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# Evaluation of yeast supplementation with urea-molasses in rice straw-based diets on *in vitro* ruminal fermentation


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**Abstract:** The effects of yeast supplementation on *in vitro* fermentation characteristics of rice straw and urea-molasses diets in Indonesian swamp buffalo were examined; five doses of yeast (0, 2.5, 5.0, 7.5, and 10 g•head$^{-1}$•d$^{-1}$) were tested. The results indicated that yeast supplementation increased dry and organic matter, neutral detergent fiber, cellulose, and hemicellulose degradability, ammonia-nitrogen and total volatile fatty acid concentration, and decreased the ruminal pH but had no effect on acid detergent fiber degradability or cellulolytic bacterial or protozoan populations. Supplementation with yeast supported ruminal fermentation of urea-molasses and rice straw-based diets, with 5.0 g yeast•head$^{-1}$•d$^{-1}$ showing the greatest response for most variables tested.


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**Key words:** Yeast, degradability, cellulolytic bacterial and protozoan populations


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## INTRODUCTION

The grazing of buffalo in nontidal swamps in Lebak, South Sumatra (Indonesia) has long since been an effort to utilize deep swamps for meat and milk production. However, the population of swamp buffalo in some subdistricts of South Sumatra have declined due to poor management and especially insufficient feed supplies during the dry season (Ali *et al*., 2013). Like other ruminants in developing countries, swamp buffalo in these subdistricts are predominantly maintained on low-grade roughage and graze on degraded range land, resulting in poor nutrient utilization and productivity. Therefore, forages must be enhanced in accordance with the swamp agroecosystem. One way to do so is through utilization of Lebak swamp rice straw to enhance forage supplies in the dry season.

Rice straw production each year is plentiful in South Sumatra and can potentially overcome the shortage of ruminant feed. The South Sumatra Central Bureau of Statistics (BPS) recorded swamp paddy production of dry unhulled rice to be around 1.65 million tons in 2011. On average, 0.83 kg of straw is produced with each kilogram of paddy grain (Trach, 1998), resulting in 1.37 milion tons of rice straw produced in swamp areas. However, there are some limitations to utilizing rice straw as ruminant feed. Rice straw consists predominantly of cellulose, hemicellulose, and lignin, and ruminant organisms need other nutrients for growth and metabolism (Hoover, 1986). Since rice straw does not contain enough sugars, amino acids, and minerals for efficient microbial growth, feeding ruminants only rice straw without further supplementation results in poor performance (Doyle *et al*., 1986). Supplementation of rice straw rations with protein, energy, and/or minerals, such as concentrates, molasses, multinutrient blocks, green leaves, crop residues, and locally available byproducts may optimize rumen function, while maximizing utilization of rice straw.

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Urea-molasses is widely used for supplementation of swamp buffalo (Ali *et al*., 2013b; Tanwar *et al*., 2013; Thu *et al*., 2000; Thu & Uden 2001; Tiwari *et al*., 1990) and other ruminants ((Vu *et al.*, 1999; Wanapat *et al*., 1999; Akter *et al*., 2004) with straw-based diets. Moreover, yeast (*Saccharomyces cereviseae*) supplementation can beneficially modify microbial activity, fermentation, and digestive functions in the rumen. Most investigators agree that yeast can have measurable effects on ruminal fermentation and results in beneficial changes in digestion. However, there are limited reports regarding yeast supplementation of high roughage ratios with urea-molasses and rice straw-based diets. The main objectives of the current study were to investigate the effect yeast supplementation on *in vitro* ruminal fermentation of urea-molasses with rice straw-based diets.

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## MATERIALS AND METHODS

**Substrate and Rumen Liquor Preparation:** The substrate for *in vitro* rumenal fermentation was a dry matter-based mixed ratio of rice straw (80%) and 20% urea-molasses supplementation (1.85% urea,  5.94% molasses, 4.83% rice bran, 3.50% tofu-waste, 2.05% cassava meal, 0.92% NaCl, 0.49% limestone flour, 0.36% trisodium phosphate, and 0.05% mineral and vitamin premix). Diets were estimated according to the requirements of a 200-kg swamp buffalo with a 5.22-kg dry matter intake and 0.62-kg weight change per day (Thu and Uden, 2001). The chemical composition of diets is reported in Table 1.

