Effects of a twin strain of *Saccharomyces cerevisiae* live cells on mixed ruminal microorganism fermentation in vitro[1]

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ABSTRACT: This experiment was designed to investigate the effects of different concentrations (0, 0.33, 0.66, 0.99, and 1.32 g/L) of a twin-strain of *Saccharomyces cerevisiae* live cells on in vitro mixed ruminal microorganism fermentation of corn starch, soluble potato starch, and sudangrass hay (60.5%, DM basis) plus concentrate mixture (39.5%, DM basis). Ruminal fluid was collected from two dairy cows, mixed with phosphate buffer (1:2), and incubated (30 mL) anaerobically at 38°C for 6 and 24 h with or without yeast supplement, using 200 mg (DM basis) of each substrate. Medium pH, ammonia-N, and numbers of protozoa were unaffected ($P = 0.38$) by yeast cells in all substrates. Molar proportion of acetate was unchanged ($P = 0.56$) with cornstarch and soluble potato starch, but increased quadratically ($P = 0.02$) with hay plus concentrate by treatment. Addition of yeast cells caused a linear increase of total VFA ($P = 0.008$) in all substrates. Excluding the soluble potato starch, supplementation of *S. cerevisiae* resulted in a quadratic increase of propionate ($P = 0.01$), with a quadratic decrease ($P = 0.04$) of acetate:propionate. When soluble potato starch was used as a substrate, a linear increase ($P = 0.006$) of the molar proportion of propionate and a quadratic decrease ($P = 0.007$) in acetate:propionate was observed by treatment. Molar proportion of butyrate was unchanged ($P = 0.35$) with cornstarch and soluble potato starch, whereas it decreased linearly ($P = 0.007$) with hay plus concentrate by yeast cell supplementation. When cornstarch and soluble potato starch were used as a substrate, minor VFA were decreased ($P = 0.05$) by treatment. Accumulation of lactate was linearly decreased by treatment ($P = 0.007$) in all substrates. During incubation with hay plus concentrate, IVDMD was linearly increased ($P = 0.006$), whereas production of methane (linear; $P = 0.02$) and accumulation of hydrogen was decreased (quadratic; $P = 0.005$) by treatment after 24 h. These results showed that a twin strain of *S. cerevisiae* live cells stimulated in vitro mixed ruminal microorganism fermentation with decreased lactate, and a small decrease of methane and hydrogen with hay plus concentrate.

Key Words: Methane, Probiotics, Protozoa, Ruminal Fermentation, Twin Strain of *Saccharomyces cerevisiae*

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Introduction

Based on growing concern over the use of antibiotics and other growth promoters in the animal feed industry, interest in the effects of microbial feed additives on animal performance has increased. Some of the benefits associated with *Saccharomyces cerevisiae* include increased DM and NDF digestion (Williams et al., 1991; Carro et al., 1992) and milk production (Williams et al., 1991; Piva et al., 1993; Kung et al., 1997). Yeast cultures also have been shown to stimulate utilization of hydrogen by ruminal acetogenic bacteria (Chaucheyras et al., 1995). Wallace and Newbold (1992) reported that the responses of yeast culture are highly variable and apparently influenced by the composition of the diet.

Saccharomyces cerevisiae (Yea-Sacc 1026; Alltech Biotechnology Center, Nicholasville, KY) increased the number of ruminal total bacteria and cellulolytic bacteria (Newbold et al., 1995), increased the proportion of propionate (Mutsvangwa et al., 1992; Newbold et al., 1995), and decreased lactate concentration (Newbold et al., 1990). Another *S. cerevisiae* culture (Diamond V XP; Diamond V Mills, Inc., Indianapolis, IN) stimulated the growth of the cellulolytic bacteria, *Fibrobacter succinogenes* and *Ruminococcus albus* (Nisbet and Martin, 1991; Callaway and Martin, 1997), and increased the

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Table 1. Composition of experimental diets

Item	Sudangrass hay	Concentrate mixture[a]
Feed composition, % DM basis	60.5	39.5
Chemical composition, % DM basis		
OM	89.8	94.4
CP	10.5	19.4
NDF	65.3	18.5

[a]Contained (DM basis) 41.3% corn, 4.5% barley, 1.2% wheat feed flower, 23.4% bran, 3.6% corn gluten feed, 8.2% rapeseed oil, 7.4% soybean meal, 6.4% corngerm meal, 0.6% molasses, 2.6% $CaCO_3$, 0.6% NaCl, and 0.2% trace mineral and vitamin premix. Trace mineral and vitamin premix contained 5.6% Mg, 0.4% Zn, 0.2% Mn, 0.08% Fe, 0.01% I, 0.002% Co, 1,500 IU of vitamin A/g, 500 IU of vitamin D/g, 9 mg of vitamin B complex/g, and 0.5 mg of α-tocopherol/g.

proportion of propionate (Sullivan and Martin, 1999) and lactate uptake by *Selenomonas ruminantium* (Martin and Nisbet, 1992). Recently, Lynch and Martin (2002) reported that the Diamond V XP yeast culture and live cells of *S. cerevisiae* (PMX70SBK; Saf Agri, Indianapolis, IN) did not affect propionate concentration, whereas lactate concentration decreased with *S. cerevisiae* live cells. This study was conducted to evaluate the effects of a twin strain of *S. cerevisiae* live cells (Yea-Sacc Twin Strain 8417 and 1026) on in vitro mixed ruminal microorganism fermentation of ground corn, soluble potato starch, and sudangrass hay plus concentrate.

