

# Effect of supplementing a *Saccharomyces cerevisiae* fermentation product during a preconditioning period prior to transit on receiving period performance, nutrient digestibility, and antioxidant defense by beef steers

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**ABSTRACT:** Forty-eight newly weaned cross-bred beef steers from a single-source were used to determine the effects of feeding a *Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe, Diamond V) on receiving period performance, nutrient digestibility, and antioxidant defense. Seven days after arrival, steers were stratified by BW ( $257 \pm 18$  kg), sorted into pens ( $n = 1$  pen/treatment), and pens assigned to dietary treatments: SCFP at 0 (CON), 12 (SCFP12), 18 (SCFP18), or  $0 \text{ g}\cdot\text{steer}^{-1}\cdot\text{d}^{-1}$  during preconditioning (PRE; days  $-19$  to  $0$ ), then  $18 \text{ g}\cdot\text{steer}^{-1}\cdot\text{d}^{-1}$  during receiving (REC; days  $0$  to  $58$ ; CON18). On day  $-1$  BW and blood were collected, steers were loaded onto a semitruck and transported  $1,748$  km over  $19$  h. Upon return, steers were weighed, stratified by BW within treatment and sorted into pens with GrowSafe bunks ( $n = 12$  steers/treatment). Steers were weighed on days  $-1$ ,  $0$ ,  $29$ ,  $30$ ,  $57$ , and  $58$ . Blood was collected from all steers on days  $-1$ ,  $1$ , and  $8$  and liver biopsies were performed on all steers on days  $-20$ ,  $-3$ , and  $59$ . Titanium dioxide was included as an indigestible marker in the diet of all steers from days  $14$  through  $29$  to determine total tract nutrient digestibility. Data were analyzed as a completely randomized design using ProcMixed of SAS with

the fixed effect of treatment. Steer was the experimental unit for REC period variables. Contrast statements compared the linear and quadratic effects of feeding SCFP throughout the trial (CON, SCFP12, and SCFP18) and the effect of supplementation at  $18 \text{ g}\cdot\text{steer}^{-1}\cdot\text{d}^{-1}$  for the entire trial or starting in REC (SCFP18 vs. CON18). Steers fed SCFP12 exhibited the greatest ADG and G:F from days  $0$  to  $30$  (quadratic  $P \leq 0.04$ ). Total tract digestibility of NDF and ADF was linearly decreased by SCFP (linear  $P \leq 0.03$ ). On day  $-3$ , SCFP12-fed steers tended to have the greatest liver concentrations of total, oxidized, and reduced glutathione (quadratic  $P = 0.06$ ). Red blood cell lysate Mn:total-superoxide dismutase activity was  $16\%$  greater  $1$  d posttransit compared with pretransit values (day  $P \leq 0.01$ ). Timing of SCFP supplementation (SCFP18 vs. CON18) did not affect any of the variables assessed herein ( $P \geq 0.19$ ). Supplementing SCFP at  $12 \text{ g}\cdot\text{steer}^{-1}\cdot\text{d}^{-1}$  tended to affect antioxidant capacity prior to transit and improved early receiving period performance; however, overall receiving period performance was not affected by SCFP supplementation. Further research is necessary to determine the optimal dose and timing of SCFP supplementation for beef cattle.

**Key words:** beef cattle, oxidative stress, *Saccharomyces cerevisiae* fermentation product, transit

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Transl. Anim. Sci. 2019.3:1227–1238  
doi: 10.1093/tas/txz140

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Received June 11, 2019.

Accepted August 16, 2019.

## INTRODUCTION

Beef cattle experience various physical and psychological stressors during the feedlot receiving period. These stressors include recent weaning, vaccination, commingling, and transportation. The combination of stress and exposure to pathogens increases disease susceptibility and decreases feedlot performance (Galyean et al., 1999; Loerch and Fluharty, 1999). Additionally, transit has been shown to increase markers of oxidative stress in cattle that were associated with increased incidence of bovine respiratory disease (Chirase et al., 2004). *Saccharomyces cerevisiae* fermentation products (SCFP) have decreased the number of first pulls and repulls as well as antibiotic usage in two retrospective analyses (NaturSafe; Diamond V, 2017a, 2017b). Feed efficiency was also improved for cattle fed SCFP throughout the entire feeding period compared with cattle that were not fed SCFP but received a metaphylactic antibiotic upon arrival (Diamond V, 2017a). The positive influence of SCFP on health and performance suggests that SCFP may be a beneficial addition to receiving cattle diets.

The first objective of this study was to determine the effects of varying doses of SCFP on receiving period performance, total tract nutrient digestibility, and oxidative stress biomarkers in beef steers. Due to the segmented nature of the beef industry, calves often change ownership prior to arrival at the feedlot and calf nutrition prior to feedlot receiving likely influences how they perform upon arrival (Duff and Galyean, 2007). Therefore, the second objective was to determine the effects of supplementing SCFP during a preconditioning phase prior to a 19-h transit event on subsequent receiving period performance. The final objective was to examine changes in markers of oxidative stress relative to transit with the hypothesis that transit would increase markers of oxidative stress and that supplementing SCFP during a preconditioning period prior to transit would have positive implications on the oxidative stress response and receiving period performance of beef steers.

