Impacts of feeding a *Saccharomyces cerevisiae* fermentation product on productive performance, and metabolic and immunological responses during a feed-restriction challenge of mid-lactation dairy cows

T. N. Marins,1 F. A. Gutierrez Oviedo,1 M. L. G. F. Costa,1 Y.-C. Chen,1 H. Goodnight,1 M. Garrick,1 D. J. Hurley,2 J. K. Bernard,1 I. Yoon,3 and S. Tao1,3*

1Department of Animal and Dairy Science, University of Georgia, Athens 30602
2Food Animal Health and Management Program, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens 30602
3Diamond V, Cedar Rapids, IA 52404

**ABSTRACT**

*Saccharomyces cerevisiae* fermentation products are commonly used in dairy cattle ration to improve production efficiency and health. However, whether these benefits will persist during feed-restriction-induced negative energy balance is unknown. The objective of this experiment was to examine the effect of a *Saccharomyces cerevisiae* fermentation product (NT, NutriTek, Diamond V) on performance, metabolic, inflammatory, and immunological responses to a feed-restriction challenge in mid-lactation dairy cows. Sixty Holstein cows were blocked by parity, days in milk, and milk yield and then randomly assigned to 1 of the 2 supplements: NT or placebo (CTL). The supplements were mixed in total mixed ration before feeding at a rate of 19 g/d per cow. The production phase of the experiment lasted 12 wk. Intake and milk yield were recorded daily, and milk composition was measured weekly. After the production trial, a subset of cows (NT: n = 16; CTL: n = 16) were immediately enrolled in a 5-d feed-restriction challenge with 40% ad libitum intake followed by a 5-d realimentation. Milk yield and composition were measured at each milking from d −2 to 10 relative to feed restriction. Blood samples were collected on d −2, −1, 1, 2, 3, 4, 5, 6, 8, and 10 relative to the initiation of feed restriction to measure circulating metabolites, insulin, cortisol, IL-10, tumor necrosis factor-α, lipopolysaccharide binding protein, and haptoglobin. Immune function assessments, including peripheral mononuclear cell proliferation and functional assays of circulating granulocytes, were performed on d −3 and 4 of the feed restriction. No differences were observed in dry matter intake, milk yield, or concentrations or yield of components except for fat yield. An interaction of parity and treatment was observed for milk fat yield that was lower for CTL than NT in primiparous cows, but no differences were observed among treatments in milk fat yield of multiparous cows. Feed restriction successfully induced negative energy balance and its associated metabolic changes, including reduced concentrations of plasma glucose and increased nonesterified fatty acids and β-hydroxybutyrate. Cows fed NT had a similar decrease in milk yield but had a more pronounced reduction in plasma glucose concentration and greater β-hydroxybutyrate concentration during feed restriction than those fed CTL. Feed restriction did not induce evidence of systemic inflammation but did reduce granulocyte functional activity. Compared with CTL, feeding NT improved the reactive oxygen species production by granulocytes after stimulation by extracellular antigens. In conclusion, feeding NT increased milk fat production of first-lactation cows but did not affect overall productive performance. However, supplementation with NT improved induced granulocyte oxidative burst. This may explain the greater glucose utilization by cows fed NT rather than CTL during feed restriction.

Key words: *Saccharomyces cerevisiae* fermentation product, feed restriction, immunity, inflammation

**INTRODUCTION**

*Saccharomyces cerevisiae* fermentation products (SCFP) are a common supplement given to dairy cattle with the goal of improving production efficiency (Poppy et al., 2012). The modes of action include, but are not limited to, improved ruminal microbial growth that favors fiber digestion and lactate utilization, increased ruminal N utilization and microbial protein synthesis, and altered ruminal nutrient digestion (Yoon and Stern, 1995; Callaway and Martin, 1997; Hristov et al., 2010; Allen and Ying, 2012). The beneficial effect of SCFP on ruminal fermentation is particularly
apparent during nutritional challenges (Li et al., 2016; Khalouei et al., 2021; Tun et al., 2020). For instance, Tun et al. (2020) reported that supplementation of SCFP prevented the reduction in the abundance of fibrolytic bacteria and ciliated protozoa and maintained the richness and diversity of the rumen microbiota during grain-induced subacute ruminal acidosis. Additionally, supplementation of SCFP has been reported to improve animal health. In preweaning dairy calves, feeding SCFP reduced the incidence and severity of scour (Magalhães et al., 2008) and alleviated the disease symptoms and lung pathology associated with a bovine respiratory syncytial virus challenge (Mahmoud et al., 2020). Similarly, feeding SCFP reduced the sickness behavior score after an intravenous LPS challenge of weaned beef calves (Burdick Sanchez et al., 2020). In mid-lactation dairy cows, supplemental SCFP resulted in a faster recovery after an intramammary Streptococcus uberis challenge compared with unsupplemented controls (Vailati-Riboni et al., 2021). These data suggest several potential immunomodulatory roles for SCFP in cattle production. Indeed, SCFP contain a significant number and quantity of biologically active compounds that are known to modulate immune and inflammation functions (Jensen et al., 2008). Some of these compounds include oligosaccharides, organic acids, amino acids, and phytosterols (Kondo et al., 2014; Gessner et al., 2017).

Short-term feed restriction has been widely used to study the impact of negative energy balance (NEB) on performance, immunity, and metabolism. This approach successfully mimics NEB without the complications associated with hormonal changes related to parturition. In mid-lactation cows, feed restriction not only results in increased adipose tissue mobilization and hepatic ketogenesis but is also associated with impaired adaptive and innate immunity (Velez and Donkin, 2005; Moyes et al., 2009; Gross et al., 2011; Vanacker et al., 2020). Kvidera et al. (2017) reported that feed restriction might also induce systemic inflammation due to a potential increase in intestinal permeability. During the transition period, supplementation of SCFP improved milk production and DMI, enhanced mucosal and humoral immunity, and modulated systemic inflammation (Poppy et al., 2012; Zaworski et al., 2014; Yuan et al., 2015). However, it is not clear if the benefits of feeding SCFP persist throughout a feed-restriction challenge in mid-lactation dairy cows. No study addressing this effect has been published. Therefore, our hypotheses were that supplementation of SCFP would improve the performance of mid-lactation dairy cows and would minimize the negative impacts of NEB on immune and inflammatory responses typically induced by short-term feed restriction. Our objective was to examine the impact of supplementing a SCFP on production performance and metabolic, inflammatory, and immunological responses during feed restriction in mid-lactation dairy cows.

MATERIALS AND METHODS

The experiment was conducted at Dairy Research Center of the University of Georgia Tifton campus (Tifton, GA) from October 2019 to March 2020. The experimental procedure and animal handling were approved by the Institutional Animal Care and Use Committee of the University of Georgia (AUP#: A2019 01-024-A2) before the experiment began.

Sixty Holstein lactating dairy cows were blocked by parity, DIM, and milk yield. Within each block, cows were randomly assigned to 1 of the 2 treatments: TMR supplemented with 19 g/d per cow placebo (CTL, the same grain composition used for NutriTek production), and TMR supplemented with 19 g/d per cow NutriTek (NT, Diamond V). The randomization was achieved by tossing a coin, sides of which represent treatments. NT is a newly formulated, novel SCFP that contains additional bioactive compounds including antioxidants and phytosterols. The experiment included 2 replicates with 30 cows each. The first replicate included 12 primiparous and 18 multiparous cows, and the second replicate included 10 primiparous and 20 multiparous cows. The cows in the first replicate were enrolled in October 2019, and those in the second replicate were enrolled in November 2019. Cows in both replicates were housed in adjacent pens of the same free stall barn. The experiment included a 1-wk baseline period, a 12-wk production trial, a 5-d feed-restriction challenge, and a 5-d realimentation. During the baseline period, all cows were fed the CTL diet. During the production trial, the feed-restriction challenge, and the realimentation period, all cows received diets based on their respective treatments. All cows were managed in the same manner throughout the experiment. The average parity and DIM of cows at the onset of the production trial were 2.0 ± 0.9 and 117.7 ± 37.9 d (mean ± SD), respectively.

