Addition of gut active carbohydrates to colostrum replacer does not improve passive transfer of immunoglobulin G in Holstein dairy calves

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ABSTRACT

The primary objective of this study was to investigate the effects of supplementing a commercial colostrum replacer (CR) with gut active carbohydrates (GAC) on passive transfer of IgG in commercial dairy calves. A secondary objective was to evaluate the effect of treatment on preweaning health and growth. A total of 240 newborn Holstein dairy calves on a commercial dairy farm were enrolled in this study. Newborn heifer and bull calves were weighed and then randomly assigned to either the treated group [GAC: 30 g of GAC mixed into 1.5 doses (150 g of IgG) of commercial colostrum replacer; $n = 119$] or the control group [CON: 1.5 doses (150 g of IgG) of CR; $n = 121$]. The assigned CR treatment was fed within 3.5 h of birth using an esophageal tube feeder. Venous blood samples were collected at 0 and 24 h of age and used to measure serum IgG (mg/mL) and serum total protein (g/dL) concentrations and to estimate the apparent efficiency of absorption of IgG (%). The 129 heifers calves enrolled (CON = 60; GAC = 69) were also followed until weaning to assess the effect of GAC addition on preweaning health and growth. Multivariable linear regression showed that the addition of GAC to CR did not influence passive transfer of IgG, as measured by apparent efficiency of absorption at 24 h of age (CON = 54.0 vs. GAC = 54.3%), serum IgG (CON = 20.3 vs. GAC = 20.2 mg/mL), and serum total protein (CON = 5.69 vs. GAC = 5.68 g/dL). Although study sample sizes were not originally derived to evaluate health outcomes, treatment had no effect on weight gain or incidence of health events (diarrhea, pneumonia, mortality) for heifer calves between birth and 7 wk of age.

Key words: colostrum replacer, gut active carbohydrates, immunoglobulin absorption, passive transfer

INTRODUCTION

The importance of colostrum feeding on survival of newborn calves has been understood for almost 100 yr. Smith and Little (1922) concluded that calves deprived of colostrum “lacked something, which permitted intestinal bacteria to invade the body of the animal and multiply in various organs.” More recent publications have stated that a good colostrum management program is the most important step in reducing disease and death (McGuirk and Collins, 2004; Smith and Foster, 2007) and in promoting morphological and functional development in newborn calves (Blum and Hammon, 2000). Adequate passive transfer in the dairy calf is generally accepted as a serum IgG concentration ≥ 10 mg/mL at 24 and 48 h after birth (Godden, 2008). The most recent USDA study reported the prevalence of failure of passive transfer (FPT) to be 19.2% among newborn heifers calves on US dairy operations (Beam et al., 2009), indicating that a significant opportunity remains for improving colostrum management programs on dairy farms.

The timing of colostrum ingestion, the volume and quality of the colostrum fed, and levels of bacterial contamination in colostrum are among several factors affecting successful passive transfer of IgG (McGuirk and Collins, 2004; Chigerwe et al., 2008; Godden, 2008; Godden et al., 2009b). A minimum mass of 150 to 200 g of IgG is needed to achieve adequate passive transfer when calves are fed using an esophageal tube within 2 h of birth (Chigerwe et al., 2008; Godden et al., 2009a). Over the last decade, researchers have investigated various approaches in attempts to increase IgG absorption from colostrum. Several experiments have reported inconsistent or equivocal results when investigating if the addition of various supplements to maternal colostrum, including selenium or sodium bicarbonate, affected IgG absorption in neonatal calves (Kamada et al., 2007; Morrill et al., 2010; Cabral et al., 2011, 2012). The addition of gut active carbony-

