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Ruminant Nutrition

Effects of *Saccharomyces cerevisiae* fermentation product on ruminal fermentation, total tract digestibility, blood proinflammatory cytokines, and plasma metabolome of Holstein steers fed a high-grain diet

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ABSTRACT

This study aimed to assess the impact of *Saccharomyces cerevisiae* fermentation product (SCFP) on digestibility, ruminal fermentation, and plasma metabolome of Holstein steers fed a high-grain diet. Steers were fed diet with 80% concentrate and 20% corn silage once daily ad libitum. Steers were stratified based on initial body weight (BW) and randomly assigned to two treatments: 1) control (CON), a basal diet without SCFP; 2) basal diet + 12 g/head/day SCFP, top-dressed. Eight rumen-cannulated Holstein steers (**BW**: 580 ± 29.2 kg) were enrolled in a crossover design study with 25-d treatment periods and a 24-d washout period. Dry matter intake (DMI) was calculated from daily feed offered and refusals. Blood was collected before morning feeding on day 25 of each period. Rumen fluid was collected at 0, 4, 8 and 12 h post-feeding on d 25. Fecal samples were collected from d 22 to 24 for digestibility measures. Statistical analyses were performed with the GLIMMIX procedure of SAS 9.4 (SAS, 2023). Supplementing SCFP had no effect on digestibility of organic matter (OM, $P = 0.63$), crude protein (CP, $P = 0.97$), neutral detergent fiber (NDF, $P = 0.59$), and acid detergent fiber (ADF, $P = 0.84$). Treatment did not affect fecal excretion of nitrogen (N, $P = 0.69$), phosphorus (P, $P = 0.24$), copper (Cu, $P = 0.71$), and zinc (Zn, $P = 0.95$). Supplementing SCFP increased (6.29 vs. 6.01, $P = 0.01$) ruminal pH compared to CON. Lactic acid concentrations were similar between treatments (P = 0.17) and low in both groups (0.09mM vs. 0.28mM). Treatment did not affect ruminal total volatile fatty acid (VFA) concentrations ($P > 0.10$) but decreased butyrate molar proportion ($P = 0.01$) and tended to increase the molar proportions of isobutyrate ($P = 0.06$) and isovalerate ($P \leq 0.10$). Treatment had no effect on the in vitro production of proinflammatory cytokines, IL-1β ($P > 0.11$) and IL-6 ($P > 0.12$), in the whole blood in response to various toll-like receptor stimulants. Plasma pathways of purine metabolism, amino sugar and nucleotide sugar metabolism, and lysine degradation were enriched ($P \leq 0.05$) by feeding SCFP. Overall, supplementing SCFP did not affect total tract digestibility, fecal excretions of macro minerals but enhanced ruminal pH in cattle fed a high-grain diet. Furthermore, feeding SCFP enriched several important plasma pathways related to protein metabolism.

Lay Summary

Previous research has shown that *Saccharomyces cerevisiae* fermentation product (SCFP) can improve the performance of beef cattle. However, research on the impact of SCFP on beef cattle fed a high-grain diet is limited. Specifically, its effects on metabolism and metabolic pathways, which are crucial for understanding its mode of action, have not been thoroughly explored. Our study investigated how SCFP influences ruminal fermentation, digestibility, macro-mineral utilization, blood proinflammatory cytokines, and plasma metabolome in beef steers fed a high-grain diet. SCFP supplementation did not affect total tract digestibility and fecal mineral excretion. However, it improved ruminal pH, suggesting potential benefits in reducing ruminal subacute acidosis linked to high-grain diets. Additionally, SCFP supplementation enriched key protein metabolism pathways, such as nitrogen metabolism, purine metabolism, pyrimidine metabolism, and lysine degradation pathways.

Key words: metabolic profiling, nutrient utilization, postbiotics, yeast fermentation product

INTRODUCTION

In beef cattle, a typical finishing phase can last around 200 d on a high grain diet (Hayek and Garrett, 2018). Due to the high palatability and fermentability of this diet, ruminal microorganisms produce and accumulate increased levels of volatile fatty acids (VFAs) (Kim et al. 2018; Plazier et al. 2018). This may lead to subacute ruminal acidosis (SARA), characterized as ruminal pH remaining below 5.6 for more than 3 h per day (Plaizier et al., 2018; Sarmikasoglou et al., 2022). During SARA, cattle can experience reduced ruminal digestion, particularly of fiber, along with decreased productivity and an increased risk of health problems such as

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rumenitis and liver abscesses (Brown et al., 2006; Plaizier et al., 2018).

Saccharomyces cerevisiae fermentation product (SCFP) is a postbiotic produced through the fermentation of the yeast *Saccharomyces cerevisiae*. It consists of amino acids, organic acids, B vitamins, residual yeast cells, yeast cell wall fragments, and fermentation media (Shen et al., 2011; Mahmoud et al., 2020). Feeding SCFP has been reported to improve ruminal pH and liver health, increase feed efficiency, and organic matter (OM) digestibility in beef cattle fed high-grain finishing diet (Shen et al., 2018, 2019; Rients et al., 2023). Additionally, earlier research has shown that SCFP supplementation enhanced nitrogen (N) balance and the retention of zinc (Zn) and iron (Fe) (Cole et al., 1992) and improved metabolizable minerals and retention for potassium (K) and Zn (Petersen et al., 1987) in lambs. Efficient utilization of these macro-minerals and elements can improve the cost-effectiveness of feed and mineral supplements and minimize the environmental impact from manure. Previous research has also reported the beneficial effects of SCFP on cytokine production in calves and cows, as well as its role in supporting various health and growth functions (Lee and Jun, 2019).

However, few studies have comprehensively investigated the effects of SCFP on ruminal characteristics, digestibility, macro-mineral utilization, and pro-inflammatory cytokine production in cattle fed a high-grain diet. Furthermore, there is a lack of understanding of how SCFP affects the metabolism or metabolic pathways of cattle, which is essential for understanding its mode of action and improving its consistency and effectiveness in the future. In recent years, the approach of metabolomics has provided insights into the metabolism of animals and offered a unique perspective in understanding the modes of action of nutritional interventions through a whole-animal approach (Ogunade et al., 2019; Wang et al., 2019; Oyebade et al., 2024).