## MATERIALS AND METHODS

### *Animals and Experimental Design*

All experimental procedures were approved by the Iowa State University Animal Care and Use Committee (#8376-B). Sixty newly weaned (bawling) crossbred beef steers ( $253 \pm 23$  kg) from a single source were transported approximately 265

km to the Iowa State University Beef Nutrition Farm (Ames, IA) where they were received into open dirt lots ( $23.6 \times 33.5$  m; 15 steers/pen) with concrete bunks (12.2 m of linear bunk space) and one automatic waterer/pen. Steers were offered long-stem hay top dressed with the preconditioning (PRE) TMR on the first day. Bunks were scored the morning after arrival and if bunks were clean an additional 0.45 kg of DM per steer was offered. The 48 steers most uniform in weight ( $257 \pm 18$  kg), disposition, and health status were utilized in this trial which consisted of two phases, PRE (days  $-19$  to  $-1$ ) followed by receiving (REC; days 0 to 58), separated by a 19-h transit event. Diet composition and nutrient analysis are shown in Table 1. Weekly control TMR samples were dried, ground, and composited within PRE and REC periods for analysis of N (AOAC, 1995b; method 990.03), NDF

**Table 1.** Composition of diets fed during preconditioning (PRE) and receiving (REC)

	PRE <sup>1</sup>	REC <sup>2</sup>
DM, %	56	61
Ingredient, % DM basis		
Corn silage	50	40
Dry-rolled corn	20	30
Dried distillers grains <sup>3</sup>	28.15	28.15
Limestone	1.4	1.4
Salt	0.31	0.31
Rumensin <sup>4</sup>	0.0135	0.0135
Vitamin A and E premix <sup>5</sup>	0.1	0.1
Trace mineral premix <sup>6</sup>	0.024	0.024
Analyzed composition <sup>7</sup> , % DM		
Crude protein	13.2	13.7
Neutral detergent fiber	27.9	24.5
Ether extract	5.1	5.1
Analyzed composition <sup>8</sup> , mg/kg DM		
Cu	15.8	13.8
Fe	69	69
Mn	38	35
Zn	68	62

<sup>1</sup>Days  $-19$  to  $-1$ .

<sup>2</sup>Days 0 to 58.

<sup>3</sup>Carrier for microingredients and *Saccharomyces cerevisiae* fermentation product (NaturSafe, Diamond V, Cedar Rapids, IA).

<sup>4</sup>Provided 200 mg monensin·steer<sup>-1</sup>·d<sup>-1</sup> (Rumensin, Elanco Animal Health, Greenfield, IN).

<sup>5</sup>Premix provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet DM.

<sup>6</sup>Provided per kilogram of diet DM: 10 mg of Cu, 30 mg of Zn, 20 mg of Mn, 0.5 mg of I, 0.1 mg of Se, and 0.1 mg of Co all from inorganic sources.

<sup>7</sup>Based on analysis of TMR from Dairyland Laboratories, Inc., Arcadia, WI.

<sup>8</sup>Analyzed mineral values reflect control diet total, which includes supplemental mineral.

(AOAC, 2005; method 2002.04), and ether extract (AOAC, 1995a; method 920.39) by a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI) as well as Cu, Fe, Mn, and Zn using inductively coupled optical emission spectroscopy as described by Richter et al. (2012).

**Preconditioning.** This period served to mimic the group feeding style of preconditioning periods common on farms and to address the second objective of this study: determining the effect of supplementing SCFP during a preconditioning period prior to a transit event on subsequent receiving period performance. Seven days after arrival (day -19) steers were weighed, vaccinated against viral infections (Pyramid 5, Presponse SQ; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO), and treated for parasites (Ivomec Eprinomex, Boehringer Ingelheim Vetmedica, Inc.). Steers were stratified by BW and sorted into open dirt lots as previously described ( $n = 1$  pen/treatment; 12 steers/pen). Pens were then randomly assigned to 1 of 4 dietary treatments: SCFP (NaturSafe, Diamond V, Cedar Rapids, IA) at 0 (CON), 12 (SCFP12), 18 (SCFP18) g·steer<sup>-1</sup>·d<sup>-1</sup> during both PRE and REC, or 0 g·steer<sup>-1</sup>·d<sup>-1</sup> during PRE then 18 g·steer<sup>-1</sup>·d<sup>-1</sup> during REC (CON18). The current manufacturer's recommended dose for receiving cattle is 12 g·steer<sup>-1</sup>·d<sup>-1</sup> or 2 kg/metric ton DM in the ration. Treatments were delivered as part of a premix using dried distillers grains as a carrier and premix inclusions were adjusted weekly based on treatment group DMI to ensure target intakes of SCFP were maintained. Intake of SCFP was back calculated based on pen DMI and premix inclusion rates; SCFP intakes for PRE were 12.0 and 18.9 g·steer<sup>-1</sup>·d<sup>-1</sup> for SCFP12 and SCFP18, respectively. On day -1, steers were weighed, blood samples collected, loaded onto a single commercial livestock trailer (Silverstar PSDCL-402; Wilson Trailer Company, Sioux City, IA), and transported 1,748 km over 19 h. Steers were stratified by dietary treatment across truck compartments to account for compartment variability.

**Receiving.** Upon return (day 0), steers were weighed, stratified by BW, and sorted into partially covered concrete pens (3.7 × 12.2 m) with one GrowSafe (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) bunk and one automatic waterer/pen (experimental unit = steer;  $n = 12$  steers/treatment; 6 steers/pen). Steers were fed the same dose of SCFP during REC as PRE, except CON18-fed steers switched from 0 to 18 g·steer<sup>-1</sup>·d<sup>-1</sup>. Intake of SCFP was back calculated based on steer DMI and premix inclusion rates; SCFP intakes for REC

were 11.4, 18.1, and 18.6 g·steer<sup>-1</sup>·d<sup>-1</sup> for SCFP12, SCFP18, and CON18, respectively. The shrunk BW collected when steers arrived back at the Iowa State University Beef Nutrition Farm after transit (day 0) was used as the final BW for PRE and the initial BW for REC. Weights were collected prior to feeding on days 0, 29, 30, 57, and 58. Feed efficiency (G:F) was calculated from days 0 to 30 (average BW collected on days 29 and 30), 30 to 58 (average BW collected on days 57 and 58), and 0 to 58 from steer DMI and weight gain. On day 1 steers were implanted with 200 mg progesterone and 20 mg estradiol (Component E-S with Tylan, Elanco, Indianapolis, IN). Morbidity was assessed daily throughout the course of the study and steers were treated with tulathromycin (Draxxin, Zoetis, Parsippany, NJ) by farm personnel if visual symptoms were observed and rectal temperature was  $\geq 39^\circ\text{C}$ .

