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Impact of a native rumen microbe supplement provided in feed on production performance and enteric methane emissions in lactating dairy cows

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ABSTRACT

The objective of this randomized controlled study was to evaluate the effects of a native rumen microbial feed supplement on production performance (milk production, milk composition, and feed efficiency). Additionally, the effects of the microbial supplement on enteric methane emissions were assessed. Forty-six second-, third-, and fourth-parity Jersey cows at 59 ± 8 DIM were blocked by baseline milk yield and randomly assigned to control (no microbial supplementation) or treatment (GF-MFS; TMR containing 5 g/cow per day of a native rumen microbial feed supplement containing a minimum of *Clostridium beijerinckii* at 2×10^6 cfu/g, *Pichia kudriavzevii* at 2×10^7 cfu/g, *Ruminococcus bovis* at 2×10^7 cfu/g, and *Butyrivibrio fibrisolvens* at 2×10^7 cfu/g). Cows were housed in a single group and fed the study diets ad libitum for 16 wk. Individual milk yield was recorded using electronic milk meters, and milk fat and protein were measured using optical in-line analyzers at each of 2 daily milkings. Individual cow TMR intake was recorded through the Biocontrol CRFI feed intake control and measurement system. Enteric methane emissions were measured using GreenFeed units. Somatic cell count was determined in milk composite samples collected at 2 consecutive milkings on a weekly basis. Treatment and treatment by time effects were assessed by multiple linear regression. Overall treatment effects were observed for ECM, milk, and fat yields; yields were (mean ± SEM) 2.19 ± 0.90, 1.64 ± 0.74, and 0.10 ± 0.03 kg/d higher for GF-MFS compared with control cows, respectively. There was also a tendency for higher milk protein yield in GF-MFS cows. No overall treatment effects were observed for milk fat and protein concentrations and DMI. Furthermore, positive treatment effects were observed on feed efficiency (0.09 ± 0.04 units higher for GF-MFS cows) and $\log_{10}$ SCC (0.13 ± 0.06 units lower for GF-MFS cows). Methane intensity tended to be lower for GF-MFS compared with control cows, but methane production and yield were similar for GF-MFS and control cows. In conclusion, the supplementation of native rumen microbes effectively improved economically important outcomes such as ECM, milk and fat yields, feed efficiency, and lowered SCC. Larger studies are required to assess the effect of the evaluated native rumen microbial feed supplement on enteric methane emissions.

Key words: probiotic, microbial feed supplement, feed additive, feed efficiency

INTRODUCTION

Enhancing productivity and feed utilization is a key component to advance the long-term sustainability of dairy farming. Feed is a major expense in dairy operations, and enteric fermentation is a major source of GHG in dairy production (Rotz, 2018). Cows that require less feed per unit of product are more efficient in converting feed into milk (e.g., feed efficient) than their counterparts and may be expected to produce less methane per unit of feed intake (methane yield, g CH_4/kg of DMI) and per unit of milk product (methane intensity, g CH_4/kg of ECM; Zehetmeier et al., 2012; Manzanilla-Pech et al., 2022). To achieve the targets of limiting global warming while both securing food supply for the growing human population and ensuring long-term economic, environmental, and social sustainability of dairy farming, it is necessary to improve the efficiency of current dairy production systems while preserving animal health and welfare. Feed efficiency is a complex multifaceted trait influenced by many biological processes, including feeding behavior, digestion, intestinal absorption, and nutrient partitioning (Kenny et al., 2018). Rumen microbes lead the fermentation process by breaking down and digesting feed and therefore play a key role in determining the feed efficiency of dairy cows (Monteiro et al., 2024).

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Supplementing cattle with live microorganisms can elicit positive effects in the host through varying modes of action. For instance, microbial feed supplements are thought to optimize rumen biochemistry to increase the availability of useful nutrients for the host, potentially through reducing host pathogens, increasing digestion of the feed, maintaining an ideal chemical environment in the rumen, or supporting biochemical pathways that favor production of energy compounds and reduce waste to carbon-containing gasses (McAllister et al., 2011). Less understood modes of action may involve more complex host-microbe interactions involving the immune system and signaling pathways (Metwaly et al., 2022). Microbes for these products have been sourced from inside or outside of the rumen, but recent research has suggested that ruminal microbes selected for their correlation with improved feed efficiency and production outcomes may also be better at colonizing the rumen environment compared with exogenous strains (Zengler and Embree, 2016; Russell et al., 2022; Monteiro et al., 2024).

Mathematical networking algorithms applied to high-throughput sequencing data from dairy cows were previously used to identify strains of native rumen microbes with high prevalence across a diverse range of diet, breed, and season and that were in addition associated with improved milk production performance (Zengler and Embree, 2016; Embree et al., 2022). These native rumen microbes compose the microbial feed supplement subject of this study and are classified as strains belonging to *Pichia kudriavzevii*, *Clostridium beijerinckii*, *Butyrivibrio fibrisolvens*, and *Ruminococcus bovis*. Recent research has evaluated the effect of this microbial feed supplement on dairy cow performance; these studies, conducted in different locations across the United States, fed the microbes daily to lactating Holstein cows at varying stages of lactation and for periods of 16 to 40 wk, overall reporting positive effects on feed efficiency (3%–5% improvement), driven mostly by positive effects of microbial supplementation on milk or ECM yield with minimally affected DMI (Dickerson et al., 2022; Valldecabres et al., 2022; Marinho et al., 2024) or by decreased DMI without a change in ECM yield (Goldsmith et al., 2023). Given that production performance is different across cattle breeds, it is plausible that different responses to this microbial feed supplement may be observed in dairy breeds other than Holstein. Compared with Holstein cattle, the Jersey breed, which is the second most popular dairy breed in the United States (USDA-NAHMS, 2014), is smaller and tends to present lower milk yields but higher concentration of solids coupled with similar or lower feed intake capacity, resulting in more milk energy relative to metabolic BW and an overall higher production efficiency (Aikman et al., 2008; Prendiville et al., 2009; Kristensen et al., 2015). Therefore, evidence of efficacy for the aforementioned microbial feed supplement is necessary to support its usage in Jersey cows.

Microbial feed supplements have the potential to reduce enteric CH_4 emissions by either reducing CH_4 production without affecting milk production (a pathway currently dominated by small molecule–based interventions) or by increasing feed efficiency or productivity. Further, microbial feed supplements have the potential to reduce enteric CH_4 production through other ecological mechanisms, such as competition with methanogens in the ruminal environment or redirection of H_2 away from CH_4 synthesis and toward acetate or propionate (Lopez et al., 1999; Jeyanathan et al., 2014). Recent meta-analyses including 26 in vitro studies (Martins et al., 2024) and 20 in vivo studies (Ncho et al., 2024) did not find evidence of microbial feed supplements consistently reducing CH_4 production; however, the treatments in this analysis compiled a variety of microbes from different domains of life, alone or in combination and at different doses, making the generalization of their findings difficult (Martins et al., 2024). When examining subgroups of these microbes, Martins et al. (2024) suggested that *Enterococcus* spp. could be effective at decreasing in vitro CH_4 production, and Ncho et al. (2024) concluded that a multistrain microbial feed supplement, sourced from beef cattle rumens and targeting reductive acetogenesis and propionate production, could be effective at decreasing in vivo CH_4 production (Pittaluga et al., 2023).

The microbial feed supplement subject of this study includes *C. beijerinckii*, which can assimilate excess CO_2 into organic acids (Sandoval-Espinola et al., 2017) and has been shown to produce extensive amounts of acetate and to interact synergistically with other complex-carbohydrate degraders (Gomez-Flores et al., 2017). Therefore, we hypothesized that the microbial feed supplement tested in this study may provide the dual benefit of animal productivity optimization and CH_4 mitigation, contributing to established climate goals and the sustainability of the dairy industry. The main objectives of the present study were to investigate the effects of a microbial feed supplement containing *C. beijerinckii* ASCUSDY20, *P. kudriavzevii* ASCUSDY21, *R. bovis* ASCUSDY10, and *B. fibrisolvens* ASCUSDY19 on multiparous Jersey cows' production performance and to provide the first examination of the treatment's effects on enteric methane emissions.

MATERIALS AND METHODS

All procedures were approved by the DairyExperts Institutional Animal Care and Use Committee (protocol number: DE220702).

Research Facility

The study was conducted at a research facility located in California's Central Valley (DairyExperts Inc., Tulare, CA). Cows were identified with radio frequency identification tag and housed in a roof-covered loose system pen with compost bedding. Feed mangers were equipped with a feed intake control and measurement system (BioControl, CRFI, Rakkestad, Norway) that allowed for control of access of cows to feed mangers with different diets, access of cows to multiple mangers within the same treatment diet, and measurement of individual cow feed intake, number of visits, and feeding time. The research facility had a TMR preparation area that included feed storage, feed mixer wagon, and concrete floors, allowing neat feed handling for the preparation of the different rations. Next to the cows' housing area was a double 10 parallel parlor where each stall was equipped with a milk meter (MPC, AfiMilk, Israel) and an optical in-line milk component analyzer (AfiLab, AfiMilk, Israel) that allowed for individual milk yield and composition determination at each milking. Feed intake, milk yield, and components measuring systems were calibrated once monthly.

