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Effect of mixed live yeast and lactic acid bacteria on in vitro fermentation with varying media pH using a high-grain or high-forage diet

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Abstract: Two experiments were conducted to assess the effects of media pH and mixtures (SCEF) of live yeast (*Saccharomyces cerevisiae*; SC) and lactic acid bacteria (*Enterococcus faecium*; EF) on gas production (GP), dry matter disappearance (DMD) and volatile fatty acid (VFA) concentrations in batch culture using either high-forage (HF) or high-grain (HG) diets. Diets were evaluated in separate experiments, each as a complete randomized design with 2 (media pH, 5.8 and 6.5) × 5 (control, 3 SCEF, monensin) factorial arrangement of treatments. The SCEF had varying ratios of SC:EF: 0:0 (control), 1.18:1 (SCEF1), 1.25:1 (SCEF2) and 1.32:1 (SCEF3), added on a log10 basis. For the HF diet, supplementation of SCEF had greater GP ($P = 0.03$) at pH 6.5, and greater DMD ($P = 0.03$) and VFA concentration ($P < 0.01$) at pH 5.8 and 6.5 than control. For the HG diet, acetate:propionate (A:P) ratio at pH 6.5 was greater ($P = 0.05$) for SCEF than control. Increasing ratio of SC to EF in SCEF linearly ($P < 0.01$) decreased GP and DMD and linearly increased acetate percentage at pH 6.5. These results suggest that optimizing the SC:EF ratio in a mixture of SCEF can help improve rumen fermentation.

Keywords: batch culture; live yeast; lactic acid bacteria; ruminal fermentation; media pH; substrates

Introduction

Inclusion of antibiotic ionophores such as monensin (Mon) in the diet improves feed efficiency and health of feedlot cattle (Baah et al. 2009). However, growing concerns regarding the development of antimicrobial resistance and the potential health risks to humans consuming animal products by the public have led to the prohibition of use of in-feed

antibiotics in raising livestock in some locations such as the E.U. Therefore, alternatives to antibiotics that promote animal performance and health are needed.

Probiotics such as lactic acid bacteria (LAB) and live yeast (LY; commonly *Saccharomyces cerevisiae*) are naturally occurring microbes, which upon administration improve animal health by competing with pathogenic microbes and providing nutrients for growth of gastrointestinal microflora (Armas et al. 2017). It has been reported that feeding LAB to ruminant livestock improves feed efficiency, growth rate and animal health by improving rumen fermentation efficiency and increasing cellulolytic bacteria (Guo et al. 2020; Seo et al. 2010). Live yeast cells fed to ruminants may scavenge oxygen to improve the anaerobic environment and provide nutrients to lactate-utilizing bacteria in the rumen, in turn stabilizing ruminal pH and enhancing the growth of cellulolytic bacteria (Chaucheyras-Durand et al. 2008; Peng et al. 2020). There is evidence that feeding LY improves ruminal pH, feed intake, digestibility and health of ruminants (Pinloche et al. 2013; Ran et al. 2018). However, the effects are inconsistent among studies conducted in growing beef cattle using these additives. The inconsistent results may be due to differences in strain, dosage, and basal diet.

Tripathi et al. (2010) found that mixed LY (*Kluyveromyces marximanus*, *Saccharomyces cerevisiae* and *Saccharomyces uvarum*; 1:1:1 ratio) improved feed intake, daily gain and feed efficiency of lambs. Moreover, supplementation of a direct-fed microbial (DFM) containing LY and two specific *Enterococcus faecium* strains enhanced forage digestibility and milk yield of dairy cows (Noeck et al. 2006). Chiquette et al. (2015) found that the DFM *E. faecium* in combination with *S. cerevisiae* had beneficial effects on pH regulation and

maintenance of protozoa populations during a subacute ruminal acidosis challenge. However, information on the effects of LY and LAB mixtures is limited for beef cattle. In growing cattle, responses to DFM have been variable possibly due to differences in the basal diet, the DFM products used, and levels of inclusion (Jiao et al. 2017). We hypothesized that a mixture of LY and LAB would exhibit additive effects on rumen microbial activity but that the effects would depend on the rumen pH and the specific SC:EF ratio in the mixture. In our previous studies, a LY strain (MUCL39865) and a LAB strain (*E. faecium*) was determined as best candidates to increase dry matter (DM) digestibility (DMD) and volatile fatty acid (VFA) concentration among five LY and five LAB products (Jiao et al. 2017; 2018). These screened strains of LY and LAB individually exhibited dose-dependent responses (Jiao et al. 2017; 2018).