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Rice straw (*Oryza sativa* var. ciherang) was harvested on August 2014 from the swamp paddy field, dried in an oven (60 ºC), and ground. Rice bran, limestone flour, and trisodium phosphate were obtained from a traditional market in the Ogan Ilir district. Solid tofu waste (local name: "ampas tahu") from the local tofu industry was dried in an oven

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(60 ºC) after being milled and extracting the soybeans. Cassava meal was prepared from bitter cassava roots, cut into thin slices, and sun-dried. All ingredients were ground and sifted through a 1-mm screen for chemical analysis. The mineral and vitamin premix (cattle mix) contained 1 g Mg•kg$^{-1}$, 1 g Co•kg$^{-1}$, 3.3 g P•kg$^{-1}$, 7 g Ca•kg$^{-1}$, 6.5 g Na•kg$^{-1}$, 1 g S•kg$^{-1}$, 50 mg Fe•kg$^{-1}$, 40 mg Mn•kg$^{-1}$, 30 mg Zn•kg$^{-1}$, 8 mg Cu•kg$^{-1}$, 500 µg I•kg$^{-1}$, 200 µg Se•kg$^{-1}$, 30,000 IU vitamin A•kg$^{-1}$, 3500 IU vitamin D•kg$^{-1}$, and 900 IU vitamin E•kg$^{-1}$. The yeast used for supplementation was Yea-Sacc$^{1026}$, a yeast culture with a declared concentration of $10^9$ CFU•g$^{-1}$, 34.58% crude protein, 7.2% crude fat, 10.44% acid detergent fiber (ADF), and 7.42% ash.

The dry matter content was determined by oven-drying at 105 °C for 24 h. The organic matter was determined by ashing at 550 °C for 4 h. Total nitrogen content was determined according to the Kjeldahl method (AOAC, 1995). The content of neutral detergent fiber (NDF), ADF, cellulose, and hemicellulose in the rice straw was determined using the method reported by Van Soest *et al*. (1991). Rumen liquor was collected from swamp buffalo rumen at a slaughter house. These buffalo were fed a diet consisting of *Oryza rupifogon*, *Eleocharis dulcis*, and *Hymenachne acutigluma* in the Rambutan subdistrict of Banyuasin district, South Sumatra province. Ruminal contents from buffalo were strained through two layers of cheese cloth and kept at 39 °C under a $CO_2$ atmosphere.

***In Vitro* Fermentation (Tilley & Terry, 1963):** The substrate (1 g) was put into a 100-ml fermentation tube, and 40 ml of McDougall buffer and 10 ml of rumen liquor were added. McDougal buffer (6 L) contained 58.8 g $NaHCO_3$, 42 g $Na_2HPO_4$•$7H_2O$, 3.42 g KCl, 2.82 g NaCl, 0.72 g $MgSO_4$•$7H_2O$, 0.24 g $CaCl_2$, and $H_2O$. The mixture was stirred and flushed

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with $O_2$-free $CO_2$, and then the tubes were sealed with a rubber fitted with the gas release valve. All fermentation tubes were incubated in a shaking water bath at 39 ºC for 48 h.

**Estimation of volatile fatty acid (VFA) and ammonia-nitrogen (N-$NH_3$) Concentration and *In Vitro* Degradability:** Measurement of total VFA content was done using a previously reported steam distillation method (General Laboratory Procedures, 1966), and the N-$NH_3$ concentration was determined using a previous microdiffusion method (Conway, 1962). The total VFA concentration in rumenal fluid was determined by Markham's distillation. To determine the *in vitro* degradability of dry and organic matter, NDF, ADF, cellulose, and hemicellulose (Van Soest *et al*., 1991), the content of the fermentation tube incubated for 48 h was transferred into a new tube and centrifuged at 2500 rpm for 20 min at room temperature. After, the supernatant was discarded, and the remaining residue was passed through a filter paper (Whatman no. 41). The residue of each fermentation tube was dried to a constant weight at 105 °C for 24 h to determine *in vitro* degradability.

**Protozoal and Bacterial Counts:** After a 48-h incubation, a 1-ml aliquot was taken from each fermentation tube for analysis of protozoan and bacterial populations. The contents of the fermentation tube were mixed properly and 1 ml of the sample was mixed with 1 ml methyl green formaldehyde saline solution containing 35% formaldehyde, distilled water, methyl green, and NaCl (Ogimoto & Imai, 1981). The stained sample was kept at room temperature, and protozoan populations were counted using a counting chamber (0.1 mm) and a microscope (40X objective). Bacterial populations were determined using a roll-tube technique (Hungate, 1969).

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**Experimental design:** The completely randomized design of the current study was chosen to evaluate five different doses of yeast (0, 2.5, 5.0, 7.5, and 10 g•head$^{-1}$•d$^{-1}$) with four replications. Data were analyzed by analysis of variance, and mean values were tested for differences using Duncan's New Multi-Range Test.

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## RESULTS

The chemical composition of the rice straw and urea-molasses, as well as buffalo diet ingredients, are presented in Table 1. pH, VFA, and N-$NH_3$ are important parameters reflecting ruminal environment. Yeast supplementation decreased the ruminal pH by 0.06 units compared to controls (Table 2). The highest pH occurred in samples with 0 g yeast supplementation, and the lowest was seen with 7.5 g yeast. Nonetheless, the ruminal pH range in all sample groups was optimal (6.0-6.9). The concentration of N-$NH_3$ was 7.57, 10.05, 11.07, 9.41, and 10.19 mM with 0, 2.5, 5.0, 7.5, and 10 g yeast, respectively ($P<0.01$; Table 2). VFA concentrations were significantly higher ($P<0.01$) in yeast-supplemented diets (74.63-94.10 mM) compared to the control diet (56.52 mM; Table 2). Results of this trial showed that yeast could not stimulate growth of cellulolytic bacterial and protozoan populations.

