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Alteration of digestive tract microbiome in neonatal Holstein bull calves by bacitracin methylene disalicylate treatment and scours[1]

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ABSTRACT: The effects of bacitracin methylene disalicylate (BMD) and scours on the fecal microbiome, animal performance, and health were studied in Holstein bull calves. Holstein bull calves ($n = 150$) were obtained from a single source at 12 to 24 h of age. Bull calves were randomly assigned to 1 of 2 treatments including CON (no BMD; $n = 75$ calves) and BMD ($n = 75$ calves). Starting 3 d after arrival, BMD was added into milk replacer (0.5 g/feeding; twice daily) and fed to the calves for 10 consecutive d. No differences ($P > 0.10$) were observed in ADG for d 0 to 28 and d 0 to 56, DMI for d 0 to 28, d 29 to 56, and d 0 to 56, or G:F for d 0 to 28, d 29 to 56, and d 0 to 56; ADG for d 29 to 56 tended to increase ($P < 0.10$) for BMD-treated calves compared with controls. Fecal samples were collected from 15 scouring calves and 10 cohorts (nonscouring calves received on the same day and administered the same treatment as the scouring calves). Animal morbidity and fecal score did not vary between the 2 treatments. Mortality was not influenced by the treatments in the BMD administration period or throughout the experiment. Fecal samples were subjected to pyrotagged 454 FLX pyrosequencing of 16S rRNA gene amplicon to examine compositional dynamics of fecal microbes. *Escherichia*, *Enterococcus*, and *Shigella* had greater ($P < 0.05$) populations in the BMD group whereas *Dorea*, *Roseburia*, *Fecalibacterium*, *Papillibacter*, *Collinsella*, *Eubacterium*, *Peptostreptococcus*, and *Prevotella* were decreased ($P < 0.05$) by BMD treatment. Genus populations were also compared between scouring and nonscouring calves. *Streptococcus* was the only genus that had notable increase ($P < 0.05$) in fecal samples from scouring calves whereas populations of *Bacteroides*, *Roseburia*, and *Eubacterium* were markedly ($P < 0.05$) greater in nonscouring calves. These results show that BMD has the ability to alter the composition of the fecal microbiome but failed to improve performance in Holstein bull calves. Discrepancy of microorganism profiles between scouring and nonscouring calves might be associated with the occurrence of scours and bacterial genera identified might be potential target of treating diarrhea.

Key words: bacitracin methylene disalicylate, Holstein bull calves, microbiome, performance

INTRODUCTION

One of the critical periods in the life of Holstein cattle is the neonatal phase, during which nutrition and management have a long-term effect on overall performance of Holstein cattle (Van Amburgh, 2003). Management factors, including limited amounts of co-lostrum (Mokhber Dezfouli et al., 2007), transportation and handling stress (Grandin, 1997; Eicher, 2001; Odore et al., 2004; Stanger et al., 2005), and adaptation to new diets (Murdock and Hodgson, 1961; Hammon and Blum, 1998; Kühne et al., 2000), can increase the susceptibility of calves to pathogenic infection. Preventative treatments that help calves maintain homeostasis and balance the hindgut microbial populations are desirable. Bacitracin methylene disalicylate (**BMD**) has been used extensively as an antimicrobial and growth promoter in swine (Dewey et al., 1999) and poultry (Huyghebaert and de Groote, 1997). Although

BMD has been approved for use in cattle by the U.S. Food and Drug Administration for more than a decade (FDA, 1998), few studies have been conducted to evaluate its growth promoting effects on cattle. The present study was conducted on Holstein bull calves because of their high availability in the market. The effect of oral-fed BMD was evaluated by measuring parameters of performance and health of animals.

16S rRNA gene deep-sequencing technique has been used more and more often to investigate system microbiome because of its powerful sequence detecting and high-throughput data generating capabilities. To our knowledge, most of the pyrosequencing studies were conducted on adult cattle, which have a considerably different diet and metabolism from preweaned calves. Here we measured fecal microbiome dynamics using 16S rRNA bacterial tag-encoded FLX amplicon pyrosequencing (**bTEFAP**), which enables the detection of bacteria from different taxa and measures their percentages of populations. Collectively, we assessed the impact of the BMD treatment and scours on the gastrointestinal tract bacteria.

