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THE EFFECTS OF CLOSTAT® (*BACILLUS SUBTILIS* PB6) SUPPLEMENTATION ON PRODUCTION PARAMETERS IN TRANSITION DAIRY COWS[1]

INTRODUCTION

The weeks surrounding parturition are critical in determining the productivity, sustainability, and welfare of a dairy cow. It has been well established that all cows, even clinically healthy ones, experience some degree of postpartum inflammation.[2,3,4] The most prominent sources of inflammation in the transition cow stem from the uterus, mammary gland, and gastrointestinal tract.[4] Further, the gastrointestinal tract is continuously exposed to toxins and pathogens[5], and microbial exposure is likely more extensive in large ruminant animals due to the overall size and microbial load of the tract. Thus, the importance of transition period nutrition and management programs to minimize inflammation, reduce pathogen load, and ultimately increase production and welfare of the dairy cow are critical during this delicate time.

Transition cow health may potentially be optimized with direct-fed microbials (DFM). Direct-fed microbials are organisms that can alter the microbiota by establishing beneficial microflora or competitively excluding opportunistic pathogens.[6,7] While the concept of DFMs is not new, they have been used as prophylactic agents against pathogens by modulating host immunity or balancing the microbiota.[8] In particular, *Bacillus subtilis* PB6 is a bacterial DFM, which is fed as a spore that bypasses the rumen and activates in the small intestine and lower gut when stimulated with a low pH and the presence of bile salts.[9] Upon supplementation, *Bacillus subtilis* PB6 decreases fecal counts and gastrointestinal concentrations of *Salmonella* in steers.[10,11] Additionally, feeding *Bacillus subtilis* PB6 improved gastrointestinal health and reduced the inflammatory response in a model of inflammatory bowel disease.[12] Promoting gastrointestinal health via DFM supplementation could be beneficial to the transitioning dairy cow by reducing the pathogen load and ultimately minimizing the risk of gut-derived inflammation. Therefore, study objectives were to evaluate the effects *Bacillus subtilis* PB6 supplementation on the inflammation, gut permeability, fecal metrics, energetic metabolism, and production parameters in transition dairy cows.

MATERIALS & METHODS

All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee (20-138). Forty-eight multiparous Holstein cows calving between November 2020 and January 2021 were stratified by previous 305 ME and parity and assigned to 1 of 2 top-dressed dietary treatments 21 d before expected calving: 1) CON (13 g/d calcium carbonate; n = 24) or 2) CST (13 g/d *Bacillus subtilis* PB6; CLOSTAT® Kemin Industries, Des Moines, IA; n = 24). Cows remained on their respective postpartum dietary treatment for the remainder of the study (i.e., 63 DIM). Immediately postpartum, cows were milked and processed according to standard operating procedures implemented at the Iowa State University Dairy Farm and moved into a freestall lactation pen. Pre and postpartum cows were individually fed a diet that was formulated to meet or exceed the predicted requirements[13] (Table 1 and 2) of energy, protein, minerals and vitamins. Samples were obtained weekly and composited into a monthly sample for nutrient analysis (Dairyland Laboratories Inc., Arcadia, WI).

Cows were milked twice daily (~1,100 and 2200 h) throughout the experiment and yield was recorded using the BouMatic SmartDairy® parlor system and reported by the PCDart dairy management software (DRMS) at the Iowa State University Dairy Farm. Throughout the experiment, samples for milk composition were obtained every Monday and Thursday from the 1,100 h milking (BouMatic SmartControl™ metering system).

Prepartum body weight (BW) and body condition scores (BCS) were obtained once weekly. Postpartum BW and BCS were obtained every Monday and Thursday following the 1,100 h milking. Body weight and BCS were condensed into weekly means throughout the

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duration of the experiment. Body condition scores for each cow were determined utilizing Wildman et al. scoring system but reported in 0.25-unit increments.[15]

