



The Use of Various Substrate Microbial Bamboo Stems for Rice Straw Fermentation and its effect on digestibility and fermentation product of Beef Cattle Ration

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HIGHLIGHT

- Bamboo stems contain mixed culture microbial, viz. lactic acid bacteria, *Bacillus*, *Endomycetes lactic* and *Staphylococcus*.
- Fermentation can improve the quality and make rice straw as probiotics carrier
- Fermented rice straw in beef cattle ration can influence digestibility and products fermentation.

ABSTRACT

This experiment investigated various substrate growth of bamboo stems microbes (BSM) in rice straw fermentation and its effect on dry matter digestibility (DMD), organic matter digestibility (OMD), NH₃-N and VFA concentration. Materials were rumen fluid of beef cattle from the slaughterhouse. Basal diets consisted of rice straw and concentrate with ratio of 40 : 60 (% dry matter) were administered to five treatments namely P0 = untreated rice straw (control), P1 = Ammoniated rice straw, fermented rice straw using microbial bamboo stems grown on ammoniated rice straw (P2), rice bran (P3) and cassava waste (P4). The experiment used in vitro method was designed with Completely Randomized Design and the data were analyzed variance. The results showed that the treatment had no significant effect on dry matter digestibility (DMD), organic matter digestibility (OMD), NH₃-N and VFA concentration (P>0,05). The fermentation process of microbial bamboo stems in rice straw with a level of 10% with different substrate did not able to increase the digestibility and products fermentation, but there tends that the rice bran and substrates can increase DMD and N-NH₃ concentration.

Keyword

Rice-straw; fermentation; microbial-bamboo; beef-cattle

1. Introduction

Indonesia is the third largest rice-producing country and rice straw after China and India (Sarnklong et al., 2010), and rice straw became the main feed of beef cattle. According to data Badan Pusat Statistik (2011) it is estimated that there are 100.965 million tons of straw production per year. Rice straw is mainly composed of polysaccharides such as cellulose (40%), hemicellulose (35%), lignin (10%), and silica (5%) (Kogo et al., 2017). The obstacles to utilizing rice straw as feed include the high

level of lignification and silicification, the slow and limited ruminal degradation of the carbohydrates, and the low content of nitrogen (Van Soest, 2006). This resulted in , rice straw is poorly fermented, bearing low rates of disappearance in the rumen and low rates of passage through the rumen, reducing feed intake (Sarnklong et al., 2010). Therefore, processing technology is needed to improve the digestibility of rice straw and fermentation using natural inoculums from bamboo stems as an alternative solution.

Various microbes in bamboo stems include *Lactobacillus*., *Streptococcus*.,

Lactococcus variants, *Bacillus cereus* and *Staphylococcus* and yeast *Endomyces lactic* (Pato, 2003). Fermentation required inoculum as a substrate for microbial growth prior to fermentation process.

Agro-industrial waste such as rice straw, rice bran, and cassava waste can be used as a substrate for microbial growth in the inoculum because it comprises of carbohydrates that become energy for microbes. Microbes need nutrients for their growth when grown on different substrates because they will secrete different hydrolytic enzymes. This research aims to evaluate microbial bamboo stems grown on different substrates as inoculums in fermented rice straw production and its effect on the digestibility and fermentation products in beef cattle ration

2. Research methods

This research was carried out at Feed and Nutrition Laboratory, Faculty of the Animal Science University of Jenderal Soedirman.

Research used in vitro method (Tilley and Terry, 1963) for variables, i.e. dry matter and organic matter digestibility (DMD and OMD) measured in AOAC method (1999), NH₃-N concentration with Conway micro-diffusion technique (Davis and Smith, 1958) and total volatile fatty acids (VFAs) product with steam distillation method (AOAC, 1999).

Experimental materials. Rice straws were collected from rice-field, air-dried to reach 10% dry matter (DM), and chopped to a theoretical length of 2 to 3 cm. Rumen fluids were obtained from the slaughterhouse as soon as the cow was slaughtered.

Production of bamboo stems inoculum. A total 100 g of each ammoniated rice straw mash, rice bran, and cassava waste was added with 1 g urea, one gram sugar, 5 g BSM, and water until 60% moisture was reached. After sterilization, the inoculums were incubated for seven days.

