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Performance of Neonatal Holstein Heifers Fed Yeast Derivatives in Milk Replacer

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Abstract: Total 40 (n = 8) Holstein heifers were used to evaluate growth and health when supplemented with coccidiostat, mannanoligosaccharide or β-glucan in milk replacer. Calves were randomly assigned to one of five treatments at birth: CX (1 g day^{-1} Deccox), MOS (10 g day^{-1} MOS), β-g (0.5 g day^{-1} β-glucan), CX+MOS (1 g day^{-1} Deccox+10 g day^{-1} MOS) and MOS+β-g (10 g day^{-1} MOS + 0.5 g day^{-1} β-glucan). Heifers received 3.8 L milk replacer (22% CP, 20% fat) once daily until 6 weeks of age but remained on trial for 56 days. Calves were fed a non-medicated starter (18% CP) at 0.9 kg day^{-1} and increased by 0.9 kg day^{-1} when orts = 0. Orts were collected and weighed daily and pooled by week within treatment. Calves had *ad lib.* access to clean water. At birth, calves were fed colostrum via esophageal tube, weighed and measured. Fecal and respiratory scores were recorded daily body measurements, blood and fecal samples were collected weekly. Fecal samples analyzed for coccidia bi-weekly after 21 days at 2, 4 and 8 weeks fecal samples were analyzed for *E. coli*. Blood samples were analyzed for CBC weight/differential. There were no significant differences in DMI ($p<0.93$), FE ($p<0.95$), ADG ($p<0.79$) or blood analysis ($p>0.10$) among treatment groups. Given similar diets fed, no changes in growth or intake were expected. No differences in fecal or respiratory scores, CBC or other health measures indicated that supplementation with MOS, β-g or a combination supported immune function similarly to CX. Fecal shedding of *E. coli* was not different across treatments ($p<0.23$), however orthogonal contrasts showed greater *E. coli* from MOS+β-g (80.4×10^4 CFU μL^{-1} feces) compared to β-g (23.2×10^4 CFU μL^{-1} feces; $p<0.04$). There was also a trend for calves fed MOS to shed more *E. coli* compared to the calves fed β-g (70.3 vs. 23.2×10^4 CFUs μL^{-1} feces, respectively, $p<0.06$). *E. coli* shedding decreased over time (114.6, 49.4 and 5.0×10^4 CFU μL^{-1} feces at weeks 2, 4 and 8, respectively; $p<0.01$).

Key words: Dairy heifers, yeast additives, milk replacer, calves, blood

INTRODUCTION

Since, the discovery of growth promoting effects of antibiotics, poultry, swine and cattle producers have utilized some antibiotics or derivatives as growth promoters and gut health enhancers. This often raises a red flag with consumers as their concerns over food quality and safety and possible translation of these antimicrobials into food products grow. Finding, alternatives for sub-clinical antibiotic use in production animals is necessary because of consumer concerns and subsequent new policies regulating the use of antimicrobials in livestock production systems. Growth promotion is defined as the administration of an antimicrobial, usually as a feed additive, over a period of time to growing animals resulting in improved physiological performance (Phillips *et al*., 2004). Advancements in biotechnology in recent years have allowed the use of microbial cultures as feed additives and growth promotants (Gorgulu *et al*., 2003). Among these additives are oligosaccahrides which have been used in both human and animal medicine to stabilize gut microflora. Mannanoligosaccharides, a derivative of yeast cell walls are thought to bind and remove pathogens from the intestinal tract which results in immune system stimulation (Spring *et al*., 2000). Three of the possible alternatives being studied currently with and for dairy calves are live yeast culture (*Saccharomyces cerevisae*), yeast cell wall products (oligosaccharides) and β-glucans (derivative of yeast cell wall). To date, the research conducted thus far involving these three yeast derivatives has been very positive.

Total 40 Holstein heifer calves (n = 8) were used in the current trial at the Bearden Dairy Research Center at Mississippi State University under IACUC approval. At birth, calves were randomly assigned to one of five

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different treatment groups. Treatments were as follows: CX (1 g day^{-1} Deccox), YST (10 g day^{-1} yeast), β-g (0.5 g day^{-1} β-glucan), CX+YST (1 g day^{-1} Deccox+ 10 g day^{-1} yeast) and YST+β-g (10 g day^{-1} yeast+ 0.5 g day^{-1} β-glucan). The YST additive contained 50% mannanoligosaccharide and 25% β-glucan fractions. Treatment additives were mixed with 100 mL warm water and then added to milk replacer at feeding. Calves were individually housed in plastic hutches made by Calf Tel® which were bedded with wheat straw. The trial was conducted over an 8 weeks period where calves were weaned at 6 weeks of age and remained in hutches until 8 weeks of age. Calves were fed 3.8 L (710 g powder) of a non-medicated milk replacer (22% CP, 20% fat; DM, land o' lakes milk) from an open pail once daily at 06:30 until day 35, at which daily milk allowance was then reduced to 1.4 L (355 g powder).

