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Evaluation of Different Yeast Species for Improving *In vitro* Fermentation of Cereal Straws

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ABSTRACT: Information on the effects of different yeast species on ruminal fermentation is limited. This experiment was conducted in a 3×4 factorial arrangement to explore and compare the effects of addition of three different live yeast species (*Candida utilis* 1314, *Saccharomyces cerevisiae* 1355, and *Candida tropicalis* 1254) at four doses (0, 0.25×10^7, 0.50×10^7, and 0.75×10^7 colony-forming unit [cfu]) on *in vitro* gas production kinetics, fiber degradation, methane production and ruminal fermentation characteristics of maize stover, and rice straw by mixed rumen microorganisms in dairy cows. The maximum gas production (*Vf*), dry matter disappearance (IVDMD), neutral detergent fiber disappearance (IVNDFD), and methane production in *C. utilis* group were less ($p<0.01$) than other two live yeast supplemented groups. The inclusion of *S. cerevisiae* reduced ($p<0.01$) the concentrations of ammonia nitrogen (NH_3-N), isobutyrate, and isovalerate compared to the other two yeast groups. *C. tropicalis* addition generally enhanced ($p<0.05$) IVDMD and IVNDFD. The NH_3-N concentration and CH_4 production were increased ($p<0.05$) by the addition of *S. cerevisiae* and *C. tropicalis* compared with the control. Supplementation of three yeast species decreased ($p<0.05$) or numerically decreased the ratio of acetate to propionate. The current results indicate that *C. tropicalis* is more preferred as yeast culture supplements, and its optimal dose should be 0.25×10^7 cfu/500 mg substrates *in vitro*. (**Key Words:** Fiber Degradation, *In vitro* Fermentation, Live Yeast, Methane, Volatile Fatty Acids)

INTRODUCTION

Supplementation with yeast products in the diets has become a common practice in improving the efficiency of feed utilization and the performance of ruminants for over 20 years (Moallem et al., 2009). It has been confirmed that yeast culture supplementation benefits digestion and metabolism of ruminants in several aspects, such as the improvement of nutrient digestibility, optimization of the proportion of volatile fatty acids (VFA) in the rumen, decrease in the ruminal ammonia nitrogen (NH_3-N), alleviation of pH fluctuation, and stimulation of ruminal microorganism population (Chaucheyras-Durand et al., 2008). Furthermore, it has been verified that yeast culture inclusion in the diets of ruminants can provide various growth factors, pro-vitamins and other stimulants to rumen microorganisms, and balance the ruminal fluid redox potential to create the optimal fermentation conditions for the rumen bacterial microflora (Jouany, 2001).

In the past few years, it had also been demonstrated that

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dietary live yeast supplementation plays a beneficial role in the improvement of ruminant's productivity. Holtshausen and Beauchemin (2010) reported that live yeast (Levucell SC-1077) supplementation had a positive effect on milk yield and milk efficiency in cows fed a barley-based diet. In another study, dietary live yeast supplementation in dairy cows during the hot season in Israel improved the rumen environment by enhancing the ruminal pH and ammonia utilization, and in consequence improved dry matter (DM) intake, productivity and conversion efficiency of feeds (Moallem et al., 2009).

However, as observed in many studies so far, the effectiveness of dietary yeast products inclusion are variable, which might be ascribed to variation between animals, experimental diets fed, method of feeding, strains of yeasts, and their viability as well. For instance, supplementing beef cattle with *Saccharomyces cerevisiae* could raise the live weight by 7.5% depending on the type of diet tested, while improvement reached 13% in feedlot for diets rich in starch and sugars (Estefan, 1999). Supplementation of yeast culture improved the rate of gas production (GP), DM and organic matter disappearances for rice straw, wheat straw and maize stover (Tang et al., 2008). Meanwhile, dietary inclusion of *S. cerevisiae* NCYC 240, NCYC 1026 and Yea-Sacc stimulated total and cellulolytic bacterial numbers, while *S. cerevisiae* NCYC 694 and NCYC 1088 exerted no influence on the numbers of bacteria *in vitro* (Newbold et al., 1995).

Up to now, there is still little available information about the variation in the ruminal fermentation resulting from the addition of different live yeast species. Hence, the objectives of this study were to explore and compare the effects of three different species of yeasts (*Candida utilis* 1314, *S. cerevisiae* 1355, and *Candida tropicalis* 1254) on the *in vitro* ruminal fermentation characteristics of rice straw and maize stover by ruminal microorganisms from dairy cows, to further understand the mode of action of live yeast species in the rumen, and to provide more valuable information on the live yeast application to ruminants' diets in practice.

MATERIALS AND METHODS

Crop straws, yeasts, and experimental design

Two types of crop straws which are most commonly used as roughage in diets for dairy cows in south China, i.e., maize stover (*Zea mays*, variety Kexiang Sweet Corn No. 1, Changsha, China) and rice straw (*Oryza sativa*, variety Xiang 125S/BAR-1, Changsha, China) were selected as *in vitro* fermentation substrates in this study. They were dried at 65°C for 24 h, and then ground through a 1 mm sieve and stored in plastic bags for assay. Maize stover and rice straw contained (DM basis): 52.3 and 62.4 g crude protein (CP)/kg, 636 and 632 g neutral detergent fiber (NDF)/kg, and 386 and 434 g acid detergent fiber (ADF)/kg, respectively.

Three different species of yeasts (*C. utilis* 1314, *S. cerevisiae* 1355, and *C. tropicalis* 1254) originally used as feed additives, were purchased from and reactivated by the China Center of Industrial Culture Collection. Yeasts were cultured and amplified using liquid malt extract medium (130 g malt extract and 0.1 g chloramphenicol/L), then their total viable numbers were counted in the form of colony-forming units (cfu) by employing the spread plate method. Afterwards, the yeasts were preserved at 4°C until the *in vitro* fermentation was started. The experiment was conducted in a 3×4 factorial arrangement, factors included yeast (three yeast species) and dose (0×10^7cfu [without addition of yeast], 0.25×10^7cfu, 0.50×10^7cfu, and 0.75×10^7cfu).