The GreenFeed System (C-Lock Inc., Rapid City, SD) was used to measure CH_4 from the eructation and exhalation of the cows in their normal housing environment. Measurements were gathered from the cows upon their voluntarily visit to the instrument, where pelletized bait feed was provided as an incentive. Two GreenFeed units were placed at the same location as feed mangers and calibrated according to manufacturer's instructions, including the automatic and gas recovery calibrations. Manual CO_2 recoveries were performed at the beginning and end of the trial, and once a month in between. The average recovery rate for the 2 units across the different times was 99.6% and the SD was 1.9%.

Study Cow Husbandry and Adaptation Period

A total of 46 Jersey cows between 25 to 54 DIM in their second, third, or fourth lactation were sourced from a large commercial dairy farm near the research facility. Upon arrival at the facility, cows were housed in the same space and allowed a 21-d adaptation period before study initiation. Two GreenFeed units were available to the cows during the adaptation period. Cows had ad libitum access to water and TMR daily. The TMR was formulated to meet or exceed the predicted requirements of energy, protein, minerals, and vitamins (NASEM, 2021; Table 1). The amount of TMR prepared daily was calculated based on the previous day's average intake plus 5%. Cows in the study were milked twice daily at approximately 0700 and 1900 h. Cows were observed daily for clinical signs of disease.

Table 1. Ingredients of study cows' TMR and nutrient composition (mean ± SD) of TMR and pellet feed offered in the GreenFeed units

Item	TMR % of DM[1]	Pellet feed % of DM[2]
TMR ingredient		
Corn silage	20.96	
Alfalfa hay	4.25	
Canola	14.98	
Rolled corn	12.30	
Ground corn	12.30	
Wheat mill	8.56	
Dried distillers grains	8.02	
Cottonseed	6.42	
Almond hulls	5.03	
Molasses	3.21	
Rumen-protected fat	1.39	
Sodium bicarbonate	0.96	
Limestone	0.95	
Urea	0.53	
Magnesium oxide	0.23	
Salt	0.21	
Selenium	0.026	
Zinc methionine complex	0.021	
Vitamin A, D, and E premix[3]	0.020	
Trace mineral mix[4]	0.016	
Ethylenediamine dihydroiodide	0.004	
Copper sulfate	0.002	
Water	0.011	
Nutrient composition		
CP	17.14 ± 0.51	16.10
Ether extract	5.63 ± 0.19	2.20
ADF	16.46 ± 1.14	33.60
Ash-free NDF	27.98 ± 1.74	45.60
NFC	42.21 ± 2.06	26.60
Starch	27.27 ± 1.48	1.00
Lignin	3.56 ± 0.35	6.58
Ash	8.07 ± 0.23	11.72
NEL (Mcal/kg)	1.65 ± 0.02	1.25

[1] Cows were fed a TMR at 60.6% of DM (average of daily determinations during the study period). Results are the average of 8 TMR samples collected at 2-wk intervals (±SD) and 2 pellet feed samples collected during the study period.

[2] Cows were offered a pellet feed at 92.8% of DM in the GreenFeed units.

[3] Pinnacle Premix (Visalia, CA). Vitamins A (3.31%), D_3 (1.10%), and E (17.64%), limestone ground (38.67%), rice hulls ground (38.67%), and mineral oil (0.61%).

[4] Pinnacle Premix (Visalia, CA). Limestone ground (38.27%), $ZnSO_4$ (24.84%), $MnSO_4$ (22.05%), Na_2SeO_3 (6.00%), $CuSO_4$ (4.55%), KCl (1.99%), MgO (0.79%), $CoSO_4$ (0.76%), mineral oil (0.50%), and ethylenediamine dihydroiodide (0.25%).

Experimental Design

On November 2022, a total of 46 cows were enrolled in a randomized controlled experimental study based on a sample size estimation of 21 cows per treatment group to allow for the detection of a 3 kg milk yield increase for treatment cows (SD = 4.5 kg; Valldecabres et al., 2022) with an α level of 0.10 and power of 0.80 performed using G*Power software (version 3.1.9.7; Faul et al., 2007). To account for possible follow-up losses, a total of 23 cows per treatment group were included in the study. Average milk yield from the last 4 d of the adaptation period (baseline period) was used to sequentially group cows in pairs. Within each pair of cows, treatments were randomly assigned using a random number generator (Microsoft Excel version 16.0; Microsoft Corp., Redmond, WA) by DairyExperts technicians upon the cows' arrival at the research facility.

Cows were randomly assigned to (1) control, TMR with no microbial supplementation (n = 23) or (2) treatment (**GF-MFS**), 5 g/cow per day of a microbial feed supplement (GALAXIS FRONTIER; Native Microbials Inc., San Diego, CA) containing a minimum of *C. beijerinckii* ASCUSDY20 at 2 × 10^6 cfu/g, *P. kudriavzevii* ASCUSDY21 at 2 × 10^7 cfu/g, *R. bovis* ASCUSDY10 at 2 × 10^7 cfu/g, and *B. fibrisolvens* ASCUSDY19 at 2 × 10^7 cfu/g mixed into the TMR (n = 23) as recommended by the manufacturer. Feed was offered for ad libitum consumption for 16 consecutive weeks (from 60 ± 8 DIM to 171 ± 8 DIM; mean ± SD). The microbes composing the microbial feed supplement are grown in pure culture, freeze-dried, and encapsulated in a lipid coating material, which maintains their viability (Embree et al., 2022). Detailed DNA extraction and sequencing procedures, as well as the genomic and virulence analysis of the microorganisms included in the evaluated product are described in a previous publication (Valldecabres et al., 2022).

A base TMR was prepared once a day in a single batch for all treatments and then this base ration was divided into 2 piles in an amount equivalent to the previous day's feed intake for each group plus 5%. The microbial feed supplement was hand-added into one of the piles, and the TMR was reloaded into the mixer wagon for further mixing and distribution into the respective mangers. Homogeneous mixing of the microbes into the TMR was validated with the use of microtracers, as described in a previous publication (Valldecabres et al., 2022). To avoid cross-contamination between batches, after delivering the feed into the mangers for each treatment, approximately 75 kg of Bermuda grass hay was loaded into the mixing wagon and augurs were run for approximately 4 min before discharging it to sweep away residues from the previous batch of TMR. A total of 24 feed mangers were assigned to control or treatment (12 mangers/treatment). The TMR with the appropriate treatment was delivered once a day into the feed mangers, and cows had ad libitum access to any of the 12 mangers containing their assigned treatment. Nutrient composition of TMR was determined from base TMR samples collected the day before microbial supplementation start and then every 2 wk up to study wk 14. A total of 200 g of fresh feed were stored at −20°C until wet chemistry analysis at Cumberland Valley Analytical Services (Hagerstown, MD). Total mixed ration ingredients and nutrient composition are presented in Table 1. Nutrient composition of pelleted feed offered in the GreenFeed units was determined by wet chemistry at Cumberland Valley Analytical Services (Hagerstown, MD; Table 1) on 2 samples collected from 2 different batches of pellet feed used during the study.

Data Collection

Production Outcomes. Individual cow milk yields and composition (concentration of fat and protein) were measured at each milking with the milk meters and optical in-line milk component analyzers and downloaded using the AfiFarm software (AfiMilk, Israel). Energy-corrected milk yield (milk yield value corrected for 3.5% fat and 3.2% true protein as [0.3246 × kg of milk] + [12.86 × kg of fat] + [7.04 × kg of true protein]), and fat and protein yield (as milk component concentration × kg of milk) were calculated for each cow and milking using the information previously described; information from the last 4 d before microbial feed supplementation start was used as the baseline.

DMI and Feed Efficiency. Individual cow feed intake was continuously recorded through the feed intake control and measurement system described in the "Research Facility" section. The mangers specifications indicate that the maximum combined error of the 2 load cells is 0.01%. Dry matter of the base TMR was measured on a daily basis to allow for individual daily DM intake determination as [kg of TMR consumed × DM of TMR] + [GreenFeed pelleted feed drops × provided pelleted feed DM per drop (35 g at 92.8% DM)]. Daily feed efficiency was defined as kilograms of ECM produced per kilogram of DM consumed on an individual basis. Information from the last 4 d before microbial feed supplementation start was used as the baseline.

Enteric Methane Emissions. Methane from cows' eructation and exhalation was measured using 2 GreenFeed units (C-Lock Inc., Rapid City, SD), which were available at all times for the study cows from arrival to the research facility. When the animal's head is in the feed trough of the unit, the system draws air in past its nose and into an air filter and samples the air to determine CH_4 and CO_2 by infrared technology. Additional data recorded by the GreenFeed included individual cow number of visits and number of feed drops. Data were automatically uploaded to the C-Lock website where it was processed by C-Lock's algorithms; only emissions recorded while the cow's head position met the criteria were used, while records of feed drops from successful and unsuccessful emission-recording visits were used for the DM and nutrient intakes calculations. A total of 5,036 emission-recording visits were available for analysis (average emission-recording visits per cow = 240). Enteric CH_4 emission yield (g/kg of DMI) and intensity (g/kg of ECM) were calculated using the DMI and ECM daily records. Information from the full last week before microbial feed supplementation start was used as the baseline.

