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# Effects of feeding a *Saccharomyces cerevisiae* fermentation product and ractopamine hydrochloride to finishing beef steers on growth performance, immune system, and muscle gene expression

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## Abstract

The objective of this study was to determine impacts on immune parameters, anti-oxidant capacity, and growth of finishing steers fed a *Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe; Diamond V, Cedar Rapids, IA) and ractopamine hydrochloride (**RAC;** Optaflexx; Elanco Animal Health, Greenfield, IN). Angus-crossbred steers ($N = 288$) from two sources were utilized in this 90-d study. Steers were blocked by source, stratified by initial body weight to pens of six steers, and pens randomly assigned to treatments (16 pens per treatment). Three treatments compared feeding no supplemental SCFP (control; **CON**) and supplemental SCFP for 57 d (**SCFP57**), and 29 d (**SCFP29**) before harvest. Supplementation of SCFP was 12 g per steer per d, and all steers were fed RAC at 300 mg per steer per d for 29 d before harvest. Blood samples were collected from3 steers per pen, and muscle samples were collected from 1 steer per pen at 57, 29 (start of RAC), and 13 (midRAC) days before harvest. Blood was analyzed from 2 steers per pen for ferric reducing anti-oxidant power (**FRAP**). Muscle gene expression of myokines, markers of anti-oxidant and growth signaling were assessed. Individual animal BW were also collected on 57, 29, 13, and 1 d before being harvested at a commercial facility (National Beef, Tama, IA). Data were analyzed using the Mixed procedure of SAS 9.4 (Cary, NC) with pen as the experimental unit. The model included fixed effects of treatment and group. Increased BW compared to CON was observed days −29, −13, and −1 in SCFP57 steers ($P \leq 0.05$), with SCFP29 being intermediate days −13 and −1. Overall G:F was improved in SCFP29 and SCFP57 ($P = 0.01$). On day −29, FRAP was greater in SCFP57 than CON ($P = 0.02$). The percent of gamma delta T cells and natural killer cells in both SCFP29 and SCFP57 was greater than CON on day −13 ($P = 0.02$). There were no treatment × day effects for muscle gene expression measured ($P \geq 0.25$). Interleukin 6 tended to decrease in SCFP29 and SCFP57 on day −13 ($P = 0.10$). No other treatment effects were observed for muscle gene expression. Muscle gene expression of interleukin 15 was increased ($P = 0.01$), and expression of interleukin 8 was decreased ($P = 0.03$) due to RAC feeding. Increased growth in SCFP-fed cattle may be related to changes in anti-oxidant capacity and the immune system.

## Lay Summary

*Saccharomyces cerevisiae* fermentation products (SCFP) can provide additional support for improved growth performance. This study investigated the effects of supplementing a SCFP (NaturSafe; Diamond V, Cedar Rapids, IA; 12 g per steer per d) for 29 (SCFP29) or 57 (SCFP57) d before harvest when also feeding ractopamine hydrochloride (RAC; 300 mg per steer per d; Optaflexx, Elanco Animal Health, Greenfield, IN) for 29 d before harvest. Compared to steers not fed SCFP (CON), SCFP29 and SCFP57 had improved gain:feed for the entire feeding period. Steers supplemented with SCFP had increased percentages of gamma delta T cells and natural killer cells 13 d before harvest compared to CON. Gene expression of cytokine and anti-oxidant signaling in muscle were changed in all treatments during RAC compared to before RAC. Improvements in growth during RAC with SCFP supplementation may be due to the changes in anti-oxidant and cytokine signaling in muscle.

**Key words:** beef cattle, beta-agonist, feedlot, immune cell populations, *Saccharomyces cerevisiae* fermentation products

**Abbreviations:** ADG, average daily gain; BW, body weight; CXCR, chemokine C-X-C receptor; DMI, dry matter intake; FRAP, ferric reducing anti-oxidant power; HCW, hot carcass weight; IGF-1, insulin like growth factor-1; IL, interleukin; NRF2, NF-E2-related factor; RAC, ractopamine hydrochloride; ROS, reactive oxygen species; SCFP, *Saccharomyces cerevisiae* fermentation products; TMR, total mixed ration

## Introduction

*Saccharomyces cerevisiae* fermentation products (**SCFP**) can positively affect performance and health of feedlot cattle. A meta-analysis reported SCFP supplementation of finishing cattle increased body weight (**BW**) and increased average daily gain (**ADG**) by 6.4% (Wagner et al., 2016). Improved cattle performance in previous studies has been attributed to positive effects of SCFP on anti-oxidant capacity and inflammatory markers (Deters and Hansen, 2019; Shen et al., 2019). Immune system improvements have also been

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observed in SCFP supplemented cattle challenged with disease or infection (Mahmoud et al., 2020; Vailati-Riboni et al., 2021).

The beta-agonist ractopamine hydrochloride (**RAC**) is commonly included in beef cattle diets the last 28 to 42 d on feed (Samuelson et al., 2016) due to improvements in final BW, hot carcass weight (**HCW**), and feed efficiency (Boler et al., 2012; Bittner et al., 2017). Changes in inflammation and oxidative stress markers have been observed in animals exposed to beta-agonists. Increases in intestinal reactive oxygen species (**ROS**) have been observed after beta-agonist exposure in nematodes (Zhuang et al., 2014). Steers fed RAC (300 mg per steer per d) had increased serum interleukin-8 (**IL-8**) concentrations and leukocyte numbers (Genther-Schroeder et al., 2016). Cytokines, like IL-8, signal for immune cells to migrate to a site of a pathogen or injury (Zhang and An, 2009). Other tissues, such as muscle, can also produce these signals, termed myokines. Changes in myokine gene expression have been observed in RAC fed cattle (Messersmith et al., 2021) and may be affecting the growth of muscle with links to muscle metabolism in other species and in vitro (Stanley Chan et al., 2004; Serrano et al., 2008).

Therefore, SCFP may have the potential to further enhance performance of RAC-fed cattle through support of anti-oxidant capacity, immune response, and nutrient availability. A 90-d study was conducted to determine the effects of feeding SCFP leading up to and during the RAC period, on feedlot performance, immune status, and muscle gene expression of beef steers. The hypothesis was inclusion of SCFP in diets of finishing steers would improve growth through modulation of the inflammatory and oxidative stress response induced by RAC.

## Materials and Methods

### Animals and experimental design

The Iowa State University Institutional Animal Care and Use Committee approved this study's procedures and protocols (IACUC # 20-115). Two hundred eighty-eight Angus-cross steers from two sources (group 1 BW = 423 ± 6.4 kg; group 2 BW = 442 ± 6.4 kg) were used in a 90-d finishing study in the fall of 2020. Upon arrival to the Iowa State University Beef Nutrition Farm (Ames, IA), steers were fed a silage-based receiving ration, and transitioned to a common finishing diet. Steers were blocked by source (group), and the experiment start was staggered by 2 wk between the groups to accommodate for sample collection logistics. Days on SCFP treatment were identical between the two groups and only differed in the calendar start date (Table 1). There were 16 pens per dietary treatment in this experiment (group 1 = 6 pens per treatment; group 2 = 10 pens per treatment).