Therefore, we aimed to evaluate the influence of SCFP supplementation on a high-grain diet using a comprehensive approach with the incorporation of metabolomics. The objective of this study was to understand the effects of SCFP on the apparent total tract digestibility, excretion and utilization of N, P, Zn, and Cu, along with the production of blood proinflammatory cytokines, and changes in the plasma metabolome of Holstein steers fed a high-grain diet. We hypothesized that SCFP supplementation would enhance digestibility and mineral utilization, improve ruminal pH, enhance blood proinflammatory cytokine production, and upregulate energy and nitrogen utilization pathways in the plasma.

MATERIALS AND METHODS

The steers used in this research received care following the protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Kentucky State University (Protocol number 23-007) and the IACUC of the University of Kentucky.

Animals, Design and Treatment

Eight rumen-cannulated Holstein steers (Body Weight, BW: 580 ± 29.2 Kg) were enrolled in a crossover design study with two 25-d treatment periods with a 24-d washout period. All steers were initially stratified by BW and randomly assigned to one of two treatments: 1) Control (**CON**)**:** a basal diet without SCFP, or **2**) basal diet with **SCFP** (NaturSafe, Diamond V, Cedar Rapids, IA, 12 g/day, top-dressed). Steers were adapted to SCFP treatment for 21 d before any measurements were taken. Steers received their assigned treatment for 25 d, followed by a 24-d washout period, during which all steers were fed the CON diet to eliminate any residual effects of SCFP. After the washout period, steers switched treatments for the second 25-d period. The basal diet was a finishing diet consisting of 20% corn silage, 55% cracked corn, 15% dried distillers' grains with solubles, 7.19% ground corn, 0.23% tallow, 0.33% urea, 1.75% limestone, 0.46% mineral premix, 0.02% vitamin premix, 0.007% tylan-40 and 0.016% rumensin-90, on a DM basis (Table 1). Steers were housed individually in 3 m × 3 m pens at the University of Kentucky C. Oran Little Research Center in Versailles, KY, USA and were fed once daily at 8 a.m. Feed was provided on an *ad libitum* basis and adjusted daily according to the previous day's refusals. Fresh water was readily available to steers through push-paddle water bowls.

Measurement of Growth Performance and Digestibility

The body weights of each steer were recorded before morning feeding on d 1 and d 25 of each period. The offered total mixed ration (TMR) and refusal were weighed and recorded

Table 1. Experimental diet and nutrient composition

Item[1]	Value
Ingredient, % of diet DM	
Corn silage	20.0
Corn grain, cracked	55.0
Distillers grain + solubles	15.0
Ground corn	7.19
Tallow	0.23
Urea	0.33
Limestone	1.75
Mineral premix[2]	0.46
Vitamin premix[3]	0.02
Tylan-40	0.007
Rumensin-90	0.016
Nutrient composition, % DM	
Crude protein, %	13.4
Neutral detergent fiber, %	22.3
Acid detergent fiber, %	12.9
Starch, %	46.0
Ether extract, %	5.36
Ash, %	5.20
Ca, %	0.83
P, %	0.43
Zn, mg/kg	77.0
Cu, mg/kg	15.5
Total digestible nutrients, %	79.9

[1]Ca: calcium; P: phosphorus; Zn: zinc; Cu: copper.
[2]Mineral premix contains 1.21% sulfur, 92.7% salt, 9280 ppm iron, 5572 ppm zinc, 4805 ppm manganese, 1837 ppm copper, 115 ppm iodine, 65.9 ppm cobalt, 18.5 ppm selenium.
[3]Vitamin premix contains 8,818,490.49 IU/kg of vitamin A, 1,763,698.1 IU/kg of vitamin D3, 1,102.31 IU/kg of vitamin E.

daily for each steer. TMR and refusal samples were collected daily to determine dry matter (DM) content by drying in a forced-air oven at 60 °C for at least 48 h until a constant weight was achieved. The daily DMI was calculated as the difference between the weight of dry TMR offered and dry refusal weight for each animal. Spot fecal samples were also collected for d 22 to 24. Approximately 100 g of fecal samples were collected each time at 0500h, 0900h, 1300h, and 1700h on d 22, 0600h, 1000h, 1400h, and 1800h on d 23, and 0700h, 1100h, 1500h, and 1900h on d 24 via rectal grabbing. The TMR, refusals, and feces were subjected to drying in a forced-air oven at 60°C to achieve constant weight and ground to pass a 2-mm screen of a Wiley mill (Model 4 Laboratory Mill, Thomas-Wiley Company, Philadelphia, PA). Equal portions of the samples were pooled to create one composite sample per steer for each sample type in each collection period. The pooled samples were analyzed in a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI) for organic matter, neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), N, P, Cu, and Zn, along with acid-insoluble ash, which served as an internal marker.

The analysis of ADF was conducted following the AOAC Official Method 973.18 (1996). The determination of NDF was performed according to the AOAC Official Methods of Analysis, Method 2002.04 (2002). The CP content was assessed using the combustion method outlined in AOAC Official Method 990.03 (1990). The concentrations of P, Cu, and Zn were determined following AOAC Official Method 2011.14 (2011), described in AOAC Official Method 2017.02 (2017). Acid insoluble ash (AIA) was measured using the method described by Van Keulen and Young (1977), which involves ashing for 12 to 18 hours at 450 °C, boiling the sample in 2 M hydrochloric acid, and then conducting a second ashing for another 12 to 18 hours at 450 °C. The digestibility of organic matter (OM), CP, NDF, NDFom, and ADF was calculated based on the approach described by Henry et al. (2015): Digestibility (%) = 100 – 100 × [(marker concentration in feed/marker concentration in feces) × (nutrient concentration in feces/nutrient concentration in feed)]. The AIA concentration in the feed and feces was utilized as an internal marker.