BW. Body weight was recorded after the morning milking once before microbial supplementation commenced, and at 3 occasions during the study, study wk 6, 12, and 16, using an electronic scale (PS-2000 scale; Salter Brecknell, Fairmont, MN). Body weight gain was calculated by subtracting consecutive weights. Body weight gain by wk 6 was calculated by subtracting baseline weight to that recorded on wk 6.

SCC. Individual cow full milking representative a.m. and p.m. milking samples for SCC determination were collected twice before the start of microbial feed supplementation (baseline) and on 9 occasions during the study (study wk 2, 3, 4, 5, 7, 8, 11, 14, and 15); SCC was determined by flow cytometry (Fossomatic, Foss, Hillerød, Denmark) at Tulare Dairy Health Improvement Association (Tulare, CA).

Statistical Analyses

All statistical analyses were performed with SAS (version 9.4; SAS Institute Inc., Cary, NC). Parity and DIM at microbial supplementation start were compared among treatments using the chi-squared test and ANOVA with the FREQ and MIXED procedures, respectively. Raw data plots were generated with the SGPANEL procedure to identify data outliers before analysis. Baseline information was summarized for each outcome and treatment group using the MEANS procedure.

Production Outcomes. Outcomes were evaluated as weekly averages (wk 1–16 from start of microbial feed supplementation) generated using the SQL procedure and daily values. Daily milk, ECM, and fat and protein yields were calculated as the sum of both morning and afternoon weights (kg), and milk fat and protein concentrations were calculated as the average of both a.m. and p.m. milking readings; these variables were only computed when observations from both morning and evening milkings were available. Two daily milk yield records (44 kg at 61 DIM; 34 kg at 136 DIM; GF-MFS: n = 2) were considered outliers according to the respective cow's previous and subsequent milk yields and were excluded along with the associated outcomes from the analysis.

Multiple linear regression was used to analyze production data with the MIXED procedure. All statistical models included the fixed effects of baseline, parity (second, third, or fourth), treatment, time (study week), and treatment by time. Time and parity were included in the models as categorical variables. For each outcome, the variance-covariance structure leading to the lowest Akaike's and Bayesian information criteria was used to model the correlation of multiple measures within cow, with cow as the random effect (subject of the repeated statement). Unstructured, compound symmetry, autoregressive 1, heterogeneous autoregressive 1, Toeplitz, and Toeplitz heterogeneous were the variance-covariance structures evaluated. To limit pairwise comparisons to those of interest (among treatments and week), customized hypothesis tests were generated using the PLM procedure with the slice option. Results are presented as LSM with the corresponding SEM, unless otherwise stated. Overall model fit was assessed with the final models' residuals plots generated with the residual option in the model statement.

DMI and Feed Efficiency. Dry matter intake and feed efficiency were evaluated as described for production data. One feed intake record was considered a measurement error and removed before data analysis (46 kg of DM at 115 DIM; control: n = 1). Baseline information was not available for 1 cow (control: n = 1), thus this cow was not included in the data analysis.

Enteric Methane Emissions. Enteric CH_4 production (g of CH_4), emission yield (g/kg of DMI) and intensity (g/kg of ECM) were evaluated as described for production data using 2 different time period definitions, weekly and monthly, to allow for a higher number of observations per cow and to decrease the uncertainty associated with the measurements. Baseline information was not available for 1 cow and no measurements were obtained from another cow during the entire study, thus, these cows were not included in the data analysis (control: n = 2).

BW. Data were evaluated as described for production data. Baseline BW information was not available for 1 cow, thus, this cow was not included in the data analysis (control: n = 1). Body weight gain was analyzed as described without including the baseline covariate.

SCC. Data were evaluated as described for production data. Milk SCC was calculated as the average of both a.m. and p.m. milking on the sampling day. Data were log-transformed before statistical analysis using the LOG10 function, and results are reported as $\log_{10}$SCC.

The accompanying figures were created with Excel (Microsoft Corp., Redmond, WA). Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Forty-six Jersey cows (23 per treatment group) were enrolled in the study at 59 ± 8 DIM (control: 60 ± 8 DIM; GF-MFS: 58 ± 8 DIM). Cows were on their second (n = 20), third (n = 18) or fourth (n = 8) lactation. Baseline parameters by treatment group are described in Table 2. On average, GF-MFS cows received 4.96 ± 0.39 g/d of the microbial feed supplement throughout the study. Estimated LSM differences between GF-MFS and control cows using the control group as the reference are presented with associated SE and 95% CI in the text. Results

Table 2. Baseline parameters (mean ± SD) by treatment group

Item	Treatment[1]	
	Control	GF-MFS
Production outcome		
Milk yield, kg/d	37.07 ± 5.39	37.53 ± 4.02
ECM yield, kg/d	43.92 ± 6.32	44.29 ± 4.76
Fat yield, kg/d	1.75 ± 0.27	1.73 ± 0.21
Protein yield, kg/d	1.27 ± 0.18	1.29 ± 0.17
Fat concentration, %	4.71 ± 0.23	4.67 ± 0.19
Protein concentration, %	3.44 ± 0.16	3.44 ± 0.16
DMI, kg/d	23.45 ± 3.08	23.49 ± 2.50
Feed efficiency	1.89 ± 0.17	1.91 ± 0.16
Enteric methane emissions		
CH_4, g/d	313.77 ± 61.63	316.57 ± 56.22
CH_4 yield, g/kg of DMI	14.39 ± 3.41	14.43 ± 2.98
CH_4 intensity, g/kg of ECM	8.03 ± 2.38	7.22 ± 1.38
BW, kg	436.19 ± 32.89	443.44 ± 31.34
Log_{10}SCC, cells/mL[2]	1.56 ± 0.24	1.81 ± 0.31

[1] Control: No microbial TMR supplementation (n = 23; DMI, feed efficiency, nutrient digestibility, fecal DM flow and BW: n = 22; enteric methane emissions: n = 21); GF-MFS: TMR containing 5 g/cow per day of a microbial feed supplement containing a minimum of *Clostridium beijerinckii* at 2 × 106 cfu/g, *Pichia kudriavzevii* at 2 × 107 cfu/g, *Ruminococcus bovis* at 2 × 107 cfu/g, and *Butyrivibrio fibrisolvens* at 2 × 107 cfu/g (n = 23; GALAXIS FRONTIER; Native Microbials Inc., San Diego, CA).

tables include the estimated LSM by treatment group and associated SEM.

Production Outcomes

Milk and ECM Yield. Treatment effects unconditional to time were observed for ECM and milk yields; these were respectively 2.19 ± 0.90 kg/d (95% CI = 0.37–4.02 kg/d; $P = 0.02$) and 1.64 ± 0.74 kg/d (95% CI = 0.14–3.14 kg/d; $P = 0.03$) higher for GF-MFS compared with control cows (Figures 1A and 1B; Table 3).

Milk Components Yield and Concentration. Cows assigned to GF-MFS produced 0.10 ± 0.04 kg fat/d (95% CI = 0.17–0.02 kg/d; $P = 0.01$) and tended to produce 0.06 ± 0.03 kg protein/d (95% CI = −0.006 to 0.12 kg/d; $P = 0.07$) more compared with control cows, irrespective of time on supplementation (Figures 1C and 1D; Table 3). No evidence of association between microbial feed supplementation and concentration of milk fat (LSM difference ± SE = 0.05 ± 0.04 percentage units; 95% CI = −0.04 to 0.13 percentage units; $P = 0.30$) or concentration of milk protein was observed (LSM difference ± SE = −0.003 ± 0.036 percentage units; 95% CI = −0.07 to 0.07 percentage units; $P = 0.94$).

DMI and Feed Efficiency

A treatment effect conditional to time was observed for DMI ($P = 0.04$); at wk 15 DMI was 1.49 ± 0.63 kg/d higher for GF-MFS compared with control cows (95% CI = 0.25–2.73 kg/d; $P = 0.02$; Table 3; Figure 2A); however, no further differences were detected.

A treatment effect that tended to be conditional to time was observed for feed efficiency (Table 3). Overall feed efficiency was 0.09 ± 0.04 units higher for GF-MFS compared with control cows (95% CI = 0.007–0.18 units; $P = 0.03$); over time, statistical differences or trends were detected at study wk 3 (0.11 ± 0.06 units; $P = 0.06$), 5 (0.13 ± 0.05 units; $P = 0.01$), 6 (0.10 ± 0.05 units; $P = 0.05$), 7 (0.12 ± 0.05 units; $P = 0.007$), 9 (0.07 ± 0.04 units; $P = 0.09$), 12 (0.10 ± 0.06 units; $P = 0.10$), 14 (0.14 ± 0.06 units; $P = 0.01$), 15 (0.08 ± 0.05 units; $P = 0.09$), and 16 (0.10 ± 0.05 units; $P = 0.02$; Figure 2B).

