

The impact of direct-fed microbials and enzymes on the health and performance of dairy cows with emphasis on colostrum quality and serum immunoglobulin concentrations in calves

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Summary

Thirty-six cows were blocked by calving date and randomly assigned to one of three treatments. Cows were on treatments 3 weeks prepartum through 8 weeks postpartum. Treatments were as follows: (i) no direct-fed microbial (DFM) or cellulase and amylase enzymes (C), (ii) 45.4 g/day of DFM (D) or (iii) 45.4 g/day of DFM and 18.2 g/day of enzyme (DE). Total mixed ration fed and refused were measured daily to determine dry matter intake (DMI). Blood samples were taken three times weekly and analysed for β -hydroxybutyrate, glucose and non-esterified fatty acids. Body weight (BW) was measured weekly. Colostrum was weighed and analysed for IgA and IgG concentration. Calves were fed 4 L of colostrum within 2 hr of birth. Calf blood samples were taken at 0 and 24 hr for analysis of IgA and IgG concentrations and apparent efficiency of absorption. Milk yield was measured daily and samples collected weekly. Initial BW was different among treatments with D being lesser than C or DE treatments. Body weight, weight gain, efficiency of gain, DMI and blood parameters were unaffected. Treatment did not affect colostrum yield. Ash percentage of colostrum tended to increase with D and DE, while IgA and total solids yield decreased with D. Colostrum fat yield was decreased in D and DE. Treatments did not impact BW, serum IgA and IgG concentrations or apparent efficiency of absorption of calves. Post-partum BW, DMI, blood parameters, milk production and composition were unaffected by treatment. However, cows on D gained more BW and tended to have greater efficiency of gain compared to those on DE, but were similar to C. Somatic cell scores were greatest for D. Results indicate that DFM and enzyme supplementation did not improve health and performance of dairy cattle during the pre- and post-partum periods under conditions of this study.

KEYWORDS

colostrum, direct-fed microbials, enzymes, immunoglobulin A, immunoglobulin G

1 | INTRODUCTION

Direct-fed microbials (DFM) and probiotics are often used interchangeably, but the U.S. Food and Drug Administration defines

DFM as “products that are purported to contain live (viable) microorganisms (bacteria and/or yeast)” (FDA, 2015). Claimed benefits of DFM supplementation to dairy cattle include increased dry matter intake (DMI), ruminal total volatile fatty acid (VFA) production

and milk yield, and decreased β -hydroxybutyrate (BHB) and non-esterified fatty acid (NEFA) concentrations post-partum (Nocek & Kautz, 2006; Nocek, Kautz, Leedle, & Block, 2003; Oetzel, Emery, Lautz, & Nocek, 2007; Wohlt, Finkelstein, & Chung, 1991). In calves, DFM supplementation increased DMI, average daily gain (ADG), body weight (BW), feed efficiency, serum immunoglobulin G (IgG) concentrations and white blood cell counts (Al-Saiady, 2010; Clymer, Daniels, Smalley, Richeson, & Madere, 2015; Jatkauskas & Vrontniakiene, 2010; Zhang et al., 2016), and decreased diarrhoea, fever and cost of treatments (Magalhães et al., 2008). Enzyme supplementation has been reported to increase milk production efficiency, milk protein content, digestibility of acid detergent fibre (ADF), crude protein (CP), dry matter (DM), neutral detergent fibre (NDF) and starch, and total ruminal VFA production (Beauchemin, Yang, & Rode, 1999; Nozière, Steinberg, Silberberg, & Morgavi, 2014; Rode, Yang, & Beauchemin, 1999; Yang, Beauchemin, & Rode, 2000). Based on these findings, it is suggested that DFM and enzymes can impact health and performance of dairy cattle. However, no studies have evaluated the effect of prepartum DFM and enzyme supplementation of cows on colostrum quality and passive transfer of immunoglobulin to calves. Calves are born agammaglobulinaemic because of the synepitheliochorial placenta, which prevents transfer of immunoglobulin in utero (Akers, 2002). As a result, cattle have evolved to transfer immunoglobulin via colostrum.

There are contrary results with supplementation of DFM to animals. For example, Al-Saiady (2010) reported that bull calves fed probiotics had greater serum IgG concentrations than those on the control. Studies with poultry have also indicated that DFM can increase IgG and IgM concentrations and/or cells responsible for IgA, IgG and IgM production (Lee, Lillehoj, & Siragusa, 2010). However, Marakoudakis et al. (2010) found no impact of DFM on plasma IgA, IgG and IgM concentrations in dairy goats, but immunoglobulin in the mammary secretions was not evaluated. Queszada-Mendoza, Heinrichs, and Jones (2011) found no effect of probiotics on plasma, faecal or salivary IgG concentrations in calves. These differing outcomes suggest that there is potential for DFM to increase IgG content in dairy cattle colostrum, which could impact the health of the calf through more IgG available for absorption, as well as impact the health and production of the cow when fed post-partum.

The objectives of this study were to evaluate the impact of feeding DFM and enzymes (amylase and cellulase) to dairy cows on: (i) the quantity and composition of the colostrum with focus on IgG and IgA; (ii) calf BW and IgG uptake; (iii) DMI both pre- and post-partum; (iv) blood glucose, BHB and NEFA concentrations; and (v) the quantity and quality of the milk produced during the first 8 weeks of lactation. Our hypothesis was that feeding DFM alone or in combination with enzymes during the transition period would increase colostrum IgG and IgA, DMI pre- and post-partum, milk production, and serum concentrations and apparent efficiency of absorption of IgG and IgA in the calf.

2 | MATERIALS & METHODS

2.1 | Experimental and treatment design

This experiment was reviewed and approved by the University of New Hampshire Institutional Animal Care and Use Committee (Protocol #141107).

Thirty-six multiparous Holstein cows were used in this study. They were grouped into 12 blocks based on expected calving date. Parity number ranged from 2 to 7. There were 12, 11, seven, three and two cows in their second, third, fourth, fifth and sixth lactation respectively. Within each block, cows were randomly assigned to one of three treatments: (i) 0 g/day DFM and enzymes (C); (ii) 45.4 g/day of DFM product (D); and (iii) 45.4 g/day of D plus 18.2 g/day of enzyme product (DE). The DFM (D) contained *Enterococcus faecium* and *Saccharomyces cerevisiae* at 1.323 billion cfu/g. The enzyme (E) was a combination of both DFM and enzymes, which contained 0.88 billion cfu/g of *E. faecium* and *S. cerevisiae*. The enzyme portion of E contained both amylase and cellulase. The ingredients of D were calcium carbonate, rice hulls, active dry yeast, mineral oil, fructooligosaccharides, dried *E. faecium* fermentation product, sodium silico aluminate, and natural and artificial flavours. The ingredients of E contained rice hull extract, sodium silico aluminate, dried *Trichoderma longibrachiatum* fermentation extract, dried *Bacillus subtilis* fermentation extract, dried *Aspergillus niger* fermentation extract, torula dried yeast, dried *E. faecium* fermentation product, *Yucca schidigera* extract, riboflavin, calcium gluconate, nicotinic acid, biotin, pyridoxine hydrochloride, thiamine mononitrate, vitamin B₁₂, citric acid, natural and artificial flavours, and mineral oil. The experimental treatments were being evaluated for combination and for eventual commercial application.