In vitro gas production and sampling

Culture solutions, i.e., macroelement solution, buffered solution and reducing solution used for *in vitro* fermentation were prepared to form artificial saliva according to the procedures modified by Tang et al. (2006). The artificial saliva was kept anaerobic by continuously pumping carbon dioxide for 2 h.

Rumen fluids were obtained from three rumen-cannulated Holstein dairy cows fed *ad libitum* a mixed diet of rice straw and concentrate (60:40, weight/weight) offered twice daily at 07:00 and 19:00 h. Concentrate contained (per 1,000 g DM): 396 g ground maize, 181 g soybean meal, 10 g $CaHPO_4$, 3 g limestone meal and 10 g premix. The rumen-cannulated Holstein dairy cows were managed according to the protocols approved by the Animal Care and Use Guidelines of the Animal Care Committee, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha, China. Rumen contents of each dairy cow were obtained from various locations within the rumen immediately before the morning feeding, mixed and strained through four layers of cheesecloth under a continuous CO_2 stream. The obtained rumen fluids were then anaerobically combined with artificial saliva in the proportion of 1 to 9 at 39°C.

Samples of strawor stoverin an amount of 500±10 mg was accurately weighed into 100-mL fermentation bottles (Wanhong Glass Instrument Factory, Haimen, China) prewarmed at 39°C, then 50 mL of the mixed fluids (artificial saliva plus rumen fluids) were introduced into each bottle using a dispenser (Varispenser 4960000.060; Eppendorf, Wesseling-Berzdorf, Germany). After that, the yeasts were respectively added according to the above-mentioned different doses when the *in vitro* fermentation was started. Blanks containing only mixed fluids, mixed fluids and substrates, mixed fluids and different doses of

yeasts were all incubated together with the treated bottles.

All fermentation bottles were connected with pressure sensors (CYG130-12; SQsensor, Kunshan, China) and incubated at 39°C. The pressure in all the bottles was recorded at 0, 1, 2, 4, 6, 12, 24, and 48 hours during the process of *in vitro* fermentation. Each time at 12, 24, and 48 h, three bottles for each treatment were respectively taken out from the incubator to stop the incubation. After termination of incubation, a 5 mL gas sample was collected into the vacuum flask (LabcoExetainer; Labco, High Wycombe, UK) with plastic syringe for CH4 determination, and then undegraded residues were immediately filtered through 2 layers of nylon cloth (40-um pore size). The incubation solutions of each treatment were sampled for determination of NH_3-N and VFA concentrations at 12, 24, and 48 h, respectively. *In vitro* fermentation was separately run three times on different days to result in nine analytical replicates (i.e., three analytical replicates per run).

Chemical analysis

The DM (method 930.15) and CP (6.25×N, method 990.03) were analyzed using the procedures of the Association of Official Analytical Chemists (AOAC, 1999). The NDF and ADF content were determined using a Fibretherm Fiber Analyzer (Gerhardt, Bonn, Germany) according to Van Soest et al. (1991) with addition of sodium sulphite and alpha-amylase in the NDF analysis. The filtered residues were dried at 105°C for 12 h and weighed for *in vitro* dry matter disappearance (IVDMD) determination. The NDF content in the dried residues was determined to calculate *in vitro* NDF disappearance (IVNDFD).

Two mL of incubation solution was centrifuged at 10,000×g at 4°C for 15 min, then 1.5 mL of supernatant solution was taken and 0.15 mL of metaphosphoric acid was added and homogenized. The mixed solution was centrifuged at 10,000×g at 4°C for 15 min again, and the supernatant solution was used to determine VFA content with a gas chromatograph (HP5890, Agilent 5890; Agilent Technologies, Palo Alto, CA, USA). A DB-FFAP column (30 m in length with a 0.25 mm inside diameter [i.d.]) was used for the separation. The attenuation was set at a nitrogen diffluent ratio of 1:50, hydrogen flow 30 mL/min, airflow 365 mL/min, injector temperature 250°C, column temperature 150°C, and detector temperature 220°C. The N_2 was used as carrier gas at a flow rate of 0.8 mL/min. The relative response factor, representing the peak of each VFA, was calculated using the standard VFA mixture, which was chromatographed with each group of 10 samples. Total molar concentration was calculated by taking the sum of individual VFA as 1.

For the determination of NH_3-N, 5 mL of incubation solution was centrifuged at 4,000×g and 4°C for 10 min, then 2 mL of the supernatant solution was taken and mixed with 8 mL 0.2 M HCl into a tube followed by homogenization. Subsequently, 0.4 mL of the mixed solution was taken and mixed with 2 mL of sodium nitroprusside solution (0.08 g sodium nitroprusside dissolved in 100 mL of 0.14 natrium salicylicum) and 2 mL of prepared solution (2 mL sodium hypochlorite solution mixed with 100 mL 0.3 M sodium hydroxide solution), then transferred into a tube followed by homogenization and equilibrated at room temperature for 10 min. The light absorption value was recorded at 700 nm using spectrophotometer (UV-2300; Shimadzu, Kyoto, Japan).

The CH_4 analysis was performed by gas chromatography (GC)-flame ionization detection using GC (GC7890A; Agilent Technologies, USA) equipped with a Hayesep Q packing column (2.44 M×1/8 in.×2.0 mm i.d.). The temperature of column and injector was respectively set at 60°C and 100°C for 3 min. The N_2 was used as carrier gas at a flow rate of 21 mL/min.