Enteric Methane Emissions

Methane Production. No evidence of microbial feed supplementation effect on CH_4 production was observed when data were analyzed on a weekly (1.47 ± 11.49 g/d; 95% CI = −21.78 to 24.72 g/d; $P = 0.90$; Figure 3A) or a monthly basis (−0.57 ± 11.67 g/d; 95% CI = −24.17 to 23.03 g/d; $P = 0.96$; Table 4).

Methane Yield. No evidence of microbial feed supplementation effect on CH_4 yield was observed when data were analyzed on a weekly basis (−0.07 ± 0.49 g/kg of DMI; 95% CI = −1.06 to 0.93 g/kg DMI; $P = 0.89$; Figure 3B) or a monthly basis (−0.20 ± 0.50 g/kg of DMI; 95% CI = −1.22 to 0.82 g/kg of DMI; $P = 0.70$; Table 4).

Methane Intensity. A trend for a reduction on CH_4 intensity associated with microbial feed supplementation was observed when data were analyzed on a monthly basis (−0.56 ± 0.33 g/kg of ECM; 95% CI = −1.22 to 0.09 g/kg ECM; $P = 0.09$; Table 4). However, the support for this trend was weaker when data were analyzed on a weekly basis (−0.51 ± 0.33 g/kg of ECM; 95% CI = −1.19 to 0.17 g/kg of ECM; $P = 0.13$; Figure 3C).

BW

A trend for a treatment effect conditional to time on BW was observed ($P = 0.06$); however, treatment comparisons within study week were not significant (Table 3; Figure 4A). Similarly, a trend for a treatment effect conditional to time on BW gain was observed ($P = 0.07$); by study wk 16 BW gain was 6.18 ± 2.55 kg lower for GF-MFS compared with control cows (95% CI = 1.03–11.34 kg; $P = 0.02$; Table 3; Figure 4B).

SCC

A treatment effect was observed for log_{10}SCC; overall log_{10}SCC was −0.13 ± 0.06 units lower for GF-MFS compared with control cows (95% CI = −0.25 to −0.005 units; $P = 0.04$; Table 3; Figure 5).

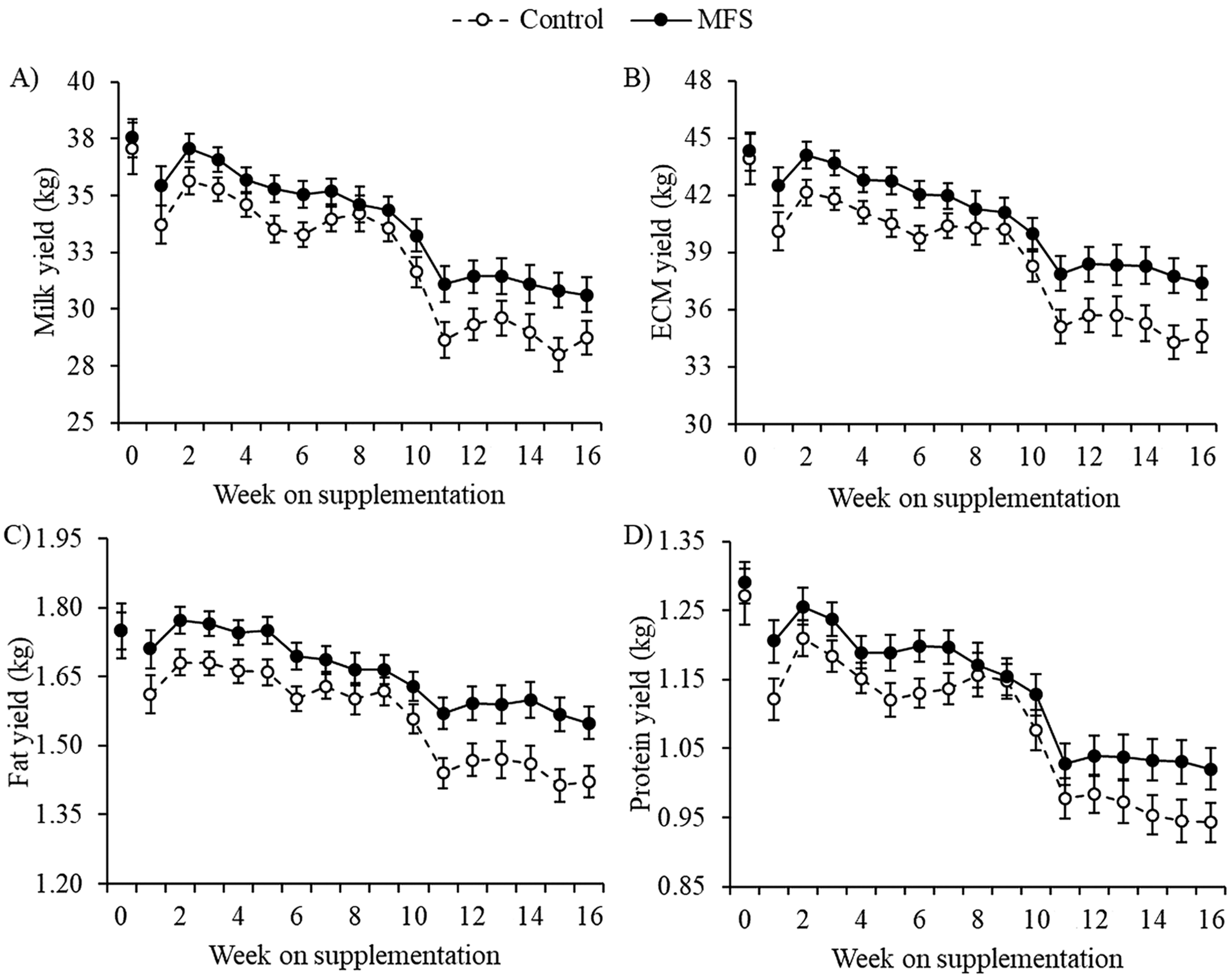

Figure 1. Milk yield (A), ECM yield (B), fat yield (C), and protein yield (D) LSM by treatment and week on supplementation for multiparous Jersey cows assigned to control or microbial feed supplementation (GF-MFS) from 59 ± 8 to 171 ± 8 DIM. Week 0 represents baseline values. Error bars represent SEM. Fixed effects included in the statistical models represented were baseline, lactation number, treatment, time, and treatment by time. Treatments were control (no microbial supplementation; n = 23) and GF-MFS (TMR containing 5 g/cow per day of a microbial feed supplement containing a minimum of *Clostridium beijerinckii* at 2 × 10^6 cfu/g, *Pichia kudriavzevii* at 2 × 10^7 cfu/g, *Ruminococcus bovis* at 2 × 10^7 cfu/g, and *Butyrivibrio fibrisolvens* at 2 × 10^7 cfu/g; n = 23; GALAXIS FRONTIER; Native Microbials Inc., San Diego, CA).

DISCUSSION

The objective of this study was to evaluate the effects of a native rumen microbial feed supplement on cows' production performance (milk production, milk composition, and feed efficiency). Additionally, the effect of the microbial supplement on enteric methane emissions was assessed. Based on the roles of the microbes provided in the evaluated product and previous research, we hypothesized that daily feeding of a TMR supplemented with the native rumen microbes *C. beijerinckii*, *P. kudriavzevii*, *R. bovis*, and *B. fibrisolvens* (GALAXIS FRONTIER; Native Microbials Inc., San Diego, CA) would increase Jersey cow milk yields and feed efficiency.

Higher ECM, as well as milk and fat yields, were observed for GF-MFS compared with control cows during the 16-wk supplementation period. Both the lower and upper ends of the 95% CI accompanying the mean treatment difference support the hypothesis of positive milk production effects of the evaluated native rumen microbial supplement, in agreement with previous research (Valldecabres et al., 2022; Bulnes et al., 2025; Marinho et al., 2024). Both in this current study and our previous study (Valldecabres et al., 2022), as well as in the study

Table 3. The LSM (±SEM) of various outcomes for mid-lactation multiparous Jersey cows assigned to control or microbial feed supplementation (GF-MFS) during 16 wk

	Treatment[1]		Fixed effects *P*-value[2]		
Outcome	Control	GF-MFS	Treatment	Time	Treatment × time
Productive outcome					
ECM yield, kg/d	38.45 ± 0.63	40.65 ± 0.67	0.02	<0.001	0.88
Milk yield, kg/d	32.05 ± 0.52	33.69 ± 0.55	0.03	<0.001	0.81
Fat yield, kg/d	1.56 ± 0.03	1.66 ± 0.03	0.01	<0.001	0.78
Protein yield, kg/d	1.08 ± 0.02	1.13 ± 0.02	0.07	<0.001	0.58
Fat concentration, %	4.91 ± 0.03	4.95 ± 0.03	0.30	<0.001	0.13
Protein concentration, %	3.35 ± 0.02	3.34 ± 0.03	0.94	<0.001	0.18
DMI, kg/d	23.80 ± 0.35	23.99 ± 0.37	0.69	<0.001	0.04
Feed efficiency	1.63 ± 0.03	1.72 ± 0.03	0.04	<0.001	0.07
BW, kg	451.1 ± 3.5	453.3 ± 3.7	0.66	<0.001	0.06
Log_{10}SCC, cells/mL[3]	1.81 ± 0.04	1.68 ± 0.04	0.04	<0.001	0.86

[1] Control: No microbial TMR supplementation (n = 23; DMI and feed efficiency: n = 22); GF-MFS: TMR containing 5 g/cow per day of a microbial feed supplement containing a minimum of *Clostridium beijerinckii* at 2 × 106 cfu/g, *Pichia kudriavzevii* at 2 × 107 cfu/g, *Ruminococcus bovis* at 2 × 107 cfu/g, and *Butyrivibrio fibrisolvens* at 2 × 107 cfu/g (n = 23; GALAXIS FRONTIER; Native Microbials Inc., San Diego, CA).