Throughout the entire experiment, air temperature and relative humidity inside the barn were monitored every 15 min by Hobo Pro Series Temp probes (Onset Computer Corporation). The temperature-humidity index (THI) was calculated as THI = (1.8 × T + 32) − [(0.55 − 0.0055 × RH) × (1.8 × T − 26)], where T = air temperature (°C) and RH = relative humidity (%) (NRC, 1971). All cows were trained to eat behind a Calan Broadbent Feeding System (American Calan Inc.) before the experiment. Cows were fed once daily (1700 h for replicate 1; 1600 h for replicate 2), and the
daily individual feed intake was recorded. The amount of feed delivered to each cow was adjusted daily to provide approximately 5 kg (as-fed basis) ors. The diet was formulated to meet or exceed the NRC (2001) requirements for a cow consuming 23.1 kg DM/d and producing 38.6 kg/d milk containing 3.5% fat and 3.0% true protein. Control or NT (19 g) was premixed with 881 g of ground corn, added to the data ranger, and mixed throughout with TMR before feeding. The CTL and NT were color coded, and all research personnel were blind to the treatment information until the experiment was completed. Dietary ingredients were sampled 3 times each week, and experimental diets were sampled daily to determine DM at 55°C for 48 h in a forced-air oven. Proportions of dietary ingredients were adjusted based on changes in DM content of the ingredients. Samples of the dried TMR were composited every 4 wk by treatment and replicate, ground to pass through a 1-mm screen using a Wiley mill (Thomas Scientific), and submitted to Cumberland Valley Analytical Services (Waynesboro, PA) for nutrient analysis. The ingredient composition and chemical content of the diets are presented in Tables 1 and 2, respectively. Cows were milked 3 times daily (0100, 0900, and 1700 h for cows in replicate 1; 0000, 0800, and 1600 h for cows in replicate 2), and individual milk yields were recorded at each milking (Delpro, DeLaval). Milk samples were collected with bronopol-B-14 as a preservative from 3 consecutive milkings, once each week, for composition (fat, protein, lactose, SNF, MUN, and SCC) analysis at Dairy One Cooperative (Ithaca, NY) using a Foss 4000 instrument (Foss North America). The BW and BCS (Wildman et al., 1982) were assessed every week after morning milking and before eating.

During the experiment, the health status of each cow was monitored and recorded daily. Lameness was identified when a cow exhibited an obvious limp. Clinical mastitis was diagnosed by physical change in the milk, such as clots and flakes, during forestripping at each milking. Subclinical mastitis was defined when milk SCC was greater than 200,000 cells/mL. Simple indigestion was defined by anorexia and ruminal hypo-motility to atony in the absence of fever and infectious disease. One cow fed NT in replicate 2 died because of clinical bovine leucosis at d 2 of the production trial. Therefore, her performance data (intake, milk yield and composition, BW, and BCS) were not included in the statistical analyses.

At the end of the production trial, a subset of cows (n = 32; replicate 1: 6 primiparous cows, 10 multiparous cows; replicate 2: 4 primiparous cows, 12 multiparous cows) were enrolled in a 5-d feed-restriction challenge and a following 5-d realimentation. The sample size of 16 cows per treatment was calculated based on the blood LPS binding protein data (4.7 vs. 8.3 µg/mL, respectively; SD = 2.88 µg/mL) of cows that received ad libitum feeding or 40% of ad libitum feeding (Kvidera et al., 2017), using a level of significance of 0.05 and 90% power. During feed restriction, the intake of the cow was restricted to 40% of her basal intake (Kvidera et al., 2017). The basal intake of each cow was calculated as the average intake (as-fed basis) of the week before feed restriction. The total amount of feed was divided into 3 equal portions and delivered 3 times each day (1700, 2400, and 0900 for replicate 1; 1600, 2300, and 0800 h for replicate 2). Treatments were top dressed onto TMR of the afternoon feeding during the feed restriction. During realimentation, feed was provided ad libitum.

| Table 1. Ingredient composition of the experimental diet; diets were supplemented with 19 g/d per cow of a placebo (CTL) or a Saccharomyces cerevisiae fermentation product (NT, NutriTek, Diamond V) |
| Ingredient, % of DM | CTL | NT |
|---------------------|-----|-----|
| Corn silage         | 42.26 | 42.26 |
| Bermudagrass hay    | 10.81 | 10.81 |
| Brewers grains, wet | 7.86  | 7.86  |
| Ground corn         | 11.49 | 11.49 |
| Placebo             | 0.07  | —    |
| NT                  | —    | 0.07  |
| Liquid supplement1  | 3.93  | 3.93  |
| Corn gluten feed    | 4.51  | 4.51  |
| Soybean hulls, pelleted | 4.51 | 4.51 |
| Urea                | 0.09  | 0.09  |
| Soybean meal        | 7.67  | 7.67  |
| Pro-Lak2            | 2.71  | 2.71  |
| Alinet1             | 0.07  | 0.07  |
| Energy booster4     | 1.35  | 1.35  |
| Calcium carbonate   | 0.36  | 0.36  |
| CalMin              | 0.36  | 0.36  |
| Salt, white         | 0.18  | 0.18  |
| Sodium sesquicarbonate | 0.45 | 0.45 |
| Magnesium oxide     | 0.18  | 0.18  |
| Dynamate6           | 0.09  | 0.09  |
| DCAD Plus7          | 0.36  | 0.36  |
| Clarify8            | 0.04  | 0.04  |
| AB-209              | 0.36  | 0.36  |
| Trace mineral-vitamin premix10 | 0.29  | 0.29  |

128% CP molasses-based liquid supplement (Quality Liquid Feeds).
2Marine and animal bypass protein supplements (H. J. Baker & Bros. LLC).
3Methionine hydroxyl analog (Novus International Inc.).
4Hydrolized animal and vegetable fatty acid (Milk Specialties).
5Callecurities maris algae (Celtic Sea Minerals).
6Potassium magnesium sulfate (Mosaic Co. Inc.).
7Potassium carbonate (Arm & Hammer Animal Nutrition, Church & Dwight Co., Inc.).
8Larvicide (Central Life Science).
9Bentonite adsorbent (Phibro Animal Health).
10Mineral-vitamin premix contained (DM basis): 29.5% Ca; 0.06% P; 0.42% Mg; 0.31% S; 377 mg/kg Co; 3.472 mg/kg Cu; 530 mg/kg Fe; 388 mg/kg I; 23,882 mg/kg Mn; 110 mg/kg Se; 13,313 mg/kg Zn; 1,221,966 IU/kg vitamin A; 129,456 IU/kg vitamin D; 2,817 IU/kg vitamin E.
The vaginal temperature was measured every 10 min from −2 to 10 d relative to the onset of feed restriction using an ibutton thermometer (Mouser Electronics) attached to a blank intravaginal implant. During the same period, all cows were equipped with activity monitors (Nedap Livestock Management) to record daily rumination activity. Milk samples were collected at each milking from all cows for composition analysis from −2 to 10 d relative to feed restriction. At −2, −1, 1, 2, 3, 4, 5, 6, 8, and 10 d relative to the onset of feed restriction, blood samples were collected (0600–0700 h) from coccygeal vessels of all cows into vacutainers containing sodium heparin or without anticlotting factors (Becton Dickinson). Samples were immediately placed on ice or at room temperature before centrifugation at 450 × g for 5 min at room temperature to generate plasma and buffy coat. The procedures for PBMC isolation and proliferation assay were based on do Amaral et al. (2010) and Perdomo et al. (2011) with modification. Briefly, PBMC were isolated and purified on a single-step density gradient by centrifugation using Fico/Lite LymphoH (Atlanta Biologicals). The cells on top of the gradient were washed in complete RPMI-1640 (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Cynto Life Sciences LLC), 200 IU/mL penicillin, and 0.2 mg/mL streptomycin (MP Biomedicals). The concentration of the PBMC was adjusted to 1 × 10⁶ cells/mL in complete RPMI-1640 and added (100 µL/well) to each well of a 96-well, flat-bottom sterile plate (Corning Inc.). Then, 100 µL of complete RPMI-1640 containing LPS (20 µg/mL, Sigma-Aldrich; Perdomo et al., 2011), concanavalin A (ConA, 20 µg/mL, Sigma-Aldrich; do Amaral et al., 2010), or without mitogens (CTL) was added into wells in triplicate. Plates were incubated 44 h at 37°C with 5% CO₂. Then, 30 µL MTT (3-[4,5-di-methylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, CellTiter 96@ nonradioactive cell proliferation assay, Promega) was added to each well and incubated an additional 4 h. One hundred µL stabilization solution (Promega) was added to each well and incubated 1 h at room temperature before reading at 570 nm on a plate reader. The PBMC proliferation was measured as stimulation index calculated as the ratio of the optical density of treated wells to the optical density of control wells.

