Active dry *Saccharomyces cerevisiae* can alleviate the effect of subacute ruminal acidosis in lactating dairy cows

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ABSTRACT

The objective of the study was to determine the effect of active dry *Saccharomyces cerevisiae* (ADSC) supplementation on dry matter intake, milk yield, milk components, ruminal pH, and microbial community during a dietary regimen that leads to subacute ruminal acidosis (SARA). Sixteen multiparous, rumen-cannulated lactating Holstein cows were randomly assigned to 1 of 2 dietary treatments that included ADSC (Biomate; AB Vista, Marlborough, UK; 8×10^{10} cfu/head per day) or control. During wk 1 to 6, all cows received a high-forage (HF) diet (77:23, forage:concentrate). Cows were then abruptly switched during wk 7 to a high-grain (HG) diet (49:51, forage:concentrate) and remained on the HG until the end of wk 10. Feed intake and milk yields were recorded daily. Ruminal pH was recorded continuously using an indwelling system for 1 to 2 d per week during the pre-experimental phase, and wk 6, 7, and 10. Ruminal digesta samples were collected at the end of the experiment and analyzed for relative change in microbial communities using real-time quantitative PCR. Cows were considered to have SARA if the duration below pH 5.6 was ≥ 300 min/d. Ruminal pH during wk 6 (HF plateau) was not different across treatments (15 ± 46 min/d at pH < 5.6). The dietary regimen successfully induced SARA during wk 7 (transition from HF to HG diet), and ruminal pH (551 ± 46 min/d at pH < 5.6) was not different across treatments. However, cows receiving ADSC had an improved ruminal pH (122 ± 57 vs. 321 ± 53 min/d at pH < 5.6) during wk 10 (HG plateau) compared with control. Additionally, cows receiving ADSC had a better dry matter intake (23.3 ± 0.66 vs. 21.6 ± 0.61 kg/d) and 4% fat-corrected milk yield (29.6 ± 1.2 vs. 26.5 ± 1.2 kg/d) than control cows during the HG phase (wk 8 to 10). During HG feeding, cows receiving ADSC had greater total volatile fatty acid and propionate concen-

trations (175 ± 7.5 vs. 154 ± 7.5 and 117 ± 6.1 vs. 94 ± 5.7 mM for ADSC and control, respectively) and lower acetate:propionate ratio (0.26 ± 0.5 vs. 0.36 ± 0.05 for ADSC and control, respectively). Microbial analyses conducted on samples collected during wk 10 showed that cows supplemented with *S. cerevisiae* had a 9-fold, 2-fold, 6-fold, 1.3-fold, and 8-fold increase in *S. cerevisiae*, *Fibrobacter succinogenes*, *Anaerovibrio lipolytica*, *Ruminococcus albus*, and anaerobic fungi, respectively, which suggested an increase in cellulolytic microbes within the rumen. Cows supplemented with ADSC had 2.2-fold reduction in *Prevotella albensis*, which is a gram-negative bacterium predominant during SARA. *Prevotella* spp. are suggested to be an important source of lipopolysaccharide responsible for inflammation within the rumen. Cows supplemented with ADSC had a 2.3-fold increase in *Streptococcus bovis* and a 12-fold reduction in *Megasphaera elsdenii*. The reduction in *M. elsdenii* may reflect lower concentration of lactic acid within the rumen for ADSC cows. In conclusion, ADSC supplementation to dairy cows was demonstrated to alleviate the condition of SARA caused by abrupt dietary changes from HF to HG, and can potentially improve rumen function, as indicated by greater numbers of cellulolytic microorganisms within the rumen.

Key words: *Saccharomyces cerevisiae*, subacute ruminal acidosis, rumen microbes, dairy cattle

INTRODUCTION

Subacute ruminal acidosis is a common digestive disorder in dairy cows caused by feeding rapidly fermentable carbohydrates. The condition is characterized by a diurnal depression in ruminal pH due to the accumulation of VFA, and to a lesser extent lactic acid, within the rumen (Oetzel, 2000). A research model using cannulated dairy cows has defined SARA as a decline in ruminal pH below 5.6 for approximately 300 min/d (AlZahal et al., 2007a). Symptoms of SARA are variable, but often include depressed intake, resulting in poor body condition and reduced production (Plaizier et al., 2008), and may predispose cows to milk fat depression (AlZahal et al., 2009, 2010).

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Ruminant nutritionists have used a spectrum of feed additives to improve ruminal pH and maximize fiber degradation, namely by directly feeding active dry bacteria and yeast and yeast culture products. Active dry yeast products are manufactured in a manner to maintain a specific number of live cells ($>1.5 \times 10^{10}$ cfu/g of DM), thus preserving the majority of the products' ability to ferment forages.

The mechanisms proposed to explain the mode of action of active dry yeast within the rumen are mainly focused on optimizing fiber digestion [see review by Chaucheyras-Durand et al. (2008)]. Active dry yeast was proposed to survive for a short period of time within the rumen by utilizing traces of dissolved oxygen, which can be directly involved in fiber digestion and (or) can create an optimal anaerobic environment for bacterial growth. Live yeast was also proposed to create optimal growth conditions for bacteria by preventing the accumulation of lactic acid within the rumen (Nocek, 1997). Most commercially available active dry yeasts are based on strains of *Saccharomyces cerevisiae*. Yeast culture products, on the other hand, do not contain a guaranteed live yeast cell level but rather yeast fermentation by-products. Those by-products have been suggested to affect the growth of ruminal microbes (Callaway and Martin, 1997).