Calculation and statistical analysis

During the initial stages of this work, the correlation between the pressure in bottle and gas volume was measured at 39°C, and the regression equation was then established:

$$y = 1.506x \ (n = 20, r^2 = 0.999, p<0.0001) \quad (1)$$

Where y represents gas volume (mL), x is the pressure in bottle (kPa), 1.506 is a constant. The measured pressure was then converted to GP (mL). *In vitro* GP at 0, 1, 2, 4, 6, 12, 24, and 48 hours was fitted to Logistic-Exponential (Wang et al., 2011):

$$GP = Vf\,(1 - \exp(d - t\times k))/(1+\exp(b - k\times t)) \quad (2)$$

Where GP represents GP at t time, Vf means the maximum GP (mL), k represents GP fraction (/h), b and d represent the shapes of the GP curve. The time ($t_{0.5}$, h) when half of the maximum GP was achieved and the initial fractional rate of degradation (/h) were calculated by respectively employing the following two equations (Wang et al., 2011; Wang et al., 2013):

$$t_{0.5} = \text{In}(\exp(b)+2\exp(d))/k \quad (3)$$

$$FRD_0 = k/(1+\exp(b)) (4)$$

GP, IVDMD, and IVNDFD were corrected by subtracting the values obtained for the blanks. Data were analyzed by two-way analysis of variance in the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA)

(SAS Institute Inc., 2001). For GP parameters, the model included species, dose, and species×dose as fixed effects. For pH, NH_3-N, CH_4 production, VFAs, IVDMD and IVNDFD, the fixed effects of species, dose, and species×dose were included in the model, with incubation time as a repeated effect. The bottle was used as the experimental unit, and run and bottle were considered as random effects in the entire study. Linear and quadratic effects of dose were analyzed using orthogonal polynomial contrasts. Cubic effects of dose were not examined for inexplicability in biology. Least squares means are reported throughout the text, and significance was declared at $p<0.05$.

RESULTS

In vitro gas production parameters

For maize stover, *in vitro* GP parameters generally were not affected by yeast species except for *Vf*, which was respectively 7% and 8% higher ($p<0.01$) for *S. cerevisiae* and *C. tropicalis* than the *C. utilis* (Table 1). All the parameters, except FRD_0, were influenced to a certain extent by yeast dose being dependent on yeast species. The *C. utilis* addition quadratically decreased ($p<0.05$) *Vf*, while linearly reduced ($p<0.01$) *k*. The addition of *C. tropicalis* showed a quadratic decreasing ($p<0.05$) effect on $t_{0.5}$. In comprehensive consideration of the effectiveness of improving GP and rate, the optimum supplemental dose of *S. cerevisiae* and *C. tropicalis* would be 0.25×10^7 cfu and 0.75×10^7 cfu, respectively.

For rice straw, the yeast species exerted significant effects ($p<0.01$) on *Vf* and FRD_0 (Table 2). Compared with the addition of *C. utilis*, *Vf* for *S. cerevisiae* and *C. tropicalis* were respectively 16% and 19% higher. Besides, FRD_0 was 43% higher for the *C. tropicalis* treatment than for the *S. cerevisiae* treatment. The dose effects of yeast addition on *in vitro* GP parameters were dependent on yeast species. The addition of *C. utilis* decreased *k* and $t_{0.5}$ in the same manner (linear, $p<0.05$), but increased FRD_0 (linear, $p<0.05$). Moreover, a quadratic ($p<0.05$) dose response to *S. cerevisiae* addition for *Vf* was positively observed. The profitable effects of *C. tropicalis* addition on FRD_0 (linear, $p<0.01$) and $t_{0.5}$ (linear, $p<0.05$) were also noted. The interactive effects of species and dose on *Vf* and $t_{0.5}$ were observed ($p<0.05$). Generally considering *in vitro* GP and rate, the optimum supplemental doses of *S. cerevisiae* and *C. tropicalis* might both be 0.25×10^7 cfu.

In vitro dry matter and neutral detergent fiber disappearance

For maize stover, the yeast species affected IVDMD and IVNDFD ($p<0.01$) (Table 3). The lowest IVDMD and IVNDFD were observed in the *C. utilis* treatment, the former was 5% and 13% less, while the latter was 11% and 21% lower, when compared with *S. cerevisiae* and *C. tropicalis* treatment, respectively. A linear decrease ($p<0.01$) both in IVDMD and in IVNDFD was noted in response to

Table 1. Effects of different yeast species addition on *in vitro* gas production kinetics for maize stover

Item	Species	Dose ($\times10^7$ colony-forming unit)					SEM[2]	Significance (>p)[3]		
		Mean [1]	0	0.25	0.50	0.75		Species	Dose	Species×dose
Vf (mL)	*Candida utilis*	67.97[f]	71.33[a]	65.50[ab]	64.25[b]	70.80[a]	2.013	<0.01	Q (<0.05)	NS
	Saccharomyces cerevisiae	72.85[e]	71.33	75.91	72.16	71.99			NS	
	Candida tropicalis	73.12[e]	71.33	72.45	72.45	76.24			NS	
	SEM [4]	1.006								
k (/h)	*Candida utilis*	0.087	0.105[a]	0.108[a]	0.091[a]	0.043[b]	0.0113	NS	L (<0.01)	NS
	Saccharomyces cerevisiae	0.010	0.105	0.010	0.096	0.098			NS	
	Candida tropicalis	0.097	0.105	0.102	0.100	0.081			NS	
	SEM	0.0056								
FRD_0 (/h)	*Candida utilis*	0.027	0.024	0.025	0.026	0.034	0.0038	NS	NS	NS
	Saccharomyces cerevisiae	0.023	0.024	0.026	0.021	0.021			NS	
	Candida tropicalis	0.027	0.024	0.028	0.026	0.031			NS	
	SEM	0.0019								
$t_{0.5}$ (h)	*Candida utilis*	17.10[e]	16.84	15.42	16.67	19.49	0.729	NS	NS	NS
	Saccharomyces cerevisiae	16.96[ef]	16.84	15.67	17.75	17.58			NS	
	Candida tropicalis	15.91[f]	16.84	15.11	15.72	15.98			Q (<0.05)	
	SEM	0.364								

SEM, standard error of the mean; NS, not significant.
[1] Mean for individual species across doses including the dose of 0.
[2] SEM for strain×dose.
[3] NS (p>0.05), L = linear effect of dose, Q = quadratic effect of dose.
[4] SEM for pooled mean of species including the dose of 0.
[a, b] Means within a row for doses that do not have a common superscript differ (p<0.05).
[e, f] Means within a column for species that do not have a common superscript differ (p<0.05).