Steers were weighed on two consecutive days approximately 90 d prior to harvest and stratified by initial BW to pens (3.7 × 12.2 m; 6 steers per pen; 16 pens per treatment). Pens were randomly assigned to one of three treatments: control (**CON**), which received no SCFP, **SCFP57** receiving SCFP (NaturSafe; Diamond V, Cedar Rapids, IA) starting 57 d prior to harvest, and **SCFP29** receiving SCFP starting 29 d prior to harvest. Supplementation of SCFP was targeted at 12 g per steer daily. Inclusions of SCFP were 11.9 and 12.8 g per steer daily for groups 1 and 2, respectively, when calculated using feed delivery and premix inclusion percentage. All steers were implanted with Component TE-200 with Tylan (donated by Elanco Animal Health, Greenfield, IN) on day 0, containing 200 mg trenbolone acetate and 20 mg estradiol USP. All treatments were fed RAC (Optaflexx, donated by Elanco Animal Health, Greenfield, IN) at 300 mg per steer daily starting 29 d before harvest. Individual, single-day BW were collected 57, 29, 13, and 1 d prior to harvest. All BW had a 4% pencil shrink applied to account for potential gut fill. Steers were restricted from feed before BW and sample collection and pens were weighed in the same order each sampling day.

**Table 1.** Experimental sampling schedule

| Activity | Days prior to harvest |
|---|---|
| Group 1: BW | 90 |
| Group 1: BW, Implant, start finishing diet | 89 |
| Group 2: BW | 92 |
| Group 2: BW, Implant, start finishing diet | 91 |
| BW, blood and SCFP57 starts SCFP[1] | 57 |
| BW, blood, muscle biopsy, SCFP29 starts SCFP[1], RAC[2] starts for all | 29 |
| BW, blood, muscle | 13 |
| BW and ship to abattoir | 1 |

[1]NaturSafe; Diamond V, Cedar Rapids, IA.
[2]Ractopamine Hydrochloride (Optaflexx, Elanco Animal Health, Greenfield, IN).

Steers were fed once daily via a wagon mixer and had ad libitum access to water. Dietary treatments were delivered as part of total mixed ration (**TMR**) using dry distillers' grain as a premix carrier. Feed bunks were scored each morning and targeted such that all the feed was consumed when the next meal was delivered. Feed deliveries were recorded daily. Pen intakes were monitored weekly, and SCFP and RAC inclusion adjustments were made as needed to meet target intakes. Total feed weigh-back and sampling occurred when there were diet changes, weigh dates, or musty feed. Weekly TMR samples were collected. All TMR and discard samples were frozen until dried in a forced-air oven at 70 °C for 48 h. Composites of ground TMR samples were analyzed at a commercial lab (Dairyland INC, Arcadia, WI) for nutrient analysis (methods 990.03 and 920.39; AOAC, 1995). Dry matter intake (**DMI**) was calculated using weekly TMR sample DM values, daily feed delivery, and bunk discards. Gain to feed ratios were calculated by dividing the ADG by the calculated DMI. The diet composition and analysis can be found in Table 2.

All steers were visually assessed for signs of illness at least once daily. Trained personnel treated steers with visual symptoms of illness and a temperature of 39.8 °C or greater with LA 200 or Draxxin (Zoetis, Parsippany-Troy Hills, NJ), depending on illness symptoms and treatment protocols. Steers were harvested at a commercial facility (National Beef, Tama, IA), and carcass data were collected after being chilled for 48 h. Yield grade was calculated using the equation:

$$YG = 2.5 + (2.5 \times BF) + (0.2 \times KPH) + (0.0038 \times HCW) - (0.32 \times REA).$$

Carcass adjusted performance was calculated using the average dressing percentage for the respective treatment and group.

**Table 2.** Composition of diets fed

| %DM | 69.4 |
|---|---|
| Ingredient, % DM basis | |
| Corn silage | 15 |
| Sweet Bran[1] | 22 |
| Corn | 48 |
| Dried distillers grains[2] | 13.05 |
| Limestone | 1.5 |
| Vitamin A and E premix[3] | 0.1 |
| Salt | 0.31 |
| Rumensin[4] | 0.0135 |
| Trace mineral premix[5] | 0.0244 |
| Analyzed composition[6], % DM | |
| Crude protein | 14.03 |
| Neutral detergent fiber | 19.43 |
| Ether extract | 4.36 |

[1]Branded wet corn gluten feed (Cargill Corn Milling, Blair, NE).
[2]Carrier for micro-ingredients and SCFP (NaturSafe; Diamond V, Cedar Rapids IA) to treatment pens and ractopamine hydrochloride (Optaflexx, Elanco Animal Health, Greenfield, IN) during RAC period.
[3]Premix provided 2,200 IU vitamin A and 25 IU vitamin E per kg diet DM.
[4]Provided monensin (Elanco Animal Health, Greenfield, IN) at 27 g/ton.
[5]Provided per kilogram of diet DM: 0.15 mg of Co, 10 mg of Cu, 20 mg of Mn, 0.1 mg of Se, 30 mg of Zn, and 0.5 mg of I.
[6]Based on analysis of TMR from Dairyland Laboratories, Inc., Arcadia, WI.

## Sample collection procedures

Blood was collected ($N$ = 3 steers per pen) via jugular venipuncture 57, 29, and 13 d before harvest in tubes containing heparin or no coagulant (Greiner Bio-One, Kremsmünster, Austria; Becton, Dickinson and Company, Franklin Lakes, NJ). Blood was centrifuged at 1,200 × *g* for 20 min at 4 °C. Blood was aliquoted and stored at −80 °C. Thirteen days before harvest, fresh blood samples were collected in 3-mL K2EDTA vacutainers (Greiner Bio-One) for complete blood count analysis at the Iowa State University Veterinary Pathology Lab (Ames, IA). Flow cytometry measures for B and T cell types were analyzed as in Mahmoud et al. (2020) from blood collected in heparin tubes 13 d prior to harvest.

Muscle biopsies were collected from 1 steer per pen on 28 and 13 d prior to harvest. The same steer was sampled at each time point, and samples were collected from opposite sides to avoid damage from repeated sampling. Muscle biopsies were collected using the methods described by Pampusch et al. (2008) from the *longissimus thoracis* muslce, flash-frozen in liquid nitrogen, and stored at −80 °C until analysis.

## Sample analysis

Blood samples from 2 steers per pen were analyzed for plasma anti-oxidant capacity and serum IL-8. A ferric reducing anti-oxidant power (**FRAP**) detection kit was used to measure the anti-oxidant status of the plasma (K043-H1; Arbor Assays, Ann Arbor, MI). The intra-assay coefficient of variation was 6.56% using a supplied ascorbic acid control. In serum samples, IL-8 was measured using the Human IL-8/CXCL8 Quantine ELISA kit (D8000C; R&D Systems, Minneapolis, MN). The intra- and inter-assay coefficient of variations were 17.6% and 10.75%, respectively. Plasma samples were also analyzed for Cu, Zn, and Fe concentrations using inductively coupled plasma optimal emission spectrometry as in Genther-Schroeder et al. (2016).

