Effects of Ruminal Crabtree-negative Yeast Ensiled Rice Straw on Feed Intake, Rumen Fermentation, and Performance in Tropical Crossbred Lactating Holstein Cows

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Abstract

This research aimed to determine the effects of ruminal Crabtree-negative yeast ensiled rice straw (RS) on feed intake, ruminal fermentation, milk production, and milk composition in tropical crossbred lactating Holstein cows. This study used 6 multiparous crossbreds between Holstein Frisian × Zebu dairy cows in their mid-lactation period (165.5 ± 44.0 of day-in-milk) with an initial body weight of 363.9 ± 55.80 kg (average milk yield 8.58 kg/d). Dairy cows were randomly allocated to three ensiled RS with various yeast species including *S. cerevisiae*, *P. kudriavzevii* KKU20, and *C. tropicalis* KKU20 according to a 3 × 3 replicated Latin square design. The ruminal yeasts were obtained by isolating, screening, and identifying the rumen of crossbred Thai-Holstein Friesian dairy cattle. The yeast species did not change the RS intake, concentrate diet, and total intake (P > 0.05). Crabtree-negative yeast (*P. kudriavzevii* and *C. tropicalis*) increased the apparent digestibility of dry matter by about 6.9% when compare with Crabtree-positive yeast (*S. cerevisiae*). Rumen pH and ammonia-nitrogen concentration were not changed among yeast species (P > 0.05). The bacterial populations at both 0 hours and 4 hours after feeding and the mean value were highest (P < 0.05) with ensiled RS with *C. tropicalis* KKU20. Ensiled RS with *P. kudriavzevii* KKU20 and *C. tropicalis* KKU20 were significantly increased with a total volatile fatty acids (VFAs) at 0 and 4 hours after feeding (P < 0.05) when compared with *S. cerevisiae*, whereas yeasts ensiled RS had no effect on the VFAs’ profile (P > 0.05). The yeast strains’ effects were not observed (P > 0.05) on actual milk yields. The treatments did not alter the milk composition (P > 0.05); except for when the protein in the milk was highest in the *C. tropicalis* KKU20 fed group. In conclusion, *C. tropicalis* KKU20 could enhance the RS’s nutrition value through increasing digestibility, the ruminal bacterial population, and total VFAs as well as could increase the milk protein.

Introduction

For several years, yeast has been the model organism used for enhancing animal efficiency and is the traditional practice for ruminant feed additives ¹. In many studies, using *S. cerevisiae* fermented with an agricultural by-product or rice straw (RS) has been shown to enhance their nutritional value, silage quality, and nutrient digestibility ²,³. The baker's yeast, *S. cerevisiae*, rapidly converts molasses and urea to provide biomass and greater nutrients from whole cell when added with oxygen (O₂) during the proliferation process ⁴. *Wanapat et al.* ⁵ stated that *S. cerevisiae* significantly increases crude protein (CP) in feedstuff via cell proliferation during the fermentation process and it provides essential amino acids, particularly lysine and methionine, for dairy cattle. In addition, previous studies explained the benefit of live yeast in that it could provide the positive effect on feed utilization and performance production in the ruminants ⁶,⁷.

Although *S. cerevisiae* has many benefits, several limitation have been reported, particularly that it produces low cell biomass ⁸. Under aerobic conditions, *S. cerevisiae* exhibits alcoholic fermentation more than producing biomass. The "Crabtree-positive yeasts" are those that represent this characteristic ⁹.

Under excessive glucose and even aerobic conditions, Van Urk *et al.* ¹⁰ revealed that *S. cerevisiae* had a
limited proliferation capacity. Moreover, Wardrop et al.\textsuperscript{11} revealed that when cultivated with excessive glucose in a media solution, \textit{S. cerevisiae} provides 7 times lower biomass compared to other strains. This phenomenon restricts the chances of animals to receive highly nutritious from yeast biomasses such as protein, essential amino acids, and vitamins. Therefore, it is important to extend the scope of research such as investigating different species that could solve this issue. However, yeasts that do not have the Crabtree effect can therefore generate greater biomass in aerobic culture than Crabtree-positive yeasts. These yeasts are called “Crabtree-negative yeasts”\textsuperscript{9}.

Recently, newly isolated ruminal yeasts have been discovered and are classified as Crabtree-negative yeasts that can generate high biomass. Suntara and Cherdthong\textsuperscript{8} reported that \textit{Candida tropicalis} KKU20 and \textit{Pichia kudriavzevii} KKU20 provide biomass more than \textit{S. cerevisiae} by about 23.7\% and 26.4\%, respectively. Furthermore, both species were found able to release cellulase enzymes during the incubation time of about 0.022 to 0.101 unit/ml, respectively. In addition, \textit{P. kudriavzevii} KKU20 can provide the maximum Carboxy methyl cellulase activity with the inoculant molasses 250 g/kg and urea 30 g/kg\textsuperscript{8}. Similarly, Sarawan\textsuperscript{12} found that yeast isolated from the jasmine plant \textit{Candida konsanensis} can release cellulase enzymes. Thus, yeast-producing cellulase enzymes might help to breakdown fiber structure and could then improve feed utilization for ruminant. In an earlier study, Suntara and Cherdthong\textsuperscript{13} suggested that, when compared to \textit{S. cerevisiae}, the ensiled RS with \textit{P. kudriavzevii} KKU20 and \textit{C. tropicalis} KKU20 had the greater ability to increase cumulative gas production and improve \textit{in vitro} degradability. However, applying potential yeast in RS fed to dairy cattle has not yet been reported. It was expected that the \textit{P. kudriavzevii} KKU20 and \textit{C. tropicalis} KKU20 from the rumen may support the feed utilization and performance production of tropical dairy cattle.

This research aimed to determine the effects of newly isolated ruminal Crabtree-negative yeast ensiled RS on feed intake, ruminal fermentation, milk production, and milk composition in tropical crossbred lactating Holstein cows.