RESULTS & DISCUSSION

Overall, prepartum DMI did not differ between CST and CON fed cows ($P > 0.97$; Table 3). As expected, postpartum DMI substantially increased from week 1 to 9 (47%; $P < 0.01$, Figure 1). Supplementing CST decreased postpartum DMI relative to CON (1.43 lb.; $P = 0.05$), and this became more pronounced after the 5th week of lactation. Milk yield progressively increased from week 1 to 5 (62%) for both treatments and remained steady from week 5 to 9 ($P < 0.01$). CST supplementation increased milk yield relative to CON (3.57 lb.; $P < 0.01$; Table 3), and this effect was consistent throughout the 9-week experiment. Compared to CON cows, dietary supplementation of CST increased ECM (Figure 2), FCM and SCM throughout the postpartum period (3.57, 3.59, 3.35 lb., respectively; $P \leq 0.02$). Feed efficiency of MY, ECM (Figure 3), FCM, and SCM were all increased in CST-fed cows relative to CON (6, 5, 5, and 5%, respectively; $P < 0.01$; Table 3).

Overall, there were no treatment differences detected for concentrations of milk fat, protein, total solids, SCC or SCS ($P > 0.18$; Table 4). Milk lactose and MUN concentrations were decreased in CST cows relative to CON (1 and 5%, respectively; $P \leq 0.02$; Table 4). Milk protein and lactose yield were increased with CST supplementation (5 and 2%, respectively; $P \leq 0.01$).

No dietary effects were observed for pre or postpartum BW and BCS ($P > 0.57$; Table 5). Body weight and BCS change from week 1 to 9 were not different among treatments ($P > 0.48$). Prepartum energy balance (Ebal) was similar between treatments ($P > 0.87$; Table 6, Figure 27); however, it declined from week -3 to -1 (39%; $P < 0.01$). Postpartum Ebal was decreased in CST cows compared to CON (2.32 Mcal/d; $P = 0.02$), likely attributed to the increased MY and decreased DMI. Additionally, postpartum Ebal decreased from week 1 to 2 (47%) before increasing 2-fold from week 2 to 9 ($P < 0.01$).

In general, CST supplemented cows increased milk yield thus resulting in improved feed efficiency of MY and ECM. Regardless of treatment, DMI significantly increased postpartum. The CST cattle had a slight decrease in DMI starting at 5 weeks postpartum compared to CON. This reduction in DMI did not reflect in lower ECM but improved feed efficiency as ECM was consistently improved through the experimental period.

Table 1. Formulated ingredients and composition of diet[1]

	% DM	
Ingredient	Prepartum[2]	Postpartum[3]
Corn Silage	26.95	43.00
Straw	16.40	-
Alfalfa Hay	12.84	15.67
Molasses	5.71	-
Ground Corn	4.60	16.53
Soybean Meal	3.42	3.66
SoyPlus®	-	4.88
Postpartum VTM Mix	-	8.42
Corn Gluten Feed	-	7.84
Prepartum VTM Pellet	30.08	-
Chemical analysis, % of DM		
Starch	19.94	29.33
CP	13.60	15.65
NDF	40.65	27.28
ADF	27.73	19.75
NE_L Mcal/lb DM	1.47	1.65

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[1]Values represent an average of ration nutrient summary reports collected throughout the trial. Pre and postpartum diet dry matter averaged 62.0 and 54.8%, respectively
[2]Average prepartum nutrient levels: 2.91% fat, 1.26% Ca, 0.33% P, 0.39% Mg, 0.39% S, 1.74% K, 0.15% Na, 0.76% Cl, 112 ppm of Zn, 91 ppm of Mn, 372 ppm of Fe, 16 ppm of Cu, 17 ppm of Boron, 477 ppm Al
[3]Average postpartum nutrient levels: 4.39% fat, 1.03% Ca, 0.33% P, 0.27% Mg, 0.16% S, 1.31% K, 0.46% Na, 115 ppm of Zn, 63 ppm of Mn, 248 ppm of Fe, 18 ppm of Cu, 15 ppm of Boron, 232 ppm Al

Table 2. Ingredients of prepartum VTM pellet and postpartum VTM Mix[1]