Muscle samples were ground using a mortar and pestle in liquid nitrogen. To isolate RNA, 50 to 100 mg of muscle tissue was homogenized with 1 mL of TRIzol reagent (Invitrogen, Waltham, MA). After incubating for 5 min at room temperature, 0.2 mL of chloroform were added, and samples were vortexed. Samples were incubated at room temperature for 3 min; and then centrifuged for 15 min at 12,000 × *g* to separate into a phenol-chloroform phase, interphase, and colorless aqueous phase containing the RNA. The aqueous phase was transferred into a new microcentrifuge tube and incubated with 0.5 mL of isopropanol for 10 min at 4 °C. The sample was centrifuged for 10 min at 12,000 × *g*. The supernatant was removed, leaving a pellet of RNA. A DNAse kit (Qiagen, Hilden, Germany) was used. The pellet was then resuspended in 1 mL of 75% ethanol, vortexed briefly, and centrifuged for 5 min at 7,500 × *g*. The supernatant was removed, and the RNA pellet was air-dried for 5 to 10 min. Using 20 µL of RNAse-free H2O, the RNA pellet was resuspended and incubated in a water bath at 55 to 60 ° C for 10 to 15 min. The quantity and quality of RNA were measured on a Cytation5 (BioTek, Winooski, VT) with a Take3Trio (BioTek) using a nucleic acid quantification protocol.

Isolated RNA samples were stored at −80 °C for no more than 1 wk before cDNA synthesis. If stored longer, RNA quantity and quality were re-evaluated before cDNA synthesis. For cDNA synthesis, 500 ng of RNA per sample was synthesized using random primers and Superscript III Reverse Transcriptase (Invitrogen, Waltham, MA; Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Briefly, 500 ng of RNA was added to 500 ng of random primers and 10mM of dNTP mix. This was incubated for 5 min at 70 °C, spun briefly then placed in ice prior to adding 4 µL of 5x first-strand buffer, 2 µL of 0.1 DTT, 1 µL of SuperScript III, and 1 µL of RNAse out. The samples were then incubated for 5 min at room temperature before being incubated for 1 h in a 50 °C water bath. The reaction was stopped by incubating on a heat block at 80 °C for 5 min. The cDNA product were stored at 20 °C until quantitative real-time polymerase chain reaction (qPCR) analysis. Quantitative real-time PCR was performed as described in McGill et al. (2016) using ribosomal protein S9 (RPS9) as a reference gene. Primers utilized for qPCR can be found in Table 3. Relative gene expression was determined by using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) and calculations of mRNA relative expression were conducted relative to CON day −29.

## Statistical analysis

Performance and carcass data were analyzed using the Mixed procedure of SAS 9.4 (Cary, North Carolina) with pen as the experimental unit. The model included fixed effects of treatment and group. Bodyweight collected at the start of SCFP57 supplementation (57 d before harvest) was used as a covariate in the analysis of subsequent BW and HCW. Treatment by day effects were tested and were not significant, so simple effects of treatment within day and day are reported. Outliers were evaluated on an individual animal basis. For BW, ADG, carcass characteristics, blood measures, and gene expression, data points for an individual were removed if more than three standard deviations from the treatment's mean.

**Table 3.** Forward and reverse primers used for quantitative real-time PCR

| Gene | Accession number | Strand | Sequence (5′-3′) |
|---|---|---|---|
| IL-8[1] | EU276073.1 | Forward | CGCTGGACAGCAGAGCTCACAAG |
| | | Reverse | GCCAAGAGAGCAACAGCCAGCT |
| CXCR1[2] | EF597244.2 | Forward | GTCCCCGTGAGATAAGCAC |
| | | Reverse | CAGGTTCAGCAGGTAGACA |
| CXCR2[3] | DQ328664.1 | Forward | CAACACTGACCTGCCCTCTA |
| | | Reverse | CAGGTTCAGCAGGTAGACA |
| IL-6[4] | NM_173921.2 | Forward | CTGAAGCAAAAGATCGCAGATCTA |
| | | Reverse | CTCGTTTGAAGACTGCATCTC |
| IL-15[5] | U42433.1 | Forward | TTTGAGAAGTACTTCCATCCAG |
| | | Reverse | GAAGTGTTGATGAACATTTGCAC |
| NRF2[6] | NM_001011678 | Forward | CCCAGTCTTCACTGCTCCTC |
| | | Reverse | TCAGCCAGCTTGTCATTTTG |
| IGF1[7] | NM_001077828.1 | Forward | TCGCATCTCTTCTATCTGGCCCTGT |
| | | Reverse | GCAGTACATCTCCAGCCTCCTCAGA |
| RPS9[8] | NM_001101152.1 | Forward | CGCCTCGACCAAGAGCTGAAG |
| | | Reverse | CCTCCAGACCTCACGTTTGTTCC |

[1]Interleukin-8.
[2]Chemokine C-X-C receptor 1.
[3]Chemokine C-X-C receptor 2.
[4]Interleukin-6.
[5]Interleukin-15.
[6] NF-E2-related factor 2.
[7]Insulin-like growth factor 1.
[8] Ribosomal protein S9.

Four steers were removed during the study due to illness not related to treatment ($N$ = 3 from SCFP29; $N$ = 1 from SCFP57). For pens in which steers were removed, DMI and G:F for the period was not used in the analysis. The least-squares means and SEM are reported. Statistical significance was determined at $P \leq 0.05$, and a statistical tendency was determined at $0.05 < P \leq 0.1$.

## Results

### Performance and carcass characteristics

Increased BW, ADG, and G:F were observed in SCFP57 29 d before harvest ($P$ = 0.01; Table 4) compared to CON and SCFP29. Increased BW for SCFP57 compared to CON was also observed at the midRAC (day −13) and final live BW (day −1) time points, with SCFP29 being intermediate ($P \leq 0.03$). Despite no difference between treatments for ADG during the RAC period (day −29 to −1; $P$ = 0.93), ADG was increased in SCFP57 for the overall 57-d treatment period compared to both SCFP29 and CON ($P$ = 0.04). Treatment did not affect DMI 57 to 29 d before harvest ($P$ = 0.49). There was a tendency for DMI to be greater in CON vs. SCFP29 during the RAC period ($P$ = 0.06) and overall ($P$ = 0.07), with SCFP57 being intermediate. Overall feed efficiency was greater for SCFP29 or SCFP57 compared to CON ($P$ = 0.01). Carcass adjusted G:F tended to be greater in SCFP29 compared to CON, with SCFP57 being intermediate ($P$ = 0.07; Table 4). Carcass adjusted final BW and ADG were not different based on treatment ($P \geq 0.19$).

Although the mean marbling score for all treatments were within the same quality grade of choice, CON had a greater marbling score than SCFP29; SCFP57 was intermediate ($P$ = 0.01; Table 5). There were no differences due to treatment for any other carcass characteristics ($P \geq 0.31$).

### Immune, anti-oxidant, and plasma trace mineral response

There were no treatment × day or day effects for plasma FRAP concentrations ($P \geq 0.44$). After 28 d of SCFP supplementation, FRAP concentrations were greater ($P$ = 0.02; Table 6) for SCFP57 than CON, with SCFP29 being intermediate though SCFP treatment had not yet begun for SCFP29. There were no treatment differences in FRAP concentrations 57 or 13 d before harvest ($P \geq 0.24$). There were no treatment × day or treatment effects for serum IL-8 ($P \geq 0.25$). However, there was an effect of day where serum IL-8 concentrations were increased prior to RAC (day −29) in comparison to midRAC (day −13; $P$ = 0.01; Table 7). There were no treatment × day or treatment effects for plasma Cu, Fe, or Zn concentrations ($P \geq 0.11$). There was, however, a day effect where plasma Cu was increased on day −13 compared to day −29 ($P$ = 0.02; Table 7). Plasma Fe and Zn did not differ by day ($P \geq 0.55$).