Studies that examined the effects of active dry yeast, exclusively, on performance and digestive characteristics of lactating ruminants are scarce and inconclusive. It was reported that active dry yeast supplementation can increase DMI and milk yield in early-lactation dairy cows (Wohlt et al., 1991) and dairy goats (Stella et al., 2007), whereas others reported no difference in dairy cattle (Swartz et al., 1994; Chiquette, 1995; Kung et al., 1997; Soder and Holden, 1999). The main variations among studies are likely due to differences in manufacturing processes, dosages and strains, and production systems. Additionally, the majority of available reviews of the literature, both the qualitative and the quantitative, did not differentiate between active dry yeast and yeast culture products, which limited the reliability of the results of such reviews. Studies evaluating the effect of active dry yeast on fermentation characteristics, including pH, are limited to in vitro methods, which were considered inappropriate for studying the effect of yeast on pH due to its high buffering capabilities (Carro et al., 1992).

To our knowledge, no studies exist in the literature that directly examined the effect of active dry *S. cerevisiae* (ADSC) on mitigating SARA by utilizing a nutritional SARA induction model, and concurrently assessed changes in ruminal microbes. Therefore, the objective of the current study was to determine the effect of *S. cerevisiae* supplementation on ruminal pH,

cow performance (DMI, milk yield, and milk components), and microbial community during a dietary regimen that leads to SARA.

MATERIALS AND METHODS

Animals, Treatments, and Feeding

Sixteen multiparous lactating Holstein cows (166 ± 30 DIM) were used in a randomized complete block design. Cows were randomly assigned into 1 of 2 blocks ($n = 8$ each) and then assigned to 1 of the 2 dietary treatments that included ADSC (Biomate; AB Vista, Marlborough, UK; strain 1242) or control. The layout of the experimental design and feeding regimen is depicted in Figure 1. The 2 blocks were conducted in a staggered manner with 1-wk difference to facilitate complex measurements. The daily allotments of ADSC were prepared weekly by mixing 4 g of the ADSC product (2×10^{10} cfu/g of DM) with 250 g of ground dry corn. The control diet contained the carrier only. Before starting the experiment, cows were maintained on a regular lactating-cow TMR fed twice daily (0700 and 1300 h) that consisted (DM basis) of 28% corn silage, 27% haylage, 6% straw, 20% high-moisture corn, and 19% protein supplement. The TMR included approximately 16% CP, 40% NFC, and 33% NDF. During the first 6 wk, all cows received a high-forage (HF) diet (Table 1). The HF diet was created by replacing 42% of the TMR (DM basis) with chopped hay. During wk 7, cows were switched abruptly to a high-grain (HG) diet (Table 1), and cows remained on the HG diet until the end of wk 10. The HG diet was created by replacing 20% of the TMR (DM basis) with wheat and barley pellets (1:1). Cows were allowed only 1 d of transition from the HF to the HG diet, where they received only 50% of the grain pellet allotment. The HF diet, transition diet, and HG diet were remixed in a Data Ranger (American Calan Inc., Northwood, NH) and fed twice daily as a TMR. The HG diet has been reported in previous studies to induce sustainable SARA [see, for example, AlZahal et al. (2009)], whereas the HF has been designed to induce optimal ruminal conditions. Cows were top-dressed during the morning feeding (wk 1 through 10) with either the ADSC or control diet. The amount of offered feed was adjusted daily to allow a maximum of 5 kg of orts/d (as-fed basis). Researchers and technical staff were blinded to the treatments. Ingredients and chemical analyses of TMR are presented in Table 1.

The cows were housed in a tie-stall facility at the Ponsonby Dairy Research Station (University of Guelph, Guelph, ON, Canada). All experimental procedures were approved by the University of Guelph

Week	Time 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8	week 9	week 10
Day	1	4				36 38		50 51			71 72 73
n = 8	TMR	High-forage + Control							High-grain + Control		
n = 7	TMR	High-forage + ADSC							High-grain + ADSC

Transition

Figure 1. Layout of the experimental design and feeding regimen. Prior to the start of the experiment, cows received no added yeast and were maintained on regular TMR. Days 1 to 3 were used as a baseline measurement. The experiment commenced on d 4 and ended on d 73. Cows were supplemented with either control (carrier only; n = 8) or active dry *Saccharomyces cerevisiae* (ADSC; 8×10^{10} cfu/head per day; n = 7) starting on d 4 and through d 73. On d 4 through 49, cows were fed the high-forage diet. On d 50, cows were transitioned to the high-grain diet by feeding only 50% of the allotted amount of grain. On d 51 and through d 73, cows were fed the high-grain diet to induce SARA. Ruminal pH was measured on days shaded in black. Ruminal digesta were assessed on days shaded in gray for VFA (d 38 and 73) and for microbial analysis (d 73).

Animal Care Committee (animal utilization protocol no. 12R050) in accordance with the Canadian Council on Animal Care (CCAC, 1993).