Cows began the study 3 weeks prior to the expected calving date and continued through 8 weeks of lactation. All feed ingredients and nutrient composition are reported in Table 1. Feed ingredients and nutrient composition of the prepartum, transition totally mixed ration (TMR) and TMR are found in Tables 2, 3, 4 and 5 respectively. Orts analyses are found in Tables 6, 7 and 8. Nutrient composition of Orts indicated that there were similar values and little sorting by treatment. All cows were fed the prepartum TMR prior to calving and then switched to the transition TMR immediately post-partum, which helped them to transition to the post-partum TMR at 2 weeks after calving. All cows were kept in tie stalls with mattresses and bedded with kiln dried sawdust from day -21 to 56. Each cow was fed in separate wooden feed tubs (90 × 90 × 90 cm). Three days prior to the expected calving date, cows were moved to individual maternity pens (4.3 × 3.7 m) until parturition and placenta delivery. At all times, the cows had access to automated water bowls (Delaval, Tumba, Sweden).

2.2 | Cow measurements

Daily DMI was recorded for each cow throughout the study. Samples of TMR and Orts were taken each day and then frozen at -20°C until

TABLE 1 Feed ingredients and nutrient composition

Nutrient composition	DM, % \pm SD		DM, %						
	Corn silage	Grass silage	RUP mix (Provaal elite) ^a	Soya/Urea mix ^b	Energy mix ^c	Fat supplement ^d	Dry cow mix ^e	Pre-fresh mineral mix ^f	Lactation mineral ^g
Dry matter (DM) %	32.5 \pm 4.7	35.4 \pm 4.7	90.0	88.6	84.3		89.7	94.9	93.3
Crude protein %	7.5 \pm 0.4	16.8 \pm 1.0	93.0	47.1	7.8		19.9	14.3	3.9
RUP %	6.0 \pm 0.3	13.6 \pm 0.9			3.9		7.4		
Acid detergent fibre %	24.5 \pm 3.0	35.1 \pm 4.7		8.3	12.5		13.9		
Neutral detergent fibre %	40.1 \pm 5.3	50.2 \pm 5.1		5.8	21.8		23.7		
Non-fibre carbohydrate %				25.4	63.9		33.4		
Starch %	35.0 \pm 5.9	2.4 \pm 0.7		5.8	47.3		16.3		
Sugar %	0.7 \pm 0.2	4.1 \pm 0.7		11.7	8.3		5.8		
Fat %	3.2 \pm 0.3	4.2 \pm 0.5	2.4	2.6	3.5	99	2.3		
Lignin %	2.8 \pm 0.4	5.9 \pm 1.3			2.1				
Ash %	3.5 \pm 0.7	9.9 \pm 0.8	7.1	2.6	3.0				
Ca %	0.2 \pm 0.03	0.7 \pm 0.10	0.7	0.5	0.4		1.8	15.3	13.0
P %	0.2 \pm 0.02	0.3 \pm 0.02	0.1	0.7	0.2		0.3	1.6	2.7
Mg %	0.1 \pm 0.02	0.2 \pm 0.02	0.2	0.4	0.2		1.2	3.3	4.7
K %	1.0 \pm 0.22	2.8 \pm 0.26	0.2	1.9	0.6		1.1	0.6	5.4
Na %			0.2	0.05	0.04		0.6	0.6	8.4
Cl %			0.2	0.08	0.2		1.6	3.5	9.5
S %	0.1 \pm 0.01	0.3 \pm 0.02	1.4	0.4	0.2		1.0	2.8	0.6
Mn (mg/kg)			8.7	40.7	27.9			1,388	1,665
Fe (mg/kg)			2,315	174	328.6			2,815	4,024
Cu (mg/kg)			8.9	13.5	5.5			288	409
Zn (mg/kg)			35	53.5	21.7			1,420	2,240

^aRUP mix (Provaal elite, Perdue, Kings Mountain, NC).

^bSoya/urea mix (Poulin grain, VT) contained 7.3% distillers grain, 69.1% soya bean meal, 21.8% canola meal and 1.8% urea.

^cEnergy mix (Poulin Grain, VT) contained 4.1% molasses, 45.8% corn meal, 14.7% stream flaked corn and 35.3% whole beet pulp.

^dBergafat F-100 (Berg + Schmidt Functional Lipids, Liberty, IL) contained 100% vegetable oils.

^eDry cow mix (Poulin grain, VT) contained 10.9% beet pulp, 21.9% soya hulls, 3.1% molasses, 14.9% soya bean meal, 1.2% salt, 1.9% calcium carbonate, 1.9% magnesium oxide, 2.6% calcium sulphate, 0.7% Vitamin E 20000, 0.2% Poulin dairy vitamin premix, 21.4% corn meal, 0.5% Rumensin 90, 8.1% Amino Plus, 0.5% Dimune trace pack, 1.1% magnesium sulphate and 9.5% Biochar.

^fPre-fresh mineral mix (Agri-King, Inc., Fulton, IL) contained 18.1% soya bean meal, 0.1% Rumensin, 1.8% magnesium oxide, 1.2% feed bond, 2.6% dicalcium phosphate, 23.4% Soya Chlor, 2.6% calcium sulphate, 6.5% magnesium sulphate, 28.0% calcium (38%), 1.5% salt and 9.8% Dry cow micro pack.

^gLactation mineral mix (Agri-King, Inc., Fulton, IL) contained 12.2% corn distillers, 0.1% Rumensin 90, 0.1% magnesium oxide, 1.1% feed bond, 16.7% sodium bicarbonate, 13.9% dical 18.5, 26.4% calcium (38%), 9.1% potassium chloride, 6.8% salt, 1.1% selenium yeast 600 and 4.9% dairy max.

further processing. All samples were then dried at 55°C in forced air ovens for 72 hr (Binder Inc., Bohemia, NY, USA and 1380 FM, VWR Scientific, Radnor, PA, USA). Orts were composited weekly by cow, and TMR samples were composited by week. Both Orts and TMR samples were ground to pass through a 1-mm sieve using Wiley mills (Thomas Wiley Laboratory Mill Model 4, Thomas Scientific, Swedesboro, NJ, USA and Wiley Mill Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA, USA). All samples were then subsampled and shipped to Agri-King, Inc. (Fulton, IL, USA) for nutrient analysis. The samples were analysed for moisture and DM (method 935.29,

AOAC International, 1999), ADF (method 973.18, AOAC International, 1999), NDF (method 2002.04, AOAC International, 1999), CP (method 990.03, AOAC International, 1999), soluble protein (Krishnamoorthy, Muscato, Sniffen, & Van Soest, 1982), heat damaged protein (method 973.18 & 976.06, AOAC International, 1999), neutral detergent insoluble protein (method 2002.04 (minus sodium sulphite) and 976.06, AOAC International, 1999), ash (method 942.05, AOAC International, 1999), fat (method 920.39, AOAC International, 1999), starch (Enzymatic method analysed on RFA using Glucose Trinder; Glucose Reagent Set, Amresco, Solon, OH, USA) and lignin (method 973.18,