[2] Models also included the effects of baseline and parity.

by Bulnes et al. (2025), treatment differences increased over time, thus, it is plausible that larger effects on ECM would be detected if GF-MFS had been provided for a longer period in the present study. In contrast to our findings, 2 previous studies evaluating the same native rumen microbial product did not find evidence of treatment effects on milk, ECM, and fat yields, or found it to be conditional to DIM at the start of supplementation (Dickerson et al., 2022; Goldsmith et al., 2023). Compared with our study, these studies statistically analyzed together 2 different native rumen microbial feed supplements, which in our experience may elicit different production responses (Valldecabres et al., 2022), and had higher variability in the enrolled cows in terms of parity and DIM at enrollment, all potentially limiting their ability to detect treatment effects. Further, in our study microbes were fed mixed in the TMR, mimicking the use of the product in commercial operations, while previous research top dressed the product on feed.

Feed efficiency was on average 5.5% higher in GF-MFS cows compared with control cows, with the true value being between 0.4% and 11.0% higher in GF-MFS cows compared with control cows. In agreement, previous research has reported on average a 3% to 5% improvement in feed efficiency (Valldecabres et al., 2022; Goldsmith et al., 2023; Marinho et al., 2024). Consistent feed efficiency response suggests that dietary nutrient utilization is improved in GF-MFS cows. The average treatment effect on DMI could range from a 3.4% decrease to a 5.1% increase in DMI (95% CI = −0.81 to 1.21 kg/d), and the observed treatment by time effect, with significance at a single study week, was likely driven by factors other than a consistent treatment effect, making it challenging to determine whether higher nutrient intake contributed to the increased milk production in GF-MFS cows. In our previous study, treatment effects on DMI increased over time along with milk production; however, GF-MFS cows still demonstrated higher feed efficiency than control cows (Valldecabres et al., 2022). In studies without evidence for treatment effects on DMI, feed efficiency among the GF-MFS cows was still increased (Bulnes et al., 2025; Marinho et al., 2024) or tended to be increased (Dickerson et al., 2022). Last, our results do not suggest that energy for milk production originated from body tissue mobilization. Treatment effects on BW during the 16-wk study period were minimal, ranging from a 1.7% decrease to a 2.7% increase (95% CI = −7.74 to 12.09 kg), and treatment effects on BW gain, even when significant by study wk 16, do not suggest BW loss for GF-MFS cows. In alignment with these findings, with the exception of Goldsmith et al. (2023), previous research has not found evidence of association between supplementation with native rumen microbes and BW (Dickerson et al., 2022; Valldecabres et al., 2022; Marinho et al., 2024). Furthermore, Bulnes et al. (2025) measured plasma nonesterified fatty acid concentration and found no evidence of a treatment effect.

Decreased methanogenesis is one potential pathway for improving feed efficiency because energy in CH_4 could be redirected to fermentation products with value to the cow (Badhan et al., 2025). However, we were not able to find evidence for treatment effects on CH_4 production in this study as discussed later in this section. Methane production in our study was similar to the average reported for US dairy cows (91% Holstein) from

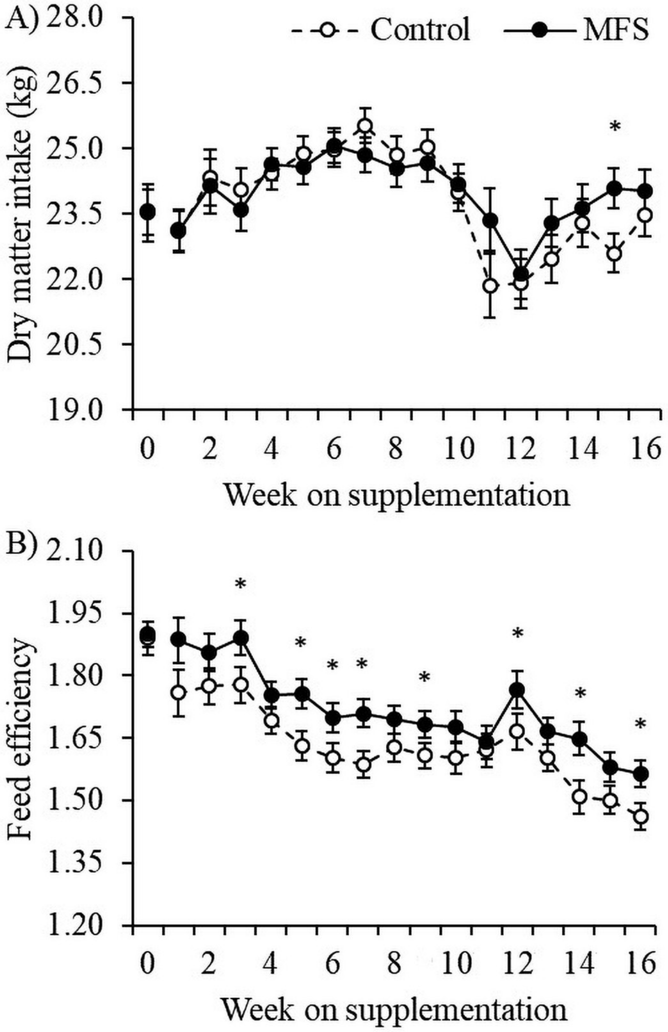

Figure 2. Dry matter intake (A) and feed efficiency (B; ECM/DMI) LSM by treatment and week on supplementation for multiparous Jersey cows assigned to control or microbial feed supplementation (GF-MFS) from 59 ± 8 to 171 ± 8 DIM. Week 0 represents baseline values. Error bars represent SEM. *Indicates differences at $P < 0.10$. Fixed effects included in the statistical models represented are baseline, lactation number, treatment, time, and treatment by time. Treatments were control (no microbial supplementation; n = 22) and GF-MFS (TMR containing 5 g/cow per day of a microbial feed supplement containing a minimum of *Clostridium beijerinckii* at 2 × 10^6 cfu/g, *Pichia kudriavzevii* at 2 × 10^7 cfu/g, *Ruminococcus bovis* at 2 × 10^7 cfu/g, and *Butyrivibrio fibrisolvens* at 2 × 10^7 cfu/g; n = 23; GALAXIS FRONTIER; Native Microbials Inc., San Diego, CA).