### Sample Collection and Analytical Procedures

**Digestibility.** To determine the effect of SCFP on total tract nutrient digestibility, titanium dioxide was included as an indigestible marker in the diet of all steers (10 g·steer<sup>-1</sup>·d<sup>-1</sup>) from days 14 through 29. Back calculated daily intakes of titanium dioxide were 10.4, 9.7, 10.2, and 10.3 g/steer for CON, SCFP12, SCFP18, and CON18, respectively. Fecal samples were collected prior to feeding on days 29 and 30 for digestibility analyses. Treatment TMR samples from the digestibility period as well as fecal samples collected on days 29 and 30 were dried and then ground to pass through a 2-mm screen in a Retsch ZM 100 grinding mill (Retsch GmbH, Haan, Germany) and analyzed for DM, OM, NDF, ADF, and N using methods described by Russell et al. (2016a). Titanium dioxide was analyzed using methods outlined by Myers et al. (2004). Nutrient and titanium dioxide concentrations were analyzed separately for consecutive day fecal samples and the average was used for final digestibility calculations as described previously (Russell et al., 2016a).

**Blood and liver.** Blood was collected from the jugular vein of all steers on days -1, 1, and 8 into vacuum tubes (sodium heparin, No. 367874, Becton Dickinson, Franklin Lakes, NJ), transported to the laboratory on ice, and centrifuged at 1,000 ×  $g$  for 10 min at 4°C. Plasma was removed, aliquoted, and stored at -80°C until analysis of malondialdehyde (MDA) concentrations (#700870, Cayman Chemical, Ann Arbor, MI); inter- and intra-assay CV were 7.7% and 6.4%, respectively. Once the remaining plasma was removed and the white buffy

layer discarded, 2 mL of the red blood cell fraction was transferred into a 30-mL Teflon tube, lysed with 8 mL of ice-cold ultrapure water, and centrifuged at  $10,000 \times g$  for 15 min at  $4^{\circ}\text{C}$ . The supernatant (red blood cell lysate; RBCL) was removed, aliquoted, and stored at  $-80^{\circ}\text{C}$  until analysis of total and manganese superoxide dismutase (SOD) activity (#706002, Cayman Chemical). Activity is reported as units (U)/g hemoglobin, where one U is defined as the amount of enzyme required to dismutate 50% of the superoxide radical. Inter- and intra-assay CV for total-SOD activity were 10.7% and 9.0%, respectively; inter- and intra-assay CV for Mn-SOD activity were 7.8 and 8.6, respectively. Copper/Zn-SOD activity was calculated by subtracting Mn-SOD from total SOD activity. Hemoglobin was determined using methods described by [Hansen et al. \(2010\)](#).

Liver biopsies were performed as described by [Engle and Spears \(2000\)](#) on all steers on 1 of 2 d prior to the start of PRE (days  $-21$  and  $-20$ ) as well as prior to shipping (day  $-3$ ) and the end of REC (day 59). Liver samples were snap frozen in liquid nitrogen and transported to the laboratory where they were stored at  $-80^{\circ}\text{C}$ . Samples were ground in liquid nitrogen prior to homogenization. Liver for SOD activity (0.15 g tissue; wet basis) was homogenized in 0.75 mL of 20 mM HEPES buffer, centrifuged at  $1,500 \times g$  for 5 min at  $4^{\circ}\text{C}$ , and the supernatant was removed, aliquoted, and stored at  $-80^{\circ}\text{C}$  until further analysis (#706002, Cayman Chemical). Liver SOD activity is reported as U/mg protein. Inter- and intra-assay CV for total-SOD activity were 10.2% and 6.5%, respectively; inter- and intra-assay CV for Mn-SOD activity were 11.4% and 9.9%, respectively. Protein concentration of the sample analyzed for SOD activity was determined using a commercially available kit (#23200, Thermo Scientific, Rockford, IL). Liver for total (tGSH) and oxidized (GSSG) glutathione concentrations (0.15 g tissue; wet basis) was homogenized in 0.75 mL of 50 mM MES buffer and centrifuged at  $10,000 \times g$  for 15 min at  $4^{\circ}\text{C}$ . Samples were then deproteinated by removing 0.5 mL of supernatant, adding 0.5 mL of MPA reagent, vortexing, and allowing to sit at room temperature for 5 min prior to centrifugation at  $3,000 \times g$  for 3 min at  $4^{\circ}\text{C}$ . The supernatant was removed, aliquoted, and stored at  $-80^{\circ}\text{C}$  until further analysis (#703002, Cayman Chemical). Inter- and intra-assay CV for tGSH were 2.8% and 1.1%, respectively; inter- and intra-assay CV for GSSG were 5.1% and 2.1%, respectively. Reduced glutathione (GSH) concentrations were calculated by subtracting GSSG from

tGSH. Glutathione concentrations are reported as  $\mu\text{M/g}$  wet tissue. Remaining liver was dried, prepared, and analyzed for Cu, Fe, Mn, and Zn concentrations using inductively coupled optical emission spectroscopy (ICP-OES) as described by [Richter et al. \(2012\)](#).