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*In vitro* degradability of dry and organic matter was increased by supplementation with yeast ($P<0.01$). Dry and organic matter degradability with 5.0 g yeast was similar to that with 7.5 and 10 g yeast but higher than with 0 and 2.5 g ($P<0.01$). Furthermore, yeast supplementation affected NDF degradability but not ADF.

## DISCUSSION

The chemical composition of rice straw was similar to results reported previously (Tan *et al*., 1996; Thalib *et al*., 2000; Van Soest 2006). This rice straw had greater NDF, ADF, cellulose, and hemicellulose and lower crude protein content compared to the others. Moreover, urea-molasses supplementation with locally available products decreased the fiber fraction and increased crude protein content in the diet.

Although there were significant differences on rumen pH among the different yeast treatments in the current study, the differences was small. Ruminal pH affects digestibility of feed stuffs. Fibrolytic bacteria are very sensitive and dependent on pH changes. In fact, the digestibility of organic matter, NDF, and nitrogen decrease at pH 5.8 and increase at pH 6.2. Production of total VFA content was shown to be highest between pH 6.2 and 6.6 in high concentrate diets (Shriver *et al*., 1986). Sung *et al*. (2007) reported increases dry matter digestion and VFA production from pH 6.2 to 6.7 after 48 h of *in vitro* rumen fermentation. Dolezal *et al*. (2011) reported that yeast supplementation increased ruminal pH in high concentrate diets, while Mao *et al.* (2013) found that ruminal pH increased in rice straw- but decreased in corn stover-based subtrate diets with yeast supplementation. The current results are consistent with results observed by Lynch and Martin (2002), where live cells decreased ruminal pH when alfalfa hay was incubated. These differences in ruminal pH were likely associated with the lactic acid concentration and differences in substrate degradation with yeast supplementation. Compared with Thu and Uden (2001), the control treatment had a similar pH but lower concentration of $NH_3$.

Ammonia is the main source of nitrogen for microbial protein synthesis (Bach *et al*., 2005). The present results showed that yeast supplementation increased the N-$NH_3$ concentration. This is in agreement with Mao *et al.* (2013) who reported a N-$NH_3$ concentration of 8.0 mg per 100 ml in controls and 8.3-10.5 mg per 100 ml in animals supplemented with rice straw. Zain *et al*. (2011) found that yeast supplementation decreased N-$NH_3$ concentrations in ammoniated rice straw. Opsi *et al.* (2012) reported that yeast supplementation increased N-$NH_3$ in high forage diets but did not affect high

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concentrate diets. It is likely that increases in N-$NH_3$ output represent microbial degradation of large amounts of yeast cells which have a high protein content.

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Supplementation of high-fiber diets with yeast additives affected total VFA production in the current study. This result is consistent with the slight decline in rumen pH discussed above and also agree with reports by Mao *et al.* (2013), Zain *et al*. (2011), and Opsi *et al.* (2012), among other *in vivo* studies, indicating stimulation of rumen microbial fermentation activity. This alteration in ruminal VFA by yeast supplementation could contribute to improved feed efficiency in swamp buffalo. Wallace and Newbold (1992) suggested that variable responses in VFA production and patterns are a consequence of the effects of yeast on rumen microbial numbers rather than a direct effect on ruminal fermentation.

Data regarding the 48-h degradability of diets in the present study are presented in Table 2; the current results generally agree with previous experiments (Lila *et al*., 2004; Tang *et al*., 2008; Zain *et al*., 2011). Lila *et al*. (2004) reported that *in vitro* dry matter degradability increased with yeast supplementation of sudangrass hay and concentrate mixtures. Zain *et al*. (2011) reported that yeast supplementation increased dry and organic matter, NDF, ADF, and cellulose degradability. Herawaty *et al*. (2013) reported that yeast supplementation increased the degradability of organic matter, NDF, and ADF more than a diet of unsupplemented rice straw alone. When yeast was supplemented at 5.0 g•$kg^{-1}$, the greatest dry matter degradability occurred for maize stover, maize stover silage, and wheat straw but generally decreased with rice straw. On the other hand, yeast supplementation increased organic matter degradability of maize stover, maize stover silage, and rice straw (Tang *et al*., 2008). Opsi *et al*. (2012) reported that supplementation of yeast had not effect on dry matter and NDF digestibility in high and low forage ratio diets.

