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 (NH₃-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...
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 cattle in practice. 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 cattle (Estefan, 1999). Supplementation of yeast culture improved the rate of gas production (GP), DM and organic matter disappearances for diets rich in starch and sugars (Estefan, 1999). Meanwhile, dietary inclusion of S. cerevisiae NCYC 240, NCYC 1026 and Yea-Sacc stimulated total and cellulytic bacterial numbers, while S. cerevisiae NCYC 694 and NCYC 1088 exerted no influence on the numbers of bacteria in vitro (Newbold et al., 1995).

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 CaHPO₄, 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 CO₂ stream. The obtained rumen fluids were then anaerobically combined with artificial saliva in the proportion of 1 to 9 at 39°C.

Samples of straw or stover in 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. Samples 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 NH3-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 according to Van Soest et al. (1991) with addition of sodium sulphite and alpha-amylase in the NDF analysis. The fibretherm fiber analyzer (Gerhardt, Bonn, Germany) 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 N2 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:

\[ y = 1.506x \quad (n = 20, r^2 = 0.999, p<0.0001) \]  (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):

\[ GP = Vf \frac{(1 - \exp(d - t\times k))/(1+\exp(b - k\times t))}{(1+\exp(b - k\times t))} \]  (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 (\( t_{0.5} \)) 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):

\[ t_{0.5} = \frac{\ln(\exp(b)+2\exp(d))}{k} \]  (3)

\[ \text{FRD}_0 = k/(1+\exp(b)) \]  (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).
RESULTS

In vitro gas production parameters

For maize stover, in vitro GP parameters generally were not affected by yeast species except for \( V_f \), 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) \( V_f \), 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 \( 0.25 \times 10^7 \text{cfu} \) and \( 0.75 \times 10^7 \text{cfu} \), respectively.

For rice straw, the yeast species exerted significant effects (p<0.01) on \( V_f \) and \( FRD_0 \) (Table 2). Compared with the addition of \( C. utilis \), \( V_f \) 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 \( V_f \) 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 \( V_f \) 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 \( 0.25 \times 10^7 \text{cfu} \).

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

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

| 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 |
| \( V_f \) (mL) | Candida utilis        | 67.97e                          | 71.33a | 65.50ab | 64.25b | 70.80a | 2.013   | <0.01 | Q (<0.05) | NS           |
|               | Saccharomyces cerevisiae | 72.85e                        | 71.33 | 75.91   | 72.16 | 71.99 | NS               |
|               | Candida tropicalis    | 73.12e                          | 71.33 | 72.45   | 72.45 | 76.24 | NS               |
| SEM           | 1.006                 |                                  |       |         |       |       |                   |
| \( k \) (h)   | Candida utilis        | 0.087                           | 0.105b | 0.108b | 0.091b | 0.043b | 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        | 17.10f                          | 16.84 | 15.42   | 16.67 | 19.49 | 0.729   | NS         | NS         | NS           |
|               | Saccharomyces cerevisiae | 16.96f                         | 16.84 | 15.67   | 17.75 | 17.58 | NS               |
|               | Candida tropicalis    | 15.91f                          | 16.84 | 15.11   | 15.72 | 15.98 | Q (<0.05) | NS         | NS         | NS           |
| 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).

^c,d 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^a | 58.46 | 53.12 | 50.31 | 51.05 | 2.564 | <0.01   | NS   | <0.05     |
|                      | Saccharomyces cerevisiae | 61.68^b | 58.46 | 69.13 | 60.30 | 58.82 |       | Q (<0.05) |       |           |
|                      | Candida tropicalis | 63.55^a | 58.46 | 67.48 | 62.86 | 65.41 |       | NS      |       |           |
|                      | SEM                | 1.282   |       |       |       |       |       |         |       |           |
| k (h)                 | Candida utilis     | 0.101^b | 0.119a | 0.110^a | 0.100^ab | 0.074^b | 0.0122 | NS      | L (<0.05) | NS   |
|                      | Saccharomyces cerevisiae | 0.115^b | 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^e | 0.013^b | 0.014^b | 0.020^ab | 0.024^f | 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^f | 0.013^b | 0.021^a | 0.021^a | 0.025^f |       | L (<0.01) |       |           |
|                      | SEM                | 0.0011   |       |       |       |       |       |         |       |           |
| t_0.5 (h)            | Candida utilis     | 19.31    | 19.98 | 19.67 | 18.25 | 19.34 | 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 | 20.07 | 17.97 | 18.26 |       | 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.
5,6 Means within a column for species that do not have a common superscript differ (p<0.05).
7,8 Means within a row for doses 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), respectively. 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 0.50×10^7 cfu, 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
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 CH4 production by 18% compared with the control. There were interactive effects of species and dose on pH (*p*<0.05), NH3-N (*p*<0.01) and CH4 production/g IVDMD (*p*<0.01).