To assess the functions of granulocytes, blood samples were centrifuged at 600 × g for 10 min at room temperature to generate plasma and buffy coat. Theuffy coat and the top 10% of the red blood cell layer were collected and washed in PBS. After centrifugation at 450 × g for 5 min at room temperature, the PBS was discarded, and the cell pellet was suspended in 2 mL sterile distilled water and vortexed for 40 s to lyse the remaining red blood cells. The reaction was stopped by adding 2 mL double-strength PBS followed by 6 mL PBS. The membrane of red blood cells and platelets was removed by washing the cells with PBS by centrifugation at 450 × g for 5 min twice. The leukocytes recovered using this isolation method were comprised of 46.9 ± 7.7% granulocytes and 53.1 ± 7.7% mononuclear cells (means ± SD). Following isolation, the total number of leukocytes was counted using an hemocytometer, and the number of granulocytes was estimated as 50% of the total leukocytes. The concentration of the leukocytes was adjusted to 3 × 10⁶ cells/mL in the RPMI-1640. A 2-color protocol was used to measure (1) phagocytosis of propidium iodide (PI)-labeled killed bacteria in red, or (2) reactive oxygen
species (ROS) production by conversion of DHR-123 to R-123 in green, or both at the same time. To evaluate phagocytosis, isolated leukocytes were incubated in individual samples with PI-labeled killed Escherichia coli or PI-labeled killed Staphylococcus aureus, each at a ratio of 40 bacteria per granulocyte. Samples for assessment of ROS activity also had 10 µL of 1 µM DHR-123 in complete medium added to each sample. The samples were incubated at 37°C for 15 min in the dark, and then 300 µL of FACS buffer (PBS with 0.5% BSA and 0.1% sodium azide) at 4°C was added into each tube. In addition, replicate samples with only phorbol 12-myristate, 13-acetate (as an intracellular cell activator of the inflammatory complex, PMA, 10−7 M, Sigma-Aldrich), killed S. aureus whole cell antigen (SAA, as an extracellular cell activator of the inflammatory complex, representing ~107 killed cfu, in-house prepared) or medium (to assess basal endogenous ROS production) plus DHR-123 were prepared, incubated, and processed as described. All samples were quantified using a C6 flow cytometer (BD-Accuri), employing the 488-nm laser, and the FL1 (530 nm) and FL2 (565 nm) detectors with predetermined forward-angle and side-scatter gates for bovine blood cells prepared using this method were employed.

Plasma or serum concentrations of glucose (Autokit Glucose, Wako Chemicals USA Inc.), BHB (Autokit 3-HB, Wako Chemicals USA Inc.), nonesterified fatty acids (NEFA; HR Series NEFA-HR(2), Wako Chemicals USA Inc.), BUN (urea nitrogen colorimetric detection kit, Arbor Assay), cortisol (cortisol ELISA kit, Arbor Assay), insulin (Mercodia Bovine Insulin ELISA, Mercodia AB), tumor necrosis factor α (TNF-α; Bovine TNF-α DIY ELISA, Kingfisher Biotech Inc.), IL-10 (Bovine IL-10 DIY ELISA, Kingfisher Biotech Inc.), and LPS binding protein (LPSBP; multispecies reactive ELISA kit, Cell Sciences Inc.) were measured using commercially available colorimetric or ELISA kits following the manufacturers’ procedures. Plasma concentrations of haptoglobin were determined by a colorimetric method described by Cooke and Arthington (2013). Samples collected from cows in one block were always arranged in one plate and placed in random order. The intra- and interassay coefficients of variation were 3.1% and 4.3% for glucose, 4.3% and 7.7% for BHB, 3.6% and 5.2% for NEFA, 3.8% and 5.0% for BUN, 3.8% and 5.0% for cortisol, 2.7% and 10.1% for insulin, 4.9% and 8.9% for TNF-α, 3.8% and 4.8% for IL-10, 3.1% and 12.9% for LPSBP, and 1.5% and 4.7% for haptoglobin, respectively.

The means ± standard deviation of the THI and the parity and DIM of cows at the onset of the production trial were summarized using the PROC UNIVARIATE procedure of SAS 9.4 (SAS Institute Inc.). Intake, BW, BCS, milk yield, and milk composition data collected during the baseline period and immunological data collected during the feed-restriction challenge (PBMC proliferation, granulocyte phagocytosis and ROS production, and hematological data) were analyzed using the PROC MIXED procedure of SAS 9.4. The statistical models included treatment, parity, time, and their respective interactions as fixed variables, with cow nested within treatment, block, and replicate as random variables. Repeated-measures data collected during the baseline period, production trial, feed-restriction challenge, and realimentation (DMI, milk yield and composition, energetic parameters, plasma or serum concentrations of metabolites, hormones, cytokines, and acute-phase protein, vaginal temperature, and rumination) were analyzed by the PROC MIXED procedure of SAS 9.4. The statistical models included treatment, parity, time, and their respective interactions as fixed variables, with cow nested within treatment, block, and replicate as random variables. During the baseline period, cows subsequently fed NT had higher milk SCC compared with CTL animals. Because milk SCC is positively correlated with a cow’s milk loss (Hand et al., 2012), the milk concentrations of SCC collected during the baseline period were included in the SAS models as covariates for the yield of milk and composition and measures related to production efficiency. Least squares means ± standard error of the mean are reported. The morbidity rate and incidence of subclinical mastitis during the production trial were analyzed using logistic regression by the PROC LOGISTIC procedure, and the incidences of diseases were summarized by the PROC FREQ procedure. The original logistic model included treatment, parity, and treatment by parity interaction. The interactions between treatment and parity were not significant (P > 0.80) and thus were excluded from the final model. The odds ratio and the 95% confidence interval were calculated and reported. Because the incidence of individual diseases (mastitis, foot problems, simple indigestion, and leukosis) was low, only descriptive data were reported. Significance and tendency were declared when P ≤ 0.05 and 0.05 < P ≤ 0.10, respectively.

RESULTS

During the baseline period, cows that were subsequently assigned to NT and CTL had similar (P ≥ 0.16) BW, BCS, DMI, and milk yield and composition (Supplemental Table S1; https://doi.org/10.5281/zenodo.5248780; Tao, 2021). However, cows that were subsequently fed NT had higher SCC (3.20 vs. 2.22, SEM = 0.32, P = 0.02) and higher incidence of subclinical mastitis (P = 0.04, Table 3) during the baseline period.
period compared with cows that were subsequently fed CTL. During the production trial, the ambient temperature, relative humidity, and THI averaged (mean ± SD) 13.6 ± 5.9°C, 77.2 ± 17.3%, and 56.7 ± 9.4 in the pen where the cows in replicate 1 were housed, and 13.3 ± 6.0°C, 79.3 ± 16.6%, and 56.4 ± 9.6 in the pen for cows in replicate 2, respectively. These data indicate that all cows were exposed to environments with minimal stress. The incidences of diseases during the production trial are reported in Table 3. No differences ($P = 0.27$) were observed in morbidity rate between cows fed NT and CTL.

Data collected during the production trial are presented in Table 4. As expected, primiparous cows had lower ($P \leq 0.05$) BW, DMI, milk yield, FCM, ECM, and yield of milk protein, lactose, and SNF compared with multiparous cows. Treatment or treatment by parity interaction had no ($P \geq 0.18$) effect on BW, BCS, DMI, milk yield, FCM, ECM, feed efficiency, energy balance, milk component concentrations, yield of milk protein, lactose, and SNF, or milk SCS. Compared with cows supplemented with CTL, cows fed NT tended ($P = 0.09$) to have greater MUN concentration (10.4 vs. 11.1 mg/dL, SEM = 0.3 mg/dL, respectively). There was a treatment by parity interaction ($P = 0.05$) for milk fat yield, because feeding NT increased the yield of milk fat for primiparous cows but not for multiparous cows (Table 4).

As designed, the DMI was reduced by ~60% during feed restriction from the basal intake (time effect: $P < 0.01$; Figure 1). Across treatments, DMI increased dramatically and exceeded the basal intake on d 6 after the onset of feed restriction (d 1 of the realimentation), but subsequently reduced to ~90% of the basal DMI from d 2 to 5 of the realimentation (time effect: $P < 0.01$; Figure 1). Without regard to treatments, milk yield decreased and reached the nadir at d 5 of the feed restriction and then gradually increased during the realimentation (time effect: $P < 0.01$; Figure 1). Regardless of treatment, milk concentrations of protein, lactose, and SNF decreased, but concentrations of fat, MUN, and SCS increased during feed restriction (time effect: $P < 0.01$; Supplemental Figure S1; https://doi.org/10.5281/zenodo.5248780; Tao, 2021). There were no interactions of treatment by parity by time observed for DMI, milk yield, and concentration and yield of milk components during feed restriction and realimentation (Supplemental Table S2; https://doi.org/10.5281/zenodo.5248780; Tao, 2021). Treatment by time interactions ($P = 0.05$) were observed for MUN and SCS (Supplemental Table S2 and Figure S1). Compared with cows fed CTL, MUN concentration of cows supplemented with NT tended to be higher ($P = 0.10$) at d 4 but lower ($P = 0.10$) at d 8 of the feed restriction.