High-forage (HF) backgrounding diets and high-grain (HG) finishing diets are commonly fed to feedlot cattle in North America and elsewhere. Due to differences in rumen fermentability of diets, ruminal pH typically ranges from 5.84 to 6.25 in growing and finishing beef cattle (He et al. 2015, Shen et al. 2018), and these differences in pH may influence the response of animals to DFM. Therefore, the objective of the study was to evaluate the effects of three mixtures of LY and LAB on in vitro gas production (GP), DMD, and fermentation characteristics for HF and HG diets, using a batch culture technique with a media pH of 5.8 or pH 6.5. As Mon is routinely used in feedlot cattle diets, it was included as a positive control.

Materials and methods

Yeast products and LAB sources

The LY (*Saccharomyces cerevisiae,* SC) was provided by AB Vista (Marlborough, UK). The number of viable LY cells was determined using a spread plate method and was 1.71 × 10^{10} cfu g^{-1} (Jiao et al. 2018). The LAB (*Enterococcus faecium,* EF) strain was provided by Chr. Hansen A/S (Horsholm, Denmark). It was kept in glycerol and grown anaerobically in 10 mL of selective culture medium (De Man, Rogosa and Sharpe agar (MRS), Oxoid Ltd., Basingstoke, Hampshire, England) at 37°C in an anaerobic incubator. Then, 1 mL of LAB was transferred into 99 mL of MRS medium and incubated at 37°C for 18 h in an anaerobic incubator. The concentration of LAB was measured by spectrometry for optical density (OD600) after incubation.

Experimental design, substrate and inoculum

High-forage and HG diets, typical of western Canadian feedlot diets, were used as substrates in Experiment 1 and 2, respectively. Each experiment was a completely randomized design with a factorial arrangement of treatments: 2 media pH (5.8 and 6.5) × 5 additives. Each experiment was conducted in 3 runs on 3 different days with 3 replications (bottles) per treatment within run. The 2 media pH treatments were achieved using 2 different buffers that varied in pH (5.8 and 6.5). The 5 additive treatments were: control (no additive), SCEF1, SCEF2, SCEF3 and Mon. The three mixtures of SCEF were prepared by varying the ratio of SC:EF within each bottle: SCEF1, 1.18:1 (1.44 × 10^8 cfu of SC and 8.4 × 10^6 cfu of EF; SCEF2, 1.25:1 (2.4 × 10^8 cfu of SC and 6 × 10^6 cfu of EF; and SCEF3, 1.32:1 (3.36 × 10^8 cfu of SC and 3.6 × 10^6 cfu of EF), on a log10 basis. Bottles without SCEF or Mon were used as negative controls. Monensin was added at dose of 0.17 mg $bottle^{-1}$ and used as a positive control for each pH level. The dose of Mon was calculated based on a daily dose of

300 mg day^{-1} for beef cattle (Yang et al. 2014). The HF diet substrate consisted of 60% barley silage, 27% dry-rolled barley grain, 10% canola meal, and 3% vitamins and minerals (DM basis). The HG diet substrate consisted of 10% barley silage, 87% dry-rolled barley grain, and 3% vitamins and minerals (DM basis). The HF and HG diets were ground to pass a 1-mm screen prior to the in vitro incubations.

Four beef heifers with permanent rumen fistula were used as rumen inoculum donors. Two heifers fed a forage-based diet (forage to concentrate ratio = 3:1) were used to provide rumen inoculum for the batch culture using the HF substrate. Two other heifers were fed a grain-based diet (forage to concentrate ratio = 1:3) to provide inoculum for the batch culture using the HG substrate. Prior to inoculum collection, the heifers were given three weeks to adapt to the experimental diets. All animal procedures outlined followed the guidelines of the Canadian Council on Animal Care (2009).

Batch culture procedure

Glass bottles (125 mL) were used in triplicate for the incubations. The ground HF and HG substrates were weighed (approximately 0.75 g) into filter bags (F57; Ankom Technology, Macedon, NY, USA) that had been washed with acetone, dried and weighed. A filter bag with substrate and appropriate dose of additive (SCEF or Mon) was added to each bottle. Two buffers were prepared with differing pH (5.8 and 6.5) by adjusting the volume of sodium bicarbonate in the solution (Yang et al. 2002). Bottles were then filled with 45 mL of freshly prepared buffer (either pH 5.8 or 6.5 as required) and 15 mL of strained ruminal fluid. Bottles were purged with carbon dioxide to remove air from the headspace. After loading, each bottle was immediately sealed with a butyl rubber stopper (14 mm) plus aluminum

crimp cap. Bottles filled with buffer and ruminal fluid in triplicate were used as blanks at each pH level. All the bottles were placed in an incubator with shaking at 39°C for 24 h.