TABLE 2 Prepartum TMR composition – Diet 1^a

Ingredient	Dry matter (%)
Corn silage	42.0
Grass silage	26.5
RUP mix (Provaal elite) ^b	2.5
Energy mix ^c	18.8
Soya/Urea mix ^c	3.4
Pre-fresh mineral mix ^d	6.8
Nutrient composition	Dry matter, % ±SD
Crude protein %	15.8 ± 1.4
Acid detergent fibre %	26.0 ± 2.3
Neutral detergent fibre %	42.3 ± 3.2
Non-fibre carbohydrate %	34.7 ± 2.5
Starch %	18.5 ± 2.7
Fat %	2.8 ± 0.3
Lignin %	3.2 ± 0.8
Ash %	8.1 ± 0.7
Ca %	0.7 ± 0.16
P %	0.3 ± 0.04
Mg %	0.6 ± 0.12
K %	1.8 ± 0.26
Na %	0.2 ± 0.07
Cl %	0.8 ± 0.16
S %	0.4 ± 0.08
Mn (mg/kg)	80.4 ± 22.2
Fe (mg/kg)	313.2 ± 85.5
Cu (mg/kg)	17.3 ± 5.6
Zn (mg/kg)	90.2 ± 35.9

^aThis TMR was fed to the prepartum cows for the first 2 months of the study, but was discontinued after 3 cases of hypocalcaemia.

^bPerdue, Kings Mountain, NC, USA.

^cPoulin Grain, Newport, VT, USA.

^dAgri-King, Inc., Fulton, IL, USA.

AOAC International, 1999). Samples were also analysed for Ca, P, Mg, K, Na, Fe, Cu, Zn, Mn (method 985.01) and S (method 923.01, AOAC International, 1999), Cl (method 915.01, AOAC International, 1999), Co (method 2006.03, AOAC International, 1999), Cr (Binnerts, Van't Klooster, & Treno, 1968).

Blood samples were taken from the cows every Monday, Wednesday and Friday at 11:00 hours starting from day -21 through 56. Two samples were taken from the coccygeal vein of each cow using 22-gauge needles and 10-ml vacutainer tubes with and without sodium heparin (Covidien LLC, Mansfield, MA, USA). The serum samples were allowed to clot and centrifuged at 1,388 g (5430 R Eppendorf, Hauppauge, NY, USA) at 4°C for 20 min. Samples were then frozen at -20°C. Plasma samples were taken in 10-ml vacutainer tubes with sodium heparin and then centrifuged at 1,388 g (5430 R Eppendorf, Hauppauge, NY, USA) at 4°C for 20 min. Both plasma and serum samples were then stored at -20°C until shipped to Agri-King (Fulton, IL,

TABLE 3 Prepartum TMR composition – Diet 2^a

Ingredient	Dry matter (%)
Corn silage	40.1
Grass silage	25.5
RUP mix (Provaal Elite) ^b	2.4
Energy mix ^c	1.0
Dry cow mix ^c	30.0
Soya urea mix ^c	1.0
Nutrient composition	Dry matter, % ±SD
Crude protein %	15.8 ± 1.4
Acid detergent fibre %	26.0 ± 2.3
Neutral detergent fibre %	42.3 ± 3.2
Non-fibre carbohydrate %	34.7 ± 2.5
Starch %	18.5 ± 2.7
Fat %	2.8 ± 0.3
Lignin %	3.2 ± 0.8
Ash %	8.1 ± 0.7
Ca %	0.7 ± 0.16
P %	0.3 ± 0.04
Mg %	0.6 ± 0.12
K %	1.8 ± 0.26
Na %	0.2 ± 0.07
Cl %	0.8 ± 0.16
S %	0.4 ± 0.08
Mn (mg/kg)	80.4 ± 22.2
Fe (mg/kg)	313.2 ± 85.5
Cu (mg/kg)	17.3 ± 5.6
Zn (mg/kg)	90.2 ± 35.9

^aThe prepartum TMR – diet 2 was fed to prepartum cows starting month 2 of the study to the end of the experiment.

^bPerdue, Kings Mountain, NC, USA.

^cPoulin Grain, Newport, VT, USA.

USA) for NEFA, glucose and BHB analysis via enzyme-linked immunosorbent assay (Wako Chemicals USA, Inc., Richmond, VA, USA). Serum samples from day of parturition were also analysed for IgG by radial immunoassay (Triple J Farms, Bellingham, WA, USA).

Urine samples were collected every Monday, Wednesday and Friday at 11:00 hours starting from day -21 through calving and analysed for pH within 1 hr after collection using either a portable (VWR SP20 SympHony; Thermo Fisher Scientific; Chelmsford, MA, USA) or stationary (Orion Star A214; Thermo Fisher Scientific; Chelmsford, MA, USA) pH meter both calibrated with specific standards.

Colostrum was harvested by machine within 60 min of calving and weighed. One 40 ml colostrum sample was frozen at -20°C until analysed for IgG and IgA by radial immunoassay (Triple J Farms, Bellingham, WA, USA), while the second 40 ml sample was preserved with 2 bromo-2-nitropropan-1, 3 diol and shipped to DairyOne Cooperative, Inc. (Ithaca, NY, USA) for analysis of fat (method 989.05,

TABLE 4 Transition TMR composition^a

Ingredient	Dry matter (%)
Corn silage	49.4
Grass silage	13.6
RUP mix (Provaal elite) ^b	1.0
Lactation mineral mix ^c	3.8
Soya/Urea mix ^d	15.5
Energy mix ^d	16.7
Nutrient composition	Dry matter (%)
Crude protein %	15.6 ± 1.0
Acid detergent fibre %	24.9 ± 2.2
Neutral detergent fibre %	40.6 ± 3.0
Non-fibre carbohydrate %	36.9 ± 2.7
Starch %	22.5 ± 2.9
Fat %	2.8 ± 0.3
Lignin %	3.1 ± 0.6
Ash %	7.6 ± 0.5
Ca %	0.6 ± 0.08
P %	0.4 ± 0.03
Mg %	0.4 ± 0.04
K %	1.6 ± 0.12
Na %	0.4 ± 0.06
Cl %	0.7 ± 0.10
S %	0.2 ± 0.02
Mn (mg/kg)	93.3 ± 17.1
Fe (mg/kg)	307.5 ± 50.0
Cu (mg/kg)	21.5 ± 4.6
Zn (mg/kg)	97.0 ± 14.5

^aTransition TMR was fed the first 2 weeks after parturition.

^bPerdue, Kings Mountain, NC, USA.

^cAgri-King, Inc., Fulton, IL, USA.

^dPoulin Grain, Newport, VT, USA.

