

The Effect of Tannin on Banana Stem Juice on In Vitro Dry Matter and Organic Matter Digestibility

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Abstract: This study aims to determine the effect of tannin on the banana stem juice to the soybean meal protein protection in optimizing the digestibility of *in vitro* dry material and organic material. The study was arranged completely randomized design (CRD) consisted of 4 treatments, and 3 replications with 2 incubation periods of 48 and 72 hours. The total experimental units were 12 units x 2 = 42 experimental units. The treatments arrangement were T0 = 100 grams of soybean meal; T1 = 100 grams of soybean meal + 25 cc of banana stem juice; T2 = 100 grams soybean meal + 50 cc of banana stem juice; and T3 = 100 grams soybean meal + 75 cc of banana stem juice. The observed parameters in this study included *in vitro* dry matter and organic matter digestibility. The results of the study showed that dry matter and organic matter digestibility decreased ($P < 0.05$) along with the increase of banana stem juice concentration level on 48-hour and 72-hour incubation period. Deviation between dry matter and organic matter digestibility between 48-hour and 72-hour incubation on the T2 treatment. As the conclusion of the study, it was reported that banana stem juice containing tannin up to the standard of 75 cc/100 grams can decrease dry matter and organic matter digestibility on soybean meal.

Keywords: protein protection, tannin, soybean meal, digestibility.

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Introduction

Soybean meal is one source of effective feed protein but it has high rumen degradation level. The amount of soybean meal protein resistant to degradation in rumen ranges from 22 to 53% and digestibility in the small intestine ranges from 86 to 100% of the amount of resistant protein to degradation in the rumen (Sriyana and Sutarto, 2004; Stern *et al.*, 2006). The main source of protein that goes into the post-rumen gastrointestinal tract is protein by-pass and synthesized microbial protein synthesized in rumen (Mathius *et al.*, 2002). To minimize protein degradation of soybean meal from microbial degradation in rumen, soybean meal will require protection before feeding the cattle as feedstuff. The applicable method to protect protein in rumen is by supplying the protection agent in feed. Agent protection proteins include formaldehyde (HCHO), tannin and chitosan (Makkar, 2005). Tannins are compounds in plants that bind proteins and other molecules, such as carbohydrates.

The effect of tannins on livestock such as increasing by-pass protein, minimizing cases of bloat, increasing milk production and decreasing internal parasites, larvae and worms (Tanner *et al.*, 1995; Athanasiadou *et al.*, 2000; Coffey *et al.*, 2007). Tannins are polyphenolic compounds that can form a complex bond with the resistant protein to microbial degradation in a neutral pH in rumen (Bunglavan and Dutta, 2013). Tannins can be used to increase by-pass protein because of its ability to bind to the protein at the condition of neutral pH. However, in conditions of acidic pH such as in the abomasal protein, tannin will be decomposed and therefore, it can be digested in the abomasum and in the small intestine (Perez-Maldonado and Norton, 1996; Kariuki and Norton, 2008). A complex bond that occurs between tannin with protein is stable at pH 4 to 7 and will be dissociated again at pH less than 4 and more than 7, whereas the normal pH of the rumen is 6.3 to 7 and the pH of the abomasums is 2 to 3. The complex tannin-protein will not be degraded in the rumen until reaching the small intestine, with a low pH and cause complex binding. The tannin-protein will be separated and can be degraded by enzymes of the gastrointestinal tract (Van Soest, 1994).

Tannins found in various types of plants, including on banana plants (Wina, 2001). It was reported that the banana stem contains a secondary tannin compound as much as 0.01-4, 96 mg/ml juice. Yulistiani *et al.*, (2010), states that protection of soybean meal using banana stem juice can decrease degradation rate in the rumen. The substitution of commercial concentrate with a protected by tannins from the banana stem juice can increase the consumption and digestibility of feed nutrients (Yulistiani *et al.*, 2011). The study aims to identify the effect of tannin on the banana stem juice in protecting soybean meal protein for optimizing in vitro dry matter and organic matter digestibility.

Materials and Methods

Sample Preparation

Rumen fluid used to test in vitro digestibility was obtained from slaughtering house. Rumen fluid collection was performed shortly after the slaughtering. Other materials consisted of banana stem juice collected from *Bulo* banana tree with 16, 2 mg/ml tannin content. Banana stem juice which was about to use is approximately 25 ml, 50 ml, and 75 ml stored into beaker glass. Another material is soybean meal weighed in 100 grams in 4 different containers. All materials were homogenized and stored in weighing paper which were already labelled. Prepared materials were dried in oven at a temperature of 60°C for 48 hours. After that, a test to identify in vitro dry matter and organic matter digestibility was performed.