	% of DM	
Ingredient	Prepartum VTM Pellet	Postpartum VTM Mix
Wheat Midds	22.63	-
SurePro®	21.67	19.45
Ground Soy Hulls	13.92	-
Dehulled Soymeal	13.32	-
Animate®	9.28	-
Calcium Carbonate	7.86	15.36
Ground Corn	4.7	-
Magnesium Sulfate	1.9	-
Calcium Sulfate	1.3	-
Mono-Dical Phos	0.7	-
Choice White Grease	0.62	1.23
Magnesium Oxide	0.52	2.65
Salt	0.26	4.71
Super-Bind® BG	0.78	-
Trace Mineral Package	0.20	-
Selenium PX 0.16%	0.10	-
Vit E 227 IU/LB	0.10	-
Biotin/Vit A/DY VT4X	0.11	0.05
Rumensin®	0.03	0.07
Blood Meal	-	13.80
Pork Meat and Bone Meal	-	9.62
Palmit 80®	-	13.24
Sodium Bicarbonate	-	11.24
Soybean Meal	-	3.07
Dairy Micro w/ Se[2]	-	2.33
Urea	-	2.44
Smartamine® M	-	0.74

[1]Pre and postpartum dry matter: 89.4 and 94.3% respectively
[2]Dairy micro premix with organic Se

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Table 3. Effect of CLOSTAT® (*Bacillus subtilis* PB6) on production parameters in transition dairy cows

	Treatment[1]			*P*		
Parameter	CON	CST	SEM	Trt	Time	Trt × Time
DMI, lb/d	58.43	57.00	0.55	0.05	<0.01	0.62
Milk Yield, lb/d	103.70	107.27	0.68	<0.01	<0.01	0.10
ECM[2], lb/d	122.08	126.11	0.97	<0.01	<0.01	0.50
DMI, lb/d	58.43	57.00	0.55	0.05	<0.01	0.62
Milk Yield, lb/d	103.70	107.27	0.68	<0.01	<0.01	0.10
FCM[3], lb/d	57.40	59.03	1.15	0.02	<0.01	0.61
SCM[4], lb/d	50.87	52.39	0.40	<0.01	<0.01	0.52
Feed Efficiency						
MY/DMI	1.80	1.90	0.02	<0.01	<0.01	0.27
ECM/DMI	2.13	2.25	0.03	<0.01	<0.01	0.31
FCM/DMI	2.21	2.32	0.03	<0.01	<0.01	0.35
SCM/DMI	1.96	2.06	0.02	<0.01	<0.01	0.31

[1]CON = 13 g/d calcium carbonate (n = 24); CST = 13 g/d CLOSTAT® (*Bacillus subtilis* PB6; n = 24)
[2]Energy corrected milk
[3]Fat corrected milk
[4]Solids corrected milk

Table 4. Effect of CLOSTAT® (*Bacillus subtilis* PB6) on milk composition parameters in transition dairy cows

	Treatment[1]			*P*		
Parameter	CON	CST	SEM	Trt	Time	Trt × Time
Milk Composition						
Fat, %	4.85	4.81	0.07	0.58	<0.01	0.73
Fat, lb	5.03	5.16	0.06	0.14	<0.01	0.75
Protein, %	3.08	3.14	0.03	0.18	<0.01	0.98
Protein, lb	3.20	3.37	0.04	<0.01	<0.01	0.20
Lactose, %	4.76	4.73	0.02	0.02	<0.01	0.71
Lactose, lb	4.94	5.07	0.02	0.01	<0.01	0.11
Total Solids, %	13.96	13.92	0.08	0.72	<0.01	0.44
MUN, mg/dL	10.86	10.36	0.15	0.01	<0.01	0.51
SCC, ×1000	73	112	29	0.32	0.66	0.93
SCS[2], log	2.60	2.89	0.20	0.28	<0.01	0.98

[1]CON = 13 g/d calcium carbonate (n = 24); CST = 13 g/d CLOSTAT® (*Bacillus subtilis* PB6; n = 24)
[2]Somatic cell score

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Table 5. Effects of CLOSTAT® (*Bacillus subtilis* PB6) on body composition in transition dairy cows