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drates (**GAC**), a mannan-oligosaccharide derived from the cell wall of yeast, has been investigated as a possible method of enhancing passive transfer of IgG from colostrum in young mammals. Lazarevic et al. (2010) fed 22.5 g of GAC over 3 feedings of colostrum to 12 newborn Holstein calves and compared their final serum IgG concentrations (mg/mL) to those of 12 control calves fed nonsupplemented colostrum. All calves in this study received the same colostrum quality, volume, and number of feedings. The authors reported that the 12 calves fed GAC-supplemented colostrum had higher circulating serum IgG levels than the 12 control calves. Although the mode of action was not clear, it was proposed that GAC might be promoting immunoglobulin uptake through a role that carbohydrates may play in communication between the gut and the immune system (Lazarevic et al., 2010). In a study using young chicks, Spring et al. (2000) hypothesized that GAC would prevent the attachment of pathogenic bacteria, such as *Escherichia coli*, by the principle of competition for attachment sites. If GAC interferes with attachment of *E. coli*, then this is one possible explanation for how GAC might enhance absorption of colostral IgG, because other studies have reported that passive transfer of IgG in calves is negatively associated with total bacteria counts, and especially total coliform counts, in colostrum (Godden et al., 2012). The latter study also reported that a reduced risk of illness was associated with improved serum IgG concentrations in calves fed heat-treated colostrum containing lower total coliform counts.

Although the results of the Lazarevic et al. (2010) study are encouraging, the study was limited in sample size, and the authors did not report on several potentially important details regarding the methods used or parameters measured, including calf weights, colostrum quality, colostrum bacteria counts, or the effect of treatment on apparent efficiency of absorption of IgG (**AEA**, %). Lazarevic et al. (2010) recommended that further studies be completed to investigate the nature and possible modes of action involved in enhancing blood IgG concentrations in young animals, and the longer term effects this may have on the development of the immune response. The primary objective of the current study was to investigate the effects of supplementing colostrum replacer with GAC on passive transfer of colostral IgG. A secondary objective was to evaluate the effect of treatment on health and growth in the preweaning period. The hypothesis was that supplementing colostrum replacer with GAC should result in improved absorption of colostral IgG, resulting in improved serum IgG concentrations and, subsequently, improved health and growth in newborn calves.

MATERIALS AND METHODS

Calf Enrollment and Sampling

The Institutional Animal Care and Use Committee of the University of Minnesota (St. Paul) approved all procedures described in this study. This study was conducted between October 2011 and May 2012 on a large commercial Holstein dairy farm in western Wisconsin. In total, 240 calves from a single herd were enrolled in this study. Before calving, dry cows from 2 milking barns were housed together in a 400-stall transition cow facility during the entire dry period and for the first 15 to 25 d after calving. Close-up cows and heifers were housed in freestall pens that were observed hourly. Animals were moved to individual maternity pens when signs of imminent calving were detected. Cows were observed throughout the entire calving process and calves were separated from their dams within 60 min of birth. Calves were observed continuously between birth and removal to ensure that no suckling of the dam occurred. To be eligible for enrollment in this study, all calves, bulls and heifers, had to be from single births, weigh ≥ 31.5 kg at birth, and have a calving ease score of ≤ 4 on a scale of 1 to 5. Calving ease score was defined using the following scale: 0 = unobserved, 1 = observed but unassisted, 2 = easy pull, 3 = moderate pull, 4 = hard pull, and 5 = caesarean section or uterine torsion. As all calves were observed during birth, no calves included in this study had a calving ease score of 0 or 5.

Before colostrum replacer (**CR**) feeding, each newborn calf was weighed using an electronic scale, identified using a unique ear tag according to its dam (**ID**), had its navel dipped in a 7% tincture of iodine solution, and then placed in a small group pen (3.6×3.6 m) bedded with fresh straw for 24 h following birth. A 10-mL prefeeding blood sample was collected from the jugular vein using a serum Vacutainer tube (Becton Dickinson, Franklin Lakes, NJ) and then the calves were randomly assigned to 1 of 2 treatment groups: GAC or control (**CON**). Randomization of calf to a CR treatment group was completed within blocks of 8 calves by birth order, using a predetermined spreadsheet. All calves were fed 1.5 doses (150 g of IgG) of 100% lacteal-based CR (Land O'Lakes Colostrum Replacer; Land O'Lakes Inc., Arden Hills, MN) within 3.5 h of life using an esophageal tube feeder. The CR powder was mixed into 3 L of warm water before tube feeding. Control calves received CR only, whereas treatment calves had 30 g of a commercial GAC product (Bio-Mos, Alltech Inc., Nicholasville, KY) mixed into the CR solution immediately before feeding. The GAC was derived from a specific strain of yeast.