**Volatile fatty acid**

The yeast species influenced (*p*<0.01) the concentrations of isobutyrate, butyrate, isovalerate, and total volatile fatty acids (TVFA) 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 3. Effects of different yeast species addition on IVDMD and IVNDFD of maize stover and rice straw |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Item            | Species         |          |          |          |          |          |          |          |
|                 |                 | Mean    | 0      | 0.25   | 0.50   | 0.75   | SEM 2   | Species | Dose | Species×dose |
|                 |                 |         |        |        |        |        |         |         |
| Maize stover    | *Candida utilis*| 0.499a  | 0.531a | 0.505ab| 0.483b | 0.478b | 0.0002  | <0.01  | L (<0.01) | <0.05 |
|                 | *Saccharomyces  | 0.526c  | 0.531  | 0.529  | 0.497  | 0.548  |          |         |         |       |
|                 | *cerevisiae*    |         |        |        |        |        |         |         |         |       |
|                 | *Candida tropicalis* | 0.574a | 0.531a | 0.589a | 0.580a | 0.597a | 0.0001  |         |         |       |
|                 | SEM 4           |         |        |        |        |        |         |         |         |       |
|                 | *Candida utilis*| 0.380c  | 0.424a | 0.356b | 0.380b | 0.359b | 0.0002  | <0.01  | L (<0.01) | <0.01 |
|                 | *Saccharomyces  | 0.428c  | 0.424  | 0.431  | 0.426  | 0.430  |          |         |         |       |
|                 | *cerevisiae*    |         |        |        |        |        |         |         |         |       |
|                 | *Candida tropicalis* | 0.484c | 0.424b | 0.500a | 0.493a | 0.518a | 0.0001  |         |         | <0.05 |
|                 | SEM 4           |         |        |        |        |        |         |         |         |       |
| Rice straw      | *Candida utilis*| 0.442c  | 0.482a | 0.453ab| 0.424bc| 0.408c | 0.0001  | <0.01  | L (<0.01) | <0.01 |
|                 | *Saccharomyces  | 0.475c  | 0.482  | 0.471  | 0.472  | 0.473  |          |         |         |       |
|                 | *cerevisiae*    |         |        |        |        |        |         |         |         |       |
|                 | *Candida tropicalis* | 0.509c | 0.482  | 0.527  | 0.523  | 0.503  | 0.0001  |         |         |       |
|                 | SEM 4           |         |        |        |        |        |         |         |         |       |
|                 | *Candida utilis*| 0.339f  | 0.402a | 0.347a | 0.298b | 0.310b | 0.0002  | <0.01  | Q (<0.01) | <0.01 |
|                 | *Saccharomyces  | 0.400c  | 0.402  | 0.398  | 0.395  | 0.405  |          |         |         |       |
|                 | *cerevisiae*    |         |        |        |        |        |         |         |         |       |
|                 | *Candida tropicalis* | 0.423c | 0.402b | 0.467a | 0.464a | 0.359b | 0.0001  |         |         | <0.01 |
|                 | SEM 4           |         |        |        |        |        |         |         |         |       |