In vitro dry matter and organic matter digestibility test were performed by modified Tilley and Terry Technique (1963).

The sample was filled into a 50 ml fermentation tube as much as 0,5 gr for each tube and 30-ml rumen fluid and 12-ml McDougall buffer solution bubbled with CO₂ were added into the tube. Anaerobic incubation was performed for 48 hours at the 39°C in a shaking water bath. pH is kept at ±6,8 by bubbling with CO₂ for each 4 hours. After 48 hours, 12 tubes were removed from the bath. The fermentation process for remaining 12 tubes was stopped for performing hydrolytic digestion process by adding 0,6-gr pepsin liquid per tube and 2-ml HCL 4 N per tube. Supernatant separation from the sediment after incubation of 48 and 72 hours was performed by filtering the sediment using sintered glass and vacuum pump. Dry matter was collected by the drying process from oven 105°C for 12 hours. Organic matter was collected by calculating the difference between dry matter and ash collected from ashing in furnace at the temperature of 60°C for 4 hours.

Research Design

The study was arranged by completely randomized design (CRD) consisted of 4 treatments and 3 replications with 2 incubation periods of 48 and 72 hours. The total experimental units were 12 units x 2 = 42 experimental units. The treatments arrangement were T0 = 100 grams of soybean meal; T1 = 100 grams of soybean meal + 25 cc of banana stem juice; T2 = 100 grams soybean meal + 50 cc of banana stem juice, and T3 = 100 grams soybean meal + 75 cc of banana stem juice.

The Data obtained were then tested with the Analysis of Variance (ANOVA). If the treatment shows a real effect, then proceed with the test region of multiple (Duncan) to determine differences between treatments (Gaspersz, 1991).

Results

The different combination of soybean meal and banana stem juice in the incubation period of 48 and 72 hours is presented in Table 1.

Table 1. In vitro dry matter and organic matter digestibility of Soybean Meal Protected Banana Stem juice

Parameters	Treatment			
	T0	T1	T2	T3
Dry Matter Digestibility				
48 hours of incubation, %	81,04 ^a ±0,44	61,59 ^b ±0,35	50,91 ^c ±0,43	49,97 ^d ±0,43
72 hours of incubation, %	95,02 ^a ±0,14	92,82 ^b ±0,08	90,13 ^c ±0,56	83,11 ^d ±0,19
Organic Matter Digestibility				
48 hours of incubation, %	80,60 ^a ±0,60	60,06 ^b ±0,63	48,20 ^c ±0,91	45,98 ^d ±0,98
72 hours of incubation, %	94,63 ^a ±0,09	92,58 ^b ±0,04	86,68 ^c ±1,55	82,21 ^d ±0,61
Description: ^{a,b,c,d} Superscript with different letter on the similar row shows significant difference. (P<0,05). T0 = 100 grams of soybean meal T2= 100 grams of soybean meal + 50 cc of banana stem juice. T3= 100 grams of soybean meal + 75 cc of banana stem juice.				

Discussion

Analysis of variance showed that the treatment had a significant effect (P<0, 05) to in vitro dry matter and organic matter digestibility on incubation for 72 and 48 hours. Digestibility on 48-hour incubation represented the value of degradation in the rumen. In this study, the treatment with significant effect can protect soybean meal protein. There was an increase from 48 hours to 72 hours for all treatments either on the in vitro dry matter digestibility or the organic matter digestibility.

The decrease in dry matter digestibility on the 48-hour incubation from treatment T0 to treatment T3 was as much as 38, 34%. However, on the 72-hour incubation, it was as much as 12, 53%. The decrease in organic matter from treatment T0 to treatment T3 was as much as 42, 95% on 48-hour incubation. However, on the 72-hour incubation was as much as 13, 12%. It can be seen that the higher the level of banana stem juice supplemented, the lower the dry matter digestibility on the 48-hour incubation period.

The decrease of dry matter digestibility was caused by tannin content in banana stem juice. Vienna (2001) reported that banana stem juice contains secondary compound of tannin as much as 0,1-4,96 mg/ml juice. According to Jayanegara and Sofyan (2008), tannin is categorized to polyphenolic compounds with characteristics that can form complex

compounds with other macromolecules. Bonding complexes formed by the tannins caused digestive processes to be inhibited. Such bond inhibits microbial activity to digest nutrient in the feed. As a result, dry matter digestibility on incubation of 48 hours was low at treatment T3.