Table 2. Effects of different yeast species addition on *in vitro* gas production kinetics for rice straw

Item	Species	Dose (×10^7 colony-forming unit)					SEM[2]	Significance (>p)[3]		
		Mean [1]	0	0.25	0.50	0.75		Species	Dose	Species×dose
Vf (mL)	*Candida utilis*	53.23[f]	58.46	53.12	50.31	51.05	2.564	<0.01	NS	<0.05
	Saccharomyces cerevisiae	61.68[e]	58.46[b]	69.13[a]	60.30[b]	58.82[b]			Q (<0.05)	
	Candida tropicalis	63.55[e]	58.46	67.48	62.86	65.41			NS	
	SEM [4]	1.282								
k (/h)	*Candida utilis*	0.101[ef]	0.119[a]	0.110[ab]	0.100[ab]	0.074[b]	0.0122	NS	L (<0.05)	NS
	Saccharomyces cerevisiae	0.115[e]	0.119	0.103	0.121	0.117			NS	
	Candida tropicalis	0.093[f]	0.119	0.078	0.099	0.077			NS	
	SEM	0.0061								
FRD_0 (/h)	*Candida utilis*	0.018[ef]	0.013[b]	0.014[b]	0.020[ab]	0.024[a]	0.0023	<0.01	L (<0.05)	NS
	Saccharomyces cerevisiae	0.014[f]	0.013	0.016	0.014	0.013			NS	
	Candida tropicalis	0.020[e]	0.013[b]	0.021[a]	0.021[a]	0.025[a]			L (<0.01)	
	SEM	0.0011								
$t_{0.5}$ (h)	*Candida utilis*	19.31	19.98[a]	19.67[a]	18.25[b]	19.34[ab]	0.412	NS	L (<0.05)	<0.05
	Saccharomyces cerevisiae	19.36	19.98	19.20	18.65	19.63			NS	
	Candida tropicalis	19.07	19.98[a]	20.07[a]	17.97[b]	18.26[b]			L (<0.05)	
	SEM	0.206								

SEM, standard error of the mean; NS, not significant.
[1] Mean for individual species across doses including the dose of 0.
[2] SEM for strain×dose.
[3] NS (p>0.05), L = linear effect of dose, Q = quadratic effect of dose.
[4] SEM for pooled mean of species including the dose of 0.
[a, b] Means within a row for doses that do not have a common superscript differ (p<0.05).
[e, f] Means within a column for species that do not have a common superscript differ (p<0.05).

the increase of *C. utilis* addition, while the *C. tropicalis* addition linearly increased IVDMD and IVNDFD (p<0.05), and the maximum IVDMD and IVNDFD were both achieved at the supplemental dose of 0.75×10^7 cfu, which were respectively 12% and 22% greater than the control.

As for rice straw, IVDMD and IVNDFD were influenced (p<0.01) by the yeast species, and the least IVDMD and IVNDFD were observed in the *C. utilis* treatment, the former was 7% and 13% less, while the latter was 15% and 20% less than those of *S. cerevisiae* and *C. tropicalis* treatment, respectively. The *C. utilis* supplementation decreased IVDMD (linear, p<0.01) and IVNDFD (quadratic, p<0.01). A quadratic (p<0.01) response in IVNDFD to *C. tropicalis* addition was noted, and the greatest IVNDFD occurred at the supplemental dose of 0.25×10^7 cfu, which was improved by 16% compared with the control.

pH, NH_3-N and CH_4 production

For maize stover, yeast species affected (p<0.01) the NH_3-N concentration of *in vitro* fermentation liquors and CH_4 production/g IVDMD (Table 4). The NH_3-N concentration of the *S. cerevisiae* treatment was respectively 6% and 8% less than those of the *C. utilis* and *C. tropicalis* treatments, while the CH_4 production for *C. utilis* addition was respectively 12% and 10% lower than the addition of *S. cerevisiae* and *C. tropicalis*. A linear reduction (p<0.05) in pH value was noted for both the *C. utilis* and *C. tropicalis* treatments, while a quadratic response and a linear increase in the concentration of NH_3-N were observed for the *S. cerevisiae* (p<0.05) and *C. tropicalis* (p<0.01) treatments, respectively. Besides, the addition of *S. cerevisiae* and *C. tropicalis* caused overall increases in CH_4 production, which were quadratic (p<0.01) and linear (p<0.01), respectively. Moreover, the greatest CH_4 production in those two yeast treatments were both achieved at the supplemental dose of 0.50×10^7cfu, which were respectively 33% and 26% greater than the control. There was an interactive effect (p<0.05) of species and dose on the NH_3-N concentration.