Ruminal fluid was collected from donor cattle 2 h post-feeding and filtered through PeCAP® polyester screen. The strained ruminal fluid was stored in a thermos bottle and immediately transferred to the laboratory for a second filtration through 4 layers of cheesecloth, then placed in a water bath at 39°C. A pH meter (B20PI, SympHony Benchtop Meters; VWR, Edmonton, AB, Canada) was used to measure the pH of strained ruminal fluid. For Experiment 1 (HF diet), the collected ruminal fluid pH was 6.08, 6.01 and 6.13 for the first, second and third runs, respectively. For Experiment 2 (HG diet), the ruminal fluid pH was 5.88, 5.70 and 5.79 for the first, second and third runs, respectively.

At 3, 6, 9, 12, and 24 h after the start of incubation, the gas pressure was recorded and corrected for blank GP (GP in buffered rumen fluid without substrate). Gas pressure at each time point, corrected for the gas released from the blanks, was used to generate gas volume estimates using the equation of Romero- Pérez and Beauchemin (2018):

Gas volume = $4.7047 \times (\text{gas pressure}) + 0.0512 \times (\text{gas pressure}^2)$.

Gas data obtained were fitted to the exponential model of France et al. (2000):

$y = a\,(1 - \exp^{-c \times [t\text{-}lag]})$, where y is the volume of GP at time t; a is the asymptotic GP (mL g^{-1} DM); c is the rate constant of GP (% h^{-1}) and lag (h) is the initial delay before GP begins.

After 24 h of incubation, the bottles were placed on ice to stop the fermentation, and pH was determined in the culture media. Samples (5 mL) of culture fluid were preserved with 1 mL of 0.25 (wt vol^{-1}) HPO_3 and 1 mL of 0.01 (vol vol^{-1}) H_2SO_4 at -20°C for determining VFA and ammonia-nitrogen (NH_3-N) concentrations, respectively. Dry matter disappearance

(DMD) was calculated as the difference between the amount of DM in the substrates before and after incubation.

Chemical analysis

The DM concentration (method 930.15) of substrates was analyzed by drying samples at 135°C for 2 h followed by hot weighing according to the procedures of the Association of Official Analytical Chemists (AOAC 2005). The VFA concentration was quantified using the gas chromatographic method (Jiao et al. 2018). The NH_3-N concentration was determined using the method described by Rhine et al. (1998).

Statistical analysis

Data were averaged for the 3 replicates within run and statistically analyzed by experiemtn using the MIXED model procedure of SAS (SAS Inst. Inc. Cary, NC) including fixed effects of media pH, treatment additives, and interaction between pH and additives, and the random effects of run. As there were numerous interactions, the effects of additives were examined for each pH. The effect of increasing ratio of SC:EF was examined using linear and quadratic orthogonal contrasts and contrasts were performed to compare control to the average of the 3 SCEF, and the average of SCEF to Mon. The PDIFF option adjusted by Tukey's method was included in the LSMEANS statement to account for multiple comparisons among treatments within pH level. Differences were declared significant at $P \leq 0.05$. Trends are discussed at $0.05 < P \leq 0.10$ unless otherwise stated.

Results

Gas production kinetics and DMD

For the HF diet, asymptotic GP (a), rate of GP (c) and DMD were consistently greater ($P <$

0.01) at media pH 6.5 than pH 5.8 (Table 1). Increasing the SC:EF ratio did not affect the volume or rate of GP at pH 5.8, whereas at media pH 6.5, the volume and rate of GP linearly ($P = 0.05$) decreased with increasing SC:EF ratio. Increasing SC:EF ratio quadratically ($P < 0.01$) decreased DMD at media pH 6.5, with no effect at pH 5.8. Asymptotic GP ($P < 0.01$) and rate of GP ($P = 0.03$) were greater for SCEF than control at media pH 6.5. Moreover, supplementation of SCEF increased DMD ($P = 0.03$) compared with control at both media pH. It is notable that the greatest GP (a and c) and DMD occurred for SCEF1, accounting for most of the significant differences between SCEF and control. In comparison with Mon, asymptotic GP ($P = 0.03$ at pH 5.8, and $P < 0.01$ at pH 6.5), rate of GP ($P < 0.01$) and DMD ($P = 0.04$) were greater for SCEF regardless of the media pH.

For the HG diet, greater ($P < 0.01$) asymptotic GP, rate of GP and DMD were observed at media pH 6.5 than pH 5.8 (Table 2). Increasing ratio of SC:EF linearly ($P < 0.01$) increased asymptotic GP and rate of GP at media pH 5.8, but the opposite trend occurred for asymptotic GP ($P = 0.02$) and rate of GP ($P < 0.01$) at pH 6.5. However, the DMD of HG was not affected by the ratio of SC:EF at pH 5.8, while there was a quadratic ($P = 0.03$) response to the ratio at pH 6.5. Supplementation of SCEF did not affect GP (a and c) compared with the control, except rate of GP at media pH 6.5 was less ($P < 0.01$) with SCEF. Asymptotic GP ($P = 0.04$) and rate of GP ($P < 0.01$) were greater at pH 6.5 with SCEF compared with Mon, without differences in DMD.