AOAC International, 2006), total protein (method 991.20, AOAC International, 2006), total solids (method 990.20, AOAC International, 2006), ash (method 942.05) and lactose (calculated by the following equation: % total solids – % fat – % total protein – % ash).

All cows were milked twice a day at 05:00 and 16:00 hours, and weights were recorded at each milking until day 56. Milk samples were taken at 16:00 hours every Tuesday and 05:00 hours every Wednesday. These a.m. and p.m. milk samples were composited by the respective yields for each milking for each cow. All samples were placed into 40-ml vials containing 2 bromo-2-nitropropan-1, 3 diol and stored at 4°C until shipped to DairyOne Cooperative, Inc. (Ithaca, NY, USA). Samples were analysed for fat, protein, ash, lactose, milk urea nitrogen (MUN) and somatic cell count (SCC) using mid-infrared reflectance spectroscopy (Foss MilkoScan 4000, Foss Electric, Hillerød, Denmark) and converted to somatic cell score (SCS, DairyOne Cooperative, Inc.).

Body weight was also recorded every Friday from day –21 to 56 for each cow using a platform scale (Cardinal Scale Manufacturing Co.,

TABLE 5 TMR composition^a

Ingredient	Dry matter (%)
Corn silage	43.1
Grass silage	7.1
Fat supplement ^b	1.0
RUP mix (Provaal Elite) ^c	1.7
Lactation mineral mix ^d	4.4
Soya/Urea mix ^e	16.0
Energy mix ^e	26.7
Nutrient composition	Dry matter, % ±SD
Crude protein %	15.7 ± 1.3
Acid detergent fibre %	22.5 ± 1.9
Neutral detergent fibre %	37.9 ± 2.8
Non-fibre carbohydrate %	38.5 ± 2.6
Starch %	24.6 ± 2.9
Fat %	3.9 ± 0.6
Lignin %	2.7 ± 0.5
Ash %	7.3 ± 0.4
Ca %	0.6 ± 0.09
P %	0.4 ± 0.04
Mg %	0.4 ± 0.04
K %	1.6 ± 0.21
Na %	0.4 ± 0.05
Cl %	0.7 ± 0.05
S %	0.2 ± 0.02
Mn (mg/kg)	101.3 ± 11.4
Fe (mg/kg)	338.6 ± 56.0
Cu (mg/kg)	24.6 ± 4.1
Zn (mg/kg)	107.1 ± 12.3

^aThe post-partum high TMR was fed from week 2 through week 8.

^bBergafat, Berg + Schmidt functional lipids, liberty, IL.

^cPerdue, Kings Mountain, NC.

^dAgri-King, Inc., Fulton, IL.

^ePoulin Grain, VT.

Hooksett, NH, USA). Calving ease was documented at calving with the scores of 1, 2 or 3 for unassisted calving, some assistance easy calving or assisted difficult calving respectively. There were 37 calves born alive on this study and 32 of those calves were born with a calving score of 1 (10 C, 11 D and 11 DE). Three other calves were born with a calving score of 2 (2 C and 1 D), and two calves were born with a score of 3 (1 C and 1 D).

2.3 | Calf measurements

All calves were removed from the cow prior to nursing immediately after calving or as early as possible. Calves were weighed using a platform scale (Salter Scales, Fairfield, NJ, USA), navel dipped with 7% iodine, and placed into 1 × 2.15 m pens with kiln dried sawdust. Heifer calves received bovine Rota-coronavirus (Calf Guard; Zoetis

TABLE 6 Nutrient composition of prepartum orts from cows fed control, direct-fed microbial (DFM) (D) or DFM + enzymes (DE) treatments during the 21 days prior to parturition

Nutrient composition	Dry matter, % \pm SD		
	Control	D	DE
Crude protein %	13.1 \pm 1.7	13.1 \pm 1.2	13.6 \pm 1.5
Acid detergent fibre %	27.6 \pm 2.6	28.3 \pm 2.0	28.0 \pm 2.4
Neutral detergent fibre %	45.2 \pm 4.0	46.2 \pm 3.8	45.9 \pm 3.9
Non-fibre carbohydrate %	32.5 \pm 4.2	31.1 \pm 3.6	31.1 \pm 3.7
Starch %	19.4 \pm 3.8	18.9 \pm 2.2	17.4 \pm 3.0
Fat %	2.4 \pm 0.4	2.3 \pm 0.5	2.3 \pm 0.4
Lignin %	3.4 \pm 0.8	3.2 \pm 0.7	3.5 \pm 1.0
Ash %	8.8 \pm 1.4	9.6 \pm 1.6	9.2 \pm 1.4
Na %	0.2 \pm 0.1	0.2 \pm 0.06	0.3 \pm 0.06
Mg %	0.5 \pm 0.1	0.6 \pm 0.11	0.6 \pm 0.11
P %	0.3 \pm 0.04	0.3 \pm 0.04	0.3 \pm 0.04
S %	0.4 \pm 0.10	0.4 \pm 0.07	0.4 \pm 0.08
K %	1.7 \pm 0.23	1.7 \pm 0.13	1.7 \pm 0.26
Ca %	0.6 \pm 0.10	0.7 \pm 0.10	0.7 \pm 0.11
Cl %	0.7 \pm 0.20	0.7 \pm 0.26	0.8 \pm 0.15
Mn (mg/kg)	71.8 \pm 23.4	69.1 \pm 21.2	75.0 \pm 23.7
Fe (mg/kg)	288.4 \pm 97.2	307.6 \pm 117.9	358.2 \pm 164.2
Cu (mg/kg)	16.1 \pm 3.9	16.5 \pm 4.1	16.6 \pm 3.1
Zn (mg/kg)	76.3 \pm 31.3	75.0 \pm 32.2	81.0 \pm 27.1

Inc.; Kalamazoo, MI, USA) and *Escherichia coli* vaccinations (Bar-Guard-99; Boehringer Ingelheim; St. Joseph, MO, USA). Blood samples were taken from the jugular vein on the calves at 0 and 24 hr using 22-gauge needles and 10-ml vacutainer tubes (Covidien LLC, Mansfield, MA, USA). Samples were left to clot followed by centrifugation at 1,388 g (5430 R Eppendorf, Hauppauge, NY, USA) at 4°C for 20. Samples were then frozen at -20°C until IgG and IgA radial immunoassays were performed (Triple J Farms, Bellingham, WA, USA). Apparent efficiency of absorption (AEA) of IgG was calculated using the following formula: (Plasma IgG [g/L] \times BW [kg] \times 0.09)/(IgG intake [g]) \times 100 as reported by Quigley, Fike, Egerton, Drewry, and Arthington (1998). Also, AEA of IgA was calculated using the following formula: (Plasma IgA [g/L] \times BW [kg] \times 0.090)/(IgA intake [g]) \times 100.