As regards rice straw, the yeast species influenced the NH_3-N concentration of fermentation liquors (p<0.01) and CH_4 production/g IVDMD (p<0.01). The NH_3-N concentration in the *S. cerevisiae* treatment was respectively 7% and 18% lower than those of *C. utilis* and *C. tropicalis* treatment, while CH_4 production supplemented with *C. utilis* was 8% less than that of *S. cerevisiae*, and 10% less than that of *C. tropicalis*. The pH value decreased in response to the addition of *C. utilis* (linear, p<0.01), *S. cerevisiae* (quadratic, p<0.05), and *C. tropicalis* (quadratic, p<0.01). As for the concentration of NH_3-N, a quadratic reduction and a linear increase (p<0.01) were observed with the increasing doses of *S. cerevisiae* and *C. tropicalis*, respectively. The CH_4 production was increased by the

Table 3. Effects of different yeast species addition on IVDMD and IVNDFD of maize stover and rice straw

Item	Species	Dose (×10^7 colony-forming unit)					SEM[2]	Significance (>p)[3]		
		Mean [1]	0	0.25	0.50	0.75		Species	Dose	Species×dose
Maize stover										
IVDMD (mg/mg)	*Candida utilis*	0.499^g	0.531^a	0.505^{ab}	0.483^b	0.478^b	0.0002	<0.01	L (<0.01)	<0.05
	Saccharomyces cerevisiae	0.526^f	0.531	0.529	0.497	0.548			NS	
	Candida tropicalis	0.574^e	0.531^b	0.589^a	0.580^a	0.597^a			L (<0.05)	
	SEM [4]	0.0001								
IVNDFD (mg/mg)	*Candida utilis*	0.380^g	0.424^a	0.356^b	0.380^{ab}	0.359^b	0.0002	<0.01	L (<0.01)	<0.01
	Saccharomyces cerevisiae	0.428^f	0.424	0.431	0.426	0.430			NS	
	Candida tropicalis	0.484^e	0.424^b	0.500^a	0.493^a	0.518^a			L (<0.05)	
	SEM	0.0001								
Rice straw										
IVDMD (mg/mg)	*Candida utilis*	0.442^g	0.482^a	0.453^{ab}	0.424^{bc}	0.408^c	0.0001	<0.01	L (<0.01)	<0.01
	Saccharomyces cerevisiae	0.475^f	0.482	0.471	0.472	0.473			NS	
	Candida tropicalis	0.509^e	0.482	0.527	0.523	0.503			NS	
	SEM	0.0001								
IVNDFD (mg/mg)	*Candida utilis*	0.339^f	0.402^a	0.347^b	0.298^b	0.310^b	0.0002	<0.01	Q (<0.01)	<0.01
	Saccharomyces cerevisiae	0.400^e	0.402	0.398	0.395	0.405			NS	
	Candida tropicalis	0.423^e	0.402^b	0.467^a	0.464^a	0.359^b			Q (<0.01)	
	SEM	0.0001								

IVDMD, *in vitro* dry matter disappearance; IVNDFD, *in vitro* neutral detergent fiber; SEM, standard error of the mean; NS, not significant.
[1] Mean for individual species across doses including the dose of 0.
[2] SEM for strain×dose.
[3] NS (p>0.05); L = linear effect of does; Q = quadratic effect of dose.
[4] SEM for pooled mean of species including the dose of 0.
[a-c] Means within a row for doses that do not have a common superscript differ (p<0.05).
[e-g] Means within a column for species that do not have a common superscript differ (p<0.05).

addition of *C. utilis* (quadratic, p<0.01), *S. cerevisiae* (quadratic, p<0.05), and *C. tropicalis* (linear, p<0.01). Addition of *C. utilis* at the dose of 0.25×10^{7}cfu decreased CH_4 production by 18% compared with the control. There were interactive effects of species and dose on pH (p<0.05), NH_3-N (p<0.01) and CH_4 production/g IVDMD (p<0.01).

Volatile fatty acid

The yeast species influenced (p<0.01) the concentrations of isobutyrate, butyrate, isovalerate, and ratio of acetate to propionate (A:P) in incubation fluids when maize stover was used as substrate (Table 5). For the addition of *S. cerevisiae*, the concentration of isobutyrate was 10% and 16% lower, while the concentration of butyrate was 20% and 12% higher, when compared to those of *C. utilis* and *C. tropicalis* treatments respectively. The maximum concentration of isovalerate and A:P were observed in *C. tropicalis* treatment, which were respectively 20% and 37%, and 9% and 6% greater than those of *C. utilis* and *S. cerevisiae* treatments. The addition of *C. utilis* linearly decreased (p<0.01) A:P, and it obtained the numerical greatest concentrations of acetate, propionate, isovalerate, and total volatile fatty acids (TVFA) at the dose of 0.50×10^{7}cfu, which were respectively 25%, 26%, 18%, and 27% greater than the control. The *S. cerevisiae* addition increased the propionate concentration (linear, p<0.05), but decreased the concentrations of isobutyrate (quadratic, p<0.05), isovalerate (quadratic, p<0.05), valerate (linear, p<0.05), and A:P (linear, p<0.05). Additionally, the *C. tropicalis* addition linearly increased (p<0.01) the concentration of isovalerate, reaching the maximum which was 36% greater than that of the control at the dose of 0.75×10^7 cfu. The interactive effects of species and dose on the concentrations of acetate (p<0.05), butyrate (p<0.05), isovalerate (p<0.01), TVFA (p<0.05), and A:P (p<0.05) were noted respectively.