### Table 3. Morbidity rate and incidences of diseases of lactating Holstein cows supplemented with 19 g/d per cow of a placebo (CTL, n = 30) or a Saccharomyces cerevisiae fermentation product (NT, NutriTek, Diamond V, n = 30)

| Item                     | Baseline period | Production trial | Morbidity 1
|--------------------------|-----------------|------------------|--------------|
|                          | NT, % (no./total) | CTL, % (no./total) | OR (95% CI) |
| Subclinical mastitis     | 36.7 (11/30)    | 46.7 (14/30)     | 0.25 (0.07–0.94) | 0.04 |
| Mastitis                 | 0.0 (0/30)      | 0.0 (0/30)       | —             | —   |
| Foot problem             | 6.7 (2/30)      | 10.0 (3/30)      | 0.67 (0.26–1.74) | 0.46 |
| Simple indigestion       | 0.0 (0/30)      | 10.0 (3/30)      | —             | —   |
| Leukosis                 | 3.3 (1/30)      | 0.0 (0/30)       | —             | —   |

1 OR = odds ratio.
2 Morbidity events include mastitis, foot problems, simple indigestion, and leukosis.
In both treatments, total rumination time per day was reduced and reached the nadir at d 4 of the feed restriction and immediately returned to normal during realimentation (time: \( P < 0.01 \); Supplemental Figure S2; Tao, 2021). Regardless of treatment, BW was lost during feed restriction but regained during realimentation (Supplemental Table S3; https://doi.org/10.5281/zenodo.5248780; Tao, 2021). Energy balance became negative during feed restriction but then returned to positive during realimentation (time: \( P < 0.01 \); Supplemental Table S3, Figure 2). Dietary treatment did not \( (P \geq 0.17) \) affect BW changes or energy balance (Supplemental Table S3). During feed restriction, concentrations of plasma glucose and insulin were reduced, whereas NEFA, BHB, and BUN increased, regardless of treatment (time: \( P < 0.01 \); Supplemental Table S3). Dietary treatment did not \( (P \geq 0.17) \) affect BW changes or energy balance (Supplemental Table S3).

### Table 4. Production parameters of lactating Holstein cows supplemented with 19 g/d per cow of a placebo (CTL, \( n = 30 \)) or a *Saccharomyces cerevisiae* fermentation product (NT, NutriTek, Diamond V, \( n = 29 \))

| Item                      | Primiparous | Multiparous | SEM\(^1\) | \( P^2 \) | D | D \( \times P \) |
|---------------------------|-------------|-------------|------------|----------|---|-----------------|
| BW, kg                    | 601.0       | 608.0       | 17.0       | \( <0.01 \) | 0.87 | 0.82 |
| BCS                       | 2.95        | 2.93        | 2.80       | 2.72     | 0.08 |                   |
| Treatment intake, g/d     | 19.3        | 19.0        | 22.5       | 21.8     | 0.5  | \( <0.01 \)       |
| DMI, kg/d                 | 23.3        | 23.0        | 27.1       | 26.2     | 1.1  | \( <0.01 \)       |
| Milk yield,\(^3\) kg/d   | 32.2        | 31.0        | 36.0       | 36.4     | 1.5  | \( <0.01 \)       |
| 3.5% FCM,\(^3,4\) kg/d   | 34.7        | 32.0        | 37.7       | 38.0     | 1.5  | \( <0.01 \)       |
| ECM,\(^5\) kg/d          | 34.6        | 32.0        | 37.4       | 37.6     | 1.41 | \( <0.01 \)       |
| Milk yield/DMI\(^6\)     | 1.38        | 1.37        | 1.33       | 1.38     | 0.05 | \( <0.01 \)       |
| FCM/DMI\(^6\)            | 1.48        | 1.42        | 1.39       | 1.44     | 0.05 | \( <0.01 \)       |
| ECM/DMI\(^6\)            | 1.48\(^a\)  | 1.42\(^b\)  | 1.38\(^a\) | 1.43\(^b\) | 0.04 | \( <0.01 \)       |
| \( E_{intake}\) McaL/d   | 36.95       | 36.49       | 43.04      | 41.85    | 1.02 | \( <0.01 \)       |
| \( E_{milk}\) McaL/d     | 23.56       | 21.70       | 25.21      | 25.41    | 0.95 | \( <0.01 \)       |
| \( E_{maintenance}\) McaL/d | 9.71      | 9.79        | 10.56      | 10.54    | 0.21 | \( <0.01 \)       |
| Energy balance,\(^6\) McaL/d | 3.84      | 4.53        | 7.35       | 5.91     | 0.69 | \( <0.01 \)       |
| \( E_{intake}/(E_{milk}+E_{maintenance})\)\(^6\) \( \% \) | 112.0       | 114.0       | 122.0      | 117.0    | 2.0  | \( <0.01 \)       |

Milk composition

| Fat, \( % \)         | 3.89        | 3.66        | 3.69       | 3.70     | 0.13 | \( <0.01 \)       |
| Fat,\(^3\) kg/d      | 1.26\(^a\)  | 1.11\(^b\)  | 1.30\(^a\) | 1.32\(^b\) | 0.05 | \( <0.01 \)       |
| Protein, \( % \)     | 3.03        | 2.98        | 2.92       | 2.88     | 0.09 | \( <0.01 \)       |
| Protein,\(^3\) kg/d  | 0.97        | 0.92        | 1.04       | 1.04     | 0.04 | \( <0.01 \)       |
| Lactose, \( % \)     | 4.97        | 4.94        | 4.82       | 4.87     | 0.04 | \( <0.01 \)       |
| Lactose,\(^3\) kg/d  | 1.62        | 1.49        | 1.75       | 1.77     | 0.09 | \( <0.01 \)       |
| SNF, \( % \)         | 8.93        | 8.84        | 8.67       | 8.68     | 0.07 | \( <0.01 \)       |
| SNF,\(^3\) kg/d      | 2.87        | 2.73        | 3.12       | 3.15     | 0.12 | \( <0.01 \)       |
| MUN, mg/dL            | 11.1        | 10.4        | 11.1       | 10.5     | 0.4  | \( <0.01 \)       |
| SCS,\(^1,0\)          | 2.43        | 2.81        | 2.51       | 2.16     | 0.27 | \( <0.01 \)       |

\(^a,b\)Means within the same row with different superscripts differ \( (P \leq 0.05) \).

\(^x,y\)Means within the same row with different superscripts tend to differ \( (0.05 < P \leq 0.10) \).

\(^1\)Pooled standard error of the mean.

\(^2\)P: parity; D: diet.

\(^3\)Data were analyzed using SCC data collected during the baseline phase as a covariate.

\(^4\)3.5% FCM = \((0.4324 \times \text{kg of milk yield}) + (16.216 \times \text{kg of milk fat yield})\).

\(^5\)ECM = \((0.327 \times \text{kg of milk yield}) + (12.95 \times \text{kg of milk fat yield}) + (7.20 \times \text{kg of milk protein yield})\).

\(^6\)Net energy intake = DMI \times \text{dietary NEL concentration}.

\(^7\)Milk energy = \([(0.0929 \times \text{fat} \% + 0.0547 \times \text{CP} \% +0.0395 \times \text{lactose} \%) \times \text{milk yield kg/d}]\). Calculated according to NRC (2001).

\(^8\)Energy used for maintenance = \(0.08 \times \text{BW}^{0.75}\). Calculated according to NRC (2001).

\(^9\)Energy balance = \(E_{intake} - (E_{maintenance} + E_{milk})\).

\(^10\)SCS = \(\log_{2}(SCC/100) + 3\).
respectively). Relative to the prechallenge level, plasma cortisol concentration remained unchanged during feed restriction but was lower \((P \leq 0.09)\) during realimentation (time effect: \(P < 0.01\); Figure 3). Similarly, serum IL-10 concentration of cows remained constant during feed restriction but was lower \((P \leq 0.09)\) at d 8 and 10 after the onset of feed restriction (d 3 and 5 of the realimentation) compared with the prechallenge level (time effect: \(P < 0.01\)). In contrast, serum TNF-\(\alpha\) concentration was higher \((P \leq 0.08)\) from d 2 to 5 of the feed restriction but returned to the prechallenge level during realimentation (time effect: \(P < 0.01\)). No differences \((P \geq 0.21)\) were observed for treatment by time or treatment by parity by time interactions in circulating cortisol and cytokines. There was a tendency \((P = 0.06)\) for a treatment by parity interaction for serum TNF-\(\alpha\) concentration. This was because primiparous cows fed NT had greater \((P = 0.01)\) TNF-\(\alpha\) concentration compared with multiparous cows fed NT, whereas both primiparous and multiparous cows fed CTL were intermediate (Supplemental Table S3). Treatments or treatment by time interaction did not \((P \geq 0.24)\) affect serum LPSBP concentration, but primiparous cows had higher serum LPSBP concentration compared with multiparous cows \((420 \text{ vs. } 113 \text{ ng/mL}, \text{SEM} = 79 \text{ ng/mL}, \text{respectively}; P < 0.01)\). Serum LPSBP concentration remained unchanged during feed restriction but increased during realimentation relative to the prechallenge level (time: \(P < 0.01\); Supplemental Table S3; Figure 3). Serum haptoglobin concentration was not \((P \geq 0.14)\) affected by treatment, parity, time, or their interactions (Supplemental Table S3, Figure 3).