### Statistical Analysis

Data were analyzed as a completely randomized design using the Mixed procedures of SAS 9.4 (SAS Inst., Inc., Cary, NC) with the fixed effect of treatment. Steer was the experimental unit for blood and liver analyses, digestibility, as well as REC performance ( $n = 12$  steers/treatment). One steer from SCFP12 died during REC from illness unrelated to treatment and was therefore removed from the analysis of all data excluding PRE performance means. Orthogonal polynomial (linear and quadratic) contrast statements were constructed to compare the effects of SCFP inclusion throughout the trial (CON, SCFP12, and SCFP18). Contrast coefficients were determined using the IML procedure of SAS based on back calculated SCFP intake (0, 11.4, and 18.1  $\text{g}\cdot\text{steer}^{-1}\cdot\text{d}^{-1}$  for CON, SCFP12, and SCFP18, respectively). An additional contrast statement (SCFP18 vs. CON18) was used to determine the effect of supplementing SCFP throughout the entire trial or just during REC. Dry matter intake from titanium dioxide feeding period (days 14 through 29) was utilized as a covariate in analysis of all nutrient digestibility data. Values from day  $-20$  (prior to treatment initiation) were utilized as covariates in analyses of liver SOD, glutathione, and trace mineral data. Plasma MDA and RBCL SOD were analyzed as repeated measures using the repeated effect of day without a covariate in the model. The autoregressive (AR1) covariance structure was used for all repeated measures analyses based on lowest Akaike's information criterion. Data were tested for normality and homogeneity of variance using the Shapiro-Wilks test; RBCL total and Mn-SOD activity were log transformed to meet the assumption of normality and back-transformed means and SEM are presented. Outliers were determined using Cook's  $D$  statistic and removed if Cook's  $D > 0.5$ ; one steer from SCFP12 was removed from liver glutathione analyses. Pearson correlations between liver mineral concentrations and liver SOD activity were determined using Proc CORR of SAS. Data are reported as least square means  $\pm$  SEM. Significance was declared at  $P \leq 0.05$  and tendencies from  $0.05 < P \leq 0.10$ .

## RESULTS AND DISCUSSION

### Feedlot Performance

The feedlot receiving period is often characterized by poor performance and increased incidence of disease. Preconditioning programs have been shown to improve the subsequent health of calves in the feedlot resulting in improved ADG and feed efficiency (Hilton, 2015). As SCFP have also been shown to positively influence cattle health (Diamond V, 2017a, 2017b), supplementing SCFP during preconditioning programs may have positive implications for subsequent feedlot health and performance. Average DMI, ADG, and G:F for the PRE period were 6.1 kg/d, 1.60 kg/d, and 0.261 kg/kg, respectively. Regardless of treatment, the 19-h transit event resulted in an average BW shrink of 7.1% (SD = 1.4%).

Receiving period performance data are presented in Table 2. Supplementing SCFP throughout the trial did not affect final BW or DMI ( $P \geq 0.32$ ). There was a quadratic effect of SCFP on ADG and G:F from days 0 to 30 ( $P \leq 0.04$ ) driven by greatest performance by SCFP12-fed steers; however, there were no effects of treatment on ADG or G:F from days 30 to 58 or overall (days 0 to 58;  $P \geq$

0.22). A retrospective study utilizing data from beef steers and heifers at a large commercial feedlot observed that cattle fed SCFP (NaturSafe, Diamond V) at 1.56 kg/metric ton DM in the starter ration and 1.62 kg/metric ton DM in the finisher ration vs. those that were not fed SCFP but received an antibiotic treatment upon arrival had numerically greater ADG (1.45 vs. 1.32 kg/d) and numerically lesser feed:gain (3.01 vs. 3.08; Diamond V, 2017a). It is possible that the performance benefits noted in the retrospective study were a result of improved performance early in the receiving period; however, results are only presented for the entire feeding period. Other sources of discrepancy between the retrospective analysis and the current study include diet type (steam flaked corn vs. corn silage), dose and duration of supplementation (entire feeding period vs. receiving period only), as well as nutritional and environmental background of the cattle (commingled vs. single-source). More work is needed to determine the influence of diet type and environment on the way cattle respond to SCFP supplementation.

Timing of SCFP supplementation may also be vital to how cattle respond. This study sought to address this question by supplementing SCFP at 18

**Table 2.** Effect of *Saccharomyces cerevisiae* fermentation product on performance of beef steers during a 58-d receiving period

	Treatment <sup>1</sup>				SEM <sup>4</sup>	Contrast <i>P</i> -value <sup>2</sup>		
	CON <i>n</i> = 12 steers	SCFP12 <i>n</i> = 11 steers <sup>3</sup>	SCFP18 <i>n</i> = 12 steers	CON18 <i>n</i> = 12 steers		Linear	Quadratic	Timing
SCFP intake <sup>5</sup>	0.0	11.4	18.1	18.9	–	–	–	–
Initial BW <sup>6</sup> , kg	289	287	284	290	5.7	0.49	0.85	0.42
Final BW, kg	382	386	375	377	6.9	0.58	0.32	0.83
DMI, kg/d								
Days 0 to 30	6.7	7.0	6.9	6.9	0.25	0.55	0.51	0.79
Days 30 to 58	8.3	8.3	8.0	8.3	0.28	0.41	0.63	0.38
Days 0 to 58	7.5	7.6	7.4	7.6	0.24	0.87	0.54	0.53
ADG, kg/d								
Days 0 to 30	1.37	1.65	1.36	1.32	0.099	0.77	0.02	0.74
Days 30 to 58	1.84	1.77	1.81	1.71	0.109	0.81	0.72	0.49
Days 0 to 58	1.60	1.71	1.58	1.51	0.081	0.98	0.24	0.53
G:F								
Days 0 to 30	0.206	0.237	0.198	0.189	0.014	0.90	0.04	0.65
Days 30 to 58	0.222	0.218	0.224	0.205	0.012	0.94	0.70	0.25
Days 0 to 58	0.213	0.226	0.211	0.197	0.009	0.97	0.22	0.25

<sup>1</sup>*Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe, Diamond V, Cedar Rapids, IA) at 0 g·steer<sup>-1</sup>·d<sup>-1</sup> (CON), 12 g·steer<sup>-1</sup>·d<sup>-1</sup> (SCFP12), 18 g·steer<sup>-1</sup>·d<sup>-1</sup> (SCFP18), or 0 g·steer<sup>-1</sup>·d<sup>-1</sup> during preconditioning (days –19 to –1) then 18 g·steer<sup>-1</sup>·d<sup>-1</sup> during receiving (days 0 to 58; CON18).

<sup>2</sup>Linear and quadratic contrast statements compare CON, SCFP12, and SCFP18; Timing contrast statement compares SCFP18 vs. CON18.

<sup>3</sup>One steer from NS12 died during the course of the study from illness unrelated to treatment.

<sup>4</sup>Highest SEM of any treatment reported.

