

## Improving Sugarcane Bagasse as Animal Feed by Ammoniation and Followed by Fermentation with *Trichoderma harzianum* (In Vitro Study)

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**Abstract.** Sugarcane bagasse is one of agro-industrial residues containing low nutrient content and difficult to be digested by animals. However, it can be recycled to produce value-added product such as protein-enriched animal feed by application feed technology such as ammoniation and fermentation. The purposes of these experiments were to evaluate the quality and in vitro digestibility of sugarcane bagasse by using two steps feed technology process: ammoniation and continued by fermentation process. Two studies were conducted in these experiments. The first study was to determine the quality of ammoniated sugarcane bagasse after incubation at room temperature at different days (0, 7, 14, 21, 28 days) and the second study was to determine in vitro digestibility of ammoniated sugarcane bagasse fermented by using the various levels of sago flour (0%, 5%, 10% and 15% from sample total) and kept at room temperature for 21 day in an-aerobic condition. Parameters measured in this study were DM, CP, CF, pH, OM, IVDMD (in vitro dry matter digestibility), IVOMD (in vitro organic matter digestibility). The model used for the statistical analysis was completely randomized design (CRD). The results of the first study indicated that length of incubation was not significantly affect ( $P>0.05$ ) on crude fiber content but had significant effect ( $P<0.05$ ) on crude protein content but only for control treatment. For the second study concluded that administration of the various levels of sago flour into ammoniated sugarcane bagasse fermented with *T.harzianum* significantly influenced ( $P<0.05$ ) on OM, IVDMD and IVOMD but had not significantly effect on pH value. The results of the study indicated that application 10% of soluble carbohydrate from sago flour was recommended for fermentation process based on the results of in vitro and fermented feed quality studies.

**Key Words:** ammoniation, fermentation, sugarcane bagasse, quality feed, in vitro

**Abstrak.** Ampas tebu adalah salah satu hasil produksi limbah industri pertanian dengan kandungan nutrisi dan daya cerna yang rendah. Tujuan dari penelitian ini adalah untuk mengevaluasi kualitas dan daya cerna secara *in vitro* ampas tebu dengan menerapkan teknologi pakan: amoniasi yang dilanjutkan dengan proses fermentasi. Dua percobaan dilakukan dalam penelitian ini. Percobaan pertama adalah menentukan kualitas dari ampas tebu yang telah diamoniasi dan difermentasi dengan lama pemeraman yang berbeda (0,7,14,21 dan 28 hari). Sementara penelitian kedua adalah penentuan daya cerna *in vitro* dari ampas tebu yang telah diamoniasi dan difermentasi selama 21 hari secara *an-aerob* dengan level karbohidrat terlarut (tepung sago) yang berbeda (0%, 5%, 10% dan 15% dari total sampel). Parameter yang diukur dalam penelitian ini adalah kandungan bahan kering, protein kasar, serat kasar, pH, pencernaan bahan kering dan organik secara *in vitro*. Rancangan yang digunakan dalam penelitian ini adalah rancangan acak lengkap (RAL). Hasil dari penelitian pertama menunjukkan bahwa lama inkubasi tidak berpengaruh ( $P>0,05$ ) terhadap kandungan serat kasar tetapi berpengaruh nyata ( $P<0,05$ ) terhadap kandungan protein kasar namun hanya pada protein kasar kontrol. Hasil penelitian kedua dapat disimpulkan bahwa penambahan berbagai level tepung sago sebagai karbohidrat terlarut pada ampas sago yang diamoniasi dan difermentasi dengan *T.harzianum* berpengaruh nyata ( $P<0,05$ ) terhadap bahan organik, daya cerna bahan kering dan daya cerna organik secara *in vitro*, tetapi tidak berpengaruh terhadap nilai pH. Hasil penelitian menunjukkan bahwa penambahan karbohidrat terlarut tepung sago sebanyak 10% direkomendasikan pada proses fermentasi berdasarkan hasil positif pada penelitian *in vitro* dan juga pada kualitas bahan pakan yang diteliti.

**Kata kunci:** amoniasi, fermentasi, ampas tebu, kualitas pakan, in vitro

### Introduction

Shortage of forages availability both quality and quantity was the main problem to improve ruminant animals productivity in Indonesia.