DMI, Feed Analysis, Milk Production, and Milk Components

Offered TMR, orts, and milk yield were recorded daily for individual cows starting 3 d before the start of the experiment [to obtain baseline measurements while on the regular TMR (time 0); Figure 1] and throughout the experiment (10 wk). Samples from TMR (HF and HG)

and orts were collected 3 times per week and frozen at -20°C until analysis. The TMR and orts samples were dried for 48 h in a forced-air oven at 60°C to determine DM content for that week. Subsamples of TMR were pooled every 2 wk and analyzed (Agri-Food Laboratories Inc., Guelph, ON, Canada) for CP, ash, ether extract, NDF (Mertens, 1997), ADF [procedures 4.2.08, 4.1.10, 4.5.01, and 4.6.03, respectively (AOAC International, 1996)], soluble CP (Licitra et al., 1996), and starch concentration (Hall, 2000). The chemical analyses of TMR are presented in Table 1. Cows were milked twice daily at 0500 and 1500 h, and milk samples were collected 3

Table 1. Ingredient composition and chemical analyses of high-forage (HF) and high-grain (HG) diets

Item	Diet	
	HF	HG
Ingredient, % of DM		
Corn silage	16.0	22.0
Alfalfa silage	16.0	22.0
Straw	3.6	4.9
High-moisture corn	11.7	16.0
Protein supplement ¹	11.0	15.1
Hay	41.7	0.0
Grain pellets ²	0.0	20.0
Chemical composition, % of DM (unless otherwise noted)		
DM, %	59.0	48.2
CP ($\text{N} \times 6.25$)	14.3	16.4
Soluble protein	6.4	5.3
ADIN	1.7	1.2
ADF	32.5	18.4
NDF	44.9	28.2
NFC ³	31.5	45.2
Starch ⁴	16.6	26.2
Ether extract	2.3	3.4
Ash	7.0	6.9
NE _L , ⁵ Mcal/kg	1.4	1.6

¹Supplement contained (as-fed basis) 9% high-protein corn gluten meal, 30% soybean meal 48 (containing 48% CP), 7% Tri-Pro Gold (Tri-County Protein Corp., Winchester, ON, Canada), 14% canola meal, 10% beat pulp, 3% herring meal, 4% dry corn distillers grains, 12% mineral mix, 5% soybean hulls, 2% molasses, and 3% tallow.

²Contained 2:1 wheat and barley.

³NFC = $100 - (\text{NDF} + \text{CP} + \text{ether extract} + \text{ash})$.

⁴Analyzed according to Hall (2000).

⁵Estimated according to NRC (2001).

times per week from morning and afternoon milkings. Milk samples were pooled by cow by day based on production, before being pooled by week using equal daily proportions, and submitted to Laboratory Services Division (Guelph, ON, Canada) for composition analysis using a near-infrared analyzer (Foss System 4000; Foss Electric A/S, Hillerød, Denmark).

Ruminal pH, Ruminal VFA, and Ruminal Digesta

Ruminal pH was measured and recorded continuously (1-min intervals) using a cordless indwelling pH electrode as described by AlZahal et al. (2007b). Briefly, a pH electrode was inserted through the cannula and connected to a data logger for data acquisition. The electrodes were attached a 0.5-kg stainless steel weight to ensure that they resided in the ventral sac of the rumen. The data were downloaded daily for pH evaluation and analysis. Ruminal pH was recorded on d 1 (regular TMR), d 36 (HF diet), d 50 (transition to the HG diet), d 51 (first day on the HG diet), and d 71 and 72 (on the HG diet).

Digesta grabs were collected from the ventral rumen at 1600 h on d 73 and filtered through 4 layers of cheesecloth into duplicate 50-mL screw-top tubes. The first allotment was snap-frozen in liquid nitrogen and stored at -20°C for VFA analysis. The concentration of VFA in ruminal digesta was quantified in duplicate using gas chromatography (Mutsaers et al., 2002). The second allotment was diluted 1:1 with 100% ethanol for microbial genomic DNA isolation.

Microbial DNA Isolation, Quality Assessment, and Real-Time Quantitative PCR

Approximately 1.5 mL of the ethanol-preserved rumen digesta samples were centrifuged at $12,000 \times g$ for 5 min at room temperature to create a pellet ($\sim 200 \mu\text{g}$). The microbial DNA was isolated from the rumen digesta pellet according to Yu and Morrison (2004). The protocol included three 1-min rounds of bead-beating (Mini-bead-beater-8; BioSpec Products Inc., Bartlesville, OK) for mechanical disruption of microbial cells. The beating was conducted at room temperature and at maximum speed with the inclusion of 0.4 g of zirconium beads (0.3 g of 0.1-mm + 0.1 g of 0.5-mm beads). The quality of total DNA extracted from digesta samples was assessed by 0.8% agarose gel electrophoresis and total DNA concentration was quantified using a NanoDrop spectrophotometer (ND-1000; NanoDrop Technologies Inc., Wilmington, DE).

The PCR primers used to amplify microbial 16S ribosomal DNA are listed in Table 2. The primers were assembled from the literature and tested for specificity

in silico. The primers that did not meet our criteria for selection were redesigned using Primer Express 3.0 software (Applied Biosystems Inc., Foster City, CA). The primers were synthesized by Sigma (The Woodlands, TX). Real-time PCR was carried out using an AB 7300 system (Applied Biosystems Inc.). Each reaction mixture was run in duplicate. Amplification reactions were carried out with Power SYBR green PCR master mix (Applied Biosystems Inc.) mixed with the selected primer set (Table 2).

