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A Randomized Clinical Trial Evaluating the Effects of Oligosaccharides on Transfer of Passive Immunity in Neonatal Dairy Calves

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Background: Bacterial contamination of colostrum is common and can decrease IgG absorption in neonatal calves. Strategies that mitigate this situation without complicating colostrum management will benefit dairy calf health and survival.

Objectives: To evaluate the effects of supplementing colostrum with oligosaccharides (OS) on serum IgG concentration and apparent efficiency of absorption of IgG (AEA%) in calves fed unpasteurized colostrum and characterize these outcomes with respect to colostrum bacterial exposures.

Animals: One hundred twenty-three neonatal dairy calves.

Methods: Randomized, blinded, controlled clinical trial conducted at a commercial dairy operation. Calves were enrolled at birth in 1 of 4 treatment groups. Data were complete for 123 calves, which were distributed across the treatment groups as follows: mannan-oligosaccharides (MOS), n = 33; *Saccharomyces* galacto-oligosaccharides (SGOS), n = 31; *Bifidobacterium* galacto-oligosaccharides (BGOS), n = 28; and lactose control (CON), n = 31. A commercial radial immunodiffusion kit was used to determine colostrum and serum IgG concentrations. Conventional microbiology methods were used to enumerate colostrum bacterial counts.

Results: Bacterial counts were not significantly different among treatment groups. Total bacterial plate counts (TPC) were relatively low for the majority of colostrum samples, but TPC had a significant negative effect on serum IgG concentration and AEA% in the lactose-supplemented control group but not the OS treatment groups.

Conclusions and Clinical Importance: These results suggest that a complement of OS structures may mitigate adverse effects of bacteria on transfer of passive immunity (TPI).

Key words: Apparent efficiency of absorption; Bovine; Colostrum; IgG; Oligosaccharide.

Dairy calves that fail to absorb sufficient IgG from colostrum are at increased risk of morbidity and mortality during the neonatal period.[1,2] Bacterial contamination of colostrum, a common colostrum quality problem, can decrease absorption of colostral IgG.[3–5] One proposed mechanism for this effect is lactogenic immunity in which colostral IgG binds to pathogens in colostrum and effectively decreases IgG mass available for absorption by the calf intestine. Bacteria also may interfere at IgG absorption sites in the gut, also effectively decreasing IgG absorption.[6–8] Supporting these findings, studies also show that decreasing live bacterial counts by pasteurization improves IgG absorption from colostrum.[4,5,9]

Colostrum management is labor-intensive, and lapses in hygiene at any stage of a colostrum program can result in significant bacterial contamination.[10,11] Supplementing colostrum with carbohydrate molecules called oligosaccharides (OS) that are capable of binding bacteria may mitigate the adverse effects of bacterial contamination on IgG absorption. Studies investigating the effects of OS supplementation on transfer of passive immunity (TPI) have focused on 1 type of OS and have produced conflicting results. In 1 study, the inclusion of mannan-oligosaccharides (MOS) in colostrum fed to dairy calves was associated with higher serum IgG concentrations compared with control calves at 24 hours of age.[12] However, colostrum IgG concentrations, AEA% of IgG, and bacterial counts were not reported, making it difficult to meaningfully assess the relationship of OS

Abbreviations:

AEA%	percent apparent efficiency of absorption (of IgG)
AER	abomasal emptying rate
B-C time	time between birth and colostrum administration
BGOS	*Bifidobacterium* galacto-oligosaccharides
CFU	colony-forming unit
CON	control group
C-V time	time between colostrum administration and venipuncture for serum IgG
IgG	immunoglobulin G
MOS	mannan-oligosaccharides
OS	oligosaccharides
PV	plasma volume
SGOS	*Saccharomyces* galacto-oligosaccharides
TCC	total coliform count
TEC	total *E. coli* count
TPC	total plate count
TPI	transfer of passive immunity

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Field work for this study was completed on a commercial dairy farm in the state of Washington. Laboratory work was completed at Washington State University, College of Veterinary Medicine, Pullman, WA 99164-6610.

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supplementation to TPI results in this study. Another study failed to document any effect of MOS on AEA% of IgG when administered in a colostrum replacer.[13] Bacterial counts were not evaluated, but colostrum replacer regulations require very low bacterial counts, and the opportunity for MOS to exert a positive effect on IgG absorption by mitigating bacterial contamination was probably low. Finally, another study showed a significant decrease in AEA% of IgG and serum IgG concentrations in calves fed raw colostrum supplemented with MOS.[14]

The objective of our study was to evaluate the effects of supplementing nonpasteurized colostrum with 3 structurally different but commercially available OS on TPI in neonatal dairy calves and determine if OS supplementation mitigates the negative impact of bacterial contamination on TPI. We hypothesized that calves supplemented with OS would have higher serum IgG concentrations and AEA% of IgG compared with calves in the control group.