Regarding to rice straw, the concentrations of isobutyrate, isovalerate, and valerate of incubation fluids were affected (p<0.01) by yeast species (Table 6). For the *S. cerevisiae* addition, the isobutyrate concentration was respectively 15% and 18% less than the addition of *C. utilis* and *C. tropicalis*, while the isovalerate concentration in the *C. tropicalis* treatment was 19% and 39% higher compared to *C. utilis*and *S. cerevisiae*. In addition, the valerate

Table 4. Effects of different yeast species addition on pH, NH_3-N concentration and CH_4 production of maize stover and rice straw *in vitro*

	Item	Species	Dose (×10^7 colony-forming unit)					SEM[2]	Significance (>p)[3]		
			Mean [1]	0	0.25	0.50	0.75		Species	Dose	Species ×dose
Maize stover	pH	*Candida utilis*	6.46	6.50^{ab}	6.51^{a}	6.43^{ab}	6.41^{b}	0.033	NS	L (<0.05)	NS
		Saccharomyces cerevisiae	6.47	6.50	6.49	6.46	6.45			NS	
		Candida tropicalis	6.44	6.50^{a}	6.44^{ab}	6.41^{ab}	6.40^{b}			L (<0.05)	
		SEM[4]	0.017								
	NH_3-N (mg/dL)	*Candida utilis*	7.57^{e}	7.30	7.92	7.41	7.65	0.269	<0.01	NS	<0.05
		Saccharomyces cerevisiae	7.14^{f}	7.30^{ab}	6.68^{b}	7.10^{ab}	7.50^{a}			Q (<0.05)	
		Candida tropicalis	7.77^{e}	7.30^{b}	7.25^{b}	7.70^{b}	8.83^{a}			L (<0.01)	
		SEM	0.135								
	CH_4 (mmol/g) IVDMD	*Candida utilis*	0.44^{f}	0.43	0.38	0.49	0.46	0.024	<0.01	NS	NS
		Saccharomyces cerevisiae	0.50^{e}	0.43^{c}	0.53^{ab}	0.57^{a}	0.48^{bc}			Q (<0.01)	
		Candida tropicalis	0.49^{e}	0.43^{b}	0.49^{ab}	0.54^{a}	0.52^{a}			L (<0.01)	
		SEM	0.013								
Rice straw	pH	*Candida utilis*	6.49	6.57^{a}	6.51^{b}	6.46^{bc}	6.42^{c}	0.019	NS	L (<0.01)	<0.05
		Saccharomyces cerevisiae	6.50	6.57^{a}	6.49^{b}	6.48^{b}	6.46^{b}			Q (<0.05)	
		Candida tropicalis	6.50	6.57^{a}	6.48^{bc}	6.44^{c}	6.52^{ab}			Q (<0.01)	
		SEM	0.010								
	NH_3-N (mg/dL)	*Candida utilis*	6.30^{f}	6.33	5.91	6.64	6.30	0.276	<0.01	NS	<0.01
		Saccharomyces cerevisiae	5.87^{g}	6.33^{a}	5.73^{ab}	5.24^{b}	6.16^{a}			Q (<0.01)	
		Candida tropicalis	7.14^{e}	6.33^{b}	7.14^{a}	7.58^{a}	7.51^{a}			L (<0.01)	
		SEM	0.138								
	CH_4 (mmol/g) IVDMD	*Candida utilis*	0.35^{f}	0.34^{b}	0.28^{c}	0.36^{b}	0.41^{a}	0.018	<0.01	Q (<0.01)	<0.01
		Saccharomyces cerevisiae	0.38^{e}	0.34^{b}	0.40^{a}	0.40^{a}	0.39^{ab}			Q (< 0.05)	
		Candida tropicalis	0.39^{e}	0.34^{b}	0.40^{a}	0.41^{a}	0.42^{a}			L (< 0.01)	
		SEM	0.009								

SEM, standard error of the mean; NS, not significant.
[1] Mean for individual species across doses including the dose of 0.
[2] SEM for strain×dose.
[3] NS (p>0.05); L = linear effect of dose, Q = quadratic effect of dose.
[4] SEM for pooled mean of species including the dose of 0.
[a-c] Means within a row for doses that do not have a common superscript differ (p<0.05).
[e-g] Means within a column for species that do not have a common superscript differ (p<0.05).

concentration in the *C. utilis* treatment was respectively 41% and 23% greater than that in the *S. cerevisiae* and *C. tropicalis* treatments. The *C. utilis* addition linearly increased (p<0.05) the concentration of isobutyrate and reached the peak which was 16% greater than that of the control, while the concentrations of isovalerate and valerate were both quadratically reduced (p<0.05) by adding *S. cerevisiae*. The *C. tropicalis* addition linearly increased the concentrations of isobutyrate (p<0.05) and isovalerate (p<0.01) reaching the maximum at the dose of 0.75×10^{7}cfu, but quadratically decreased (p<0.05) the valerate concentration. Besides, the A:P reached the numerical minimum with the addition of *C. utilis* and *S. cerevisiae* both at the doses of 0.25×10^{7}cfu, while it was obtained at the dose of 0.50×10^{7}cfu in the *C. tropicalis* treatment, which were respectively 14%, 9%, and 11% less than that of the control. The interactive actions of species and dose on the concentrations of isobutyrate (p<0.05) and isovelerate (p<0.01) were also observed.

DISCUSSION

As a matter of fact, the effectiveness of yeast addition on *in vitro* GP parameters is somehow inconsistent in some previous studies. Mutsvangwa et al. (1992) reported that *in vitro* GP of a barley diet for beef cattle supplemented with yeast culture (Yea-Sacc1026) was on average less than that in the control, while Tang et al. (2008) found that supplementation of yeast culture (Original XP; Diamond V Mills Inc., Cedar Rapids, IA, USA) increased the cumulative GP, theoretical maximum of GP and the rate of GP of low quality roughages. This disparity might be caused by the difference in the yeast species used in their studies, fermentation substrate and experimental conditions. In the present study, adding *C. utilis* at all the designated doses decreased *in vitro* GP compared to the control, which

Table 5. Effects of different yeast species addition on volatile fatty acids concentration of *in vitro* incubation fluids for maize stover