**Results**

**Chemical composition of feeds**

The ensiled RS with different yeast species contained CP at 58.9 to 71.2 g/kg DM and 8.4 to 8.5 MJ/kg DM of ME, while the concentrate diet contained CP at 178.0 g/kg DM and 12.3 MJ/kg DM of ME. The NDF content in ensiled RS with \textit{P. kudriavzevii} KKU20 and \textit{C. tropicalis} KKU20 were lower than in \textit{S. cerevisiae} at 12.7\% and 12.1\%, respectively (Table 1). Fermentation quality of ensiled RS such as pH were ranged from 4.19 to 4.30, while lactic acid, C\textsubscript{2}, C\textsubscript{4}, and NH\textsubscript{3}-N were ranged from 19.8 to 21.9, 5.1 to 5.4, 0.81 to 0.82, and 1.8 to 2.0 g/kg DM, respectively.
Table 1
Dietary ingredients and chemical composition of different yeast species in ensiled rice straw and concentrate diet. Premix = Vitamins and minerals; A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g. Hemicellulose = NDF-ADF Cellulose = ADF-lignin Metabolizable energy calculated according to the equation described by Robinson et al. (2004).

| Item                  | Ensiled rice straw (g/kg fresh matter) | Concentrate diet (g/kg DM) |
|-----------------------|----------------------------------------|---------------------------|
|                       | S. cerevisiae(KKU20) | P. kudriavzevii (KKU20) | C. tropicalis (KKU20) |
| Ingredients           |                         |                           |                         |
| Cassava ship          | -                       | -                         | -                       |
| Corn                  | -                       | -                         | -                       |
| Palm kernel meal      | -                       | -                         | -                       |
| Rice bran             | -                       | -                         | -                       |
| Soybean meal          | -                       | -                         | -                       |
| Molasses              | 500.0                   | 500.0                     | 500.0                   |
| Premix                | -                       | -                         | -                       |
| Di-calcium phosphate  | -                       | -                         | -                       |
| Urea                  | 20.0                    | 20.0                      | 20.0                    |
| Salt                  | -                       | -                         | 5.5                     |
| Chemical composition (g/kg DM) | | | |
| Dry matter (g/kg as fed) | 279.3                  | 275.7                     | 278.7                   |
| Organic matter        | 871.4                   | 879.0                     | 874.1                   |
| Ether extract         | 7.8                     | 8.2                       | 7.4                     |
| Crude protein         | 60.8                    | 58.9                      | 71.2                    |
| Neutral detergent fiber | 715.3                | 624.6                     | 629.3                   |
| Acid detergent fiber  | 452.2                   | 390.8                     | 396.6                   |
| Acid detergent lignin | 64.6                    | 60.6                      | 61.6                    |
| Hemicellulose         | 263.1                   | 233.9                     | 232.8                   |
| Cellulose             | 387.6                   | 330.1                     | 335.0                   |
| Energy content (MJ/kg DM) | | | |
| Gross energy          | 15.1                    | 15.1                      | 15.0                    |

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| Item                          | Ensiled rice straw (g/kg fresh matter) | Concentrate diet (g/kg DM) |
|-------------------------------|----------------------------------------|---------------------------|
|                               | S. cerevisiae KKU20                      |                           |
| Metabolizable energy          | 8.4                                    | 8.4                        | 8.5                        | 12.3                        |
|                               |                                        |                           |                            |                            |
| **Fermentation quality**      |                                        |                           |                            |                            |
| pH                            | 4.20                                   | 4.30                       | 4.21                       | -                          |
| Lactic acid (g/kg DM)         | 19.8                                   | 21.9                       | 22.1                       | -                          |
| Acetic acid (g/kg DM)         | 5.4                                    | 5.4                        | 5.1                        | -                          |
| Butyric acid (g/kg DM)        | 0.82                                   | 0.82                       | 0.81                       | -                          |
| Ammonia-N (g/kg DM)           | 2.0                                    | 2.0                        | 1.8                        | -                          |

**Feed intake, nutrient intake, and nutrient apparent digestibility**

The impacts of different yeast species ensiled RS on the effectiveness of feed utilization in dairy cattle is illustrated in Table 2. The yeast species did not change the RS intake, concentrate diet, and total intake (P > 0.05). Total intake ranged from 111.7 to 121.1 g/kg BW^{0.75}. OM and CP intake were 8.9 to 9.6 kg/day and 1.3 to 1.4 kg/day, respectively, which was not altered among treatments (P > 0.05). Crabtree-negative yeast (P. kudriavzevii KKU20 and C. tropicalis KKU20) increased the apparent digestibility of DM by about 6.9% when compare with Crabtree-positive yeast (S. cerevisiae). However, the data achieved in this study showed that apparent digestibility of OM (OMD), CP (CPD), NDF (NDFD), and ADF (ADFD) were not altered among yeast species and ranged from 762.1 to 791.5, 752.0 to 791.5, 601.5 to 641.3, and 492.8 to 525.4 g/kg, respectively. Furthermore, the total digestible nutrients were the same among yeast species and ranged from 734.8 to 767.7 g/kg (P > 0.05).
Table 2
Effect of different yeast species in ensiled rice straw on dry matter intake (DMI), nutrient intake and digestibility in tropical crossbred lactating dairy cows. a, b Means in the same row with different superscript letters differ (P < 0.01, P < 0.05). S. cerevisiae = Saccharomyce cerevisiae, P. kudriavzevii = Pichia kudriavzevii, C. tropicalis = Candida tropicalis.