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In the present study, yeast supplementation did not significantly affect bacterial and protozoan numbers in the *in vitro* fermentation test even though they tended to increase. Previous studies have reported that yeast supplementation increased cellulolytic bacteria and protozoa significantly *in vitro* (Mao *et al*., 2013; Newbold *et al*., 1995; Zain *et al*. 2011) and *in vivo* in buffalo (Kumar *et al*., 2013). However, no significant effect of yeast on protozoa was observed (Hristov *et al*. 2010; Yoon and Stern, 1996). Increased dry and organic matter, NDF, cellulose, and hemicellulose degradability, as well as VFA production with different substrates could be attributed to an increased fiber-digesting bacterial population.

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## CONCLUSIONS AND IMPLICATIONS

It is concluded that yeast supplementation of urea-molasses and rice straw diets increases the degradability of dry and organic matter, NDF, cellulose, and hemicellulose, the concentration of N-$NH_3$ and VFA, but decreases the rumen pH. The current results also showed that supplementation with 5.0 g yeast•head$^{-1}$•d$^{-1}$ provides the greatest response for most variables tested. *In vivo* studies of yeast supplementation should be implemented in future to optimize the utilization of dietary nutrients and improve production in buffalo fed low-quality roughage.

## ACKNOWLEDGEMENTS

Financial support for this work was provided by a competitive research grant from the Sriwijaya University (Palembang, Indonesia) in 2014. The authors would like to thank the Research Center for Sub-Optimal Lands, and Laboratory of Animal Nutrition and Feed Science of Sriwijaya University, as well as the Laboratory of Dairy Science and Technology, Bogor Agricultural University (Bogor, Indonesia) for use of their facilities.

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**Table 1.** Chemical composition of rice straw and urea-molasses, as well as buffalo diets.

| **Item** | **DM** | **OM** | **CP** | **NDF** | **ADF** | **Lignin** | **C** | **HC** |
|---|---|---|---|---|---|---|---|---|
| Rice straw80%+UMS20% | 86.03 | 90.07 | 10.14 | 63.37 | 41.15 | 6.31 | 29.67 | 22.22 |
| Rice straw | 92.98 | 83.76 | 4.83 | 76.14 | 47.97 | 7.07 | 37.50 | 28.17 |
| UMS | 80.69 | 66.64 | 31.41 | 15.61 | 11.42 | 2.78 | 6.66 | 4.19 |
| Rice bran | 90.67 | 74.41 | 6.36 | 55.79 | 47.03 | 11.85 | 23.95 | 8.76 |
| Solid Tofu Wastes | 96.00 | 93.05 | 20.29 | 48.25 | 23.60 | 2.64 | 20.46 | 24.65 |
| Cassava meal | 84.41 | 83.19 | 1.85 | 22.34 | 5.08 | 0.82 | 4.32 | 17.26 |

UMS, urea-molasses supplementation; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; C, cellulose; HC, hemicellulose

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**Tabel 2.** Degradability, rumen pH, N-$NH_3$, volatile fatty acid (VFA) concentration, bacterial and protozoan populations with yeast supplementation.

| | **Yeast Supplementation (g.head$^{-1}$.day$^{-1}$)** | | | | | **SE** | **Sig.** |
|---|---|---|---|---|---|---|---|
| | **0** | **2.5** | **5.0** | **7.5** | **10** | | |
| Degradability | | | | | | | |
| DM | 34.35$^{a}$ | 43.22$^{b}$ | 48.25$^{cd}$ | 46.99$^{c}$ | 49.87$^{d}$ | 1.32 | 0.000 |
| OM | 33.08$^{a}$ | 42.27$^{b}$ | 46.86$^{c}$ | 46.28$^{c}$ | 47.94$^{c}$ | 1.31 | 0.000 |
| NDF | 43,29$^{a}$ | 44,70$^{ab}$ | 45,75$^{b}$ | 46,17$^{b}$ | 48,61$^{c}$ | 0,62 | 0,010 |
| ADF | 48,22 | 49,52 | 48,28 | 49,58 | 51,04 | 0,43 | 0,192 |
| Cellulose | 2,99$^{a}$ | 6,09$^{b}$ | 5,53$^{b}$ | 4,51$^{ab}$ | 5,47$^{b}$ | 0,37 | 0,028 |
| Hemicellulose | 32,65$^{a}$ | 40,83$^{b}$ | 40,22$^{b}$ | 38,41$^{ab}$ | 50,28$^{c}$ | 1,67 | 0,001 |
| pH | 6.84$^{c}$ | 6.82$^{bc}$ | 6.83$^{c}$ | 6.78$^{a}$ | 6.80$^{ab}$ | 0.01 | 0.000 |
| N-$NH_3$ | 7.57$^{a}$ | 10.05$^{b}$ | 11.07$^{b}$ | 9.41$^{ab}$ | 10.19$^{b}$ | 0.37 | 0.001 |
| Total VFA | 56.52$^{a}$ | 92.05$^{b}$ | 85.88$^{b}$ | 74.63$^{ab}$ | 94.10$^{b}$ | 4.06 | 0.000 |
| Cellulolitic | | | | | | | 0.348 |
| Bacteria | 5.36 | 5.38 | 6.09 | 4.30 | 5.89 | 0.29 | |
| Protozoa | 4.83 | 5.10 | 5.17 | 4.85 | 5.05 | 0.06 | 0.175 |