Materials and Methods

Study Design and Farm Management

This study was a blinded, randomized, controlled trial that was performed between the months of July and September on a commercial dairy farm in the US Pacific Northwest. Consent for this study was obtained from the farm owner, and the Institutional Animal Care and Use Committee approved use of the animals. The primary outcomes of the study were serum IgG concentration and AEA% in dairy calves approximately 24 hours after colostrum administration. The farm reared Holstein, Jersey, and Holstein-Jersey crossbred heifer calves. Cows and heifers calved in a joint dry lot maternity pen that was monitored 24 hours per day. Calving scores were recorded by farm personnel according to the farm's protocol as follows: 1 = no assistance, 2 = easy pull with or without mild repositioning, 3 = moderately difficult repositioning and pull, and 4 = severe dystocia requiring veterinary assistance. Calves generally were removed from the pen within an hour of birth and weighed with a mechanical beam scale.[a] Colostrum was obtained from cows with a bucket milker at regular, twice daily milking intervals, poured into 3.79 L jugs, chilled in a −20°C freezer, and then stored in a 4°C refrigerator for ≤24 hours before administering to calves. Although calves may have received colostrum pooled from >1 cow, colostrum from heifers generally was avoided. Colostrum was warmed in a hot water bath and administered with an esophageal feeder. Calves received 3.79 L of colostrum unless farm personnel determined a calf to be too small for this volume to be administered safely, in which case 2.84 L was administered.

Sample Size and Enrollment

Sample size calculations for F tests a priori by a free-source software package[b] indicated 24 calves per treatment group were required to meet a type I error = 0.05, a type II error = 0.20 and relative 20% difference in mean AEA% between control and OS-supplemented calves. Enrollment with random assignment to OS treatment groups was continued until at least 30 heifer calves were assigned to each treatment group to account for loss to follow-up because of neonatal deaths before 24-hour blood sampling, missing data, and potential sample damage during transport and storage. All newborn heifer calves were enrolled into 1 of 4 treatment groups: mannan-OS (MOS),[c] *Saccharomyces* galacto-OS (SGOS),[d] *Bifidobacterium* galacto-OS (BGOS),[e] or lactose control (CON).[f] Simple randomization of calves to treatment groups was achieved with the following 3 steps: (1) before the onset of the clinical trial, OS powders (MOS, SGOS, and BGOS) and lactose powder were weighed out into 10 g treatment doses, packaged into sterile Whirl-Pak[g] bags, and assigned an enrollment number with a random number generator,[h] (2) treatment packages were arranged sequentially by enrollment number, and (3) calves were assigned a treatment sequentially according to birth order. Colostrum administration to newborn calves proceeded as follows: (1) a 3.79 L jug of colostrum was removed from the refrigerator and placed in a hot water bath; (2) once warm, the colostrum was aseptically sampled for analysis; (3) immediately after sampling, the contents of the treatment package assigned to the calf were poured into the colostrum jug and mixed by inverting the jug 3 times; and, (4) once sampled and treated, the colostrum was immediately administered to the calf with an esophageal feeder. A color code was assigned to each treatment type to blind farm and study personnel to the treatment assignments. Lactose was administered to the control group to ensure farm and study personnel remained blinded to the treatments and so that the colostrum handling and administration processes remained the same for all calves. Lactose was chosen because it is a normal constituent of colostrum and is not known to possess bacterial binding properties documented for OS.[15–18]

Colostrum and Serum Samples

Colostrum samples were obtained from each jug of colostrum after it had been warmed in a hot water bath, before feeding and before the addition of OS or lactose powders. Samples were collected directly from the 3.79 L jug into 3 sterile tubes. These samples were frozen on the farm at −20°C, transported on dry ice, and stored at −80°C until analyzed. To obtain peak serum IgG concentration, whole blood was collected from calves between 24 and 36 hours of age by jugular venipuncture.[19,20] Blood samples were allowed to clot at room temperature and then centrifuged. Serum was harvested, divided into multiple aliquots and stored at −20°C, and then transferred and stored at −80°C until analyzed for IgG concentration.

Microbiology

Colostrum was thawed at room temperature and samples were repeatedly inverted until well mixed. Samples were diluted with a minimum of 4 serial 10-fold dilutions with 0.9% physiologic saline. One milliliter of each serial dilution was applied to petri-film[i] for total bacterial plate counts (TPC) and total coliform counts (TCC). To enumerate total *E. coli* counts (TEC), 100 μL of each serial dilution was bead-plated onto MacConkey[j] agar. Petri-film and MacConkey plates were incubated at 37°C for 24 hours. Colonies on petri-film and lactose-positive (ie, pink) colonies on MacConkey agar were counted and multiplied by the appropriate 10-fold dilution factor to determine the number of bacterial colony-forming units per milliliter (CFU/mL). The CFUs from serial dilutions were averaged and $\log_{10}$ transformed.