There was no difference in the frequency of total gamma delta T cells between treatments ($P$ = 0.50). In SCFP supplemented groups, however, activated gamma delta T cells (CD45RO pos GD) were increased compared to CON at the midRAC timepoint ($P$ = 0.02; Table 8). Steers receiving SCFP (SCFP29 or SCFP57) had increased frequencies of total natural killer (NK) cells ($P$ = 0.02) but no difference in the percentage of activated natural killer cells (CD45RO pos NK) compared to CON ($P$ = 0.41). Bright, dim and negative populations of CD5 T cells tended to differ where SCFP57 had greater populations of bright and negative, and fewer dim CD5 T cells in comparison to CON ($P \leq 0.08$). Other immune

**Table 4.** The effects of supplementation with *Saccharomyces cerevisiae* fermentation product (SCFP) on performance measures in finishing feedlot steers

| Days relative to harvest | Treatments[1] | | | SEM[2] | *P*-value |
|---|---|---|---|---|---|
| | CON | SCFP29 | SCFP57 | | |
| Pens | 16 | 16 | 16 | | |
| Body weight[3], kg | | | | | |
| Day −57 | 520 | 516 | 517 | 1.56 | 0.19 |
| Day −29[4] | 576[b] | 578[b] | 583[a] | 1.21 | 0.01 |
| Day −13[4] | 616[b] | 619[a,b] | 622[a] | 1.72 | 0.02 |
| Day −1[4] | 641[b] | 645[a,b] | 649[a] | 2.04 | 0.03 |
| Average daily gain, kg | | | | | |
| Days −57 to −29 | 1.95[b] | 2.02[b] | 2.2[a] | 0.042 | 0.01 |
| Days −29 to −1 | 2.32 | 2.35 | 2.33 | 0.057 | 0.93 |
| Days −57 to −1 | 2.14[b] | 2.19[b] | 2.27[a] | 0.037 | 0.04 |
| Dry matter intake, kg | | | | | |
| Days −57 to −29 | 13.16 | 12.96 | 13.16 | 0.143 | 0.49 |
| Days −29 to −1 | 12.70[x] | 12.56[y] | 12.61[x,y] | 0.138 | 0.06 |
| Days −57 to −1 | 12.93[x] | 12.56[y] | 12.88[x,y] | 0.123 | 0.07 |
| Gain:feed | | | | | |
| Days −57 to −29 | 0.148[c] | 0.158[b] | 0.167[a] | 0.0032 | 0.01 |
| Days −29 to −1 | 0.182 | 0.192 | 0.183 | 0.0038 | 0.16 |
| Days −57 to −1 | 0.165[b] | 0.173[a] | 0.175[a] | 0.0023 | 0.01 |
| CA performance[5] | | | | | |
| Final body weight, kg | 639 | 638 | 644 | 3.29 | 0.33 |
| Average daily gain, kg | 2.1 | 2.15 | 2.23 | 0.051 | 0.19 |
| Gain:feed | 0.163[y] | 0.174[x] | 0.173[x,y] | 0.0034 | 0.07 |

[1]CON = No SCFP supplementation; SCFP29= SCFP (NaturSafe; Diamond V, Cedar Rapids, IA) at 12 g per steer per d 29 d prior to harvest; SCFP57 = SCFP at 12 g per steer per d 57 d prior to harvest; all treatments received ractopamine hydrochloride for final 29 d at 300 mg per steer per d.
[2]Highest SEM of any treatment reported.
[3]Body weight with 4% pencil shrink applied.
[4]Day 57 body weight covariant used.
[5]Carcass adjusted (CA) performance was calculated using the average dressing percent for the group and treatment Group 1 CON: 63.2%, NS29: 62.3, NS57: 63.0%; Group 2 CON: 62.9%, NS29: 63.3%, NS57: 62.5.
[a,b] Within a row, treatment means with different superscripts differ $P \leq 0.05$.
[x,y] Within a row, treatment means with different superscripts differ $0.1 \geq P > 0.05$.

cell phenotypic measures were not different due to treatment ($P \geq 0.18$).

Greater hematocrit for SCFP29 was observed on day −13 ($P = 0.04$; Table 9) compared to CON or SCFP57 ($P = 0.04$). In addition, hemoglobin tended to be greater for SCFP29 compared to CON ($P = 0.07$), with SCFP57 being intermediate. No other complete blood count measures were affected by treatment ($P \geq 0.15$).

## Relative gene expression

Relative gene expression of markers of growth, inflammation, cytokines, and receptors was assessed in the muscle of steers before (day −29) and during (day −13) RAC feeding. There were no treatment × day effects for any of the genes measured ($P \geq 0.25$); therefore, data were analyzed for day effects (Figure 1) and treatment effects within day (Figures 2 and 3). After 16 d of RAC supplementation, insulin-like growth factor 1 (**IGF-1;** Figure 1D) and interleukin 15 (**IL-15;** Figure 1E) expression was increased ($P \leq 0.02$). NF-E2-related factor (**NRF2**) expression tended to increase after 16 d of RAC (Figure 1G; $P = 0.09$). However, interleukin 8 (**IL-8**) expression was decreased (Figure 1A; $P = 0.03$) and the IL-8 receptor

**Table 5.** Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) supplementation on the carcass characteristics of steers

| Pens | Treatments[1] | | | SEM[2] | *P*-value |
|---|---|---|---|---|---|
| | CON | SCFP29 | SCFP57 | | |
| | 16 | 16 | 16 | | |
| Hot carcass wieght[3], kg | 404 | 407 | 407 | 1.6 | 0.37 |
| Marbling[4] | 483[a] | 433[b] | 453[ab] | 11 | 0.01 |
| Backfat, cm | 1.37 | 1.29 | 1.33 | 0.04 | 0.41 |
| Kidney, pelvic, and heart fat, % | 2.0 | 1.9 | 2.0 | 0.03 | 0.31 |
| Ribeye area, cm$^2$ | 87.9 | 89.1 | 88.8 | 0.81 | 0.51 |
| Yield grade | 3.26 | 3.16 | 3.19 | 0.064 | 0.52 |
| Dress, % | 63.0 | 63.0 | 62.7 | 0.21 | 0.59 |

[1]CON = No SCFP supplementation; SCFP29 = SCFP(NaturSafe; Diamond V, Cedar Rapids, IA) at 12 g per steer per d 29 d prior to harvest; SCFP57 = SCFP at 12 g per steer per d 57 d prior to harvest; all treatments received ractopamine hydrochloride for final 29 d at 300 mg per steer per d.
[2]Highest SEM of any treatment reported.
[3]Day −57 body weight covariant applied.
[4]Marbling scores: slight = 300, small = 400, modest = 500, moderate = 600.
[a,b]Within a row, treatment means with different superscripts differ $P \leq 0.05$.