One of strategies to solve the problem was to utilize agricultural and agro-industrial by products as animal feed. However, agricultural and agro-industrial by product feed contained

high percentage of fiber and difficult to be digested by animals (Suwandystuti and Rimbawanto, 2011). Researchs conducted by scientiests indicated that pre-treatment of high fiber feed were able to improve nutrient content and digestibility of feed (Bisaria et al., 1997; Tang et al., 2008; Wanapat et al., 2009 ; Rahman et al., 2011 ; Shrivastava et al., 2011 ; Wajizah et al., 2015 ; Samadi et al., 2015).

One of agro-industrial residues is sugarcane bagasse containing about 50% cellulose, 25% hemicellulose and 25% lignin (Pandey et al., 2000). Sugercane bagasse can be recycled to produce value-added product such as protein-enriched animal feed, enzymes, amino acids, organic acids and compounds of pharmaceutical importance (Pandey et al., 2000). Hartadi et al. (1990) informed that sugarcane bagasse contained low protein content with amount of 3,1%. Application of sugarcane bagasse as animal feed has been reported by Okana et al. (2006), in which bagasse cultured with four white-rot fungi (*Lentinula edodes*, *Pleurotus eryngii*, *Pleurotus salmoneostramineus*, *Ceriporiopsis subvermispota*) at 26°C for 8, 12 or 16 weeks influencing the in vitro organic matter digestibility (IVOMD) and in vitro neutral detergent fiber digestibility (IVNDFD) with 45.6 and 40.3% for untreated bagasse respectively and with 68.6 and 59.1%, 60.6 and 49.9% and 59.9 and 49.0% for untreated bagasse respectively. Permana et al. (2000) stated that application of sugarcane bagasse supplemented with soyabean meal or wheat bran as substrate of *Pleurotus sajor-caju*, *P. eryngii* and *Agrocybe aegerita* can influence lignin degradation, body production and improve in vitro digestibility.

Research conducted by Soejono (1988) indicated that untreated sugercane bagasse had very low digestibility with 16.8–22.2% due to its high level of lignin. Feed technology should be applied to improve the quality of sugarcane bagasse. Fermentation is one of the methods can be applied to increase animal feed quality besides chemical tretament such as

ammoniation. Marjuki (2013) stated that chemichal pre-treatment such as ammoniation before fermentation was able to optimize microorganism activities. *Trichoderma harzianum* (*T. harzianum*) has been used in fermentation process since it produces cellulotyc enzym that can break down crude fibers to be more available compounds. Research conducted by Mahmilia (2005) indicated that *T. harzianum* was able to reduce crude fiber of water hyacinth (*Eichhornia crassipes*) about 4.79% from 26.61% to 21.82% after fermentation. Based on the research results, it was concluded that feed technology such as ammoniation and fermentation to be a practical and promising alternative to increase nutritional value of agro residues by product into valuable animal feed. The purposes of these experiments were to evaluate the quality and in vitro digestibility of sugarcane bagasse by using two steps feed technology process: ammoniation and continued by fermentation process.

## Materials and Method

### Ammoniation and Fermentation Process of sugarcane bagasse process

Sugarcane bagasse utilized in this study was collected from local traders at campus area of Syiah Kuala University without determining variety and age of sugercane. Sugarcane bagasse was chopped (Restch SM 200 Rostfrei, Germany) and air-dry up to 10% DM. Then, chopped sugarcane bagasse was ammoniaed with 4% of urea ( $\text{CO}(\text{NH}_2)_2$ ) and solved in water with the comparation of water 1:1 from DM sugarcane bagasse and kept at an-aerobic condition for 21 days. The first study to determine the quality of amoniated sugarcane bagasse after incubation at room temperature at different day. The ammoniaed sugarcane bagasse was fermented by 10% of *T.harzianum* from total DM ammoniaed sugarcane bagasse. *T.harzianum* utilized in this study come from

plantation protection office in Banda Aceh and it has been cultured by using corn as medium. Before mixing *T.harzianum* with ammoniated sugarcane bagasse, it was dissolved with aquadest (1:6) and added 10% molases based on DM. Each 300 g of sample was added 10% of sago flour and kept at room temperature for 0, 7, 14, 21, 28 day in an-aerobic condition. For the second study, The ammoniated sugarcane bagasse was fermented by using the various levels of sago flour (0%, 5%, 10% and 15% from sample total) and kept at room temperature for 21 day in an-aerobic condition.