Determination of Rumen pH, VFA and NH_3-N Concentration

Baseline rumen fluid samples were collected on day 0 at 4 h post-feeding to determine pH, VFAs, and NH_3-N. On d 25 of each period, rumen samples were collected via rumen cannulas right before feeding (0 h) and at 4, 8, and 12 h after feeding. For each sampling, approximately a total of 500 g whole rumen content was collected from 5 different locations within the rumen. The composited rumen sample was strained through four layers of cheesecloth to extract the liquid fraction. The pH of the extracted liquid portion was measured using a pH probe (Orion star A1111, Thermo Scientific, Chelmsford, USA). Rumen fluid was stored at –80 °C for analysis. For the analysis of VFA concentrations, 5 mL of rumen fluid was combined with 0.5 mL of 2-ethylbutyrate (85 mM) as an internal standard and 0.5 mL of 500 g/L metaphosphoric acid (Trotta et al. 2018, 2023). The resulting mixture was centrifuged at 39,000 × g for 15 min at 23 °C. After centrifugation, the supernatant was transferred to vials, sealed, and analyzed for VFA via gas chromatography with flame-ionization detection (GC-FID) using an Agilent HP6890 Plus GC equipped with an Agilent 7683 Series Injector and Auto Sampler (Agilent Technologies, Inc., Santa Clara, CA, USA), as outlined by Harmon et al. (1985). A separate 5 mL aliquot of rumen fluid was treated with 0.5 mL of 25 g/kg metaphosphoric acid and analyzed for NH_3-N using a Konelab 20XTi clinical analyzer (Thermo Fisher Scientific Inc., Beverly, MA, USA) following the procedure described by Kim et al. (2014). Additionally, untreated centrifuged rumen fluid was measured for L(+)-lactate concentration utilizing the L(+)-lactate dehydrogenase assay (Engel and Jones, 1978), with absorbance measurements taken at 340 nm using a plate reader (Model Gen5, Biotech, Winooski, VT, USA).

Blood Collection and Processing

Blood was collected from coccygeal vein of the tail into sodium heparin tubes before morning feeding on d 25 of each period. Tubes were gently inverted to ensure proper mixing with anticoagulant and kept in ice until they reached the laboratory for further processing. One set of fresh blood samples was shipped overnight on ice and analyzed for proinflammatory cytokine production using an in vitro whole blood immune assay at Iowa State University, as described below. Additional whole blood samples were centrifuged at 2,500 × g at 4°C for 20 min. The resulting plasma samples were stored at –80 °C for metabolomics analysis.

In Vitro *Proinflammatory Cytokine Production*

Samples were stimulated using the protocol described by Mahmoud et al. (2020) with some adaptations. In this in vitro experiment, 100 µl whole blood were plated into 96-well plates in triplicate and stimulated with 10 µg/mL of Pam3CSK4 (a synthetic tripalmitoylated lipopeptide acting as a toll-like receptor TLR1/2 agonist), 10 µg/mL of polyinosinic:polycytidylic acid (poly I:C, a TLR3 agonist), 1 µg/mL of lipopolysaccharide 4 (LPS 4, a TLR4 agonist), 1 µg/mL of LPS 5 (a TLR4 agonist) or cRPMI media (the cell culture media, negative control, Mock). All stimulants were obtained from InvivoGen (San Diego, CA, USA). The selected TLR stimulants were chosen for their ability to mimic diverse infectious stimuli. Pam3CSK4 and LPS were used to model Gram-positive and Gram-negative bacterial infections, respectively, while poly I:C was used to simulate viral infections (Fortier et al., 2004; Nguyen et al., 2010; Farhana and Khan, 2023). The plates were then incubated at 37 °C for 48 h. Following incubation, the plates were subjected to centrifugation at 2000 × g for 5 min. The supernatants were preserved at –80 °C for analyzing cytokine secretion. The IL-6 and IL-1β concentrations in the supernatants were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits from Kingfisher Biotech (Minneapolis, MN, USA), according to the manufacturer's instructions. The intra and inter-assay coefficients of variability for IL-6 analysis were 7% and 13% respectively, while the intra and inter-assay coefficients of variability for IL-1β analysis were 8% and 11%, respectively.

Plasma Metabolome Analysis

The analysis of the plasma metabolome included several steps: pretreatment and normalization of samples, labeling with chemical isotopes, liquid chromatography-mass spectrometry (LC-MS) analysis, processing of data, identification of metabolites, and statistical analysis, following the methodology outlined by Zhao et al. (2019). To minimize batch effects, samples were randomized prior to any procedure. The

normalization process involved determining the total concentration of metabolites using a metabolome quantification kit (NMT-6001-KT, Nova Medical Testing Inc., Edmonton, AB, Canada). Subsequently, samples were dried down to a uniform concentration of 1.2 mM and stored at −80 °C. The samples were chemical isotope labeled using dansylation for amine- and phenol-containing metabolites (NMT-4101-KT, Nova Medical Testing Inc., Edmonton, AB, Canada) and dansylhydrazine for carbonyl-containing metabolites (NMT-4167-KT, Nova Medical Testing Inc., Edmonton, AB, Canada). The labeling procedures followed the methods described by Guo and Li (2009) and Zhao et al. (2017). Following labeling, each sample labeled with ^{12}C was combined in a 1:1 ratio with a ^{13}C-labeled reference sample and analyzed using LC-MS. For quality control, a pooled mixture containing equal volumes of ^{12}C-labeled and ^{13}C-labeled samples was injected after every ten samples.

The LC-MS analyses were performed using an Agilent 1290 LC system (Agilent Technologies, Inc., Santa Clara, CA, USA) linked to a Bruker Impact II QTOF Mass Spectrometer (Bruker Daltonics, Billerica, MA, USA). The analysis utilized an Agilent Eclipse Plus reversed-phase C18 column (150 × 2.1 mm, 1.8 µm particle size), which was kept at a temperature of 40 °C. For the mobile phases, 0.1% formic acid in water served as Mobile Phase A, and 0.1% formic acid in acetonitrile was used for Mobile Phase B. The gradient settings were t = 0 min, 25% B; t = 10 min, 99% B; t = 15 min, 99% B; t = 15.1 min, 25% B; t = 18 min, 25% B. The flow rate used was 400 µL/min. Mass spectral data was collected at a rate of 1 Hz, spanning a mass-to-charge (m/z) range of 220 to 1000.