<sup>5</sup>Back calculated SCFP intake; g·steer<sup>-1</sup>·d<sup>-1</sup>.

<sup>6</sup>Initial BW = shrunk BW after 19-h transit event.

g·steer<sup>-1</sup>·d<sup>-1</sup> during both PRE and REC (SCFP18) or supplementing SCFP at 18 g·steer<sup>-1</sup>·d<sup>-1</sup> only during REC (CON18). No effects of supplementation timing were observed for receiving period performance ( $P \geq 0.25$ ). However, improved performance early in the receiving period for cattle supplemented SCFP at 12 g·steer<sup>-1</sup>·d<sup>-1</sup> during both PRE and REC suggests that these cattle were better equipped to handle the stress of transit as well as a novel diet and environment. This suggests that timing of supplementation may have influenced receiving period performance if a lower dose had been utilized to address this objective.

### Total Tract Nutrient Digestibility

Cattle performance is influenced by diet digestibility and SCFP have been shown to affect gastrointestinal tract microflora. Because SCFP contain no live yeast, the effects of this product on gastrointestinal tract microbial communities are likely due to the unique metabolites, including vitamins, amino acids, organic acids, and oligosaccharides, that are produced during the fermentation process. Feye et al. (2016) reported that concentrations of *Salmonella* and *E. coli* in the feces of heifers fed SCFP (NaturSafe, Diamond V) were decreased by 74% and 58%, respectively, compared with heifers fed monensin, tylosin, and a direct-fed microbial. *Saccharomyces cerevisiae* fermentation products have also shown variable effects on nutrient digestibility. In the current study, there were no effects of treatment on total tract digestibility of DM, OM, or CP ( $P \geq 0.19$ ; Table 3); however, SCFP linearly decreased NDF and ADF digestibility ( $P \leq 0.03$ ). This was unexpected because several studies have

reported an increase in ruminal cellulolytic bacteria due to SCFP (Wiedmeier et al., 1987; Callaway and Martin, 1997) which would aid in ruminal fiber digestion. In support of this, Shen et al. (2018) observed improved ruminal and total NDF digestibility when SCFP (NaturSafe, Diamond V) was delivered directly to the rumen of cannulated beef heifers fed high-grain diets in a Latin square design with 28-d periods (21 d for adaptation and 7 d for data collection). Although NDF content of the barley grain-based diet fed by Shen et al. (2018) and the corn-silage based receiving diet fed in the current study were similar (29.7% vs. 24.5%), the physical nature of this fiber would be very different and likely contribute to differences in fiber digestibility by rumen microbes. Additionally, total tract digestibility analysis via titanium dioxide may be more susceptible to individual animal variation as opposed to a Latin square design in which each animal is exposed to both control and SCFP treatments.

### Antioxidant Measures

Oxidative stress occurs when cellular oxidants exceed antioxidants (Sies, 2007) and can result in damage to cell components including lipids, proteins, and nucleic acids. *Saccharomyces cerevisiae* fermentation products manufactured in a similar manner as the SCFP used in the current study (NaturSafe, Diamond V) have been shown to exhibit high ROS scavenging activity (Schauss and Vojdani, 2006), have demonstrated antioxidant properties in vitro (Original XP, Diamond V; Jensen et al., 2008), and have increased antioxidant capacity in vivo (EpiCor, Embria Health Sciences; Jensen et al., 2011). Additionally, anti-inflammatory

**Table 3.** Effect of *Saccharomyces cerevisiae* fermentation product on total tract nutrient digestibility by beef steers on d 29 and 30

	Treatment <sup>1</sup>				SEM <sup>3</sup>	Contrast <i>P</i> -value <sup>2</sup>		
	CON	SCFP12	SCFP18	CON18		Linear	Quadratic	Timing
DMI <sup>4</sup> , kg	7.7	7.9	7.8	7.9	0.28	0.91	0.65	0.72
Nutrient, %								
DM	70.2	69.4	71.2	71.5	1.17	0.61	0.33	0.85
OM	71.3	70.7	72.6	72.9	1.23	0.53	0.35	0.85
NDF	57.6	52.8	53.5	54.9	1.56	0.03	0.24	0.49
ADF	55.3	49.2	50.3	53.1	1.85	0.02	0.19	0.24
CP	68.8	69.2	69.4	67.9	0.91	0.58	0.99	0.19

<sup>1</sup>*Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe, Diamond V, Cedar Rapids, IA) at 0 g·steer<sup>-1</sup>·d<sup>-1</sup> (CON), 12 g·steer<sup>-1</sup>·d<sup>-1</sup> (SCFP12), 18 g·steer<sup>-1</sup>·d<sup>-1</sup> (SCFP18), or 0 g·steer<sup>-1</sup>·d<sup>-1</sup> during preconditioning (days -19 to -1) then 18 g·steer<sup>-1</sup>·d<sup>-1</sup> during receiving (days 0 to 58; CON18).

<sup>2</sup>Linear and quadratic contrast statements compare CON, SCFP12, and SCFP18; Timing contrast statement compares SCFP18 vs. CON18.

<sup>3</sup>Highest SEM of any treatment reported.

<sup>4</sup>Dry matter intake (DMI) during titanium dioxide feeding period (days 14 through 29) was utilized as a covariate in analysis of all nutrients.

properties demonstrated by SCFP (EpiCor, Embria Health Sciences; Jensen et al., 2007) would likely decrease the production of free radicals by phagocytic immune cells. Therefore, it was expected that SCFP supplementation would positively influence antioxidant status in the current study.