Changes in targeted populations (fold changes) were calculated using a relative quantification calculation and the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001), with general bacteria (Denman and McSweeney, 2006) cycle threshold (C_t) values used as the reference and average ΔC_t of the control group as the calibrator value.

Statistical Analysis

Weekly average of DMI, milk yield, and milk components were analyzed using PROC MIXED of SAS (SAS Institute, 2011), using a model that included treatment (ADSC or control), time (wk 1, . . . , 10), treatment $\times$ time interaction, block (1 or 2), and treatment $\times$ block interaction as fixed effects. Measurements collected during time 0 (average of d 1 to 3) were used as a covariate. Week was used as a repeated measurement with cow as the subject. For each analyzed variable, cow was subjected to 5 covariance structures: compound symmetry, heterogeneous compound symmetry, autoregressive order 1 [AR(1)], heterogeneous autoregressive order 1, and unstructured covariance structure. The covariance that gave the smallest Bayesian information criterion (BIC) was used (Littell et al., 1996). Compound symmetry gave the smallest BIC values for milk component content and yield, whereas AR(1) gave smallest values for DMI and milk yield.

Ruminal pH data were downloaded from individual loggers and the time (min/d) below a pH threshold of 5.6 (<5.6) during any given day $\times$ cow was counted. The same model was used to analyze pH data on d 36 (HF diet), d 50 (transition to the HG diet), d 51 (first day on the HF diet), and average of d 71 and 72 (SARA plateau). The covariate consisted of d 1 measurement (on regular TMR; Figure 1). The covariance AR(1) gave the smallest BIC values and was used.

Orthogonal polynomial contrasts were used to compare means of treatment (ADSC vs. control) for DMI, milk yield and components, and ruminal pH within each dietary phase (HF, HG, or transition where applicable).

The PROC TTEST (SAS) was used to test whether mean ruminal pH <5.6 (min/d) of a given group was different from 0 and ≥ 300 min/d (for example, 300,

Table 2. Primers used for real-time PCR quantification

Target organism	Primer	Sequence (5'→3')	T _m	Amplicon size, bp	Source of primer
General bacteria	1114	CGGCAACGAGCGCAACCC	60	130	Denman and McSweeney (2006)
	1275	CCATTGTAGCACGTGTGTAGCC	60		
General anaerobic fungi	Fungi_F	GAGGAAGTAAAAGTCGTAACAAGGTTTC	60	120	
	Fungi_R	CAAATTCAAAAAGGGTAGGATGATT	60		
Ciliate protozoa	P-SSU-316f	GCTTTCGWTGGTAGTGATT	55	223	Sylvester et al. (2004)
	p-SSU-539r	ACTTGCCCTCYAATCGTWCT	55		
Yeast (<i>Saccharomyces cerevisiae</i>)	Yeast_F	ACAGACACAGAGCCCGAACAT	60	72	This study ¹
	Yeast_R	TGCCCAGAGGGAGTATTCACA	60		
<i>Anaerovibrio lipolytica</i>	AnaLip2F	TGGGTGTTAGAAATGGATTCTAGTG	56.6	109	Khafipour et al. (2009)
	AnaLip2R	GCACGTCATTTCGGTATTAGCAT	57.7		
<i>Butyrivibrio fibrisolvens</i>	BfiBF	ACACACCGCCGTCACA	55	64	Klieve et al. (2003)
	BfiBR	TCCTTACGGTTGGGTCACAGA	55		
<i>Escherichia coli</i>	EcoliFimH2F	GCCGGTGGCGCTTTATTTG	57.3	114	Khafipour et al. (2009)
	EcoliFimH2R	TCATCGCTGTTATAGTTGTTGGTCT	58.4		
<i>Fibrobacter succinogenes</i>	FibSuc4F	GGAGCGTAGGCGGAGATTCA	58.7	97	
	FibSuc4R	GCCTGCCCCGTAACATATCCA	58.5		
<i>Lactobacillus</i> spp.	Ula16S1F	AGCAGTAGGGAATCTTCCA	51.5	345	Lan et al. (2004)
	Ula16S1R	ATTCCACCGCTACACATG	51.1		
<i>Megasphaera elsdenii</i>	MegEls1F	GACCGAAACTGCGATGCTAGA	57.7	129	Ozutsumi et al. (2006)
	MegEls1R	CGCCTCAGCGTCAGTTGTC	58.2		
<i>Prevotella albensis</i>	ProAlb4F	GCGCCACTGACGCTGAAG	58.3	110	Khafipour et al. (2009)
	ProAlb4R	CCCCAAATCCAAAAGGACTCAG	56.6		
<i>Prevotella brevis</i>	PreBre2F	GCGAACTGGTTTCTTGAGTGTATT	58.8	153	
	PreBre2R	ACCTTCGAGCTTTAGCGTCAGTTAT	57.6		
<i>Prevotella bryantii</i>	ProBry4F	GAAGGCAGCTCGCTGTAGTGTT	60.6	145	
	ProBryR	CTTAACGCTTTCGCTTAGCCACT	59.4		
<i>Ruminococcus albus</i>	RumAlb1F	CCCTAAAAGCAGTCTTAGTTTCG	54.3	176	
	RumAlb1R	CCTCCTTGCGGTTAGAACA	53.8		
<i>Ruminococcus flavefaciens</i>	RF_new_F	CGAACGGAGATAATTTGAGTTTACTTAGG	60	132	Denman and McSweeney (2006)
	RF_new_R	CGGTCTCTGTATGTTATGAGGTATTACC	60		
<i>Selenomonas ruminantium</i>	SelRum2F	CAATAAGCATTCGCGCTGGG	61	71	Stevenson and Weimer (2007)
	SelRum2R	TTCACCTCAATGTCAAGCCCTGG	61		
<i>Streptococcus bovis</i>	StrBov2F	TTCCTAGAGATAGGAAGTTTCTTCGG	59	82	
	StrBov2R	ATGATGGCAACTAACAAT AGGGGT	59		
<i>Succinimonas amylolytica</i>	SucAmy2F	CGTTGGGCGGTCATTTGAAAC	55.2	139	Khafipour et al. (2009)
	SucAmy2R	CCTGAGCGTCAGTTACTATCCAGA	56.2		
<i>Succinivibrio dextrinosolvens</i>	SucDex1F	CGTCAGCTCGTGTCGTGAGA	60	80	Stevenson et al. (2007)
	SucDex1R	CCCGCTGGCAACAAAGG	60		