Compared with the pre-feed-restriction challenge (d \(-3\)), feed-restricted cows (d 4) had higher \((P < 0.01)\) blood lymphocyte concentration \((4.66 \text{ vs. } 5.36 \times 10^3 \text{ cells/µL}, \text{SEM} = 0.52 \times 10^3 \text{ cells/µL}, \text{respectively})\) but tended \((P = 0.08)\) to have lower neutrophil concentration \((3.11 \text{ vs. } 2.78 \times 10^3 \text{ cells/µL}, \text{SEM} = 0.15 \times 10^3 \text{ cells/µL}, \text{respectively})\) (Table 5). Treatment, parity, time, or their interactions did not \((P = 0.12)\) affect blood concentrations of white blood cells, eosinophils, or basophils (Table 5). There was an interaction of treatment by parity by time for blood monocyte concentration \((P < 0.01)\) because primiparous cows fed
NT had higher \( P \leq 0.02 \) blood monocyte concentration than other groups at d 4 of the feed restriction (Table 5). No effects \( P \geq 0.13 \) of treatment, parity, time, or their interactions were observed in PBMC proliferative responses to either LPS or ConA ex vivo (Table 5). However, feed restriction reduced \( P \leq 0.02 \) endogenous [without stimulation in the samples, 4.78 vs. 5.21 log(MFI)/cell, \( \text{SEM} = 0.07 \) log(MFI)/cell, respectively; MFI = mean fluorescence intensity] and PMA-stimulated [6.01 vs. 6.32 log(MFI)/cell, \( \text{SEM} = 0.29 \) log(MFI)/cell, respectively] ROS production per granulocyte and tended \( P = 0.06 \) to lower SAA-stimulated [5.54 vs. 5.68 log(MFI)/cell, \( \text{SEM} = 0.06 \) log(MFI)/cell, respectively] ROS production per granulocyte compared with prechallenge levels (Table 5). Compared with CTL, supplementation of NT tended
(P = 0.08) to increase SAA-stimulated ROS production per granulocyte [5.53 vs. 5.69 log(MFI)/cell, SEM = 0.07 log(MFI)/cell, respectively] but did not (P ≥ 0.19) affect the endogenous and PMA-stimulated ROS production per granulocyte. Treatment, parity, time, or their interactions did not (P ≥ 0.12) affect the percentage of granulocytes that phagocytosed E. coli or S. aureus. However, S. aureus intensity (number of bacteria within the cell) per granulocyte was lower (P < 0.01) on d 4 of the feed restriction compared with the prechallenge level [4.90 vs. 5.01 log(MFI)/cell, SEM = 0.06 log(MFI)/cell, respectively] (Table 5). Additionally, there was a parity by time interaction (P = 0.04) for E. coli intensity per granulocyte because

Figure 3. Vaginal temperature, plasma cortisol concentration, and serum concentrations of IL-10, tumor necrosis factor α (TNF-α), lipopolysaccharide binding protein (LPSBP), and haptoglobin of lactating Holstein cows supplemented with 19 g/d per cow of a placebo (CTL, n = 16) or a Saccharomyces cerevisiae fermentation product (NT, NutriTek, Diamond V, n = 16) during a 5-d feed restriction with 40% of ad libitum intake and a following 5-d realimentation. Vaginal temperatures were recorded every 10 min from 2 d before until 10 d after the feed restriction and averaged to daily means for statistical analysis. The blood samples were collected in the morning before milking (0600–0700 h) on d −2, −1, 1, 2, 3, 4, 5, 6, 8, and 10 relative to feed restriction. Error bars represent SEM.
multiparous cows at d 4 had lower \( (P < 0.01) \) E. coli intensity per granulocyte compared with d \(-3\) of the feed restriction \([4.31 \text{ vs. } 4.43 \log(MFI)/\text{cell}, \text{SEM} = 0.05 \log(MFI)/\text{cell}, \text{respectively}]\). No differences \( (P \geq 0.54) \) were observed in the intensity of both E. coli and S. aureus per granulocyte between treatments (Table 5). Although not directly evaluated following cell isolation, the hematological analysis suggested that circulating granulocytes were comprised of 85.2% neutrophils, 10.9% eosinophils, and 3.9% basophils (Table 5).

**DISCUSSION**

Previous reports on the effects of SCFP on productive performance of dairy cows have been inconsistent. In mid-lactation dairy cows (average DIM >100 d), supplementation of SCFP had no effect on DMI or milk yield but improved feed efficiency expressed as FCM/DMI or ECM/DMI (Schingoethe et al., 2004; Cooke et al., 2007). In late lactation (average DIM >180 d), feeding SCFP increased milk yield without any effect on DMI (Zhu et al., 2016, 2017). Similarly, SCFP supplementation, from early to mid-lactation, did not affect DMI but increased milk yield with an inconsistent impact on feed efficiency (Bruno et al., 2009; Zhang et al., 2013; Dias et al., 2018). Only a few published long-term production trials have evaluated the product used in this present experiment (NT, Diamond V). Acharya et al. (2017) reported that supplementation with NT of mid- to late-lactation cows (average DIM = 164 d) increased milk yield but did not affect DMI. This resulted in a higher ratio of milk yield to DMI compared with the unsupplemented CTL. During the transition period, Shi et al. (2019) reported that supplementation with NT tended to reduce DMI while maintaining milk yield over the period of 24 to 44 DIM, leading to higher feed efficiency. However, similar effects were not observed during the freshening period from calving to 23 DIM (Shi et al., 2019). In contrast, feeding NT to transition dairy cows did not affect feed efficiency although it led to more stable DMI and numerical increase in ECM by 2.03 kg/d during the postpartum period (up to 42 DIM) compared with those without supplementation (Olagaray et al., 2019). In this current experiment, feeding NT to mid-lactation cows had no effect on milk yield, DMI, or feed efficiency. The inconsistent results among studies may be attributed to the sources of SCFP, stage of lactation (Yoon and Stern, 1995; Poppy et al., 2012), level of DMI (Allen and Ying, 2012), diet composition and fermentability (i.e., NDF, CP, and starch; Longuski et al., 2009; Robinson and Erasmus, 2009), statistical power, or the presence of aflatoxin contamination in the diet (Jiang et al., 2018).

Interestingly, although not statistically differently, supplementation of NT numerically improved ECM of primiparous cows. Indeed, in a separate analysis that only included primiparous cows, feeding NT significantly increased ECM (34.7 vs. 31.5 kg/d, SEM = 1.3 kg/d, \( P = 0.05 \)) compared with CTL. In this current study, the experimental diet was formulated to meet the requirements of a mid-lactation cow consuming 23.1 kg DM/d and producing 38.5 kg/d of ECM. Primiparous cows had a similar DMI as formulated but produced less ECM, while multiparous cows had substantially greater DMI than formulated but produced slightly lower ECM than projected. All cows were under positive energy balance, and the energy provided by the diet was approximately 13% and 20% higher than the combined energy cost for maintenance and milk synthesis for primiparous and multiparous cows, respectively. Because primiparous cows did not reach their mature BW, part of the dietary energy consumption would be used for growth (NRC, 2001), and the actual excessive energy would be lower than 13%. It is possible that the additional (20%) extra energy and nutrients provided by the diet to multiparous cows masked any potential benefits of the SCFP on rumen fermentation and production efficiency under minimal stress in the current study. In contrast, the beneficial effect of SCFP was still detectable when the growing primiparous cows consumed a diet closer to their requirements. This hypothesis warrants further evaluation in future studies.

