Effects of Saccharomyces cerevisiae fermentation product on in vitro fermentation and microbial communities of low-quality forages and mixed diets

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J ANIM SCI 2013, 91:3291-3298.
doi: 10.2527/jas.2012-5851 originally published online April 9, 2013

Effects of *Saccharomyces cerevisiae* fermentation product on in vitro fermentation and microbial communities of low-quality forages and mixed diets[1]

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ABSTRACT: Two experiments were conducted to investigate the effects of a *Saccharomyces cerevisiae* fermentation product (XP, Diamond V, Cedar Rapids, IA) on in vitro ruminal fermentation of single forage and mixed diets. In Exp. 1, an in vitro test was used to determine the effects of various concentrations (0, 1, 2, and 3 g/L) of XP on ruminal fermentation of the major forage sources of China (rice straw, RS; corn stover, CS; corn silage without grain, CSNG; and corn silage with grain, CSG). Total VFA reached a peak at 1 g/L XP for RS, CSNG, and CSG and increased linearly ($P < 0.01$) for CS. The molar proportion of acetate decreased and propionate increased linearly ($P < 0.01$) with an increasing amount of XP for RS, CS, and CSNG. Microbial protein (MCP) increased linearly ($P < 0.01$) with an increasing level of XP for RS, and it reached peak values at 1 and 2 g/L XP for CSG and CSNG, respectively. Fungi population was increased ($P < 0.05$) with 1 g/L XP for all forages except CSNG. The population of *Ruminococcus flavefaciens* increased ($P < 0.05$) at 1 or 2 g/L XP for RS, CSNG, and CSG. In Exp. 2, the effects of 3 concentrations of XP (0, 1, and 2 g/L) were tested on in vitro ruminal fermentation of 3 mixed diets with various ingredient combinations: 1) CSC (corn:soybean meal:corn stover = 33:22:45), 2) CSCC (corn:soybean meal:corn stover:corn silage = 33:22:22.5:22.5), and 3) CSCCA (corn:soybean meal:corn stover:corn silage:alfalfa = 33:22:19:21:5). Total VFA concentrations were influenced by diets ($P < 0.01$) and were enhanced linearly by increasing concentrations of XP ($P < 0.01$). The molar proportion of acetate was reduced ($P < 0.01$), but the propionate proportion was enhanced with increasing concentrations of XP ($P < 0.01$). Ammonia N was decreased and MCP was increased by the addition of XP (linear, $P < 0.01$; quadratic, $P < 0.05$). The fungi population was greater with XP addition (quadratic, $P < 0.01$). The percentage of *R. albus* was affected by diets ($P < 0.01$), the level of XP (linear and quadratic, $P < 0.01$), and their interaction ($P < 0.01$). From these 2 in vitro studies, it is inferred that the addition of XP could improve the rumen fermentation of forages and mixed diets by stimulating the number of fiber-digesting rumen microbes, especially fungi populations.

Key words: low-quality forages, microbial population, mixed diets, rumen fermentation, yeast culture

J. Anim. Sci. 2013.91:3291–3298
doi:10.2527/jas2012-5851

INTRODUCTION

The increasing demand for greater milk production for human consumption requires cows to consume energy-dense diets. It is critical to provide the proper amount of good quality forages to maintain rumen health and production efficiency of cows. Failure to do so could result in clinical and subclinical forms of metabolic diseases. Nutritional strategies need to be developed to maintain increased milk production without causing greater incidences of metabolic diseases. This is particularly of importance when availability of good quality forages is limited.

A *Saccharomyces cerevisiae* fermentation product (**XP**, Diamond V, Cedar Rapids, IA) has been shown to improve ruminal fermentation and fiber digestion in a cost-effective manner. The XP contains residual yeast cells, fermentation metabolites, and growth media. Its basic product composition is 12% CP, 3%

[1]This study was funded partly by the Diamond V, Cedar Rapids, IA, and China Agriculture Research System (CARS-37).
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Received September 12, 2012.
Accepted March 20, 2013.