The liver plays a pivotal role in systemic homeostasis of the endogenous antioxidant glutathione by exporting much of the glutathione it synthesizes into the plasma for utilization by other tissues (Lu, 2013). The oxidized to reduced glutathione ratio is an indicator of cellular redox state and a ratio greater than 0.1 is indicative of oxidative stress (Ithayaraja, 2011). Based on this threshold, steers in the current study were experiencing some degree of oxidative stress regardless of sampling day or treatment (Table 4). At the end of PRE (day -3), there was a tendency for a quadratic effect of SCFP on total, oxidized, and reduced glutathione concentrations ( $P = 0.06$ ) driven by greatest concentrations observed in SCFP12-fed steers. Additionally, there was a tendency for a linear decrease in the oxidized to reduced glutathione ratio due to SCFP ( $P = 0.07$ ). Greater concentrations of reduced glutathione, the form in which glutathione can function as an antioxidant, suggest that these

steers had greater antioxidant capacity prior to the transit event. This greater antioxidant capacity may have contributed to the greater ADG and G:F for SCFP12-fed steers early in the REC period as oxidative damage is energetically expensive and has been associated with decreased production efficiency (Iqbal et al., 2004, 2005). No treatment effects on day 59 glutathione concentrations were observed ( $P \geq 0.29$ ). Deters et al. (2018) measured RBCL glutathione concentrations in newly weaned beef steers receiving SCFP (Original XPC, Diamond V) and observed that steers supplemented SCFP at 14 g·steer<sup>-1</sup>·d<sup>-1</sup> had greater concentrations of reduced glutathione vs. steers supplemented SCFP at 0 or 28 g·steer<sup>-1</sup>·d<sup>-1</sup>. The similar dose response observed in these two studies may be due to a proinflammatory state stimulated by the presence of more cell wall components ( $\beta$ -glucans and mannan-oligosaccharides) in the greater SCFP dose, though inflammation was not measured in the present study. Inflammation can increase the production of prooxidant species due to an increase in neutrophil oxidative burst (Babior, 1984) and contribute to depletion of antioxidant status.

Although activity of the antioxidant enzyme SOD was not affected by SCFP supplementation

**Table 4.** Effect of *Saccharomyces cerevisiae* fermentation product on liver glutathione concentrations of beef steers

	Treatment <sup>1</sup>				SEM <sup>3</sup>	Contrast <i>P</i> -value <sup>2</sup>		
	CON	SCFP12	SCFP18	CON18		Linear	Quadratic	Timing
Liver glutathione <sup>4</sup> , $\mu$ M								
Day -20 <sup>5</sup>								
Total	1.54	1.91	1.69	1.86	—	—	—	—
Oxidized	0.17	0.22	0.19	0.21	—	—	—	—
Reduced	1.37	1.67	1.52	1.65	—	—	—	—
Ratio <sup>6</sup>	0.114	0.130	0.122	0.124	—	—	—	—
Day -3								
Total	2.14	2.26	1.96	1.97	0.103	0.27	0.06	0.92
Oxidized	0.27	0.28	0.23	0.24	0.017	0.11	0.06	0.54
Reduced	1.87	1.99	1.73	1.73	0.090	0.34	0.06	0.94
Ratio	0.143	0.139	0.131	0.138	0.006	0.07	0.65	0.29
Day 59								
Total	2.14	1.93	2.02	2.08	0.101	0.29	0.31	0.63
Oxidized	0.25	0.22	0.23	0.24	0.015	0.35	0.31	0.76
Reduced	1.89	1.72	1.78	1.84	0.088	0.28	0.34	0.59
Ratio	0.132	0.128	0.131	0.129	0.004	0.77	0.55	0.80

<sup>1</sup>*Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe, Diamond V, Cedar Rapids, IA) at 0 g·steer<sup>-1</sup>·d<sup>-1</sup> (CON), 12 g·steer<sup>-1</sup>·d<sup>-1</sup> (SCFP12), 18 g·steer<sup>-1</sup>·d<sup>-1</sup> (SCFP18), or 0 g·steer<sup>-1</sup>·d<sup>-1</sup> during preconditioning (days -19 to -1) then 18 g·steer<sup>-1</sup>·d<sup>-1</sup> during receiving (days 0 to 58; CON18).

<sup>2</sup>Linear and quadratic contrast statements compare CON, SCFP12, and SCFP18; Timing contrast statement compares SCFP18 vs. CON18.

<sup>3</sup>Highest SEM of any treatment reported.

<sup>4</sup>Glutathione concentrations reported as  $\mu$ M per gram of wet tissue.

<sup>5</sup>Values from days -20 (prior to treatment initiation) utilized as a covariate in analysis.

<sup>6</sup>Ratio calculated by dividing oxidized by reduced glutathione concentrations.

in the current study ( $P \geq 0.24$ ), liver Cu/Zn-SOD activity increased throughout the trial (Table 5) and liver Cu concentrations followed a similar trend (Table 6). Indeed, liver Cu/Zn-SOD activity and liver Cu were positively correlated ( $r = 0.51$ ,  $P \leq 0.01$ ) while liver concentrations of Zn and Mn were not correlated with their respective liver SOD isoforms ( $P \geq 0.22$ ). Similarly, Russell et al. (2016b) did not observe correlations between RBCL mineral-dependent antioxidant enzyme activities and liver mineral concentrations. This could be a result of RBCL and liver differing in both the magnitude and timing of an oxidative stress response or due to animals being adequate in trace minerals. Steers in the current study were adequate in Cu, Fe, Mn, and Zn (Kincaid, 2000).

Transit appears to affect oxidative stress biomarkers in various livestock species including cattle, horses, and sheep (Chirase et al., 2004; Onmaz et al., 2011; Piccione et al., 2013). Therefore, it was expected that the 19-h transit event in the current study would elicit changes in plasma MDA, a product of lipid peroxidation, and RBCL SOD activity. Regardless of treatment, plasma concentrations of MDA were greatest immediately prior

to and 1 d posttransit vs. 8 d posttransit (day  $P \leq 0.01$ ; Figure 1). In contrast, Chirase et al. (2004) observed a 3-fold increase in serum MDA concentrations of crossbred beef steers immediately after an approximately 20-h transit event vs. 3 d prior to transit. Blood was not collected immediately posttransit in the current study to avoid possible effects of decreased blood volume from dehydration on markers of oxidative stress. Therefore, it is possible that MDA concentrations had already returned to pretransit values when blood was collected 1 d posttransit.