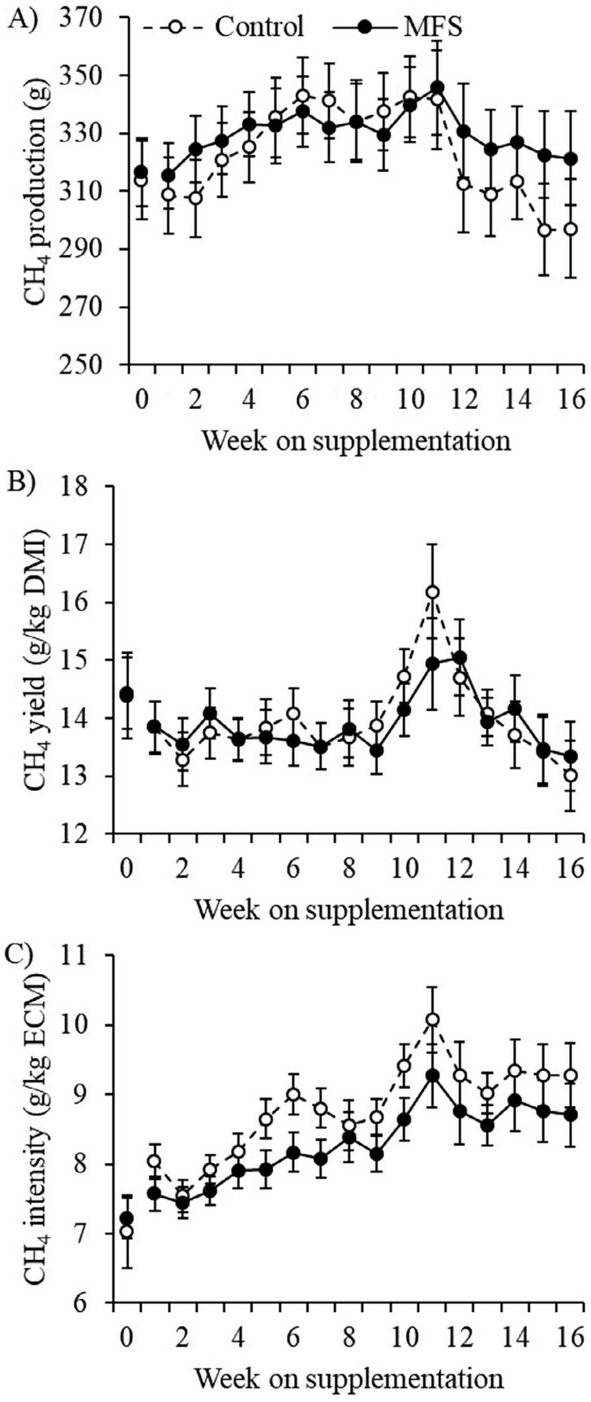

Figure 3. Methane production (A), yield (B), and intensity (C) LSM by treatment and week on supplementation for multiparous Jersey cows assigned to control or microbial feed supplementation (GF-MFS) from 59 ± 8 to 171 ± 8 DIM. Week 0 represents baseline values. Error bars represent SEM. Fixed effects included in the statistical models represented were baseline, lactation number, treatment, time, and treatment by time. Treatments were control (no microbial supplementation; n = 21) and GF-MFS (TMR containing 5 g/cow per day of a microbial feed supplement containing a minimum of *Clostridium beijerinckii* at 2 × 10^6 cfu/g, *Pichia kudriavzevii* at 2 × 10^7 cfu/g, *Ruminococcus bovis* at 2 × 10^7 cfu/g, and *Butyrivibrio fibrisolvens* at 2 × 10^7 cfu/g; n = 23; GALAXIS FRONTIER; Native Microbials Inc., San Diego, CA).

45 studies (340 g/d; Niu et al., 2018), although we note that CH_4 production may be expected to vary by breed. For example, King et al. (2011) reported differences in rumen methanogen populations between Jersey and Holstein cows managed under the same conditions, and Lahart et al. (2024) reported lower CH_4 production in Jersey cows compared with Holstein cows, likely due to lower feed intake. We did not find evidence for a treatment effect or lack thereof on enteric CH_4 produc-

Table 4. The LSM (± SEM) of enteric methane emissions outcomes for mid-lactation multiparous Jersey cows assigned to control or microbial feed supplementation (GF-MFS) during 16 wk

	Treatment[1]		Fixed effects *P*-value[2]		
Outcome	Control	GF-MFS	Treatment	Time	Treatment × time
Weekly					
CH_4, g/d	328.97 ± 8.23	330.44 ± 8.42	0.90	<0.001	0.89
CH_4 yield, g/kg of DMI	13.96 ± 0.35	13.89 ± 0.36	0.89	<0.001	0.94
CH_4 intensity, g/kg of ECM	8.82 ± 0.24	8.30 ± 0.24	0.13	<0.001	0.84
Monthly					
CH_4, g/d	326.97 ± 8.36	326.40 ± 8.51	0.96	<0.001	0.21
CH_4 yield, g/kg of DMI	13.85 ± 0.36	13.65 ± 0.37	0.70	<0.001	0.47
CH_4 intensity, g/kg of ECM	8.82 ± 0.23	8.26 ± 0.24	0.09	<0.001	0.51

[1] Control: No microbial TMR supplementation (n = 21); GF-MFS: TMR containing 5 g/cow per day of a microbial feed supplement containing a minimum of *Clostridium beijerinckii* at 2 × 106 cfu/g, *Pichia kudriavzevii* at 2 × 107 cfu/g, *Ruminococcus bovis* at 2 × 107 cfu/g, and *Butyrivibrio fibrisolvens* at 2 × 107 cfu/g (n = 23; GALAXIS FRONTIER; Native Microbials Inc., San Diego, CA).

[2] Models also included the effect of baseline and parity.

tion and CH_4 yield. In contrast, we observed a trend for lower CH_4 intensity for GF-MFS compared with control cows, with the lower end of the true mean 95% CI reaching a reduction of 1.22 g CH_4/kg of ECM. This effect was driven by the previously described higher ECM yields associated with microbial feed supplementation without differences in enteric CH_4 production for GF-MFS and control cows. However, larger studies with more cows are needed to further understand the potential effect of the evaluated microbial feed supplement on enteric CH_4 emissions.

Although the feed efficiency response suggests that dietary nutrient utilization is improved in GF-MFS cows, a lack of evidence for reduced CH_4 production means that the specific mode of action remains unknown. Prior studies show no improvements in apparent digestibility of DM, organic matter, NDF, starch, and CP with the GF-MFS (Goldsmith et al., 2023), although total-tract digestibility analyses may further this understanding. Improvements in feed efficiency might be due to changes in ruminal fermentation patterns with GF-MFS supplementation, as evidenced by trending increases in total short-chain fatty acids and the gluconeogenic precursor propionate (Marinho et al., 2024). Alternatively, the observed increase in feed efficiency in cows supplemented with GF-MFS may involve hormonally-driven changes in nutrient partitioning, as suggested by lower blood insulin levels (Goldsmith et al., 2023) and increased mammary epithelial cells and alveoli count number in mammary biopsies from cows supplemented with GF-MFS at parturition (Native Microbials, Inc., San Diego, CA; unpublished data). Further research is required to better understand the mechanisms responsible for the observed treatment effects.

We observed 0.3% to 13.8% decrease in $\log_{10}$SCC for GF-MFS compared with control cows. The biological implications of this treatment effect depend on whether the true value is closer to or further from 0. Recent evidence suggests potential biological mechanisms by which GF-MFS could contribute to lower SCC. Tabor et al. (2025) reported an increase in neutrophil oxidative burst following supplementation with GF-MFS and Bulnes et al. (2025) identified mild increases in plasma reactive oxygen metabolites and the acute phase proteins ceruloplasmin and haptoglobin, all indicative of heightened innate immune responsiveness. These observations are consistent with the broader literature showing that the rumen microbiome can modulate host immunity (Trevisi et al., 2018; Bronzo et al., 2020). Our findings, however, contrast with previous research that did not report statistical evidence for such an association (Dickerson et al., 2022; Valldecabres et al., 2022; Goldsmith et al., 2023; Marinho et al., 2024). Literature reports higher SCC in Jersey cows compared with Holstein cows, while mastitis incidence may be lower in Jerseys (Washburn et al., 2002; Sewalem et al., 2006; Magro et al., 2023). Thus, breed differences may account for the variability observed among studies. It is important to note that the studies included cows at different stages of the lactation, which may influence SCC values (Magro et al., 2023). No cases of clinical mastitis were observed during our study.

Considering findings from previous research and our efforts to minimize bias in the present study, we accept the alternative hypothesis that the TMR supplementation with a native rumen microbial feed supplement containing *C. beijerinckii*, *P. kudriavzevii*, *R. bovis*, and *B. fibrisolvens* (GALAXIS FRONTIER; Native Microbials Inc., San Diego, CA) is associated with improved milk, ECM, and fat yields, as well as with improved feed efficiency. Weaker or nonsignificant evidence was found for treatment-associated changes in milk compo-