Table 1. Chemical composition of mixed diets

Treatment[1]	Composition,[2] %			
	DM	CP	NDF	ADF
CSC	87.4	15.2	41.8	20.5
CSCC	88.9	15.4	40.2	15.0
CSCCA	89.0	15.7	39.1	15.2

[1]CSC = mixture of corn, soybean meal, and corn stover at ratios of 33%, 22%, and 45%; CSCC = mixture of corn, soybean meal, corn stover, and corn silage at ratios of 33%, 22%, 22.5%, and 22.5%; CSCCA = mixture of corn, soybean meal, corn stover, corn silage, and alfalfa at ratios of 33%, 22%, 19%, 31%, and 5%.

[2]The chemical compositions of mixed diets were calculated according to the chemical analysis and inclusion rate of ingredients as above. Chemical composition (DM as %; other composition as % DM) of individual components are as follows: corn: DM 89.8, CP 8.93, ADF 6.4, and NDF 19.5; soybean meal: DM 89.9, CP 42.3, ADF 9.4, and NDF 24.7; corn stover: DM 84.4, CP 6.5, ADF 36.2, and NDF 66.5; corn silage: DM 91.2, CP 7.57, ADF 11.9, and NDF 59.6; alfalfa: DM 88.7, CP 13.2, ADF 32.8, and NDF 41.6.

crude fat, 6.5% crude fiber, 6.5% ash, and 11% moisture (product profile of Original XP, Diamond V). A recent meta-analysis conducted by Poppy et al. (2012) has shown that XP increases milk yield, DMI, and feed use of lactating dairy cows. These effects of XP may be due to the stimulating growth of fiber-digesting bacteria (Callaway and Martin, 1997) and lactate-utilizing bacteria and increasing microbial protein flow to the duodenum (Hristov et al., 2010). Supplementing growth factors, such as organic acids, B vitamins, and AA from XP, might provide greater benefits for rumen microbes under some conditions compared with others.

The limited supply of high-quality forage is a major challenge for the Chinese dairy industry. Improved utilization of local forage sources would enhance the production efficiency by maintaining a high level of milk production and cow health and reducing production costs. Therefore, 2 in vitro studies were designed to examine the effects of XP on ruminal fermentation and microbial communities when major forage sources from China were used as substrates individually or in combination.

MATERIALS AND METHODS

Experimental Design

In Exp. 1, the effects of varying levels of XP on ruminal fermentation of major forage sources from China (rice straw, **RS**; corn stover, **CS**; corn silage without grain, **CSNG**; and corn silage with grain, **CSG**) were determined in an in vitro experiment with a single factorial arrangement of treatments. Additions were designed to contain 1 of 4 concentrations of XP (0, 1, 2, and 3 g/L of fermentation liquid) for each forage type. These amounts are approximately equivalent to 0, 60, 120, and 180 g/d feeding rates for dairy cows.

Table 2. Polymerase chain reaction primers for real-time PCR assay

Target species	Direction	Primer sequence
Total bacteria[1]	Forward	CGGCAACGAGCGCAACCC
	Reverse	CCATTGTAGCACGTGTGTAGCC
Methanogens[2]	Forward	TTCGGTGGATCDCARAGRGC
	Reverse	GBARGTCGWAWCCGTAGAATCC
Fungi[1]	Forward	GAGGAAGTAAAAGTCGTAACAAGGTTTC
	Reverse	CAAATTCACAAAGGGTAGGATGATT
Protozoa[3]	Forward	GCTTTCGWTGGTAGTGTATT
	Reverse	CTTGCCCTCYAATCGTWCT
Ruminococcus flavefaciens[1]	Forward	CGAACGGAGATAATTTGAGTTTACTTAGG
	Reverse	CGGTCTCTGTATGTTATGAGGTATTACC
R. albus[4]	Forward	CCCTAAAAGCAGTCTTAGTTCG
	Reverse	CCTCCTTGCGGTTAGAACA
Fibrobacter succinogenes[1]	Forward	GTTCGGAATTACTGGGCGTAAA
	Reverse	CGCCTGCCCCTGAACTATC

[1]Cited from Denman and McSweeney (2006).

[2]Cited from Denman et al. (2007).

[3]Cited from Sylvester et al. (2004).

[4]Cited from Koike and Kobayashi (2001).

In Exp. 2, the effects of 3 levels of XP (0, 1, and 2 g/L) were examined on ruminal fermentation of 3 substrates of mixed ingredients: 1) **CSC** (corn:soybean meal:corn stover = 33:22:45), 2) **CSCC** (corn:soybean meal:corn stover:corn silage = 33:22:22.5:22.5), and 3) **CSCCA** (corn:soybean meal:corn stover:corn silage:alfalfa = 33:22:19:21:5). The chemical composition of the mixed diet is shown in Table 1. In both experiments, XP was mixed with the substrates before the commencement of the experiment.