Item [1]	Species	Dose (×10^7 colony-forming unit)					SEM[3]	Significance (>p)[4]		
		Mean [2]	0	0.25	0.50	0.75		Species	Dose	Species×dose
Acetate (mM)	*Candida utilis*	18.08	17.97	16.56	22.39	15.40	1.260	NS	NS	<0.05
	Saccharomyces cerevisiae	18.55	17.97	17.36	18.91	19.96			NS	
	Candida tropicalis	18.79	17.97	19.43	18.03	19.75			NS	
	SEM [5]	0.630								
Propionate (mM)	*Candida utilis*	8.51	8.07	7.90	10.20	7.86	0.561	NS	NS	NS
	Saccharomyces cerevisiae	8.79	8.07^b	8.44^{ab}	8.95^{ab}	9.72^a			L (<0.05)	
	Candida tropicalis	8.38	8.07	8.48	8.20	8.77			NS	
	SEM	0.280								
Isobutyrate (mM)	*Candida utilis*	0.30^e	0.31	0.26	0.33	0.29	0.020	<0.01	NS	NS
	Saccharomyces cerevisiae	0.27^f	0.31^a	0.24^b	0.25^b	0.27^{ab}			Q (<0.05)	
	Candida tropicalis	0.32^e	0.31	0.32	0.32	0.34			NS	
	SEM	0.010								
Butyrate (mM)	*Candida utilis*	1.73^f	1.83	1.52	2.18	1.39	0.150	<0.01	NS	<0.05
	Saccharomyces cerevisiae	2.08^e	1.83	2.14	2.18	2.18			NS	
	Candida tropicalis	1.85^f	1.83	1.93	1.73	1.91			NS	
	SEM	0.075								
Isovalerate (mM)	*Candida utilis*	0.40^f	0.39	0.35	0.46	0.40	0.022	<0.01	NS	<0.01
	Saccharomyces cerevisiae	0.35^g	0.39^a	0.32^b	0.33^{ab}	0.35^{ab}			Q (<0.05)	
	Candida tropicalis	0.48^e	0.39^b	0.50^a	0.50^a	0.53^a			L (<0.01)	
	SEM	0.011								
Valerate (mM)	*Candida utilis*	0.46	0.56	0.36	0.48	0.46	0.069	NS	NS	NS
	Saccharomyces cerevisiae	0.37	0.56^a	0.30^b	0.30^b	0.32^b			L (<0.05)	
	Candida tropicalis	0.41	0.56	0.34	0.35	0.38			NS	
	SEM	0.034								
A:P	*Candida utilis*	2.03^f	2.21^a	2.04^b	1.95^{bc}	1.90^c	0.048	<0.01	L (<0.01)	<0.05
	Saccharomyces cerevisiae	2.09^f	2.21^a	2.05^b	2.09^{ab}	2.02^b			L (<0.05)	
	Candida tropicalis	2.21^e	2.21	2.27	2.14	2.23			NS	
	SEM	0.024								
TVFA (mM)	*Candida utilis*	29.70	29.11	26.95	36.87	25.84	1.903	NS	NS	<0.05
	Saccharomyces cerevisiae	30.40	29.11	28.78	30.92	32.79			NS	
	Candida tropicalis	30.22	29.11	31.00	29.12	31.67			NS	
	SEM	0.962								

SEM, standard error of the mean; NS, not significant.
[1] TVFA = total short chain fatty acids, A:P = ratio of acetate to propionate.
[2] Mean for individual species across doses including the dose of 0.
[3] SEM for strain×dose.
[4] NS (p>0.05), L = linear effect of dose, Q = quadratic effect of dose.
[5] SEM for pooled mean of species including the dose of 0.
[a-c] Means within a row for doses that do not have a common superscript differ (p<0.05).
[e-g] Means within a column for species that do not have a common superscript differ (p<0.05).

was in agreement with the results obtained by Mutsvangwa et al. (1992). Meanwhile, maize stover or rice straw supplemented with *S. cerevisiae* and *C. tropicalis* achieved greater GP than that supplemented with *C. utilis*, suggesting that the selection of yeast species should be taken into consideration when live yeast was applied to improve *in vitro* fermentation efficiency of forages. Indexes of FRD_0 and $T_{0.5}$ usually reflect the rate of degradation at early incubation stages of '<12 h' and the incubation time of reaching half of the maximum GP, respectively. In general, the FRD_0 is inversely proportional to $t_{0.5}$. The addition of *C. utilis* and *C. tropicalis* decreased $t_{0.5}$ but increased FRD_0 of rice straw fermentation, indicating that the rate of degradation would be faster at the early stage of *in vitro* fermentation. Moreover, the two reverse responses of $t_{0.5}$ in *S. cerevisiae* and *C. tropicalis* treatments for maize stover indicated that the influence on the rate of degradation would be dependent on the yeast species, but this hypothesis required further research to be conducted. The alteration in the rate of degradation in response to yeast culture addition has also been verified in some previous studies. For instance, Newbold et al. (1995) suggested that *S. cerevisiae*

culture stimulated the rate rather than the extent of degradation by ruminal micro-organisms. Sullivan and Martin (1999) found *S. cerevisiae* culture filtrate stimulated the initial rate of cellulose degradation. In addition, the decrease of *Vf* and *k* caused by *C. utilis* addition in comparison with control suggested that this yeast species might not be suitable for dietary supplement.

It was noted in the study that the increase or decrease of IVDMD and IVNDFD of maize stover and rice straw was depended upon different yeast species, as *C. utilis* reduced both IVDMD and IVNDFD while *C. tropicalis* improved IVDMD and IVNDFD, and *S. cerevisiae* did not affect IVDMD and IVNDFD being dose-dependent. Furthermore, we found that the two higher supplemental doses of *C. utilis* did not always ensure the higher IVDMD and IVNDFD compared to the dose of 0.25×10^7cfu/500 mg, which was similar to the findings of Tang et al. (2008). In the study of Tang et al. (2008), the greatest values of IVDMD occurred for maize stover, maize stover silage, and wheat straw when yeast culture was supplemented at the level of 5.0 g/kg rather than the higher level of 7.5 g/kg. However, this phenomenon lacked sufficient explanation and needs to be fully studied in further research. Considering both IVDMD and IVNDFD could be more closely related to *in vivo* conditions, it is suggested that *C. tropicalis* should be more appropriate for supplements at the dose of 0.25×10^7cfu/500 mg substrates.

