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The Characteristics of Rumen Fluid and Digestibility Cocoa Leaves Fermentation With Microorganisme Indigenus Method In -Vitro

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Abstract— *The ruminant animal feed ingredients consisting of forage with the combination of grass and legume which were almost limited in availability, the cocoa leaves as agricultural wastes or wastes containing low nutrient plantation low used as alternative feed ingredients. To overcome these problems required a simple technology that is fermentation using microorganisms indigenus so that the effluent quality cocoa can be improved. Indigenus microorganisms were microbes that exist in individuals without disturbing the activities.*

The purpose of this research was to improve the quality of rumen fluid characteristics and digestibility of cocoa leaves fermented by microorganisms indigenus. The method used in this study was completely randomized design factorial with three replications, treatment factors consist of A as the level of microbes (A1 = 5%, A2 = 10%, A3 = 15%) and factor B as the duration of fermentation (B1 = 2 day, B2 = 4 days, B3 = 6 days) and the variables; the nutrient content and digestibility of cocoa leaves before and after being fermented and the characteristics of rumen fluid in vitro methods.

The results showed that the increasing levels of use of microbes and fermentation time can enhance the quality of cocoa leaves that reduce the content of dry material from 31.389 to 25.488% and increase the protein content of the coarse 6.153 to 7.483%. Dry Material digestibility increased from 27.379 to 32.942% and 27.432% Protein 21,435- rough. Rumen fluid characteristics were rumen fluid pH ranges from 7.427 to 7.603, NH3 production ranges from 16.820 - 19,975mg / 100ml, and Volatyl Fatty Acid production from 83.667 to 101.000 mM. The conclusion that increasing the digestibility quality cocoa fruit leather could be made by fermentation by microorganisms indigenus.

Keywords— Cocoa leaves, fermentation, digestibility, microorganisme, in-vitro.

I. INTRODUCTION

The main ingredient is a ruminant livestock forage food in general, which consists of grass and legumminosa which in the present availability is limited because the land began to grow grass and legumminosa already widely used for residential and business premises where food crops. Efforts to resolve this problem do the utilization of agricultural waste or agricultural waste products of low nutritional value as animal feed, the use of alternative feed ingredient does not change the ability of livestock production.(Fridarti.at.all, 2016)

Waste food crops and plantations have an important role and potential in the supply of green feed for ruminansia livestock such as cattle, goats, sheep and buffalo, especially in the dry season. In addition ruminant rearing system is still largely dependent on forage, feed in the form of grasses and other forage feed with little or no additional feed. (Fridarti at.all, 2016). To overcome the problem of shortage of forage, farmers were expected to utilize agricultural wastes which were abundantly available in the vicinity included cocoa fruit skin, cocoa plant leaves, shoots of cane, rice straw, hay soybean and peanut hay through a certain treatment. consisting of chocolate fruit rind of 73.73%, 2% of the placenta, and 24.20% seeds (Haryati and Hardjosuwito, 1984). Cocoa fruit skin was agro-industry waste produced by cocoa plant (Theobroma cacao L). The results of proximate analysis cocoa rind contains dry matter (DM) 8%, coarse fibers (CF) 40.1%, Protein rough (CP) 8% and 50.8% TDN and Used by ruminants 30-40% (IPPTP, 2001). To improve the nutritional value of the cocoa fruit skin with technologies such as physical treatment, chemical and biological, one of the technologies used are fermented. In the process of fermentation occurs solving complex compounds such as cellulose, hemicellulose, silica, by certain enzymes that reduce the content of crude fiber and improve the digestibility of the material. Fridarti research results (2013) that fermented fruit peels that use Basil sp chocolate could lower crude fiber and increase the crude protein. Muzir study (2005) that the cocoa fruit skin

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fermented with yeast tape shows the physical shape and texture well, written by Izzati (2011) that fermented cocoa rind with Local microorganims (MOL) rind of cocoa could increase crude protein and lower crude fiber.

Indigenus microorganisms were microorganisms that exist in living things that did not interfere with life. Several studies had isolated one of microbial indigenus by isolation and identification of bacteria of lactic acid and probiotic bacteria were generally investigated derived from fruits, animals and trash. Khairati (2011) had managed to characterize molecular Lactic Acid Bacteria from the cocoa-producing Bacteriocin green. Novianty research results (2011) also characterize molecular amylolytic lactic acid bacteria as probiotic potential of cocoa. Yunensi (2011) had managed to isolate and identify isolation of probiotic bacteria Pediococcus pentosceus origin cocoa.