**Table 6.** Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) supplementation on plasma and serum analytes in beef steers

| Days relative to harvest | Treatments[1] | | | SEM[2] | *P*-value |
|---|---|---|---|---|---|
| | CON | SCFP29 | SCFP57 | | |
| Pens | 16 | 16 | 16 | | |
| Plasma FRAP [3,4], µM | | | | | |
| Day −57 | 357.0 | 343.3 | 343.7 | 11.8 | 0.64 |
| Day −29[5] | 340.7[b] | 360.0[a,b] | 373.3[a] | 8.8 | 0.02 |
| Day −13[5] | 358.8 | 354.4 | 371.5 | 4.5 | 0.24 |
| Serum interleukin-8[3], pg/mL | | | | | |
| Day −29 | 131.4 | 147.7 | 78.0 | 43.0 | 0.25 |
| Day −13 | 52.2 | 44.3 | 40.6 | 13.5 | 0.79 |
| Plasma trace mineral[6], mg/L | | | | | |
| Cu, Day −29 | 0.86 | 0.90 | 0.89 | 0.039 | 0.74 |
| Fe, Day −29 | 2.13 | 2.25 | 2.42 | 0.141 | 0.35 |
| Zn, Day −29 | 1.40 | 1.34 | 1.30 | 0.057 | 0.61 |
| Cu, Day −13 | 0.98 | 0.97 | 0.93 | 0.047 | 0.71 |
| Fe, Day −13 | 2.22 | 2.11 | 2.49 | 0.135 | 0.11 |
| Zn, Day −13 | 1.41 | 1.32 | 1.30 | 0.057 | 0.34 |

[1]CON = No SCFP supplementation; SCFP29 = SCFP (NaturSafe; Diamond V, Cedar Rapids, IA) at 12 g per steer per d 29 d prior to harvest; SCFP57 = SCFP at 12 g per steer per d 57 d prior to harvest; all treatments received ractopamine hydrochloride for final 29 d at 300 mg per steer per d.
[2]Highest SEM of any treatment.
[3] Two steers per pen were analyzed.
[4]FRAP = ferric reducing ability of plasma.
[5] Day −57 FRAP used as covariate in analysis.
[6] 1e steer per pen was analyzed.
[a,b]Within a row, means with unlike superscripts differ $P \leq 0.05$.

**Table 7.** Effects of day on serum and plasma analytes in beef steers

| | Days relative to harvest[1] | | SEM[2] | *P*-value |
|---|---|---|---|---|
| | Day −29 | Day −13 | | |
| Serum interleukin-8[3], pg/mL | 105[a] | 45.1[b] | 17.17 | 0.01 |
| Plasma Cu[4], mg/L | 0.90[b] | 0.95[a] | 0.026 | 0.02 |
| Plasma Fe[4], mg/L | 2.25 | 2.31 | 0.081 | 0.54 |
| Plasma Zn[4], mg/L | 1.38 | 1.36 | 0.036 | 0.60 |

[1]All steers received ractopamine hydrochloride for final 29 d at 300 mg per steer per d
[2]Highest SEM of days relative to harvest.
[3]Pen means base on analysis from 96 steers (2 steers per pen).
[4]Pen means based on analysis from 48 steers (1 steer per pen).
[a,b]Within a row, means with unlike superscripts differ $P \leq 0.05$.

**Table 8.** Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) supplementation on immune cell populations of beef steers 13 d prior to harvest (16 d after the start of ractopamine hydrochloride supplementation)

| Pens[3] | Treatment[1] | | | SEM[2] | *P*-Value |
|---|---|---|---|---|---|
| | CON | SCFP29 | SCFP57 | | |
| | 16 | 16 | 16 | | |
| % CD4 T cell | 29.8 | 30.5 | 29.0 | 0.756 | 0.38 |
| % CD45RO pos CD4 | 51.1 | 50.1 | 54.2 | 1.837 | 0.25 |
| % CD8 T cell | 11.3 | 11.0 | 10.2 | 0.746 | 0.57 |
| % CD45RO pos CD8 | 15.7 | 14.7 | 14.5 | 1.135 | 0.73 |
| % GD[4] T cell | 12.5 | 12.8 | 11.6 | 0.722 | 0.50 |
| % CD45RO pos GD[4] | 78.8[b] | 83.7[a] | 85.0[a] | 1.569 | 0.02 |
| % NK[4] cells | 3.2[b] | 3.9[a] | 4.0[a] | 0.225 | 0.02 |
| % CD45RO pos NK[4] | 46.2 | 47.4 | 50.7 | 2.491 | 0.41 |
| % B cells of lymphocytes | 26.8 | 25.1 | 23.5 | 0.129 | 0.18 |
| CD5 bright[5] | 60.2[x] | 58.5[x,y] | 56.0[y] | 1.257 | 0.07 |
| CD5 dim[5] | 30.6[y] | 31.4[y] | 33.8[x] | 0.994 | 0.08 |
| CD5 negative[5] | 4.5[b] | 5.5[a,b] | 5.6[a] | 0.309 | 0.04 |

[1]CON = No SCFP supplementation; SCFP29 = SCFP (NaturSafe; Diamond V, Cedar Rapids, IA) at 12 g per steer per d 29 d prior to harvest; SCFP57 = SCFP at 12 g per steer per d 57 d prior to harvest; all treatments received ractopamine hydrochloride for final 29 d at 300 mg per steer per d.
[2]Highest SEM of any treatment.
[3]Analyzed in 3 steers per pen.
[4]GD = gamma delta; NK = natural killer.
[5]Only measured in group 2.
[a,b]Within a row, means with unlike superscripts differ $P \leq 0.05$.
[x,y]Within a row, means with unlike superscripts differ $0.1 \geq P > 0.05$.

chemokine C-X-C receptor 2 (**CXCR2**) expression tended to be decreased (Figure 1C; $P = 0.07$) relative to preRAC. Treatment did not affect preRAC (day −29) expression of genes measured in this study (Figure 2; $P \geq 0.38$). However, after 16 d of RAC supplementation, interleukin-6 (**IL-6**) gene expression tended to be decreased in SCFP supplemented steers compared to CON (Figure 2E; $P = 0.10$). There were no other effects of treatment on the muscle gene expression measured 13 d before harvest ($P \geq 0.22$).

## Discussion

Ractopamine is commonly fed to improve efficiency and muscle gain in late stage finishing cattle. Since SCFP has been shown to provide anti-oxidant and immune support (Jensen et al., 2008; Deters and Hansen, 2019), this experiment assessed the impact of SCFP supplementation before and during ractopamine supplementation of finishing beef steers.

In the current study, supplementing late-stage finishing steers with SCFP improved performance. Supplementing SCFP prior to the RAC-period improved preRAC ADG but had minimal impact on ADG during the RAC feeding period. Cattle receiving SCFP for any length of time had better feed efficiency overall, suggesting this additive may be a useful tool for optimizing feed resources. The effects of SCFP on cattle growth in individual studies have been inconsistent (Hinman et al., 1998; Geng et al., 2016; Shen et al., 2019). Other studies have observed increased growth with SCFP, but no differences in carcass characteristics (Swyers et al., 2014), similar to the current study. A meta-analysis reported increased ADG in cattle supplemented SCFP in the finishing period, but also no differences in HCW on average (Wagner et al., 2016).

