1998. Effects of a colostrum replacement product derived from serum on immunoglobulin G absorption by calves. *J. Dairy Sci.* 81:1936–1939.

- Quigley, J. D., 3rd, C. J. Kost, and T. M. Wolfe. 2002. Absorption of protein and IgG in calves fed a colostrum supplement or replacer. *J. Dairy Sci.* 85:1243–1248.
- Robichaud, M., S. M. Godden, D. M. Haines, D. B. Haley, D. L. Pearl, J. Rushen, and S. LeBlanc. 2014. Addition of gut active carbohydrates to colostrum replacer does not improve passive transfer of immunoglobulin G in Holstein dairy calves. *J. Dairy Sci.* 97:5700–5708.
- Robison, J. D., G. H. Stott, and S. K. DeNise. 1988. Effects of passive immunity on growth and survival in the dairy heifer. *J. Dairy Sci.* 71:1283–1287.
- Spring, P., C. Wenk, K. A. Dawson, and K. E. Newman. 2000. The effects of dietary mannan oligosaccharides on caecal parameters and the concentrations of enteric bacteria in the caeca of salmonella-challenged broiler chicks. *Poult. Sci.* 79:205–211.
- Steele, M. L., W. B. McNab, C. Poppe, M. W. Griffiths, S. Chen, S. A. Degrandis, L. C. Fruhner, C. A. Larkin, J. A. Lynch, and J. A. Odumeru. 1997. Survey of Ontario bulk tank raw milk for food-borne pathogens. *J. Food Prot.* 60:1341–1346.
- Streeter, R. N., G. F. Hoffsis, S. Bech-Nielsen, W. P. Shulaw, and D. M. Rings. 1995. Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows. *Am. J. Vet. Res.* 56:1322–1324.
- USDA. 2012a. United States Department of Agriculture. Center for Veterinary Biologics Policy, Evaluation, and Licensing Reviewer's Manual. Accessed Dec. 20, 2012. http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/animalhealth/sa_vet_biologics/sa_biologics_regulations/ct_vb_pelmanual_toc.
- USDA. 2012b. United States Department of Agriculture. Center for Veterinary Biological Products. Ames, IA. Accessed Dec. 20, 2012. http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/animalhealth/sa_vet_biologics.
- Walz, P. H., T. P. Mullaney, J. A. Render, R. D. Walker, T. Mosser, and J. C. Baker. 1997. Otitis media in preweaned Holstein dairy calves in Michigan due to *Mycoplasma bovis*. *J. Vet. Diagn. Invest.* 9:250–254.
- Weaver, D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler, and G. M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. *J. Vet. Intern. Med.* 14:569–577.
- Wells, S. J., D. A. Dargatz, and S. L. Ott. 1996. Factors associated with mortality to 21 days of life in dairy heifers in the United States. *Prev. Vet. Med.* 29:9–19.