Treatment did not affect the yield or concentration of milk protein and lactose. This is consistent with previous studies conducted in both early- and mid-lactation cows (Acharya et al., 2017; Shi et al., 2019; Al-Qaisi et al., 2020). Although no effects were observed for milk fat content, supplementation of NT increased milk fat yield in primiparous cows. This is likely due to the observed higher milk yield and fat content of primiparous cows fed NT over CTL. The reason for the higher milk fat yield in NT-fed primiparous cows is not clear. Olagaray et al. (2019) reported a trend toward an increase in milk fat yield when feeding NT during the transition period. They also found that NT increased the number of meals consumed and reduced the intermeal interval for primi- but not multiparous cows before calving and for both primi- and multiparous cows after calving relative to the unsupplemented CTL. Because meal frequency is positively associated with milk fat yield (Johnston and DeVries, 2018), this suggests that supplementation with NT could influence milk fat yield by altering feeding behavior. In this experiment, supplementation with NT tended to increase MUN relative to CTL. This is consistent with Acharya et al. (2017), who reported that feeding NT increased ruminal ammonia and BUN concentrations in mid-lactation dairy cows.
Table 5. Hematological profile, peripheral blood mononuclear cells (PBMC) proliferative responses to concanavalin A (ConA) or LPS, reactive oxygen species production, and phagocytosis of granulocytes in lactating Holstein cows supplemented with 19 g/d per cow of a placebo (CTL, n = 16) or a *Saccharomyces cerevisiae* fermentation product (NT, NutriTek, Diamond V, n = 16) at d −3 and 4 relative to a feed-restriction challenge.

| Item                                              | Primiparous | Multiparous | Primiparous | Multiparous | SEM¹ | P² | D | T | D × P | P × T | D × T | D × P × T |
|---------------------------------------------------|-------------|-------------|-------------|-------------|------|----|---|---|------|-------|-------|-----------|
| Hematological profile, 10⁶ cells/µL               |             |             |             |             |      |    |   |   |      |       |       |           |
| White blood cells                                 | NT          | CTL         | NT          | CTL         |      |    |   |   |      |       |       |           |
| Lymphocyte                                        | 6.43        | 4.55        | 4.21        | 3.68        |      |    |   |   |      |       |       |           |
| Neutrophil                                        | 3.33        | 3.25        | 3.03        | 2.87        |      |    |   |   |      |       |       |           |
| Monocyte                                          | 0.59<sup>a</sup> | 0.79<sup>a</sup> | 0.52<sup>b</sup> | 0.49<sup>b</sup> | 0.77<sup>a</sup> | 0.47<sup>b</sup> | 0.51<sup>b</sup> | 0.49<sup>b</sup> | 0.08   | 0.03   | 0.57   | 0.36   | 0.81    | <0.01   | <0.01 |
| Eosinophil                                        | 0.33        | 0.42        | 0.46        | 0.29        | 0.38  | 0.48 | 0.43 | 0.31 | 0.09  | 0.73  | 0.79  | 0.39    | 0.20    | 0.21  | 0.52    | 0.71    |
| Basophil                                          | 0.15        | 0.13        | 0.11        | 0.11        | 0.16  | 0.13 | 0.12 | 0.11 | 0.03  | 0.23  | 0.47  | 0.50    | 0.71    | 0.37  | 0.91    |
| PBMC proliferation, stimulation index             |             |             |             |             |      |    |   |   |      |       |       |           |
| ConA                                              | 2.41        | 2.39        | 2.71        | 2.85        | 2.76  | 2.33 | 2.74 | 2.62 | 0.28  | 0.33  | 0.67  | 0.85    | 0.66    | 0.34  | 0.17    | 0.73    |
| LPS                                               | 1.25        | 1.22        | 1.40        | 1.01        | 1.16  | 1.28 | 1.30 | 1.10 | 0.13  | 0.79  | 0.30  | 0.90    | 0.17    | 0.86  | 0.13    | 0.87    |
| Granulocyte reactive oxygen species production, log(MFI) |             |             |             |             |      |    |   |   |      |       |       |           |
| Endogenous                                        | 5.25        | 5.04        | 5.35        | 5.20        | 4.70  | 4.69 | 4.89 | 4.87 | 0.11  | 0.12  | 0.19  | <0.01   | 0.88    | 0.70  | 0.21    | 0.80    |
| PMA stimulated                                    | 6.08        | 6.15        | 6.56        | 6.48        | 6.05  | 5.98 | 6.03 | 6.00 | 0.33  | 0.11  | 0.83  | 0.02    | 0.83    | 0.12  | 0.85    | 0.72    |
| SAA stimulated                                    | 5.70        | 5.50        | 5.78        | 5.74        | 5.60  | 5.28 | 5.67 | 5.60 | 0.11  | 0.06  | 0.08  | 0.06    | 0.24    | 0.82  | 0.59    | 0.79    |
| Granulocyte phagocytosis                          |             |             |             |             |      |    |   |   |      |       |       |           |
| *Escherichia coli*, % of cell                      | 60.75       | 57.77       | 60.63       | 60.83       | 60.17 | 59.37 | 60.74 | 59.01 | 6.09  | 0.80  | 0.38  | 0.60    | 0.49    | 0.46  | 0.58    | 0.76    |
| *E. coli*, log(MFI)                               | 4.36        | 4.30        | 4.43        | 4.43        | 4.34  | 4.32 | 4.31 | 4.31 | 0.06  | 0.46  | 0.54  | 0.03    | 0.49    | 0.04  | 0.80    | 0.79    |
| *Staphylococcus aureus*, % of cell                 | 82.80       | 78.40       | 70.64       | 75.18       | 79.00 | 72.80 | 71.36 | 71.64 | 3.79  | 0.12  | 0.59  | 0.18    | 0.17    | 0.46  | 0.50    | 0.78    |
| *S. aureus*, log(MFI)                             | 5.08        | 5.02        | 4.97        | 4.98        | 4.93  | 4.87 | 4.89 | 4.92 | 0.07  | 0.26  | 0.62  | <0.01   | 0.24    | 0.23  | 0.90    | 0.91    |

¹Means within the same row with different superscripts differ (P ≤ 0.05).
²Pooled standard error of the mean.
³P: parity; D: diet; T: time.
⁴MFI = mean fluorescence intensity; PMA = phorbol 12-myristate, 13-acetate; SAA = *S. aureus* whole cell antigen.
By design, feed restriction resulted in NEB in mid-lactation cows. Energy balance reached its nadir at d 1 of feed restriction. The energy balance gradually increased due to the reduction in milk yield on subsequent days. As in other feed-restriction trials (Ferraretto et al., 2014; Acharya et al., 2017), milk fat concentration increased. This perhaps was mainly a result of the enhanced synthesis of preformed fatty acid resulting from the increased circulating NEFA in response to NEB (Abdelatty et al., 2017). Feed restriction has been shown to downregulate gene expression related to de novo fatty acid synthesis but concomitantly to upregulate gene expression associated with fatty acid uptake in the mammary gland of mid-lactation cows (Abdelatty et al., 2017). The decreases in milk protein and lactose content during feed restriction may reflect the reduced mammary synthetic capacity resulting from decreased nutrient supply. Unlike in previous feed-restriction experiments (Velez and Donkin, 2005; Kvidera et al., 2017), MUN and BUN increased after feed restriction in the present study. Because N intake was reduced by feed restriction, the increases in MUN and BUN may reflect increased muscle mobilization and hepatic amino acid deamination and transamination. Previous research has reported that diet-induced NEB in mid-lactation dairy cows did not affect the milk concentrations of pro- and anti-inflammatory cytokines (Perkins et al., 2002; Moyes et al., 2009). This indicates that feed restriction did not induce inflammatory responses in the mammary gland. Therefore, the increased SCS following feed restriction may have been due to the dilution effect associated with reduced milk yield. The greater SCS of cows fed NT at d 3 of feed restriction may reflect the numerically greater reduction in milk yield than in cows fed CTL.

During feed restriction, total rumination time per day decreased. This would be expected because of the positive relationship between DMI and rumination time (Johnston and DeVries, 2018). The loss of BW, increased plasma NEFA and BHB concentrations, and decreased circulating glucose and insulin levels are typical responses to NEB and consistent with previous feed-restriction experiments (Gross et al., 2011; Ferraretto et al., 2014; Kvidera et al., 2017). Interestingly, cows supplemented with NT had a more severe reduction in plasma glucose concentration during feed restriction and greater plasma BHB concentration than did cows fed CTL. With similar responses in yield of milk lactose, these data appear to suggest that cows fed NT had enhanced glucose utilization during nutrient restriction than cows fed the CTL diet. The mechanism driving this phenomenon is not clear. One potential route to higher glucose usage by cows fed NT compared with CTL was the need to support the greater innate immune function as discussed below.