¹Designed based on National Center for Biotechnology Information (NCBI) accession no. Y08934 adopted from (Castillo-Lopez et al., 2010) using Primer Express 3.0 software (Applied Biosystems Inc., Foster City, CA).

400, . . . , n min/d). A given treatment group was considered as having SARA if ruminal pH <5.6 (min/d) was different from 0 but not different from ≥ 300 min/d and vice versa.

Ruminal VFA data were analyzed using PROC MIXED of SAS, the model included treatment (ADSC or control), time (d 38 and 73 on the HF and HG diet, respectively), block, and their interactions as fixed effects. Orthogonal polynomial contrasts were used to separate the means. The PROC TTEST of SAS was used to test if the relative fold change in the concentration of individual microbial 16S ribosomal DNA was significant from 0 (no change due ADSC). Data were logarithmically ($\log_2$) transformed before analysis.

RESULTS

Ruminal pH

Ruminal pH was assessed during time 0 (on regular TMR), wk 5 (HF), and wk 11 (HG; Figure 1). Ruminal pH measurement during time 0 was used as a covariate in the analysis, and also was used to exclude any individual cow with preexisting SARA. This criteria was used to comply with our objective, which was to examine the effect of ADSC supplementation during SARA induction. Cows were considered to have SARA if the duration below pH 5.6 was ≥ 300 min/d (AlZahal et al., 2007a). One cow in the ADSC group (cow no. 3641) was deemed to have preexisting SARA and was removed from the trial. Ruminal pH (min/d <5.6 ; Figure 2) during wk 6 (HF plateau) was not different across treatments and both treatment groups were not different from 0 min/d but different from ≥ 300 min/d ($P < 0.001$), which indicated that both treatment groups did not have SARA. The dietary regimen successfully induced SARA during wk 7 (transition from the HF to HG diet) in both treatment groups (pH <5.6 were not different from ≥ 300 min/d). Additionally, ruminal pH (min/d <5.6) was not different across treatments. The dietary model to induce SARA has been used successfully in previous studies (AlZahal et al., 2009, 2010) and has been shown to induce sustainable depression in ruminal pH without causing a drastic impairment of rumen function or intake. Furthermore, after 3 wk of HG feeding (wk 10), cows receiving ADSC had higher ruminal pH compared with controls (122 ± 57 vs. 321 ± 53 min/d <5.6 , respectively; $P = 0.01$). Cows supplemented with ADSC were considered to have recovered from SARA within 3 wk (pH <5.6 not different from 0 min/d but different from ≥ 300 min/d; $P = 0.02$); whereas cows receiving the control diet did not recover from SARA

(pH <5.6 not different from ≥ 300 min/d but different from 0 min/d; $P < 0.001$).

DMI, Milk Yield, and Milk Components

During HF feeding (wk 1 through 6), average weekly DMI (Figure 3a) and milk yield and parameters (Table 3) did not differ across treatments, with the exception of lactose content, as cows receiving ADSC had a slightly greater ($P = 0.04$) lactose content compared with controls. Nonetheless, lactose yield (kg/d) did not differ across treatments. During the transition week to HG (SARA induction week), milk yield and parameters were not different across treatments and, similarly to the previous phase, lactose content was greater ($P = 0.01$) in cows receiving ADSC compared with controls (Table 3). However, during wk 7, cows that received the control diet had lower DMI (Figure 3a; $P = 0.01$). During the HG feeding phase (wk 8, 9, and 10), cows receiving the control diet continued to have lower ($P = 0.03$) DMI (kg/d; Figure 3a). The observed difference in intake was reflected by a reduction in fat yield (kg/d; Table 3; $P = 0.01$) and 4% FCM (kg/d; Figure 3b; $P = 0.03$). No difference was detected in other measured milk parameters.

