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The Fermentation Products and Digestibility (*In Vitro*) Fermented of Cacao Pod Husk (*Theobroma cacao* L.)

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ABSTRACT

The study aimed to determine the fermentation products and digestibility in vitro of fermented cocoa pods husk. The research was used completely randomized design. The experiment consisted of one factor, namely the doses of EM-4 plus, comprised of K_0: fresh cacao pod husk; K_1: fresh cacao pod husk + 2% EM-4 plus; K_2: fresh cacao pod husk + 4% EM-4 plus and K_3: fresh cacao pod husk + 6% EM-4 plus. Cacao pod husk was fermented for 5 days. After that, the rumen fermentation product (pH, N-NH3 and total VFA), dry matter digestibility (DMD) and organic matter digestibility (OMD) were measured. The result of this research showed that the fermentation products namely pH and total VFA were not significantly different between the treatments. The level of N-NH3 in K_3 was significantly different with $K_1$and K_2, but not significantly different with K_0. The dry matter digestibility (DMD) and organic matter digestibility (OMD) in K_0, K_1, and K_2 were not significantly different between the treatments, while DMD and OMD in K_3 were significantly higher than those of K_0, K_1, and K_2.In concluding that the pH and total VFA did not show any difference, whereas the highest DMD and OMD were produced in K_3 treatment or by the addition of 6% EM-4 plus.

***KEYWORDS**: fermented cocoa pod husk, in vitro digestibility*

INTRODUCTION

Feed is one of the main determinants that influence the success of a farm. The availability of animal feeds is decreasing because of the shrinking of land for the forage production. The decline occurred because most of the land was used as housing and food needs. Therefore, it is necessary to find new resources that potential to use as alternative livestock feed that can replace part or all of the forage.

One alternative that can be done is to utilize waste of food or plantation crops. Mastika (2006) stated that one of the plantation commodities that produced substantial biomass or by-products is the cacao plant (*Theobroma cacao* L). Cacao is one of the plantation crops that has grown very rapidly. In Bali, the area of cacao plants reached 14 470 ha and produced 38 013 ton of cacao in 2015 (Indonesian Plantation Statistics, 2015). According to Suparjo*et al*. (2011), the percentage of cocoa fruits peel was 75% of cocoa fruits as a whole, so the total waste was 28 509.75 ton in a year. The utilization of cocoa waste in agro-industrial was as a source of fibrous food for ruminants (Mujnisa, 2007; Puastuti and Susana, 2014).

The nutritional value of plantation waste is very low, especially of the protein content. Besides that, the plantation waste contains high crude fiber, which causes low digestibility. Suharto (2004) reported that the cacao pod husk contained crude protein, fatty, crude fiber,

and TDN that were 9.15%, 1.25%, 32.7%, and 50.3%, respectively. The limiting factors in the use of cacao pod husk are the high value of crude fiber, it also contains tannin and 1.0% of theobromine (3,7-dimethylxanthine) alkaloid(Ginting, 2004). Theobromine and tannin have a strong affinity to protein and carbohydrates (Amirroenes *et al*., 2005), so that can inhibit rumen microbial growth. The fermentation technology that included the physical, chemical, and biological processes was needed to reduce the crude fiber and increase the nutritional value of agricultural waste (Pasaribu, 2007).

Fermentation is a process of chemical change in an organic substrate that requires enzymes produced by certain microbes as a biochemical catalyst. The process can simplify the nutrient in animal feeds such as lignocellulose, so that it can be digested easily (Aji *et al*., 2013; Darmawan*et al*., 2014; Hermawan*et al*., 2017). Fermentation of cacao pod husk can be done using cellulolytic microorganisms, such as Effectiveness of Microorganisms 4 (EM-4).EM-4 was kind of microbes that can be degraded the crude fiber because of the ability to produce laccase and peroksidaseenzymes. Those enzymes can be used to remodel and dissolve lignin in animal feeds so that can act as an energy source for the livestock. The used of EM-4 can increase the digestibility, microbial protein synthesis, improved flavor, and aroma, reduced odor, increased vitamin and mineral and are environmentally friendly (Mangisah*et al*., 2009).

Based on the description above, it is necessary to do an in vitro study of the waste of cocoa pods that are fermented using EM-4, which can later be used as a fiber feed for ruminants.

METHODOLOGY

Location and Length of Research

The research was conducted at the Laboratory of Animal Nutrition and Food, Faculty of Animal Science, Udayana University, Bali.