The raw LC-MS data were converted into.csv format with DataAnalysis 4.4 (Bruker Daltonics, Billerica, MA, USA). The. csv files were then imported into IsoMS Pro 1.2.12 (Nova Medical Testing Inc., Edmonton, AB, Canada) for processing the data and identifying the metabolites. The process involved extracting and calculating the intensity ratios of $^{12}C/^{13}C$ peak pairs from each sample (Zhou et al., 2014). During this phase, any redundant data, such as adduct ions and dimers, were eliminated to keep only one pair for each metabolite, followed by aligning the same metabolite (peak pair) across all samples and imputing missing ratio values using the software. Further data cleaning steps included the removal of peak pairs found in blank samples or those not present in at least 80% of the samples within any group. Post-acquisition data normalization was carried out based on ratio of the total useful signal, which is the sum of all useful ^{12}C peaks over the total useful ^{13}C-peaks (Wu and Li, 2016). The identification of metabolites was conducted through a two-tiered approach with the NovaMT Metabolite Databases 2.0 (Nova Medical Testing Inc. Edmonton, AB, Canada) (Zhao et al., 2019). In the first tier, we focused on matching peak pairs with a labeled library (LL) of metabolites, utilizing their accurate mass and retention times for positive identification. The second tier assessed the remaining peak pairs against a linked identity library (ILL), which includes over 9,000 pathway-related metabolites. This process yielded high-confidence putative identifications based on matches in accurate mass and predicted retention times.

Calculations and Statistical Analysis

All data except for the metabolomics data were analyzed using the GLIMMIX procedure of SAS (SAS, 2023). Each animal was considered the experimental unit. For non-repeated measures including performance, digestibility and cytokine data, and the model used was:

$$Y = \mu + T_i + P_j + A_k + (T \times P)_{ij} + \varepsilon_{ijk},$$

where μ is the overall mean, T_i is the fixed effects of treatment, P_j is the effect of period and $(T \times P)_{ij}$ is the interaction between treatment and period, and A_k is random effects of the animal, and ε_{ijk} is the residual error.

For repeated measures, ruminal fermentation data, the model used was:

$$Y = \mu + T_i + P_j + A_k + H_l + (T \times P)_{ij} + (T \times H)_{il} + \varepsilon_{ijkl},$$

where μ is the overall mean; T_i, P_j, H_l, $(T \times P)_{ij}$, and $(T \times H)_{il}$ are effects of treatment, period, hour, interaction of treatment and period, and interaction of treatment and hour, respectively. A_k is random effects of the animal, and ε_{ijk} is the residual error. Animal within period was used as the subject, and an autoregressive order 1 [AR(1)] was selected as the time-series covariance structure based on the smallest Akaike Information Criterion (AIC) values.

Plasma metabolomic data were analyzed with MetaboAnalyst 6.0. The metabolites falling in lower 25% of Interquartile Range (IQR) ranking were filtered out. Autoscaling was applied to the filtered data for normalization. Volcano plot analysis with the Wilcoxon rank-sum test was performed to identify metabolites with differing concentrations between the two treatments. Principal Component Analysis (PCA) was conducted to enable efficient visualization and pattern recognition within the metabolomes of the treatments. Furthermore, Metabolite Set Enrichment Analysis (MSEA), using the Kyoto Encyclopedia of Genes and Genomes (KEGG) reference library, were conducted to identify the enrichment of metabolic pathways.

Significant effects were declared at $P \leq 0.05$, while tendencies were considered when $0.05 < P \leq 0.10$.

RESULTS

Digestibility and Fecal Excretions

No treatment effects were observed on the digestibility of OM ($P = 0.63$, Table 2), CP ($P = 0.97$), NDF ($P = 0.59$), and ADF ($P = 0.84$). Feeding SCFP did not affect the intake, fecal concentration, fecal output and absorption rate of N ($P \geq 0.17$, Table 3), P ($P \geq 0.23$), Cu ($P \geq 0.13$), and Zn ($P \geq 0.24$).

Rumen Fermentation

Steers supplemented with SCFP had greater (6.29 vs 6.01, $P = 0.01$, Figure 1) overall ruminal pH than the CON. At 12 hours post-feeding, the ruminal pH of CON steers was 5.62, while SCFP-supplemented steers had a ruminal pH of 6.01. Supplementation with SCFP had no effect on total ruminal VFA concentration ($P = 0.15$, Table 4). Ruminal butyrate proportion decreased ($P = 0.01$, 12.8 vs. 11.2%) while ruminal isobutyrate ($P = 0.06$) and isovalerate proportions tended to increase ($P = 0.10$) with SCFP supplementation. The molar proportions of acetate, propionate, and valerate in the rumen were unaffected ($P \geq 0.22$) by treatment. Feeding SCFP did not affect ($P = 0.16$) NH_3-N concentration in the rumen. Ruminal lactic acid concentrations were negligible (< 1 mM) in both CON and SCFP steers.

Table 2. Effects of supplementing *Saccharomyces cerevisiae* fermentation product (SCFP) compared to a non-supplemented control on apparent digestibility of Holstein steers fed a high-grain diet

Item[1]	CON	SCFP[2]	SEM[3]	*P*-value [4]	
				TRT	Period
OM digestibility, %	83.5	84.9	2.60	0.63	0.40
CP digestibility, %	81.7	81.8	2.33	0.97	0.18
NDF digestibility, %	75.8	74.3	3.42	0.59	0.34
ADF digestibility, %	79.0	78.5	2.78	0.84	0.36

[1] OM: organic matter, CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber.
[2]SCFP, daily feeding of NaturSafe (Diamond V, 12 g/head/day).
[3]Standard error of the mean.
[4]Significance levels for effects of treatment and period. TRT × Period interaction is not significant (P > 0.10) and not presented in the table.