Radial Immunodiffusion and AEA

A commercial radial immunodiffusion (RID) kit[k] was used to quantify IgG concentrations of colostrum and serum. Colostrum samples were thawed at room temperature and repeatedly inverted to mix thoroughly before 1.0 mL was removed and diluted 1 : 4 with deionized, distilled water. A 5.0 μL aliquot of each diluted colostrum sample was transferred into wells of the

immunodiffusion plate. Serum samples also were thawed at room temperature and repeatedly inverted to mix thoroughly before 5-μL aliquots of serum were removed and transferred into the wells of the immunodiffusion plate. All colostrum and serum samples were run in duplicate. Three IgG reference standards provided with the RID kit were run on each plate to generate standard curves. Concentrations of reference standards were 196 mg/dL, 1402 mg/dL, and 2748 mg/dL. Any colostrum sample with a precipitin diameter greater than the highest reference standard was diluted 1 : 5 with deionized, distilled water and rerun in duplicate. Any serum sample with a precipitin diameter greater than the reference standard was diluted 1 : 2 with sterile 0.9% physiologic saline and rerun in duplicate. Plates were allowed to incubate for 24 hours at room temperature (approximately 22°C) before precipitin diameters were measured. Precipitin diameters for each sample were measured in 3 different locations around the precipitin circumference with digital calipers calibrated to 0.01 mm. These measurements were averaged, and IgG concentrations were calculated using the standard curve equation generated for each immunodiffusion plate and multiplied by the appropriate dilution factor. The AEA% was calculated using the measured serum IgG concentrations in grams per liter, estimated plasma volume (PV) in liters, and IgG intake in grams according to the following equations: AEA = ((Serum [IgG]) × PV)/(IgG intake), where PV: 0.089 × birth weight (kg) and intake of IgG was calculated by multiplying the colostrum IgG concentration (g/L) by the volume of colostrum administered (L).[21,22]

Statistical Analysis

All statistical analyses were performed in SAS[l] and statistical significance was inferred when $P < .05$. Descriptive statistics, expressed as means and standard deviations, were generated for calf birth weight (kg), time between birth and colostrum administration (B-C time), time between colostrum administration and venipuncture for whole blood sampling (C-V time), serum IgG concentration (mg/mL), colostrum IgG concentration (g/L), mass of colostral IgG fed (g), AEA%, and colostrum microbiology variables (TPC $\log_{10}$ CFU/mL, TCC $\log_{10}$ CFU/mL, TEC $\log_{10}$ CFU/mL). Distributions of these variables were examined for deviations from a Gaussian distribution with the Shapiro-Wilk test. Parametric and nonparametric one-way analysis of variance tests for unbalanced data were used to evaluate the distribution of independent variables among treatment groups. Fisher's exact test was used to evaluate treatment groups for differences in calf breed and volume of colostrum fed (3.79 L versus 2.84 L). Linear models (PROC MIXED) were used to describe the relationship between treatment group (independent variable) and dependent variables (serum IgG concentration and AEA%). The relationship of the following independent variables—colostral IgG mass fed (standardizing total IgG consumed by a calf), birth weight, B-C time, C-V time, calving score, and $\log_{10}$ transformed colostrum bacterial counts (TPC, TCC, and TEC)—to the dependent variables, serum IgG concentration and AEA% by treatment group, was assessed with analysis of covariance. These models were initially constructed by PROC HPREG and the "forward-swap" procedure to identify significant predictors and overall best-fit models for serum IgG concentration and AEA%. All significant independent variables identified in this model were then forced into final models by treatment group by PROC GLM. Because the mode of action for mitigating the effect of TPC on serum IgG and AEA% was different among the tested OS and the lactose control, we modeled each treatment group separately to control the interaction between treatment group and TPC. Statistical diagnostics for model fit and influential data points between independent and dependent variables included plots for standardized residuals, Cook's D parameter, and leverage.