Treatments. Bamboo stem inoculum was used 10% as much as DM rice straw. The ration was prepared using concentrate and rice straw with a ratio of 60: 40 (% DM) for five treatments as follow:

P0 = 60% concentrate + 40% untreated rice straw. P1= 60% concentrate + 40% ammoniated rice straw, P2 = 60% concentrate + 40% fermented rice straw produce by ammoniated rice straw inoculums, P3 = 60% concentrate + 40% fermented rice straw produce by rice brain inoculums, P4 = 60% concentrate + 40% fermented rice straw produce by cassava waste inoculums.

Statistical Analysis. The data were analyzed in a completely randomized design using ANOVA with five treatments and five replicated. The results were presented as the mean values and standard error of the means.

3. Result and Discussion

3.1. Dry Matter and Organic Matter Digestibility (DMD and OMD)

The highest dry matter digestibility (DMD) and organic matter digestibility (OMD) values were found on P1 (ammoniated rice straw inoculums), namely $33,87 \pm 0,72\%$ dan $40,27 \pm 0,72\%$, while the lowest was P3 (fermented rice straw with rice bran inoculums) $29,51 \pm 1,95\%$ and $32,61 \pm 2,23\%$ (Table 1 and 2). Analysis of variances informed that the use of inoculum from bamboo stems microbes grown on various substrates for rice straw fermentation had no significant effect ($P > 0.05$) on DMD and KBO.

Various types of bacteria and yeast reported to live in the stem of bamboo include *Lactobacillus*., *Streptococcus*., *Lactococcus* variants, *Bacillus cereus*, *Staphylococcus* and yeast *Endomyces lactic* (Pato, 2003). *Lactobacillus*, *Streptococcus*, and *Lactococcus* are lactic acid bacteria (LAB) which play an important role in various food fermentations. LABs are protease and amylase producing bacteria; they have no cellulose or lignin degrading enzyme. While *Staphylococci* is a facultative anaerobe, it mainly produces lactic acid in the fermentation process (Plata, 2009). Pogoku et al., (2010) reported a bacterial strain isolated from an oil-contaminated soil identified as *Staphylococcus* sp. Lp12 and it was secreted lipase. *Endomyces* sp. IFO 0111 yeast is known to produce an extracellular amylase which was proved to production of glucose from starch. This yeast used hydrolysis of starch and *Rhizopus* sp. (Yamamoto et al., 1964). Brimer et al., (1998) reported that another *Endomyces* namely *E. fibuliger* LU677 produced an extracellular beta-glycosidase activity when grown in malt extract broth. *Bacillus cereus* is a group of ubiquitous facultative anaerobic spore-forming gram-positive rods commonly found in soil (Tallent, 2012). *Bacillus* sp. is known as a producer of various types of hydrolytic enzymes, but it is unknown whether the bacteria produce degrading lignin enzymes.

Accordingly, fermentation of rice straw using microbial inoculum bamboo stem is evident to produce no significant effect on DMD and OMD due to the presence of cellulose and lignin-degrading microbial in bamboo stems, i.e. hydrolyzed lignocellulose in rice straw. MacDonald et al., (1991) informed the growth of LAB via fermentation requires adequate WSC supplement, and the addition of homofermentative LAB in silages did not effectively promote the fermentation process. Meanwhile, Li et al., (2016) reported that inoculation with hetero and homofermentative LAB had no effects on the DM and ADF content of rice straw silages

Similarly, Amin (2013) on probiotic *Bacillus* for fermenting rice straw reported 33,22% DMD. However, Suryapratama (2012) reported that 2% *Saccharomyces cerevisiae* in the basal ration contained fermented rice straw using

Phanerochaete chrysosporium produced 49,83±1,80% DMD and 52,69 ± 1,69 % OMD. *Phanerochaete chrysosporium* is white-rot fungi that can break down lignin, but it has a low ability to degrade cellulose and hemicellulose so that both cellulose and hemicellulose can be used by rumen microbes and subsequently by the host animal. White-rot fungi, as a wood-decaying basidiomycete and lignocellulolytic microorganism, can decompose and metabolize

Table 1. Dry matter and Organic matter digestibility of beef cattle ration containing untreated ammoniated and fermented rice straw.