Table 3. Effects of supplementing *Saccharomyces cerevisiae* fermentation product (SCFP) compared to a non-supplemented control on intake, fecal output, and absorption of zinc (Zn), copper (Cu), phosphorus (P), and nitrogen (N) of Holstein steers fed a high-grain diet

Item[1]	CON	SCFP[2]	SEM[3]	*P*-value[4]	
				TRT	Period
Fecal output, kg/d	1.90	1.65	0.359	0.50	0.88
Zn					
Zn intake, mg/d	881	827	42.7	0.24	0.81
Fecal Zn conc., mg/kg	197	214	16.7	0.44	0.93
Fecal Zn output, mg/d	347	350	55.0	0.95	0.74
Absorbed Zn, mg/d	534	479	57.9	0.41	0.90
Absorbed Zn, % intake	60.6	57.9	5.92	0.75	0.89
Cu					
Cu intake, mg/d	178	167	9.4	0.28	<0.01
Fecal Cu conc., mg/kg	44.5	50.0	3.2	0.13	0.96
Fecal Cu output, mg/d	76.7	80.1	10.7	0.71	0.78
Absorbed Cu, mg/d	102	87.6	9.84	0.22	<0.01
Absorbed Cu, % intake	57.3	52.5	5.03	0.26	0.04
P					
P intake, g/d	49.5	46.4	2.39	0.23	0.03
Fecal P conc., g/kg	0.71	0.71	0.056	0.99	0.01
Fecal P output, g/d	13.2	11.9	2.53	0.24	0.10
Absorbed P, g/d	36.4	35.0	2.38	0.54	0.01
Absorbed P, % intake	73.5	75.4	4.45	0.55	0.01
N					
N intake, kg/d	248	230	11.7	0.17	0.02
Fecal N conc., g/kg	2.38	2.50	0.108	0.30	0.77
Fecal N output, kg/d	43.3	40.7	6.97	0.69	0.82
Absorbed N, kg/d	204	189	9.04	0.12	0.01
Absorbed N, % intake	82.3	82.2	2.33	0.90	0.38

[1]Zn: zinc; Cu: copper; P: phosphorus; N: nitrogen.
[2]SCFP, daily feeding of NaturSafe (Diamond V, 12 g/head/day).
[3]Standard error of the mean.
[4]Significance levels for effects of treatment and period. TRT × Period interaction is not significant (*P* > 0.10) and not presented in the table.

Plasma Proinflammatory Cytokines and Metabolome

The in vitro production of blood proinflammatory cytokines, IL-1β and IL-6, were unaffected ($P > 0.11$ and 0.18, respectively, Table 5) by treatment when Pam3CSK, Poly(I:C), LPS4, or LPS5 were used as stimulants.

A total of 579 metabolites were detected in the plasma of the steers. Volcano plot analysis showed that 18 compounds were significantly higher ($P \leq 0.05$), while 41 compounds were significantly lower ($P \leq 0.05$) in the SCFP group as compared to the CON (Figure 2). The differentially abundant compounds ($P < 0.05$) between treatments are listed in the supplementary table (Table S1). Principal Component Analysis (PCA) indicated some overlap between the two treatment groups (Figure not shown). This suggests that, overall, plasma metabolomic profiles did not show significant distinctions between treatments. The explained variances for the first and second principal components were 19.9% and 14.8%, respectively. Three metabolic pathways were enriched ($P \leq 0.05$, Figure 3) following SCFP supplementation. These pathways include purine metabolism, amino sugar and nucleotide sugar metabolism, and lysine degradation. Tryptophan metabolism and pyrimidine metabolism tended to be enriched ($0.05 < P \leq 0.07$) by feeding SCFP. Xanthine, an essential metabolite in purine metabolism, was elevated by 55% in the SCFP group (Table S1, P = 0.004).

DISCUSSION

The lack of impact on nutrient digestibility in our study aligns with the findings of Lehloenya et al. (2008), who reported similar results when yeast culture was added to the diets of finishing beef steers at a dosage of 56 g/steer. However, this contrasts with Shen et al. (2018), who observed that SCFP supplementation at 18 g/day increased ruminal and total tract NDF digestibility in beef heifers fed a high-concentrate diet containing 90% grain. In dairy cows, the varying effects of SCFP supplementation on digestibility were also reported, such as by Yoon and Stern (1996) at 57 g/day and Hristov et al. (2010) at 56 g/day. These discrepancies can be attributed to several factors, including diet composition, yeast strain and dosage, and baseline rumen health (Hristov et al., 2010).

It is possible that animal stress status interacts with the way SCFP affects mineral utilization, especially considering that SCFP has been reported to have beneficial effects on the inflammatory response and metabolism in health-challenged animals (Ogunade et al., 2019; Sanchez et al., 2020). The unaffected mineral absorption in our study contrasts to the findings of Cole et al. (1992), who observed increased N retention and numerically higher retention of Cu, Fe, and Zn in lambs that had been fasted for 3 d to mimic transportation stress. Research on mineral excretion following SCFP supplementation remains limited. Further studies are recommended to examine the effects of SCFP on digestibility and mineral utilization over a longer experimental period and to explore how stress may interfere with mineral utilization.

At 12 hours post-feeding, CON steers had a ruminal pH of 5.62, nearing subclinical acidosis levels (Thrune et al., 2009), while the ruminal pH in the SCFP-supplemented group was 6.01. The consistently higher ruminal pH in the SCFP-supplementation after 25 d of feeding indicated that SCFP may prevent subacute acidosis in finishing beef cattle, which agrees with numerous previous studies. A meta-analysis by Desnoyers et al. (2009) analyzed data from 110 papers and reported yeast culture supplementation in dairy cows increased the ruminal pH by an average of 0.03 units, from 6.31 in the control group to 6.34 in yeast-supplemented animals. Shen et al. (2018) reported higher minimum ruminal pH and

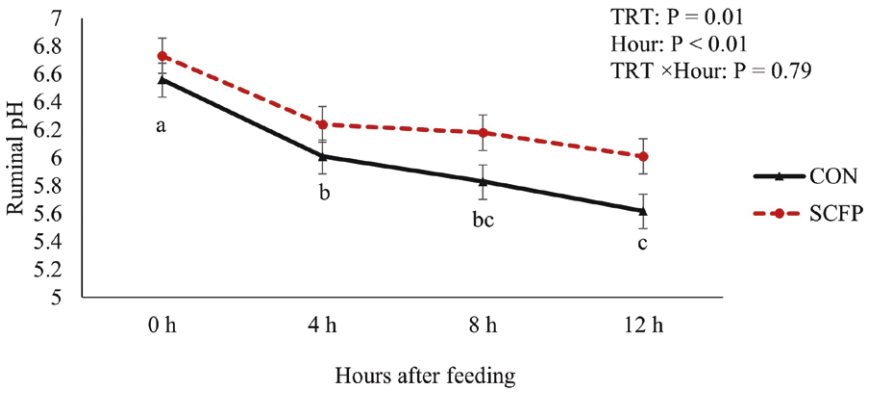

Figure 1. Effects of supplementing *Saccharomyces cerevisiae* fermentation product (SCFP) compared to a non-supplemented control on ruminal pH of Holstein steers fed a high-grain diet. SCFP, daily feeding of NaturSafe (Diamond V, 12 g/head/day). TRT, treatment. The solid line represents the CON while the dashed line represents the SCFP. Error bars represent the standard error of the mean (SEM). Hours without common superscripts are significantly different.