At the time of enrollment, information recorded included calf ID, dam ID, birth date, birth time, sex, calving ease score (1 to 4), birth weight (kg), time at CR feeding (min), and CR treatment group assigned (GAC or CON). Between the single CR feeding and 24 h of age, calves were offered 1.9 L of a commercially available milk replacer twice daily using a nipple bottle (Amplifier Max, Land O' Lakes Animal Milk Products, Arden Hills, MN; 22% all-milk CP, 20% crude fat). At 24 h of age, a postfeeding 10-mL blood sample was collected from the jugular vein using a serum Vacutainer tube (Becton Dickinson). At this point, all calves were moved from the group pen and placed into individual freshly bedded hutches. Bull calves were sold and removed from the farm once per week and therefore exited the trial after the 24-h blood sample was collected.

Collection of Growth and Health Records for Preweaned Heifer Calves

Heifer calves were raised in individual hutches and fed 3.8 L of pasteurized waste milk supplemented with a pasteurized milk balancer (Land O' Lakes Pasteurized Milk Balancer, 25% CP, 10% crude fat; Land O' Lakes Inc.), added as a fixed quantity for a given volume of milk, twice daily. Starting at 3 d of age, heifers were offered free-choice water and commercial starter pellet (20% CP).

Fecal scores were recorded by the study technician 3 times per week until 14 d of age. Fecal scores were based on a 4-point scale as previously described by McGuirk (2008): 1 = normal consistency, 2 = soft, semi-formed feces, 3 = loose feces, and 4 = watery feces that sift through bedding material. Clinical disease occurrence, including treatments for pneumonia, diarrhea, and other diseases, plus death events were recorded daily by farm staff using standardized farm protocols. Farm personnel were blinded to CR treatment groups. Weaning weights were measured by the study technician at 7 wk of age using a heart girth measuring tape. Average daily gain (kg/d) was calculated by calculating the gain in weight between birth and wk 7 of age, and then dividing this value by the interval in days.

Sample Analysis Procedure

Pre (0 to 1 h)- and post (24 h)-CR feeding venous blood samples collected from all calves were refrigerated for a minimum of 6 h and allowed to clot. They were then centrifuged (model DSC-158T Digisystem Laboratory Instruments Centrifuge; Digisystem Laboratory Instruments Inc., New Taipei City, Taiwan) at 10,000 rpm for 10 min and serum was separated. Serum samples were frozen at -20°C until submission on ice to

the Saskatoon Colostrum Co. Ltd. Quality Assurance Laboratory (Saskatoon, Canada) for determination of serum IgG concentration (mg/mL) and serum total protein (STP; g/dL) by means of radial immunodiffusion as described by Chelack et al. (1993). Apparent efficiency of absorption of IgG (%) was calculated to estimate the percentage of the mass of IgG consumed that was absorbed by the calf. The equation used assumes that the calf plasma volume is 9.9% of its birth weight in kilograms (Quigley et al., 1998, 2002). The equation was (serum IgG concentration at 24 h – serum IgG concentration at 0 h) $\times$ weight of the blood of the calf/150 g of IgG fed $\times$ 100.

Statistical Analyses

The a priori sample size calculations for this study were determined based on the primary outcome of serum IgG at 24 h of age. It was estimated that 125 calves per treatment group would provide 95% confidence and 80% power to detect a predicted difference in serum IgG of 2 mg/mL (CON = 20 mg/mL, GAC = 22 mg/mL; SD = 5.5 mg/mL; 2-tailed test). For the secondary outcomes (effect of treatment on health and growth), it was known that group sizes would be reduced by approximately half due to the loss to follow-up of bull calves. As such, a treatment effect would be detected only if large differences existed between CON and GAC groups. An estimated 62 heifer calves per group would provide 95% confidence and 80% power to detect a 50% difference in risk for scours (assumptions: CON = 30% with scours, GAC = 15% with scours, 2-tailed test).