| Items                | S. cerevisiae | P. kudriavzevii | C. tropicalis | SEM  | P-value |
|----------------------|---------------|-----------------|---------------|------|---------|
|                      | KIU20         | KIU20           |               |      |         |
| Dry matter intake    |               |                 |               |      |         |
| Rice straw silage    |               |                 |               |      |         |
| kg/d                 | 3.7           | 3.8             | 3.1           | 0.41 | 0.17    |
| % BW                 | 1.0           | 1.0             | 0.8           | 0.10 | 0.11    |
| g/kgBW<sup>0.75</sup> | 43.4          | 45.0            | 36.5          | 4.54 | 0.11    |
| Concentrate diet     |               |                 |               |      |         |
| kg/d                 | 5.9           | 6.3             | 6.2           | 0.19 | 0.11    |
| % BW                 | 1.7           | 1.8             | 1.7           | 0.06 | 0.27    |
| g/kgBW<sup>0.75</sup> | 72.3          | 76.1            | 75.2          | 2.53 | 0.21    |
| Total intake         |               |                 |               |      |         |
| kg/d                 | 9.6           | 10.1            | 9.3           | 0.55 | 0.25    |
| % BW                 | 2.7           | 2.8             | 2.6           | 0.15 | 0.27    |
| g/kgBW<sup>0.75</sup> | 115.7         | 121.1           | 111.7         | 4.54 | 0.11    |
| Nutrient intake, kg/d|               |                 |               |      |         |
| Organic matter       | 9.1           | 9.6             | 8.9           | 0.49 | 0.24    |
| Ether extract        | 0.3           | 0.3             | 0.3           | 0.01 | 0.11    |
| Crude protein        | 1.3           | 1.4             | 1.4           | 0.05 | 0.35    |
| Neutral detergent fiber | 5.1         | 4.9             | 4.5           | 0.32 | 0.13    |
| Acid detergent fiber  | 2.5           | 2.4             | 2.1           | 0.18 | 0.08    |
| Apparent digestibility, g/kg |     |                 |               |      |         |
| Dry matter           | 701.4<sup>b</sup> | 746.5<sup>a</sup> | 753.1<sup>a</sup> | 15.3 | P<0.05  |
| Organic matter       | 762.1         | 763.4           | 791.5         | 24.4 | 0.30    |
| Crude protein        | 752.0         | 754.8           | 786.4         | 29.6 | 0.34    |
Effect on rumen pH, NH$_3$-N, blood metabolites and microbial communities

Table 3 illustrates the influence of ensiled RS with various yeast species fed to crossbred lactating dairy cows on ruminal pH, NH$_3$-N, BUN, and microbial communities. Rumen pH was not changed among yeast species, and the pH values were 6.4 to 6.8 (P > 0.05). Ruminal NH$_3$-N and BUN ranged from 16.5 to 22.1 mg/dL and 13.3 to 17.3 mg/dL, respectively (P > 0.05). The bacterial populations at both 0 hours and 4 hours after feeding and the mean value were highest (P < 0.05) with ensiled RS with *C. tropicalis* KKU20 by 9.9, 12.5 and 11.2 Log$_{10}$ cell/ml, respectively. However, the fungal zoospore and protozoa populations were not affected by any treatments (P > 0.05).
Table 3

Effect of different yeast species in rice straw ensiled on ruminal pH, NH$_3$-N concentration, blood urea-nitrogen concentration, and microbial communities in crossbred lactating dairy cows. $^a, b$ Means in the same row with different superscript letters differ (P < 0.01, P < 0.05). $S$. cerevisiae = Saccharomyces cerevisiae, $P$. kudriavzevii = Pichia kudriavzevii, $C$. tropicalis = Candida tropicalis.

| Items                        | $S$. cerevisiae KKU20 | $P$. kudriavzevii KKU20 | $C$. tropicalis KKU20 | SEM | P-value |
|------------------------------|-----------------------|-------------------------|-----------------------|-----|---------|
| Ruminal pH                   |                       |                         |                       |     |         |
| 0 hours-after feeding        | 6.8                   | 6.8                     | 6.8                   | 0.11| 0.71    |
| 4 hours-after feeding        | 6.4                   | 6.5                     | 6.4                   | 0.19| 0.98    |
| mean                        | 6.6                   | 6.6                     | 6.7                   | 0.14| 0.86    |
| Ruminal NH$_3$-N, mg/dL      |                       |                         |                       |     |         |
| 0 hours -after feeding       | 17.7                  | 16.5                    | 16.7                  | 1.32| 0.52    |
| 4 hours -after feeding       | 20.7                  | 18.9                    | 22.1                  | 1.40| 0.06    |
| mean                        | 19.2                  | 17.7                    | 19.4                  | 1.11| 0.18    |
| BUN, mg/dL                   |                       |                         |                       |     |         |
| 0 hours -after feeding       | 13.8                  | 13.5                    | 13.3                  | 2.45| 0.97    |
| 4 hours -after feeding       | 15.8                  | 16.0                    | 17.3                  | 1.13| 0.26    |
| mean                        | 14.8                  | 14.8                    | 15.3                  | 1.45| 0.87    |
| Rumen microbes, cells/ml     |                       |                         |                       |     |         |
| Bacteria, Log10 cell/ml      |                       |                         |                       |     |         |
| 0 hours -after feeding       | $9.2^b$               | $9.4^{ab}$              | $9.9^a$               | 0.28| $p < 0.05$ |
| 4 hours -after feeding       | $11.9^b$              | $12.2^a$                | $12.5^a$              | 0.22| $p < 0.05$ |
| mean                        | $10.5^b$              | $10.8^{ab}$             | $11.2^a$              | 0.19| $p < 0.01$ |
| Fungi zoospore,              |                       |                         |                       |     |         |
| Log10 cell/ml                |                       |                         |                       |     |         |
| 0 hours -after feeding       | 7.8                   | 8.1                     | 8.1                   | 0.29| 0.31    |
| 4 hours -after feeding       | 6.9                   | 6.9                     | 6.9                   | 0.33| 0.43    |
| mean                        | 5.9                   | 5.7                     | 5.6                   | 0.24| 0.27    |
| Protozoa, Log10 cell/ml      |                       |                         |                       |     |         |
| Items                  | S. cerevisiae | P. kudriavzevii | C. tropicalis | SEM | P-value |
|-----------------------|---------------|-----------------|--------------|-----|---------|
|                      |               | KKU20           | KKU20        |     |         |
| 0 hours -after feeding| 4.3           | 4.6             | 4.6          | 0.30| 0.38    |
| 4 hours -after feeding| 5.9           | 6.2             | 6.2          | 0.27| 0.39    |
| mean                  | 5.1           | 5.4             | 5.3          | 0.17| 1.00    |