### Chemical Analysis

Chemical analysis was conducted at Animal Nutrition Laboratory of Bogor Institute Agriculture and Animal Nutrition Laboratory of Syiah Kuala University, Banda Aceh. The chemical content was analyzed for DM, CF, CP (AOAC, 1990). The method to measure in vitro analysis was based on Tilley dan Terry (1963) in which samples were incubated for 24 hours and continued for post rumen digestibility by adding of pepsin for the next 24 hours. Concentration of pH for in vitro analysis was determined according to Soejono (1996). Measurement of rumen pH was conducted at the end of every incubation period.

### Statistical analysis

Statistical analyses ( $P \leq 0.05$ ) was run with the program Statistical Software Package SPSS. The model used for the analysis was completely randomized design (CRD). The following is formula applied for statistical analysis:  $Y_{ij} = \mu + T_i + e_{ij}$ , where,  $Y_{ij}$  was an observation of the dependent variable  $ij$ ;  $\mu$  was the population mean for the variable;  $T_i$  was the effect of the fermentation, as a fixed effect, and  $e_{ij}$  was the random error associated with the observation  $ij$ . Differences between variables were compared by a one-way analysis of variance (ANOVA). Verification of variance homogeneity and identification of statistical significance was applied by Duncan multiple range test. Observations with ( $P \leq 0.05$ ) were considered to be statistical significant and trends were declared at  $P \leq 0.10$ .

## Results and Discussion

### Crude Fiber and Crude Protein

Based on results, incubation time of sugarcane bagasse was not significantly effect on ( $P > 0.05$ ) on ammoniated crude fiber fermented with *T. harzianum* (Figure 1).

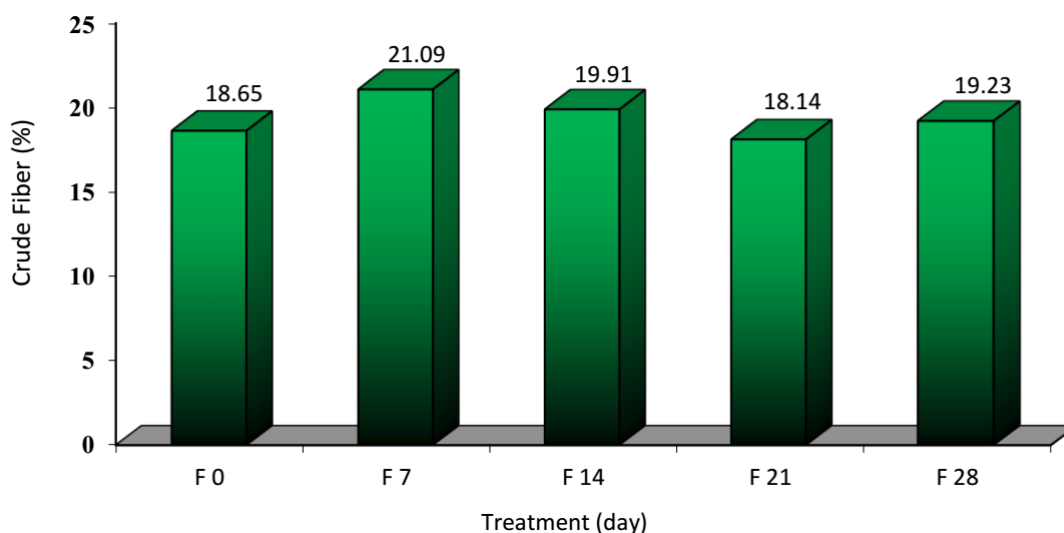


Figure 1. Crude fiber content of ammoniated sugarcane bagasse followed by fermentation with *T. harzianum* at different incubation time (F<sub>0</sub>= 0 d; F<sub>7</sub>=7 d; F<sub>14</sub>= 14 d; F<sub>21</sub>=21 d; F<sub>28</sub>=28 d) n=4.