As pH value is a main index reflecting the internal homeostasis of rumen environment, therefore maintaining a relatively stable ruminal pH is vital to assuring efficient rumen fermentation. Ruminants usually possess highly developed systems to maintain ruminal pH within a physiological range of about 5.5 to 7.0 (Krause and Oetzel, 2006). In this study, although adding *C. utilis*, *S. cerevisiae*, and *C. tropicalis*, respectively lowered pH value to different extents, whilst pH value across all treatments ranged from 6.40 to 6.57, which still kept a suitable condition for fermentation, growth of microorganism, and fiber degradation in the rumen (Stewart et al., 1997). Satter and Slyter (1974) suggested that the lowest NH_3-N concentration of rumen liquor should not be less than 5 mg/dL to maintain the higher growth rate of bacteria. Deficiency of NH_3-N restricts the microbial protein synthesis, while an overly high NH_3-N concentration also inhibits the microbial utilization of NH_3-N (Hristov et al., 2002). Concentration of NH_3-N across three yeast treatments ranged from 5.24 to 8.83 mg/dL in this study, indicating that the growth and protein synthesis of microorganisms was not restricted. Fadel Elseed et al. (2007) reported yeast (*S. cerevisiae*) supplementation resulted in a numerical increase in ammonia-N concentration in rumen fluid of Nubian goat's kids. Similarly, the inclusion of *C. utilis* and *C. tropicalis* could enhance NH_3-N concentration to different extents with maize stover as fermented substrate, while for rice straw, only *C. tropicalis* addition elevated NH_3-N concentration of incubation fluids.

Methanogenesis is an essential metabolic pathway for hydrogen elimination and subsequently for efficient degradation of plant cell wall carbohydrates in the rumen (Wolin et al., 1997). Since yeast, especially *S. cerevisiae*, is the most frequently used direct-fed microbial in ruminant production, its influence on methanogenesis has been investigated in a few studies both *in vitro* and *in vivo*, but the results of these studies are inconsistent. In present study, the addition of *S. cerevisiae* and *C. tropicalis* respectively increased CH_4 production/g IVDMD of crop straws. The elevation of CH_4 production might be due to the increased disappearance of fiber under the *in vitro* closed anaerobic environment. Qiao and Shan (2006) found that addition of *S. cerevisiae* and *Saccharomycopsis fibuligera* also increased *in vitro* methane production, while *C. tropicalis* addition decreased CH_4 production with cornstarch, soybean, and wheat bran plus concentrate (3:1) mixture as the fermented substrates. It was inferred that the reduced CH_4 production might be attributed to the suppressed methanogens in the rumen, while the enhanced CH_4 production could be caused by the stimulated methanogens. Nevertheless, the inconsistency in results from different studies necessitates further research on this topic.

A number of trials have been conducted to examine the effects of yeast culture supplements on VFA in the rumen. Dawson et al. (1990) reported that the VFA patterns were not altered by yeast supplement (*S. cerevisiae*) in either rumen-simulating cultures or in the rumens of steers, while Mutsvangwa et al. (1992) found the addition of yeast culture (Yea-Sacc1026) increased the concentration of acetate and TVFA both *in vitro* and *in vivo*. In this study, not only *Vf*, *k*, IVDMD, and IVNDFD, but also the production of VFA was decreased by supplementing *C. utilis*, which indicated that *C. utilis* might be unsuitable as an additive for enhancing *in vitro* fermentation of cereal straws. Besides, supplementing *S. cerevisiae* and *C. tropicalis* elevated CH_4 production without significantly increasing VFA concentrations could be regarded as a disadvantage for *in vitro* fermentation of cereal straws. In addition, it was found that *S. cerevisiae* and *C. tropicalis* addition increased the propionate concentration, but decreased the concentrations of isovalerate and valerate*in vitro* with the increasing dose with maize stover and rice straw as fermented substrates. The decline of isovalerate and valerate concentrations suggested that *S. cerevisiae* and *C. tropicalis* addition have potential to stimulate plant cell wall digestion and ammonia utilization by mixed ruminal bacteria, as it was found that cell wall digestion and ammonia utilization were increased by low concentrations of isovalerate and valerate (Gorosito et al., 1985). Additionally, our results showed that the

addition of these three yeasts decreased or numerically decreased A:P, this was in agreement with the *in vitro* finding of Martin et al. (1989). In contrast, Mutsvangwa et al. (1992) pointed out that yeast culture addition did not alter A:P *in vitro* and *in vivo*, whereas Arambel et al. (1987) reported that A:P in the *in vitro* rumen fermentation supplemented with a yeast culture increased. This variation could be attributed to different species or strains of yeast used in different studies or the distinction between live yeast and yeast culture, and it needs to be investigated via further experiments.

CONCLUSION

In conclusion, both *S. cerevisiae* and *C. tropicalis* are more desirable than *C. utilis* as yeast culture supplements. Further, *C. tropicalis* is preferred compared to *S. cerevisiae* and its optimal dose should be 0.25×10^7 cfu/500 mg substrates, as *C. tropicalis* enhanced IVDMD and IVNDFD, and digestibility was viewed as the most fundamental parameter reflecting *in vitro* fermentation of cereal straws. The finding obtained from this study provides the dairy farmers with a practicable reference on the selection of live yeast species as feed additives. However, the verification of effects on these three live yeast additives *in vivo*, the evaluation of more live yeast additives for improving rumen fermentation, and the explanation of mechanisms of live yeast additives in ruminants all require further research in future.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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