In vitro digestibility assessment of banana stem silage (Musa paradisiaca) inoculated with EM-4 and different accelerators added as ruminant feed

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Abstract. Utilization of banana (Musa paradisiaca) stem as ruminant feed is limited by low crude protein and high crude fiber content. High water content of banana stems accelerates the decay process. Implementation of ensilage technology with the addition of inoculants and accelerators is intended to increase the nutritional value of the banana stem and its storability. The purpose of this study was to evaluate in vitro digestibility of banana stem silage inoculated with EM-4 and different accelerators addition as ruminant feed. This study was design as a completely randomized design consisted of 4 treatments, namely R0 (control without accelerator), addition of rice bran (R1), cassava (R2) and sago (R3) with 4 replications each. Parameters measured in this study were in vitro pH value, N-NH₃ concentration, dry matter (DM) and organic matter (OM) digestibility. The result showed that cassava added silage produced the lowest (P<0.01) pH value compared to other treatments and increased (P<0.01) in vitro N-NH₃ concentration, DM and OM digestibility compared to control. It was concluded that addition of cassava as accelerators improved the fermentation quality of banana stem, as shown by the lowest pH value and the highest in vitro N-NH₃ concentration, DM and OM digestibility.

1. Introduction
Banana (Musa paradisiaca) stems are by-product of banana plantations which production continues to increase with banana production each year. Total production of banana stems in fresh weight reaches 100 times the production of banana fruit. This large biomass has the potential as energy source feedstuffs, especially for ruminants [1]. On the other hand, utilization of banana stems as component of ruminant ration has limitations because of high crude fiber and low protein content, resulting in low digestibility. High water content of banana stems accelerates the decay process. Because of these reasons, utilization of banana stems as ruminant feed needs further processing to improve its storage capacity and nutritional quality [2], one of them with ensilage.

The ensilage process can be optimized by addition of accelerators into the substrate. Accelerators such as either an inoculant or soluble carbohydrate can also act as a preservative to facilitate the ensilage process and accelerate the pH decrease [3]. However, application of inoculants together with soluble carbohydrate and complex carbohydrates at the same time is rarely reported. The purpose of
this study was to evaluate in vitro digestibility of banana stem silage inoculated with EM-4 and different accelerators added in the form of complex carbohydrates as ruminant feed.

2. Material and methods

2.1. Material
The materials used in this study were banana stems derived from one-year old banana trees, accelerators (sago, cassava, and rice bran), Effective Microorganisms (EM-4), molasses and urea.

2.2. Methods

2.2.1. Sample preparation and silage production. Banana stems chopped 3 to 5 cm in size were sun-dried to decrease water content to around 11%. The substrate prepared were added with 7% (fed basis) accelerator according to the four treatments as follows: R1 (control without accelerator), R2 (rice bran), R3 (cassava) and R4 (sago), 5% molasses and 0.1% urea dissolved in water. Water was added into the substrate until the moisture content reaches 40%. Each treatment had 4 replications to obtain 16 experimental units. Ensiling was conducted in the silos of double plastic bags at room temperatures for 21 days and protected from sunlight. At the end of fermentation, the silos were opened and the samples were oven-dried at temperature 60°C for 48 days, finely ground and analyzed to determine of dry matter (DM) and organic matter (OM) contents.

2.2.2. In vitro fermentation. In vitro digestibility trial was conducted based on modification of Tilley & Terry method [4]. Fresh rumen fluid of fistulated beef cattle was filtered with four layers cheese cloth. The 0.5 g sample was added into the 100 ml incubation tube containing 50 mL mixed solution consisting of 10 mL rumen fluid and 40 mL McDougall buffer, resazurin and reduction solution. The tubes were closed before incubation, CO₂ gas was purged for 30 sec and incubated at 39°C. After incubation for 24 hours, the pH was measured and subsequently 2 to 3 drops of H₂SO₄ 9M was added into the cultures. Furthermore, the incubation tubes were centrifuged at 3578 g for 10 min. Supernatant was used for determination of N-NH₃ concentration by Conway micro diffusion technique, then the residue was added 50 mL pepsin-HCl 0.2% solution and incubated for 48 h. The residue was filtered by Whatman no 41, dried at 60°C for 48 h for determination of in vitro digestibility of DM and OM.