**Table 9.** Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) supplementation on the complete blood count of steers13 d prior to harvest (16 d after the start of ractopamine hydrochloride supplementation)

| Pens[3] | Treatment[1] | | | SEM[2] | *P*-value |
|---|---|---|---|---|---|
| | CON | SCFP29 | SCFP57 | | |
| | 16 | 16 | 16 | | |
| White Blood cells, $\times 10^3/\mu L$ | 9.6 | 9.1 | 9.7 | 0.29 | 0.38 |
| Red Blood cells, $\times 10^6/\mu L$ | 8.5 | 8.5 | 8.5 | 0.13 | 0.97 |
| Hemoglobin, g/dL | 13.7[y] | 14.2[x] | 13.9[x,y] | 0.16 | 0.07 |
| Hematocrit, % | 37.5[b] | 38.9[a] | 37.7[b] | 0.41 | 0.04 |
| MVC, fl | 44.7 | 45.6 | 44.9 | 0.54 | 0.48 |
| MCH, pg | 16.3 | 16.7 | 16.5 | 0.20 | 0.53 |
| MCHC, g/dL | 36.6 | 36.5 | 36.7 | 0.11 | 0.28 |
| RDW, % | 20.1 | 19.9 | 19.9 | 0.21 | 0.79 |
| Platelet, $\times 10^3/\mu L$ | 333.8 | 298.0 | 307.9 | 13.55 | 0.15 |
| MPV, fl | 7.3 | 7.6 | 7.5 | 0.32 | 0.77 |
| Neutrophil, $\times 10^3/\mu L$ | 2.9 | 2.8 | 2.9 | 0.12 | 0.55 |
| Lymphocyte, $\times 10^3/\mu L$ | 5.6 | 5.2 | 5.5 | 0.17 | 0.25 |
| Monocyte, $\times 10^3/\mu L$ | 0.52 | 0.53 | 0.52 | 0.019 | 0.79 |
| Eosinophil, $\times 10^3/\mu L$ | 0.45 | 0.38 | 0.38 | 0.045 | 0.43 |
| Basophils, $\times 10^3/\mu L$ | 0.11 | 0.10 | 0.11 | 0.005 | 0.59 |
| Absolute AUC, $\times 10^3/\mu L$ | 0.05 | 0.06 | 0.05 | 0.0032 | 0.79 |
| Neutrophil: lymphocyte | 0.54 | 0.55 | 0.54 | 0.024 | 0.97 |

[1]CON = No SCFP supplementation; SCFP29 = SCFP (NaturSafe; Diamond V, Cedar Rapids, IA) at 12 g per steer per d 29 d prior to harvest; SCFP29 = SCFP at 12 g per steer per d 57 d prior to harvest; all treatments received ractopamine hydrochloride for final 29 d at 300 mg per steer per d.
[2]Highest SEM of any treatment reported.
[3]Analyzed in 3 steers per pen.
[a,b]Within a row, means with unlike superscripts differ $P \leq 0.05$.
[x,y]Within a row, means with unlike superscripts differ $0.1 \geq P > 0.05$.

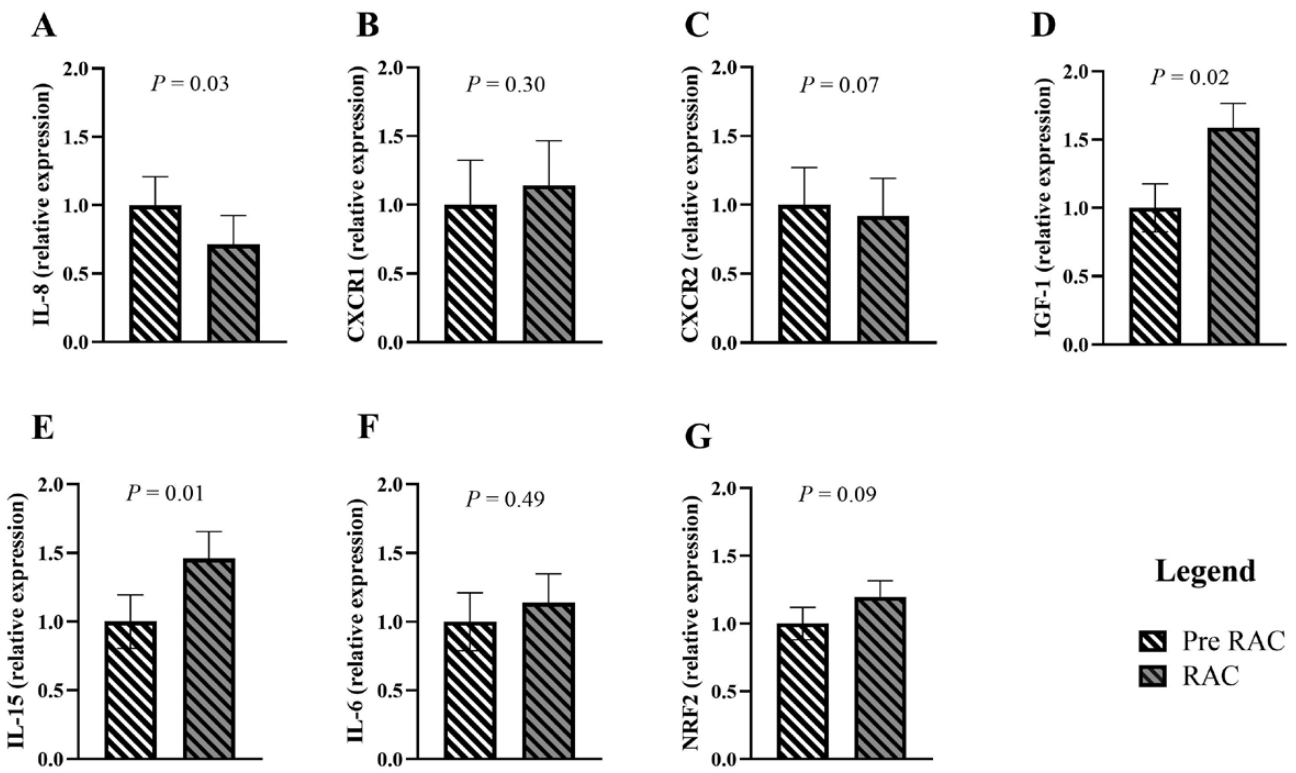


**Figure 1.** Relative gene expression (quantitative real-time PCR) of markers of growth, inflammation, cytokines, and receptors in the muscle of steers before and during ractopamine supplementation (300 mg per steer per d). Muscle samples (*longissimus thoracis*) were collected 29 (preRAC) and 13 (RAC) d before harvest. Gene expression was calculated relative to 29 d before harvest. Interleukin-8 (IL-8) gene expression (A) decreased with RAC ($P$ = 0.03). Chemokine C-X-C motif receptor 1 (CXCR1) gene expression (B) did not change with RAC. Chemokine C-X-C motif receptor 2 (CXCR2) gene expression (C) tended to decrease with RAC ($P$ = 0.07). Insulin-like growth factor-1 (IGF-1) (D) increased with RAC ($P$ = 0.02). Interleukin-15 (IL-15) gene expression (E) increased with RAC ($P$ = 0.01). Interleukin-6 (IL-6) gene expression (F) did not change with RAC ($P$ = 0.49). NF-E2-related factor 2 (NRF2) gene expression (G) tended to increase with RAC ($P$ = 0.09).