At day 42, calves were weaned. After milk feeding, buckets were rinsed and filled with water, allowing calves *ad libitum* access to water until next feeding. A non-medicated starter grain (18% CP) was offered in increments of 0.9 kg day^{-1} starting at 1 day of age. When a calf had no feed refusals, grain allowance was increased by 0.9 kg day^{-1}. All 40 calves used for this study were born between September, 2009 and February, 2010.

MATERIALS AND METHODS

Growth performance: Body Weight (BW), Hip Height (HH), Hip Width (HW), Wither Height (WH) and Body Length (BL) was measured at birth, once weekly and the final day of trial (Fig. 1). Rectal body temperatures were also recorded weekly. Orts were collected and weighed daily and then pooled by treatment for weekly feed analysis. Analyses of feed samples were completed in the Scales Nutrition Laboratory in the Animal and Dairy Sciences Department at Mississippi State University for dry matter, nitrogen, fat, neutral detergent fiber and acid detergent fiber (according to AOAC methods).

Health status and blood sampling: Calves were separated from dams within 12 h after birth and then received 2.83 L of colostrum via esophageal feeding tube. A single blood sample was collected via jugular venipuncture between day 2 and 7 after birth to ensure adequate IgG absorption. Immunoglobulin G (IgG) concentrations were determined using a single radial immunodiffusion kit (VMRD Inc., Pullman, WA). Respiratory and fecal scores were evaluated and recorded daily at feeding according to Larson. Briefly, fecal scores were determined based on a 5 point scale where 1 represented normal (soft, solid, no fluid), 2 soft (semi-solid), 3 runny (soft, mostly fluid), 4 watery (fluid) and 5 bloody. Respiratory scores were as follows: 1: Normal, 2: Runny nose, 3: Heavy breathing, 4: Moist cough and 5: Dry cough. Two blood samples were collected once weekly, 4 h after feeding; one 5 mL vaccutainer containing EDTA for Complete Blood Count (CBC) analysis with differential and one 10 mL vaccutainer containing no anticoagulant. Samples were immediately transported (11 km) to the laboratory. The whole blood samples were analyzed for CBC in the Animal Pathophysiology Laboratory of the College of Veterinary Medicine at Mississippi State University. The blood samples containing no anticoagulant were processed in the Animal Physiology Lab at Mississippi State University; centrifuged at 3000×g at 4°C for 30 min and then serum was stored in 1.5 mL polypropylene tubes at -20°C until further analysis of cortisol concentrations. Cortisol samples were analyzed via radioimmunoassay (Coat-A-Count, Siemens Healthcare Diagnostics Inc., Los Angeles, CA).

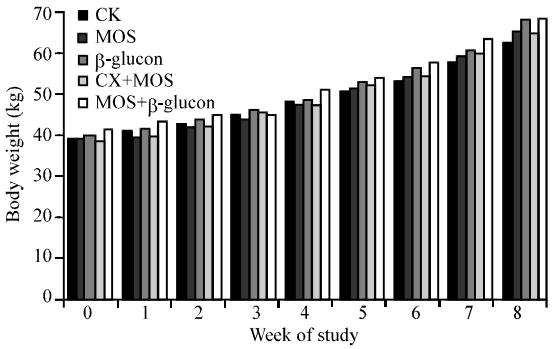

Fig. 1: Growth performance of Holstein heifers fed CX, YST, β-glucan or some combination

Fecal sampling: Fecal samples were collected weekly until calves reached 21 days of age and then twice weekly to analyze for the presence of Coccidia. Analyses were conducted in the Animal Pathophysiology Laboratory of the College of Veterinary Medicine at Mississippi State University. Samples were graded subjectively based on the number of oocyst present: None (0), rare (1-5), few (10-25), moderate or many (>25). Twice weekly fecal samples were collected once calves reached 21 days of age due to the 21 days lifecycle of coccidia. Fecal *E. coli* shedding was recorded and analyzed by taking a fecal sample from each calf at weeks 2, 4 and 8. A sterile 10 μL loop of fecal material was placed into 990 μL of Luria-Bertani (LB) broth. A serial dilution was conducted to yield a 10^{-4} dilution and was then plated on MacConkey agar. Plates were incubated for 24 h at 37°C. After incubation time was complete, Colony Forming Units (CFU's) were counted.