Formulation of the problem : was cocoa leaves could be improved digestibility by using microorganisms indigenus?

Research purposes : Improve the quality of cocoa leaves nutrients from waste cacao using microorganisms indigenus.

Research hypothesis : Miroorganisme indigenus could increase digestibility of cocoa leaves

Benefits of research :

The results of this study could reduce the waste problem of cocoa used as the building blocks for livestock rations ruminasia by breeders.

II. MATERIALS AND METHODS

Research material

The material used in the research was a microorganism indigenus derived from the cocoa leaves cultured on an agar seaweed medium and then made inoculum using cocoa leaves that had been finely ground and then stored for 4 days, the results were used for fermentation cocoa leaves, scale research laboratory using 100 grams cocoa leaves that had been chopped up and added to the level of microorganisms according to treatment (5, 10, 15%) and stored for 2, 4, 6 days, after it was dried in an oven with a temperature of 60 ° C so that the water content reached 10-12%.

Research methodology

The design was completely randomized design (CRD) factorial pattern of 3 x 3 with three replications, where factor A was the concentration of microorganisms (A1 = 5%, A2 = 10% ,, 15%), factor B was a long fermentation (B1 = 2 days, 4 days B2 =, B3 = 6 days).

The design models used in the experiment were:

$$Yijk = \mu + \alpha i + \beta j + \Sigma ijk$$

Where:

Yijk = value observations on satua perobaan who gets treated first with the standard of all replications k ke-

μ = Mean General

αi = Influence ith level of factor A

βj = Effect extent to - j of Factor B

Σijk = Effect of errors on experimental unit that received the standard of all i Factor A, level j of factor B, with replicates all k

To determine the effect of treatment in early stages of the statistical analysis of variance. If there was a real effect of different treatments to do LSD (Steel and Torrie, 1995).

Implementation of the research.

1. Preparing cocoa leaves which had been cut into small pieces with a size of 2-5 cm.
2. Combining all ingredients in accordance with the treatment of each in plastic bags as a fermentation and tied up airtight.

Then treatment was stored according to treatment after that the samples were taken to the laboratory to be analyzed according to the variables.

Parameters measured

The variables measured were:

1. Nutritional content and digestibility of the substrate after fermentation with the method of the proximate analysis and in vitro
2. Characteristics of rumen fluid (pH, NH3, VFA)

III. RESULTS AND DISCUSSION

Nutritional content of Fermentation of Cocoa leaves

Results of the study the mean content of the cocoa leaves fermentasi Nutrition during the study were shown in Table 1.

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Table.1: Mean Nutritional content Dried Materials (DM), Organic Matter (OM), Crude Fiber (CF), Crude Fat (CF), of leaves cocoa Frementation

No	Content	Factor A (Microbial Concentration)	Factor B (old fermentation)			Mean
			B1 (2 days)	B2 (4 days)	B3 (6 days)	
1	Dry Material	A1 (5%)	31,389	26,974	25,933	28,098
		A2 (10%)	31,068	26,583	25,900	27,850
		A3 (15%)	31,042	25,488	25,629	27,386
mean			31,166[a]	26,348[b]	25,821[b]	
2	Organic Matter	A1 (5%)	80,723	82,083	83,121	81,975
		A2 (10%)	80,238	82,444	82,852	81,845
		A3 (15%)	80,302	81,175	82,931	81,469
	mean		80,421	81,901	82,968	
3	Protein Rough	A1 (5%)	6,726	6,720	6,299	6,582
		A2 (10%)	6,913	7,483	7,363	7,253
		A3 (15%)	6,153	7,378	7,264	6,932
Mean			6,597	7,194	6,976	
4	Crude Fiber	A1 (5%)	23,289	21,500	21,315	22,034[a]
		A2 (10%)	21,676	21,800	19,832	21,103[b]
		A3 (15%)	21,563	20,994	20,424	20,993[b]
Mean			22,176[a]	21,431[a]	20,524[b]	
5	Fat Rough	A1 (5%)	1,052	0,943	1,096	1,030
		A2 (10%)	1,140	1,001	1,135	1,092
		A3 (15%)	1,238	1,075	1,067	1,127
Mean			1,143	1,006	1,099	