Results

Data were complete for 123 heifer calves, which were distributed across treatment groups as follows: MOS (n = 33), SGOS (n = 31), BGOS (n = 28), and CON (n = 31). Descriptive statistics expressed as means and standard deviations for independent and dependent variables for the study population and stratified by treatment group are presented in Table 1. No statistically significant differences were detected among treatment groups for any of these variables. Most of the calvings, 118 of 123 (96%), were unassisted (score 1/4). Four of 123 (3%) calvings required minor assistance (score 2/4), and only 1 calf (1%) experienced severe dystocia (score 4/4). Calves were generally removed from the calving pen within 1 hour of parturition, and most of the calves (104/123, 85%) received colostrum within 2 hours of birth. Treatment groups were not statistically significantly different with respect to the distribution of dairy calf breeds ($P = .4336$) or volume of colostrum fed ($P = .667$). The number of Holstein, Jersey, and Holstein-Jersey cross calves across the treatment groups was as follows: MOS (n = 29, 3, 1), SGOS (n = 24, 6, 1), BGOS (n = 23, 5, 0), and CON (n = 29, 2, 0). The

Table 1. Means and standard deviations describing calves and data associated with colostrum intake and assessments stratified by treatment group from a randomized clinical trial evaluating the effects of oligosaccharide supplementation on serum IgG concentration and apparent efficiency of IgG absorption in neonatal dairy calves.

Treatment Group	Birth Weight (kg) Mean (SD)	B-C Time (hours) Mean (SD)	C-V Time (hours) Mean (SD)	Serum IgG (mg/mL) Mean (SD)	AEA% Mean (SD)	N
MOS	36.0 (6.0)	1.55 (0.67)	27.02 (4.28)	23.92 (8.26)	29.6 (8.7)	33
SGOS	35.3 (3.8)	1.30 (0.63)	25.98 (2.78)	24.61 (8.57)	29.5 (9.8)	31
BGOS	35.8 (6.5)	1.37 (0.52)	27.65 (3.63)	23.36 (9.85)	25.4 (8.6)	28
CON	35.8 (5.5)	1.27 (0.69)	26.48 (2.37)	25.69 (10.60)	29.9 (10.3)	31
All calves	35.7 (5.4)	1.38 (0.64)	26.77 (3.37)	24.41 (9.26)	28.7 (9.4)	123
P	.41	.3	.17	.69	.26	

B-C time = time between birth and colostrum administration; C-V time = time between colostrum administration and venipuncture for serum IgG concentration; AEA% = apparent efficiency of absorption of IgG; MOS = mannan-oligosaccharides, SGOS = *Saccharomyces* galacto-oligosaccharides; BGOS = *Bifidobacterium* galacto-oligosaccharides, and CON = lactose control, P = P-value for statistical significance for differences among treatment groups.

Table 2. Descriptive statistics for colostrum IgG quantity and microbiological quality stratified by treatment group from a randomized clinical trial evaluating the effects of oligosaccharide supplementation on serum IgG concentration and apparent efficiency of IgG absorption in neonatal dairy calves.

Treatment Group	TPC $\log_{10}$ CFU/mL Mean (SD)	TCC $\log_{10}$ CFU/mL Mean (SD)	TEC $\log_{10}$ CFU/mL Mean (SD)	Colostrum IgG (g/L) Mean (SD)	Colostrum IgG Mass (g) Mean (SD)	N
MOS	4.6 (1.2)	2.9 (1.7)	3.3 (2.1)	72.09 (19.86)	264.91 (76.25)	33
SGOS	4.6 (1.1)	2.6 (1.8)	3.0 (2.2)	72.74 (21.58)	268.96 (74.96)	31
BGOS	4.7 (1.1)	3.8 (1.5)	3.0 (1.9)	80.05 (20.84)	294.04 (78.84)	28
CON	4.5 (0.70)	3.1 (1.3)	3.0 (1.9)	74.93 (16.69)	270.63 (61.48)	31
All samples	4.6 (1.1)	3.1 (1.6)	3.1 (2.0)	74.78 (19.80)	274.00 (73.05)	123
P	.75	.27	.86	.41	.42	

MOS = Mannan-oligosaccharides; SGOS = *Saccharomyces* galacto-oligosaccharides; BGOS = *Bifidobacterium* galacto-oligosaccharides, and CON = lactose control; TPC = total plate count; TCC = total coliform count; TEC = total *E. coli* count; *P* = *P*-value for statistical significance for differences among treatment groups.

number of calves which received 3.79 L and 2.84 L volumes of colostrum across the treatment groups was as follows: MOS (n = 29 and 4), SGOS (n = 29 and 2), BGOS (n = 25 and 3), and CON (n = 26 and 5).