MATERIALS AND METHODS

Animals

This research was approved by the University of Arizona Animal Care and Use Committee (protocol number 07-113). One hundred fifty 1- to 3-d-old Holstein bull calves were obtained from a commercial dairy. Calves were transported to the University of Arizona Calf Research facility (131 km; 2 h in transit). Before transportation, each calf was fed at least 4 L of pooled colostrum and vaccinated with TSV-2 (Pfizer Animal Health, New York, NY). Calves were received at the Calf Research facility at 1100 h daily in 5 loads over a span of 8 d. Upon arrival, animals were immediately weighed and placed in housing crates.

Housing and Feed

Calves were housed in commercial California brand calf crates. Crates were constructed of wood and their sizes were 1.60 m high (cover included) by 2.45 m wide by 1.50 m long, with each crate housing 3 calves. Calves within the same crate were separated by a fence. Wooden slatted floors allowed for drainage of urine and feces. The tilted opening of the tops faced north and could be closed at night. Individual nipple waterers and feed buckets were provided for each animal. Each calf was bottle fed twice daily from the day of arrival (d 0) for 56 d with 1.89 L of milk replacer [12% milk replacer powder (Lawley's All Star Milk Replacer; Turlock, CA; 22% CP, 20%

Table 1. Composition (DM basis) of concentrate diet fed to Holstein bull calves from d 3 to the end of the trial.

Item	Content
Ingredient (% of DM)	
Steam-flaked corn	59
Beet pulp	10
Molasses	6
Bovatec CalfPellets[1]	25
Chemical analysis[2]	
DM, %	89.7
CP, %	18.4
Ash,%	1.35
NEm, Mcal/kg	1.74
NEg, Mcal/kg	1.19
NDF, %DM	14.4
ADF, %DM	5.0

[1]Obtained from Dairy Nutrition Services, Inc., Chandler, AZ. The 45% CP supplement contained vitamins, trace minerals, and lasalocid [Bovatec, Alpharma Animal Health (now Zoetis Animal Health) Florham Park, NJ].

[2]Dry matter, ash, NDF, and ADF were analyzed and CP, NEm, and NEg were calculated using values from National Research Council (1996).

crude fat, 15% crude fiber, 5.6% ash, 33,000 IU vitamin A/kg, 18,700 IU vitamin D/kg, 330 IU vitamin E/kg, on a DM basis) mixed with water (88%; wt/wt)]. In addition, calves were provided free access to a concentrate diet (Table 1) from d 3 to the end of the experiment.

Treatments

The 150 Holstein bull calves were grouped by their calving dates. Calves within the same groups were stratified from heaviest to lightest BW and then randomly assigned to 1 of 2 experimental treatments, Control (**CON**, no BMD supplement) or BMD. Soluble BMD (BMD-soluble; Alpharma, Inc. Zoetis Animal Health, Florham Park, NJ) was added into milk replacer at 1 $g^{-1} \cdot calf^{-1} \cdot d^{-1}$ (0.5 g twice daily) for 10 d, from d 3 to 12. Weighed BMD powder was mixed in water before adding into milk replacer using a repeat syringe.

Data Collection, Sample Collection, and Statistical Analysis

Bull calves were individually weighed on d 0, 28, and 56 of the experiment after the morning feeding. Certified weights (22.7 kg) were used to ensure scale accuracy. Fecal scores were recorded daily following a 4-point scale (1 = normal, firm stool; 2 = soft, does not hold form; 3 = runny, spreads easily; 4 = liquid, devoid of solid matter). Fecal scores greater than 2 were considered as scours. In addition, morbidity scores were recorded daily with a 3-point scale (1 = normal respiratory, normal hydration appearance, alert, normal ears; 2 = rhinitis, sunken eyes, depressed attitude, head tilt, cough-

ing, skin tented 5 to 10s, nonresponsive, droopy ears; 3 = moribund: heavy thoracic breathing, skin tented >10s, unable to stand without assistance). Daily consumption of milk replacer of each calf was also recorded as well as the weight of dry feed fed to each calf and orts. Samples of milk replacer and feed were collected weekly, stored at –20°C, and then dried in a forced air oven at 100°C overnight for DM calculation.