Cattle in the present study exhibited high rates of gain and SCFP may have provided additional support to these rapidly growing cattle.

Supplementation of SCFP did not affect expression of genes in muscle measured prior to (day −29) or midway through (day −13) RAC feeding, except for IL-6. IL-6 gene expression tended to be decreased in SCFP fed cattle compared to CON during the RAC period. IL-6 is a regulator of muscle hypertrophy, as seen through decreased hypertrophy in IL-6 deficient mice (Serrano et al., 2008), but also may have negative effects when in excess as seen through increased IL-6 concentrations and gene expression in muscular dystrophy modeled mice (Wada et al., 2017). Interleukin-6 also plays a role in glucose and fatty acid metabolism (Wolsk et al., 2010). Overall, IL-6 can have both positive and negative effects when concentrations and gene expression are deficient or in excess. In other studies, SCFP supplementation tended to decrease concentrations of IL-6 in serum (Burdick Sanchez et al., 2020) or had no effect (Vailati-Riboni et al., 2021; Valigura et al., 2021), when assessed during an immune or exercise challenge.

IL-8 serves as a pro-inflammatory signal by binding to G-coupled protein receptors (CXCR1 and CXCR2) and inducing neutrophil movement to an affected area (Hammond et al., 1995). While circulating IL-8 concentrations were not different due to treatment in this study, others have reported lesser serum IL-8 concentrations in cattle and horses following SCFP supplementation (Deters et al., 2018; Valigura et al., 2021). There was a decrease in serum IL-8 concentrations from days −29 to −13 which aligns with decreased muscle gene expression of IL-8 and a tendency for decreased CXCR2 expression during RAC supplementation. Alternatively, Messersmith et al. (2021) observed increased gene expression of IL-8, CXCR1, and CXCR2 in the muscle of rapidly growing steers 6 d after the start of RAC supplementation, suggesting cytokine signaling may change throughout the course of RAC supplementation. Cytokine expression may also contribute to desensitization of beta agonist signals through cyclic adenosine monophosphate (Chung et al., 1990; Hall et al., 1999). Further investigation into the role of IL-8 and other cytokine signaling in RAC-induced growth is needed.

Feeding ractopamine increased ADG and feed efficiency, regardless of treatment and affected expression of several genes of interest in muscle. Insulin-like growth factor 1 stimulates protein synthesis (Fang et al., 1997) and was increased during the RAC period, aligning with increased rates of ADG during the RAC period for all treatments. Similarly, Zheng et al. (2018) found muscle IGF-1 expression to be correlated with improved steer ADG.

IL-15 decreases adipose tissue deposition when administered in mice (Carbó et al., 2001) and increases myotube development when administered to human muscle cells in vitro (Furmanczyk and Quinn, 2003; O'Leary et al., 2017). In the current study, IL-15 expression was increased during the RAC period, a time associated with increased rates of

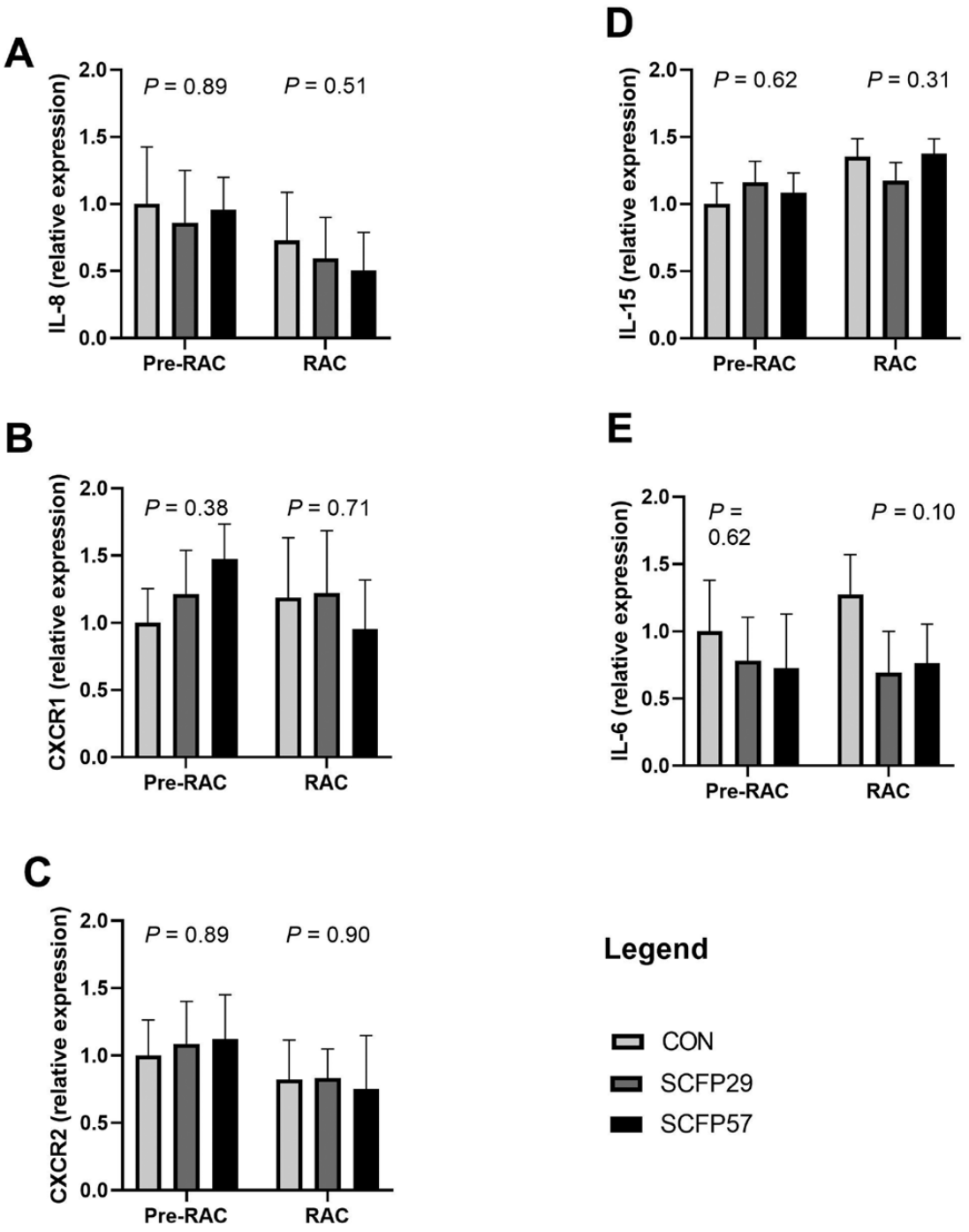


**Figure 2.** Relative gene expression of cytokines and receptors in muscle of steers supplemented with *Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe, Diamond V, Cedar Rapids, IA; 12 g per steer per d) before (SCFP57) and during (SCFP29) ractopamine hydrochloride supplementation (300 mg per steer per d). Muscle samples (*longissimus thoracis*) were collected 29 (preRAC) and 13 (RAC) d before harvest. Gene expression is reported relative to day −29 CON samples. There were no treatment × day interactions ($P \geq 0.25$). Expression of the following genes was not impacted by treatment within a day ($P \geq 0.31$): Interleukin-8 (IL-8) (A), (B) Chemokine C-X-C motif receptor 1 (CXCR1), (C) Chemokine C-X-C motif receptor 2 (CXCR2). Interleukin-15 (IL-15) (D), Interleukin-6 (IL-6) gene expression (E) was affected by treatment on day −29 ($P = 0.62$), but SCFP tended to decrease expression on day −13 ($P = 0.10$).

lipolysis and protein synthesis. In a study by Messersmith et al. (2021), RAC-fed cattle with greater ADG and G:F also exhibited lesser muscle expression of IL-15 and greater expression of the of IL-15 receptor (IL-15Rα). Like with IL-8, the current study observed different gene expression of IL-15 compared to Messersmith et al. (2021), potentially because of differences between studies in sampled time relative to RAC supplementation.