Material

The cocoa pods husk waste obtained from Cau hamlet, Tua village, Marga sub-district, Tabanan regency. The starter used in the fermentation process was EM-4 plus which was purchased in the Tabanan area. The content of EM-4 plus is composed of EM-4 solution and molasses. The fermentation had done in 5 days.

Experimental Design

The experiment was arranged in Completely Randomized Design, with single factor with dosage of EM-4 plus as a treatments, comprised of K_0: fresh cocoa pod husk; K_1: fresh cocoa pod husk + 2% EM-4 plus; K_2: fresh cocoa pod husk + 4% EM-4 plus and K_3: fresh cocoa pod husk + 6% EM-4 plus. Each treatment has 4 repetitions so that there were 16 experimental units.

Research Variable

Rumen Fermentation Products

In vitro fermentation process was carried out for 4 hours. After 4th hours of incubation, samples were taken for the measurement of pH value, ammonia (N-NH3) and total volatile fatty acid (VFA total). The pH samples were measured by using pH meter. Ammonia (N-

NH3) concentration was measured using Phenolhypochloritemethod (Solarzano, 1969) and the total VFA contents were determined by steam distillation method (AOAC, 1990).

Digestibility

Digestibility observed was carried out in two different observation times, 48 hours fermentative and hydrolytic. The method used was according to Minson and Mc Leod Method (1972) with modification. Dry matter digestibility (DMD) and organic matter digestibility (OMD) was calculated with the following equation:

$$DMD\ (\%) = \frac{DM\ sample\ (g) - [DM\ residue\ (g) - DM\ blank\ residue\ (g)]}{DM\ sample\ (g)} \times 100\%$$

$$OMD\ (\%) = \frac{OM\ sample\ (g) - [OM\ residue\ (g) - OM\ blank\ residue\ (g)]}{OM\ sample\ (g)} \times 100\%$$

Data Analyses

Data that obtained were analyzed with ANOVA if there were significantly different among treatments ($P<0.05$) the analyses would be continued with the Duncan Multiple Range Test (Steel and Torrie, 1993).

RESULT AND DISCUSSION

In vitro fermentation products for 4 hours

The results showed that the cocoa pod husk that fermented for 4 hours had the pH between 7.74-8.06 (Table 1). The addition of *EM-4 plus* doses was not affected by rumen pH. The degree of acidity (pH) was a factor that influences the population growth and rumen microbial activity. Utomo (2001) stated that rumen pH was affected by the type of food eaten, especially non-structural carbohydrates. Animal feeds that contained lots of non-structural carbohydrates would reduce the pH of rumen fluid quickly.

The highest N-NH3 substrate was K_0 treatment and the value was 4.91 mMol (Table 1). A concentration of N-NH3 substrate in K_0 was not significantly different with K_3 but was significantly different with $K_1$and K_2. The presence of N-NH3 in the rumen was an indication of protein degradation. A protein that degraded in the rumen was a source of nitrogen for rumen microbes. The average of N-NH3 concentration in this research ranged from 1.85 to 4.91 mMol. These results were still lower than Sutardi (1979) recommendation. He stated that N-NH3 concentration was in between 4-12 mMol. McDonald *et al*. (2002) stated that the normal range of N-NH3 concentration to support rumen microbial growth was around 6-21 mMol. The concentration of N-NH3 reflected the amount of substrate protein available in the rumen. Its value was strongly influenced by the ability of the rumen microbes to degrade the substrate protein. Ammonia was an important source of N for rumen-living micro organisms

and it also used for microbial protein synthesis (Darmawan *et al*., 2014). The low concentration of N-NH3 in K_2 and K_3 was likely to have been utilized by microbes to support its growth.

Total VFA in cacao pod husk was not significantly different in all treatments (Table 1). Carbohydrates in rumen would be fermented by microbes into energy, which consists of acetate, propionate and butyrate and a small portion of valeric acid. VFA was the main energy for ruminants (Preston and Leng, 1987). In this study, the average total VFA was lower than the previous studies. Sutardi (1979) stated thatthe range of VFA levels sufficient for rumen microbial growth was 80-160 mMol.Aji*etal*. (2013) reported that the ranged of total VFA was 83.6-87.2 mM in cacao pod husk that fermented by *Aspergillusniger*.