Fecal samples were collected from 15 calves with fecal scores of 3 and 4 from both CON and BMD groups on the last day of treatment (d 12). At the same time, fecal samples from 10 cohorts (calves received on the same day and receiving the same treatment but without signs of scouring) were collected from both treatments. Available sample amounts used in analysis: $n = 14$ for BMD × scouring, $n = 9$ for BMD × nonscouring, $n = 13$ for CON × scouring, and $n = 10$ for CON × nonscouring. Samples were stored at –20°C and sent to the Research and Testing Laboratory, Lubbock, TX, where samples underwent bTEFAP to verify major bacterial genera and species. The following summarizes the procedures as reported by Dowd et al. (2008). Fecal samples were first homogenized and total genomic DNA was extracted using a QIAamp stool DNA mini kit (Qiagen, Valencia, CA) and quantified using a Nanodrop spectrophotometer (Nyxor Biotech, Paris, France). Samples then entered into the bTEFAP process (Dowd et al., 2008). One hundred nanograms DNA of each sample was used in a 50 μL PCR reaction. Universal primers targeting a region (530 to 1,100 bp) of the 16S rRNA gene (forward: 5′-GTGCCAGCMGCNGCGG and reverse: 5′-GGGTTNCGNTCGTTG) were used to amplify the 600 bp region of 16S rRNA genes with PCR (94°C for 3 min followed by 32 cycles of 94°C for 30 s, 60°C for 40 s, and 72°C for 1 min and a final elongation at 72°C for 5 min). The PCR products were purified and a secondary PCR for FLX (Roche, Nutley, NJ) followed with the same conditions but using fusion primers (LinkerA-Barcodes-Forward and LinkerB-Reverse) with unique barcode sequences in forward primer for each sample. This was to prevent any potential bias that might be generated by the inclusion of the noncomplimentary barcodes and linkers during the initial template amplification. After the 2 PCR reactions, all amplification products from different samples were mixed in equal volumes and purified with Agencourt Ampure beads (Agencourt Bioscience Company, Beverly, MA). The DNA fragments size and concentration of purified products were measured by using Experion DNA chips (Bio-Rad Laboratories, Hercules, CA) for preparation of FLX sequencing. After measurement, DNA fragments were combined with DNA capture beads and amplified by emulsion PCR. The bead-attached DNAs were denatured with NaOH and sequencing primers were attached. The sequenc-

ing was run on a PicoTiterPlate by using a 454 Genome Sequencer FLX System (Roche, Nutley, NJ). Quality trimmed sequences generated from FLX system were grouped from the multi-text-based format of nucleotide sequences files into individual sample specific files according to assigned barcodes. Sequences without 100% homology to designated barcodes and sequences that were less than 150 bp after quality trimming were discarded. Sample specific files were assembled by CAP3 (Huang and Madan, 1999). Files obtained from CAP3 were processed to generate a secondary text-based format of nucleotide sequences (**FASTA**) containing tentative consensus. Tentative consensus FASTA of each sample was evaluated using BLASTn against database derived from the RDP-II database (Maidak et al., 2001) and GenBank to identify DNA sequences.

Weight of concentrate diet feed and milk replacer given to each calf was used to measure DMI. Body weight, ADG, DMI, and G:F were calculated for d 0 to 28, d 29 to 56, and d 0 to 56. Fecal and attitude scores collected from d 1 to 13 were averaged within treatment group before analysis.

Body weight, DMI, ADG, G:F, average fecal, and attitude scores were analyzed using PROC MIXED of SAS 9.2 (SAS Inst. Inc., Cary, NC). Models included treatments (CON vs. BMD), scours (no vs. yes), and treatments × scours interaction. Effects were considered significant at $P < 0.05$, with tendencies declared when $0.05 \leq P < 0.10$. Bacterial analysis results contain population percentages of different genera and species. Results of bacterial genera from bTEFAP were rearranged according to levels of prevalence from highest to lowest. Thirty genera with the highest prevalences were subjected to SAS (Proc Mixed, Tukey adjustment for nonorthogonal pair comparisons) to analyze the effects of treatments, scours, and their combinations. Individual animals were experimental units for all analyses.