Although RBCL total and Cu/Zn-SOD activity were decreased 1 d posttransit, Mn-SOD activity was increased (day  $P \leq 0.01$ ; Figure 2), resulting in a greater Mn:total-SOD activity ratio on day 1 (day  $P \leq 0.01$ ; Figure 3). Several studies have reported a decrease in SOD activity due to transportation of livestock (Onmaz et al., 2011; El-Deeb and El-Bahr, 2014; Polycarp et al., 2016), possibly due to increased ROS production and subsequent consumption of the enzyme for antioxidant reactions. However, these studies only reported total SOD activity rather than specific SOD isoforms. Expression of Mn-SOD mRNA has been shown to

**Table 5.** Effect of *Saccharomyces cerevisiae* fermentation product on liver superoxide dismutase (SOD) activity of beef steers

	Treatment <sup>1</sup>				SEM <sup>3</sup>	Contrast $P$ -value <sup>2</sup>		
	CON	SCFP12	SCFP18	CON18		Linear	Quadratic	Timing
Liver SOD activity <sup>4</sup>								
Day -20 <sup>5</sup>								
Total	215	212	221	180	—	—	—	—
Mn	121	123	137	108	—	—	—	—
Cu/Zn	94	89	85	72	—	—	—	—
Ratio <sup>6</sup>	0.56	0.60	0.62	0.62	—	—	—	—
Day -3								
Total	289	292	287	311	19.6	0.98	0.87	0.41
Mn	152	168	153	163	10.4	0.80	0.24	0.48
Cu/Zn	142	127	139	135	19.5	0.85	0.58	0.88
Ratio	0.52	0.58	0.55	0.56	0.043	0.51	0.48	0.86
Day 59								
Total	336	360	350	303	30.4	0.64	0.67	0.26
Mn	137	146	143	127	12.8	0.68	0.71	0.38
Cu/Zn	202	214	206	173	28.6	0.87	0.78	0.40
Ratio	0.43	0.41	0.43	0.43	0.046	0.92	0.76	0.90

<sup>1</sup>*Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe, Diamond V, Cedar Rapids, IA) at 0 g·steer<sup>-1</sup>·d<sup>-1</sup> (CON), 12 g·steer<sup>-1</sup>·d<sup>-1</sup> (SCFP12), 18 g·steer<sup>-1</sup>·d<sup>-1</sup> (SCFP18), or 0 g·steer<sup>-1</sup>·d<sup>-1</sup> during preconditioning (days -19 to -1) then 18 g·steer<sup>-1</sup>·d<sup>-1</sup> during receiving (days 0 to 58; CON18).

<sup>2</sup>Linear and quadratic contrast statements compare CON, SCFP12, and SCFP18; Timing contrast statement compares SCFP18 vs. CON18.

<sup>3</sup>Highest SEM of any treatment reported.

<sup>4</sup>SOD activity is reported as units (U)/mg protein where one U is defined as the amount of enzyme required to dismutate 50% of the superoxide radical.

<sup>5</sup>Values from day -20 (prior to treatment initiation) utilized as a covariate in analysis.

<sup>6</sup>Ratio calculated by dividing Mn-SOD activity by total SOD activity.

**Table 6.** Effect of *Saccharomyces cerevisiae* fermentation product on liver mineral concentrations of beef steers

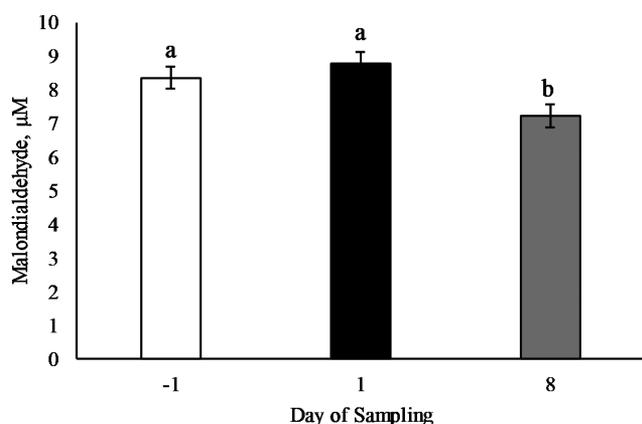
	Treatment <sup>1</sup>				SEM <sup>3</sup>	Contrast <i>P</i> -value <sup>2</sup>		
	CON	SCFP12	SCFP18	CON18		Linear	Quadratic	Timing
Liver mineral, mg/kg DM								
Day -20 <sup>4</sup>								
Cu	119	142	171	143	–	–	–	–
Fe	172	157	171	165	–	–	–	–
Mn	8.5	8.0	8.6	8.6	–	–	–	–
Zn	120	124	117	110	–	–	–	–
Day -3								
Cu	203	219	202	215	11.0	0.92	0.20	0.34
Fe	163	170	171	167	7.5	0.30	0.85	0.61
Mn	9.0	7.9	8.5	8.2	0.47	0.19	0.14	0.50
Zn	137	130	138	145	9.3	0.99	0.48	0.48
Day 59								
Cu	332	351	327	303	20.9	0.98	0.38	0.42
Fe	157	158	162	154	7.1	0.58	0.78	0.41
Mn	10.5	9.5	10.0	9.7	0.48	0.25	0.23	0.67
Zn	124	146	139	123	11.9	0.22	0.32	0.31

<sup>1</sup>*Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe, Diamond V, Cedar Rapids, IA) at 0 g·steer<sup>-1</sup>·d<sup>-1</sup> (CON), 12 g·steer<sup>-1</sup>·d<sup>-1</sup> (SCFP12), 18 g·steer<sup>-1</sup>·d<sup>-1</sup> (SCFP18), or 0 g·steer<sup>-1</sup>·d<sup>-1</sup> during preconditioning (days -19 to -1) then 18 g·steer<sup>-1</sup>·d<sup>-1</sup> during receiving (days 0 to 58; CON18).

<sup>2</sup>Linear and quadratic contrast statements compare CON, SCFP12, and SCFP18; Timing contrast statement compares SCFP18 vs. CON18.