Feed restriction reduced body temperature regardless of diet or parity. This was likely due to the lower metabolic heat production by reduced nutrient intake and milk production. Interestingly, the vaginal temperature of cows supplemented with NT was lower than that of those fed CTL. This suggests a lower metabolic heat production, enhanced heat dissipation, or both. In contrast, Al-Qaisi et al. (2020) reported that feeding NT did not affect rectal temperature of mid-lactation cows under either thermal neutral condition or induced hyperthermia by an electric heating blanket. The reasons for the different responses in terms of body temperature between these studies are unknown. As with Fisher et al. (2002), feeding level did not affect circulating cortisol. This suggested that feed restriction did not elicit a stress response in mid-lactation cows. Yet Moyes et al. (2009) reported that diet-induced NEB elevated plasma cortisol concentration in mid-lactation cows. The discrepancy may be explained by the magnitude of induced NEB. The energy balance of cows ranged from −7 to −14 Mcal/d in the present experiment but ranged from approximately −12 to −25 Mcal/d in the experiment reported by Moyes et al. (2009). The lack of stress response during feed restriction may account for the similar plasma cortisol concentration between cows fed NT and CTL. Al-Qaisi et al. (2020) reported that supplementation of NT prevented circulating cortisol from increasing during hyperthermia compared with the unsupplemented CTL. This suggests that a potential function of NT is to alleviate stress (Al-Qaisi et al., 2020).

Feed restriction did not affect the proliferative responses of PBMC stimulated by either LPS or ConA ex vivo. These data suggested that nutrient restriction did not have a direct effect on the overall proliferative capacity of PBMC. However, it is important to note that altered serum composition due to NEB could indirectly influence PBMC proliferation. Vanacker et al. (2020) reported that the proliferative responses of PBMC were reduced when the cell culture media was supplemented with serum collected from cows under NEB by feed restriction. These inhibitory effects were attributed to the elevated NEFA concentration in the serum samples (Vanacker et al., 2020). Feed restriction reduced endogenous ROS production, and PMA- and SAA-stimulated ROS production on a per granulocyte basis. This suggested a reduced capacity for oxidative burst. Interestingly, feed restriction did not affect the percentage of granulocyte that phagocytosed bacteria. However, a reduced number of both E. coli and S. aureus were found inside the average cell in these studies. Vanacker et al. (2020) reported that restricting feed
intake to 75% of the ad libitum intake did not affect the percentage of neutrophils that performed phagocytosis but did reduce the percentage of neutrophils producing ROS after PMA stimulation in cells from mid-lactation cows. Moyes et al. (2009) also reported that mid-lactation cows had a lower percentage of phagocytosing neutrophils at d 5 of feed restriction compared with cows fed ad libitum. Our data provided further evidence that nutrient restriction directly impaired the functionality of granulocytes in mid-lactation cows. Coupled with the lower blood neutrophil number per mL, cows under feed restriction may be at greater risk for infectious diseases due to the impaired state of innate immunity.

The dietary treatments had no effect on PBMC proliferation when stimulated with either ConA or LPS. This indicated that NT supplementation did not affect the proliferative capacity of lymphocytes under stimulation in the culture. Although diet did not affect granulocyte phagocytosis or PMA-stimulated ROS production, granulocytes collected from cows fed NT tended to have greater production of ROS after stimulation with SAA compared with those collected from CTL cows. Similarly, NT supplementation did not affect neutrophil endogenous or PMA-stimulated ROS production compared with unsupplemented CTL during the production period (Sivinski, 2018). In contrast, feeding preweaning dairy calves a calf starter containing SCFP improved the number of phagocytized bacteria per neutrophil when cells were cultured with a pathogenic strain of E. coli (Magalhães et al., 2008). Additionally, Mahmoud et al. (2020) reported that adding SCFP into milk replacer and calf starter did not affect the ability of neutrophils to phagocytose latex beads or endogenous or PMA-stimulated ROS production. The results observed in the current experiment suggested that supplementation of NT better primed granulocytes and potentially improved the killing capacity of granulocytes after encountering bacteria in mid-lactation cows. Importantly, NEB induced by feed restriction did not diminish these beneficial effects. In this study, cows assigned to NT had higher incidence of subclinical mastitis during the baseline period before NT was supplemented but had similar incidence during the production period compared with those fed CTL. These data may suggest that feeding NT maintained similar incidence of subclinical mastitis, while CTL failed to prevent the increase in subclinical mastitis during the production period. This may be partially explained by the improved granulocyte functions due to NT supplementation.

Feed restriction gradually increased serum TNF-α concentration. This may be due to the upregulated TNF-α production by adipose tissue during lipolysis (reviewed by Contreras et al., 2017). However, circulating LPSBP, haptoglobin, and IL-10 remained unchanged during feed restriction. It is possible that the magnitude of increase in circulating TNF-α was not sufficient to elicit a significant acute-phase response. The feed restriction failed to induce systemic inflammation in mid-lactation dairy cows in this experiment. These data are consistent with previous studies where feed restriction did not elicit systemic inflammation in mid-lactation cows or in close-up dry cows (Perkins et al., 2002; Moyes et al., 2009; Pascottini et al., 2019). In contrast, Kvidera et al. (2017) reported a linear increase in circulating endotoxin, LPSBP, haptoglobin, and serum amyloid A as the intake of mid-lactation cows was reduced from 100% to 40% of the ad libitum intake. The reasons for the discrepancies between these studies are not clear. On the first day of realimentation, the DMI of the cows increased 2.6-fold compared with d 5 of the feed restriction. This large increase in nutrient consumption, including grain, may temporarily induce subacute ruminal acidosis (reviewed by González et al., 2012), leading to translocation of LPS into the peripheral circulation. This is associated with an increase in circulating LPSBP (Khafipour et al., 2009). However, this inflammatory stimulus did not seem to be strong enough to increase circulating TNF-α or haptoglobin.

Supplementation of SCFP has been reported to influence the inflammatory responses of cattle. Supplementing SCFP in milk replacer and calf starter increased the proinflammatory cytokine production of PBMC in preweaning calves but decreased the secretion of inflammatory cytokines from leukocyte collected from bronchoalveolar lavage after toll-like receptor stimulation ex vivo (Mahmoud et al., 2020). These data suggested a different set of effects of SCFP on immune cell inflammatory responses for cells from the peripheral blood relative to the responses of cells resident in mucosal tissues (Mahmoud et al., 2020). Using an intravenous LPS challenge model, Burdick Sanchez et al. (2020) reported that weaned beef calves fed SCFP had lower serum concentrations of TNF-α, IL-6, and IFN-γ compared with unsupplemented CTL, indicating an attenuation of the inflammatory response. Similarly, feeding NT minimized the increase in circulating serum amyloid A during hyperthermia induced by an electric heating blanket in mid-lactation dairy cows (Al-Qaisi et al., 2020). In addition, NT appeared to reduce the expected increase in circulating haptoglobin in early-lactation cows (Knoblock et al., 2019). Unfortunately, the lack of systemic inflammation during the feed-restriction challenge in this present experiment prevented us from observing any of these potential benefits of NT in modulating inflammation.

In conclusion, except for increased milk fat yield in primiparous cows, supplementing NT had no effects on...
productive performance of mid-lactation cows. In this experiment, cows were fed 12% to 22% more energy than the combined energy cost for maintenance and milk synthesis and exposed to environments with minimal stress. This may explain why the beneficial effects of SCFP on performance during nutritional challenges, such as subacute ruminal acidosis, were not apparent in the current experiment. However, the granulocyte function data suggested that supplementing NT better primed the granulocyte-mediated innate responses relative to bacterial stimulation. This may have a positive effect on how mid-lactation cows cope with conditions leading to bacterial infection. This was evidenced by the fact that cows supplemented with NT maintained a similar incidence of subclinical mastitis, while CTL cows had an increased incidence from baseline to the production periods. Because glucose is the primary energy source for neutrophil function (Kumar and Dikshit, 2019), the improved oxidative burst of granulocytes may also explain the higher level of glucose utilization by cows fed NT during feed restriction compared with those fed CTL. In this experiment, feed restriction successfully induced NEB and the associated metabolic changes. However, it failed to stimulate systemic inflammation. This may have prevented us from observing the effects of NT in modulating systemic inflammation.

ACKNOWLEDGMENTS

The authors thank the staff of the Dairy Research Center of the University of Georgia (Tifton, GA) for animal care. This project was partially supported by Diamond V (Cedar Rapids, IA) and the National Institute of Food and Agriculture (Washington, DC) Hatch project (GE000749). Ilkyu Yoon is employed with Diamond V. The remaining authors are affiliated with the University of Georgia. The authors have not stated any conflicts of interest.

REFERENCES

Abdelatty, A. M., M. E. Iwaniuk, M. Garcia, K. M. Moyes, B. B. Teter, P. Delmonte, A. K. G. Kadegowda, M. A. Tony, F. F. Mohamad, and R. A. Erdman. 2017. Effect of short-term feed restriction on temporal changes in milk components and mammary lipogenic gene expression in mid-lactation Holstein dairy cows. J. Dairy Sci. 100:4000–4013. https://doi.org/10.3168/jds.2016-11130.