In Vitro Fermentation

In vitro fermentation studies were conducted in triplicate. In each incubation run, 3 blanks were included simultaneously to provide a baseline for all results. Rumen fluid was collected from 3 donor sheep fed a mixed diet of lucerne hay and a concentrate mixture (50:50, wt/wt) fed twice daily. Ingredients (% DM) of the concentrate mixture are as follows: corn (50), wheat bran (15), soybean meal (15), rapeseed meal (13), dicalcium phosphate (2.0), salt (1.5), calcium carbonate (1.5), and vitamin–trace mineral premix (2) formulated to provide (per kg DM) 1,200,000 IU of vitamin A, 280,000 IU of vitamin D, 5,000 mg of vitamin E, 14,000 mg of Zn, 100 mg of Se, 200 mg of I, 3,000 mg of Fe, 60 mg of Co, 3,500 mg of Mn, and 3,000 mg of Cu.

Rumen fluid (10 mL) was injected into 180-mL bottles containing 90 mL of buffered medium (Theodorou et al., 1994) and 750 mg forage substrates (1 mm) at 39°C under anaerobic conditions.

Table 3. Effect of a *Saccharomyces cerevisiae* fermentation product (XP, Diamond V, Cedar Rapids, IA) on pH and VFA production on low-quality forage substrates (Exp. 1)

	XP, g/L					Effect	
Items	0	1	2	3	SEM	Linear	Quadratic
Rice straw							
pH	6.67[b]	6.73[b]	6.72[b]	6.82[a]	0.015	ns	**
Total VFA, mmol/L	14.9[ab]	19.6[a]	17.5[a]	10.1[b]	1.53	*	**
Molar proportion, %							
Acetate	68.3[a]	66.3[b]	65.4[b]	66.2[b]	0.38	**	*
Propionate	26.3[c]	27.3[bc]	27.6[b]	28.9[a]	0.36	**	ns
Butyrate	5.39[b]	6.38[a]	6.99[a]	4.84[b]	0.178	ns	**
Acetate:propionate	2.60[a]	2.43[b]	2.37[b]	2.29[b]	0.044	**	ns
Corn stover							
pH	6.71[a]	6.67[b]	6.69[ab]	6.62[c]	0.012	*	**
Total VFA, mmol/L	18.7[b]	24.0[b]	26.9[ab]	37.8[a]	3.49	**	ns
Molar proportion, %							
Acetate	75.6[a]	72.4[b]	70.8[c]	67.9[d]	0.30	**	ns
Propionate	19.9[d]	21.7[c]	23.0[b]	25.4[a]	0.35	**	ns
Butyrate	4.55[b]	5.93[a]	6.15[a]	6.75[a]	0.281	**	ns
Acetate:propionate	3.80[a]	3.35[b]	3.07[c]	2.67[d]	0.064	**	ns
Corn silage without grain							
pH	6.74[b]	6.68[c]	6.69[c]	6.79[a]	0.012	**	ns
Total VFA, mmol/L	21.1[b]	27.8[a]	26.6[a]	16.7[c]	1.02	*	**
Molar proportion, %							
Acetate	68.9[a]	66.1[a]	55.0[b]	52.4[b]	0.86	**	ns
Propionate	25.6[b]	27.5[b]	35.6[a]	38.6[a]	0.87	**	ns
Butyrate	5.46[c]	6.42[b]	9.45[a]	9.31[a]	0.221	**	*
Acetate:propionate	2.69[a]	2.42[a]	1.55[b]	1.36[b]	0.101	**	ns
Corn silage with grain							
pH	6.75[a]	6.64[b]	6.64[b]	6.62[b]	0.017	**	*
Total VFA, mmol/L	23.7[b]	31.3[a]	25.9[b]	28.9[ab]	1.59	*	**
Molar proportion, %							
Acetate	65.5[ab]	64.0[b]	62.2[c]	66.5[a]	0.45	ns	**
Propionate	27.0[bc]	28.0[ab]	28.9[a]	26.4[c]	0.349	ns	**
Butyrate	7.54[bc]	8.05[b]	8.86[a]	7.11[c]	0.146	ns	**
Acetate:propionate	2.42[ab]	2.29[bc]	2.16[c]	2.52[a]	0.043	ns	**

[a–d]Means within a row with different superscripts differ ($P < 0.05$).