DM, dry matter; OM, organic matter; NDF, neutral detergent fiber; ADF, acid detergent fiber

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# Evaluation of yeast supplementation with urea-molasses in rice straw-based diets on *in vitro* ruminal fermentation

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**Abstract:** The effects of yeast supplementation on *in vitro* fermentation characteristics of rice straw and urea-molasses diets in Indonesian swamp buffalo were examined; five doses of yeast (0, 2.5, 5.0, 7.5, and 10 $g{\bullet}head^{-1}{\bullet}d^{-1}$) were tested. The results indicated that yeast supplementation increased dry and organic matter, neutral detergent fiber, cellulose, and hemicellulose degradability, ammonia-nitrogen and total volatile fatty acid concentration, and decreased the ruminal pH but had no effect on acid detergent fiber degradability or cellulolytic bacterial or protozoan populations. Supplementation with yeast supported ruminal fermentation of urea-molasses and rice straw-based diets, with 5.0 g $yeast{\bullet}head^{-1}{\bullet}d^{-1}$ showing the greatest response for most variables tested.

**Key words:** Yeast, degradability, cellulolytic bacterial and protozoan populations

## INTRODUCTION

The grazing of buffalo in nontidal swamps in Lebak, South Sumatra (Indonesia) has long since been an effort to utilize deep swamps for meat and milk production. However, the population of swamp buffalo in some subdistricts of South Sumatra have declined due to poor management and especially insufficient feed supplies during the dry season (Ali *et al*., 2013). Like other ruminants in developing countries, swamp buffalo in these subdistricts are predominantly maintained on low-grade roughage and graze on degraded range land, resulting in poor nutrient utilization and productivity. Therefore, forages must be enhanced in accordance with the swamp agroecosystem. One way to do so is through utilization of Lebak swamp rice straw to enhance forage supplies in the dry season.

Rice straw production each year is plentiful in South Sumatra and can potentially overcome the shortage of ruminant feed. The South Sumatra Central Bureau of Statistics (BPS) recorded swamp paddy production of dry unhulled rice to be around 1.65 million tons in 2011. On average, 0.83 kg of straw is produced with each kilogram of paddy grain (Trach, 1998), resulting in 1.37 milion tons of rice straw produced in swamp areas. However, there are some limitations to utilizing rice straw as ruminant feed. Rice straw consists predominantly of cellulose, hemicellulose, and lignin, and ruminant organisms need other nutrients for growth and metabolism (Hoover, 1986). Since rice straw does not contain enough sugars, amino acids, and minerals for efficient microbial growth, feeding ruminants only rice straw without further supplementation results in poor performance (Doyle *et al*., 1986). Supplementation of rice straw rations with protein, energy, and/or minerals, such as concentrates, molasses, multinutrient blocks, green leaves, crop residues, and locally available byproducts may optimize rumen function, while maximizing utilization of rice straw.

Urea-molasses is widely used for supplementation of swamp buffalo (Ali *et al*., 2013b; Tanwar *et al*., 2013; Thu *et al*., 2000; Thu & Uden 2001; Tiwari *et al*., 1990) and other ruminants ((Vu *et al.*, 1999; Wanapat *et al*., 1999; Akter *et al*., 2004) with straw-based diets. Moreover, yeast (*Saccharomyces cereviseae*) supplementation can beneficially modify microbial activity, fermentation, and digestive functions in the rumen. Most investigators agree that yeast can have measurable effects on ruminal fermentation and results in beneficial changes in digestion. However, there are limited reports regarding yeast supplementation of high roughage ratios with urea-molasses and rice straw-based diets. The main objectives of the current study were to investigate the effect yeast supplementation on *in vitro* ruminal fermentation of urea-molasses with rice straw-based diets.

## MATERIALS AND METHODS

**Substrate and Rumen Liquor Preparation:** The substrate for *in vitro* rumenal fermentation was a dry matter-based mixed ratio of rice straw (80%) and 20% urea-molasses supplementation (1.85% urea, 5.94% molasses, 4.83% rice bran, 3.50% tofu-waste, 2.05% cassava meal, 0.92% NaCl, 0.49% limestone flour, 0.36% trisodium phosphate, and 0.05% mineral and vitamin premix). Diets were estimated according to the requirements of a 200-kg swamp buffalo with a 5.22-kg dry matter intake and 0.62-kg weight change per day (Thu and Uden, 2001). The chemical composition of diets is reported in Table 1.