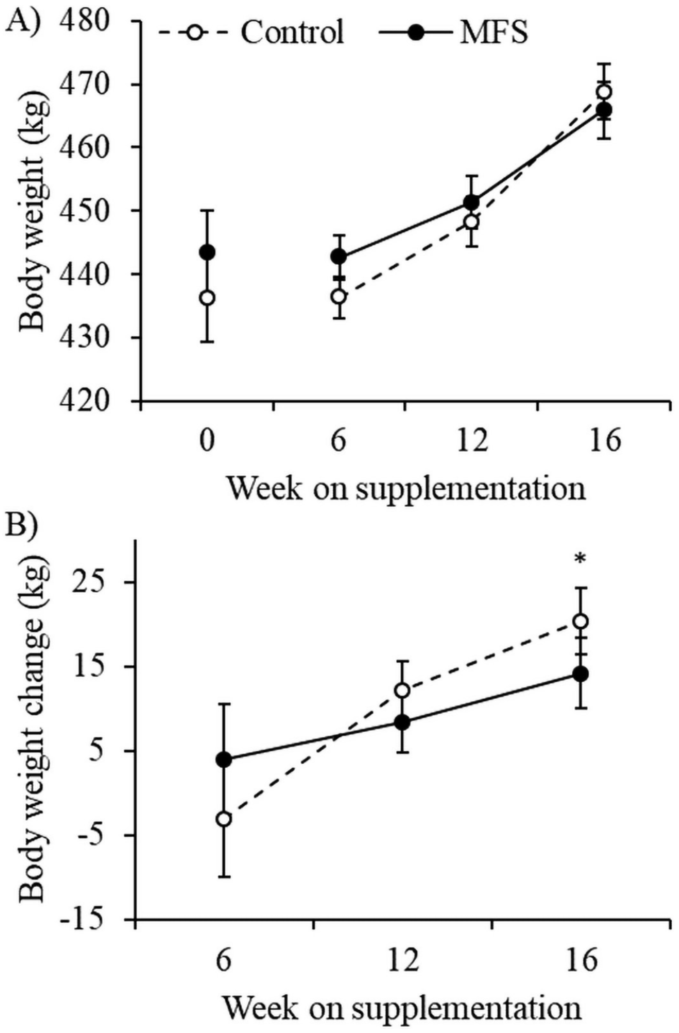

Figure 4. Body weight (A) and BW change (B) LSM by treatment and week on supplementation for multiparous Jersey cows assigned to control or microbial feed supplementation (GF-MFS) from 59 ± 8 to 171 ± 8 DIM. Week 0 represents baseline values. Error bars represent SEM. *Indicates differences at $P \leq 0.05$. Fixed effects included in the statistical models represented are baseline (only for BW), lactation number, treatment, time, and treatment by time. Treatments were control (no microbial supplementation; n = 22) and GF-MFS (TMR containing 5 g/cow per day of a microbial feed supplement containing a minimum of *Clostridium beijerinckii* at 2×10^6 cfu/g, *Pichia kudriavzevii* at 2×10^7 cfu/g, *Ruminococcus bovis* at 2×10^7 cfu/g, and *Butyrivibrio fibrisolvens* at 2×10^7 cfu/g; n = 23; GALAXIS FRONTIER; Native Microbials Inc., San Diego, CA).

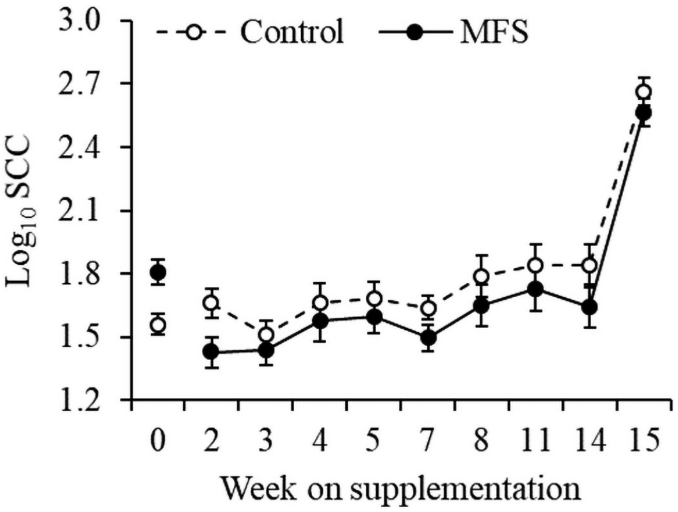

Figure 5. Log_{10} milk SCC LSM by treatment and week on supplementation for multiparous Jersey cows assigned to control or microbial feed supplementation (GF-MFS) from 59 ± 8 to 171 ± 8 DIM. Week 0 represents baseline values. Error bars represent SEM. Fixed effects included in the statistical models represented were baseline, lactation number, treatment, time, and treatment by time. Treatments were control (no microbial supplementation; n = 23) and GF-MFS (TMR containing 5 g/cow per day of a microbial feed supplement containing a minimum of *Clostridium beijerinckii* at 2×10^6 cfu/g, *Pichia kudriavzevii* at 2×10^7 cfu/g, *Ruminococcus bovis* at 2×10^7 cfu/g, and *Butyrivibrio fibrisolvens* at 2×10^7 cfu/g; n = 23; GALAXIS FRONTIER; Native Microbials Inc., San Diego, CA).

sition, protein yield, DMI, and enteric CH_4 emissions, and further research is needed if conclusions in this regard are to be reached.

CONCLUSIONS

Total mixed ration supplementation with a native rumen microbial feed supplement containing *C. beijerinckii*, *P. kudriavzevii*, *R. bovis*, and *B. fibrisolvens* at 5 g/cow per day was associated with higher milk, ECM, and fat yields, as well as higher feed efficiency and lower $\log_{10}$SCC. In addition, supplementation was associated with trends for higher milk protein yield and lower CH_4 intensity. We did not find evidence of treatment effects or lack thereof for milk fat and protein concentrations, DMI, and enteric CH_4 production and yield. Thus, supplementation of the evaluated native rumen microbes in TMR is a promising strategy to improve dairy production efficiency and sustainability, but larger studies are required to assess the effect of the evaluated native rumen microbial feed supplement on enteric CH_4 emissions.

NOTES

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Valldecabres) and senior author (A. Lago) of this study have not stated any conflicts of interest.

Nonstandard abbreviations used: GF-MFS = treatment group, GALAXIS FRONTIER (Native Microbials, Inc., San Diego, CA) at 5 g/cow per day.

REFERENCES

Aikman, P. C., C. K. Reynolds, and D. E. Beever. 2008. Diet digestibility, rate of passage, and eating and rumination behavior of Jersey and Holstein cows. J. Dairy Sci. 91:1103–1114. https://doi.org/10.3168/jds.2007-0724.

Badhan, A., Y. Wang, S. Terry, R. Gruninger, L. L. Guan, and T. A. McAllister. 2025. *Invited review:* Interplay of rumen microbiome and the cattle host in modulating feed efficiency and methane emissions. J. Dairy Sci. 108:5489–5501. https://doi.org/10.3168/jds.2024-26063.

Bronzo, V., V. Lopreiato, F. Riva, M. Amadori, G. Curone, M. F. Addis, P. Cremonesi, P. Moroni, E. Trevisi, and B. Castiglioni. 2020. The role of innate immune response and microbiome in resilience of dairy cattle to disease: The mastitis model. Animals (Basel) 10:1397. https://doi.org/10.3390/ani10081397.

Bulnes, M., J. Bonilla, M. Suazo, T. C. Michelotti, A. Paz, J. Lefler, C. Marotz, M. Embree, G. Begalli, J. Halfen, T. Fernandes, E. Trevisi, M. E. Uddin, and J. S. Osorio. 2025. Effects of peripartal rumen-derived direct-fed microbials supplementation on lactation performance, metabolism, ruminal fermentation, and microbial abundance in dairy cows. J. Dairy Sci. 108:2354–2370. https://doi.org/10.3168/jds.2024-25058.

Dickerson, A. M., F. Yang, H. B. Green, M. M. Embree, and J. K. Drackley. 2022. Feeding native rumen microbial supplements increases energy-corrected milk production and feed efficiency by Holstein cows. JDS Commun. 3:239–244. https://doi.org/10.3168/jdsc.2022-0210.

Embree, M., L. Picking, G. Gogul, and J. Tarasova. 2022. Microbial compositions and methods of use for improving milk production. Native Microbials Inc. US Pat. No. 20220287330 A1.

Faul, F., E. Erdfelder, A.-G. Lang, and A. Buchner. 2007. GPower 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39:175–191. https://doi.org/10.3758/BF03193146.

Goldsmith, K., J. Lefler, M. Embree, and M. J. VandeHaar. 2023. The effect of supplementing native rumen microbes on milk production of dairy cows. JDS Commun. 4:31–34. https://doi.org/10.3168/jdsc.2022-0250.

Gomez-Flores, M., G. Nakhla, and H. Hafez. 2017. Hydrogen production and microbial kinetics of *Clostridium termitidis* in mono-culture and co-culture with *Clostridium beijerinckii* on cellulose. AMB Express 7:84. https://doi.org/10.1186/s13568-016-0256-2.

Jeyanathan, J., C. Martin, and D. P. Morgavi. 2014. The use of direct-fed microbials for mitigation of ruminant methane emissions: A review. Animal 8:250–261. https://doi.org/10.1017/S1751731113002085.

Kenny, D. A., C. Fitzsimons, S. M. Waters, and M. McGee. 2018. Invited review: Improving feed efficiency of beef cattle—The current state of the art and future challenges. Animal 12:1815–1826. https://doi.org/10.1017/S1751731118000976.

King, E. E., R. P. Smith, B. St-Pierre, and A. D. G. Wright. 2011. Differences in the rumen methanogen populations of lactating Jersey and Holstein dairy cows under the same diet regimen. Appl. Environ. Microbiol. 77:5682–5687. https://doi.org/10.1128/AEM.05130-11.

Kristensen, T., C. Jensen, S. Østergaard, M. R. Weisbjerg, O. Aaes, and N. I. Nielsen. 2015. Feeding, production, and efficiency of Holstein-Friesian, Jersey, and mixed-breed lactating dairy cows in commercial Danish herds. J. Dairy Sci. 98:263–274. https://doi.org/10.3168/jds.2014-8532.

Lahart, B., F. Buckley, J. Herron, R. Fitzgerald, E. Fitzpatrick, N. Galvin, and L. Shalloo. 2024. Evaluating enteric methane emissions within a herd of genetically divergent grazing dairy cows. J. Dairy Sci. 107:383–397. https://doi.org/10.3168/jds.2022-22646.