2.2.3. Statistical analysis. The data obtained were analyzed using analysis of variance (ANOVA) and followed by Duncan Multiple Range Test (DMRT) for the significant results [5].

3. Results and discussion

3.1. Rumen fermentation characteristics
The addition of different accelerators in banana stem silage significantly affect (P <0.01) the pH value of rumen fluid in vitro (Table 1). The dynamics of different pH values describe different levels of hydrolysis. Decreasing pH values in silage with cassava indicated a high fraction of soluble carbohydrates (non-structural carbohydrates) in cassava was used by microorganisms to support optimal fermentation which produce a number of organic acids. Whereas in silage with sago added, high pH values indicated the low fermentation activities, resulting low organic acids production due to high lignocellulose content in sago which inhibited the energy availability for microorganisms to develop and ferment the substrate. Availability of non-structural soluble carbohydrates is needed to stimulate the development of rumen microbes at the beginning of the fermentation, and highly affect the effectiveness of the fermentation in the reticulo-rumen system [6].
Table 1. Fermentation characteristics of banana stem silage

| Parameter       | Control       | Rice bran     | Cassava       | Sago          |
|-----------------|---------------|---------------|---------------|---------------|
| pH              | 6.893±0.004   | 6.876±0.004   | 6.809±0.010   | 6.924±0.001   |
| NH₃ (mM)        | 6.424±0.110   | 7.342±0.098   | 9.653±0.104   | 5.942±0.114   |

Note: Means in the same row with different superscript differ highly significant (P<0.01).

Protein degradation can determine N-NH₃ concentration in the rumen. The energy availability for rumen microbes, suitable rumen pH for proteolytic microbial activity, the availability of readily soluble energy and the energy produced from fiber degradation affect N-NH₃ concentration [7]. Protein deamination process occurs faster in the media rich in monosaccharides and disaccharides compared with polysaccharides [6], showed by increased (P<0.01) of N-NH₃ concentration in silage with cassava added compared to other treatments. Availability of N-NH₃ in rumen is very important as a source of N for cellulase synthesis by cellulolytic microbes [8] which was shown by high digestibility of DM and OM of silage with cassava.

3.2. In vitro digestibility
The addition of different accelerators in banana stem silage affect (P<0.01) the in vitro DM and OM digestibility as presented in Table 2.

Table 2. Digestibility of banana stem silage

| Parameter       | Control       | Rice bran     | Cassava       | Sago          |
|-----------------|---------------|---------------|---------------|---------------|
| % IVDMD         | 71.47±0.537   | 73.17±0.242   | 75.75±0.218   | 69.76±0.141   |
| % IVOMD         | 67.97±0.546   | 71.11±0.414   | 73.66±0.276   | 66.53±0.536   |

Note: Means in the same row with different superscript differ highly significant (P<0.01). IVDMD= In vitro dry matter degradability; IVOMD=In vitro organic matter degradability.

The highest digestibility of DM and OM was found in silage with cassava compared to other accelerators, indicated that the soluble carbohydrates in cassava can be used by rumen microorganisms in the beginning stages of their growth. Energy available during fermentation can be used by microbes to improve their capability to degrade crude fiber [9]. Previous study reported, the effect of accelerator types on physical, chemical, and biological qualities of buffalo grass obtained that the highest digestibility value in the silage with cassava flour added, most possible because of high content of soluble carbohydrates in cassava flour [10]. Mechanism of increasing feed digestibility due to fermented products was due to the formation of compounds during the fermentation process used by rumen microorganisms for their growth, and improves rumen capability to digest feed [11].

4. Conclusion
Addition of cassava as accelerators improved fermentation quality of banana stem, as shown by the lowest pH value and the highest in vitro N-NH₃ concentration, dry matter and organic matter digestibility.

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