*$P < 0.05$; **$P < 0.01$; ns = not significant.

Sampling and Measurement

After incubation at 39°C for 24 h, fluid was sampled to determine pH, ammonia N, VFA, and microbial CP (**MCP**). To determine the relative quantity to total bacterial 16S rDNA of protozoa, fungi, *Fibrobacter succinogenes*, *Ruminococcus albus*, and *R. flavefaciens*, two 1-mL aliquots of fluid from each bottle were sampled under oxygen-free CO_2 and stored at –80°C immediately. Fermentation parameters such as pH, ammonia N, VFA, and MCP were determined by using methods described by Hu et al. (2005).

Total DNA Extraction and Real-Time Quantitative PCR

Total DNA was extracted from rumen fluid by the bead-beating method as described by Zhang et al. (2008). The amplifying primer sets of total bacteria, fungi, protozoa, *F. succinogenes*, *R. albus,* and *R. flavefaciens* are listed in Table 2, as described by Koike and Kobayashi (2001), Sylvester et al. (2004), Denman and McSweeney (2006), and Denman et al. (2007). Real-time quantitative PCR was performed using the ABI 7500 real-time PCR system (Applied Biosystems, Foster, CA) as described by Mao et al. (2010), with fluorescence detection of SYBR Green dye.

Calculations and Statistical Analysis

Quantification for protozoa, fungi, *F. succinogenes, R. albus,* and *R. flavefaciens* were expressed as a ratio to total rumen bacterial 16S rDNA according to the following equation: relative quantification of target = $2^{-(Ct\ target - Ct\ total\ bacteria)}$, where **Ct** represents threshold cycle.

Table 4. Effect of a *Saccharomyces cerevisiae* fermentation product (XP, Diamond V, Cedar Rapids, IA) on ammonia N and microbial protein production on low-quality forage substrates (Exp. 1)

Items	XP, g/L				SEM	Effect	
	0	1	2	3		Linear	Quadratic
Rice straw							
Ammonia N, mg/L	80	89	83	105	7.2	ns	ns
Microbial protein, mg/mL	3.09^{b}	3.67^{b}	4.03^{b}	6.51^{a}	0.672	**	ns
Corn stover							
Ammonia N, mg/L	137^{c}	202^{a}	169^{b}	153^{bc}	8.9	ns	**
Microbial protein, mg/mL	4.59	4.36	5.90	5.93	0.574	ns	ns
Corn silage without grain							
Ammonia N, mg/L	142	127	158	137	7.8	ns	ns
Microbial protein, mg/mL	6.76^{a}	8.60^{a}	8.68^{a}	3.50^{b}	0.568	**	**
Corn silage with grain							
Ammonia N, mg/L	201^{a}	134^{b}	147^{b}	208^{a}	11.1	ns	**
Microbial protein, mg/mL	4.40^{b}	5.80^{a}	3.80^{bc}	2.90^{c}	0.257	**	**

$^{a–c}$Means within a row with different superscripts differ ($P < 0.05$).

*$P < 0.05$; **$P < 0.01$; ns = not significant.

The general linear models procedure (SAS Inst. Inc., Cary, NC) was used to analyze the data. Data from Exp. 1 were analyzed as 1-way ANOVA, and data from Exp. 2 were analyzed as 2-way ANOVA. An orthogonal polynomial contrast was used to examine the responses (linear or quadratic) to increasing concentrations of XP. Multiple comparisons of means among treatments were performed by Duncan's multiple range tests.

RESULTS

Experiment 1: Single Forages

Fermentation Characteristics. Effects of XP on the fermentation parameters of 4 single forages are presented in Tables 3 and 4.

Rice Straw. Supplementing XP influenced total VFA production (linear, $P < 0.05$; quadratic, $P < 0.01$), with the greatest value observed at 1 g/L XP addition. With increasing concentrations of XP, the proportion of acetate decreased (linear, $P < 0.01$; quadratic, $P < 0.05$), but propionate proportion increased (linear, $P < 0.01$), resulting in linear reduction ($P < 0.01$) in the acetate to propionate ratio. Compared with the control (0 g/L XP), 1 and 2 g/L XP increased the butyrate proportion by 18.4% and 29.7%, respectively (quadratic, $P < 0.01$). The MCP increased linearly as XP concentration increased ($P < 0.01$).