	Treatment[1]			*P*		
Parameter	CON	CST	SEM	Trt	Time	Trt × Time
Body Weight, lb						
Prepartum	1747	1787	37	0.97	<0.01	0.85
Postpartum	1536	1562	33	0.57	<0.01	0.68
BCS[2]						
Prepartum	3.68	3.68	0.09	0.99	0.16	0.34
Postpartum	3.39	3.41	0.08	0.86	<0.01	0.99
BW Change[3], lb	-211	-224	15	0.83	-	-
BCS Change[4]	-0.29	-0.27	0.05	0.48	-	-
Ebal[5], Mcal/d						
Prepartum	7.98	8.12	0.67	0.87	<0.01	0.65
Postpartum	-4.99	-7.31	0.71	0.02	<0.01	0.94

[1]CON = 13 g/d calcium carbonate (n = 24); CST = 13 g/d CLOSTAT® (*Bacillus subtilis* PB6; n = 24)
[2]Body condition score
[3]Body weight change wk 1 - 9
[4]Body condition score change wk 1 - 9
[5]Energy balance

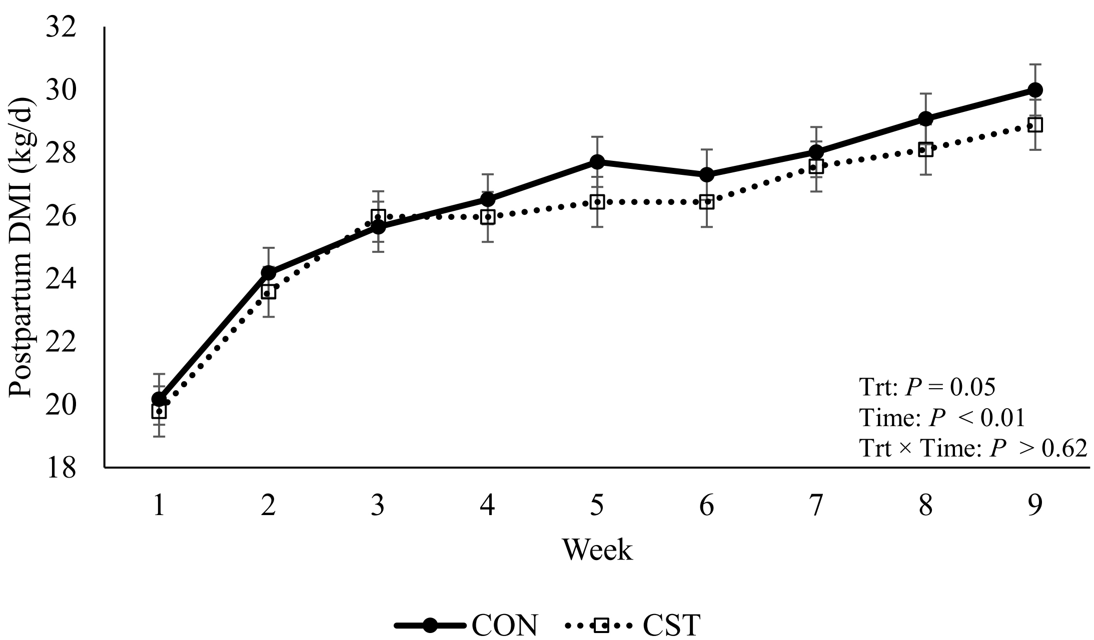

Figure 1. Effect of CLOSTAT® (*Bacillus subtilis* PB6) on postpartum dry matter intake (DMI)