Colostrum Microbiology

Descriptive statistics expressed as means and standard deviations for $\log_{10}$ CFU/mL are summarized in Table 2. Mean $\log_{10}$ CFU/mL and standard deviations for TPC, TCC, and TEC for the study were 4.6 (SD, 1.1), 3.1 (SD, 1.6), and 3.1 (SD, 2.0), respectively. The mean values for these variables stratified by treatment group were similar to overall means and not statistically different. Total plate counts ranged from 27.8 CFU/mL to 65.3 million CFU/mL. More than half (71/123) of the samples had a TPC <40,000 CFU/mL and nearly three-fourths (91/123) of the samples had a TPC lower than the recommended level of 100,000 CFU/mL.[23] Nearly half (58/123) of the samples had total coliform counts less than the recommended level of 10,000 CFU/mL.[23] The majority (94/123) of samples had very low *E. coli* counts with ECCs less than the recommended level of 1000 CFU/mL for more than half (69/123) of the samples and no growth for one-fifth (25/123) of the samples.[23]

Colostrum IgG, Serum IgG, and AEA%

The mean coefficient of determination (R^2) for IgG precipitin standard curves generated for each RID plate was 0.98 indicating excellent fit of the data to a straight line. Coefficients of variation for the precipitin diameters for each of the 3 reference standards were 2.18, 1.80, and 3.01% (n = 29) indicating that plate running conditions and performance of reference standards were consistent among RID plates. Mean colostrum IgG concentration was 74.78 g/L (SD, 19.80 g/L; Table 2) and ranged from 29.88 g/L to 128.68 g/L. Mean colostrum IgG mass fed was 274.00 g (SD, 73.05 g; Table 2) and ranged from 84.83 g to 487.04 g. The majority of colostrum administered in this study was of high quality with 116/123 (94%) of colostrum samples exceeding IgG concentrations of 50 g/L. Mean serum IgG concentration was 24.41 mg/mL (SD, 9.26 mg/mL; Table 1) and ranged from 4.43 mg/mL to 49.09 mg/mL. The rate of failure of transfer of passive immunity using an IgG concentration <1,000 mg/dL was very low at 2.4% (3/123). Mean AEA% was 28.7% (SD, 9.4%; Table 1) and ranged from 4.9 to 58.6%. The mean values for these variables stratified by treatment group were similar to the overall means and not significantly different (Tables 1 and 2).

Results of models for each treatment group, which control the interaction between treatment group and TPC, are presented in Tables 3 and 4. The TPC was statistically significant only for the CON group for which the model indicated that every $\log_{10}$ CFU increase in TPC produced a 5.9 mg/mL decrease in serum IgG concentration ($P = .03$) and 6.9 percentage point decrease in AEA% ($P = .02$). For all the treatment groups, a positive effect of increasing mass of colostral IgG fed on serum IgG concentration was significant, but extremely small (<1%). A small but negative effect of increasing mass of colostral IgG fed on AEA% was observed for OS groups but not for the control group. Increasing birth weight had a significant but small effect on IgG concentration for all treatment groups except for SGOS. Birth weight had no significant effect on AEA% in any of the treatment groups.

Discussion

Although there was no significant difference in mean serum IgG concentration or AEA% among the control and OS-supplemented groups, a substantial and significant negative effect of TPC on serum IgG concentration and AEA% was detected for the control group, but not the OS-supplemented groups. Feeding raw colostrum with TPCs as low as 40,000 CFU/mL has been associated with lower serum IgG concentrations in neonatal dairy calves.[4] Approximately 50% of the samples in this study however had TPC counts <40,000 CFU/mL, suggesting that bacterial contamination <40,000 CFU/mL also may have adverse effects. These results indicate that the adverse effects of bacterial contamination on absorption of colostral IgG can be mitigated by

Table 3. Summary of multivariate linear models evaluating the relationship of independent variables to serum IgG concentration (mg/mL) stratified by treatment group from a randomized clinical trial evaluating the effects of oligosaccharide supplementation on serum IgG concentration and apparent efficiency of IgG absorption (AEA%) in neonatal dairy calves.

Treatment Group	Parameters	Estimates	Standard Error	*P*	R^2
MOS	Intercept	31.7	12.5	.0167	0.3
	Colostrum IgG Mass (g)	0.04	0.02	.02	
	Total Plate Count (log_{10} CFU/mL)	−0.4	1.1	.8	
	Birth Weight (kg)	−0.2	0.1	.03	
SGOS	Intercept	33.2	14.6	.03	0.4
	Colostrum IgG Mass (g)	0.06	0.02	.002	
	Total Plate Count (log_{10} CFU/mL)	−1.4	1.2	.2	
	Birth Weight (kg)	−0.2	0.2	.1	
BGOS	Intercept	54.3	12.8	.003	0.3
	Colostrum IgG Mass (g)	0.02	0.02	.4	
	Total Plate Count (log_{10} CFU/mL)	−2.0	1.5	.2	
	Birth Weight (kg)	−0.3	0.1	.008	
CON	Intercept	70.8	19.2	.001	0.4
	Colostrum IgG Mass (g)	0.08	0.03	.008	
	Total Plate Count (log_{10} CFU/mL)	−5.9	2.6	.03	
	Birth Weight (kg)	−0.5	0.1	.002	