Acharya, S., J. P. Pretz, I. Yoon, M. F. Scott, and D. P. Casper. 2017. Effects of Saccharomyces cerevisiae fermentation products on the lactational performance of mid-lactation dairy cows. Transl. Anim. Sci. 1:221–228. https://doi.org/10.2727/tas.2017.0028.

Al-Qaisy, M., E. A. Horst, E. J. Mayorga, B. M. Goetz, M. A. Abeysta, I. Yoon, L. L. Timms, J. A. Appuhamy, and L. H. Baumgard. 2020. Effects of a Saccharomyces cerevisiae fermentation product on heat-stressed dairy cows. J. Dairy Sci. 103:9634–9645. https://doi.org/10.3168/jds.2020-18721.

Allen, M. S., and Y. Ying. 2012. Effects of Saccharomyces cerevisiae fermentation product on ruminal starch digestion are dependent upon dry matter intake for lactating cows. J. Dairy Sci. 95:6591–6605. https://doi.org/10.3168/jds.2012-5377.

Bruno, R. G., S. M. Rutigliano, R. L. Cerri, P. H. Robinson, and J. E. P. Santos. 2009. Effect of feeding Saccharomyces cerevisiae on performance of dairy cows during summer heat stress. Anim. Feed Sci. Technol. 150:175–186. https://doi.org/10.1016/j.anifeedsci.2008.09.001.

Burdick Sanchez, N. C., J. A. Carroll, P. R. Broadway, T. S. Edrington, I. Yoon, and C. R. Belknap. 2020. Some aspects of the acute phase immune response to a lipopolysaccharide (LPS) challenge are mitigated by supplementation with a Saccharomyces cerevisiae fermentation product in weaned beef calves. Transl. Anim. Sci. 4:txaa156. https://doi.org/10.1093/tas/txaa156.

Callaway, E. S., and S. A. Martin. 1997. Effects of a Saccharomyces cerevisiae culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci. 80:2035–2044. https://doi.org/10.3168/jds.S0022-0302(97)76148-4.

Contreras, G. A., C. Strieder-Barboza, and W. Raphael. 2017. Adipose tissue lipolysis and remodeling during the transition period of dairy cows. J. Anim. Sci. Biotechnol. 8:41. https://doi.org/10.1186/s40104-017-0174-1.

Cooke, K. M., J. K. Bernard, and J. W. West. 2007. Performance of lactating dairy cows fed whole cottonseed coated with gelatinized starch plus urea or yeast culture. J. Dairy Sci. 90:360–364. https://doi.org/10.3168/jds.S0022-0302(07)72637-1.

Cooke, R. F., and J. D. Arthington. 2013. Concentrations of haptoglobin in bovine plasma determined by ELISA or a colorimetric method based on peroxidase activity. J. Anim. Physiol. Anim. Nutr. (Berl.) 97:531–536. https://doi.org/10.1111/j.1439-0396.2012.01298.x.

Dias, A. L. G., J. A. Freitas, B. Micaí, R. A. Azevedo, L. F. Greco, and J. E. P. Santos. 2018. Effects of supplementing yeast culture to diets differing in starch content on performance and feeding behavior of dairy cows. J. Dairy Sci. 101:186–200. https://doi.org/10.3168/jds.2017-13240.

do Amaral, B. C., E. E. Connor, S. Tao, J. Hayen, J. Bubolz, and G. E. Dahl. 2010. Heat stress abatement during the dry period influences prolactin signaling in lymphocytes. Domest. Anim. Endocrinol. 38:38–45. https://doi.org/10.1016/j.domaniend.2009.07.005.

Ferraretto, L. F., H. Gencoglu, K. S. Hackbart, A. B. Nascimento, F. Della Costa, R. W. Bender, J. N. Guenther, R. D. Shaver, and M. C. Willbank. 2014. Effect of feed restriction on reproductive and metabolic hormones in dairy cows. J. Dairy Sci. 97:754–763. https://doi.org/10.3168/jds.2013-6925.

Fisher, A. D., G. A. Verkerk, C. J. Morrow, and L. R. Matthews. 2002. The effects of feed restriction and lying deprivation on pituitary-adrenal axis regulation in lactating cows. Livest. Prod. Sci. 73:255–263. https://doi.org/10.1016/S0301-6226(01)00269-9.

Gessner, D. K., R. Ringsius, and K. Eder. 2017. Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. J. Anim. Physiol. Anim. Nutr. (Berl.) 101:605–628. https://doi.org/10.1111/jpn.12579.

Gonzalez, L. A. X., M. Manteca, S. Salamiglia, K. S. Schwartzkopf-Genswein, and A. Perret. 2012. Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). Anim. Feed Sci. Technol. 172:66–79. https://doi.org/10.1016/j.anifeedsci.2011.12.009.

Gross, J., H. A. van Dorland, R. M. Bruckmaier, and F. J. Schwarz. 2011. Performance and metabolic profile of dairy cows during a lactational and deliberately induced negative energy balance with subsequent realimentation. J. Dairy Sci. 94:1820–1830. https://doi.org/10.3168/jds.2010-3707.

Hand, K. J., A. Godkin, and D. F. Kelton. 2012. Milk production and somatic cell counts: A cow-level analysis. J. Dairy Sci. 95:1358–1362. https://doi.org/10.3168/jds.2011-4927.

Hristov, A. N., G. Varga, T. Cassidy, M. Long, K. Heyler, S. K. Karnati, B. Corl, C. J. Howe, and I. Yoon. 2010. Effect of Saccharomyces cerevisiae fermentation product on ruminal fermentation
Velez, J. C., and S. S. Donkin. 2005. Feed restriction induces pyruvate carboxylase but not phosphoenolpyruvate carboxykinase in dairy cows. J. Dairy Sci. 88:2938–2948. https://doi.org/10.3168/jds.S0022-0302(05)72974-X.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt Jr., and T. N. Lesch. 1982. A dairy-cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495–501. https://doi.org/10.3168/jds.S0022-0302(82)82223-6.

Yoon, I. K., and M. D. Stern. 1995. Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants—a review. Asian-Australas. J. Anim. Sci. 8:533–555. https://doi.org/10.5713/ajas.1995.553.

Yuan, K., L. G. Mendonca, L. E. Hulbert, L. K. Mamedova, M. B. Muckey, Y. Shen, C. C. Elrod, and B. J. Bradford. 2015. Yeast product supplementation modulated humoral and mucosal immunity and uterine inflammatory signals in transition dairy cows. J. Dairy Sci. 98:3236–3246. https://doi.org/10.3168/jds.2014-8469.

Zaworski, E. M., C. M. Shriver-Munsch, N. A. Fadden, W. K. Sanchez, I. Yoon, and G. Bobe. 2014. Effects of feeding various dosages of Saccharomyces cerevisiae fermentation product in transition dairy cows. J. Dairy Sci. 97:3081–3098. https://doi.org/10.3168/jds.2013-7692.

Zhang, R. Y., I. Yoon, W. Y. Zhu, and S. Y. Mao. 2013. Effect of Saccharomyces cerevisiae fermentation product on lactation performance and lipopolysaccharide concentration of dairy cows. Asian-Australas. J. Anim. Sci. 26:1137–1143. https://doi.org/10.5713/ajas.2013.13181.

Zhu, W., Z. Wei, N. Xu, F. Yang, I. Yoon, Y. Chung, J. Liu, and J. Wang. 2017. Effects of Saccharomyces cerevisiae fermentation products on performance and rumen fermentation and microbiota in dairy cows fed a diet containing low quality forage. J. Anim. Sci. Biotechnol. 8:36. https://doi.org/10.1186/s40104-017-0167-3.

Zhu, W., B. X. Zhang, K. Y. Yao, I. Yoon, Y. H. Chung, J. K. Wang, and J. X. Liu. 2016. Effects of supplemental levels of Saccharomyces cerevisiae fermentation product on lactation performance in dairy cows under heat stress. Asian-Australas. J. Anim. Sci. 29:801–806. https://doi.org/10.5713/ajas.15.0440.

ORCIDs
T. N. Marins • https://orcid.org/0000-0001-9430-036X
Y.-C. Chen • https://orcid.org/0000-0002-8231-5596
D. J. Hurley • https://orcid.org/0000-0003-2108-4496
J. K. Bernard • https://orcid.org/0000-0001-9703-3498
I. Yoon • https://orcid.org/0000-0003-1891-1585
S. Tao • https://orcid.org/0000-0002-9447-2994