There was no reduction of crude fiber content after 28 d incubation probably due to strong and very complex bonding system in crude fibers such as cellulose and lignocellulose.

In contrast with the experiments conducted by Nelson and Suparjo (2011) cacao pods fermented with *Panerochaeta chrysosporium* reduced crude fiber contents as the results of cellulolytic microorganism's activities. In our experiment, before fermentation process the bagasse was ammoniated with 4% of urea, it might be the cause of no reduction crude fiber in fermented bagasse in this study. Imsya (2005) stated that microorganism population reduced due to poisonous  $\text{NH}_3$  and it had negative effect on activities of cellulose enzymes. The high content of crude fiber after incubation period has been reported by Hilakore (2008) by using putak fermented with *A. niger* and *T. reesei* in which the growth of yeast contributed crude fiber content by improving mycelium yeast. In addition, reducing of water content at fermented substrates during fermentation process improved total dry matter content of fermented material (Krisnan, 2006).

In this study, incubation period significantly effect on crude protein content of ammoniated bagasse fermented with *T. harzianum* ( $P < 0.05$ ) as shown in Figure 2. But the effect was only at control treatment. The research conducted by Yang et al. (2001) corn straw fermented by solid state fermentation (SSF) improved the level of protein from 6.7% to 14.7% and decreased the cellulose by 38.0% and hemicellulose by 21.2%. In addition, urea contributed not only as crude protein improvement but also functioned as protein source for microorganisms to produce true protein. However, it was not measured the true protein content in this study.

In this study (Figure 2) the content of crude protein was higher compared to other

treatments. The high level of crude protein content in control was not true protein content instead of crude protein coming from non-protein nitrogen (NPN) added during ammoniation process before fermentation treatments. This ammonia was not released before fermentation treatments and it was suggested to dry-air longer to release urea before fermentation process. It was indicated from 7 and 28 d after fermentation, the level of crude protein significantly decreased ( $P < 0, 05$ ) compared to control. In accordance with the research conducted by Sujana (2014) ammoniated palm oil fronds fermented with EM4 decreased crude protein content by using urea more than 3%.

Pasaribu et al., (2001) also reported that reducing of crude protein content after 28 weeks incubation period, however after 12 weeks incubation protein content increased to 29.09%. It was probably due to degradation of carbohydrate and other organic materials and resulted in improving the concentration of protein content in the substrate.

The second study was the effect of ammoniated sugarcane bagasse followed by fermentation with *T. harzianum* at various levels of sago flour (0%, 5%, 10% and 15% from sample total) for 21 day in an-aerobic condition (in vitro study). The results of the second study is presented at Table 1. There was no significant effect ( $P > 0.05$ ) fermentation of sugarcane bagasse at various levels of sago flour on pH rumen liquor with the range of required rumen fermentation. Usman (2013) stated that crude fiber was effectively degraded at the pH of 6.8 – 7.2 and microorganisms activities will be reduced at the pH of 6.2. During fermentation process, there were two important factors namely pH and incubation temperature. pH optimal for crude fiber degradation was 6.8 to 7.0 with the temperature of 38-39°C (Tilley and Terry, 1963).

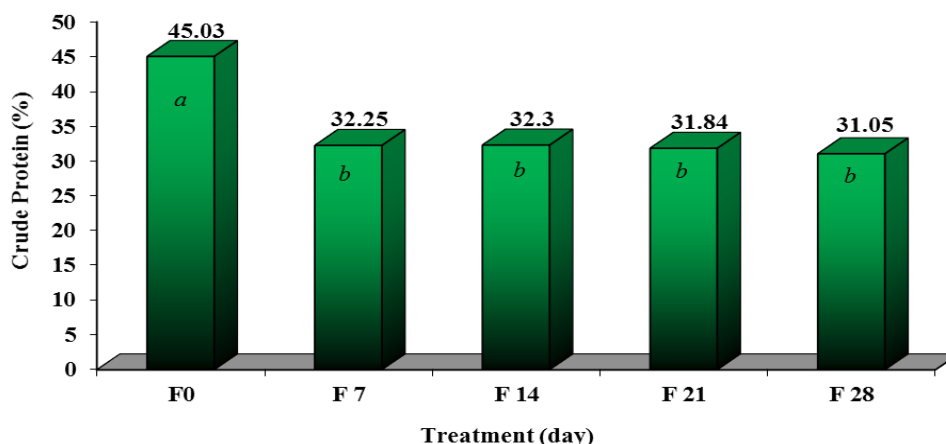


Figure 2. Crude protein content of ammoniated sugarcane bagasse followed by fermentation with *T. harzianum* at different incubation time (F<sub>0</sub>= 0 d; F<sub>7</sub>=7 d; F<sub>14</sub>= 14 d; F<sub>21</sub>=21 d; F<sub>28</sub>=28 d) n=4.