Feed analysis: Grain refusals (orts) were collected and measured daily and then pooled by treatment for weekly feed analysis. Analyses of feed samples were completed in the H.W. Essig Nutrition Laboratory in the Animal and Dairy Sciences Department at Mississippi State University. All samples were first dried for 48 h in a 64°C drying oven. Samples were then ground in a Willy Mill through a 2 mm screen. Samples were analyzed in duplicate for DM, N, fat, NDF and ADF (AOAC, 1984).

Statistical analysis: Class variables for statistical analysis included calf, treatment and week. All data were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC) with week being the repeated measure. Orthogonal contrast statements were used to determine differences between treatments if present, the contrast statements were: YST vs. β-glucan, YST vs. YST+β-glucan, β-glucan vs. YST+β-glucan and CX vs. all. Treatment differences with $p \leq 0.05$ were considered significant while $p \leq 0.10$ were considered a tendency.

RESULTS AND DISCUSSION

Calf IgG concentrations were adequate for passive transfer of immunity in all calves in the present study. Nutrient intake was not different and as expected, growth measures among treatment groups were similar ($p \leq 0.05$; Table 1). Calves were fed the same basal diet, therefore no differences were expected among treatments due to diet. Similar to findings of Lesmeister *et al.* (2004), Morrill *et al.* (1995) and Galvao *et al.* (2005), no differences were observed between treatment groups with respect to blood parameters ($p \geq 0.05$). The effects of 1 or 2% live yeast culture (Lesmeister *et al.*, 2004; Galvao *et al.*, 2005) or probiotics did not change total plasma protein concentrations when compared to control fed calves. Weekly body measurements were recorded and analyzed and no differences were found among treatments for BW, WH, HH, HW or BL ($p = 0.52, 0.21, 0.19, 0.53$ and 0.39, respectively). Hill *et al.* (2008, 2009) and Heinrichs *et al.* (2003) all supplemented Holstein calves with MOS and compared to calves with no supplementation of antibiotics, yeast or coccidiostat, there were no differences in growth. Heinrichs *et al.* (2003) did however, report differences in grain intake during week 6 compared to calves fed antibiotics while Hill *et al.* (2009) saw no change in starter intake due to supplementation of MOS in Holstein calves. Improved grain intakes ($p<0.05$) were observed in calves supplemented yeast culture in starter grain compared to control while a tendency ($p<0.10$) for greater starter grain intake was shown by calves supplemented with yeast culture in milk replacer compared to control (Galvao *et al.*, 2005). It can be noted that grain intake for calves fed yeast culture in both milk replacer and starter grain did not differ from other treatment groups. Lesmeister *et al.* (2004) found similar results when calves were supplemented 1 or 2% yeast culture in starter grain. Overall (week 1-6) intakes were significantly higher ($p<0.01$) for calves fed 2% yeast culture. No differences were found in fecal and respiratory scores or

Table 1: Nutrient intake and growth measures in Holstein heifers fed CX, YST, β-glucan or some combination

							p-value	
Treatments	CX	YST	β-glucan	CX+YST	YST+β-glucan	SEM	Trt	Period[1]
DMI[2] (kg day^{-1})								
Period 1	0.81	0.88	0.91	0.85	0.88	0.08	0.25	<0.001
Period 2	0.93	1.16	1.15	0.99	1.28	0.09	-	<0.001
Period 3	1.61	1.88	1.73	1.63	1.90	0.08	-	<0.001
CP intake; total (kg day^{-1})								
Period 1	0.16	0.17	0.18	0.17	0.17	0.02	0.29	<0.001
Period 2	0.18	0.23	0.23	0.20	0.25	0.02	-	<0.001
Period 3	0.31	0.36	0.32	0.31	0.38	0.02	-	<0.001
NDF intake; starter (kg day^{-1})								
Period 1	0.02	0.04	0.03	0.03	0.03	0.02	0.26	<0.001
Period 2	0.12	0.17	0.14	0.12	0.17	0.02	-	<0.001
Period 3	0.34	0.40	0.32	0.31	0.32	0.02	-	<0.001
ADF intake; starter (kg day^{-1})								
Period 1	0.01	0.02	0.02	0.01	0.01	0.01	0.28	<0.001
Period 2	0.05	0.07	0.06	0.05	0.08	0.01	-	<0.001
Period 3	0.13	0.16	0.14	0.12	0.13	0.01	-	<0.001
Initial BW (kg)	39.20	39.10	39.50	38.10	41.10	1.23	>0.05	-
Final BW (kg)	61.90	64.90	67.70	64.40	67.30	2.67	>0.05	-
ADG (kg day^{-1})	0.41	0.46	0.50	0.47	0.48	0.04	>0.05	-
Feed efficiency[3]	0.39	0.40	0.44	0.42	0.40	0.02	>0.05	-