Ration	DMD (%)	OMD (%)
60% concentrate + 40% untreated rice straw (P0)	31.40 ± 1.22	36,50 ± 1.31
60% concentrate + 40% ammoniated rice straw/ars (P1)	33,87 ± 0,72	40,27± 0.72
60% concentrate + 40% fermented rice straw (ars inoculums/P2)	30.34 ± 1.92	33,75±1.95
60% concentrate + 40% fermented rice straw (rice bran inoculums/P3)	29,51 ± 1,95	32,61 ± 2.23
60% concentrate + 40% fermented rice straw (cassava waste inoculums/P4)	32.91 ± 1.24	35,91 ± 1.38

all plant cell constituents (cellulose, hemicellulose, and lignin) by their enzymes (Eriksson et al., 1990). Karunanandaa et al. (1995) reported that 30-day incubation of rice straw with three white-rot fungi resulted in an enhanced IVDMD by *Pleurotus sajor-caju* (Table 1)

Rice straw mostly contains cell walls comprised of cellulose, hemicellulose, silica, and lignin. It needs several enzymes to hydrolyze these components, viz. cellulase, hemicellulase and ligninase (Sarnklong et al., 2010). Lignin cannot be broken down in the rumen due to the lack of ligninase. Lignin located between the cellulose microfibrils is regarded as the most abundant natural aromatic organic polymer (Iiyama et al., 1990). Silica reduces palatability and degradability of rice straw in the rumen due to its direct action in preventing colonization by ruminal microorganisms (Agbagla-Dohnani et al., 2003).

P1 (ammoniated rice straw) obtained the highest DMD and OMD values when compared to other treatments (Table 1). Treatment P1 used ammoniated rice straw that contained 4% urea which effectively broke the lignin-cellulose bond and improved the digestibility. Increasing the digestibility of rice straw using urea can stretch the ester bonds between lignin and hemicellulose

as well as polysaccharide bonds, thereby allowing rumen or microbial enzymes to ferment rice straw (Bata, 2008).

3.2. NH₃-N concentration and VFA production

NH₃-N and VFA are the main products of microbial fermentation in the rumen derived from feed protein and carbohydrates. VFA is the main energy source for ruminants, and for rumen microbes growth and activity, while NH₃-N is for microbial protein synthesis. Sutardi (1977) stated that microbial growth was supported by approximately 4-12 mM NH₃-N and 80-160 mM VFA. Van Soest (2006) claimed that the growth of rumen microbe declined when N-NH₃ was below 3.57 mM.

Analysis of variance showed that various substrates in a bamboo stem microbial inoculum for rice straw fermentation had no significant effect ($P < 0.05$) on NH₃-N and VFA despite the ideal amount to support microbial growth and activity (Table 2).

However, fermented rice straw (P2, P3, and P4) may produce higher NH₃-N and VFA concentrations than untreated (P0) and ammoniated (P1) rice straw (Table 2). Fermented rice straw was suspected to increase microbial population and fermentative process in rumen so it increases fermentation products despite the statistical non-significant effect on N-NH₃ and VFA. Seo et al. (2010) informed that microorganisms in direct-fed microbes for ruminants may be classified as lactic acid producing bacteria (LAB), lactic acid utilizing bacteria (LUB), or other microorganisms including species of *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, *Bacillus* and *Propionibacterium*, strains of *Megasphaera elsdenii* and *Prevotella bryantii* and yeast products containing *Saccharomyces* and

Aspergillus. LAB such as lactobacilli and enterococci might prevent ruminal acidosis in dairy cows (Weiss et al., 2008) by facilitating the growth of ruminal microorganisms adapted to the presence of lactic acid in the rumen (Raeth-Knight, 2007) and by stimulating lactic acid utilizing bacteria. Lynch et al. (2002) suggested that yeasts remove oxygen in the rumen and maintain the anaerobic rumen environment. Fermented rice straw that contained microbial bamboo stems in ration may improve ruminal degradation which subsequently increases N-

Tabel 2. NH₃-N and VFA concentrations of beef cattle ration containing untreated ammoniated and fermented rice straw.

Ration	NH ₃ -N (mM)	VFA (mM)
60% concentrate + 40% untreated rice straw (P0)	6.64 ± 0.86	152 ± 13.04
60% concentrate + 40% ammoniated rice straw/ars (P1)	7.44 ± 0.38	166 ± 8.94
60% concentrate + 40% fermented rice straw (ars inoculums/P2)	8.16 ± 0.48	196 ± 16.73
60% concentrate + 40% fermented rice straw (rice bran inoculums/P3)	9.40 ± 0.55	203 ± 18.54
60% concentrate + 40% fermented rice straw (cassava waste inoculums/P4)	10.80 ± 0.84	182 ± 14.83



NH₃ and VFA concentrations.

4. Conclusion

Ammoniated rice straw, rice bran, and cassava waste can be used as a substrate in BSM inoculums for rice straw fermentation.

The use of 10% of cassava waste in BSM inoculums for rice straw fermentation tends to increase dry matter digestibility and NH₃-N of beef cattle ration

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