After taking the 0-hr blood samples, 4 L of maternal colostrum was fed to the calves via bottle or oesophageal tubing. A total of 13 calves did not receive 4 L of maternal colostrum. Six of these 13 calves did not receive the amount of colostrum intended because of difficulties in bottle feeding and stomach tubing 4 L of colostrum. Another three of these 13 calves did not receive 4 L of colostrum due to the cow producing less than the amount intended. Finally, four calves did not receive any maternal colostrum because of dam being leukosis positive or produced <3 L of colostrum. As a result, these four calves did

TABLE 7 Nutrient composition of transition orts from cows fed control, direct-fed microbial (DFM) (D) or DFM + enzymes (DE) treatments

Nutrient composition	Dry matter, % \pm SD		
	Control	D	DE
Crude protein %	13.3 \pm 1.8	12.8 \pm 1.2	13.3 \pm 1.0
Acid detergent fibre %	26.0 \pm 2.7	26.3 \pm 2.3	26.5 \pm 1.6
Neutral detergent fibre %	43.4 \pm 4.1	43.9 \pm 4.1	44.0 \pm 2.2
Non-fibre carbohydrate %	34.6 \pm 4.1	34.4 \pm 3.8	33.9 \pm 2.0
Starch %	22.4 \pm 3.5	22.1 \pm 3.2	21.7 \pm 2.1
Fat %	2.3 \pm 0.4	2.3 \pm 0.3	2.3 \pm 0.3
Lignin %	3.0 \pm 0.9	2.8 \pm 0.4	3.1 \pm 0.7
Ash %	8.4 \pm 1.4	8.5 \pm 1.1	8.5 \pm 0.9
Na %	0.4 \pm 0.07	0.3 \pm 0.06	0.4 \pm 0.09
Mg %	0.3 \pm 0.05	0.3 \pm 0.03	0.3 \pm 0.05
P %	0.4 \pm 0.04	0.4 \pm 0.04	0.4 \pm 0.04
S %	0.2 \pm 0.03	0.2 \pm 0.02	0.2 \pm 0.02
K %	1.6 \pm 0.18	1.5 \pm 0.13	1.7 \pm 0.15
Ca %	0.6 \pm 0.11	0.5 \pm 0.09	0.6 \pm 0.11
Cl %	0.6 \pm 0.22	0.6 \pm 0.08	0.6 \pm 0.24
Mn (mg/kg)	83.0 \pm 16.6	83.0 \pm 13.3	91.4 \pm 18.5
Fe (mg/kg)	288.9 \pm 94.1	310.7 \pm 56.4	392.1 \pm 179.1
Cu (mg/kg)	21.3 \pm 4.7	21.0 \pm 3.3	22.7 \pm 5.3
Zn (mg/kg)	85.8 \pm 14.7	84.8 \pm 14.1	93.8 \pm 18.6

not have any blood samples taken at 24 hr of age as they received good quality colostrum from other cows not on the study or colostrum replacer.

2.4 | Statistical analysis

For all data, differences among treatments were determined using the LSMEANS option for all procedures in SAS 9.4 (2013). Significant interactions and treatment effects were defined as $p \leq .05$ and trends as $.05 \leq p \leq .10$. The covariate structure used was either autoregressive 1, compound symmetry or unstructured and was dependent on which had the least Bayesian information criterion (BIC). The covariate of expected parity was included in the models for all variables with the exception of pH where initial urine pH was used, blood metabolites where pre-treatment blood values were used and prepartum BW, BW gain and feed efficiency where initial BW was used. The covariate was removed from the model if $p > .25$.

2.5 | Prepartum data

Colostrum concentrations of IgG (g/L) and IgA (g/L), as well as concentrations and yields of total protein, fat, lactose and ash, were analysed

TABLE 8 Nutrient composition of post-partum Orts from cows fed control, direct-fed microbial (DFM) (D) or DFM + enzymes (DE) treatments

Nutrient composition	Dry matter, % \pm SD		
	Control	D	DE
Crude protein %	12.8 \pm 1.5	12.5 \pm 1.8	12.3 \pm 1.5
Acid detergent fibre %	24.2 \pm 3.1	24.3 \pm 2.8	25.9 \pm 3.1
Neutral detergent fibre %	40.9 \pm 4.5	41.8 \pm 4.7	44.5 \pm 4.8
Non-fibre carbohydrate %	36.9 \pm 3.9	36.4 \pm 3.6	34.7 \pm 4.0
Starch %	24.2 \pm 3.7	24.3 \pm 3.6	22.5 \pm 4.0
Fat %	3.2 \pm 0.8	3.1 \pm 0.8	2.5 \pm 0.7
Lignin %	2.7 \pm 0.9	2.7 \pm 0.7	3.1 \pm 1.2
Ash %	7.9 \pm 1.2	8.0 \pm 1.1	8.0 \pm 1.6
Na %	0.4 \pm 0.10	0.4 \pm 0.08	0.3 \pm 0.05
Mg %	0.3 \pm 0.05	0.3 \pm 0.06	0.3 \pm 0.06
P %	0.4 \pm 0.06	0.4 \pm 0.05	0.4 \pm 0.04
S %	0.2 \pm 0.04	0.2 \pm 0.03	0.2 \pm 0.05
K %	1.5 \pm 0.26	1.4 \pm 0.13	1.4 \pm 0.15
Ca %	0.6 \pm 0.12	0.5 \pm 0.13	0.6 \pm 0.12
Cl %	0.6 \pm 0.08	0.6 \pm 0.18	0.6 \pm 0.15
Mn (mg/kg)	87.6 \pm 16.1	90.6 \pm 18.8	84.9 \pm 11.4
Fe (mg/kg)	334.0 \pm 99.6	345.5 \pm 119.7	333.3 \pm 87.1
Cu (mg/kg)	21.5 \pm 4.0	23.2 \pm 5.8	24.0 \pm 21.8
Zn (mg/kg)	91.9 \pm 17.7	93.2 \pm 19.3	88.1 \pm 16.2

using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC, USA) according to the following model:

$$Y_{ijk} = \mu + B_i + T_j + P_k + E_{ijk}$$

where Y_{ijk} = the dependent variable, μ = the overall mean; B_i = the random effect of block ($i = 1, \dots, 12$), T_j = the effect of treatment ($j = C, D, DE$), P_k = the covariate measure of expected parity ($k = 2, \dots, 7$) and E_{ijk} = the residual error.

Initial urine pH data were analysed using the MIXED procedure in SAS 9.4 according to the following model:

$$Y_{ij} = \mu + B_i + T_j + E_{ij}$$

where Y_{ij} = the dependent variable, μ = the overall mean, B_i = the random effect of block ($i = 1, \dots, 12$), T_j = the effect of treatment ($j = C, D, DE$), and E_{ij} = the residual error.

Weekly urine pH data were analysed using repeated measures in the MIXED procedure in SAS 9.4 according to the following model:

$$Y_{ijkl} = \mu + B_i + T_j + W_k + TW_{jk} + C_l + E_{ijkl}$$

where Y_{ijkl} = the dependent variable, μ = the overall mean, B_i = the random effect of block ($i = 1, \dots, 12$), T_j = the effect of treatment ($j = C, D, DE$), W_k = the effect of week ($k = -3, -2, -1$), TW_{jk} = the treatment by week interaction, C_l = the covariate measure of initial urine H^+ and E_{ijkl} = the residual error.