MOS = Mannan-oligosaccharides; SGOS = *Saccharomyces* galacto-oligosaccharides; BGOS = *Bifidobacterium* galacto-oligosaccharides, and CON = lactose control. Parameter estimates, variance, and *P*-values for statistical significance are shown for colostrum IgG mass = amount of colostrum fed in grams, total plate count = total bacterial plate count obtained on colostrum log_{10} CFU/mL, and birth weight in kg. *P* = *P*-value for statistical significance of the parameter in the model. R^2 = proportion of variance explained by the model.

Table 4. Summary of multivariate linear models evaluating the relationship of independent variables to apparent efficiency of IgG absorption (AEA %) stratified by treatment group from a randomized clinical trial evaluating the effects of oligosaccharide supplementation on serum IgG concentration and AEA% in neonatal dairy calves.

Treatment Group	Parameters	Estimates	Standard Error	*P*	R^2
MOS	Intercept	31.0	13.2	.03	0.3
	Colostrum IgG Mass (g)	−0.06	0.02	.007	
	Total Plate Count (log_{10} CFU/mL)	0.4	1.2	.8	
	Birth Weight (kg)	0.1	0.1	.2	
SGOS	Intercept	36.2	19.5	.07	0.2
	Colostrum IgG Mass (g)	−0.05	0.02	.03	
	Total Plate Count (log_{10} CFU/mL)	−1.6	1.6	.3	
	Birth Weight (kg)	0.2	0.2	.4	
BGOS	Intercept	54.1	11.4	<.0001	0.3
	Colostrum IgG Mass (g)	−0.05	0.02	.008	
	Total Plate Count (log_{10} CFU/mL)	−1.9	1.3	.2	
	Birth Weight (kg)	−0.05	0.1	.6	
CON	Intercept	81.2	21.5	.0008	0.2
	Colostrum IgG Mass (g)	−0.01	0.03	.7	
	Total Plate Count (log_{10} CFU/mL)	−6.9	2.9	.02	
	Birth Weight (kg)	−0.2	0.2	.2	

MOS = Mannan-oligosaccharides, SGOS = *Saccharomyces* galacto-oligosaccharides, BGOS = *Bifidobacterium* galacto-oligosaccharides, and CON = lactose control. Parameter estimates, variance, and *P*-values for statistical significance are shown for colostrum IgG mass = amount of colostrum fed in grams, total plate count = total bacterial plate count obtained on colostrum log_{10} CFU/mL, and birth weight in kg. *P* = *P*-value for statistical significance of the parameter in the model. R^2 = proportion of variance explained by the model.

supplementing colostrum with mannan-OS (MOS) or galacto-OS (SGOS and BGOS). Generally, across all treatment groups colostral IgG mass fed affected both serum IgG concentration (except for BGOS) and AEA% (except for CON). Birth weight also affected serum IgG concentration (except for SGOS) but did not affect AEA%. Despite statistical significance, the effects of these predictors on these outcomes for transfer of passive immunity were minor. Colostral IgG mass fed was positively associated with serum IgG concentration, but negatively associated with AEA%, which is consistent with a potential saturation effect previously suggested for higher colostrum IgG concentrations.[24] Birth weight, when significant, was negatively associated with serum IgG concentration, consistent with previous work suggesting a

potential dilutional effect of higher plasma volumes in larger calves.[5]