Materials and Methods

Substrates and Additives

Soluble potato starch and cornstarch (Wako Pure Chemical Industries, Ltd., Tokyo, Japan), and sudangrass hay plus concentrate (1.5:1) mixture were used as substrates on a DM basis for in vitro incubation. Live *S. cerevisiae* cells (Yea-Sacc Twin Strain 8417 and 1026 [**YST**], Bussan Biotech Co. Ltd. Tokyo, Japan) were used as a probiotics, and contained 5×10^9 live organisms/g, plus the carrier (medium) on which it was grown. The carrier contained 28% CP, 14% crude fiber, 6% crude fat, and 8% ash (DM basis). The sudangrass hay plus concentrate mixture was ground in a high-speed grinder (Retsch ZM 100, Haan, Germany) to pass through a 1-mm screen. The chemical composition of the sudangrass hay and concentrate mixture is shown in Table 1.

In Vitro Batch Fermentation

Two lactating Holstein dairy cows (655 ± 26 kg BW), fed (DM basis) 5 kg of sudangrass hay and 5 kg of concentrate mixture twice daily at 0900 and 1700, were used as the donor animals for ruminal fluid. The same concentrate mixture was used in our in vitro study. The dairy cows were cared for in accordance with the Guide for the Care and Use of Laboratory Animals prepared by Tokyo University of Agriculture and Technology. Before the morning feeding (0900), approximately 500 mL of ruminal fluid was drawn from each of the dairy cows with a stainless steel stomach tube (Bayaru et al., 2001), deposited into a vacuum flask that had been previously flushed with O_2-free CO_2, mixed, and immediately transported to the laboratory. The mixed sample was strained through four layers of surgical gauze into an Erlenmeyer flask under continuous flushing with CO_2, and efforts were made to maintain the temperature at 38°C. The fluid was then mixed with buffer (pH 6.9; containing [per liter] 292 mg of K_2HPO_4, 240 mg of KH_2PO_4, 480 mg of $(NH_4)_2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_4 \cdot 7H_2O$, 64 mg of $CaCl_2 \cdot 2H_2O$, 4,000 mg of Na_2CO_3, and 600 mg of cysteine hydrochloride) in a 1:2 ratio (Russell and Martin, 1984). After mixing, 30 mL of diluted ruminal fluid was anaerobically transferred to 60-mL serum bottles containing 200 mg of each substrate on a DM basis. Weighed amounts of YST were added to achieve final concentrations of 0, 0.33, 0.66, 0.99, and 1.32 g/L. Two sets of bottles were sealed anaerobically under CO_2 atmosphere with butyl rubber stoppers and capped with aluminum. One set was incubated at 38°C for 6 h, and another set for 24 h with shaking at 30 rpm. Every set contained five levels of supplement, and for each level, duplicate bottles were incubated. Consequently, the fermentation was conducted in 30 fermentation bottles for either 6 or 24 h. An additional 10 bottles were also incubated with hay plus concentrate for 24 h to determine IVDMD. Incubations containing only YST were also run for 24 h.

Determination of the Number of Total Viable Counts and Cellulolytic Bacteria

After 24 h of incubation, a 1-mL sample was collected, and serial 10-fold dilutions in an anaerobic mineral solution (Bryant and Burkey, 1953) were prepared. The Hungate (1969) anaerobic technique was used to prepare media and to cultivate microorganisms. Total viable counts were determined in roll-tubes (triplicate) with the complete medium described by Leedle and Hespell (1980). Cellulolytic bacteria were cultivated in Halliwell and Bryant (1953) medium. Dilutions of 10^6 to 10^9 were used to cultivate total viable bacteria and cellulolytic bacteria. One milliliter of diluted sample from each tube was used to inoculate 5 mL of culture media in Hungate tubes. Counts (five tubes for each dilution) were estimated by the most probable number method (Alexander, 1982). The existence of cellulolytic bacteria was assumed by the degradation of a filter paper strip (qualitative filter paper No. 1, ashless; Toyo Roshi Kaisha, Ltd., Tokyo; Morvan et al., 1996).

Analyses

At the end of incubation, total gas was measured by insertion of the glass syringe needle through the butyl rubber stopper, and a volume of gas exceeding 1 atmo-

spheric pressure was measured through the displacement of the syringe plunger (Callaway and Martin, 1996). The gas was injected back into the serum bottle, and a 0.5-mL sample of gas was removed from each bottle with a gas-tight syringe, and methane and hydrogen were measured by a gas chromatograph (model GC-8A, Shimadzu Co., Ltd., Kyoto, Japan) using a molecular sieve 5A column (1.6 m × 3.2 mm i.d., 60 to 80 mesh, Shinwakako, Kyoto, Japan) and thermal conductivity detector (column temperature = 60°C, injector and detector temperature = 80°C). The carrier gas (Ar) flow rate was 50 mL/min. The bottles were uncapped, and pH was immediately determined in the culture fluid with a portable pH meter (ATC pH meter Piccolo2, Hannah Instruments, Arvore-Vila do Conde, Portugal). One milliliter of the incubated fluid was diluted with 4 mL of methylgreen-formalin-saline (formalin, 100 mL; NaCl, 8.5 g; methyl green, 0.3 g; and distilled H_2O, 900 mL), and protozoa were counted using a Fuchs-Rosenthal counting chamber as described previously (Ogimoto and Imai, 1981). For analysis of ammonia-N and VFA, 1 mL of 25% meta-phosphoric acid (wt/vol) was added to 5 mL of fermentation fluid, and stored at –30°C until analyzed. One milliliter of thawed fermentation fluid was centrifuged (10,000 × *g* for 10 min at 4°C), and VFA were analyzed by gas chromatography (model GC-14B, Shimadzu Co. Ltd.) using a Thermon-3000 5% Shincarbon A column (1.6 m × 3.2 mm i.d., 60 to 80 mesh, Shinwakako) and a flame ionization detector (column temperature = 130°C, injector and detector temperature = 200°C). The carrier gas (N_2) flow rate was 50 mL/min. Ammonia-N was determined by a microdiffusion method (Conway, 1962). One milliliter of thawed sample was centrifuged (27,000 × *g* for 20 min at 4°C), filtered (0.45 μm pore size), and the lactic acid was analyzed by HPLC (Shimadzu Co. Ltd.) using a Shodex Rspak KC-811 column (8 mm i.d. × 300 mm length; Showa Denko, Tokyo, Japan). The mobile phase used for isocratic elution was 3 m*M* $HClO_4$. The flow-rate was 1.0 mL/min, column temperature was 50°C, and the monitoring wavelength was 210 nm, with a UV absorbance detector. To determine IVDMD, the bottles' contents were transferred into a tube and centrifuged at 11, 000 ×*g* for 15 min at 4°C after 24 h incubation. The supernatants were passed through a filter paper (qualitative filter paper No. 1, ashless; Tokyo Roshi Kaisha Ltd., Tokyo, Japan), washed twice with distilled water and once with acetone, and then dried to a constant weight at 105°C. The IVDMD was calculated as the original weight of hay plus concentrate DM (added in each incubation) minus dry residue weight (after incubation) divided by the original sample weight. These values were then multiplied by 100 to derive the percentage of IVDMD.