Body weight, efficiency of gain, DMI, serum beta-hydroxybutyric acid (BHBA), serum NEFA and serum glucose were analysed using repeated measures in the MIXED procedure of SAS 9.4 according to the following model:

$$Y_{ijkl} = \mu + B_i + T_j + W_k + TW_{jk} + P_l + E_{ijkl}$$

where Y_{ijkl} = the dependent variable, μ = the overall mean, B_i = the random effect of block ($i = 1, \dots, 12$), T_j = the effect of treatment ($j = C, D, DE$), W_k = the effect of week ($k = -3, -2, \text{ and } -1$), TW_{jk} = the treatment by week interaction, P_l = the covariate measure of initial BW for BW, efficiency of gain and DMI was used. For BHBA, NEFA and glucose, initial pre-treatment serum concentration of the specific metabolite being tested served as a covariate. The residual error term was E_{ijkl} . Repeated measures were not used for the calculation of initial BW and BW gain. Body weight gain was calculated as final weight - initial weight.

2.6 | Calf data

Calf data for serum IgG, IgA, AEA and BW were analysed using the MIXED procedure of SAS 9.4 according to the following model:

$$Y_{ijk} = \mu + B_i + T_j + P_k + E_{ijk}$$

where Y_{ijk} = the dependent variable, μ = the overall mean, B_i = the random effect of block ($i = 1, \dots, 12$), T_j = the effect of dam treatment ($j = C, D, DE$), P_k = the covariate measure of parity ($k = 2, \dots, 7$) and E_{ijk} = the residual error.

2.7 | Post-partum data

Body weight, BW gain, efficiency of gain, DMI, serum BHBA, serum NEFA and serum glucose were analysed using the REPEATED procedure of SAS 9.4 according to the following model:

$$Y_{ijkl} = \mu + B_i + T_j + W_k + TW_{jk} + P_l + E_{ijkl}$$

where Y_{ijkl} = the dependent variable, μ = the overall mean, B_i = the random effect of block ($i = 1, \dots, 12$), T_j = the effect of treatment ($j = C, D, DE$), W_k = the effect of week ($k = -3, -2, \text{ and } -1$); TW_{jk} = the treatment by week interaction, P_l = the covariate measure of parity ($k = 1, \dots, 6$) and E_{ijkl} = the residual error.

Post-partum DMI, milk yield, energy-corrected milk (ECM) yield, milk efficiency, total protein yield (kg), total protein content (%), fat yield (kg), fat content (%), lactose yield (kg), lactose content (%), ash yield (kg) and ash content (%), SCS, serum NEFA, serum BHBA and serum glucose were analysed using the REPEATED procedure of SAS 9.4 according to the following model:

$$Y_{ijkl} = \mu + B_i + T_j + W_k + TW_{jk} + P_l + E_{ijkl}$$

where Y_{ijkl} = the dependent variable, μ = the overall mean, B_i = the random effect of block ($i = 1, \dots, 12$), T_j = the effect of treatment ($j = C, D, DE$), W_k = the effect of week ($k = 1, \dots, 8$), TW_{jk} = the treatment by week interaction, P_l = the covariate measure of current parity ($k = 2, \dots, 7$), and E_{ijkl} = the residual error.

TABLE 9 Body weight (BW), dry matter intake (DMI), urine pH, blood metabolites, and colostrum yield and colostrum composition for cows fed control, direct-fed microbial (DFM) (*D*) or DFM + enzymes (DE) treatments during the 21 days prior to parturition

Parameter	Treatments ¹			SE	p-value
	Control	<i>D</i>	DE		
Initial BW (kg) ^{2, 3}	812.9 ^a	784.5 ^b	821.5 ^a	8.12	.003
BW (kg) ⁴	830.6	804.1	821.4	14.9	.17
BW gain (kg)	29.2	22.2	4.5	9.15	.65
Efficiency of gain (BWG/DMI) ⁵	0.12	0.17	-0.01	0.12	.51
DMI (kg)	15.6	15.3	16.0	0.9	.85
Initial urine pH	8.24	8.25	8.22	0.02	.63
Urine pH	7.71	7.85	7.81	0.14	.73
BHBA (mmol/L)	0.51	0.65	0.59	0.05	.22
Glucose (mg/dl)	63.6	62.7	64.3	1.5	.73
Non-esterified fatty acid (μmol/L)	93.5	64.4	93.3	17.1	.34
Colostrum					
Yield (kg)	10.7	6.6	7.8	1.5	.12
IgG content (mg/ml)	79.1	88.2	91.1	6.0	.34
IgG yield (g)	836.8	562.0	734.2	116.5	.21
IgA content (mg/ml)	6.3	5.7	6.8	0.5	.34
IgA yield (g) ³	65.6 ^a	36.8 ^b	51.9 ^{ab}	7.9	.04
Fat percentage (%)	5.8	5.1	4.9	0.9	.75
Fat yield (kg) ³	0.68 ^a	0.37 ^b	0.36 ^b	0.12	.05
TP percentage (%)	13.7	16.1	16.2	1.0	.93
TP ⁶ yield (kg)	1.4	1.1	1.3	0.2	.44
TS ⁶ percentage (%)	26.0	25.3	24.9	2.2	.93
TS ^{6,7} yield (kg)	2.7 ^x	1.7 ^y	1.9 ^{xy}	0.4	.07
Ash percentage (%) ⁷	1.07 ^y	1.16 ^{xy}	1.25 ^x	0.05	.07
Ash yield (kg)	0.11	0.06	0.08	0.02	.22
Lactose percentage (%)	3.2	2.9	2.7	1.6	.25
Lactose yield (kg)	0.47	0.19	0.19	0.12	.11

TP = true protein.

¹Treatments: control; *D* = 45.40 g/day of DFM; DE = 45.40 g/day of *D* and 18.16 g/day of enzymes.

²Initial body weight.

³Means in superscripts significantly differ ($p \leq .05$).

⁴LS means for average BW during prepartum phase.

⁵Efficiency of gain = body weight gain (kg)/dry matter intake (kg).

⁶TS = total solids, means in superscript differ ($p \leq .10$).

⁷Means in superscript differ ($p \leq .10$).

3 | RESULTS

3.1 | Prepartum cow results

Prepartum cow data are presented in Table 9. Initial BW was different among treatments where cows supplemented with *D* weighed less at the start of the study compared to *C* or *DE* cows ($p = .003$). However, there were no differences in average BW or BW gain during the prepartum period. No effect of treatments was found on efficiency of gain during the prepartum period. There was no effect of treatments on DMI of cows 21 days prior to parturition. There was no effect of treatments on prepartum urinary pH. No effect of treatments was observed for serum BHBA,

NEFA or glucose concentrations during the prepartum period. Colostrum yield was not altered by treatments. There was also no impact on colostrum composition with the exception of IgA yield, fat yield, ash concentration and total solids yield. Treatment *D* decreased IgA yield compared to *C*. Both the *D* and *DE* treatments decreased fat yield in comparison with cows fed *C*. Ash concentration was greater for *D* than *DE*, while total solids yield tended to decrease in cows fed *D* and *DE* than those fed *C*.