The data were analyzed using Stata 11 statistical software (StataCorp LP, College Station, TX). Descriptive statistics were generated to describe, by treatment group, calf characteristics at enrollment [serum IgG (mg/mL) and STP (g/dL) at 0 h, sex ratio, calving ease score, birth weight, time to CR feeding], the primary outcomes measured for all calves describing passive transfer of IgG [AEA IgG (%), serum IgG (mg/mL), and STP (g/dL) at 24 h], and the secondary outcomes measured between birth and weaning for heifer calves [BW at 7 wk, ADG between birth and 7 wk, proportion treated for scours by farm staff between birth and 7 wk, proportion with severe scours (fecal score = 4) as diagnosed by the study technician in the first 14 d, proportion treated for pneumonia by farm staff between birth and 7 wk, and proportion died between birth and 7 wk]. One calf (CON) with a calculated AEA of 93% was considered to be an outlier, based on lack of biological plausibility, and was therefore removed from the data set before analysis.

Linear regression was used to describe the relationship between CR treatment (CON vs. GAC) and each

of the following continuous descriptor or outcome variables measured: birth weight (kg), calving ease score (0 to 4), age at CR feeding (min), STP at 0 and 24 h (g/dL), serum IgG at 0 and 24 h (mg/mL), AEA IgG (%), ADG (kg/d), and BW at 7 wk (kg).

Logistic regression was used to describe the relationship between CR treatment (CON vs. GAC) and each of the following dichotomous descriptor or outcome variables measured: sex (male/female), risk for treatment for scours by farm staff between birth and 7 wk, risk for severe scours (fecal score = 4) as diagnosed by the study technician during the first 14 d, risk for treatment for pneumonia by farm staff between birth and 7 wk, and risk for death between birth and 7 wk. In addition to CR treatment group (CON vs. GAC), which was forced into all logistic and linear regression models, additional parameters offered into all models to improve model efficiency or control for potential confounding included calf sex, birth weight (kg), age at CR feeding (min), and calving ease score (1 to 4). An initial cut-off value of $P < 0.2$ was used as a prerequisite to include one of these additional covariates into a multivariable model that included CR treatment group. A backward stepwise technique was then used to remove all nonsignificant terms, with the exception of CR treatment group, which was forced. Interactions between CR treatment group and any other remaining significant covariates were investigated. Confounding effects were assessed according to biological plausibility, with confounding parameters retained in the multivariable model according to significance and impact on the estimated coefficient for CR treatment effect greater than 20%. The assumption of linearity was assessed visually with the residuals distribution for all linear and logistic regression models. The existence of collinearity between predictors was also tested for all linear and logistic regression models. The assumptions of homoscedasticity and normality were assessed visually using the residuals distribution. Final significance of variables was declared at $P < 0.05$.

RESULTS

Calf Characteristics

In total, 240 newborn Holstein calves (129 heifers, 111 bulls) were enrolled in the study. The mean birth weight (kg), age at CR feeding (min), 0-h STP (g/dL), and 0-h serum IgG (mg/mL) were not different between CR treatment groups (Table 1; $P > 0.05$). Serum total protein at 0 h ranged from 3.8 to 5.5 g/dL and serum IgG concentration at 0 h ranged from 0.2 to 2.4 mg/mL. Immunoglobulin G concentrations at 0 h >1.0 mg/

mL were found in 13 calves (5.4%) and >2.0 mg/mL in 4 calves (1.7%). As calves were continuously observed from birth and removed before any suckling of their dams, those calves were not excluded from the analysis. Calving ease score was different between treatments, with calves that received the CR supplemented with GAC having slightly easier calving on average than control calves (Table 1; $\beta = -0.23$, $P = 0.04$). This difference resulted in calving ease being offered as a covariate in each regression model. Apart from this, we observed no other differences in enrollment characteristics between CON and GAC calves.

Effects of Treatment on Passive Transfer of IgG

Based on the results of multivariable regression models, the addition of 30 g of GAC to 1.5 doses (150 g of IgG) of CR did not influence passive immune transfer in newborn Holstein dairy calves as indicated by similar AEA IgG (%), STP (g/dL), and serum IgG (mg/mL) concentrations between groups at 24 h of age (Table 2; $P > 0.05$). The average (range) serum IgG and STP measures for all calves in the trial were 20.2 (7.4 to 33.5 mg/mL) and 5.7 (4.7 to 7.0 g/dL), respectively. Only 3 calves (1.3%) did not achieve adequate passive transfer (serum IgG <10 mg/mL), with 1 and 2 calves in the CON and GAC groups, respectively. The average AEA IgG observed in this trial was 54.1%, ranging from 20.7 to 80.5%.