Statistical Analyses

Data were analyzed using the GLM procedure of SAS (SAS Inst., Inc. Cary, NC). The statistical model included the fixed effects of treatment, replication (day), the interaction of treatment × day and the time effect. The model as fitted was as follows:

$$y_{ijk} = m + T_i + R_j + TR_{ij} + e_{ij} + H_k + (TH)_{ik} + (RH)_{jk} + (TRH)_{ijk} + e_{ijk}$$

where y_{ijk} = the dependent variable, m = overall mean, T_i = treatment effect (i = 1, ..., 5), R_j = replication effect (j = 1,2,3), TR_{ij} = interaction effect, e_{ij} = random residual error, and H_k = time effect (k = 1, 2). Total viable bacteria, cellulolytic bacteria, protozoa, and concentration of lactate were analyzed using the following model:

$$y_{ijk} = m + T_i + R_j + TR_{ij} + e_{ij}$$

where m, T, and R are as defined above. Linear and quadratic contrasts examined the effect of increasing YST concentration. Data are presented as least squares means, and the contrast *P*-values are the observed significance levels and are considered statistically different when $P < 0.05$. Differences of $P < 0.15$ to $P < 0.05$ are discussed as trends.

Results

In the absence of added substrates, YST had no effect on medium pH, ammonia-N, minor VFA, protozoa, hydrogen, and methane (Table 2). The concentration of total VFA in the medium and the concentration CH_4 in the total gas fluctuated with time ($P = 0.03$). Total VFA was linearly increased ($P = 0.04$) by YST. Molar proportion of acetate tended to decrease ($P = 0.07$) quadratically, whereas the proportion of propionate increased ($P = 0.05$) linearly, and the proportion of butyrate tended to be increase linearly ($P = 0.08$) with YST. Total gas production tended to increase ($P = 0.08$) quadratically with YST.

Table 3 shows the in vitro effects of YST on microbial fermentation with cornstarch. Most responses by YST seemed to be similar after 6 or 24 h of incubation. The concentration of ammonia-N and total VFA in the medium, and the concentration of H_2 and CH_4 in the total gas fluctuated with time ($P = 0.02$). Ammonia-N and pH were not affected ($P = 0.38$) by the treatment. Increasing the concentration of yeast supplement resulted in a linear increase ($P = 0.008$) in total VFA concentration. There were no differences among treatments in the molar proportion of acetate ($P = 0.56$). Compared with untreated cultures, increasing the concentration of yeast caused a quadratic increase ($P = 0.007$) of molar proportion of propionate, whereas proportions of butyrate were unchanged ($P = 0.73$). Minor VFA were decreased (quadratic effect for isobutyrate and valerate, $P = 0.05$; linear effect for isovalerate, $P = 0.05$) by treatment. Increasing the concentration of YST caused a linear increase in total gas production ($P = 0.007$). The numbers of protozoa and concentrations of methane and hydrogen were unchanged ($P = 0.26$) by the treat-

Table 2. Effects of Yea-Sacc Twin-Strain 8417 and 1026 (YST) *Saccharomyces cerevisiae* live cells on in vitro mixed ruminal microorganism fermentation in the absence of added substrate

	YST g/L						Contrast[b]		
Item	0	0.33	0.66	0.99	1.32	SEM[a]	L	Q	Time
pH	6.23	6.23	6.24	6.22	6.24	0.03	0.69	0.75	0.38
6 h	6.25	6.25	6.27	6.24	6.28	0.03	0.74	0.79	
24 h	6.21	6.21	6.20	6.19	6.20	0.03	0.63	0.71	
Ammonia-N, mg/dL	1.28	1.21	1.21	1.32	1.27	0.09	0.65	0.69	0.51
6 h	1.15	1.09	1.12	1.12	1.11	0.08	0.62	0.67	
24 h	1.41	1.32	1.30	1.52	1.43	0.09	0.68	0.70	
Total VFA, m*M*	16.2	16.2	16.7	16.8	17.4	0.25	0.04	0.47	0.03
6 h	12.4	12.6	12.8	13.1	13.5	0.20	0.05	0.43	
24 h	9.9	19.8	20.5	20.5	21.3	0.29	0.03	0.51	
Individual VFA, mol/100 mol									
Acetate	68.9	68.3	66.7	65.3	64.9	2.05	0.49	0.07	0.18
Propionate	15.6	16.2	17.0	17.8	17.9	0.34	0.05	0.48	0.09
Butyrate	10.6	11.1	12.2	12.5	12.7	0.21	0.08	0.42	0.17
Isobutyrate	1.01	0.76	0.83	0.96	0.89	0.07	0.28	0.34	0.29
Valerate	2.62	2.53	2.19	2.31	2.40	0.38	0.52	0.61	0.31
Isovalerate	1.26	1.06	1.07	1.15	1.22	0.19	0.44	0.51	0.38
Acetate:propionate	4.42	4.22	3.91	3.68	3.63	0.63	0.32	0.07	0.18
Protozoa, $\times 10^4$/mL									
6 h	2.3	2.2	2.4	2.5	2.7	1.04	0.67	0.72	
Total gas, mL	1.47	1.46	1.52	1.60	1.77	0.08	0.42	0.08	0.008
6 h	0.62	0.61	0.61	0.60	0.63	0.05	0.48	0.70	
24 h	2.32	2.30	2.43	2.60	2.91	0.10	0.35	0.08	
H_2, m*M*	ND[c]	ND	ND	ND	ND	ND			
CH_4, m*M*	0.71	0.71	0.71	0.72	0.72	0.07	0.45	0.50	0.007
6 h	0.28	0.27	0.28	0.26	0.26	0.08	0.53	0.58	
24 h	1.14	1.15	1.14	1.17	1.17	0.06	0.37	0.42	