3.2 | Calf results

Calf results are presented in Table 10. No treatment effects were observed for calf BW. There was also no impact of treatments on the

TABLE 10 Calf weight, serum IgG and IgA concentrations, and IgG and IgA apparent efficiency of absorption of calves from cows fed Control, DFM (*D*) or DFM + enzymes (*DE*) treatments 21 days prior to parturition

Parameter	Treatment ^a			SE	<i>p</i> -value
	Control	<i>D</i>	<i>DE</i>		
Calf weight (kg)	44.1	44.7	45.8	2.0	.83
Serum IgG 0 hr (mg/ml)	0.2	0.4	1.3	1.0	.20
Serum IgG 24 hr (mg/ml)	24.6	25.9	26.3	3.4	.92
Serum IgA 0 hr (mg/ml)	0.03	0.00	0.00	0.02	.32
Serum IgA 24 hr (mg/ml)	1.43	1.54	1.50	0.15	.86
IgG AEA ^b (%)	34.1	31.1	33.5	3.4	.80
IgA AEA ^c (%)	29.3	28.1	29.5	2.9	.93

^aTreatments: Control; *D* = 45.40 g/day of DFM; *DE* = 45.40 g/day of *T* and 18.16 g/day of enzymes.

^bApparent efficiency of absorption of IgG = (Plasma IgG [g/L] × BW [kg] × 0.09)/IgG Intake [g] × 100.

^cApparent Efficiency of Absorption of IgA = (Plasma IgA [g/L] × BW [kg] × 0.090/IgA Intake [g]) × 100.

serum IgG and IgA concentrations in calves at 0 hr. The serum IgG and IgA concentrations at 24 hr, as well as the AEA of IgG and IgA in calves, were similar across all treatments.

3.3 | Post-partum cow results

Post-partum cow results are presented in Table 11. No effect of treatments was observed for overall BW. Body weight change was different among treatments with cows on the *D* treatment showing lesser BW losses than those on the *DE* treatment (*p* = .03). Both treatments were similar to cows on *C*. Dry matter intake during the 8-week post-partum period was not different among treatments. Efficiency of gain tended to be greater (*p* = .09) for cows on the *D* treatment in comparison with those on the *DE* treatment. Treatments did not alter serum glucose, BHBA and NEFA concentrations. Milk and ECM yields were not affected by the *D* or *DE* treatments, likely due to similar DMI across treatments. Milk efficiency of cows on the *D* and *DE* treatments was similar to those of *C* cows. There also was no effect of treatments on milk composition, with the exception of SCS. The SCS of cows on the *D* and *DE* treatments were not different from cows on the *C* treatment. However, *D* was different from the *DE* treatment (*p* = .04).

4 | DISCUSSION

Dry matter intake was similar among treatments during the prepartum period. This coincides with previous studies which found that DFM containing *S. cerevisiae* and *E. faecium* fed at either 2 g/day or 90 g/day during the 21-days prepartum period had no impact on DMI (Nocek

et al., 2003; Oetzel et al., 2007). Prepartum DMI data in this study do not agree with that of Nocek and Kautz (2006) who found that the supplementation of DFM containing both *S. cerevisiae* and *E. faecium* at a rate of 2 g/day increased DMI during the prepartum period. Also, Dann, Drackley, McCoy, Hutjens, and Garrett (2000) found that supplementation of *S. cerevisiae* culture at 60 g/day increased DMI of prepartum Jersey cows.

Previous studies have not indicated that prepartum supplementation of DFM and enzymes impacts urine pH. This might be due to the fact that both of these additives do not have enough of an anion capacity to bring about a change in the metabolic pH. Our results for glucose and NEFA concentrations are supported by previous studies which found no impact of DFM supplementation on prepartum glucose and NEFA (Nocek & Kautz, 2006; Oetzel et al., 2007). However, DFM supplementation has been reported to decrease prepartum BHBA which is in contrast to what was reported in this study (Nocek & Kautz, 2006; Oetzel et al., 2007). Defrain, Hippen, Kalscheur, and Tricarico (2005) also reported increased BHB concentrations for cows supplemented with enzymes which is in contrast to what was reported in this study.

No studies to date have reported the impact of DFM and enzymes on colostrum yield, quality and composition. Colostrum yield was similar among treatments. The lack of impact of the *D* and *DE* treatments in this study on colostrum yield is perhaps due to the fact that there was no effect on prepartum DMI. There was a decrease in IgA yield, fat yield and total solids yield of colostrum in cattle fed the *D* treatment in comparison with those fed either the *C* or *DE* treatments. Also, the ash concentration of colostrum increased in cattle fed the *D* and *DE* in comparison with those cows fed *C*. However, it is not fully understood why these results were observed and further research is needed. It can be hypothesized that decreased yields of IgA, fat and total solids may be linked to increased post-partum SCC in cows fed the *D* treatment. Specifically, milk components such as IgA, fat and other solids may have been more degraded due to a greater enzymatic activity of the somatic cells or the presence of bacteria within the mammary gland.

There were no treatment effects on calves in this experiment. A previous study showed that calves born from cows supplemented with a 105 mg of Se-yeast/day had greater serum IgG and absorption of IgG than those from cows on the control diet (Hall et al., 2014). Al-Saiady (2010) observed greater serum IgG concentration and greater 5-week BW in calves supplemented with a probiotic. However, there were no impacts on serum IgG concentrations in calves provided with a prebiotic supplement (Queszada-Mendoza et al., 2011). In both these studies, calves were over 24 hr old and AEA was not evaluated.

4.1 | Post-partum data

Body weight gain was different among treatments, suggesting that there was a greater partitioning of nutrients towards body condition in cows on the *D* treatment in comparison with those on the *DE* treatment. The reason for this difference in nutrient partitioning is not fully understood, and further research is needed to elucidate specific mechanisms.

Parameter	Treatment ¹			SE	p-value
	Control	D	DE		
BW(kg)	741.9	723.7	739.5	18.3	.71
BW Gain (kg/week) ²	-5.7 ^{a,b}	-1.2 ^a	-7.8 ^b	1.8	.03
Efficiency of Gain (BWG/ DMI) ³	-0.04 ^{xy}	-0.02 ^x	-0.05 ^y	0.01	.09
DMI (kg)	24.7	24.3	24.0	0.8	.78
BHBA (mmol/L)	0.80	0.70	0.70	0.06	.34
Glucose (mg/dl)	52.6	56.6	52.8	1.8	.38
Non-esterified fatty acid (μ mol/L)	224.7	179.5	200.5	23.8	.34
Milk					
Yield (kg)	48.4	43.4	47.7	2.1	.17
ECM yield (kg) ⁴	45.2	41.4	43.7	2.0	.34
Milk efficiency ⁵	1.87	1.75	1.83	0.08	.50
Fat content (%)	3.03	3.21	2.76	0.17	.17
Fat yield (kg)	1.44	1.34	1.33	0.08	.49
True protein content (%)	2.96	3.07	3.03	0.09	.64
True protein yield (kg)	1.41	1.30	1.42	0.05	.19
Lactose content (%)	4.82	4.81	4.84	0.04	.86
Lactose yield (kg)	2.3	2.1	2.3	0.1	.13
Total solid content (%)	11.7	12.0	11.6	0.3	.43
Total solid yield (kg)	5.6	5.1	5.5	0.2	.23
Milk urea nitrogen (mg/dl)	12.3	12.9	13.2	1.0	.80
Somatic cell score ⁶	1.7 ^{a,b}	2.7 ^a	0.9 ^b	0.5	.04

¹Treatments: Control; T = 45.40.24 g/day of DFM; TZ = 45.40 g/day of T and 18.16 g/day of enzymes.