<sup>3</sup>Highest SEM of any treatment reported.

<sup>4</sup>Values from day -20 (prior to treatment initiation) utilized as a covariate in analysis.



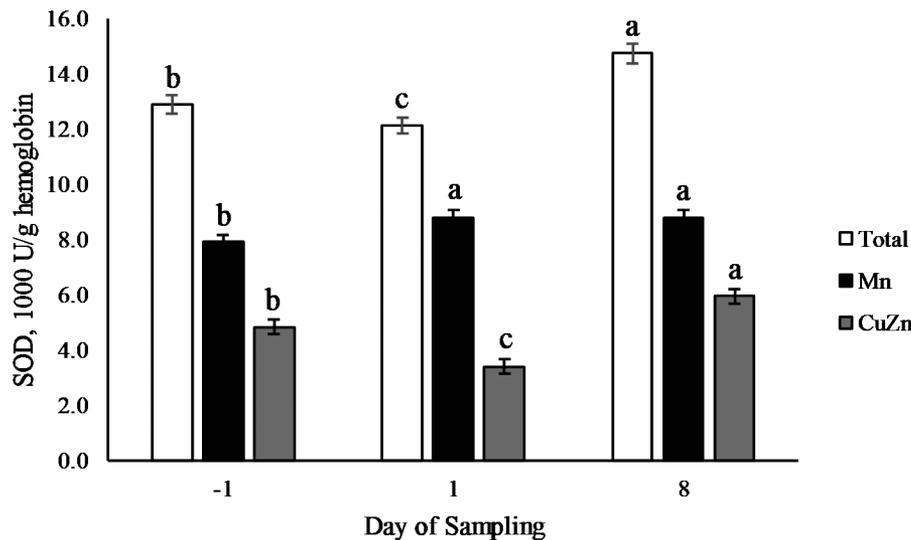
**Figure 1.** Effect of day on plasma malondialdehyde concentrations of beef steers in relation to a 19-h transit event; initial blood samples were collected immediately prior to transit on day -1. Based on repeated measures analysis, bars with unlike superscripts indicate a difference ( $P \leq 0.05$ ) between days (day  $P < 0.01$ ; treatment  $\times$  day  $P \geq 0.94$ ).

be induced by adrenocorticotrophic hormone (Chinn et al., 2002) which is secreted in response to a perceived psychological stressor and stimulates the release of cortisol, a potent glucocorticoid that acts on various tissues to increase cellular metabolism (Brockman and Laarveld, 1986). Although cortisol was not measured in the current study, it has been well established that transit increases concentrations of circulating cortisol in cattle (Crookshank

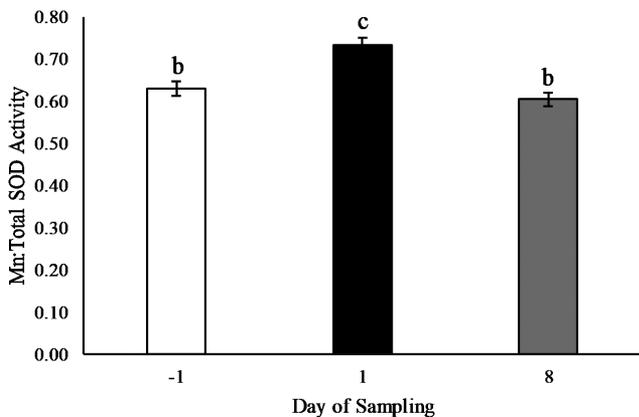
et al., 1979; Marques et al., 2012; Cooke et al., 2013). It is possible that increased secretion of adrenocorticotrophic hormone in response to transit stress resulted in upregulation of the Mn-SOD gene and contributed to the greater RBCL Mn-SOD activity observed 1 d posttransit, as a strong relationship exists between Mn-SOD gene expression, protein expression, and enzyme activity (Tiedge et al., 1997).

## IMPLICATIONS

In summary, supplementing SCFP at 12 g·steer<sup>-1</sup>·d<sup>-1</sup> was optimal compared with SCFP at 0 or 18 g·steer<sup>-1</sup>·d<sup>-1</sup> as evidenced by a tendency for greater antioxidant capacity prior to transit and improved performance early in the receiving period (days 0 to 30). Although no effects of supplementation timing were observed utilizing the dose of 18 g·steer<sup>-1</sup>·d<sup>-1</sup>, given the positive effects noted in steers receiving 12 g·steer<sup>-1</sup>·d<sup>-1</sup> throughout PRE and REC, further research should evaluate supplementation prior to entering the feedlot utilizing lesser doses. Additionally, activity of the powerful antioxidant enzyme Mn-SOD was increased posttransit and due to the detrimental effects of oxidative stress on animal health and performance, further



**Figure 2.** Effect of day on red blood cell lysate total, Mn, and Cu/Zn-superoxide dismutase (SOD) activity of beef steers in relation to a 19-h transit event; initial blood samples were collected immediately prior to transit on day -1. One unit of SOD activity (U) is defined as the enzyme required to dismutate 50% of the superoxide radical. Activity is reported as 1,000 U/g hemoglobin. Values for total and Mn-SOD were log transformed prior to statistical analysis; back-transformed means and SEM are presented. Within SOD type, bars with unlike superscripts indicate a difference ( $P \leq 0.05$ ) between days (day  $P < 0.01$ ; treatment  $\times$  day  $P \geq 0.51$ ).



**Figure 3.** Effect of day on red blood cell lysate ratio of Mn to total superoxide dismutase (SOD) activity of beef steers in relation to a 19-h transit event; initial blood samples were collected immediately prior to transit on day -1. Based on repeated measures analysis, bars with unlike superscripts indicate a difference ( $P \leq 0.05$ ) between days (day  $P < 0.01$ ; treatment  $\times$  day  $P = 0.71$ ).

investigation of the oxidative stress response in beef cattle posttransit is warranted.

## FUNDING

This study was partially supported by Diamond V, Cedar Rapids, IA.

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