Table 1. pH, dry matter, organic matter, IVDMD and IVDOM of ammoniated sugarcane bagasse followed by fermentation with *T. harzianum* at various levels of sago flour (0%, 5%, 10% and 15% from sample total) for 21 day in an-aerobic condition (in vitro study, n=4).

Variables	Treatments			
	0% SF <sup>1)</sup>	5% SF <sup>1)</sup>	10% SF <sup>1)</sup>	15% SF <sup>1)</sup>
pH	7,088 ± 0,038	6,975 ± 0,025	7,000 ± 0,029	7,075 ± 0,048
Dry matter (%)	81,94 ± 0,243	82,59 ± 0,550	82,57 ± 0,456	83,35 ± 0,093
Organic matter (%)	97,97 <sup>b</sup> ± 0,054	98,03 <sup>b</sup> ± 0,039	98,05 <sup>b</sup> ± 0,032	98,22 <sup>a</sup> ± 0,056
IVDMD (%) <sup>2)</sup>	56,690 <sup>c</sup> ± 0,793	62,575 <sup>b</sup> ± 2,201	69,934 <sup>a</sup> ± 1,141	68,170 <sup>a</sup> ± 1,436
IVOMD (%) <sup>2)</sup>	57,66 <sup>c</sup> ± 0,788	63,93 <sup>b</sup> ± 2,392	71,00 <sup>a</sup> ± 1,086	68,79 <sup>a</sup> ± 1,380

<sup>1)</sup>SF= sago flour

<sup>2)</sup>IVDMD (in vitro dry matter digestibility) and IVOMD (in vitro organic matter digestibility)

Means within a row with different superscript letters differ significantly (p<0.05)

### The value of pH, dry and organic matters, IVDMD (in vitro dry matter digestibility) and IVOMD (in vitro organic matter digestibility)

In vitro study of ammoniated sugarcane bagasse followed by fermentation with *T. harzianum* at various levels of sago flour (0%, 5%, 10% and 15% from sample total) for 21 day in an-aerobic condition had no significant effect on (P>0,05) on dry matter of ammoniated and fermented sugarcane bagasse. However, IVDMD was significantly different by application of various levels of sago flour (Table 1 and Figure 3). Administration of various sago flour into fermentation media significantly improved (P<0,05) IVDMD of ammoniated and fermented sugarcane bagasse. The highest of IVDMD was at the application of 10% of sago flour at media

with 69.93%. But application of sago flour above 10% had the same results as 10% of sago flour at media fermentation.

Research conducted by Wajizah et al. (2015) application of soluble carbohydrates into fermentation media improved IVDMD. It was probably due to availability of nutrients in the in vitro media and resulted in optimum growth of rumen microbial. Harry (2007) stated that availability of energy during fermentation process could be used to improve microbial activities to degraded crude fiber. Increase of rumen bacteria population was strong correlated to fermentation process in using nutrient feed such as cellulose, hemicellulose and other soluble nutrients (Bata, 2008). In addition, Simanihuruk et al. (2008) stated that