²Means in same row with superscripts a, b significantly differ ($p < .05$).

³Efficiency of gain = body weight gain (kg)/dry matter intake (kg); Means in same row with superscripts x, y differ ($p < .10$).

⁴ECM yield = energy-corrected milk yield; ECM yield = $(12.86 \times \text{fat kg}) + (7.04 \times \text{protein kg}) + (0.3246 \times \text{milk kg})$.

⁵Milk efficiency = energy-corrected milk yield (kg)/dry matter intake (kg).

⁶Means in same row with superscripts a, b significantly differ ($p < .05$).

Efficiency of gain tended ($p = .09$) to be greater for cows on the D treatment in comparison with cows on the DE treatment. This confirms that there were more nutrients being partitioned to the body condition of these cows and that cows on the D treatment lost less BW during the post-partum period. Under the conditions of this experiment, feeding enzymes in combination with DFM were not beneficial. The underlying mechanism responsible for this is not known and further research will need to be performed.

Dry matter intake was similar among treatments. This lack of effect of treatments on DMI is in contrast to previous studies with DFM supplementation. An array of studies with various types and levels of DFM supplementation have been shown to increase DMI during early lactation (Dann et al., 2000; Moallem, Lehrer, Livshitz, Zachut, & Yakoby, 2009; Nocek & Kautz, 2006; Nocek et al., 2003; Oetzel et al., 2007). However, Schingoethe et al. (2004) found that supplementation of *S. cerevisiae* culture did not affect DMI of lactating dairy cows.

TABLE 11 Post-partum BW, dry matter intake (DMI), blood metabolites, and milk yield and composition for cows fed control, direct-fed microbial (DFM) (D) or DFM + enzymes (DE) treatments during the first 8 weeks of lactation

As for studies with enzyme supplementation, several researchers have shown that there was no impact of enzymes supplemented at various rates on DMI of lactating cows (Reddish & Kung, 2007; Rode et al., 1999; Yang et al., 2000). This suggests that treatment did not have an impact on the digestibility of the TMR.

Blood metabolite concentrations were similar among treatments. In contrast, Nocek et al. (2003) reported that post-partum concentrations of glucose increased and NEFA decreased with DFM supplementation. Defrain et al. (2005) also reported that enzyme supplementation increased post-partum glucose concentrations. However, no effect of DFM and enzyme supplementation on post-partum BHB and NEFA concentrations was reported by Defrain et al. (2005) and Oetzel et al. (2007). Conversely, Nocek and Kautz (2006) reported that DFM increased post-partum BHBA concentrations in transition cows, which is undesirable due to possible development of ketosis.

In this study, there was no effect of treatment on milk yield. This is probably due to the fact that there was no effect of treatment on DMI. Previous studies with DFM and enzymes have shown similar results. Dann et al. (2000) and Oetzel et al. (2007) found no difference in milk yield of cows supplemented with or without DFM. In addition, AlZahal, Dionissopoulos, Laarman, Walker, and McBride (2014) found no impact of DFM on milk yield during the first 6 weeks of lactation. Between week 7 and 10, an increase in milk yield of cows supplemented with DFM was observed (AlZahal et al., 2014). Other studies have found increases in milk yield with DFM supplementation during early lactation (Moallem et al., 2009; Nocek & Kautz, 2006; Nocek et al., 2003; Wohlt et al., 1991). In addition, it has been shown that enzyme supplementation increased milk production (Yang, Beauchemin, & Rode, 1999). However, most studies with enzyme supplementation have indicated that it does not impact milk production (Beauchemin et al., 1999; Holtshausen, Chung, Gerardo-Cuervo, Oba, & Beauchemin, 2011; Reddish & Kung, 2007).

Our results were corroborated by Schingoethe et al. (2004) who reported no impact on ECM yield with yeast supplementation. Boyd, West, and Bernard (2011) found an increase in ECM with supplementation of DFM containing *Lactobacillus acidophilus* and *Propionibacterium freudenreichii*, which was likely due to an increase in the yield of milk components such as fat and protein. However, these bacteria were not present in the DFM supplement used in the current study.

There was no impact of treatment on milk efficiency. This was expected because treatment did not impact ECM yields or DMI. Beauchemin et al. (1999) and Boyd et al. (2011) found no effect of DFM supplementation on milk efficiency as well. In contrast, Schingoethe et al. (2004) did find a 7% increase in milk efficiency with yeast supplementation. In addition, Holtshausen et al. (2011) found that cows in early lactation fed an enzyme product had greater milk production efficiency than those fed the control. However, in the study by Holtshausen et al. (2011), the enzyme product contained mainly xylanase and endoglucanase activity, whereas the enzyme product used in present study primarily contained amylase and cellulase activity. In this study, there was no impact of treatment on milk components, with the exception of SCS. Our data concur with those of other researchers regarding milk components. Dann et al. (2000) found no impact of DFM on the concentrations of fat, protein, lactose, total solids and MUN, which agree with our results. Moallem et al. (2009) found no impact on milk fat and protein concentrations between cattle supplemented with DFM or without DFM. Nocek and Kautz (2006) found no impact of DFM supplementation on milk fat and protein yields, as well as milk protein concentration. A similar response has been observed for enzyme supplementation (Reddish & Kung, 2007). Oetzel et al. (2007) found that DFM supplementation only increased milk fat concentration for cows in their first lactation.

Dann et al. (2000) did not find any impact of DFM supplementation on SCC. However, SCS was reduced in the milk of cows when the enzymes were fed in combination with the DFM alone, but similar to control cows. These data suggest that adding cellulase and amylase to the diet of a cow fed DFM reduces SCS. However, Reddish and

Kung (2007) reported no impact of enzyme supplementation containing cellulase and xylanase activity on SCC. As a result, it can be concluded that there is some other underlying factor contributing to the differences between treatments and further research would need to be performed to determine these discrepancies.

5 | CONCLUSION

Data from this experiment show no benefits of supplementing DFM or enzymes or a combination of both to pre- or post-partum dairy cows. As a result, it can be concluded that DFM and enzyme supplementation was not beneficial for improving the health and performance of dairy cattle during the transition period and early lactation under the conditions of the present experiment.

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