Effect of yeast (*Saccharomyces cerevisiae*) on fermentability, microbial population and digestibility of low quality roughage *in vitro*

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SUMMARY

The aim of this experiment was to obtain the best level of supplementation of *Saccharomyces cerevisiae* in improving the digestibility and fermentability of ammoniated rice straw in the rumen. This experiment used completely randomized design, with 4 treatments and 4 replications. The experimental substrates composed of 50% ammoniated rice and 50% concentrate, and this substrate was used as a control substrate (A). The rice straw was previously treated with 4% urea. The crude protein of the substrate was 12%. *S. cerevisiae* was added in the diet at 0.25, 0.5 and 0.75% on dry matter in diet B, C and D respectively. In vitro fermentability and degradability of nutrients were determined following the first stage of the Tilley and Terry procedure (1969). Ruminal fluid was obtained from a cannulated steer. The parameters measured were dry matter digestibility, organic matter, cellulose, neutral detergent fiber (NDF), acid detergent fiber (ADF), pH, concentration of ammonia (NH_3), volatile fatty acid (VFA) and rumen microbial population. The results indicated that the addition of *S. cerevisiae* significantly increased the nutrient digestibility, fermentability rations and rumen microbial population. Digestibility of dry matter increased from 53% to 61%, 65% and 66% and rumen microbial population increased from 0.29 x 10^9 cfu/ml rumen fluid to 4.99 x 10^9, 5.12 x 10^9 and 6.01 x 10^9 cfu/ml rumen fluid, respectively, in treatments A, B, C and D. Supplementation of 0.5 and 0.75 % *S. cerresiveae* did not significantly affect digestibility. It can be concluded that the best supplementation of *S. cerevisiae* is 0.5% in dry matter substrate.

Keywords: ammoniated rice straw, digestibility, fermentability, *Saccharomyces cerevisiae*

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INTRODUCTION

Agricultural by-products such as cereal straws are carbohydrate-rich residues representing a large potential source of dietary energy for ruminants. Rice straw is the predominant dry season feed for cattle, despite its low nutritive value. It is deficient in readily fermentable energy, nitrogen, minerals and vitamins, and cannot provide for optimum microbial growth in the rumen. The high fiber content prevents the access of ruminal hydrolytic enzymes to cellulose and hemicellulose (Tan et al., 1995).

During recent years, yeast culture has been used to improve the nutritive value and utilization efficiency of low-quality roughages (Tang et al, 2008). Yeast culture supplementation in ruminant diets can increase dry matter intake (DMI), production performance, cellulose degradation, and nutrient digestibility (Lesmeister et al., 2004).

Sacharomyces cerevisiae has been extensively used as a dietary supplement in ruminants. The benefits associated with *S. cerevisiae* include increased dry matter and neutral detergent fiber (NDF) ingestion, increased initial rate of fiber digestion (Williams et al., 1991), improved in situ crude protein (CP) and NDF degradation and microbial efficiency (Olson et al., 1994), and increased milk production (Fadel, 2007; Haddad and Goussous, 2005). In vitro studies have also shown that yeast culture favorably modified the mixed ruminal microorganism fermentation and stimulated cellulose digestion by pure cultures of predominant ruminal bacteria (Lynch and Martin, 2002; Miller et al., 2002).

The current experiment was designed to investigate the effect of direct addition of *S. cerevisiae* at different doses on in vitro fermentation characteristics and digestibility of ammoniated rice straw because there are no data available for ammoniated rice straw.