Corn Stover. Increasing XP concentrations increased total VFA, propionate, and the butyrate proportion and decreased acetate and the acetate to propionate ratio linearly ($P < 0.01$). Ammonia N reached the greatest value at 1 g/L XP (quadratic, $P < 0.01$). The MCP was not affected by XP supplementation.

Corn Silage without Grain. Total VFA was increased (linear, $P < 0.05$; quadratic, $P < 0.01$) 31.8% by the addition of 1 g/L XP compared with the control. The effects of additional XP on individual VFA and the ratio of acetate to propionate were similar when CS was used as the substrate. The greatest value of MCP was observed when XP was added at 2 g/L (linear and quadratic, $P < 0.01$).

Corn Silage with Grain. Total VFA reached the greatest value at 1 g/L XP (linear, $P < 0.05$; quadratic, $P < 0.01$). The acetate proportion and acetate to propionate ratio reached the lowest value, but the proportion of propionate and butyrate reached the greatest value when XP was added at 2 g/L (quadratic, $P < 0.01$). Ammonia N was decreased in response to XP addition at 1 and 2 g/L (quadratic, $P < 0.01$). Microbial protein reached the greatest value at 1 g/L XP (linear and quadratic, $P < 0.01$).

Rumen Microbial Population. The changes in microbial populations of the 4 individual forages in response to XP addition are presented in Table 5.

Rice Straw. Compared with the control, the addition of 1 g/L XP increased fungi (linear and quadratic, $P < 0.01$) and *F. succinogenes* (linear, $P < 0.01$) populations by 236% and 5.4%, respectively. The percentage of protozoa was increased linearly in response to XP addition ($P < 0.01$). The addition of 2 g/L XP increased the population of *R. flavefaciens* (quadratic, $P < 0.01$).

Corn Stover. The effect of XP addition on fungi population was similar to RS as a substrate. The population of *R. flavefaciens* increased linearly ($P < 0.05$) as XP concentration increased. The greatest value for *R. albus* was observed for 2 g/L XP (linear and quadratic, $P < 0.01$).

Corn Silage without Grain. A smaller value for fungi population was observed for 2 g/L XP than for the

Table 5. Effect of a *Saccharomyces cerevisiae* fermentation product (XP, Diamond V, Cedar Rapids, IA) on rumen microbial population (ratio to total bacterial 16S rDNA) on low-quality forage substrates (Exp. 1)

	XP, g/L					Effect	
Items	0	1	2	3	SEM	Linear	Quadratic
Rice straw							
Fungi, 10^{-4}	0.72^{c}	2.42^{a}	1.91^{b}	1.61^{b}	0.097	**	**
Protozoa, 10^{-2}	1.35^{b}	1.46^{b}	2.04^{b}	2.40^{a}	0.264	**	ns
Fibrobacter succinogenes, 10^{-2}	7.27^{a}	7.66^{a}	3.29^{b}	4.71^{b}	0.531	**	ns
Ruminococcus flavefaciens, 10^{-3}	4.25^{bc}	6.10^{ab}	7.01^{a}	3.28^{c}	0.678	ns	**
R. albus,10^{-4}	4.06	3.88	4.92	2.73	0.607	ns	ns
Corn stover							
Fungi, 10^{-4}	0.95^{b}	1.44^{a}	0.75^{b}	0.70^{b}	0.083	**	*
Protozoa, 10^{-2}	4.75	5.17	6.67	6.03	0.528	ns	ns
F. succinogenes, 10^{-2}	5.03	4.85	6.62	5.94	0.422	ns	ns
R. flavefaciens, 10^{-3}	1.55^{b}	2.78^{ab}	3.42^{a}	3.33^{a}	0.376	*	ns
R. albus,10^{-4}	5.02^{b}	7.91^{ab}	9.07^{a}	8.13^{ab}	0.672	**	**
Corn silage without grain							
Fungi, 10^{-4}	1.76^{a}	1.55^{a}	0.18^{b}	1.40^{a}	0.164	*	ns
Protozoa, 10^{-2}	5.17^{a}	6.06^{a}	0.83^{b}	1.13^{b}	0.783	**	ns
F. succinogenes, 10^{-2}	3.82^{a}	2.69^{b}	1.13^{c}	2.60^{b}	0.300	**	**
R. flavefaciens, 10^{-3}	1.89^{b}	2.86^{a}	1.57^{b}	1.97^{b}	0.160	ns	*
R. albus,10^{-4}	2.82^{ab}	4.96^{a}	3.61^{a}	1.04^{b}	0.479	**	**
Corn silage with grain							
Fungi, 10^{-4}	0.97^{b}	2.29^{a}	2.20^{a}	0.83^{b}	0.333	ns	**
Protozoa, 10^{-2}	3.95^{ab}	7.83^{a}	4.27^{ab}	3.76^{b}	0.825	ns	*
F. succinogenes, 10^{-2}	0.86^{b}	3.13^{ab}	4.38^{a}	1.79^{b}	0.564	ns	**
R. flavefaciens, 10^{-3}	0.16^{b}	1.35^{ab}	1.80^{a}	0.82^{ab}	0.317	**	**
R. albus,10^{-4}	1.36	1.72	1.36	1.35	0.345	ns	ns