Although CR treatment group was not associated with passive transfer measures, other covariates were found to be associated with some of these outcomes. The serum IgG level at 24 h after CR ingestion decreased with increased birth weight and increased significantly in calves born with a calving ease score of 3 or 4 compared with calves with unassisted birth (calving score 1; Table 2). Serum total protein at 24 h after CR feeding was positively associated with STP at 0 h; calves with higher initial STP also demonstrated higher STP at 24 h (Table 2). Serum total protein at 24 h was also associated with birth weight and time between birth and CR feeding; heavier calves had lower STP, and calves with a longer interval (min) between birth and feeding had a small increase in STP at 24 h (Table 2). However, it should be noted that all calves were fed CR within 3.5 h of birth. The linear regression model evaluating the effects of treatment on AEA of IgG used robust standard errors, as homoscedasticity could not be achieved, even when using a variety of transformations. Using this model with robust standard error, AEA was not affected by the CR treatment but was associated with calving ease score: calves from a more difficult calving (scores 3 and 4) had significantly

Table 1. Characteristics of newborn calves fed 1.5 doses (150 g of IgG) of colostrum replacer by esophageal tube feeder with addition of 30 g of gut active carbohydrates (GAC; n = 119) or without GAC (control, CON; n = 121) based on univariable linear or logistic models

Model/treatment	Mean (SD, range)	β (SE)	95% CI	P-value
Model 1: Y = Time at colostrum replacer feeding, min				
Treatment				
GAC	79 (33; 30–180)	−2.133 (4.453)	−10.905 to 6.639	0.63
CON	81 (36; 25–195)	Referent	—	—
Model 2: Y = Birth weight, kg				
Treatment				
GAC	41.9 (5.5; 30.6–58.1)	0.295 (0.679)	−1.043 to 1.633	0.66
CON	41.6 (5.1; 31.5–60.3)	Referent	—	—
Model 3: Y = Calving ease score ¹				
Treatment				
GAC	1.6 (0.8; 1–4)	−0.254 (0.109)	−0.470 to −0.039	0.02
CON	1.9 (0.9; 1–4)	Referent	—	—
Model 4: Y = Serum IgG at 0 h, mg/mL				
Treatment				
GAC	0.39 (0.29; 0.2–2.0)	−0.039 (0.045)	−0.128 to 0.050	0.39
CON	0.43 (0.40; 0.2–2.4)	Referent	—	—
Model 5: Y = Serum total protein at 0 h, g/dL				
Treatment				
GAC	4.53 (0.31; 3.9–5.5)	−0.018 (0.039)	−0.095 to 0.058	0.64
CON	4.55 (0.30; 3.8–5.2)	Referent	—	—

¹Calving ease score: 1 = observed but unassisted, 2 = easy pull, 3 = moderate pull, 4 = hard pull.

higher AEA (Table 2). Calving ease was therefore offered to all regression models to ensure that it was not influencing or confounding the effects of CR treatments. Numerically, we observed no difference in AEA IgG observed in the 2 treatment groups (CON = 54.0 vs. GAC = 54.3%).

Effects of Treatment on Health and Growth

Health. The overall proportions of all heifer calves experiencing a scours event, a pneumonia event, or a death event were 30.2, 50.4, and 9.3%, respectively, from birth to 7 wk of age. The addition of GAC in CR did not affect any measure of calf health (Table 3; $P > 0.05$). The median age at diagnosis of a first pneumonia event for the heifer calves was 19 d, ranging from 3 to 48 d of age. The median age at diagnosis of a first diarrhea event was 13 d, ranging from 2 to 40 d of age. The proportion of heifers exhibiting a fecal score of 4 (severe scours) during the first 14 d of life was 18.6%. A total of 12 (9.3%) heifer calves (6 CON, 6 GAC) died in the interval between birth and 7 wk of age.

Growth. The mean weight for all heifer calves at 7 wk of age was 82.4 kg, ranging from 46.4 to 105.3 kg. The mean ADG for all heifer calves between birth and 7 wk was 0.84 kg/d, ranging from 0.3 to 1.2 kg/d. Based on the results of the multivariable regressions, neither BW at 7 wk of age nor ADG between birth and 7 wk of age was affected by CR treatment (Table 4). Not surprisingly, birth weight was positively associated with BW at 7 wk of age (Table 4).