**Effect on ruminal volatile fatty acid**

The total VFA, acetic acid (C₂), propionic acid (C₃), butyric acid (C₄) proportions, and acetic acid to propionic acid ratio are illustrated in Table 4. Ensiled RS with *P. kudriavzevii* KKU20 and *C. tropicalis* KKU20 were significantly increased with a total VFAs at 0 hours (5.15 and 5.06%, respectively), and 4 hours (5.07 and 8.83%, respectively) after feeding (P < 0.05) when compared with *S. cerevisiae*, whereas yeasts ensiled RS had no effect on the VFAs' profile (P > 0.05). The mean value of C₂, C₃, and C₄ were 67.4, 22.3, and 10.3 mol/100 mol, respectively.
Table 4
Effect of different yeast species in rice straw ensiled on concentrations of total volatile fatty acid (VFAs) and their profiles in crossbred lactating dairy cows. \(^{a,b}\) Means in the same row with different superscript letters differ (P < 0.01, P < 0.05). S. cerevisiae = Saccharomyces cerevisiae, P. kudriavzevii = Pichia kudriavzevii, C. tropicalis = Candida tropicalis.

| Items                             | S. cerevisiae | P. kudriavzevii | C. tropicalis | SEM  | P-value |
|----------------------------------|---------------|-----------------|---------------|------|---------|
|                                  | KKK20         | KKK20           |               |      |         |
| Total VFA, mmol/L                |               |                 |               |      |         |
| 0 hours -after feeding           | 106.7\(^b\)   | 112.2\(^a\)     | 112.1\(^a\)   | 2.38 | p < 0.05|
| 4 hours -after feeding           | 122.3\(^b\)   | 128.5\(^{ab}\)  | 133.1\(^a\)   | 4.07 | p < 0.05|
| mean                            | 114.5\(^b\)   | 120.4\(^a\)     | 122.6\(^a\)   | 2.11 | p < 0.01|
| Volatile fatty acid profiles,    |               |                 |               |      |         |
| mol/100 mol                      |               |                 |               |      |         |
| Acetic acid                      |               |                 |               |      |         |
| 0 hours -after feeding           | 64.9          | 65.8            | 65.9          | 0.86 | 0.35    |
| 4 hours -after feeding           | 68.4          | 68.8            | 70.6          | 1.45 | 0.20    |
| mean                            | 66.7          | 67.3            | 68.3          | 0.97 | 0.19    |
| Propionic acid                   |               |                 |               |      |         |
| 0 hours -after feeding           | 21.0          | 21.9            | 20.7          | 1.03 | 0.36    |
| 4 hours -after feeding           | 22.6          | 24.1            | 23.2          | 1.13 | 0.32    |
| mean                            | 21.8          | 23.0            | 21.9          | 0.93 | 0.27    |
| Butyric acid                     |               |                 |               |      |         |
| 0 hours -after feeding           | 14.0          | 12.2            | 13.3          | 1.11 | 0.20    |
| 4 hours -after feeding           | 8.9           | 7.1             | 6.2           | 1.53 | 0.14    |
| mean                            | 11.5          | 9.7             | 9.8           | 0.72 | 0.02    |
| Acetic:Propionic acid ratio      |               |                 |               |      |         |
| 0 hours -after feeding           | 3.1           | 3.0             | 3.2           | 0.17 | 0.48    |
| 4 hours -after feeding           | 3.1           | 2.9             | 3.1           | 0.18 | 0.34    |
| mean                            | 3.1           | 2.9             | 3.1           | 0.17 | 0.36    |
Effect on milk production, milk composition, and feed efficiency

The effects of ensiled RS with various yeast species on milk production, composition of milk, and feed efficiency in dairy cows are shown in Table 5. The yeast strains’ effects were not observed (P > 0.05) on actual milk yields (8.5 to 8.8 kg/h/d), 4.0% FCM (7.6 to 8.3 kg/h/d), and ECM (7.7 to 8.3 kg/h/d). The treatments did not alter the milk composition (P > 0.05); except for when the protein in the milk was highest in the *C. tropicalis* KKU20 fed group at 35.6 g/kg (P < 0.01). Feed efficiency did not changed for any diets (P > 0.05).
Table 5
Effect of different yeast species in rice straw ensiled on milk yield, milk composition, feed efficiency and economic efficiency in crossbred lactating dairy cows.  

\( a, b \) Means in the same row with different superscript letters differ (P < 0.01, P < 0.05).  

\( S. \) cerevisiae = Saccharomyce cerevisiae,  

\( P. \) kudriavzevii = Pichia kudriavzevii,  

\( C. \) tropicalis = Candida tropicalis.  

FCM = fat corrected milk (calculated from 0.432 (kg of milk/d) + 16.23 (kg of fat)).  

ECM = energy-corrected milk = 7.20×protein (kg/d) + 12.95×fat (kg/d) + 0.327×milk (kg/d).  

DMI = dry matter intake.  