Rice straw (*Oryza sativa* var. ciherang) was harvested on August 2014 from the swamp paddy field, dried in an oven (60 $^{o}$C), and ground. Rice bran, limestone flour, and trisodium phosphate were obtained from a traditional market in the Ogan Ilir district. Solid tofu waste (local name: “ampas tahu”) from the local tofu industry was dried in an oven

(60 $^{o}$C) after being milled and extracting the soybeans. Cassava meal was prepared from bitter cassava roots, cut into thin slices, and sun-dried. All ingredients were ground and sifted through a 1-mm screen for chemical analysis. The mineral and vitamin premix (cattle mix) contained 1 g Mg•kg$^{-1}$, 1 g Co•kg$^{-1}$, 3.3 g P•kg$^{-1}$, 7 g Ca•kg$^{-1}$, 6.5 g Na•kg$^{-1}$, 1 g S•kg$^{-1}$, 50 mg Fe•kg$^{-1}$, 40 mg Mn•kg$^{-1}$, 30 mg Zn•kg$^{-1}$, 8 mg Cu•kg$^{-1}$, 500 µg I•kg$^{-1}$, 200 µg Se•kg$^{-1}$, 30,000 IU vitamin A•kg$^{-1}$, 3500 IU vitamin D•kg$^{-1}$, and 900 IU vitamin E•kg$^{-1}$. The yeast used for supplementation was Yea-Sacc[1026], a yeast culture with a declared concentration of $10^9$ CFU•g$^{-1}$, 34.58% crude protein, 7.2% crude fat, 10.44% acid detergent fiber (ADF), and 7.42% ash.

The dry matter content was determined by oven-drying at 105 °C for 24 h. The organic matter was determined by ashing at 550 °C for 4 h. Total nitrogen content was determined according to the Kjeldahl method (AOAC, 1995). The content of neutral detergent fiber (NDF), ADF, cellulose, and hemicellulose in the rice straw was determined using the method reported by Van Soest *et al*. (1991). Rumen liquor was collected from swamp buffalo rumen at a slaughter house. These buffalo were fed a diet consisting of *Oryza rupifogon*, *Eleocharis dulcis*, and *Hymenachne acutigluma* in the Rambutan subdistrict of Banyuasin district, South Sumatra province. Ruminal contents from buffalo were strained through two layers of cheese cloth and kept at 39 °C under a $CO_2$ atmosphere.

***In Vitro* Fermentation (Tilley & Terry, 1963):** The substrate (1 g) was put into a 100-ml fermentation tube, and 40 ml of McDougall buffer and 10 ml of rumen liquor were added. McDougal buffer (6 L) contained 58.8 g $NaHCO_3$, 42 g $Na_2HPO_4$•$7H_2O$, 3.42 g KCl, 2.82 g NaCl, 0.72 g $MgSO_4$•$7H_2O$, 0.24 g $CaCl_2$, and $H_2O$. The mixture was stirred and flushed with $O_2$-free $CO_2$, and then the tubes were sealed with a rubber fitted with the gas release valve. All fermentation tubes were incubated in a shaking water bath at 39 $^{o}$C for 48 h.

**Estimation of volatile fatty acid (VFA) and ammonia-nitrogen (N-$NH_3$) Concentration and *In Vitro* Degradability:** Measurement of total VFA content was done using a previously reported steam distillation method (General Laboratory Procedures, 1966), and the N-$NH_3$ concentration was determined using a previous microdiffusion method (Conway, 1962). The total VFA concentration in rumenal fluid was determined by Markham's distillation. To determine the *in vitro* degradability of dry and organic matter, NDF, ADF, cellulose, and hemicellulose (Van Soest *et al*., 1991), the content of the fermentation tube incubated for 48 h was transferred into a new tube and centrifuged at 2500 rpm for 20 min at room temperature. After, the supernatant was discarded, and the remaining residue was passed through a filter paper (Whatman no. 41). The residue of each fermentation tube was dried to a constant weight at 105 °C for 24 h to determine *in vitro* degradability.

**Protozoal and Bacterial Counts:** After a 48-h incubation, a 1-ml aliquot was taken from each fermentation tube for analysis of protozoan and bacterial populations. The contents of the fermentation tube were mixed properly and 1 ml of the sample was mixed with 1 ml methyl green formaldehyde saline solution containing 35% formaldehyde, distilled water, methyl green, and NaCl (Ogimoto & Imai, 1981). The stained sample was kept at room temperature, and protozoan populations were counted using a counting chamber (0.1 mm) and a microscope (40X objective). Bacterial populations were determined using a roll-tube technique (Hungate, 1969).

**Experimental design:** The completely randomized design of the current study was chosen to evaluate five different doses of yeast (0, 2.5, 5.0, 7.5, and 10 g•head$^{-1}$•d$^{-1}$) with four replications. Data were analyzed by analysis of variance, and mean values were tested for differences using Duncan's New Multi-Range Test.