Table 4. Effects of supplementing *Saccharomyces cerevisiae* fermentation product (SCFP) compared to a non-supplemented control on ruminal VFA concentration and profile of Holstein steers fed a high-grain diet

Item[1]	CON	SCFP[2]	SEM[3]	*P*-value [4]	
				TRT	Hour
Total VFA, mM	102.5	96.8	6.17	0.15	<0.01
Acetate, molar %	54.9	56.0	1.27	0.22	< 0.01
Propionate, molar %	27.8	27.9	1.90	0.96	<0.01
Butyrate, molar %	12.8	11.2	0.96	0.01	<0.01
Isobutyrate, molar %	0.90	1.03	0.08	0.06	<0.01
Valerate, molar %	1.25	1.31	0.09	0.43	<0.01
Isovalerate, molar %	2.29	2.62	0.31	0.10	0.02
Acetate: Propionate	2.11	2.17	0.18	0.63	<0.01
NH_3-N, mM	6.25	5.01	0.96	0.16	<0.01
Lactate, mM	0.09	0.28	0.17	0.17	0.10

[1]VFA: volatile fatty acids; NH_3-N: ammonia nitrogen.
[2]SCFP, daily feeding of NaturSafe (Diamond V, 12 g/head/day).
[3]Standard error of the mean.
[4]Significance levels for effects of treatment and period. TRT × Period interaction is not significant ($P > 0.10$) and not presented in the table.

Table 5. Effects of supplementing *Saccharomyces cerevisiae* fermentation product (SCFP) compared to a non-supplemented control on blood proinflammatory cytokine production of Holstein steers fed a high-grain diet

Item[1]	CON	SCFP[2]	SEM[3]	*P*-value[4]	
				TRT	Period
IL-1β, ng/mL					
Mock	0.00	0.00	-	-	-
Pam3CSK	0.00	0.00	-	-	-
Poly(I:C)	1.95	1.58	0.314	0.23	0.26
LPS4	0.37	0.23	0.089	0.28	0.11
LPS5	0.06	0.00	0.024	0.11	0.67
IL-6, ng/mL					
Mock	0.16	0.17	0.164	0.96	0.18
Pam3CSK	1.96	2.30	0.740	0.55	0.21
Poly(I:C)	3.69	3.78	0.779	0.93	0.13
LPS4	4.01	4.35	0.723	0.73	0.97
LPS5	3.71	3.26	0.225	0.12	0.17

[1]In vitro production of proinflammatory cytokines IL-1β and IL-6. In 96-well plates, 100 µl of whole blood were stimulated with 10 µg/mL of Pam3CSK (toll-like receptor, TLR1/2 agonist), 10 µg/mL of polyinosinic:polycytidylic acid (poly I:C, a TLR3 agonist), 1 µg/mL of lipopolysaccharide, LPS4 (a TLR4 agonist), 1 µg/mL of LPS5 (a TLR4 agonist), or the cell culture media as negative control (Mock).
[2]SCFP, daily feeding of NaturSafe (Diamond V, 12 g/head/day).
[3]Standard error of the mean.
[4]Significance levels for effects of treatment and period. TRT × Period interaction is not significant ($P > 0.10$) and not presented in the table.
Columns with dash sign (–) means that cytokines were not detected.

shorter duration of ruminal pH < 5.6 in finishing beef heifers supplemented SCFP. Similarly, Dias et al. (2018) observed that yeast culture increased ruminal pH, reduced the duration of pH below 6 and reduced ruminal lactate of dairy cows fed a high starch diet. Ruminal lactic acid concentrations were low (0.09mM vs 0.28mM) in both treatments, likely because monensin was included in the basal diet, which is known to suppress lactate production (Dennis et al. 1981; Burrin and Britton, 1986). The higher ruminal pH resulting from feeding SCFP is likely due to a numerically lower total VFA concentration, as elevated VFA levels play a major role in reducing pH and can cause subacute acidosis (Bevans et al., 2005; González et al., 2012).

In addition to improved ruminal pH, supplementing SCFP increased branched-chain VFAs, isobutyrate and isovalerate, which are reported to promote the growth of cellulolytic bacteria, a process further supported by higher ruminal pH (Russell and Wilson, 1996; Liu et al., 2008; Tun et al., 2020). Cellulolytic bacteria have a strong preference for NH_3-N for microbial protein synthesis (Bryant and Robinson, 1961), and their improved growth with SCFP supplementation has been reported (Callaway and Martin, 1997; Tun et al., 2020). However, in this study, we observed only a numerically lower NH_3-N concentration (5.01 vs. 6.24 mM, *P*-value = 0.16). Hristov et al. (2010) previously reported lower ruminal NH_3-N levels alongside increased microbial protein synthesis in SCFP-supplemented dairy cows. Future research should explore the effects of SCFP supplementation on cellulolytic

bacterial growth, microbial protein synthesis, and nitrogen metabolism in high-grain diets.

Previous studies suggested that the enhanced cattle performance with SCFP was attributed in part to its effects on improving immunity, antioxidant capacity, and inflammatory responses (Jiang et al. 2018; Deters and Hansen, 2019; Shen et al., 2019). Past research also highlighted the positive impact of SCFP on cytokine production in calves and cows facing various pathogenic disease challenges (Alugongo et al., 2017; Mahmoud et al., 2020; Vailati-Riboni et al., 2021), which is vital for various health and growth functions (Lee and Jun, 2019). Mahmoud et al. (2020) reported that immune cells isolated from calves fed SCFP during the first 21 d of life exhibited increased proinflammatory cytokine secretion compared to non-supplemented controls. Similarly, Henige et al. (2024) found that supplementing SCFP to dairy cows increased production of proinflammation cytokine, IL-1β and IL-6, by peripheral blood mononuclear cells in vitro during digital dermatitis. In contrast, our previous study revealed no effects of SCFP supplementation on in vitro proinflammatory cytokine production, potentially due to the use of healthy, non-stressed animals (Odunfa et al., 2024). In the current study, healthy, non-stressed animals were also used, which may explain why no effects of SCFP on proinflammatory cytokines were observed.