DCP = digestible crude protein.

| Items                     | \( S. \) cerevisiae KKU20 | \( P. \) kudriavzevii KKU20 | \( C. \) tropicalis KKU20 | SEM | P-value  |
|---------------------------|--------------------------|----------------------------|--------------------------|-----|----------|
| Actual milk yield, kg/h/day| 8.5                      | 8.8                        | 8.6                      | 0.47| 0.09     |
| 4.0% FCM, kg/h/day        | 7.8                      | 7.6                        | 8.3                      | 0.39| 0.11     |
| ECM, kg/h/d               | 7.8                      | 7.7                        | 8.3                      | 0.30| 0.07     |
| Protein, g/kg             | 34.5\(^{b}\)             | 34.1\(^{b}\)               | 35.6\(^{a}\)             | 0.02| p < 0.05 |
| Protein, g/d              | 292.7                    | 302.2                      | 303.4                    | 12.4| 0.54     |
| Fat, g/kg                 | 33.9                     | 32.0                       | 37.9                     | 3.89| 0.23     |
| Fat, g/d                  | 288.8                    | 269.7                      | 325.6                    | 28.9| 0.11     |
| Lactose, g/kg             | 44.7                     | 45.1                       | 44.5                     | 0.05| 0.44     |
| Lactose, g/d              | 382.6                    | 399.3                      | 382.5                    | 12.3| 0.22     |
| Solids-not-fat, g/kg      | 87.2                     | 86.9                       | 87.9                     | 0.71| 0.27     |
| Total solids, g/kg        | 122.7                    | 121.2                      | 126.9                    | 2.91| 0.09     |
| Somatic cell count, ×10\(^{5}\) | 5.3                     | 4.0                        | 6.6                      | 1.25| 0.10     |
| Milk urea nitrogen, mg/dL | 12.9                     | 13.1                       | 14.7                     | 0.85| 0.06     |

**Feed efficiency**

| Milk / DMI | 0.90 | 0.87 | 0.92 | 0.05 | 0.62 |
| ECM / DMI  | 0.82 | 0.76 | 0.89 | 0.06 | 0.07 |
| DCP / Milk | 93.4 | 91.1 | 97.3 | 4.02 | 0.22 |

**Discussions**

From NRC\(^{36}\), CP and ME requirement of our animal (BW 364 kg, milk yield 8.6 kg/d) increased by about 1,190 g/d and 60.9 MJ/d, respectively. Therefore, the nutrient composition in feed, especially CP (provide 1,300-1,400 g CP/d) and ME (provide 79.05–84.14 MJ/d) values, were sufficient in our study for supporting dairy cows’ performance. Furthermore, ensiled RS with \( P. \) kudriavzevii KKU20 and \( C. \) tropicalis KKU20 (Crabtree-negative yeast) were established as having a low fiber content when compared with...
adding S. cerevisiae (Crabtree-positive yeast). The low fiber content can be clarified by the yeast's ability to release cellulase enzymes and digest fiber during the fermentation process. Suntara and Cherdthong confirmed that C. tropicalis KKU20 and P. kudriavzevii KKU20 were more capable to releasing cellulase enzymes than S. cerevisiae by about 0.7 to 6.8 times, respectively. Moreover, the experiment on in vitro gas production of ensiled RS at 14 days with the P. kudriavzevii KKU20 could decrease the NDF content by about 6.7% when compared with S. cerevisiae. Ilmén et al. discovered yeast isolated from a plant named C. konsanensis species could excrete cellulase enzymes and digests fiber, and it is a new yeast strain that had not been reported previously. Similar with our study, C. tropicalis KKU20 and P. kudriavzevii KKU20 are great potential yeasts to improve feedstuffs and this study is the first report in ruminant nutrition feed research.

The fermentation quality of ensiled RS with different yeast species indicated that the silage was well preserved. The ensiled RS still maintained appropriate pH, high lactic acid content, and a low NH$_3$-N level. Acceptable silage was defined by the pH value and the composition of their fermentation products. The pH is the main indicator for evaluating silage quality and our study showed ensiled RS still has a satisfactory score of about 4.1 to 4.3. In addition, pH is highly related with lactic acid content, which in this study showed a consistent range of about 19.8–22.1 g/kg DM. Lactic acid content in silage should range between 21 to 25 g/kg DM to be considered of high quality, according to Flieg's score; therefore, it is close to the high quality of silage. In addition, our result showed lactic acid content similar to an earlier study by Suntara et al. who revealed that about 20.53 to 26.14 g/kg DM of lactic acid was produced when ensiled RS with C. tropicalis KKU20 and P. kudriavzevii KKU20 at 14 day. NH$_3$-N concentration in ensiled RS within the range of 1.80 to 2.00 g/kg DM indicated the normal standards for estimating silage. These results are similar to those of Li et al., who collected information on various types of RS parameters and concluded that RS silage has a NH$_3$-N concentration of approximately 1.61 to 2.36 g/kg DM. Other parameter such as C$_2$ show great value for preserved silage within range 20 to 25 g/kg DM. Moreover, after the fermentation process, the moisture content should range from 650 to 750 g/kg to be optimum, which in our study showed an average of 722.1 g/kg. Therefore, our study proposes that the nutrients in ensiled RS are still well preserved.

Crabtree-negative or –positive yeast has no effect on the dry matter intake (DMI). Our results showed that the DMI (range from 2.6 to 2.8 %BW) was similar to previous experiments, which is that feeding separate ensiled RS with a concentrate diet to dairy cows creates a DMI range from 2.5 to 3.2 %BW. Generally, the amount of RS that an animal intakes daily is limited to around 2.0 % BW or less BW. Because RS is rich in polysaccharides and has a high lignin and silica content, and thus it limits the voluntary intake. However, Aquino et al. reported that the amount of RS that ruminants can consume can be as high as 1.2% BW, which is similar with our result of 0.8–1.0%BW. The intake of OM, EE, NDF, and ADF was similar with previous studies of lactating crossbred dairy cows. The CP intake (CPI) in this study was also similar with Wanapat, et al., which used lactating crossbred dairy cows (50% Holstein Frisian × 50% Thai native cows) and BW around 365.5 kg, and the CPI was about 1.0 to 1.2 kg/d. Typically, the CP
found in tropical forage plants is often relatively low \textsuperscript{49}. Especially in RS (3\%CP) when using a roughage source it can have an effect on the animal's yield adequacy \textsuperscript{50}. However, our study showed that ensiled RS with yeast could support protein from yeast to low quality roughage as RS, and the enhanced intake of protein were high enough to meet the requirement of tropical lactation dairy cows.