[a]n = 15.
[b]L = linear effect due to dose of YST; Q = quadratic effect due to dose of YST.
[c]ND = Not detectable.

ment. A quadratic increase ($P = 0.03$) of total viable bacteria was observed with increasing YST concentration. Concentration of lactate linearly decreased ($P = 0.007$) with increasing YST concentration.

Table 4 shows the effects of YST on microbial fermentation of soluble potato starch. Most responses by YST seemed to be similar after 6 or 24 h of incubation. Concentration of ammonia-N and total VFA in the medium and concentration of H_2 and CH_4 in the total gas fluctuated with time ($P = 0.02$). Similar to results with cornstarch, YST did not alter ($P = 0.44$) pH or ammonia-N. Increasing the YST concentration caused a linear increase ($P = 0.001$) in total VFA concentration. The addition of YST had no effect ($P = 0.57$) on proportion of acetate. Increasing the concentration of YST caused a linear increase ($P = 0.006$) in the proportion of propionate, whereas butyrate and the numbers of protozoa were unaffected ($P = 0.35$). Minor VFA were decreased (quadratic effect for isobutyrate, $P = 0.05$; linear effect for valerate, $P = 0.05$; and isovalerate, $P = 0.05$) by treatment. Increasing the YST concentration caused a linear increase ($P = 0.007$) in total gas production. Methane and hydrogen production were unchanged ($P = 0.30$) by the treatment. A quadratic increase ($P = 0.005$) in total viable bacteria was observed with increasing YST concentration. Lactate concentration was linearly decreased ($P = 0.006$) with increasing YST concentration.

Table 5 shows the effects of YST on the fermentation of hay plus concentrate. Most responses to YST seemed to be similar after either 6 or 24 h of incubation. The concentration of ammonia-N and total VFA in the medium and the concentration of H_2 and CH_4 in the total gas fluctuated with time ($P = 0.04$). Similar to the results described above, the pH of the medium and ammonia-N were unchanged ($P = 0.40$) by treatment. Supplementation of YST caused a linear increase ($P = 0.008$) in total VFA. Increasing the YST concentration caused a quadratic increase ($P = 0.04$) in the proportions of acetate and propionate, and a linear decrease ($P = 0.007$) in the proportion of butyrate. The addition of YST resulted in a quadratic decrease ($P = 0.05$) of other VFA. Increasing the YST concentration linearly increased ($P = 0.008$) total gas production. Numbers of protozoa were unchanged ($P = 0.40$) by treatment. Methane production decreased linearly ($P = 0.04$), whereas hydrogen accumulation decreased quadratically ($P = 0.02$) after 24 h with increasing YST concentration. A linear increase ($P = 0.007$) was observed on total viable bacteria and cellulolytic bacteria with increasing YST concentration. Lactate concentration lin-

Table 3. Effects of Yea-Sacc Twin-Strain 8417 and 1026 (YST) *Saccharomyces cerevisiae* live cells on the fermentation of cornstarch by mixed ruminal microorganisms

	YST g/L						Contrast[b]		
Item	0	0.33	0.66	0.99	1.32	SEM[a]	L	Q	Time
pH	6.07	6.07	6.06	6.05	6.03	0.02	0.38	0.55	0.07
6 h	6.18	6.19	6.17	6.16	6.14	0.02	0.44	0.62	
24 h	5.96	5.95	5.94	5.93	5.92	0.02	0.38	0.35	
Ammonia-N, mg/dL	5.2	5.0	5.2	5.3	5.2	0.31	0.40	0.51	0.02
6 h	5.6	5.6	5.7	5.8	5.7	0.30	0.41	0.55	
24 h	4.8	4.4	4.7	4.7	4.7	0.38	0.44	0.42	
Total VFA, m*M*	58.4	61.2	62.6	64.0	65.3	0.84	0.008	0.34	0.006
6 h	56.4	58.8	59.3	60.6	62.6	0.66	0.007	0.25	
24 h	60.3	63.6	65.9	67.4	68.0	0.92	0.009	0.38	
Individual VFA, mol/100 mol									
Acetate	57.2	57.1	57.3	57.1	57.1	0.61	0.67	0.56	0.15
Propionate	26.4	26.8	27.0	27.4	27.8	0.63	0.28	0.007	0.02
Butyrate	12.2	12.3	12.1	12.2	12.1	0.51	0.79	0.73	0.19
Isobutyrate	1.50	1.40	1.50	1.30	1.15	0.24	0.17	0.05	0.28
Valerate	1.70	1.55	1.55	1.30	1.15	0.24	0.17	0.05	0.34
Isovalerate	0.75	0.80	0.55	0.60	0.65	0.08	0.05	0.16	0.45
Acetate:propionate	2.18	2.14	2.12	2.08	2.06	0.47	0.22	0.006	0.03
Protozoa, $\times 10^4$/mL									
6 h	15.5	15.3	15.6	15.2	15.4	0.41	0.55	0.52	
Total gas, mL	28.0	31.1	35.0	37.5	40.0	0.63	0.007	0.57	0.001
6 h	17.0	20.1	24.0	28.0	31.0	0.65	0.006	0.62	
24 h	39.0	42.0	46.0	47.0	49.0	0.56	0.007	0.55	
H_2, m*M*	0.18	0.17	0.18	0.18	0.18	0.004	0.26	0.31	0.001
6 h	0.32	0.31	0.32	0.32	0.32	0.003	0.28	0.30	
24 h	0.03	0.03	0.03	0.03	0.03	0.004	0.28	0.30	
CH_4, m*M*	14.5	14.6	14.4	14.4	14.6	0.52	0.48	0.54	0.04
6 h	15.7	15.9	15.4	15.6	15.9	0.42	0.48	0.55	
24 h	13.3	13.2	13.3	13.2	13.4	0.56	0.51	0.48	
Total viable bacteria, $\times 10^8$/mL									
24 h	3.25	4.11	4.46	4.57	4.84	0.95	0.46	0.03	
L-Lactate, m*M*									
24 h	0.28	0.21	0.18	0.14	0.09	0.16	0.07	0.22	