DISCUSSION

This is the first study to evaluate the effect of supplementing CR with GAC on measures of passive transfer, health, and growth in commercial dairy calves. The use of 1.5 doses of CR, providing 150 g of IgG, resulted in high levels of passive transfer, with overall mean serum IgG and STP concentrations at 24 h averaging 20.2 mg/mL and 5.7 g/dL, respectively, but did not differ between CR treatments. Among all calves enrolled, only 1.2% had FPT, compared with an FPT rate of approximately 19.2% on US dairy operations (Beam et al., 2009). The average AEA of IgG (%) for this study, at 54.1%, was higher than in previous reports in the literature. For example, in earlier studies, Godden et al. (2009a,b) reported AEA of IgG varying from 36 to 51% when feeding the same CR replacer as was used in the current study. The high levels of passive transfer might be explained by the application of rigorous protocols for CR feeding management of newborns (although the aforementioned studies used similar enrollment and feeding protocols), and by the fact that this study was conducted during winter, whereas others were during summer when heat stress might negatively affect IgG absorption.

Interestingly, Lazarevic et al. (2010) reported serum IgG concentration at 24 h of 43 to 58 mg/mL. These serum IgG concentrations are considerably higher than those reported in the current study and higher than concentrations typically reported in other colostrum studies in dairy calves, with mean IgG concentrations often ranging between 12.1 and 20.7 mg/mL, depend-

Table 2. Effects of supplementing colostrum replacer with 30 g of gut active carbohydrates (GAC; n = 119) or without GAC (control, CON; n = 121) on passive transfer measures for newborn calves at 24 h of age

Model/treatment/level	Mean (SD, range)	β (SE)	95% CI	P-value
Model 1: Y = Serum IgG at 24 h, mg/mL				
Treatment				
GAC	20.24 (4.16; 7.4–33.5)	0.216 (0.512)	–0.793 to 1.225	0.67
CON	20.21 (3.80; 9.4–28.4)	Referent	—	—
Birth weight, kg	—	–0.266 (0.045)	–0.356 to –0.177	<0.001
Calving ease score				
4	22.50 (1.68; 20.3–26.3)	2.649 (1.300)	0.088 to 5.211	0.04
3	21.68 (3.59; 14.7–29.1)	1.805 (0.762)	0.304 to 3.306	0.02
2	19.76 (3.80; 8.4–27.9)	–0.124 (0.573)	–1.254 to 1.005	0.83
1	19.91 (4.21; 7.4–33.5)	Referent	—	—
Model 2: Y = STP ¹ at 24 h, g/dL				
Treatment				
GAC	5.68 (0.44; 4.7–7.0)	0.015 (0.049)	–0.081 to 0.111	0.76
CON	5.69 (0.41; 4.8–6.7)	Referent	—	—
STP at 0 h	—	0.511 (0.082)	0.350 to 0.672	<0.001
Birth weight, kg	—	–0.024 (0.005)	–0.033 to –0.015	<0.001
Time at feeding ²	—	0.002 (0.425)	0.000 to 0.003	0.01
Model 3: Y = AEA IgG, ³ %				
Treatment				
GAC	54.3 (10.38; 20.7–80.5)	0.862 (1.332)	–1.764 to 3.487	0.52
CON	54.0 (10.42; 22.4–78.5)	Referent	—	—
Calving ease score				
4	62.7 (4.38; 55.9–70.8)	9.786 (1.671)	6.494 to 13.079	<0.001
3	58.2 (8.62; 41.1–75.4)	5.216 (1.778)	1.713 to 8.719	0.004
2	52.7 (10.39; 20.7–80.5)	–0.401 (1.557)	–3.468 to 2.666	0.80
1	53.2 (10.65; 20.7–78.9)	Referent	—	—

¹Serum total protein (g/dL).²Time between birth and colostrum feeding (min).³Apparent efficiency of absorption of IgG at 24 h (%) with robust standard errors reported due to lack of homoscedasticity.

ing on a variety of factors including the type, quality, volume, and timing of colostrum administered (Arthington et al., 2000; Swan et al., 2007; Godden et al., 2009b). Because the assays used to measure serum IgG among the different studies were not standardized between laboratories, it is unknown how the absolute levels measured in the 2 studies would compare.