The dry matter digestibility (DMD) was increased when ensiled RS with Crabtree negative yeast was offered to animals. This strain is outstanding in terms of high proliferation ability and its high yield of cellulase enzymes \textsuperscript{15}. The improved digestion may be due to the potential of how rumen microflora are promoted for better digestibility. Yeast is an important biological responder in the rumen fermentation, live yeast cells improve microorganisms in rumen \textsuperscript{51} and stabilizes pH in the rumen \textsuperscript{52}. Habeeb \textsuperscript{53} stated that yeast could provide rumen with biological stimulants, which is necessary for microorganisms' growth in the rumen. Therefore, yeast contributes to establishing microbiota \textsuperscript{54} and is why the digestibility was apparently enhanced. This is consistence with Wang et al. \textsuperscript{6}, who found that Crabtree-negative yeast as \textit{C. tropicalis} could increase digestion in the \textit{in vitro} technique and that it generated 3.03\% more gas production than did \textit{S. cerevisiae}.

However, Crabtree-negative yeast did not change the apparent digestibility of OM, CP, NDF, and ADF. The digestibility of NDF and ADF are similar among Cabtree-negative and positive yeast (601.50 vs 650.05 g/kg DM and 492.8 vs 518.15 g/kg DM, respectively). Noticeable changes occurred after the silage process was complete, but when the animal intakes the feed, its digestion was not altered. The reason for this is still not clear, but it is possible that yeast does not react directly on RS. Rather, digestion in the rumen occurred by the cooperation of microbes' synergy until the resulting values were not statistically different. This is similar with an experiment by Suntara \textit{et al.} \textsuperscript{15}, who compared the effect of Crabtree-negative and -positive yeast on ensiled RS on the \textit{in vitro} gas and confirmed that in the rumen, there was no difference among yeast species in the digestibility of NDF and ADF (705.2 vs 703.6 and 464.8 vs 464.4 g/kg DM).

Ensiled RS with the \textit{P. kudriavzevii} KKU20 and \textit{C. tropicalis} KKU20 (Crabtree-negative yeast) could increase bacterial populations when compared to \textit{S. cerevisiae} (Crabtree-positive yeast) by about 4.76\%. The ruminal bacterial populations depend on sufficient nutrients or stimulants supply \textsuperscript{53}. Yeast is a great supply to stimulate bacteria because it is enriched in essential substances \textsuperscript{55}. Previous studies have confirmed that yeast could supply essential amino acids, vitamins, and minerals to increase the ruminal bacteria more than without yeast \textsuperscript{2,56}. The key explanation is that under aerobic conditions, Crabtree-negative yeast may proliferate more than Crabtree-positive yeast since the enzyme mechanism functions differently \textsuperscript{9,57}. Suntara and Cherthong \textsuperscript{8} found that at 72 h of incubation time, \textit{P. kudriavzevii} KKU20, \textit{C. tropicalis} KKU20, and \textit{S. cerevisiae} had growth by about 10.02, 9.6, and 8.87 Log cells/ml, respectively. The high amount of Crabtree-negative yeast creates a greater supply of essential nutrients to the rumen bacteria \textsuperscript{15}, thus the amount of rumen bacteria is increased in response to the Crabtree-negative yeast.
The ensiled RS with Crabtree-negative yeast has more effect on the total VFAs than with Crabtree-positive yeast by about 6.1% at the mean value. The high production of total VFAs in rumen fluids is related to the amount of ruminal bacteria. The great bacterial population could enhance carbohydrate digestion and then the animal obtains the greater VFAs. This is similar to Castillo-González et al., who stated that the expansion of rumen microorganisms could increase the quantity of rumen VFAs. Certainly, a high bacterial population in our experiment was related with the Crabtree-negative yeast’s effect. Nonetheless, the direct influence of the Crabtree-negative yeast on rumen bacterial populations was unclear and this hypothesis required further research to be conducted. However, expanding the Crabtree-negative yeast population (during fermentation process) may be more effective than expanding that of the Crabtree-positive yeast (S. cerevisiae). This suggests that animals have a greater chance of obtaining stimulants for activate rumen bacteria. In agreement with our results, Wang et al. compared the effect between Crabtree-negative yeast (C. tropicalis) and Crabtree-positive yeast (S. cerevisiae) for in vitro gas technique and found that the inclusion of $0.25 \times 10^7$ of Crabtree-negative yeast could enhanced the total VFAs by 7.7%. Moreover, Suntara et al. reported that Crabtree-negative yeast (P. kudriavzevii KKU20) increased the total VFAs by 2.3% for in vitro gas study more than Crabtree-positive yeast.