Concentration of VFA and NH_3-N

Incubation of a HF diet resulted in greater concentration of total VFA ($P < 0.01$), molar proportions of acetate ($P < 0.01$), propionate ($P < 0.01$) and butyrate ($P = 0.02$), NH_3-N

concentration ($P = 0.02$), and ratio of acetate to propionate (A:P) at media pH 6.5 than pH 5.8 (Table 3). Total VFA concentration tended ($P = 0.08$) to linearly decrease at pH 5.8, but linearly ($P = 0.03$) increased at pH 6.5 with increasing SC:EF ratio. At media pH 6.5, molar proportion of acetate increased (linear; $P = 0.05$) while proportions of propionate decreased (linearly; $P < 0.01$), and as a result, ratio of A:P linearly ($P < 0.01$) increased with increasing SC:EF ratio. Total VFA concentration was greater ($P = 0.01$) at pH 5.8 or tended ($P = 0.06$) to be greater at pH 6.5 with supplemented SCEF than control, without differences in molar proportions of individual VFA. The difference between SCEF and control was mainly due to the high VFA concentration for SCEF1 at pH 5.8, and for SCEF3 at pH 6.5. In comparison with Mon, supplementation of SCEF had greater ($P < 0.01$) total VFA concentration, acetate proportion ($P = 0.02$ at pH 5.8, and $P < 0.01$ at pH 6.5), A:P ratio and less ($P < 0.01$) propionate proportion at both pH 5.8 and 6.5. The NH_3-N concentration did not differ between control and SCEF, and was not affected by SC:EF ratio, but was greater ($P < 0.01$) for SCEF than Mon.

Using a HG diet, total VFA concentration, acetate proportion, and A:P ratio were greater ($P < 0.01$), whereas, proportions of propionate and butyrate, and NH_3-N concentration were less ($P < 0.01$) at media pH 6.5 than pH 5.8 (Table 4). The total VFA concentration was not affected by treatment additives at pH 5.8, but it quadratically ($P = 0.03$) changed with SC:EF ratio at pH 6.5. Molar proportion of acetate linearly ($P = 0.02$) decreased with SC:EF ratio at pH 5.8, but the proportion of acetate ($P = 0.04$) and ratio of A:P ($P = 0.02$) linearly increased at pH 6.5. Butyrate proportion linearly ($P < 0.01$) increased at low pH, and tended ($P = 0.06$) to linearly decrease at high pH with increasing ratio of SC:EF. Using a media pH

of 5.8, there were no differences in total VFA concentration or individual VFA molar proportions between SCEF and control, except the proportion of propionate which was less ($P = 0.03$) for SCEF. With media pH 6.5, the total VFA concentration also did not differ between SCEF and control, but it was greater for SCEF1 than control. Furthermore, at pH 6.5 there was greater ($P < 0.01$) acetate proportion, less ($P < 0.01$) propionate proportion, and thus greater ($P < 0.01$) ratio of A:P for SCEF than control. In comparison with Mon, propionate proportion was less ($P < 0.01$), and A:P ratio was greater ($P = 0.03$ at pH 5.8, and $P < 0.01$ at pH 6.5) with supplemented SCEF regardless of media pH. Supplementation of SCEF also led to greater ($P < 0.01$) total VFA concentration and acetate proportion compared with Mon at media pH 6.5. Increasing SC:EF ratio did not influence NH_3-N concentration, but SCEF treatments had greater ($P \leq 0.05$) NH_3-N than control ($P = 0.05$ at pH 5.8, and $P < 0.01$ at pH 6.5) or Mon ($P < 0.01$).

Discussion

Media pH level

Supplementation of ruminant diets with LY or LAB individually has previously been shown to stabilize ruminal pH and reduce the occurrence of acute ruminal acidosis (Chaucheyras-Durand et al. 2008; Malekkhahi et al. 2016). The present study provides further information on the combined effects of LY and LAB on rumen fermentation under variable ruminal pH conditions, as would occur for animals fed HF and HG diets. In the current study, after 24 h of incubation, the final measured media pH was on average 5.88 when a buffer with pH 5.8 was used and 6.61 when a buffer with pH 6.5 was used with the HF diet. For the HG diet, the final measured media pH was on average 5.72 and 6.44 for the

two pH treatments, respectively. Thus, the final pH of the incubations were relatively similar to the original buffer pH, as intended, providing a range of pH at which to evaluate the effects of SCEF.