[a]n = 15.
[b]L = linear effect due to dose of YST; Q = quadratic effect due to dose of YST.

early decreased ($P = 0.006$), whereas IVDMD linearly increased ($P = 0.006$) with treatment.

Discussion

No effect of yeast treatment on pH was observed in this experiment. In vitro and in vivo effects of *S. cerevisiae* on ruminal pH has been varied. Results of in vitro studies suggested that Yea-Sacc 1026 strain (25 g/kg of diet; Newbold et al., 1995) and Diamond V XP yeast culture (0.35 to 0.73 g/L; Sullivan and Martin, 1999; Lynch and Martin, 2002) did not modify mean pH. In contrast, Williams et al. (1991) reported that *S. cerevisiae* NCYC 1026 (10 g/d; Nutfield, Surrey, U.K.) also decreased the individual variability of pH in dairy cows fed energy-rich diets. Martin and Nisbet (1992) reported that the utilization of lactic acid by ruminal bacteria *S. ruminantium* was enhanced by Diamond V XP yeast culture, thereby maintaining a constant pH. Ammonia-N concentration was not modified by the addition of YST, which was similar to the results of Yea-Sacc 1026 in vitro (Newbold et al., 1995). In an in vivo study, Chademana and Offer (1990) and Newbold et al. (1995) reported that Yea-Sacc 1026 did not affect ruminal ammonia-N concentration in sheep fed the supplement at 4 g/d and 1.3 g/kg of diet, respectively. Erasmus et al. (1992) and Mutsvangwa et al. (1992) found a similar effect in lactating dairy cows (10 g/d) and in bulls (8 to 10 g/d). Decrease of lactate concentration with YST is consistent with the results of Yea-Sacc 1026 in sheep (4 g/d; Newbold et al., 1990). Other yeast culture (Diamond V XP) also decreased the lactate concentration in vitro (Callaway and Martin, 1997; Sullivan and Martin, 1999). Total gas was increased with YST; this may have resulted from the increased production of propionate because carbon dioxide is produced when propionate is made by some ruminal bacteria via the succinate:propionate pathway (Wolin and Miller, 1988). Ciliate protozoa were composed of *Entodinium* spp., *Dasytricha* sp., and *Isotricha* sp. The total number and composition of protozoa were not affected by treatment. Few experiments have dealt with the effect of direct-fed microbials on the protozoa population. Kumar et al. (1994) and Newbold et al. (1995) reported that *S. cerevisiae* did not modify protozoa in buffalo and in sheep, respectively. In contrast, Plata et al. (1994)

Table 4. Effects of Yea-Sacc Twin-Strain 8417 and 1026 (YST) *Saccharomyces cerevisiae* live cells on the fermentation of soluble potato starch by mixed ruminal microorganisms

	YST g/L						Contrast[b]		
Item	0	0.33	0.66	0.99	1.32	SEM[a]	L	Q	Time
pH	5.83	5.82	5.80	5.79	5.78	0.03	0.51	0.44	0.08
6 h	5.90	5.88	5.86	5.85	5.84	0.03	0.48	0.40	
24 h	5.76	5.75	5.74	5.73	5.72	0.03	0.51	0.45	
Ammonia-N, mg/dL	7.7	7.6	7.8	7.8	7.7	0.42	0.51	0.48	0.005
6 h	5.9	5.7	5.8	5.9	5.7	0.44	0.53	0.47	
24 h	9.5	9.4	9.7	9.6	9.7	0.47	0.56	0.52	
Total VFA, m*M*	51.3	54.3	55.6	57.2	58.4	0.53	0.001	0.41	0.02
6 h	46.3	48.8	49.4	50.9	52.6	0.53	0.001	0.38	
24 h	56.3	59.7	61.8	63.5	64.1	0.59	0.001	0.43	
Individual VFA, mol/100 mol									
Acetate	58.7	58.7	58.5	58.5	58.8	0.79	0.62	0.57	0.23
Propionate	25.5	26.5	26.9	27.6	28.0	0.61	0.006	0.34	0.08
Butyrate	10.5	10.6	10.5	10.5	10.2	0.35	0.40	0.35	0.34
Isobutyrate	1.70	1.50	1.40	1.55	1.20	0.22	0.17	0.05	0.38
Valerate	1.60	1.25	1.45	1.10	1.15	0.20	0.05	0.19	0.32
Isovalerate	1.00	1.00	0.75	0.75	0.80	0.14	0.05	0.19	0.25
Acetate:propionate	2.30	2.22	2.18	2.13	2.10	0.64	0.27	0.007	0.04
Protozoa, $\times 10^4$/mL									
6 h	13.4	13.2	13.6	13.3	13.5	0.35	0.42	0.44	
Total gas, mL	31.0	34.6	37.5	39.0	41.1	0.44	0.007	0.49	0.001
6 h	25.0	28.1	31.0	32.0	34.1	0.45	0.006	0.47	
24 h	37.0	41.0	44.0	46.0	48.0	0.46	0.009	0.51	
H_2, mL/incubation	0.11	0.11	0.10	0.11	0.10	0.005	0.30	0.34	0.007
6 h	0.18	0.18	0.17	0.18	0.17	0.005	0.29	0.32	
24 h	0.03	0.03	0.03	0.03	0.03	0.004	0.31	0.35	
CH_4, mL/incubation	12.0	12.1	12.0	11.8	12.1	0.42	0.73	0.74	0.04
6 h	12.4	12.6	12.5	12.3	12.6	0.47	0.64	0.71	
24 h	11.60	11.54	11.42	11.26	11.63	0.34	0.77	0.81	
Total viable bacteria, $\times 10^8$/mL									
24 h	2.08	3.14	3.56	3.73	3.88	0.87	0.35	0.005	
L-Lactate, m*M*									
24 h	18.70	16.33	15.76	14.34	13.12	0.65	0.006	0.33	