[1]Period 1 = Day 1-34, Period 2 = Day 35-41; Period 3 = Day 42-55; [2]Total DMI = Starter DMI+Milk DMI, [3]Feed efficiency = Gain/Feed

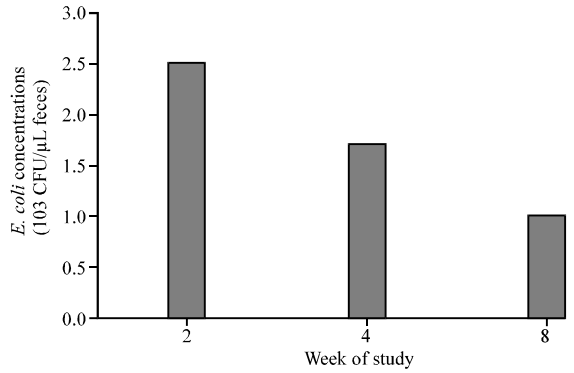

Fig. 2: Fecal *E. coli* concentrations of Holstein heifers fed CX, YST, β-glucan or some combination

rectal temperatures ($p \geq 0.05$) among any treatment groups. These observations were similar to Galvao *et al.* (2005) who found the addition of live yeast products in calf diets had no effect ($p > 0.10$) before or after weaning. In contrast, Heinrichs *et al.* (2003), Hill *et al.* (2009) and Magalhaes *et al.* (2008), found calves fed MOS or yeast had a higher probability for more normal feces, lower average fecal scores and fewer days scouring. Similar fecal coccidia scores ($p = 0.77$) were observed between treatment groups from weeks 0-8. The findings are different from Heinrichs and Bush (1990) Heinrichs *et al.* (1991) and Waggoner *et al.* (1994) who all found differences in coccidia shedding in calves treated with a anticoccidial with shedding rates being greater for calves treated compared to control. Among all 40 calves in this study, cortisol concentrations ranged from 0.22-126.19 ng mL^{-1} over the course of the trial while there were no differences between treatments ($p = 0.92$) the effect of week, was significantly different ($p < 0.01$) over the 8 weeks course of the trial. These findings are similar to those of Cummins and Brunner (1991) who conducted a housing trial with colostrum-deprived Holstein calves. No differences ($p \geq 0.05$) were observed in blood nitrate or nitrite concentrations among treatment groups. Heifers fed YST or YST+β-glucan had higher ($p = 0.02$ and $p < 0.01$, respectively) fecal concentrations of *E. coli* compared to heifers fed CX, CX+YST or β-glucan alone. Fecal *E. coli* concentrations were lower ($p < 0.05$) in week 8 when compared to week 2 and 4 (Fig. 2). This decrease in shedding may be a result of weaning when additives were no longer included in the diet.

CONCLUSION

Based on the results found, feeding these products at the given dosages, had no beneficial impact on growth and health performance which includes feed intake and efficiency, fecal and respiratory health and blood parameters, however these products had no negative effects on calf performance and are comparable to performance of calves supplemented with coccidiostats. All calves were healthy throughout the duration of the trial and no clinical signs of coccidiosis or respiratory infections were observed. Feeding these products may serve as a viable source of gut health enhancers, therefore reducing the need for non-therapeutic antibiotic use. The *E. coli* shedding results in the current study may be an indication of the gram (-) binding and non-digestible properties of YST which increased *E. coli* shedding, serving as a suitable product to aid in balancing gut microflora which ultimately leads to a healthy, productive calf. Further investigation is needed to determine the optimum dose and feeding method (example: Milk/milk replacer or starter grain) of these products to maximize efficacy while being an economical solution for producers to increase calf productivity.

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