The greater GP, DMD, total VFA concentration at high versus low pH with either HF or HG diets demonstrated higher ruminal microbial activity with elevated ruminal pH. Furthermore, the greater molar proportion of acetate and A:P ratio suggested an improvement of fibre digestion at media pH 6.5 than pH 5.8. Rumen cellulolytic microorganisms are sensitive to low ruminal pH. Jiao et al. (2019) observed that the populations of the two main cellulolytic microorganisms, *Fibrobacter succinogenes* and *Ruminococcus flavefaciens,* were less abundant at media pH 5.8 versus pH 6.5 in an in vitro ruminal fermentation. Petri et al. (2013) found that increasing dietary forage levels increased copy numbers of ruminal fibrolytic bacteria, suggesting that microbial populations were affected by the composition of the diet. It is noteworthy that the increase of A:P ratio due to the higher pH was more pronounced with the HG (+19%) than with the HF diet (+6%), indicating that an increase in ruminal pH was more efficient in improving fibre digestion of the HG diet.

The interactions between media pH and SCEF on fermentation characteristics are of interest because ruminal pH of cattle varies with diet composition and feeding management, thus can therefore impact feed additive activity. It is notable that within the total 18 combinations (i.e., 2 substrates × 9 variables measured), the significant linear or quadratic responses with increasing SC:EF ratio were more frequent at media pH 6.5 (10/18) than pH 5.8 (4/18), indicating that the dose effect of SCEF was more pronounced at high media pH than low media pH.

High-forage diet

Greater DMD of the HF diet with SCEF than control was consistent with our previous finding that adding LY led to greater DMD of a HF diet than control (Jiao et al. 2019). However, the magnitude of DMD increase due to LY was slightly less in the present study (+2.8% at pH 5.8; 3.9% at pH 6.5) than in the previous study (+5.7% at pH 5.8; +5.8% at pH 6.5). The lower improvement in DMD of the HF diet in the present study might be due to the inclusion of LAB in SCEF, as supplementation of LAB did not affect DMD of a HF diet in the study of Jiao et al. (2017). Consistent with the current study, Perdomo et al. (2020) reported that increasing the dose of LY (0, 0.5 and 1.0 g d^{-1}; 3.76×10^{10} cfu g^{-1}) linearly increased the apparent digestibility of DM in dairy cows. Adding LY to the rumen has been proposed to stimulate the growth of anaerobic bacteria, improve fibre digestion and enhance rumen microbial protein production. In contrast, LAB such as *Lactobacillus* and *Enterococcus* species may have beneficial effects in preventing ruminal acidosis (Krehbiel et al. 2003), potentially by allowing the ruminal microorganisms to adapt to the presence of lactate in the rumen (Ghorbani et al. 2002; Yoon et al. 1995). However, the incidence of subacute ruminal acidosis (SARA) under the current experimental conditions was minor. Unlike acute acidosis, SARA results mainly from high concentration of VFA rather than an accumulation of lactate. The results from our study suggest that the improvement of DMD was likely due to the LY addition, rather than LAB addition. In addition, the increased ratio of LY to LAB in SCEF did not affect DMD of the HF diet at media pH 5.8, which agrees with the previously reported lack of effect of LY dose on DMD (Jiao et al. 2019). In contrast, the linear decrease in DMD of the HF diet at pH 6.5 with increasing SCEF ratio was

somewhat unexpected because increasing the ratio of LY to LAB in SCEF would be expected to increase DMD. Nevertheless, the linearly increased acetate proportion at the expense of propionate proportion and hence linearly increased ratio of A:P with increasing ratio of LY to LAB in SCEF confirms the mode of action of LY on promoting fibrolytic activity and fibre digestion. Decreasing the dose of EF by increasing the ratio of SC to EF, would have been expected to decrease lactic acid production and consequently decrease the population of lactate utilizing bacteria (LUB). Chaucheyras-Durand et al. (2008) stated that LY can stimulate the growth of LUB by providing growth factors such as amino acids, peptides, vitamins and organic acids. Therefore, we speculated that the increased ratio of LY to LAB could offset the impact of decreased population of EF on rumen fermentation.

High-grain diet

The similar DMD and VFA concentration of HG for SCEF and control treatments is consistent with our previous studies using LAB and LY with a HG diet (Jiao et al. 2017; 2018). The responses of ruminal digestibility to yeast supplementation in the literature are inconsistent. Lynch et al. (2002) found lower in vitro DMD of hay at 48 h of incubation by adding SC live cells at doses of 0.35 and 0.73 g L^{-1} comparted with the control. However, increased ruminal microbial digestion by adding LY, or no effect of yeast culture on in situ DMD of a non-lactating cow diet were also reported (Enjalbert et al. 1999; Yoon et al. 1996). Desnoyers et al. (2009) concluded that a higher dose of some yeast strains was needed to be effective when used in HG diets. Regarding LAB addition, Baah et al. (2009) reported that the effect of LAB supplementation on in vitro VFA concentration at 12 h of incubation was less pronounced with HG compared with HF diets. Both LY and LAB have been shown to