We hypothesized that the GAC additive evaluated in the current study would improve passive transfer of IgG and, subsequently, the preweaning health and growth of treated calves. Based on earlier studies, we hypothesized that this effect, if it truly existed, might be mediated through the relationship between the gut and the immune system by the effect of competition in the gut

Table 3. Effects of supplementing colostrum replacer with 30 g of gut active carbohydrates (GAC; n = 69) or without GAC (control, CON; n = 60) on health measures for heifer calves between birth and 7 wk of age

Model/treatment ¹	% (no./total)	Odds ratio (SE)	95% CI	P-value
Model 1: Y = Fecal score of 4: birth to 14 d				
Treatment				
GAC	21.7 (15/69)	1.57 (0.731)	0.633 to 3.913	0.33
CON	15.0 (9/60)	Referent	—	—
Model 2: Y = Pneumonia event: birth to 7 wk				
Treatment				
GAC	50.7 (35/69)	1.06 (0.378)	0.531 to 2.134	0.86
CON	50.0 (30/60)	Referent	—	—
Model 3: Y = Scours event: birth to 7 wk				
Treatment				
GAC	29.0 (20/69)	0.93 (0.360)	0.435 to 1.988	0.85
CON	31.7 (19/60)	Referent	—	—
Model 4: Y = Death: birth to 7 wk				
Treatment				
GAC	8.7 (6/69)	0.86 (0.520)	0.261 to 2.813	0.80
CON	10.0 (6/60)	Referent	—	—

¹No additional variables were statistically significant or acted as confounder.

Table 4. Effects of supplementing colostrum replacer with 30 g of gut active carbohydrates (GAC; n = 63) or without GAC (control, CON; n = 54) on growth measures for heifer calves between birth and 7 wk of age

Parameter	Mean (SD, range)	β (SE)	95% CI	P-value
Model 1: Y = BW at 7 wk of age, kg				
Treatment				
GAC	83.0 (9.54; 51.3–105.3)	1.205 (1.742)	–2.246 to 4.655	0.49
CON	81.8 (10.69; 46.4–105.3)	Referent	—	—
Birth weight	—	0.827 (0.192)	0.446 to 1.207	<0.001
Model 2: Y = ADG over 7 wk, ¹ kg/d				
Treatment				
GAC	0.84 (0.18; 0.3–1.2)	0.006 (0.032)	–0.058 to 0.069	0.86
CON	0.84 (0.17; 0.3–1.2)	Referent	—	—

¹No additional variables were statistically significant or acted as confounder.

between pathogenic bacteria and GAC (Spring et al., 2000); pathogenic bacteria in the gut have been found to reduce the uptake of immunoglobulin in newborn calves (James et al., 1981). Furthermore, feeding colostrum with high total bacteria counts, especially high coliform counts, has been negatively associated with IgG absorption (Godden et al., 2012). As such, the supplementation of colostrum with GAC could theoretically interfere with the attachment of pathogenic bacteria in the gut, thereby allowing for greater absorption of IgG. However, according to the results of the current experiment, the addition of GAC to a commercial CR did not improve passive transfer of IgG, health, or growth in commercial dairy calves. These findings conflict with those of Lazarevic et al. (2010), who reported higher serum IgG levels in calves fed 22.5 g of GAC mixed into 4.5 L of whole maternal colostrum (5 g/L) fed in 3 feedings at 2, 12, and 24 h after birth (Lazarevic et al., 2010). The difference in study findings could be attributed to one or more factors that differed between the Lazarevic study and the current study, including the type of colostrum fed (whole maternal colostrum vs. CR), the mass of IgG fed (unknown for Lazarevic study; 150 g for current study), the number of feedings (3 vs. 1), colostrum total bacteria or coliform bacteria concentrations (undetermined for Lazarevic study; total plate count $<1 \times 10^5$, no coliforms or *Salmonella* in the CR used in the current study), route of colostrum administration (unknown vs. esophageal tube feeder), study size (24 vs. 240 calves), and regional or seasonal differences. Moreover, the study presented here fed a slightly higher dose of GAC (30 g), averaging 0.7 g/kg of BW compared with the Lazarevic study, which fed GAC at 0.6 g/kg of BW.