The use of lactose in the CON group could have affected the results of this study, most plausibly by altering colostrum bacterial populations, nutrient density, or colostrum osmolality. To avoid introducing changes in colostrum bacterial populations, lactose and the OS powder treatments used in this study were added to colostrum immediately before feeding colostrum to prevent bacteria from utilizing metabolizable supplements for bacterial replication and growth. As for nutrient density and osmolality, increases in both have been shown to decrease abomasal emptying rate (AER), and decreased AER has been shown to decrease AEA% of colostral IgG in neonatal dairy calves.[25–27] However, we contend that the 10 g dose of lactose used in this study would be insufficient to induce clinically relevant changes in either of these colostrum attributes. Regarding nutrient density, 10 g of additional lactose represents $1/10^{th}$ of the total dose of lactose a calf would have received in colostrum with an average lactose concentration of 26 g/L. Given that the variation in lactose concentration of colostrum can range from 26 to 44 g/L, and considering lactose is just one of the several components that contribute to the nutrient density of colostrum, it seems unlikely that this small dose of lactose would introduce substantial differences in nutrient density.[15–18,25] The effects of colostrum osmolality on AER or AEA% have not been investigated directly, but evidence for a colostrum replacer indicates that hyperosmolar concentrations of 560 mOsm/L can significantly decrease AEA% of IgG.[28] This effect is presumably because of decreased AER and therefore delayed delivery of IgG to the small intestine where it is absorbed. This deduction is supported by 2 lines of evidence. First, it is well established that oral-gastric administration of hyperosmolar fluids to dairy calves decreases AER; and, second, the effects of prokinetic drug-induced changes in AER in dairy calves fed colostrum indicate that decreased AER decreases AEA% of IgG.[26,27,29,30] On the basis of mathematical estimation of the osmolality of pure lactose, a 10-g dose would be expected to contribute 29 mOsm to colostrum osmolality. Thus, we anticipated that this dose would increase osmolality by 8–10 mOsm/L of colostrum for calves fed 3.79 and 2.84 L of colostrum, respectively. Finally, osmolality measurements made with a commercial osmometer[m] confirmed that a 10-g dose of the lactose formulation used in this study would have contributed 32 mOsm to the overall colostrum osmolality and that 10 g doses of MOS, SGOS, and BGOS would have contributed 22, 33, and 34 mOsm, respectively. Consequently, if this small increase in osmolality had any effect on AEA% in this study, it would have been similar for all 4 treatment groups.

The mitigating effect of OS identified in this study presumably is caused by OS binding to bacteria in colostrum. Oligosaccharide structure is a strong determinant of biological function, including binding affinity for various types of bacteria. Several studies have shown that mannan-based OS are capable of binding to a variety of bacteria, including bacteria expressing type 1 fimbriae, and respiratory and enteric pathogens.[31,32] *Saccharomyces* galacto-oligosaccharides, which are enzymatically produced by S*accharomyces* have also been shown to bind to enteropathogenic *E. coli* and prevent the adherence of these bacteria to gut epithelial cells in vitro.[33] *Bifidobacterium* galacto-oligosaccharides, the second commercially available GOS formulation used in this study, are enzymatically produced by *Bifidobacterium* and are more complex, consisting of 3 OS molecules as opposed to a single OS molecule as found in MOS and SGOS.[34] Similar to SGOS, BGOS has been shown to be a prebiotic OS and thus is capable of promoting growth of beneficial gut bacteria.[34] To our knowledge, however, the bacterial binding capacity of the OS molecules in the BGOS formulation has not been reported. Lack of a substantial and significant negative effect of TPC in the BGOS group suggests that it was capable of exerting a mitigating effect. However, TPC parameter estimates for BGOS models were larger than for SGOS and least for MOS suggesting diminished mitigation in the GOS groups relative to the MOS group.

Colostrum and milk from cattle and other species naturally contain a variety of OS, but the biological functions of these have yet to be completely determined.[17,35–41] Some milk OS have been shown to bind to bacterial exotoxins and pathogens including *Campylobacter jejuni*, pathogenic *E. coli*, and rotavirus.[39,42–46] Oligosaccharides from bovine milk have been shown to have strong affinity for several enteropathogenic strains of *Escherichia coli* isolated from dairy calves.[43] These findings have led some to hypothesize that mammary-secreted OS are a component of lactogenic immunity and function to help protect the neonate from pathogens and bacterial translocation while the gut is open. If so, OS potentially could decrease the role that immunoglobulins play in lactogenic immunity, thereby maximizing the amount of colostral IgG available for absorption. The concentration of OS found in colostrum can be up to 10-fold higher than in milk, which would seem logical if OS were predominantly present to protect the neonate while the gut was open.[17,35,37,40] The concentration of OS in colostrum and milk from dairy cattle has been shown to be relatively low compared with that of other species and therefore this role may be easily overwhelmed when calves are born into and housed in contaminated environments.[41,47,48]

The 10 g dose of OS used in this study was chosen because it approximated mean doses of OS naturally secreted in human milk and colostrum, which is 10 times that of colostrum from Holstein and Jersey dairy cattle.[41,47,48] In another study, supplementation with 30 g of MOS administered in raw colostrum produced decreases in serum IgG concentration and AEA% when calves were fed raw colostrum.[14] This larger dose of MOS may have decreased IgG absorption by physically blocking IgG absorption sites. Other mechanisms not yet elucidated also may have been responsible for the negative effects. This amount of MOS would seem insufficient to increase the osmolality of colostrum to a

hyperosmolar concentration sufficient to affect AER and subsequently AEA%. Doses of 10 g, as administered in our study, may be optimal for counteracting the adverse effects of bacteria without impeding IgG absorption.