MATERIAL AND METHODS

Ammoniated rice straw plus concentrate (1:1) mixture were used as substrates on a dry matter basis for *in vitro* incubation. The rice straw was previously treated with 4% urea. Ammoniated rice straw and concentrate mixture (rice bran, coconut cake (*Cocos nucifera*), cassava leaves (*Manihot esculenta*), NaCl and mineral sulfur and phosphorus), were ground in mills to pass a 1 mm sieve prior to chemical analysis and *in vitro* fermentation. The chemical composition of ammoniated rice straw and concentrate mixture are 12 % CP and 62% total digestible nutrient (TDN). Four levels of *S. cerevisiae* (0 % (A), 0.25% (B), 0.5% (C), and 0.75% (D) on dry matter basis) were added to the substrates, respectively. Live *S. cerevisiae* cells (Collection of Biotechnology Laboratory of Gajah Mada University, Jokyakarta Indonesia, strain Meyen ex

Hansen) was used as a probiotics, and contained 4 x 10^8 live organisms/g, plus the carrier (medium) on which it was grown. The carrier contained 25% CP, 15% crude fiber, 6% crude fat, and 7% ash (DM basis). A completely randomized design was used with 4 treatments and 4 replicates.

In vitro fermentability and degradability of nutrients were determined using the first stage of the Tilley and Terry procedure (Tilley and Terry, 1969). Ruminal fluid was obtained from a cannulated steer. Fermentation tubes contained of 10 ml of ruminal fluid and 40 ml of McDougall buffer solution and 0.5 g samples were incubated in 100 ml polyethylene tubes at 39^{o}C in a shaken water bath for 48 h. Treatments were replicated four times within an experiment and the experiment was repeated twice. Two fermentation tubes that did not contain diets were also incubated as blanks. Fermentation was terminated after 48 h incubation by injecting the tubes with 1 ml of $HgCl_2$. Tubes were then centrifuged at 14000 x g for 15 min and the supernatant was collected and stored. Tubes with residue were dried at 60^{o}C for 48 h and weighed and the data were used for degradability determination. These residues were also analyzed for their dry matter (DM), organic matter (OM) and nitrogen content by using standardize procedures (AOAC, 1990), NDF, acid detergent fiber (ADF), and cellulose of residues were determined according to Goering and Van Soest (1970). Supernatants were used in order to determine NH_3 concentration (microdiffusion Conway method), total volatile fatty acids (VFA) concentration (Gas chromatography) and rumen fluid pH. Total bacterial populations were determined in the methods described by Ogimoto and Imai (1981) using selective media for growth the rumen bacteria only.

Data were analyzed by ANOVA using the general linear model (GLM) procedure. Differences between the control treatment and *S. cerevisiae* supplementation treatment were analyzed by Duncan Multiple Range Test (DMRT) (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Table 1 shows results of *S. cerevisiae* supplementation effects on bacterial population and other variables of rumen fermentability and degradability nutrient of ammoniated rice straw. Effects of treatments were significant (P<0.05) for rumen pH, concentration of ammonia, total VFA concentrations, rumen bacterial population and degradability variables.

The rumen pH with supplementation of *S. cerevisiae* was relatively more stable and met the needs of rumen microbes to perform its activity compared to the control. Relatively stable rumen pH with supplementation *S. cerevisiae* could happen due to low concentrations of lactic acid in the rumen because *S.*

cerevisiae can stimulate the growth of *lactic acid bacteria* users. This is in agreement with research conducted by Erasmus (1992) and El Ghani (2004).

Total VFA production showed that the highly significant effect of *S. cerevisiae* supplementation (*P* <0.01). Total VFA concentration increased with the supplementation. It is also reported by El-Waziry *et al*. (2000) and El-Ghani (2004) where the total VFA production increased in the ration supplemented with *S. cerevisiae.* Increased VFA production is associated with high activities of microbes in the rumen because *S. cerevisiae* produces growth factors for microbial growth in the form of an organic acid, vitamin B and amino acids that can stimulate rumen microbial growth and activity. The highest VFA obtained on the addition of *S. cerevisiae* by 0.5% of ration dry matter on treatment C.