Dry matter digestibility on the incubation period of 72 hours was in line with incubation period of 48 hours. Dry matter digestibility during the incubation of 72 hours also showed a decrease in T3 treatment. The increase of dry matter digestibility from the incubation period of 48 hours and 72 hours was different among all of the treatments. If the change on dry matter digestibility between incubation period of 48 hours and 72 hours was different, it can be seen that T2 treatment reached the highest difference and treatment T0 at the lowest position.

Dry matter digestibility on treatment T0 only increased 17,25% from incubation of 48 hours which was from 81,04% to 95,02% at the incubation of 72 hours. The increase of digestibility on T1 treatment was 50,70% while T3 treatment was 66,31%. The increase in high dry matter digestibility on T2 treatment was caused by the treatment at the incubation period of 48 hours was set according to rumen characteristic with pH kept at $\pm 6,8$. In contrast, the incubation period of 72 hours replicate the characteristics of abomasum and intestines. The normal acidity level of rumen was from 6,3 to 7 and the abomasum pH was from 2 to 3 (Cheeke and Shull, 1985). Tannin bond will be decomposed in abomasum at pH lower than 3 (El-Wzyri *et al.*, 2005) and in the small intestine at pH more than 7. Tannin protein complex will not be degraded in the rumen but after reaching the small intestine, with a low pH and cause the bonding of the complex and the tannin-protein will be decomposed and can be degraded by enzymes of the gastrointestinal tract.

In vitro, organic matter digestibility was in line with dry matter digestibility on the incubation period of 48 hours or 72 hours. The highest digestibility was in T0 treatment T0 without any supplementation of banana stem juice. This study performs the supplementation of banana stem juice in soybean meal intending to protect protein of soybean meal from the synthesis of the rumen microbes. Although the protection was only performed on the feed protein, it can be seen that the treatment also had an impact on the value of organic matter digestibility and the dry matter. It was caused by that protein is a part of the organic matter and dry matter which mutually affect one another. Tillman *et al.*, (1998) stated that the feed material containing a substance of nutrients from the water, dry materials, organic materials. Organic matter consisted of proteins, carbohydrates, fats, and vitamins. Dry matter consisted of organic matter and inorganic matter. Sutardi (1979), stated that digestibility of dry matter is affected by feed protein content because each protein source has different solubility and resistance to degradation.

The increase of organic matter digestibility from incubation of 48 hours to 72 hours was not significantly different with the value of the highest dry matter digestibility on T2 treatment. The increase in organic matter digestibility in treatment T2 was 79,83% from the incubation period of 48 hours accounted for 48,20 % to the incubation period of 72 hours accounted for 86,68%. The increase in organic matter digestibility in T0 treatment was 17,40% followed by T1 treatment for 54,14%, while in T3 treatment was accounted for 78,79%. This suggested that the increase in the value of the organic matter digestibility was not in line with the increase in banana stem juice supplementation. T2 treatment with the supplementation of banana stem juice was as much as 50 cc and was considered to be the most optimal in the protein protection using tannins. This was caused by intolerable tannin dose on T3 treatment

to the microbes in the gastrointestinal tract in the rumen. Makkar (2003), stated that tannin compound has the positive and negative effect to cattle at once, the effect of which will be resulted depending on its concentration, its nature, its composition in the ration, the type and physiological status of livestock that consume it. Wahyuni *et al.*, (2014) reported that feed supplemented with tannins capable of showing the profile of better digestibility on the low-dose supplementation.

The increase in high organic matter digestibility on banana stem juice supplementation after an incubation period of 72 hours was caused by the disintegration of tannin-protein bond at the setting replicating the abomasum and intestines. Tannin affects on digestibility by binding digestive enzymes or by binding feed nutrients (Barman and Rai, 2000). The use of tannin as protein protection was also reported by Wahyuni *et al.*, (2014) that digestibility of organic matter on feed supplemented with tannin was higher compared to the control at the level of 2%. Puastuti *et al.*, (2006) reported THAT protection of soybean meal with the use of banana stem juice with a ratio of 1:1 (w/v) and heated at a temperature of 60 °C is able to decrease the rate of protein degradation in the rumen.

Conclusion

The application of banana stem juice that contains tannin up to the standard of 75 cc/100 grams (T3) had significant effect ($P < 0.05$) on the decrease of degradation level and the dry and organic matter digestibility on soybean meal in vitro

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