Ruminal VFA

The concentration of ruminal VFA was assessed during wk 5 and 10. No difference across treatments were detected during HF feeding in total VFA (115 ± 7.5 vs. 107 ± 7.5 mM for the ADSC and control diet, respectively) and most measured VFA (mM; Figure 4), with the exception of butyrate and the acetate:propionate (A:P) ratio, as cows receiving ADSC had greater butyrate concentration (mM; Figure 4; $P = 0.04$) and a smaller A:P ratio (0.88 ± 0.5 vs. 1.09 ± 0.05 for ADSC and control diet, respectively; $P = 0.01$) compared with controls. The shift to HG increased the concentration (mM) of total VFA ($P < 0.001$; data shown below) and also increased the concentration (mM) of propionate ($P < 0.001$), butyrate ($P = 0.001$), valerate ($P < 0.001$), and isovalerate ($P = 0.01$), and reduced the concentration of acetate ($P < 0.001$; Figure 4). However, the change with HG feeding was more pronounced for cows receiving ADSC on total VFA (175 ± 7.5 vs. 154 ± 7.5 mM for the ADSC and control diet, respectively) and propionate concentration (mM; Figure 4). Furthermore, the A:P ratio was reduced ($P < 0.001$) with HG feeding and cows receiving ADSC maintained a significantly smaller A:P ratio (0.26 ± 0.5 vs. 1.36 ± 0.05 for the ADSC and control diet, respectively). The HG feeding significantly reduced the concentration (mM)

Figure 2. Least squares means of ruminal pH (time below 5.6; min/d) during high-forage feeding, transition (50:50), and high-grain feeding. Cows were either supplemented with control (carrier only; $n = 8$; white bars) or active dry *Saccharomyces cerevisiae* (8×10^{10} cfu/head per day; $n = 7$; gray bars). Cows were considered as having SARA when the pH exceeded 300 min/d below 5.6 (dotted line). Error bars represent SE.

of isobutyrate only for those cows receiving the ADSC supplement (Figure 4).

Ruminal Microbes

Microbial analyses conducted on samples collected during wk 10 showed that cows supplemented with ADSC had a 9-fold difference in *S. cerevisiae* population ($P = 0.003$) compared with controls. Additionally, cows supplemented with ADSC had a 6-fold increase in *Anaerovibrio lipolytica* ($P = 0.05$) and a 2-fold increase in *Fibrobacter succinogenes* ($P = 0.03$), and had a tendency for a 1.3-fold increase in *Ruminococcus albus* ($P = 0.01$) and an 8-fold increase in anaerobic fungi ($P = 0.09$) relative to controls. On the other hand, cows supplemented with ADSC had a 2.2-fold reduction in *Prevotella albensis* ($P = 0.01$). Cows supplemented with ADSC had a 2.3-fold increase in *Streptococcus bovis* ($P = 0.05$) and a tendency ($P = 0.08$) for a 12-fold reduction in *Megasphaera elsdenii*.

DISCUSSION

We hypothesized that supplementing cows with ADSC would mitigate SARA. This mitigation was proposed to alleviate the effect of abrupt changes from the HF to HG diet on ruminal pH, intake, and production. The experiment was designed to allow the cows to acclimatize to an HF diet and thus create optimum

rumen conditions before inducing SARA. Cows were either supplemented with the ADSC or control diet while on the HF diet and before switching to HG to assess the ability of the treatment groups to withhold SARA. Ruminal pH measured during HF feeding did not decrease below 5.6 (Figure 2), which indicated that cows did not have SARA (AlZahal et al., 2007a). Dry matter intake and milk yield and parameters were similar between the treatment groups and were typical for mid-lactation Holstein cow (Table 3; Figure 1). Cows were abruptly changed from HF to HG to mimic field conditions where, in most of the cases, managerial errors or sudden dietary changes (i.e., postcalving) were to blame for causing SARA. We allowed only 1 d of transition to ensure that cows did not get severe depression in ruminal pH and (or) get off feed. The SARA induction regimen relied on feeding a TMR twice daily; however, this TMR contained a high proportion of grains (high-moisture corn, wheat, and barley pellets). This model has been shown in previous experiments to induce chronic SARA (AlZahal et al., 2009, 2010; Dionissopoulos et al., 2013). Ruminal pH measured on d 51 (first day on the HG diet; Figure 2) were decreased for over 500 min/d to below 5.6, with no differences between treatments. The first few days after dietary shifts are considered critical as cows attempt to cope with the new condition via several physiological processes, such as limiting intake to reduce ruminal acid load (Plai-zier et al., 2008). However, although both treatments

Figure 3. Least squares means of DMI (a; kg/d) and 4% FCM (b; kg/d) during high-forage feeding, transition week (50:50), and high-grain feeding. Cows were either supplemented with control (carrier only; n = 8; white bars) or active dry *Saccharomyces cerevisiae* (8×10^{10} cfu/head per day; n = 7; gray bars). Error bars represent SE.