Lopez, S., F. M. McIntosh, R. J. Wallace, and C. J. Newbold. 1999. Effect of adding acetogenic bacteria on methane production by mixed rumen microorganisms. Anim. Feed Sci. Technol. 78:1–9. https://doi.org/10.1016/S0377-8401(98)00273-9.

Magro, S., A. Costa, M. Santinello, M. Penasa, and M. De Marchi. 2023. Udder health-related traits in cow milk: Phenotypic variability and effect on milk yield and composition. Animal 17:100823. https://doi.org/10.1016/j.animal.2023.100823.

Manzanilla-Pech, C. I. V., R. B. Stephansen, G. F. Difford, P. Løvendahl, and J. Lassen. 2022. Selecting for feed efficient cows will help to reduce methane gas emissions. Front. Genet. 13:885932. https://doi.org/10.3389/fgene.2022.885932.

Marinho, M. N., M. C. Perdomo, B. S. Simões, A. Husnain, U. Arshad, C. C. Figueiredo, and J. E. P. Santos. 2024. Dietary supplementation of rumen native microbes improves lactation performance and feed efficiency in dairy cows. J. Dairy Sci. 107:7918–7931. https://doi.org/10.3168/jds.2024-24795.

Martins, L. F., S. F. Cueva, C. F. A. Lage, M. Ramin, T. Silvestre, J. Tricarico, and A. N. Hristov. 2024. A meta-analysis of methane-mitigation potential of feed additives evaluated in vitro. J. Dairy Sci. 107:288–300. https://doi.org/10.1016/S0022-0302(23)00819-6.

McAllister, T. A., K. A. Beauchemin, A. Y. Alazzeh, J. Baah, R. M. Teather, and K. Stanford. 2011. Review: The use of direct fed microbials to mitigate pathogens and enhance production in cattle. Can. J. Anim. Sci. 91:193–211. https://doi.org/10.4141/cjas10047.

Metwaly, A., S. Reitmeier, and D. Haller. 2022. Microbiome risk profiles as biomarkers for inflammatory and metabolic disorders. Nat. Rev. Gastroenterol. Hepatol. 19:383–397. https://doi.org/10.1038/s41575-022-00581-2.

Monteiro, H. F., C. C. Figueiredo, B. Mion, J. E. P. Santos, R. S. Bisinotto, F. Peñagaricano, E. S. Ribeiro, M. N. Marinho, R. Zimpel, A. C. da Silva, A. Oyebade, R. R. Lobo, W. M. Coelho Jr, P. M. G. Peixoto, M. B. Ugarte Marin, S. G. Umaña-Sedó, T. D. G. Rojas, M. Elvir-Hernandez, F. S. Schenkel, B. C. Weimer, C. T. Brown, E. Kebreab, and F. S. Lima. 2024. An artificial intelligence approach of feature engineering and ensemble methods depicts the rumen microbiome contribution to feed efficiency in dairy cows. Anim. Microbiome 6:5. https://doi.org/10.1186/s42523-024-00289-5.

NASEM. (National Academies of Sciences, Engineering, and Medicine). 2021. Nutrient Requirements of Dairy Cattle. 8th rev. ed. The National Academies Press.

Ncho, C. M., S. H. Kim, S. A. Rang, and S. S. Lee. 2024. A meta-analysis of probiotic interventions to mitigate ruminal methane emissions in cattle: Implications for sustainable livestock farming. Animal 18:101180. https://doi.org/10.1016/j.animal.2024.101180.

Niu, M., E. Kebreab, A. N. Hristov, J. Oh, C. Arndt, A. Bannink, A. R. Bayat, A. F. Brito, T. Boland, D. Casper, L. A. Crompton, J. Dijkstra, M. A. Eugène, P. C. Garnsworthy, M. N. Haque, A. L. F. Hellwing, P. Huhtanen, M. Kreuzer, B. Kuhla, P. Lund, J. Madsen, C. Martin, S. C. McClelland, M. McGee, P. J. Moate, S. Muetzel, C. Muñoz, P. O'Kiely, N. Peiren, C. K. Reynolds, A. Schwarm, K. J. Shingfield, T. M. Storlien, M. R. Weisbjerg, D. R. Yáñez-Ruiz, and Z. Yu. 2018. Prediction of enteric methane production, yield, and intensity in dairy cattle using an intercontinental database. Glob. Chang. Biol. 24:3368–3389. https://doi.org/10.1111/gcb.14094.

Pittaluga, A. M., F. Yang, J. R. Gaffney, M. Embree, and A. E. Relling. 2023. Effect of supplementation with ruminal probiotics on growth performance, carcass characteristics, plasma metabolites, methane emissions, and the associated rumen microbiome changes in beef cattle. J. Anim. Sci. 101:skac308. https://doi.org/10.1093/jas/skac308.

Prendiville, R., K. M. Pierce, and F. Buckley. 2009. An evaluation of production efficiencies among lactating Holstein-Friesian, Jersey, and Jersey × Holstein-Friesian cows at pasture. J. Dairy Sci. 92:6176–6185. https://doi.org/10.3168/jds.2009-2292.

Rotz, C. A. 2018. Modeling greenhouse gas emissions from dairy farms. J. Dairy Sci. 101:6675–6690. https://doi.org/10.3168/jds.2017-13272.

Russell, B. J., S. D. Brown, N. Siguenza, I. Mai, A. R. Saran, A. Lingaraju, E. S. Maissy, A. C. D. Machado, A. F. M. Pinto, C. Sanchez, L.-A. Rossitto, Y. Miyamoto, R. A. Richter, S. B. Ho, L. Eckmann, J.

Hasty, D. J. Gonzalez, A. Saghatelian, R. Knight, and A. Zarrinpar. 2022. Intestinal transgene delivery with native E. coli chassis allows persistent physiological changes. Cell 185:3263–3277.e15. https://doi.org/10.1016/j.cell.2022.06.050.

Sandoval-Espinola, W. J., M. S. Chinn, M. R. Thon, and J. M. Bruno-Bárcena. 2017. Evidence of mixotrophic carbon-capture by n-butanol-producer *Clostridium beijerinckii*. Sci. Rep. 7:12759. https://doi.org/10.1038/s41598-017-12962-8.

Sewalem, A., F. Miglior, G. J. Kistemaker, and B. J. Van Doormaal. 2006. Analysis of the relationship between somatic cell score and functional longevity in Canadian dairy cattle. J. Dairy Sci. 89:3609–3614. https://doi.org/10.3168/jds.S0022-0302(06)72400-6.

Tabor, E., A. R. Guadagnin, M. Guenther, L. R. Cangiano, F. Panisa, B. Anderson, L. Niehues, C. Marotz, M. Embree, and J. Laporta. 2025. Effects of feeding a rumen-native microbial live supplement during pre- and postpartum on health, performance, and blood metabolites of Holstein cows. J. Dairy Sci. 108:12705–12721. https://doi.org/10.3168/jds.2025-26677.

Trevisi, E., F. Riva, J. F. S. Filipe, M. Massara, A. Minuti, P. Bani, and M. Amadori. 2018. Innate immune responses to metabolic stress can be detected in rumen fluids. Res. Vet. Sci. 117:65–73. https://doi.org/10.1016/j.rvsc.2017.11.008.

USDA-NAHMS (USDA National Animal Health Monitoring System). 2014. Dairy cattle management practices in the United States.

USDA. 2016. Dairy 2014: Dairy cattle management practices in the United States, 2014. USDA, Animal and Plant Health Inspection Service, Veterinary Services, Center for Epidemiology and Animal Health, Fort Collins, CO. Accessed Nov. 28, 2025. https://www.aphis.usda.gov/sites/default/files/dairy14_dr_parti_1.pdf.

Valldecabres, A., S. P. Gilmore, J. J. Embree, I. Z. Zhelev, J. R. Gaffney, C. A. Marotz, F. Yang, A. S. Izzo, M. M. Embree, and A. Lago. 2022. Effects of rumen-native microbial feed supplementation on milk yield, composition, and feed efficiency in lactating dairy cows. J. Anim. Sci. 100:skac275. https://doi.org/10.1093/jas/skac275.

Washburn, S. P., S. L. White, J. T. Green Jr., and G. A. Benson. 2002. Reproduction, mastitis, and body condition of seasonally calved Holstein and Jersey cows in confinement or pasture systems. J. Dairy Sci. 85:105–111. https://doi.org/10.3168/jds.S0022-0302(02)74058-7.

Zehetmeier, M., J. Baudracco, H. Hoffmann, and A. Heißenhuber. 2012. Does increasing milk yield per cow reduce greenhouse gas emissions? A system approach. Animal 6:154–166. https://doi.org/10.1017/S1751731111001467.

Zengler, K., and M. Embree. 2016. Methods, apparatuses, and systems for analyzing microorganism strains from complex heterogeneous communities, predicting and identifying functional relationships and interactions thereof, and selecting and synthesizing microbial ensembles based thereon. Native Microbials Inc. US Pat. No. 20160376627A1.

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