$^{a\text{-}c}$Means within a row with different superscripts differ ($P < 0.05$).

* $P < 0.05$; ** $P < 0.01$; ns = not significant.

Table 6. Effect of a *Saccharomyces cerevisiae* fermentation product (XP, Diamond V, Cedar Rapids, IA) on fermentation parameters of mixed diets (Exp. 2)

Item				Molar proportion, %			Ammonia N,	Microbial protein,
	XP, g/L	pH	Total VFA, mmol	Acetate	Propionate	Butyrate	mg/L	mg/mL
Diets[1]								
CSC	0	6.39^{a}	35.2^{e}	66.4^{a}	23.4^{f}	10.1^{a}	221^{bc}	4.07^{c}
	1	6.35^{abcd}	38.3^{cd}	65.6^{cd}	24.3^{e}	10.2^{a}	183^{f}	4.79^{ab}
	2	6.33^{cd}	42.1^{b}	64.5^{e}	25.4^{b}	10.0^{ab}	217^{bc}	4.99^{ab}
CSCC	0	6.39^{a}	37.3^{d}	66.2^{ab}	24.3^{e}	9.5^{c}	200de	3.99^{c}
	1	6.36^{abc}	39.4^{c}	65.4^{cd}	24.8^{cd}	9.8^{abc}	187^{ef}	5.19^{a}
	2	6.32^{d}	41.8^{b}	64.0^{e}	26.3^{a}	9.7^{bc}	181^{f}	5.06^{ab}
CSCCA	0	6.39^{a}	38.0^{d}	65.9^{abc}	24.5^{de}	9.6^{c}	240^{a}	4.63^{b}
	1	6.37^{ab}	42.9^{b}	65.7^{bcd}	24.9^{cd}	9.4^{c}	232^{ab}	4.86^{ab}
	2	6.34^{bcd}	45.6^{a}	65.2^{d}	25.0^{bc}	9.8^{abc}	211^{cd}	5.02^{ab}
Pooled SEM		0.021	0.43	0.18	0.16	0.13	5.2	0.155
Effects								
Diets		ns	**	ns	**	**	**	ns
XP								
Linear		**	**	**	**	ns	**	**
Quadratic		**	ns	ns	ns	ns	*	*
Diets × XP		ns	*	*	**	ns	**	ns

$^{a\text{–}f}$Means within a column with different superscripts differ ($P < 0.05$).

[1]CSC = mixture of corn, soybean meal, and corn stover at ratios of 33%, 22%, and 45%; CSCC = mixture of corn, soybean meal, corn stover, and corn silage at ratios of 33%, 22%, 22.5%, and 22.5%; CSCCA = mixture of corn, soybean meal, corn stover, corn silage, and alfalfa at ratios of 33%, 22%, 19%, 31%, and 5%.

*$P < 0.05$; **$P < 0.01$; ns = not significant.