RESULTS AND DISCUSSION

Performance data is shown in Table 2. Initial BW did not differ ($P = 0.97$) between CON and BMD treatments. Similarly, BW at d 28 ($P = 0.68$) and 56 ($P = 0.19$) did not differ between treatments. Daily DMI were similar for d 0 to 28 ($P = 0.96$), d 28 to 56 ($P = 0.91$), and d 0 to 56 ($P = 0.87$) between treatments. Average daily gain for d 0 to 28 ($P = 0.38$) and d 0 to 56 ($P = 0.34$) did not differ. However, ADG for d 29 to 56 tended to be greater ($P < 0.10$) for BMD-treated calves than that of control. No difference in G:F was observed between treatments throughout the trial ($P = 0.30$ for d 0 to 28, $P = 0.15$ for d 29 to 56, and $P = 0.70$ for d 0 to 56). Attitude ($P = 0.26$) and fecal scores ($P = 0.54$) did not differ between treatments (Table 3). Mortality was not influenced by treatments during the BMD

Table 2. Effect of control or bacitracin methylene disalicylate treatments on performance of Holstein bull calves

Item	Treatment[1]		SEM[2]	*P*-value
	CON	BMD		
BW, kg				
d 0	41.4	41.4	0.80	0.98
d 28	48.1	47.7	0.90	0.68
d 56	64.5	66.3	1.72	0.29
DMI,[3] kg				
d 0 to 28	0.6	0.6	0.024	0.97
d 28 to 56	1.4	1.4	0.103	0.92
d 0 to 56	1.0	1.0	0.055	0.88
ADG, kg				
d 0 to 28	0.25	0.22	0.036	0.39
d 28 to 56	0.58	0.66	0.047	0.09
d 0 to 56	0.42	0.44	0.028	0.35
G:F[4]				
d 0 to 28	0.39	0.34	0.042	0.31
d 28 to 56	0.43	0.48	0.033	0.15
d 0 to 56	0.42	0.43	0.022	0.71

[1]Treatments included a control (CON; no bacitracin methylene disalicylate) or BMD [bacitracin methylene disalicylate; BMD-soluble; Alpharma, Inc. (Zoetis Animal Health, Florham Park, NJ]) included in the milk replacer from d 3 to 12 at 1 g/d.

[2]*n* = 75 for d 0, *n* = 72 for CON at d 28 and *n* = 69 for BMD at d 28, and *n* = 70 for CON at d 56 and *n* = 69 for BMD at d 56.

[3]DMI for dry feed. Milk replacer was fed at 0.45 kg/d for each bull calf.

[4]G:F for both dry feed and milk replacer.

Table 3. Effect of control or bacitracin methylene disalicylate treatments on health of Holstein bull calves

Item	Treatment[1]		SEM[2]	*P*-value
	CON	BMD		
Morbidity score[3]	1.06	1.04	0.017	0.26
Fecal score[4]	1.55	1.59	0.059	0.55
Death loss,[5] head				
d 3 to 12	2	2	–	–
Mortality d 3 to 12	2.67%	2.67%	0.026	1.00
Death loss, head				
d 3 to 56	5	6	–	–
Mortality d 3 to 56	6.67%	8.00%	0.043	0.76

[1] Treatments included a control (CON; no bacitracin methylene disalicylate) or BMD (bacitracin methylene disalicylate) included in the milk replacer from d 3 to 12 at 1 g/d.

[2]*n* = 75 for d 0, *n* = 71 for CON at d 28 and *n* = 70 for BMD at d 28, and *n* = 70 for CON at d 56 and *n* = 70 for BMD at d 56.

[3]Morbidity scores are a set of numbers representing animal attitude. They were determined with clinical criteria of 1 = normal respiratory, normal hydration appearance, alert, normal ears; 2 = rhinitis, sunken eyes, depressed attitude, head tilt, coughing, skin tented 5 to 10 s, nonresponsive, droopy ears; 3 = moribund: heavy thoracic breathing, skin tented >10 s, unable to stand without assistance.