Naturally-derived bovine OS secreted by the mammary gland and with strong affinity for bacteria and pathogens present in the environments in which calves are born would likely be the ideal candidates for binding bacteria in colostrum and preventing exposures to pathogens while the gut is open. Unfortunately, commercial sources of bovine lactogenic OS are produced in relatively small quantities, primarily marketed for bench-top research, and are cost-prohibitive for supplementation trials. However, natural bovine milk OS currently are being extracted from whey processing streams.[49] Therefore, wider implementation of these processing methods may offer upscaled production of bioactive bovine milk OS in the near future.

Most colostrum fed to calves in our study was of a high quality, exceeding 50 g/L of IgG, and was likely an important factor contributing to the very low rate of failure of transfer of passive immunity during the study. Other factors that likely contributed to the success of the colostrum program on this farm included management practices such as rapid removal of calves from the calving pen, prompt administration of colostrum to newborns, and protocols aimed at preventing bacterial contamination and bacterial growth in colostrum, which included good hygiene during colostrum collection, storage, and feeding; prompt chilling; and, relatively short colostrum storage times.

Other strategies shown to effectively decrease bacterial counts in colostrum include pasteurization and the addition of preservatives. Lower-temperature, longer-duration batch pasteurization has been shown to be effective at decreasing bacterial counts while preserving colostrum IgG.[4] However, pasteurization requires considerable financial investment and human resources to be effectively implemented. Preservatives such as potassium sorbate have been shown to decrease colostrum bacterial counts and effectively prevent bacterial growth for 96 hours when used in conjunction with refrigeration.[11] Several other chemical additives including sodium benzoate, sorbitol, propionic acid, and formaldehyde also have been used to effectively preserve colostrum.[11,18] Some of these additives, such as formaldehyde, are dangerous to handle, and unlike pasteurization, preservatives cannot substantially decrease a bacterial load once it is introduced.[18] Supplementing colostrum with OS to mitigate bacterial contamination may offer some unique benefits. Unlike pasteurization, it does not require any capital investment or management commitment to implement. It is easy to do and can be carried out immediately before colostrum feeding. This approach could have obvious benefit because bacterial contamination and growth can occur at any point up to the time of colostrum administration. It also offers a logistic advantage because OS could be used as an adjunct to other control measures without disrupting the existing colostrum program. Finally, OS have been shown to possess other beneficial bioactive properties such as immunomodulation and prebiotic effects that may result in other beneficial effects in the neonatal calf gut.[31]

Although colostrum management on our study farm was excellent, we were able to demonstrate a positive role of OS supplementation to improve IgG uptake from colostrum. Additional research is warranted to understand if the OS effect on AEA% may change with higher bacterial loads. Research to clarify optimal OS inclusion rates and to clarify effects of inclusion rates on colostrum osmolality, AER, and AEA% are also indicated. The mechanism by which OS had a positive effect in our study is unknown. In addition to the binding bacteria, both MOS and GOS have been shown to possess many bioactive effects, which may have consequences for IgG absorption across the open gut in neonatal calves. Additional research to clarify the mechanism by which bacteria affect IgG absorption from colostrum and to describe the interactions that various commercial and natural bovine milk OS have with bacteria as well as the bioactive roles of these in the neonatal gut will help determine the actual benefits of supplementing colostrum with OS.

Footnotes

[a] Paul Scale, Duncan, OK
[b] GPower 3.1, Heinrich Heine University Düsseldorf (HHU), Düsseldorf, Germany. © Franz Faul, Edgar Erdfelder, Albert-Georg Lang, and Axel Buchner, 2006, 2009
[c] Bio-Mos, Alltech Inc., Nicholasville, KY
[d] Oligomate 55NP, Yakult, Japan & Kanematsu USA Inc., Somerset, NJ
[e] Bimmuno, Clasado Limited, Wolverton Mill, Milton Keynes, MK, UK
[f] Lactose, Brewcraft USA, Vancouver, WA.
[g] Whirl-Pak® sterile sample collection bag, Nasco Inc., Modesto, CA
[h] Microsoft® Office Excel 2011 Mac 14.4.8, Microsoft, Redmond, WA
[i] 3M™ Petri-film™, Forest City, IA
[j] MacConkey agar, Fisher Scientific, Pittsburgh, PA
[k] Radial Immunodiffusion bovine IgG, Triple J Farms, Bellingham, WA
[l] SAS9.3©, SAS Institute Inc., Cary, NC
[m] Advanced® Model 3320 Micro-Osmometer, Advanced Instruments Inc., Norwood, MA

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Conflict of Interest Declaration The authors declare no conflict of interest.

Off-label Antimicrobial Declaration The authors declare no off-label use of antimicrobials.

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