The milk yield and milk composition of ensiled RS with Crabtree-negative yeast did not have any impact. Our study showed that the actual milk yields are about 8.5 to 8.8 kg/h/d, which are slightly lower than previous trials using early to mid-lactation cows (12.6 kg/h/d according to Supapong and Cherdthong; 11.1 kg/h/d according to Wanapat et al.). To produce milk, cows must calve and split its lactation cycle into four phases (early, mid, late lactation and dry period). The milk yield response was greater in the early lactation, and in the mid-lactation period, the milk yield begins to decline from its peak. Therefore, the lower actual milk yields in this study may be because dairy cows were in mid to late lactation (DIM 165.5 to 186.5). Our study indicated that daily protein yields in milk of the C. tropicalis KKU20 group was highest at 35.6 g/kg and lowest when applied with S. cerevisiae and P. kudriavzevii KKU20 in ensiled RS at 34.5 and 34.1 g/kg, respectively. Milk protein is associated with the feed degradation energy supply as VFAs and microbial protein (MCP) synthesis. High amounts of microorganisms in rumen could affect the MCP synthesis. This will be the supply protein and amino acids (AA) in the small intestine and could enhance the milk protein yields. Our result clearly demonstrated that C. tropicalis KKU20 was unique in the highest bacterial population (11.2 Log10 cell / ml), which is why the increase in milk protein yields occurred. Furthermore, there were no differences in milk proteins between S. cerevisiae and P. kudriavzevii KKU20. This suggests that the influence of Crabtree-negative yeast may play different roles in terms of milk quality. This thought is support by Intanoo et al., who compared different yeast strains that were in the same group of Crabtree-negative, and found that P. kudriavzevii KKU20 decreased daily protein yields in milk by 14.9% when compared with Kluyveromyces marxianus in crossbred lactating cows. This yeast species could provide high biomass, which possibly supplies more amino acid sources for milk protein synthesis. This is similar to Wardrop et al. who stated that K. marxianus has an outstanding ability to provide high biomass when compared with other strain. The explanation is limited in regard to P. kudriavzevii’s impact on daily protein yields in
milk. A few studies have focused on applying non-\textit{S. cerevisiae} to dairy cows and further research about the influence of each strain is required.

Based on this study, we conclude that Crabtree-negative yeast-treated RS, especially \textit{C. tropicalis} KKU20, could enhance the RS’s nutrition value through increasing DMD, the ruminal bacterial population, and total VFAs. In addition, \textit{C. tropicalis} KKU20 could increase the milk protein when compared with other groups. However, there are certain drawbacks associated with the high-producing lactating cows influenced by \textit{C. tropicalis} KKU20 treated RS, which requires further investigation.

\textbf{Methods}

The animals participating in this study have been certified by the Khon Kaen University Animal Ethics Committee (Record No. IACUC-KKU 38/62), based on the Ethics of Animal Experimentation of the National Research Council of Thailand. In addition, we confirmed that all methods were performed in accordance with the relevant guidelines and regulations.

\textbf{Animals and experimental design}

This study used 6 multiparous crossbreds between Holstein Frisian \texttimes Zebu dairy cows in their mid-lactation period (165.5 \textpm 44.0 of day-in-milk) with an initial body weight of 363.9 \textpm 55.80 kg (average milk yield 8.58 kg/d) and a mean age of 5 years. The milk yield reported was slightly higher than the previous studies, which Holstein Frisian \texttimes Zebu cow’s milk yields were 2,897 kg/year or 8.05 kg/d\textsuperscript{14}. Dairy cows were randomly allocated to three ensiled RS with various yeast species including \textit{S. cerevisiae}, \textit{P. kudriavzevii} KKU20, and \textit{C. tropicalis} KKU20 according to a 3 \texttimes 3 replicated Latin square design.

\textbf{Ensiling rice straw with yeast from rumen fluid}

The ruminal yeasts were obtained by isolating, screening, and identifying the rumen of crossbred Thai-Holstein Friesian dairy cattle\textsuperscript{8}. The \textit{P. kudriavzevii} KKU20 and \textit{C. tropicalis} KKU20 were tested for their high-potential on \textit{in vitro} study, which has an outstanding benefit for feed digestion and \textit{in vitro} gas production\textsuperscript{15}. The \textit{S. cerevisae} was obtained from the commercial baker’s yeast (Perfect yeast Co., Ltd, Ubon Ratchathani, Thailand). Isolated homogenous yeast suspension from rumen (about 10\textsuperscript{6} cells per ml) were multiplied in media solution including 250 g molasses (Khon Kaen Dairy cooperative Co., Ltd., Khon Kaen, Thailand) plus 10 g urea per 1000 ml of water. After that, the solution's pH was then modified using formic acid (L.C. industrial Co., Ltd, Nakhon Pathom, Thailand) to reach a final pH of 3.5\textsuperscript{2}. Media solution directly into electromagnetic air compressor (Hailea aco-318 oxygen pump, Sagar aquarium \textregistered, Gujarat, India) flushed with oxygen to complete respiration for maximum cell growth at 72 hours (final estimated yeast as 1 \times 10\textsuperscript{9} cfu/ml,\textsuperscript{15}. The media solution was mix on the RS (2:1 ratio) and adjusted with a moisture content of 650–750 g/kg to provide sufficient ensilage conditions\textsuperscript{16}. Fifteen kilograms of ensiled RS were put into plastic bags (size 24 \times 42 inch, PP Plastic Pagchong Co., Ltd, Nakhonrachasrima, Thailand), and sealed with a vacuum machine (Imaflex 1400W VC-921, Imarflex
Industrial Co., Ltd., Bangkok, Thailand). To ensure anaerobic environment, the bags were securely sealed and fermented at room temperature for 14 days.

**Feeding and samples collection**

The feeding trial lasted for 63 days (21 days/period with 3 periods); dairy cows were held in independent pens and individually fed roughage and concentrate diets at 07:00 and 16:00. Ensiled RS offered ad libitum for all cows. The experimental diet was formulated by using the KCF 2006 Program. The ingredients and nutrient composition of ensiled RS and concentrate diet were provided in Table 1. During the experiment, mineral blocks and fresh water were accessible. The experiment was performed over 3 periods with double squares. The period lasted for 21 days, the first 14 days for treatment adjustments and the last 7 days for sample intake and collection assessment.