[4]Fecal scores are a set of numbers describing consistency of feces. They are judged by using a scale of 1 = normal feces to 4 = watery stool, devoid of solid material.

[5]BMD administered from d 3 to 12.

administration period (*P* = 1.000) or the entire experimental period (*P* = 0.76). The present study showed that BMD did not significantly improve Holstein bull calves' ADG, G:F, or prevention of scours.

Fecal bacteria community identified in this study shared taxa with the community from adult dairy and beef cattle (Dowd et al., 2008; Callaway et al., 2010; Durso et al., 2010; Shanks et al., 2011). However, the relative abundances of the major bacterial groups differed greatly. At the phylum level, Firmicutes occupied an average 51.6% of total bacteria population. Proteobacteria (18.5%), Bacteroidetes (14.2%), and Fusobacteria (12.6%) were the following highest prevalent phyla identified. Verrucomicrobia (1.6%) and Actinobacteria (1.4%) were also present. The rest of other phyla composed only 0.05% of the total population. Collectively, 19 phyla were identified. Figure 1 depicts the frequencies of different phylum organized in treatment × scours combinations. The percentage of Firmicutes changed between treatments and along with scours occurrence. Proteobacteria abundance shrunk without BMD treatment whereas Bacteroidetes decreased when scours happened.

It is believed that the lower intestinal bacteria community of adult cattle is dominated by obligate anaerobes such as *Bacteroides*, *Clostridium*, and *Bifidobacterium* and facultative anaerobes have been reported to oc-

cur 1/100 in number compared with obligate anaerobes (Dowd et al., 2008). The finding of the present study, on the other hand, showed a discrepancy of microflora composition between neonate calves and adult cattle. Facultative anaerobes (e.g., *Streptococcus*, *Escherichia*) were a great portion of the total bacterial population. Among major bacteria groups, another observable change is that lactic acid bacteria, such as *Streptococcus*, *Lactobacillus*, and *Enterococcus*, increased their percentages of population compared with those found
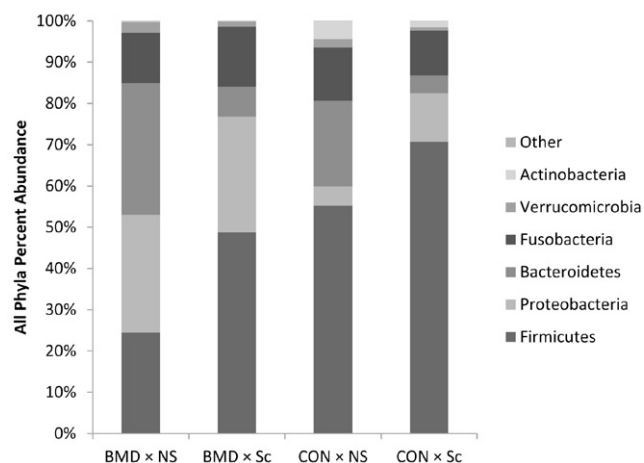

Figure 1. Stacked histogram depicting the relative abundances of different bacterial phyla identified from feces of scouring (Sc) or nonscouring (NS) Holstein bull calves fed milk replacer with bacitracin methylene disalicylate (BMD) or without BMD (CON).

Table 4. Most common genera (as a percentage of the total bacterial population) identified from feces of scouring (Sc) or nonscouring (NS) Holstein bull calves fed milk replacer with bacitracin methylene disalicylate (BMD) or without BMD (CON)[1]