Table 7. Effect of a *Saccharomyces cerevisiae* fermentation product (XP, Diamond V, Cedar Rapids, IA) on rumen microbial population (ratio to total bacterial 16S rDNA) of mixed diets (Exp. 2)

Item	XP, g/L	Fungi, 10^{-5}	Protozoa, 10^{-2}	*Fibrobacter succinogenes*, 10^{-2}	*Ruminococcus flavefaciens*, 10^{-4}	*R. albus*, 10^{-4}
Diets[1]						
CSC	0	1.99^{c}	1.23^{b}	12.0^{a}	1.42^{ab}	2.77^{d}
	1	4.26^{a}	1.44^{ab}	9.27^{b}	1.52^{a}	6.25^{c}
	2	2.23^{bc}	1.55^{ab}	11.9^{a}	1.50^{a}	6.92^{c}
CSCC	0	3.17^{abc}	1.98^{a}	5.25^{cd}	1.23^{ab}	1.94^{de}
	1	3.49^{ab}	1.66^{ab}	8.41^{b}	1.67^{a}	2.51^{de}
	2	2.39^{bc}	1.87^{a}	6.78^{c}	1.19^{abc}	1.59^{e}
CSCCA	0	2.39^{bc}	1.56^{ab}	4.49^{d}	0.64^{cd}	8.41^{b}
	1	3.03^{abc}	1.83^{a}	6.01^{cd}	0.59^{d}	13.0^{a}
	2	2.64^{bc}	1.66^{ab}	4.88^{d}	0.90^{bcd}	12.8^{a}
Pooled SEM		0.301	0.152	0.451	0.160	0.292
Effects						
Diets		ns	*	**	**	**
XP						
Linear		ns	ns	ns	ns	**
Quadratic		**	ns	ns	ns	**
Diets × XP		ns	ns	**	ns	**

$^{a–e}$Means within a column with different superscripts differ ($P < 0.05$).

[1]CSC = mixture of corn, soybean meal, and corn stover at ratios of 33%, 22% and 45%; CSCC = mixture of corn, soybean meal, corn stover, and corn silage at ratios of 33%, 22%, 22.5%, and 22.5%; CSCCA = mixture of corn, soybean meal, corn stover, corn silage, and alfalfa at ratios of 33%, 22%, 19%, 31%, and 5%.

*$P < 0.05$; **$P < 0.01$; ns = not significant.

other 3 treatments (linear, $P < 0.05$). The percentage of protozoa was greater for 1 g/L XP than for 2 and 3 g/L XP (linear, $P < 0.01$). The addition of XP decreased the *F. succinogenes* population (linear and quadratic, $P < 0.01$) and increased *R. flavefaciens* (quadratic, $P < 0.05$). The greatest values of *R. albus* (linear and quadratic, $P < 0.01$) were observed when XP was added at 1 g/L.

Corn Silage with Grain. Populations of fungi (quadratic, $P < 0.01$) and protozoa (quadratic, $P < 0.05$) were the greatest at 1 g/L XP, and the addition of XP at 2 g/L caused the greatest increase in value of *F. succinogenes* (quadratic, $P < 0.01$) and *R. flavefaciens* (linear and quadratic, $P < 0.01$).

Experiment 2: Mixed Diets

Fermentation Characteristics. The average rumen fluid pH in all treatments was between 6.32 and 6.39 (Table 6). Total VFA concentrations were enhanced linearly by XP ($P < 0.01$) and also influenced by diets ($P < 0.01$), with the greatest concentration observed for CSCCA. The molar proportion of acetate was reduced, and the propionate proportion was enhanced markedly with increasing concentrations of XP (linear, $P < 0.01$). Ammonia N was decreased with increasing concentrations of XP (linear, $P < 0.01$; quadratic, $P < 0.05$). A Diet × XP concentration interaction was observed ($P < 0.01$) for ammonia N. The addition of XP also increased MCP (linear, $P < 0.01$; quadratic, $P < 0.05$).

Rumen Microbial Population. Fungi population was affected by the level of XP (quadratic, $P < 0.01$; Table 7). The greatest value of fungi population was observed when 1 g/L XP was added to CSC ($P < 0.05$). The addition of XP did not influence ($P > 0.05$) the populations of protozoa, *F. succinogenes,* or *R. flavefaciens*. The percentage of *R. albus* was increased by the addition of XP (linear and quadratic, $P < 0.01$) and reached the greatest values with 1 g/L XP.

