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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/d) were tested. The results indicated that yeast supplementation increased dry and organic matter, neutral detergent fiber (NDF), 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 (ADF) 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/d 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.*, 2013a). Like other ruminants in developing countries, swamp buffalo in these sub districts are predominantly maintained on low-grade roughage, 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 recorded swamp paddy production of dry unhulled rice to be around 1.65 million tons in 2011 (Biro Pusat Statistik, 2012). On average, 0.83 kg of straw is produced with each kilogram of paddy grain (Trach, 1998), resulting in 1.37 million 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 molasses, multi nutrient blocks, green leaves, crop residues and locally available agro-industrial by products 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 and Uden, 2000, 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 cerevisiae*) 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 rations 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* ruminal fermentation was a dry matter-based mixed rations 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 in the Rambutan subdistrict of Banyuasin district, chopped, dried in an oven (60°C) and ground to 1 mm. 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°C) after being milled and extracting the soybeans. Cassava meal was prepared from bitter cassava roots from the traditional market, cut into thin slices and sun-dried. All ingredients were ground and sifted through a 1 mm. The mineral and vitamin premix (Cattle Mix) contained 1 g Mg/kg, 1 g Co/kg, 3.3 g P/kg, 7 g Ca/kg, 6.5 g Na/kg, 1 g S/kg, 50 mg Fe/kg, 40 mg Mn/kg, 30 mg Zn/kg, 8 mg Cu/kg, 500 µg I/kg, 200 µg Se/kg, 30,000 IU vitamin A/kg, 3500 IU vitamin D/kg and 900 IU vitamin E/kg. The yeast used for supplementation was Yea-Sacc⁰²⁹, a yeast culture with a declared concentration of 10⁹ CFU/g, 34.58% crude protein, 7.2% crude fat, 10.44% 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 NDF, ADF, cellulose and hemicellulose 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 rufipogon*, *Eleocharis dulcis* and *Hymenachne acutigluma* in the Rambutan subdistrict of Banyuasin district, South Sumatra province. Rumen contents from buffalo were strained through two layers of cheese cloth and kept at 39°C under a CO₂ atmosphere.

***In vitro* fermentation (Tilley and 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. McDougall buffer solution (6 L) contained 58.8 g NaHCO₃, 42 g Na₂HPO₄•7H₂O, 3.42 g KCl, 2.82 g NaCl, 0.72 g MgSO₄•7H₂O, 0.24 g CaCl₂. The mixture was stirred and flushed with O₂-free CO₂ 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°C for 48 h.

Estimation of volatile fatty acid (VFA) and ammonia-nitrogen (N-NH₃) 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-NH₃ concentration was determined using a previous microdiffusion method (Conway, 1962). The total VFA concentration in ruminal 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 and 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). Statistical Analysis. 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/d) 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-NH₃ 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-NH₃ 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 no significant effects of yeast supplementation on growth of cellulolytic bacterial and protozoan populations.

Table 1: Chemical composition of rice straw and urea-molasses, as well as buffalo diets (% of DM)

Item	DM	OM	CP	NDF	ADF	Lignin	C	HC
Rice straw (80%)+UMS (20%)	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	86.64	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 Waste	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

Table 2: Degradability, rumen pH, N-NH₃, volatile fatty acid (VFA) concentration, bacterial and protozoan populations with yeast supplementation

	Yeast supplementation (g/head/day)					SE	p-value
	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 ^d	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 ^{bc}	5.47 ^b	0.37	0.028
Hemicellulose	32.65 ^a	40.83 ^b	40.22 ^b	38.41 ^{bc}	50.28 ^d	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 ^{bc}	10.19 ^b	0.37	0.001
Total VFA	56.52 ^a	92.05 ^b	85.88 ^b	74.63 ^{cd}	94.10 ^d	4.06	0.000
Cellulolytic 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

^aMeans within a row with different superscripts differ

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 previously reported by Tan *et al.*, 1996; Thalib *et al.*, 2000 which showed that rice straw had greater NDF, ADF, cellulose and hemicellulose and lower crude protein content compared to the other cereal straw. 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. Rumenal pH affects digestibility of feed stuffs. Fibrolytic bacteria are very sensitive and dependent on pH changes; the digestibility of organic matter, NDF and nitrogen decrease at pH 5.8 and increase at pH 6.2. Production of total VFA content of continuous culture system was shown to be highest between pH 6.2 and 6.6 in high concentrate diets (Shiver *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 substrate diets with yeast supplementation. The current results are consistent with results observed by Lynch and Martin (2002), where live cells of yeast decreased ruminal pH when alfalfa hay was *in vitro*-incubated. These differences in ruminal pH of Lynch and Martin (2002) 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 of the current study had a similar pH but lower concentration of N-NH₃. 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-NH₃ concentration. This is in agreement with Mao *et al.* (2013) who reported a N-NH₃ concentration of 8.0 mg per 100 ml in controls and 8.3-10.5 mg per 100 ml in yeast-supplemented treatments with rice straw based diet. Zain *et al.* (2011) found that yeast supplementation decreased N-NH₃ concentrations in ammoniated rice straw. Opsi *et al.* (2012) reported that yeast supplementation increased N-NH₃ in high forage diets but did not affect in high concentrate diets. It is likely that increases in N-NH₃ 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 the reports of 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 rice straw alone.

When yeast was supplemented at 5.0 g/kg, 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 ration 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 *in vitro* studies (Mao *et al.*, 2013; Newbold *et al.*, 1995; Zain *et al.*, 2011) and *in vivo* study in buffalo (Kumar *et al.*, 2013). However, Hristov *et al.* (2010) and Yoon and Stern (1996) showed that no significant effect of yeast supplementation on protozoan number was observed. Increased dry and organic matter, NDF, cellulose and hemicellulose degradability, as well as VFA production with different yeast supplementation level in the present study 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-NH₃ and VFA, but decreases the rumen pH. The current results also showed that supplementation with 5.0 g yeast/head/d 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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