| Genus name | Population of total bacteria population, % | | | | P-value[2] | |
	BMD × NS (n = 9)	BMD × Sc (n = 14)	CON × NS (n = 10)	CON × Sc (n = 13)	Treatment	Scours
Streptococcus	7.623 ± 8.189	27.581 ± 6.566	16.926 ± 7.769	28.657 ± 6.814		0.037
Bacteroides	31.667 ± 6.859[a]	7.030 ± 5.499[b]	18.603 ± 6.507[ab]	3.930 ± 5.707[b]		0.003
Fusobacterium	12.110 ± 7.667	14.272 ± 6.147	12.816 ± 7.273	10.812 ± 6.379		
Escherichia	16.892 ± 4.022[ab]	19.934 ± 3.224[a]	2.930 ± 3.815[b]	7.893 ± 3.346[ab]	0.001	
Lactobacillus	0.346 ± 4.295	4.318 ± 3.444	4.724 ± 4.075	12.764 ± 3.574		
Enterococcus	6.017 ± 2.656[ab]	10.015 ± 2.129[a]	1.066 ± 2.519[b]	3.388 ± 2.210[ab]	0.020	
Ruminococcus	2.073 ± 3.570	2.379 ± 2.862	2.108 ± 3.387	9.009 ± 2.970		
Shigella	6.692 ± 1.269[a]	6.239 ± 1.018[a]	1.047 ± 1.204[b]	2.429 ± 1.056[ab]	<0.001	
Clostridium	3.720 ± 1.415	0.854 ± 1.134	3.935 ± 1.342	5.221 ± 1.177	0.079	
Dorea	0.017 ± 1.300[a]	0.014 ± 1.042[a]	5.676 ± 1.233[b]	3.908 ± 1.082[ab]	<0.001	
Akkermansia	2.691 ± 1.200	1.321 ± 0.962	2.050 ± 1.138	0.745 ± 0.998		
Roseburia	0.187 ± 0.846[a]	0.084 ± 0.679[a]	5.474 ± 0.803[b]	0.816 ± 0.704[a]	<0.001	0.003
Fecalibacterium	0.000 ± 0.953[a]	0.017 ± 0.764[a]	4.468 ± 0.905[b]	1.220 ± 0.793[a]	0.002	0.067
Papillibacter	0.320 ± 0.554	0.266 ± 0.444	1.991 ± 0.526	1.721 ± 0.461	0.003	
Leuconostoc	0.729 ± 0.339	1.510 ± 0.272	0.728 ± 0.321	0.710 ± 0.282		
Collinsella	0.000 ± 0.391[a]	0.003 ± 0.314[a]	1.667 ± 0.371[b]	1.193 ± 0.326[ab]	<0.001	
Gallibacterium	0.857 ± 0.528	1.015 ± 0.423	0.075 ± 0.501	0.624 ± 0.439		
Klebsiella	2.680 ± 0.873	0.058 ± 0.700	0.006 ± 0.829	0.017 ± 0.727	0.091	
Megasphaera	1.318 ± 0.693	0.213 ± 0.556	0.750 ± 0.658	0.003 ± 0.577		
Veillonella	0.872 ± 0.348	0.552 ± 0.279	0.104 ± 0.330	0.234 ± 0.289	0.090	
Eubacterium	0.272 ± 0.308[ab]	0.058 ± 0.247[b]	1.264 ± 0.293[a]	0.236 ± 0.257[ab]	0.041	0.031
Olsenella	0.191 ± 0.729	0.002 ± 0.585	1.691 ± 0.692	0.006 ± 0.607		
Turicibacter	0.000 ± 0.644	0.007 ± 0.516	1.725 ± 0.611	0.041 ± 0.536		
Sporobacter	0.012 ± 0.367	0.025 ± 0.294	0.404 ± 0.348	0.937 ± 0.305	0.055	
Mogibacterium	0.000 ± 0.414[a]	0.002 ± 0.332[a]	1.438 ± 0.393[b]	0.034 ± 0.344[a]	0.055	0.067
Peptostreptococcus	0.000 ± 0.302	0.000 ± 0.243	0.226 ± 0.287	0.881 ± 0.252	0.048	
Sutterella	0.362 ± 0.177	0.279 ± 0.142	0.098 ± 0.168	0.272 ± 0.147		
Eggerthella	0.025 ± 0.433	0.002 ± 0.347	0.998 ± 0.411	0.061 ± 0.360		
Lactococcus	0.179 ± 0.074	0.364 ± 0.060	0.154 ± 0.070	0.154 ± 0.062	0.086	
Prevotella	0.007 ± 0.164[a]	0.070 ± 0.131[a]	0.722 ± 0.156[b]	0.118 ± 0.136[a]	0.013	0.074

[a,b]Values within a row that do not share a common superscript differ by $P < 0.05$.