[a]n = 15.
[b]L = linear effect due to dose of YST; Q = quadratic effect due to dose of YST.

reported that supplementation of *S. cerevisiae* in steers (10 g/d) resulted in an increase in the number of total protozoa.

Other major effects of YST on ruminal fermentation included increased concentrations of propionate and total VFA. Published reports of the effect of yeast culture on concentrations of VFA are variable. Chademana and Offer (1990) reported that Yea-Sacc 1026 had no effect on total VFA or VFA composition, but others found stimulation in the proportion of propionate at the expense of acetate (Newbold et al., 1990) or even an increase in the proportion of acetate (Mutsvangwa et al., 1992). Supplementation of other yeast cultures (Diamond V XP) to lactating dairy cows (114 g/d; Harrison et al., 1988) and steers (14.8 g/d; Adams et al., 1981) also increased the proportion of propionate, whereas proportions of acetate and isovalerate and acetate:propionate ratios were lower. In view of the variability in the response of ruminal VFA concentrations to yeast culture, Wallace and Newbold (1992) concluded that it was unlikely that the production benefits seen when yeast culture is added to the diet arise from changes in the stoichiometry of VFA formation.

The IVDMD of hay plus concentrate was increased with YST. In vivo studies reported that supplementation of Yea-Sacc 1026 did not affect the apparent digestibility of DM, OM, NDF, and CP of hay plus concentrate at different ratios, but did tend to be higher with the control diet (Chademana and Offer, 1990). Previous studies (Dowson, 1990; Williams et al., 1991) have reported that the stimulation of cellulose degradation by yeast culture is associated with a decreased lag time, which results in increased initial rates of digestion, but not in increased extent of digestion by ruminal microorganisms. Williams et al. (1991) reported that yeast culture stimulated DM digestion in the rumen of hay-fed steers when barley was absent. They attributed this difference to a stabilization of ruminal pH by yeast culture in animals receiving barley. In a subsequent study, Newbold et al. (1995) reported that some yeast cultures increased the number of total and cellulolytic bacteria in the rumen and, in some cases, increased cellulose degradation. They also suggested that *S. cerevisiae* culture stimulated the rate rather than the extent of fiber digestion by ruminal microorganisms. In a later experiment, Callaway and Martin (1997) reported

Table 5. Effects of Yea-Sacc Twin-Strain 8417 and 1026 (YST) *Saccharomyces cerevisiae* live cells on the fermentation of hay plus concentrate by mixed ruminal microorganisms

	YST g/L						Contrast[b]		
Item	0	0.33	0.66	0.99	1.32	SEM[a]	L	Q	Time
pH	6.22	6.20	6.18	6.19	6.21	0.01	0.45	0.40	0.12
6 h	6.42	6.38	6.37	6.36	6.39	0.01	0.42	0.38	
24 h	6.02	6.01	5.98	6.01	6.02	0.01	0.52	0.45	
Ammonia-N, mg/dL	15.6	15.4	15.4	15.4	15.6	0.57	0.65	0.70	0.04
6 h	14.6	14.2	14.3	14.4	14.4	0.56	0.66	0.72	
24 h	16.5	16.5	16.4	16.3	16.7	0.52	0.69	0.72	
Total VFA, m*M*	73.9	76.8	77.9	80.6	82.3	0.84	0.008	0.25	0.006
6 h	67.4	69	69.7	72.9	74.6	0.84	0.007	0.30	
24 h	80.3	84.6	86.1	88.2	89.9	0.88	0.009	0.28	
Individual VFA, mol/100 mol									
Acetate	60.2	60.4	60.7	60.9	61.2	0.53	0.52	0.02	0.29
Propionate	24.3	24.7	24.9	25.1	25.2	0.61	0.29	0.01	0.10
Butyrate	11.9	11.7	11.2	10.8	10.7	0.25	0.007	0.37	0.31
Isobutyrate	1.50	1.35	1.20	1.35	1.25	0.14	0.17	0.05	0.46
Valerate	1.25	1.25	1.15	1.25	1.10	0.14	0.17	0.05	0.48
Isovalerate	0.60	0.55	0.50	0.50	0.60	0.08	0.19	0.05	0.53
Acetate:propionate	2.49	2.45	2.44	2.25	2.19	0.44	0.41	0.04	0.07
Protozoa, $\times 10^4$/mL									
6 h	12.5	12.4	12.8	12.4	12.5	0.35	0.40	0.57	
Total gas, mL	29.5	36.5	38.5	41.5	43.0	0.26	0.008	0.38	0.001
6 h	15.0	24.0	25.0	29.0	31.0	0.26	0.007	0.38	
24 h	44.0	49.0	52.0	54.0	55.0	0.22	0.008	0.32	
H_2, mL/incubation	0.18	0.15	0.16	0.16	0.15	0.005	0.35	0.02	0.008
6 h	0.32	0.28	0.29	0.29	0.28	0.005	0.48	0.04	
24 h	0.03	0.03	0.03	0.03	0.03	0.004	0.28	0.005	
CH_4, mL/incubation	17.1	16.5	16.4	16.3	16.3	0.96	0.04	0.43	0.03
6 h	17.7	17.7	17.7	17.7	17.8	0.75	0.62	0.59	
24 h	16.4	15.4	15.1	14.8	14.8	0.96	0.04	0.30	
IVDDM, %									
24 h	45.0	46.1	47.4	48.8	49.7	0.54	0.006	0.28	
Total viable bacteria, $\times 10^8$/mL									
24 h	6.98	7.46	7.73	7.98	8.44	0.71	0.007	0.36	
Cellulolytic bacteria, $\times 10^6$/mL									
24 h	4.77	5.34	5.81	5.97	6.49	0.88	0.007	0.37	
L-Lactate, m*M*									
24 h	0.19	0.11	0.09	0.06	0.04	0.23	0.006	0.32	