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.
5 Means within a row for doses that do not have a common superscript differ (*p*<0.05).
6 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 $0.75 \times 10^7$ 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 $0.25 \times 10^7$ cfu, while it was obtained at the dose of $0.50 \times 10^7$ 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 isovalerate (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 4. Effects of different yeast species addition on pH, NH3-N concentration and CH4 production of maize stover and rice straw *in vitro*

| Item       | Species          | Dose ($\times 10^7$ colony-forming unit) | SEM4 | Significance (>p)3 |
|------------|------------------|------------------------------------------|------|-------------------|
| Maize stover |                 | Mean 1 0 0.25 0.50 0.75 |   | Species | |
| pH         | Candida utilis   | 6.46 6.50 6.51 6.43 6.41 | 0.033 | NS         |
|            | Saccharomyces cerevisiae | 6.47 6.50 6.49 6.46 6.45 | 0.033 | NS         |
|            | Candida tropicalis | 6.44 6.50 6.44 6.41 6.40 | 0.033 | NS         |
| NH3-N (mg/dL) | Candida utilis   | 7.57 7.30 7.92 7.41 7.65 | 0.269 | <0.01 NS      |
|            | Saccharomyces cerevisiae | 7.14 7.30 6.68 7.10 7.50 | 0.269 | Q<0.05 NS    |
|            | Candida tropicalis | 7.77 7.30 7.25 7.70 8.83 | 0.269 | L<0.01 NS    |
| CH4 (mmol/g) | Candida utilis   | 0.135 |   | | |
| IVDMD      | Saccharomyces cerevisiae | 0.50 0.43 0.53 0.57 0.48 | 0.242 | Q<0.01 NS    |
|            | Candida tropicalis | 0.49 0.43 0.49 0.54 0.52 | 0.242 | Q<0.01 NS    |
| Rice straw | Candida utilis   | 6.49 6.57 6.51 6.46 6.42 | 0.019 | NS         |
| pH         | Saccharomyces cerevisiae | 6.50 6.57 6.49 6.48 6.46 | 0.019 | Q<0.05 NS    |
|            | Candida tropicalis | 6.50 6.57 6.48 6.44 6.52 | 0.019 | Q<0.01 NS    |
| NH3-N (mg/dL) | Candida utilis   | 6.30 6.33 5.91 6.64 6.30 | 0.276 | <0.01 NS      |
|            | Saccharomyces cerevisiae | 5.87 6.33 5.73 5.24 6.16 | 0.276 | Q<0.01 NS    |
|            | Candida tropicalis | 7.14 6.33 7.14 7.58 7.51 | 0.276 | L<0.01 NS    |
| CH4 (mmol/g) | Candida utilis   | 0.35 0.34 0.28 0.36 0.41 | 0.018 | Q<0.01 NS    |
| IVDMD      | Saccharomyces cerevisiae | 0.38 0.34 0.40 0.40 0.39 | 0.018 | Q<0.05 NS    |
|            | Candida tropicalis | 0.39 0.34 0.40 0.41 0.42 | 0.018 | Q<0.05 NS    |
| 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.

5 Means within a row for doses that do not have a common superscript differ (p<0.05).

6 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 Mutsangwa et al. (1992). Meanwhile, maize stover or rice straw supplemented with \textit{S. cerevisiae} and \textit{C. tropicalis} achieved greater GP than that supplemented with \textit{C. utilis}, suggesting that the selection of yeast species should be taken into consideration when live yeast was applied to improve \textit{in vitro} fermentation efficiency of forages. Indexes of \textit{FRD}_{0} and \textit{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 \textit{FRD}_{0} is inversely proportional to \textit{T}_{0.5}. The addition of \textit{C. utilis} and \textit{C. tropicalis} decreased \textit{T}_{0.5} but increased \textit{FRD}_{0} of rice straw fermentation, indicating that the rate of degradation would be faster at the early stage of \textit{in vitro} fermentation. Moreover, the two reverse responses of \textit{T}_{0.5} in \textit{S. cerevisiae} and \textit{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 \textit{S. cerevisiae}.