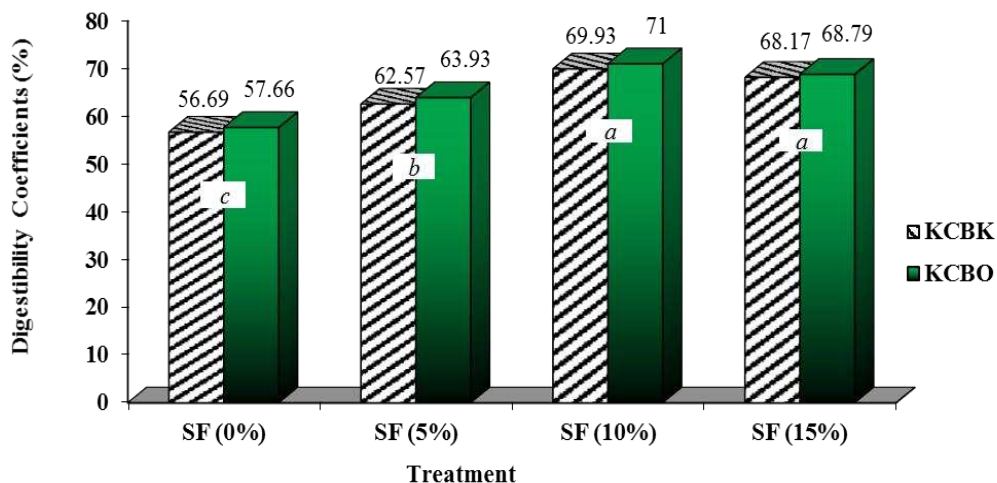


Figure 3. In Vitro Digestibility on various levels of sago flour (KCBK = IVDMD; KCBO = IVOMD)

good results of fermentation influenced by the availability of soluble carbohydrates. Treatments on high fiber contents such as sugarcane bagasse was very crucial to help degradation process since degradability of sugarcane bagasse was very low with 16.8 to 22.29% (Soejono, 1988). Chandrasari (2011) also stated that digestibility of untreated sugarcane bagasse was low with dry matter digestibility of 21.19% and organic matter digestibility of 20.92%.

In vitro study of ammoniated sugarcane bagasse followed by fermentation with *T. harzianum* at various levels of sago flour (0%, 5%, 10% and 15% from sample total) for 21 day in an-aerobic condition had significant effect ( $P < 0.05$ ) on organic matter of ammoniated and fermented sugarcane bagasse (Table 1 and Figure 4). The highest organic matter content was application of 10% soluble carbohydrate with the average value of 98.23%. Research concocted by Wajizah et al. (2015) also indicated that application of soluble carbohydrates significantly effect on organic matter due to crude protein improvement in the substrate. Luh (1991) reported that during fermentation process, yeast growth and microbial protein synthesis from metabolic process caused higher protein content in the substrate.

Administration of various levels of sago flour on ammoniated and fermented sugarcane bagasse significantly influenced ( $P < 0.05$ ) IVOMD at all levels of sago flour. The highest level of IVOMD was by adding 10% of sago flour in the fermented sugarcane bagasse and tended to reduce after adding of 15% of sago flour in the fermented sugarcane bagasse. Sabda (2015) reported that administration of more soluble carbohydrates for fermentation might increase crude fiber. Improvement of crude fiber was probably caused by high growth of yeast and it could contribute the high level crude fiber from mycelium (Martieje, 2008).

*T. harzianum* also produced amylase enzyme to degrade amylopectin compound from sago flour. Okada (1988) reported that *T. harzianum* was not only function for cellulolytic activities but for hemicellulolytic activity and amylase as well. Application of 15% sago flour tended to reduce organic matter digestibility, it was due to increase of amylolytic enzymes. In this study, application of 10% sago flour was the optimum for microorganism degradation both during feed processing and in vitro results. Before study by Sabda (2015) reducing of crude fiber was in accordance with increasing of IVDMD and IVODM at the level of 10% sago flour. It was probably that by adding 10% of sago flour will be balance between required energy and

nitrogen to support the growth of microorganism to degrade substrate. Microbe's activity during fermentation process was influenced by the nutrient availability of substrate and added nutrients in the fermentation media (Khuluq, 2012).

## Conclusions

The results of the first study indicated that length of incubation was not significantly affect ( $P>0.05$ ) on crude fiber content but had significant effect ( $P<0.05$ ) on crude protein content but only for control treatment. For the second study concluded that administration of the various levels of sago flour into ammoniated sugarcane baggase fermented with *T.harzianum* significantly influenced ( $P<0.05$ ) on OM, IVDMD and IVOMD but had not significantly effect on pH value. The results of the study indicated that application 10% of soluble carbohydrate from sago flour was recommended for fermentation process based on the results of in vitro and fermented feed quality studies.

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