Anti-oxidants play a crucial role in protecting cells from oxidative damage and may be important in skeletal muscle growth and repair from injury (Shela et al., 2016). *Saccharomyces cerevisiae* fermentation products contain vitamins,

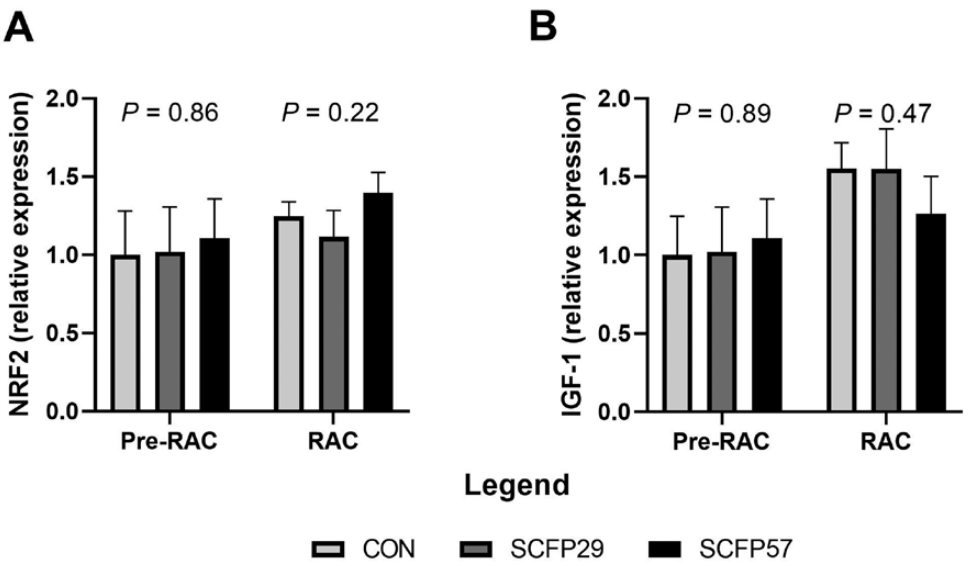


**Figure 3.** Relative gene expression of anti-oxidant and growth markers in muscle of steers supplemented with *Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe, Diamond V, Cedar Rapids, IA; 12 g per steer per d) before (SCFP57) and during (SCFP29) ractopamine hydrochloride supplementation (300 mg per steer per d). Muscle samples (*longissimus thoracis*) were collected 29 (pre-RAC) and 13 (RAC) d before harvest. Gene expression was calculated relative to day −29 CON samples. There were no treatment × day interactions ($P \geq 0.57$). Neither (A) NF-E2-related factor 2 (NRF2) or (B) Insulin-like growth factor-1 (IGF-1) was different by treatment on either sampling day ($P \geq 0.22$).

yeast cell wall components and other metabolites that may increase anti-oxidant potential (Jensen et al., 2008). Deters and Hansen (2019) reported supplementing SCFP to steers prior to a long-distance transit event increased anti-oxidant capacity which was associated with better ADG in the post-transit period. These data suggest feeding SCFP increases anti-oxidant capacity and may support cattle growth during times of oxidative insult. In the current study, supplementation with RAC tended to increase muscle gene expression of NRF2 compared to the preRAC period. Expression of NF-E2-related factor 2 expression is upregulated by ROS and increases intracellular anti-oxidant capacity by signaling for a variety of proteins with anti-oxidant function (Chen et al., 2006; Xiao et al., 2019; Zhang et al., 2022). This supports the hypothesis that RAC induces some degree of oxidative stress in the muscle. Plasma FRAP did not change due to RAC supplementation, suggesting the oxidative stress signal was limited to the muscle, or that endogenous anti-oxidants such as glutathione peroxidase and quinone oxidoreductase were upregulated to counteract a drop in anti-oxidant capacity. After 28 d of SCFP supplementation, FRAP was increased for SCFP57, as was ADG. However, improvements in anti-oxidant capacity did not persist during the RAC period. The current study observed increases in plasma Cu concentrations during the RAC period which may reflect a response to generalized inflammation or oxidative stress, as Cu-dependent ceruloplasmin is the likely culprit for this increased plasma Cu and is part of the acute phase protein response or can be an anti-oxidant. Further investigation of muscle anti-oxidant mechanisms and signaling in beef cattle is warranted given the role of redox signaling in muscle differentiation and development (Langen et al., 2002) and changes in anti-oxidant signaling observed in the current study.

Several studies have suggested SCFP supplementation modulates immune responses (Jensen et al. 2007, 2008; Mahmoud et al., 2020). Since energy demands increase when an animal is sick, decreasing the likelihood of illness and duration of sickness can be beneficial to the overall performance of cattle (Kvidera et al., 2016). Increased frequency of activated gamma delta T cells and of natural killer cells in circulation in SCFP supplemented steers at the midpoint of RAC supplementation suggests changes in the innate immune system. As the first line of defense against invading pathogens, the innate immune system acts broadly on pathogens. Gamma delta T cells are an innate T cell population that bridge the innate and adaptive arms of the immune system through cytokine production, lysis of infected or stressed cells and priming of other cells for antigen presentation (Vantourout and Hayday, 2013; Paul et al., 2015). NK cells are also part of the innate immune system and play an important role in recognition and lysis of virally infected or damaged host cells (Vivier et al., 2004). Mahmoud et al. (2020) observed increased gamma delta T cells in bronchoalveolar lavage fluid of SCFP supplemented calves and increases in innate immune function by cells in the peripheral blood. While changes in immune cell populations may not be specifically related to growth performance improvements, enhanced innate immune function may be important for preventing or controlling viral or bacterial infections. With this in mind, supplementing SCFP may provide additional immune support and decrease the risk of infection in vulnerable or high-risk cattle.

Understanding the nutritional requirements of fast-growing cattle is becoming increasingly important as the industry strives to optimize efficiency. In the current study, SCFP supplementation improved live cattle performance, potentially through positive changes in innate immunity and anti-oxidant capacity. Myokine and anti-oxidant gene expression were also altered during RAC, indicating changes in oxidative stress and potentially inflammation during this period of high growth. Determining the cytokine and inflammatory response within target tissues such as muscle and adipose may help to elucidate the mechanisms of SCFP and RAC-induced growth in finishing cattle. This understanding may help increase performance through strategic supplementation and management strategies.

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## Conflict of Interest Statement

Iowa State University authors have no conflicts of interest. Craig Belknap is employed by Diamond V.

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