groups had a depressed ruminal pH during the first week of HG feeding; cows receiving ADSC had greater intake, which may indicate that ADSC-supplemented sustained better rumen function. To fulfill the objective of this study, cows remained approximately for 3 wk on the HG diet, and the cows' performance and ruminal parameters were assessed toward the end of the third week (plateau phase). Interestingly, results showed that cows supplemented with ADSC had a significantly higher pH compared with nonsupplemented cows. Ruminal pH of ADSC-supplemented cows, as measured by the duration of pH <5.6, was not indicative of SARA, whereas nonsupplemented cows had a depressed ruminal pH indicative of SARA (≥ 300 min/d at pH <5.6; AlZahal et al., 2007a). Our results were in agreement

Table 3. Least squares means of weekly averages (where applicable) of milk yield (kg/d) and composition during high-forage (HF) feeding, transition to high-grain (HG) diet (cows were offered 50% of allotted grains on d 4 of wk 7 and 100% of the HG diet on d 5 through the 7 of that week), and HG feeding phases

Item	Period and treatment					Period and treatment					Period and treatment				
	wk 1 to 6 (HF)		wk 7 (transition to HG)			wk 8 to 10 (HG)		wk 7 (transition to HG)			wk 8 to 10 (HG)		wk 7 (transition to HG)		
	Control	ADSC	SE ²	P-value		Control	ADSC	SE	P-value		Control	ADSC	SE	P-value	
Milk yield, kg/d	28.7	28.5	1.05	0.83		28.6	28.4	1.05	0.87		32.2	33.4	2.10	0.35	
Fat, %	3.68	3.69	0.17	0.93		3.96	3.80	0.18	0.53		2.82	3.05	0.34	0.22	
Protein, %	3.05	3.00	0.03	0.10		3.09	3.08	0.03	0.82		3.29	3.29	0.06	0.99	
Lactose, %	4.50	4.57	0.032	0.04		4.40	4.53	0.032	0.01		4.57	4.62	0.06	0.16	
Fat yield, kg/d	1.06	1.12	0.06	0.27		1.16	1.15	0.06	0.93		0.91	1.08	0.125	0.01	
Protein yield, kg/d	0.90	0.88	0.03	0.57		0.91	0.90	0.03	0.89		1.08	1.13	0.07	0.22	
Lactose yield, kg/d	1.34	1.35	0.05	0.88		1.30	1.33	0.05	0.69		1.52	1.61	0.11	0.24	

¹Cows were either supplemented with active dry *Saccharomyces cerevisiae* (ADSC; 8×10^{10} cfu/head per day; n = 7) or control (carrier only; n = 8).

²SE = pooled SE.

Figure 4. Rumen concentration (mM) of acetate (a), propionate (b), butyrate (c), isobutyrate (d), valerate (e), and isovalerate (f) during high-fiber (measured on d 38) and high-grain diet (measured on d 73) feeding. Cows were either supplemented with control (carrier only; n = 8; white bars) or active dry *Saccharomyces cerevisiae* (8×10^{10} cfu/head per day; n = 7; gray bars). Bars labeled with different letters within a given chart are different ($P < 0.05$). Error bars represent SE.

with Al-Ibrahim et al. (2012), who demonstrated that ADSC supplementation (2.5×10^9 cfu/head per day) increased ruminal pH in cows when abruptly or gradually switched to pasture postcalving compared with nonsupplemented cows (2.3 vs. 3.5 h at pH <5.8, respectively). Contrary to our results, Chung et al. (2011) tested 2 different strains of ADSC (1×10^{10} cfu/head per day) and reported that strain 1 had no effect on ruminal pH, whereas strain 2 reduced ruminal pH compared with controls (1.0, 7.5, and 3.3 h/d at pH <5.6, respectively). However, variations in ruminal pH among studies may be attributed to the use of different sampling techniques (rumenocentesis and stomach tubing vs. cannulation), number of samples collected (few per day vs. continuous recording every minute), or methodology of summarizing pH data (daily mean, minimum vs. duration below a given pH threshold). Nonetheless, it is well documented that using continuous recording of ruminal pH via an indwelling probe and summarizing ruminal pH as duration below 5.6 is the most reliable method to define ruminal pH (Plaizier et al., 2008).

The greater intake observed in the current study for ADSC-supplemented cows was sustained as the mean weekly average (wk 8 through 10) was significantly greater than for control cows. It is noticeable that the cows, in general, consumed significantly more feed while on HG compared with HF (week main effect), likely due to the higher fiber content in the HF diet (Table 1; Allen, 1997). The improved ruminal conditions and intake was associated, in the current study, with an increase in fat yield and 4% FCM (Figure 3). The effect of ADSC on DMI and milk yield as was demonstrated in the Introduction section has been shown to be highly variable. Increased intake or diet fermentability can translate to greater OM available for ruminal fermentation and, thus, greater VFA concentration and lower ruminal pH within the rumen (Allen, 1997). In the current study, feeding HG increased total VFA concentration and consequently reduced ruminal pH. However, cows supplemented with ADSC had less pH depression (Figure 2) than nonsupplemented cows despite their greater increase in total VFA concentration, suggesting an increase in ruminal buffering capacity (Giger-Reverdin et al., 2004) in ADSC-supplemented cows.