Table 1 Effect of *Sacharomyces cerevisiae* supplementation on total bacterial population and fermentation in the rumen and in vitro degradability of ammoniated rice straw (%)

Variables	*S. cerevisiae* supplementation (%DM)			
	0	0.25	0.50	0.75
pH	6.46^{b}	6.68^{a}	6.93^{a}	6.70^{a}
Total bacterial population (x 10^{10} cfu/ml)	0.29^{b}	4.99^{a}	5.12^{a}	6.01^{a}
N-NH_3 (mM)	12.31^{b}	10.48^{a}	10.31^{a}	10.21^{a}
Total VFA (mM)	135^{b}	140^{b}	157.50^{a}	160.50^{a}
Dry matter degradability (%)	52.5^{c}	61.0^{b}	64.7^{a}	65.5^{a}
Organic matter degradability (%)	54.1^{b}	63.2^{a}	63.0^{a}	64.2^{a}
NDF degradability (%)	32.7^{c}	38.4^{b}	43.5^{a}	45.8^{a}
ADF degradability (%)	30.9^{c}	37.5^{b}	40.9^{a}	41.4^{a}
Cellulose degradability (%)	34.1^{b}	36.2^{b}	40.3^{a}	42.9^{a}

a, b, c: means within rows with different superscript are significantly different (P<0.05)

NH_3 concentration of rumen fluid obtained in this study was significantly affected by treatment (P <0.01). NH3 produced decreased from 12.31 to 10.12 mM due to *S. cerevisiae* supplementation. A decrease in rumen ammonia production due to an increase incorporation of ammonia into the protein microbial occurred when the microbial populations were also increasing. It is also founded by El-Waziry *et al*. (2000) and El-Ghani (2004). While ammonia is the main compound for the synthesis of microbes in the rumen.

Rumen microbial population of the control diet from this study was significantly lower than *S. cerevisiae* supplementation treatment. Supplementation of *S. cerevisiae* was able to increase rumen microbial population. But increased levels of *S. cerevisiae* from 0.25% to 0.75% in dry matter ration did not significantly affect rumen microbial population. Increased bacterial numbers in the rumen have been one of the most consistently reported effects in animals fed another yeast culture (Harrison *et*

al., 1988). It has been suggested that increased bacterial flora in animals fed *S. cerevisiae* is central to the action of yeast in the rumen, and increased bacterial population leads to an increase in both the degradation of fiber in the rumen and the flow of microbial protein from the rumen (Wallace and Newbold, 1992). Besides that, *S. cerevisiae* also produces growth factors that are essential for rumen microbial growth (Chiquette, 2009).

Addition of *S. cerevisiae* at different levels significantly affected nutrient digestibility ($P<0.05$). The increased digestibility of dry matter and organic materials with the addition of *S. cerevisiae* caused by increasing rumen microbial population. Increased digestibility also increased the production of VFA in the rumen. Increased dry matter digestibility and organic matter was also reported by Fadel (2007) and Paryad and Rashidi (2009), who states that the nutrient digestibility of goat and sheep rations supplemented with yeast was significantly increased compared to controls. Average dry matter digestibility ranged from 52.5% to 65.5%. While the average organic matter digestibility ranged from 54.1% to 64.2%.

Digestibility NDF, ADF and cellulose obtained in this study was also significantly affected by *S. cerevisiae* supplementation. NDF, ADF and cellulose were fiber from the carbohydrate fraction of potential as a source of energy for ruminants. Increased digestibility of NDF, ADF and cellulose with supplementation *S. cerevisiae* caused by increased population especially rumen cellulolytic microbes (Callaway and Martin, 1997). The relatively stable rumen pH for growth of rumen microbes, especially cellulolytic bacteria causing growth to be better so the fiber fraction digestibility also increased. It is also reported by several researchers such as Miller *et al*., (2002) and Fadel (2007).

The results obtained showed that supplementation of *S. cerevisiae* gave the best in improving the digestibility and fermentability of rations based on ammoniated rice straw at the level 0.5% of dry matter ration. Increased level up to 0.75% was no longer affected digestibility and feed fermentability significantly. This showed that *S. cerevisiae* have optimum levels of 0.5% of diet dry matter.

CONCLUSIONS

Supplementation of *Sacharomyces cerevisiae* to substrates containing ammoniated rice straw and concentrate influenced rumen fermentability and nutrient degradability. The effects occurred through reduction in ammonia concentration, increased in total bacterial population, total VFA concentration, and degradability of dry matter, organic matter, and fibrous fractions. The best level of *S. cerevisiae* supplementation is obtained at a 0.5% of diet dry matter.

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