[1]The genera are ordered by highest overall percentage.

[2]Overall treatment (BMD vs. CON) or scours (Sc vs. NS) effect were also tested.

in other studies conducted in adult cattle (Dowd et al., 2008; Callaway et al., 2010).

In this study, genera significantly affected ($P < 0.05$) by BMD treatment (Table 4), included *Escherichia*, *Enterococcus*, *Shigella*, *Dorea*, *Roseburia*, *Fecalibacterium*, *Papillibacter*, *Collinsella*, *Eubacterium*, *Peptostreptococcus*, and *Prevotella*. Among the affected genera, populations of *Escherichia*, *Enterococcus*, and *Shigella* increased under BMD treatment whereas the others decreased. Compared with CON, prevalence of gram-positive bacteria are lower in BMD treated samples, being consistent with previous study (Butaye et al., 2003). The greater percentages observed in gram-negative genera, such as *Enterococcus* and *Shigella*, could have resulted from BMD's suppressing effect on gram-positive genera,

enabling a colonization dynamic that favors environmentally prolific microbes with short generation times. Two other gram-negative genera, *Klebsiella* and *Veillonella*, were more prevalent in BMD-treated calves. *Dorea*, a less studied genus, was susceptible to BMD. *Salmonella*, usually acknowledged as a common causative agent of diarrhea (Bicknell and Noon, 1993; Costello, 2005; Powell, 2004), rarely (2 of the 46 calves; population percentages are 0.015 and 0.019%) appeared in samples collected during this study.

Genera with differing population percentages ($P < 0.05$) between scouring and nonscouring (Table 4) were *Streptococcus*, *Bacteroides*, *Roseburia*, and *Eubacterium*. Surprisingly, *Streptococcus*, as a gram-positive genus, was not affected by BMD treatment but was the

only genus that had notable increase in fecal samples from scouring calves. Both negative and positive influences had been reported regarding the role played by *Streptococcus* in the host (Busconi et al., 2008; Herrera et al., 2009). *Streptococcus bovis* is considered to be responsible for causing ruminal acidosis and bloat (Herrera et al., 2009). The rapid introduction of easily digestible carbohydrates can lead to overgrowth of *S. bovis*, which produce excessive lactic acid. As lactic acid accumulates, the osmolarity of the ruminal contents increases and can lead to dehydration and diarrhea (Herrera et al., 2009; Wahrmund et al., 2012). Therefore, *Streptococcus* might be a potential causative factor in Holstein calves suffering diarrhea. *Bacteroides* is normally commensal in the gut flora and has the ability to degrade plant polysaccharides in the host intestine (Hooper et al., 2002; Wexler, 2007; Sonnenburg et al., 2010). *Bacteroides* spp. have been shown to ferment hemicellulosic polymers and their major sugar xylose in the human colon (Reddy et al., 1983; Chassard et al., 2008). The decrease of *Bacteroides* of scouring calves observed in this study might create a chance for colonization of less favorable bacteria and subsequently lead to mucosal and epithelial invasion and subsequently diarrhea.

A limitation of this procedure is that bacteria collected from feces may not accurately represent the microbiome composition of the rumen and other areas of digestive system. Biogeographical variation in microbiome composition exists between ingesta and feces (Savage, 1977; Callaway et al., 2010) and could not be covered by these data. Multiple samplings from different portions of gastrointestinal tract would be needed to determine the effects on the microbiome in their entirety. In fact, several rumen microbiome diversity studies point out that *Prevotella* is the dominant genus of bacteria in cattle (Edwards et al., 2004; Stevenson and Weimer, 2007; Uyeno et al., 2007).

Conclusion

In this study, we hypothesized that BMD may prevent scours of Holstein calves and improve performance. The results suggest that BMD significantly changed fecal bacterial microbiome but did not improve Holstein bull calf performance or prevention of scours. Fecal microbiome from scouring and healthy calves differed substantially. Whether the alteration of feces form initiates the change of fecal bacteria profile or the microbiome change subsequently causes different feces is unknown. Further studies have to be conducted to clarify the causative relationship. Compared to data generated from adult cattle studies, neonatal calves have a distinct composition of fecal bacteria.

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