Table 1. In Vitro Fermentation Products for 4 Hours

Variable	Treatment [1)]				SEM[3)]
	K_0	K_1	K_2	K_3	
pH	7.74[a2)]	7.82[a]	8.06[a]	7.95[a]	0.07
N-NH_3 (mMol)	4.91[a]	1.85[b]	2.41[b]	3.81[a]	0.38
Total VFA (mMol)	80.32[a]	64.19[a]	76.71[a]	77.19[a]	8.52
Degradation of DM (%)	3.16[d]	10.14[c]	16.60[b]	24.89[a]	1.86
Degradation of OM (%)	11.62[d]	17.76[c]	23.76[b]	28.26[a]	0.83

Note: [1)]K_0: fresh cacao pod husk; K_1: fresh cacao pod husk + 2% *EM-4 plus*; K_2: fresh cacao pod husk + 4% *EM-4 plus* and K_3: fresh cacao pod husk + 6% *EM-4 plus*; [2)] Superscript that are on the same line have significantly different on $P<0.05$; [3)]SEM: Standard Error of the Treatment Means

Degradation of dry matter (DM) and organic matter (OM) were significantly different in all treatments (Table 1). The highest DM degradation was produced in K_3 was 24.89% and the lowest in K_0 was 3.16%. A similar result as DM degradation was also found in OM degradation. The highest OM degradation resulted in K_3 was 28.26% and the lowest in K_0 was 11.62%. Putra (1999) reported that the degradation of DM and OM influenced by animal feeds and types of microbes. The higher dosage of EM-4 plus increased the degradation of DM and OM with values between 3.16-24.89% and 11.62-28.26%, respectively.

It is showed that in vitro fermentation for 4 hours was able to degrade cacao pod husk, where the degradation was caused by the enzyme that produced by rumen microbial. After that process, VFA and NH3 produced as a source of energy and N for the growth and synthesis of microbial protein.

In Vitro Digestibility

The results showed that dry matter digestibility (DMD) and organic matter digestibility (OMD) of fermented cacao pod husk increased with increasing the number of EM-4 plus (Table 2). The highest DMD and OMD was K_3, while the lowest was K_0 and the difference between those treatments were 8.76% and 11.07%, respectively. The percentage of DMD in

K_1 and K_2 increased 2.64% and 8.04% of K_0, but it was not significantly different ($P > 0.05$). DMD percentage in K_3 was 22.07%, 18.92%, and 13.40% higher than K_0, K_1, and K_2, respectively.

Table 2. In Vitro Digestibility

Variable	Treatment[1)]				SEM[3)]
	K_0	K_1	K_2	K_3	
DMD (%)	39.70[b2)]	40.75[b]	42.89[b]	48.46[a]	1.64
OMD (%)	43.66[b]	45.42[b]	48.42[b]	54.73[a]	1.61

Note: [1)]K_0: fresh cacao pod husk; K_1: fresh cacao pod husk + 2% EM-4 plus; K_2: fresh cacao pod husk + 4% EM-4 plus and K_3: fresh cacao pod husk + 6% EM-4 plus; [2)] Superscript that are on the same line have significantly different on $P<0.05$; [3)]SEM: Standard Error of the Treatment Means

In this research, the best results were obtained by adding 6% EM-4 plus (K_3), which was increased the level of DMD and OMD (Table 2). The addition of EM-4 plus until 4% could increase the OMD but the value was not significantly different with K_0. It can be seen that the longer fermentation time, the DMD and OMD in vitro were higher than the degradation of DM and OM in 4 hours fermentation (Table 1). These results were in line with Suryani et al. (2013) and Mariani and Suryani (2016), they reported that the longer the digestive process, the DM and OM digestibility increases. These results were reinforced Putra (2006), that feed was digested fermentative, the longer the fermentation process takes place, the higher the dry and organic matter degraded.

Muhtarudin and Liman (2006) stated that the higher DMD and OMD could increase the chance of livestock to utilize the nutrition for production and vice versa. Cacao pod husk fermented with EM-4 plus produce high of DMD and OMD values, which means that the value of nutrients from cocoa pod husk can be absorbed and utilized by livestock. The determining of digestibility is to get the quality of feed ingredients, because of not every feed digested well by livestock. The level of digestibility was very influential on the value of the benefits of a feed ingredient. When the feed ingredients have a high level of digestibility then it would have a high value of benefits.

CONCLUSIONS

It can be concluded that the addition of EM-4 plus until 6% increased the level of $N\text{-}NH_3$, but it had no effect on fermentation products for 4 hours, total pH and VFA. K_3 was the best treatment that gave the highest dry and organic matter digestibility.

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