## RESULTS

The chemical composition of the rice straw and urea-molasses, as well as buffalo diet ingredients, are presented in Table 1. pH, VFA, and N-$NH_3$ are important parameters reflecting ruminal environment. Yeast supplementation decreased the ruminal pH by 0.06 units compared to controls (Table 2). The highest pH occurred in samples with 0 g yeast supplementation, and the lowest was seen with 7.5 g yeast. Nonetheless, the ruminal pH range in all sample groups was optimal (6.0-6.9). The concentration of N-$NH_3$ was 7.57, 10.05, 11.07, 9.41, and 10.19 mM with 0, 2.5, 5.0, 7.5, and 10 g yeast, respectively ($P<0.01$; Table 2). VFA concentrations were significantly higher ($P<0.01$) in yeast-supplemented diets (74.63-94.10 mM) compared to the control diet (56.52 mM; Table 2). Results of this trial showed that yeast could not stimulate growth of cellulolytic bacterial and protozoan populations.

*In vitro* degradability of dry and organic matter was increased by supplementation with yeast ($P<0.01$). Dry and organic matter degradability with 5.0 g yeast was similar to that with 7.5 and 10 g yeast but higher than with 0 and 2.5 g ($P<0.01$). Furthermore, yeast supplementation affected NDF degradability but not ADF.

## DISCUSSION

The chemical composition of rice straw was similar to results reported previously (Tan *et al*., 1996; Thalib *et al*., 2000; Van Soest 2006). This rice straw had greater NDF, ADF, cellulose, and hemicellulose and lower crude protein content compared to the others. Moreover, urea-molasses supplementation with locally available products decreased the fiber fraction and increased crude protein content in the diet.

Although there were significant differences on rumen pH among the different yeast treatments in the current study, the differences was small. Ruminal pH affects digestibility of feed stuffs. Fibrolytic bacteria are very sensitive and dependent on pH changes. In fact, the digestibility of organic matter, NDF, and nitrogen decrease at pH 5.8 and increase at pH 6.2. Production of total VFA content was shown to be highest between pH 6.2 and 6.6 in high concentrate diets (Shriver *et al*., 1986). Sung *et al*. (2007) reported increases dry matter digestion and VFA production from pH 6.2 to 6.7 after 48 h of *in vitro* rumen fermentation. Dolezal *et al*. (2011) reported that yeast supplementation increased ruminal pH in high concentrate diets, while Mao *et al.* (2013) found that ruminal pH increased in rice straw- but decreased in corn stover-based subtrate diets with yeast supplementation. The current results are consistent with results observed by Lynch and Martin (2002), where live cells decreased ruminal pH when alfalfa hay was incubated. These differences in ruminal pH were likely associated with the lactic acid concentration and differences in substrate degradation with yeast supplementation. Compared with Thu and Uden (2001), the control treatment had a similar pH but lower concentration of $NH_3$.

Ammonia is the main source of nitrogen for microbial protein synthesis (Bach *et al*., 2005). The present results showed that yeast supplementation increased the N-$NH_3$ concentration. This is in agreement with Mao *et al.* (2013) who reported a N-$NH_3$ concentration of 8.0 mg per 100 ml in controls and 8.3-10.5 mg per 100 ml in animals supplemented with rice straw. Zain *et al*. (2011) found that yeast supplementation decreased N-$NH_3$ concentrations in ammoniated rice straw. Opsi *et al.* (2012) reported that yeast supplementation increased N-$NH_3$ in high forage diets but did not affect high concentrate diets. It is likely that increases in $N\text{-}NH_3$ output represent microbial degradation of large amounts of yeast cells which have a high protein content.

Supplementation of high-fiber diets with yeast additives affected total VFA production in the current study. This result is consistent with the slight decline in rumen pH discussed above and also agree with reports by Mao *et al.* (2013), Zain *et al.* (2011), and Opsi *et al.* (2012), among other *in vivo* studies, indicating stimulation of rumen microbial fermentation activity. This alteration in ruminal VFA by yeast supplementation could contribute to improved feed efficiency in swamp buffalo. Wallace and Newbold (1992) suggested that variable responses in VFA production and patterns are a consequence of the effects of yeast on rumen microbial numbers rather than a direct effect on ruminal fermentation.

Data regarding the 48-h degradability of diets in the present study are presented in Table 2; the current results generally agree with previous experiments (Lila *et al*., 2004; Tang *et al*., 2008; Zain *et al*., 2011). Lila *et al*. (2004) reported that *in vitro* dry matter degradability increased with yeast supplementation of sudangrass hay and concentrate mixtures. Zain *et al*. (2011) reported that yeast supplementation increased dry and organic matter, NDF, ADF, and cellulose degradability. Herawaty *et al*. (2013) reported that yeast supplementation increased the degradability of organic matter, NDF, and ADF more than a diet of unsupplemented rice straw alone. When yeast was supplemented at 5.0 $g{\bullet}kg^{-1}$, the greatest dry matter degradability occurred for maize stover, maize stover silage, and wheat straw but generally decreased with rice straw. On the other hand, yeast supplementation increased organic matter degradability of maize stover, maize stover silage, and rice straw (Tang *et al*., 2008). Opsi *et al*. (2012) reported that supplementation of yeast had not effect on dry matter and NDF digestibility in high and low forage ratio diets.