[a]n = 15.
[b]L = linear effect due to dose of YST; Q = quadratic effect due to dose of YST.

that Diamond V XP yeast culture filtrate increased cellulose disappearance as much as 11% after 24 h of incubation, but no change in cellulose disappearance was found after 48 or 72 h when incubated with predominant ruminal bacteria *F. succinogenes* and *R. flavefaciens*. They concluded that the *S. cerevisiae* culture filtrate stimulated the initial rate of cellulose degradation by these two predominant cellulolytic bacteria without influencing the extent of degradation.

In the present study, YST also increased the numbers of total viable bacteria and cellulolytic bacteria. Newbold et al. (1995) reported that Yea-Sacc 1026 increased the numbers of total viable bacteria and cellulolytic bacteria both in vitro and in vivo studies. Increased bacterial numbers in the rumen have been one of the most consistently reported effects in animals fed another yeast culture, Diamond V XP (Wiedmeier et al., 1987; Harrison et al., 1988). It has been suggested that increased bacterial flora in animals fed *S. cerevisiae* is central to the action of yeast in the rumen, and increased bacterial population leads to an increase in both the degradation of fiber in the rumen and the flow of microbial protein from the rumen (Wallace and Newbold, 1992). The increase in bacterial numbers in our study with cornstarch and soluble potato starch was relatively small compared with hay plus concentrate, which might have been the result of the induction of cellulolytic bacteria growth in the presence of forage.

In the presence of ground corn and soluble potato starch, YST had no effect on the concentration of hydrogen and methane. But after 24 h of incubation with hay plus concentrate, there was a small decrease in methane with increasing concentration of YST. Methane production was also decreased by Yea-Sacc 1026 in vitro (Mutsvangwa et al., 1992). Recent research reported that the live cells of *S. cerevisiae* (PMX70SBK; Saf Agri) decreased methane significantly from alfalfa hay (Lynch and Martin, 2002). The decrease in methane

production may be due to the utilization of metabolic hydrogen by acetogenic bacteria to produce acetate in the present study. Coculture of *S. cerevisiae* strain (CNCM I-1077, Institut Pasteur, Paris, France) with methanogen and acetogen, enhanced the metabolism of acetogenic strain and its acetate production, which supports the present results (Chaucheyras et al., 1995).

Literature Cited

Adams, D. C., M. L. Galyean, H. E. Kiesling, J. D. Wallace, and M. D. Finkner. 1981. Influence of viable yeast culture, sodium bicarbonate, and monensin on liquid dilution rate, rumen fermentation, and feedlot performance of growing steers and digestibility in lambs. J. Anim. Sci. 53:780–785.

Alexander, M. 1982. Most probable number method for microbial populations. Pages 815–820 in Methods of Soil Analysis. 2nd ed. A. L. R. H. Miller and D. R. Keeney, ed. Am. Soc. Agron., Madison, WI.

Bayaru, E., S. Kanda, T. Kamada, H. Itabashi, S. Andoh, T. Nishida, M. Ishida, T. Itoh, K. Nagara, and Y. Isobe. 2001. Effect of fumaric acid on methane production, rumen fermentation, and digestibility of cattle fed roughage alone. Anim. Sci. 72:139–146.

Bryant, M. P., and L. A. Burkey. 1953. Cultural methods and some characteristics of some more numerous groups of bacteria in the bovine rumen. J. Dairy Sci. 36:205–217.

Callaway, T. R., and S. A. Martin. 1996. Effects of organic acid and monensin treatment on in vitro mixed ruminal microorganism fermentation of cracked corn. J. Anim. Sci. 74:1982–1989.

Callaway, E. S., and S. A. Martin. 1997. Effects of *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci. 80:2035–2044.

Carro, M. D., P. Lebzien, and K. Rohr. 1992. Effects of yeast culture on rumen fermentation, digestibility and duodenal flow in dairy cows fed a silage based diet. Livest. Prod. Sci. 32:219–229.

Chademana, I., and N. W. Offer. 1990. The effect of dietary inclusion of yeast culture on digestion in the sheep. Anim. Prod. 50:483–489.

Chaucheyras, F., G. Fonty, G. Bertin, and P. Gouet. 1995. In vitro H_2 utilization by a ruminal acetogenic bacterium cultivated alone or in association with an archea methanogen is stimulated by a probiotic strain of *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 61:3466–3469.