play a role in reducing incidence of SARA and improving fibre digestion in the rumen via various modes of action (Weinberg et al. 2007). Therefore, the lack of overall improvement of DMD and VFA concentration when supplementing a HG diet with SCEF may have been due to the low fibre concentration in the HG diet. As such, any improvement in fibre digestibility would have had minimal impact on DMD. Although DMD of the HG diet was not affected by SC:EF ratio, the volume and rate of GP were linearly increased at pH 5.8, and linearly decreased at pH 6.5, with a quadratic effect on VFA concentration at pH 6.5. This inconsistency suggests differences in relative partitioning of nutrients for GP, microbial mass and VFA production from fermented DM, depending upon media pH. These results confirm that the response of microbial digestion to yeast and LAB strains is media pH dependent (Jiao et al. 2017; 2018). Alterations of ruminal VFA profile are indicative of shifts in fermentation patterns (Kenney 2013). In the present study, acetate proportion linearly decreased without changing A:P ratio at pH 5.8, while both acetate proportion and A:P ratio linearly increased at pH 6.5. The greater ratio of A:P suggests improved fiber degradation but only at a media pH of 6.5, which is similar to the results for the HF diet in this study. In short, the results confirm the impact of LY and LAB on rumen fermentation compared with a control, whereas the effect of supplementing mixtures varying in ratio of LY and LAB appeared to be minor.

SCEF vs. Mon

The greater propionate proportion, lower A:P ratio and lower NH_3-N concentration with supplementation of Mon compared with control, confirm the mode of action of Mon in the rumen to improve fermentation efficiency and reduce proteolytic activity (Domescik et al. 1999; Quinn et al. 2009). The GP kinetics of both HF and HG were greater with SCEF than

Mon supplementation, and the DMD of the HF diet was greater only by adding SCEF. As truly digested substrates are divided among VFA, gas and microbial biomass in a fermentation system, the DMD more accurately reflects truly digested substrate. The suggestion of improved fibre digestion of the low-fibre HG diet with SCEF compared with Mon is supported by greater A:P ratio but not by the DMD, possibly due to the low fibre content of the HG diet for which improved fibre digestibility would have limited effects on total DMD. Furthermore, SCEF and Mon appeared to act differently within diet and media pH. Adding SCEF vs. Mon increased total VFA concentration and molar proportion of acetate at both low and high media pH for the HF diet, but only at high pH for the HG diet. The results suggest that the mixture of SCEF may not have potential beneficial effects vs. Mon for a HG diet because ruminal pH of cattle fed a HG diet is often low (~5.8). The greater ratio of A:P with addition of SCEF compared with Mon, suggests a different fermentation pattern between SCEF and Mon in the rumen. Additionally, the mode of action on proteolytic activity appears different between SCEF and Mon. The SCEF may increase proteolytic activity as shown by greater NH_3-N concentration compared with Mon. The lower concentration of NH_3-N with Mon in the current study can be explained by its inhibitory effect on hyper ammonia-producing bacteria in the rumen (Wang et al. 2015). The mechanism by which adding SCEF increased ruminal NH_3-N concentration, suggesting increased proteolytic activity, is not clear, but it is unlikely a beneficial effect because high ruminal protein degradation reduces protein use efficiency.

Conclusions

Using a lower media pH to reflect the rumen environment of beef cattle fed high grain

diets generally inhibited ruminal microbial activity in the batch culture incubations. The effects of SCEF were more pronounced at high versus low media pH. Supplementation of SCEF stimulated rumen fermentation, and primarily altered fermentation pattern with greater A:P due to increased acetate proportion and deceased propionate proportion compared with the control for a HG diet. The results confirm the beneficial effects on rumen fermentation when adding LY and LAB to cattle diets. The effects of increasing SC:EF ratio in the mixture of SCEF on GP kinetics, DMD, and fermentation characteristics varied with diet and media pH. Among the mixtures of SCEF, a SC:EF ratio of 1.18:1 demonstrated greater beneficial effects on DMD (HF, pH 5.8), greater total VFA concentration (HF, pH 5.8; HG, pH 6.5), and lower GP without differences in DMD, suggesting improved feed efficiency (HG, pH 5.8). Supplementation of SCEF and Mon had different modes of action on ruminal fermentation, and SCEF may be used as alternatives to Mon to stimulate rumen fermentation. These results showed that manipulating the mixture of LY and LAB could be a new strategy to improve rumen fermentation. Further research is warranted to confirm the mechanism by which LY and EF improve rumen fermentation in beef cattle, and whether cattle performance and animal health are improved.