The enriched plasma metabolic pathways, purine metabolism, amino sugar and nucleotide sugar metabolism, lysine degradation, tryptophan metabolism and pyrimidine metabolism, are all related to N metabolism (Røjen et al., 2011; Stentoft et al., 2015; Mariz et al., 2018). Purine metabolism and pyrimidine metabolism are related to microbial protein production in cattle. Purines and pyrimidines are regarded as internal biomarkers for ruminal microbial protein synthesis (Schelling et al., 1982). Ruminal microbes utilize nitrogenous compounds in feed to synthesize microbial protein and nucleic acids. These are later digested and absorbed in the small intestine and converted to purine and pyrimidine derivatives in the liver (McAlian and Smith, 1973; Fujihara and Shem, 2011; Stentoft et al., 2015). In our study, the increased xanthine concentrations in the plasma of SCFP-fed steers also indicate the increased microbial protein synthesis in the rumen since xanthine is a purine derivative and is regarded as a biomarker of bacterial cell degradation in the rumen (Ametaj et al., 2010). Amino sugar and nucleotide sugar metabolism pathway was enriched with SCFP supplementation, indicating a significant impact on carbohydrate metabolism. Amino sugars are key constituents of biomacromolecules and microbial secondary metabolites, where they influence protein activity through their incorporation into protein-linked sugar chains (Skarbek and Milewska, 2016). Similarly, nucleotide sugars, the activated forms of monosaccharides, are vital intermediates in carbohydrate metabolism, contributing to processes such as protein glycosylation, glycogen biosynthesis, and cell wall synthesis (Bülter and Elling, 1999; Freeze and Aebi, 2005; Moretti and Thorson, 2008). Enrichment of this pathway suggests an enhancement in the synthesis of key molecules involved in protein regulation and vital processes like

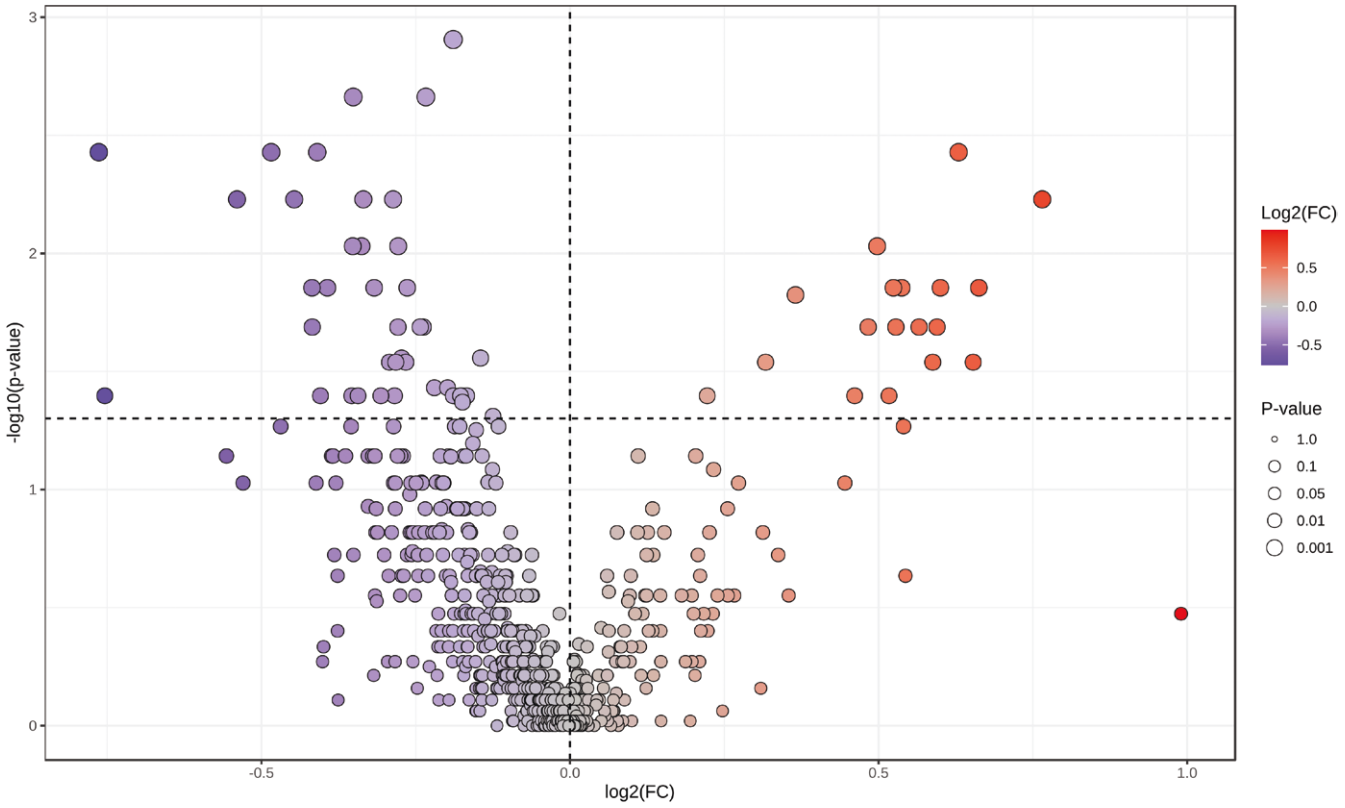

Figure 2. Volcano plot analysis of plasma metabolome of Holstein steers fed a high-grain diet. The color scale represents the fold change of the detected compounds in the *Saccharomyces cerevisiae* fermentation product (SCFP) group compared to the non-supplemented control (CON). Blue dots indicate plasma metabolites with decreased concentrations in SCFP-fed steers, while red dots indicate metabolites with increased concentrations. SCFP, daily feeding of NaturSafe (Diamond V, 12 g/head/day).