DISCUSSION

The pH, VFA, ammonia N, and MCP are important parameters reflecting the ruminal environment. In the current study, terminal pH of various substrates was differed. However, the values were all within the normal range (>6.3), and because of that the potential effect of XP on stabilizing rumen pH was not determined. Although the effect of XP on total VFA production varied somewhat with different substrates, the addition of XP at 1 or 2 g/L increased the total VFA concentration, indicating stimulated rumen microbial fermentation activities. In addition, supplemental XP manipulated the molar proportion of individual VFA toward increased propionate production. This result agrees with the report of Miller-Webster et al. (2002), who observed supplemental XP increased propionate and total VFA. The changes in molar proportions of VFA were also observed by others (Carro et al., 1992; Corona et al., 1999). This alteration in ruminal VFA

proportion by XP could contribute to the improved feed efficiency in lactating dairy cows (Schingoethe et al., 2004). Wallace and Newbold (1992) suggested that the variable response in VFA production and patterns is a consequence of the effects of yeast culture on microbial numbers in the rumen rather than a direct effect on ruminal fermentation. Lascano and Heinrichs (2009) also reported that substrates or diets influenced the growth of different species of rumen microbes that are the responsible for the VFA production and pattern when yeast culture was supplemented.

Ammonia is the main source of N for microbial protein synthesis (Bach et al., 2005). In the current study, the response to XP of ammonia N varied with forage types. The different effects of XP may be attributed to the characteristics of the diets. It appears that the addition of XP at a suitable level is beneficial for more efficient utilization of the N source for MCP synthesis when N availability is not limited. This result is consistent with the report of Hristov et al. (2010), who found an increment in overall utilization of ammonia N and increased MCP synthesis with the addition of XP.

Numerous studies (e.g., Yoon and Stern, 1996; Miller-Webster et al., 2002) have documented the positive effects of XP on ruminal fermentation and microbial activities. The addition of XP could provide important nutrients or nutritional cofactors that stimulate microbial activities (Callaway and Martin, 1997). In addition, XP supplementation could provide vitamins such as biotin and thiamine, which are reported to be required for fungal growth and activity (Akin and Borneman, 1990). In the current study, the addition of XP increased the rumen fungi population at the lower inclusion level, with the effect decreasing as XP concentration increased.

No significant effect of XP on protozoa was observed. Hristov et al. (2010) reported that the ruminal protozoa population increased by 14% with supplemental XP, but the change was insignificant ($P > 0.05$). However, changes in the proportion of different genera of protozoa may be more important than changes in total protozoa population. Arakaki et al. (2000) reported that XP increased the proportions of *Dasytricha* and *Isotricha* that have an efficient O_2-scavenging capability (Lloyd et al., 1989). They also reported that the proportion of the predominant genus, *Entodinium,* decreased from 87.7% to 69.9% with the addition of XP. *Entodinium* protozoa are responsible for bacterial engulfment and reducing microbial protein synthesis (Ivan et al., 2000). The increased MCP with XP in the present study could partially be attributed to the changes in the generic composition of rumen protozoa.

Results of this trial showed that XP could stimulate growth of certain cellulolytic bacterial population. Previous studies also reported that XP increased cellulolytic bacteria either significantly (Wiedmeier et al., 1987; Harrison et al., 1988) or numerically (Yoon and Stern, 1996). Increased VFA production on forage substrates could be attributed to an increased fiber-digesting bacterial population.

In general, the greatest responses to XP were observed at the 1 or 2 g/L level. One possible explanation for the diminishing response with a greater level of XP in this study is at least partially due to the nature of the in vitro procedure. For the batch culture in vitro model, the substrate amount relative to the rumen liquid volume is much less than in the rumen of a cow (<1% vs. 12%). Therefore, when a rumen modulator like XP is supplemented at a greater rate, it could increase the fermentation rate and cause substrate depletion more quickly, resulting in diminished response as the fermentation length is elongated. A multiple-time-point study would address the situation more accurately.

Conclusion

Although responses to supplemental XP varied among the substrates tested, the results of this study suggest that the addition of XP can support ruminal fermentation of low-quality forages and mixed diets. The improved rumen fermentation with the addition of XP was attributed to the ability of XP to stimulate the number of functional rumen microbes, especially fungi populations. In general, XP added at 1 or 2 g/L (approximately equivalent to 60 or 120 g/d, respectively) showed the greatest responses in most variables tested.

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