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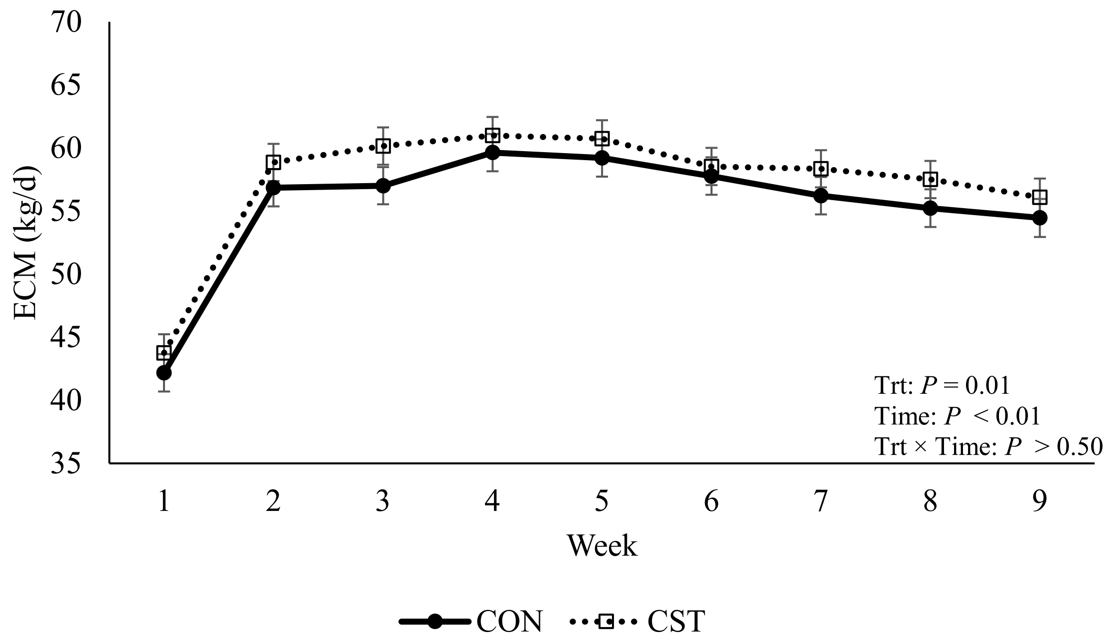

Figure 2. Effect of CLOSTAT® (*Bacillus subtilis* PB6) on postpartum energy corrected milk (ECM) yield

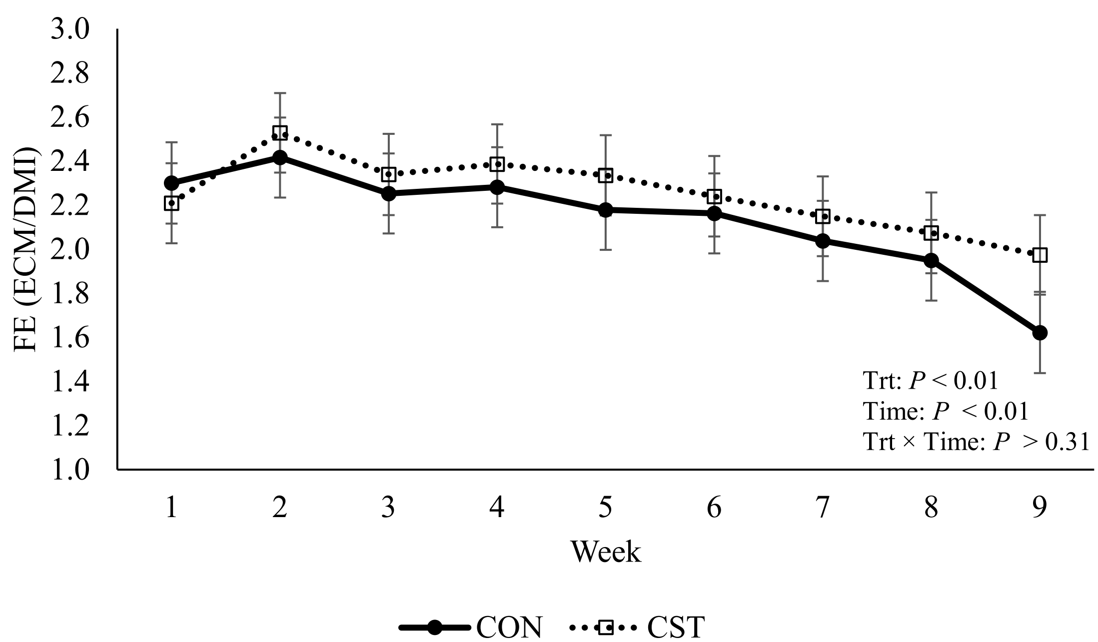

Figure 3. Effect of CLOSTAT® (*Bacillus subtilis* PB6) on postpartum feed efficiency (FE; ECM/DMI)

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