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Table 1. Effect of mixtures (SCEF) of *Saccharomyces cerevisiae* (SC) and *Enterococcus faecium* (EF) and monensin (Mon) compared with control (Ctr) on gas kinetics (asymptotic gas volume, a; rate constant, c) and DM disappearance (DMD) at different media pH using a high-forage diet.

	Treatments[a]						*P*-value[b]				
Item	Ctr	SCEF1	SCEF2	SCEF3	Mon	SEM	pH	L	Q	S vs. C	S vs. M
a, mL g^{-1} DM											
pH 5.8	113	120	114	116	110	3.7	<0.01	0.41	0.37	0.16	0.03
pH 6.5	203bc	240a	222ab	210bc	186c	11.8		0.05	0.80	<0.01	<0.01
c, % h^{-1}											
pH 5.8	5.59a	5.57a	5.49a	5.86a	4.57b	0.42	<0.01	0.39	0.46	0.80	<0.01
pH 6.5	9.70bc	11.01a	10.75ab	9.76bc	8.73c	0.64		0.05	0.47	0.03	<0.01
DMD, %											
pH 5.8	45.4	47.2	46.3	47.5	45.6	1.30	<0.01	0.67	0.11	0.03	0.04
pH 6.5	51.8b	55.6a	52.5b	53.4b	51.8b	2.48		<0.01	<0.01	0.03	0.04

Note: SEM = standard error of the mean. Within a row, values that do not share a common lowercase letter are different ($P < 0.05$).

[a]Ctr = control; SCEF1, SCEF2, SCEF3 = SC:EF ratio of 1.18:1, 1.25:1 and 1.32:1, respectively; Mon = monensin (0.17 mg $bottle^{-1}$).

[b]pH = pH 5.8 vs. 6.5; L, Q = linear or quadratic effect of SCEF1, SDEF2 and SCEF3 within media pH; S vs. C = contrast between average of SCEF and Ctr within media pH; S vs. M = contrast between average of SCEF and Mon within media pH.

Table 2. Effect of mixtures (SCEF) of *Saccharomyces cerevisiae* (SC) and *Enterococcus faecium* (EF) and monensin (Mon) compared with control (Ctr) on gas kinetics (asymptotic gas volume, a; rate constant, c) and DM disappearance (DMD) at different media pH using a high-grain diet.

	Treatments[a]						*P*-value[b]				
Item	Ctr	SCEF1	SCEF2	SCEF3	Mon	SEM	pH	L	Q	S vs. C	S vs. M
a, mL g^{-1} DM											
pH 5.8	142ab	126c	134bc	151a	129c	5.9	<0.01	<0.01	0.33	0.22	0.06
pH 6.5	235ab	247a	222bc	221bc	214c	6.6		0.02	0.21	0.37	0.04
c, % h^{-1}											
pH 5.8	7.35ab	6.30c	7.35ab	7.88a	6.77bc	0.21	<0.01	<0.01	0.24	0.47	0.09
pH 6.5	10.91	10.79	10.08	9.43	9.20	0.27		<0.01	0.91	0.01	<0.01
DMD, %											
pH 5.8	45.4	44.8	46.0	46.5	45.0	1.81	<0.01	0.35	0.81	0.64	0.38
pH 6.5	55.5	56.2	53.5	55.9	53.8	1.89		0.80	0.02	0.87	0.28

Note: SEM = standard error of the mean. Within a row, values that do not share a common lowercase letter are different ($P < 0.05$).

[a]Ctr = control; SCEF1, SCEF2, SCEF3 = SC:EF ration of 1.18:1, 1.25:1 and 1.32:1, respectively; Mon = monensin (0.17 mg $bottle^{-1}$).

[b]pH = pH 5.8 vs. 6.5; L, Q = linear or quadratic effect of SCEF1, SDEF2 and SCEF3 within media pH; S vs. C = contrast between average of SCEF and Ctr within media pH; S vs. M = contrast between average of SCEF and Mon within media pH.

Table 3. Effect of mixtures (SCEF) of *Saccharomyces cerevisiae* (SC) and *Enterococcus faecium* (EF) and monensin (Mon) compared with control (Ctr) on rumen fermentation characteristics at different media pH using a high-forage diet.