Conway, E. J. 1962. Microdiffusion Analysis and Volumetric Error. 5th ed. Crosby Lockwood, London, U.K.

Dowson, K. A. 1990. Designing the yeast culture of tomorrow-mode of action of yeast culture for ruminants and non-ruminants. Page 59 in Biotechnology in the Feed Industry. Proc. Alltech's 6th Annu. Symp., Lexington, KY. Alltech Tech. Publ., Nicholasville, KY.

Erasmus, L. J., P. M. Botha, and A. Kistner. 1992. Effect of yeast culture supplement on production, rumen fermentation, and duodenal nitrogen flow in dairy cows. J. Dairy Sci. 75:3056–3065.

Halliwell, M. E., and M. P. Bryant. 1953. The cellulolytic activity of pure strains of bacteria from the rumen of cattle. J. Gen. Microbiol. 32:441–448.

Harrison, G. A., R. W. Hemken, K. A. Dawson, R. J. Harmon, and K. B. Barker. 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial populations. J. Dairy Sci. 71:2967–2975.

Hungate, R. E. 1969. A roll-tube method for cultivation of strict anaerobes. Methods Microbiol. 3B:117–132.

Kumar, U., V. K. Sareen, and S. Singh. 1994. Effect of *Saccharomyces cerevisiae* yeast culture supplement on ruminal metabolism in buffalo calves given a high concentrate diet. Anim. Prod. 59:209–215.

Kung, Jr., L. E., M. Kreck, and R. S. Tung. 1997. Effects of a live yeast culture and enzymes on in vitro ruminal fermentation and milk production of dairy cows. J. Dairy Sci. 80:2045–2051.

Leedle, J. A. Z., and R. C. Hespell. 1980. Differential carbohydrate media and anaerobic replica plating techniques in delineating carbohydrate-utilizing subgroups in rumen bacteria populations. Appl. Environ. Microbiol. 34:709–719.

Lynch, H. A., and S. A. Martin. 2002. Effects of *Saccharomyces cerevisiae* culture and *Saccharomyces cerevisiae* live cells on in vitro mixed ruminal microorganism fermentation. J. Dairy Sci. 85:2603–2608.

Martin, S. A., and D. J. Nisbet. 1992. Effect of direct-fed microbials on rumen microbial fermentation. J. Dairy Sci. 75:1736–1744.

Morvan, B., F. Bonnemoy, G. Fonty, and P. Gouet. 1996. Quantitative determination of H_2-utilizing acetogenic and sulfate-reducing bacteria and methanogenic archaea from digestive tract of different mammals. Curr. Microbiol. 32:129–133.

Mutsvangwa, T., I. E. Edwards, J. H. Topps, and G. F. M. Paterson. 1992. The effect of dietary inclusion of yeast culture (Yea-Sacc) on patterns of rumen fermentation, food intake and growth of intensively fed bulls. Anim. Prod. 55:35–41.

Newbold, C. J., R. J. Wallace, and F. M. McIntosh. 1995. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers in vitro and in sheep. J. Anim. Sci. 73:1811–1818.

Newbold, C. J., P. E. V. Williams, N. McKain, A. Walker, and R. J. Wallace. 1990. The effects of yeast culture on yeast numbers and fermentation in the rumen of sheep. Proc. Nutr. Soc. 49:47A. (Abstr.)

Nisbet, D. J., and S. A. Martin. 1991. Effects of *Saccharomyces cerevisiae* culture on lactate utilization by the ruminal bacterium *Selenomonas ruminantium.* J. Anim. Sci. 69:4628–4633.

Ogimoto, K., and S. Imai. 1981. Atlas of Rumen Microbiology. Japan Scientific Societies Press, Tokyo, Japan.

Piva, G., S. Belladonna, G. Fusconi, and F. Sicbaldi. 1993. Effects of yeast on dairy cow performance, ruminal fermentation, blood components, and milk manufacturing properties. J. Dairy Sci. 76:2717–2722.

Plata, P. F., M. G. D. Mendoza, J. R. Barcena-Gama, and M. S. Gonzalez. 1994. Effect of a yeast culture (*Saccharomyces cerevisiae*) on neutral detergent fiber digestion in steers fed oat straw based diets. Anim. Feed. Sci. Technol. 49:203–210.

Russell, J. B., and S. A. Martin. 1984. Effects of various methane inhibitors on the fermentation of amino acids by mixed rumen microorganisms in vitro. J. Anim. Sci. 59:1329–1338.

Sullivan, H. M., and S. A. Martin. 1999. Effects of *Saccharomyces cerevisiae* culture on in vitro mixed ruminal microorganism fermentation. J. Dairy Sci. 82:2011–2016.

Swartz, D. L., L. D. Muller, G. W. Rogers, and G. A. Varga. 1994. Effect of yeast cultures on performance of lactating dairy cows: A field study. J. Dairy Sci. 77:3073–3079.

Wallace, R. J., and C. J. Newbold. 1992. Probiotics for ruminants. Page 317 in Probiotics: The Scientific Basis. R. Fuller, ed. Chapman and Hall, London, U.K.

Wiedmeier, R. D., M. J. Arambel, and J. L. Walters. 1987. Effect of yeast culture and *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestibility. J. Dairy Sci. 70:2063–2066.

Williams, P. E. V., C. A. G. Tait, G. M. Innes, and C. J. Newbold. 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of cows on milk yield and forage degradation and fermentation patterns in the rumen of sheep and steers. J. Anim. Sci. 69:3016–3026.

Wolin, M. J. and T. L. Miller. 1988. Microbe-microbe interactions. Page 343 in Rumen Microbial Ecosystem. P. N. Hobson, ed. Elsevier Science, Essex, U.K.