In the present study, yeast supplementation did not significantly affect bacterial and protozoan numbers in the *in vitro* fermentation test even though they tended to increase. Previous studies have reported that yeast supplementation increased cellulolytic bacteria and protozoa significantly *in vitro* (Mao *et al*., 2013; Newbold *et al*., 1995; Zain *et al*. 2011) and *in vivo* in buffalo (Kumar *et al*., 2013). However, no significant effect of yeast on protozoa was observed (Hristov *et al*. 2010; Yoon and Stern, 1996). Increased dry and organic matter, NDF, cellulose, and hemicellulose degradability, as well as VFA production with different substrates could be attributed to an increased fiber-digesting bacterial population.

## CONCLUSIONS AND IMPLICATIONS

It is concluded that yeast supplementation of urea-molasses and rice straw diets increases the degradability of dry and organic matter, NDF, cellulose, and hemicellulose, the concentration of N-$NH_3$ and VFA, but decreases the rumen pH. The current results also showed that supplementation with 5.0 g yeast•head$^{-1}$•d$^{-1}$ provides the greatest response for most variables tested. *In vivo* studies of yeast supplementation should be implemented in future to optimize the utilization of dietary nutrients and improve production in buffalo fed low-quality roughage.

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**Table 1.** Chemical composition of rice straw and urea-molasses, as well as buffalo diets.

| Item | DM | OM | CP | NDF | ADF | Lignin | C | HC |
|---|---|---|---|---|---|---|---|---|
| Rice straw80%+UMS20% | 86.03 | 90.07 | 10.14 | 63.37 | 41.15 | 6.31 | 29.67 | 22.22 |
| Rice straw | 92.98 | 83.76 | 4.83 | 76.14 | 47.97 | 7.07 | 37.50 | 28.17 |
| UMS | 80.69 | 66.64 | 31.41 | 15.61 | 11.42 | 2.78 | 6.66 | 4.19 |
| Rice bran | 90.67 | 74.41 | 6.36 | 55.79 | 47.03 | 11.85 | 23.95 | 8.76 |
| Solid Tofu Wastes | 96.00 | 93.05 | 20.29 | 48.25 | 23.60 | 2.64 | 20.46 | 24.65 |
| Cassava meal | 84.41 | 83.19 | 1.85 | 22.34 | 5.08 | 0.82 | 4.32 | 17.26 |

UMS, urea-molasses supplementation; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; C, cellulose; HC, hemicellulose

**Tabel 2.** Degradability, rumen pH, N-$NH_3$, volatile fatty acid (VFA) concentration, bacterial and protozoan populations with yeast supplementation.

| | **Yeast Supplementation (g.head$^{-1}$.day$^{-1}$)** | | | | | **SE** | **Sig.** |
|---|---|---|---|---|---|---|---|
| | **0** | **2.5** | **5.0** | **7.5** | **10** | | |
| Degradability | | | | | | | |
| DM | 34.35$^{a}$ | 43.22$^{b}$ | 48.25$^{cd}$ | 46.99$^{c}$ | 49.87$^{d}$ | 1.32 | 0.000 |
| OM | 33.08$^{a}$ | 42.27$^{b}$ | 46.86$^{c}$ | 46.28$^{c}$ | 47.94$^{c}$ | 1.31 | 0.000 |
| NDF | 43,29$^{a}$ | 44,70$^{ab}$ | 45,75$^{b}$ | 46,17$^{b}$ | 48,61$^{c}$ | 0,62 | 0,010 |
| ADF | 48,22 | 49,52 | 48,28 | 49,58 | 51,04 | 0,43 | 0,192 |
| Cellulose | 2,99$^{a}$ | 6,09$^{b}$ | 5,53$^{b}$ | 4,51$^{ab}$ | 5,47$^{b}$ | 0,37 | 0,028 |
| Hemicellulose | 32,65$^{a}$ | 40,83$^{b}$ | 40,22$^{b}$ | 38,41$^{ab}$ | 50,28$^{c}$ | 1,67 | 0,001 |
| pH | 6.84$^{c}$ | 6.82$^{bc}$ | 6.83$^{c}$ | 6.78$^{a}$ | 6.80$^{ab}$ | 0.01 | 0.000 |
| N-$NH_3$ | 7.57$^{a}$ | 10.05$^{b}$ | 11.07$^{b}$ | 9.41$^{ab}$ | 10.19$^{b}$ | 0.37 | 0.001 |
| Total VFA | 56.52$^{a}$ | 92.05$^{b}$ | 85.88$^{b}$ | 74.63$^{ab}$ | 94.10$^{b}$ | 4.06 | 0.000 |
| Cellulolitic Bacteria | 5.36 | 5.38 | 6.09 | 4.30 | 5.89 | 0.29 | 0.348 |
| Protozoa | 4.83 | 5.10 | 5.17 | 4.85 | 5.05 | 0.06 | 0.175 |

DM, dry matter; OM, organic matter; NDF, neutral detergent fiber; ADF, acid detergent fiber