	Treatments[a]						*P*-value[b]				
Item	Ctr	SCEF1	SCEF2	SCEF3	Mon	SEM	pH	L	Q	S vs. C	S vs. M
Total VFA, mM											
pH 5.8	66.1bc	72.6a	68.7ab	69.2ab	62.4c	5.76	<0.01	0.08	0.19	0.01	<0.01
pH 6.5	76.5b	75.9b	80.7ab	90.3a	72.0b	5.42		0.03	0.64	0.06	<0.01
mol 100 mol^{-1}											
Acetate (A)											
pH 5.8	45.0	46.8	44.3	45.8	42.6	4.36	<0.01	0.42	0.08	0.59	<0.02
pH 6.5	50.3abc	48.2c	51.2bc	52.5ab	47.4a	1.73		0.05	0.64	0.79	<0.01
Propionate (P)											
pH 5.8	21.2b	23.5b	20.7b	20.6b	27.9a	1.52	<0.01	0.07	0.32	0.75	<0.01
pH 6.5	22.5bc	23.1b	22.5bc	21.9c	25.6a	0.89		<0.01	0.95	0.99	<0.01
Butyrate											
pH 5.8	15.5	14.8	15.1	14.8	15.1	1.99	0.02	0.93	0.51	0.15	0.65
pH 6.5	15.9	17.0	15.7	15.4	15.7	1.45		0.11	0.60	0.81	0.53
A:P											
pH 5.8	2.13a	2.01a	2.15a	2.23a	1.57b	0.24	<0.01	0.21	0.96	0.08	<0.01
pH 6.5	2.25ab	2.09b	2.28a	2.41a	1.86c	0.07		0.01	0.74	0.88	<0.01
NH_3-N, mM											
pH 5.8	13.7a	14.5a	14.9a	14.4a	11.0b	0.43	0.02	0.87	0.42	0.85	<0.01
pH 6.5	14.5	14.2	15.1	14.4	12.7	0.97		0.87	0.55	0.86	<0.01

Note: SEM = standard error of the mean. Within a row, values that do not share a common lowercase letter are different ($P < 0.05$).

[a]Ctr = control; SCEF1, SCEF2, SCEF3 = SC:EF ratio of 1.18:1, 1.25:1 and 1.32:1, respectively; Mon = monensin (0.17 mg bottle^{-1}).

[b]pH = pH 5.8 vs. 6.5; L, Q = linear or quadratic effect of SCEF1, SDEF2 and SCEF3 within media pH; S vs. C = contrast between average of SCEF and Ctr within media pH; S vs. M = contrast between average of SCEF and Mon within media pH.

Table 4. Effect of mixtures (SCEF) of *Saccharomyces cerevisiae* (SC) and *Enterococcus faecium* (EF) and monensin (Mon) compared with control (Ctr) on rumen fermentation characteristics at different media pH using a high-grain diet.

	Treatments[a]						*P*-value[b]				
Item	Ctr	SCEF1	SCEF2	SCEF3	Mon	SEM	pH	L	Q	S vs. C	S vs. M
Total VFA, mM											
pH 5.8	60.3	57.8	56.9	63.2	60.2	4.13	<0.01	0.18	0.30	0.65	0.68
pH 6.5	79.3ab	85.9a	78.0bc	82.7ab	72.0c	4.56		0.29	0.03	0.33	<0.01
mol 100 mol^{-1}											
Acetate (A)											
pH 5.8	35.7	37.8	36.7	34.6	35.5	1.08	<0.01	0.02	0.63	0.56	0.42
pH 6.5	39.4b	40.8a	41.8a	41.7a	37.6c	2.35		0.04	0.18	<0.01	<0.01
Propionate (P)											
pH 5.8	35.5ab	35.0bc	33.7c	34.5bc	36.6a	2.85	<0.01	0.48	0.10	0.03	<0.01
pH 6.5	34.2b	33.4bc	32.2c	32.7c	35.7a	3.27		0.27	0.12	<0.01	<0.01
Butyrate											
pH 5.8	13.1ab	12.3	12.9	13.9	12.4	0.54	<0.01	<0.01	0.63	0.96	0.17
pH 6.5	11.6	11.2	11.6	10.9	11.2	0.31		0.06	0.96	0.27	0.24
A:P											
pH 5.8	1.02	1.09	1.10	1.02	0.98	0.11	<0.01	0.13	0.25	0.21	0.03
pH 6.5	1.16c	1.25b	1.31a	1.30ab	1.05d	0.17		0.02	0.03	<0.01	<0.01
NH_3-N, mM											
pH 5.8	22.9ab	23.2a	24.0a	24.0a	21.9b	0.41	<0.01	0.16	0.45	0.05	<0.01
pH 6.5	20.3b	20.7ab	21.5a	21.3a	19.5b	0.97		0.28	0.23	<0.01	<0.01

Note: SEM = standard error of the mean. Within a row, values that do not share a common lowercase letter are different ($P < 0.05$).

[a]Ctr = control; SCEF1, SCEF2, SCEF3 = SC:EF ratio of 1.18:1, 1.25:1 and 1.32:1, respectively; Mon = monensin (0.17 mg bottle^{-1}).

[b]pH = pH 5.8 vs. 6.5; L, Q = linear or quadratic effect of SCEF1, SDEF2 and SCEF3 within media pH; S vs. C = contrast between average of SCEF and Ctr within media pH; S vs. M = contrast between average of SCEF and Mon within media pH.