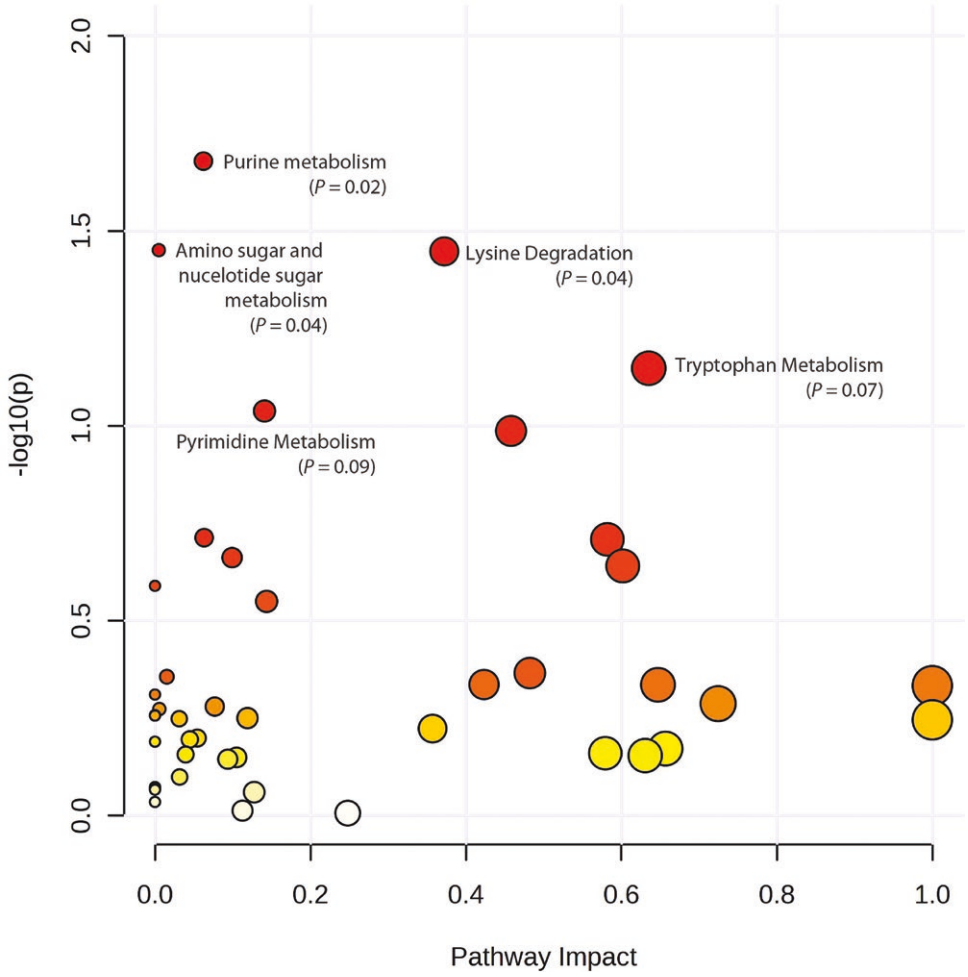

Figure 3. Pathway analysis of the plasma metabolome of Holstein steers supplemented with *Saccharomyces cerevisiae* fermentation product (SCFP). Purine metabolism, amino sugar and nucleotide sugar metabolism, and lysine degradation pathways were affected ($P \leq 0.05$). Tryptophan metabolism and pyrimidine metabolism' tended to be affected ($0.05 < P \leq 0.10$) by feeding SCFP. [1]SCFP, daily feeding of NaturSafe (Diamond V, 12 g/head/day). Each dot represents a pathway, with its size representing the enrichment ratio and the color (yellow to red) representing the *P*-value in decreasing order.

glycosylation and glycogen biosynthesis following SCFP supplementation.

Lysine degradation, tryptophan metabolism pathways are important for N metabolism since lysine and tryptophan are considered essential amino acids, and lysine is regarded as one of the two most limiting amino acids for cows (Vyas and Erdman, 2009). Feeding SCFP may enhance their supply and metabolism and improve N utilization by cattle. The enrichment of the abovementioned pathways may be related to improved ruminal pH, branched chain VFA, and possible improvement in microbial protein synthesis by feeding SCFP. Hristov et al. (2010) previously found increased microbial protein synthesis in SCFP-supplemented dairy cows. Similarly, Dias et al. (2018) reported that yeast culture supplementation increased both milk true protein yield and microbial N synthesis in dairy cows. Future studies should investigate the effects of SCFP on the ruminal and duodenum amino acids profile, as well as ruminal microbial protein synthesis efficiency, and N metabolism in cattle fed high grain diet.

CONCLUSION

Supplementing SCFP did not affect total tract digestibility. The fecal mineral excretions of Zn, Cu, P and N were not affected by feeding SCFP. The ruminal pH was increased by feeding SCFP, indicating the potential of SCFP in alleviating ruminal subacute acidosis associated with a concentrate-rich finishing diet. Furthermore, important nitrogen metabolism pathways, purine metabolism, pyrimidine metabolism, and lysine degradation, were enriched with SCFP supplementation. Further studies are needed to investigate how SCFP affects ruminal microbial protein synthesis, amino acid profiles, and nitrogen metabolism in cattle fed a high-grain diet.

Supplementary Data

Supplementary data are available at *Translational Animal Science* online.

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Author Contributions

Yun Jiang (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing—original draft, Writing—review & editing), Anjan Dhungana (Formal analysis, Writing—original draft, Writing—review & editing), Oluwaseun Odunfa (Data curation, Formal analysis, Writing—review & editing), Megan McCoun (Data curation, Writing—original draft, Writing—review & editing), Jodi McGill (Formal analysis, Writing—review & editing), Ilkyu Yoon (Conceptualization, Methodology, Writing—review & editing), and Ibukun Ogunade (Conceptualization, Methodology, Writing—review & editing)

Conflict of Interest statement

The products evaluated in this study were provided by Diamond V, Inc. Ilkyu Yoon, who is employed by Diamond V, participated in the research. Diamond V has provided additional funding to Yun Jiang's research program for sample analysis. Jodi McGill received payment or honoraria from Diamond V for delivering expert lectures in 2019 and 2020, which focused on the immune system and research findings from collaborations with Diamond V. The remaining authors report no conflicts of interest. All authors have reviewed and consented to the submission of the manuscript.

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