In the time of the feeding trial, orts were obtained and weights were collected every day, and the feeding rate was adjusted daily to yield orts between 50 to 100 g/kg of intake. Individual voluntary feed determined consumption difference between the feed offered and orts. Around 5 g/kg of the overall fresh fecal samples were split into two parts; the first part of each day for DM analysis and the second part were pooled at the end of each period. The pooled fecal samples (500 g) were stored at −20 °C until analysis. At 60 °C, composite samples were dried, pressed through a steel filter of 1 mm for grinding (Wiley mill, Arthur H. Thomas Co., Ltd., Philadelphia, PA, USA), and then analyzed for dry matter (DM; ID 967.03), ash (ID 492.05), ether extract (EE; ID 455.08), crude protein (CP; ID 984.13) content, NDF, acid detergent fiber (ADF), and acid-insoluble ash (AIA). Body weights were measured every period. The calculation of metabolizable energy (ME) was based on the equation defined by:

\[ \text{ME (MJ/kg DM)} = 0.82 \times [2.4 \times \text{CP} + 3.9 \times \text{EE} \times 1.8 \times \text{the rest of the OM}] \times \text{in vitro organic matter digestibility (IVOMD)}, \]

where CP, EE, and OM are in g per kg of DM and IVOMD with the mean values received from our recent in vitro study with mean values of 682.5 g/kg DM. The 10 g of fresh silage was blended with 90 ml of sterilized water and stored at 4 °C. The pH of the ensiled RS was measured by a pH meter using cold-water extracts (Hanna HI-8424 Portable pH/ORP Meter, Woonsocket, USA) according to. Silage fluid subsamples were centrifuged for 15 minutes at 16,000 rpm and the liquid above the solid residue was filtered using a 0.45 micron syringe filter. High-performance liquid chromatography (HPLC) devices (Shimadzu LC-20A, Shimadzu Industrial Systems Co., Ltd, Kyoto, Japan) were used to conduct lactic acid (LA), acetic acid (C₂), propionic acid (C₃), and butyric acid (C₄) analyses. The ammonia-nitrogen (NH₃-N) concentration was calculated according to the Kjeldahl process.

Jugular blood and rumen fluid samples were obtained at 0 and 4 hours after feeding on the last day of each period. A blood sample (approximately 10 mL) was obtained in tubes containing 12 mg of ethylene diamine tetra-acetic acid (EDTA) from the jugular vein. The plasma was isolated by centrifugation for 10 min at 500 × g and preserved at −20 °C until blood urea-nitrogen (BUN) analysis, according to Crocker. Approximately 200 mL of rumen fluid was collected from the rumen by a stomach tube connected to a vacuum pump. Rumen fluid was assessed immediately by the pH meter (Hanna Instruments HI 8424 microcomputer, Hanna Instruments (Thailand) Ltd, Bangkok, Thailand) for determining the pH and
temperature. Rumen fluid samples were then filtered through 4 cheesecloth layers. A fluid sample containing 5 mL of 1 mol/L of H$_2$SO$_4$ applied to 45 mL of rumen fluid was put into the bottle. The rumen fluid mixture was centrifuged for 15 min at 16,000 × g and used for analyzing the NH$_3$-N (AOAC, 1998) and volatile fatty acid (VFA) (gas chromatography, Model HP6890-Hewlett, NY, USA; $^{26}$). Methane (CH$_4$) production was calculated using VFA profiles following the equation CH$_4$ (g/d) = 0.45 × C$_2$ (mmol/L) − 0.275 × C$_3$ (mmol/L) + 0.40 × C$_4$ (mmol/L) according to Moss et al.$^{27}$. Ruminal bacteria, protozoa, and fungal zoospores were numbered under a hemocytometer using the direct counting method$^{28}$.

During the last 7 days of each experimental period, milk samples were taken according to the yield for morning and afternoon milking, preserved with 2-bromo-2 nitropropane-1, 3-dial, and stored at 4°C until analysis by using Milko-Scan (Foss Electric, Hillerod, Demark) for fat, true protein, lactose, total solids (TS), and solids-not-fat (SNF) content. Milk urea nitrogen (MUN) was estimated by the diacetyl monoxime method using UV/Vis-spectrophotometer (PG Instruments Ltd., London, UK) according to Ochei and Kolkhtar$^{29}$. Fat, protein, lactose, TS, and SNF concentrations were measured as weighted media depending on morning and afternoon milk yields per each test day by infrared methods using Milko-Scan 33 (Foss Electric, Hillerod, Demark). Yields of 4.0% fat-corrected milk (FCM) were calculated according to$^{30}$Gaines$^{31}$, while yields of energy-corrected (ECM) were calculated as described by Krause and Combs$^{32}$. For each cow and period, feed conversion efficiencies were determined by dividing the average yield of actual milk and ECM by the respective dry matter intake (DMI) and digestible protein per yield of actual milk$^{33}$.

**Statistical analysis**

All data from the experiment were analyzed according to a 3 × 3 replicated Latin square design using the GLM procedure$^{34}$ according to the model:

\[ Y_{ijkl} = \mu + S_i + M_j + A_k + P + \varepsilon_{ijkl} \]

where \( Y_{ijkl} \), observation from cow \( j \), receiving ensiled RS \( i \), in period \( k \); \( \mu \), the overall of mean; \( S_i \), the effect of square \( (i = 1, 2) \); \( M_j \), effect of yeast species in RS silage \( (j = 1, 2, 3) \); \( A_k \), the effect of cows \( (j = 1, 2, 3, 4, 5, 6) \); \( P_k \), the effect of period \( (k = 1, 2, 3) \); and \( \varepsilon_{ijkl} \), the residual effect. Significant differences between individual means were evaluated using the Duncan’s multiple comparison tests when a significant (\( P < 0.05 \)) effect was detected$^{35}$. Standard errors of means were calculated from the residual mean squares in the analysis of variance.

**Declarations**

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Author contributions

C. Suntara, A. Cherdthong and S. Uriyapongson: Investigation, Methodology; C. Suntara, A. and Cherdthong: Data curation, Formal analysis, Software, and Project administration, Conceptualization, Methodology, and Project administration, Funding acquisition; C. Suntara A. Cherdthong, and S. Uriyapongson: Resources, Supervision, Validation; Visualization; C. Suntara: Roles/Writing – original draft; C. Suntara, A. Cherdthong, M. Wanapat and P. Chanjula: Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no conflict of interest.

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