Of the differences in study methods listed above that could explain the conflicting study findings, one of the most intriguing is the question of different levels of colostrum total bacteria and perhaps different colostrum coliform levels between the whole (pooled) maternal colostrum fed in the Lazarevic et al. (2010)

study compared with the commercial CR fed in the current study. Although those authors did not report bacterial culture results for the colostrum fed, it is known from observational studies of colostrum fed on US dairy farms that a high proportion of colostrum fed to calves has excessively high total bacteria concentrations, exceeding 100,000 cfu/mL (Morrill et al., 2012). Unfortunately, Lazarevic et al. (2010) did not complete bacterial culture and were unable to report levels of bacterial contamination in the colostrum. In contrast, the commercial colostrum replacer fed in the current study is licensed under the USDA Center for Veterinary Biologics (CVB). As such, its purity, potency, and efficacy are carefully regulated, tested, and guaranteed. Purity requirements, described in 9CFR 113.450(h)(2) (USDA-APHIS, 2014), allow a specified total bacteria count, but coliforms, *Salmonella*, and fungi may not be present. As such, the coliform bacteria count in the CR fed in the current study would be expected to be negligible, with only some minor contamination possible during the CR mixing and feeding processes on farm, compared with the potentially high total bacteria and total coliform counts in the pooled maternal colostrum fed in the Lazarevic et al. (2010) study. Although we cannot investigate this hypothesis with the data collected in the current study, if it is true that GAC acts positively on passive transfer of IgG by interfering with attachment of bacteria such as *E. coli* in the gut, and if *E. coli* or other bacteria interfere with IgG absorption, as has been suggested by previous studies (James et al., 1981; Godden et al., 2012), then it is possible that differences in levels of colostrum bacterial contamination, and especially coliform bacteria, could explain differences in study findings concerning the effect of GAC addition on passive transfer parameters. Specifically, when combining commercial CR and GAC, benefits of GAC addition were not found, potentially due to a low level of bacterial contamination of the CR. Conversely, if the maternal colostrum fed in the Lazarevic et al. (2010) study did have high levels of bacterial contami-

nation, then perhaps a greater opportunity existed for GAC to demonstrate a positive effect on passive transfer by interfering with bacterial colonization of the gut, if this treatment effect does truly exist. This hypothesis should be further investigated by repeating this study to evaluate the effect of supplementing colostrum with GAC when using whole maternal colostrum with higher, and more variable, concentrations of bacteria in the colostrum.

Health, growth, and survival measures were evaluated as secondary outcomes of this study. No effect of GAC addition to colostrum replacer was found for any of the health or growth parameters measured. However, given that sample sizes were not originally calculated with health outcomes in mind, readers should interpret these results with caution. The relatively small sample size of 129 heifer calves (CON = 60; GAC = 69) might not have provided sufficient power to detect modest differences in calf health. Nonetheless, we did not observe biologically important numerical differences between treatment groups for any of the health outcomes evaluated. These results are in contrast to what was originally hypothesized: if GAC enhanced serum IgG concentrations or reduced colonization of the gut by pathogens such as *E. coli* or *Salmonella* spp., then addition of GAC to the CR would be expected to reduce calfhood disease events, and particularly enteric disease, as well as mortality. One limitation of the current study was that diagnostic testing was not performed to describe the etiology of the scours observed in study calves. In future, researchers investigating the supplementation of colostrum with GAC might consider conducting fecal analysis to document the effect of treatment on the incidence of scours caused by specific pathogens (e.g., *E. coli* or *Salmonella* spp.) and the level of fecal shedding of various enteric pathogens.

CONCLUSIONS

The addition of 30 g of GAC to 1.5 doses (150 g of IgG) of colostrum replacer, fed by esophageal tube within 3.5 h of birth, did not affect passive transfer of IgG or health and growth measures between birth and 7 wk of age in Holstein dairy calves reared on one commercial US dairy farm. Further studies are warranted to investigate if the use of GAC in whole maternal colostrum would affect passive transfer, health, and performance of neonatal calves.

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