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

| Item | Species | Dose ($10^7$ colony-forming unit) | SEM$^5$ | Species | Significance (>p)$^4$ |
|------|---------|----------------------------------|--------|---------|----------------------|
|      |         | Mean | 0 | 0.25 | 0.50 | 0.75 | Species | Dose | Species×dose |
| Acetate (mM) | \textit{Candida utilis} | 18.08 | 17.97 | 16.56 | 22.39 | 15.40 | 1.260 | NS | NS | <0.05 |
|          | \textit{Saccharomyces cerevisiae} | 18.55 | 17.97 | 17.36 | 18.91 | 19.96 | NS | | |
|          | \textit{Candida tropicalis} | 18.79 | 17.97 | 19.43 | 18.03 | 19.75 | NS | | |
| Propionate (mM) | \textit{Candida utilis} | 8.51 | 8.07 | 7.90 | 10.20 | 7.86 | 0.561 | NS | NS | |
|          | \textit{Saccharomyces cerevisiae} | 8.79 | 8.07$^a$ | 8.44$^b$ | 8.95$^a$ | 9.72$^a$ | L (<0.05) | | |
|          | \textit{Candida tropicalis} | 8.38 | 8.07 | 8.48 | 8.20 | 8.77 | NS | | |
| Isobutyrate (mM) | \textit{Candida utilis} | 0.30$^e$ | 0.31 | 0.26 | 0.33 | 0.29 | 0.020 | <0.01 | NS | |
|          | \textit{Saccharomyces cerevisiae} | 0.27$^f$ | 0.31$^a$ | 0.24$^b$ | 0.25$^b$ | 0.27$^b$ | Q (<0.05) | | |
|          | \textit{Candida tropicalis} | 0.32$^e$ | 0.31 | 0.32 | 0.32 | 0.34 | NS | | |
| Butyrate (mM) | \textit{Candida utilis} | 1.73$^f$ | 1.83 | 1.52 | 2.18 | 1.39 | 0.150 | <0.01 | NS | <0.05 |
|          | \textit{Saccharomyces cerevisiae} | 2.08$^e$ | 1.83 | 2.14 | 2.18 | 2.18 | NS | | |
|          | \textit{Candida tropicalis} | 1.85$^f$ | 1.83 | 1.93 | 1.73 | 1.91 | NS | | |
| Isovalerate (mM) | \textit{Candida utilis} | 0.40$^f$ | 0.39 | 0.35 | 0.46 | 0.40 | 0.022 | <0.01 | NS | <0.01 |
|          | \textit{Saccharomyces cerevisiae} | 0.35$^e$ | 0.39$^a$ | 0.32$^b$ | 0.33$^b$ | 0.35$^b$ | Q (<0.05) | | |
|          | \textit{Candida tropicalis} | 0.48$^e$ | 0.39$^a$ | 0.50$^a$ | 0.50$^a$ | 0.53$^a$ | L (<0.01) | | |
| Valerate (mM) | \textit{Candida utilis} | 0.46 | 0.56 | 0.36 | 0.48 | 0.46 | 0.69 | NS | NS | |
|          | \textit{Saccharomyces cerevisiae} | 0.37 | 0.56$^a$ | 0.30$^b$ | 0.30$^b$ | 0.32$^b$ | L (<0.05) | | |
|          | \textit{Candida tropicalis} | 0.41 | 0.56 | 0.34 | 0.35 | 0.38 | NS | | |
| A:P | \textit{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 |
|          | \textit{Saccharomyces cerevisiae} | 2.09$^f$ | 2.21$^a$ | 2.05$^b$ | 2.09$^{ab}$ | 2.02$^b$ | L (<0.05) | | |
|          | \textit{Candida tropicalis} | 2.21$^e$ | 2.21 | 2.27 | 2.14 | 2.23 | NS | | |
| TVFA (mM) | \textit{Candida utilis} | 29.70 | 29.11 | 26.95 | 36.87 | 25.84 | 1.903 | NS | NS | <0.05 |
|          | \textit{Saccharomyces cerevisiae} | 30.40 | 29.11 | 28.78 | 30.92 | 32.79 | NS | | |
|          | \textit{Candida tropicalis} | 30.22 | 29.11 | 31.00 | 29.12 | 31.67 | NS | | |

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.
6 Mean for individual species across doses including the dose of 0.
7 SEM for pooled mean of species including the dose of 0.
8 Means within a row for doses that do not have a common superscript differ (p<0.05).
9 Means within a column for species that do not have a common superscript differ (p<0.05).
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 degradation in the rumen (Stewart et al., 1997). Satter and others (1985) suggested that both IVDMD and IVNDFD while *C. utilis* should be more appropriate for supplements at the dose of $0.25\times10^8$cfu/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 rumen fermentation. Ruminants usually possess highly 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 Saccharomyces fibuligerus 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 Mutsvanga 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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