Several mechanisms have been proposed to explain the mode of action of live ADSC within the rumen. The most acceptable mechanism was optimizing fiber digestion through eliminating dissolved oxygen within the rumen and improving lactate metabolism (production and utilization), thus creating an optimal environment for cellulolytic microbes (Chaucheyras-Durand et al., 2008). Although the rumen environment is strictly

anaerobic, Newbold (1995) reported that 16 L/d can enter the rumen through feed and water consumption and salivation. In the current experiment, *S. cerevisiae* numbers were 9-fold greater relative to nonsupplemented cows (Figure 4), which confirms that *S. cerevisiae* can indeed proliferate within the rumen. This increase in *S. cerevisiae* was associated with an increase in bacterial species known to degrade fiber within the rumen, such as *F. succinogenes*, *A. lipolytica*, and *R. albus*. It has been shown that the activity and growth of 3 major cellulolytic bacteria (*F. succinogenes*, *R. albus*, and *Ruminococcus flavefaciens*) in the rumen of sheep were stimulated by ADSC supplementation (Fonty and Chaucheyras-Durand, 2006). This is in agreement with the current study, with the exception of *R. flavefaciens*. The current study showed no effect of ADSC supplementation on that bacterium. Additionally, the current results demonstrated an increase in anaerobic fungi with ADSC supplementation, which further supports the effect of ADSC supplementation in improving fiber degradation. However, the relationship between *S. cerevisiae* and anaerobic fungi growth has not been confirmed in vivo (Chaucheyras-Durand et al., 2008). The available literature on in vitro studies suggests that ADSC can enhance the colonization of specific anaerobic fungal strains (Chaucheyras et al., 1995).

It was suggested that ADSC can improve rumen function and increase ruminal pH by increasing lactate utilization (Nocek, 1997). In vitro studies have reported that live ADSC can stimulate the growth of lactate-utilizing bacteria such as *M. elsdenii* and *Selenomonas ruminantium* and consequently reduce lactate concentration [see review by Chaucheyras-Durand et al. (2008)]. However, it was reported that the level of lactate within the rumen of dairy cattle plays little or no role in the etiology of SARA, given its low accumulation within the rumen (Plaizier et al., 2008). For example, Chung et al. (2011) induced acidosis in dairy cattle and reported that ruminal lactate concentration, on average, never exceeded 0.5 mM throughout the day. Lactate was not measured in the current study but the high variation in *M. elsdenii* bacteria observed among animals suggests that *M. elsdenii* was not affected by ADSC supplementation. A recent study by Pinloche et al. (2013) demonstrated that cows fed a diet that consisted of 61% corn silage and 39% concentrate (DM basis) and supplemented with either ADSC (0.5 to 5×10^{10} cfu/head per day) had greater VFA concentration, higher pH, and lower lactate compared with the control, and that those changes were associated with increased abundance of a main fibrolytic group (*Fibrobacter* and *Ruminococcus*) and, contrary to our results, lactate-utilizing bacteria (*Megasphaera* and *Selenomonas*). Those authors supported the hypothesis proposed to explain

Figure 5. Changes ($\log_2$) in ruminal microbes (stated at the y-axis) comparing cows supplemented with control (carrier only; $n = 8$) with cows receiving *Saccharomyces cerevisiae* (8×10^{10} cfu/head per day; $n = 7$) during high-grain feeding (samples collected on d 73). Error bars represent SE.

the modes of action of ADSC, being improved fiber degradation and reduced lactate within the rumen. However, our results lend support only for improved fiber degradation.

Additionally, ADSC (4×10^{10} cfu/head per day) was reported to enhance the numbers of ciliate protozoa within the rumen of sheep (Brossard et al., 2006). Ciliate protozoa can compete with amylolytic bacteria on

substrate (starch), digest it slower, and produce VFA rather than lactate as an end product (Williams and Coleman, 1997). Therefore, it was suggested that ADSC may improve ruminal pH by enhancing the numbers of ciliate protozoa (Chaucheyras-Durand et al., 2008). Our results (Figure 5) showed no effect of ADSC on ciliate protozoa abundance.

Furthermore, in the current study, cows supplemented with ADSC had 2.2-fold reduction in *P. albensis*, which is a gram-negative bacterium predominant during SARA (Khafipour et al., 2009). *Prevotella* spp. are suggested to be an important source of LPS responsible for inflammation within the rumen and likely the incidence of lameness (Plaizier et al., 2008). Therefore, a reduction in gram-negative bacteria with ADSC supplementation is expected to have positive consequences on cow health. The ADSC supplementation increased *S. bovis*, a lactate-producing bacterium in spite of the increase in ruminal pH. Cows supplemented with ADSC had greater intake and, thus, greater starch intake. Therefore, it is speculated that this increase was a reflection of substrate availability.

More studies are needed to evaluate the effect of live yeast on rumen ecology and function, taking into consideration several factors that might interact with yeast, such as diet type, stage of lactation, and environment. Differences between strains and dosage should also be considered. The interaction between yeast and the host animal should be examined, namely during the onset of SARA. Recent studies have suggested that variation among animals during SARA may be explained by differences in ruminal epithelium structure (Steele et al., 2011), gene expression related to FA metabolism (Penner et al., 2009; Steele et al., 2012), or innate immune responses (Dionissopoulos et al., 2014). In conclusion, our results demonstrated that supplementing lactating dairy cows with a live yeast product can reverse the progress of SARA and its associated negative effect on DMI and milk production. The study suggested a positive effect of live yeast on fiber-degrading bacteria as the mode of action, but provided no evidence of enhanced lactate utilization within the rumen.

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