Description: The value of different superscript in the same row and column in the treatment factor for each different variables showed highly significant effect (P <0.01)

The mean of the results of research content of dry material ranging from 25.488 to 31.389%, Organic Material from 80.238 to 83.121%, coarse Protein 6.153 to 7.483%, rough Fiber 19.832 to 23.289%, coarse fat from 0.943 to 1.238%, cocoa leaf fermentation. Statistical test result analysis of variance addressed that long fermentation gave a significant influence (P <0.01) on dry matter content, Crude Fiber cocoa leaf fermentation, but the concentration level of influence and not significant (P> 0.05) on ingredients dry, protein, fat, coarse cocoa leaf fermentation. Results of research found that the average of dry matter, crude fiber cocoa fruit skin tends to decline, indicating that the fermentation time could affect the water content of the substrate, during the fermentation process took place water content would increase with longer fermentation, because during the fermentation process took place would happen increasing cell water from fermented materials, due to the increasing microbial activity in the overhaul of feed material into simpler forms. Fardiaz (1988) said that microorganism using carbohidrat after broken down into glucose. So that the decrease in dry matter during the fermentation process due to a respiration of the bacteria that produce the energy, water and carbon dioxide. Microorganisms decomposed organic matter and remains alive into the elements simpler

The results of this study showed that the longer of the fermentation process would increase the content of Organic Matter, This was in accordance with the opinion of Sukara and Admowidjodo (1987) that microorganisms having the growth and proliferation of good and could turn more components of the media into a mass of cells, resulting in the formation of proteins those from the body itself enhances the microbial crude protein. Added by Buckle (1987), that the fermentation was generally result in partial loss carbohidrat of food, but the loss was covered by the gains of protein.

This was presumably related with the formation of methane gas and hydrogen gas generated during fermentation occurs, due to the disintegration of crude protein. CO2, methane and hydrogen gas was a form of energy that was not useful for animals to be removed from the rumen through eructing process. Product methane could be reduced if the levels of propionic acid in rumen was high. Proteins were organic substances containing carbon, hydrogen, nitrogen, oxygen, sulfur and

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phosphorus (Anggorodi, 1994). This was along with the opinion of Fardiaz (1988), that mikroorganism using carbohidrat after broke down into glucose. Fermentation could hydrolyze the protein, fat, cellulose, lignin and other polysaccharides, so that the fermented material would have a higher digestibility.

The mean digestibility Dry Materials (DM), Crude Protein (CP), Crude Fat (CF), Crude Fiber (CF) Cacao Leaves Fermentation

The results of the study the average digestibility of the food substance cocoa leaves to ferment for research could be seen in Table 2. The mean digestibility Ingredients Dried cocoa rind ranging from 27.379 to 32.942%, Organic Material from 65.144 to 73.519%, 27.432% Protein 21,435- rough, coarse fibers 19.117 - BETN 20.385% and 38.653 to 46.086%. Having analyzed the results statistically significant variance indigenus stated that the concentration of microorganisms and fermentation time and interaction between the treatments showed different effects that were not significant (P <0.01) on dry matter, organic materials, coarse protein, coarse fiber, BETN cocoa leaves.

Table.2: digestibility Dry Materials (MD), Crude Protein (PC), Crude Fiber (FC), Crude Fat (FC), Organic Matter (MO) and BETN of Cocoa Leaves Fermentation

No	Digestibility	Factor A (Microbial Concentration)	Factor B (old fermentation)			Mean
			B1 (2 days)	B2 (4 days)	B3 (6 days)	
1	Dry Materials	A1 (5%)	32,089	27,911	27,782	29,261
		A2 (10%)	32,285	27,762	27,379	29,142
		A3 (15%)	32,942	29,484	29,692	30,706
Rataan			32,439	28,386	28,284	
2	Organic Matter	A1 (5%)	72,041	64,572	65,144	67,252
		A2 (10%)	72,616	66,082	69,022	69,240
		A3 (15%)	73,519	68,341	71,177	71,012
Mean			72,725	66,332	68,448	
3	Crude Protein	A1 (5%)	26,771	25,919	21,435	24,708
		A2 (10%)	27,360	27,317	26,995	27,224
		A3 (15%)	27,432	27,265	27,303	27,334
Mean			27,188	26,834	25,244	
4	Crude Fiber	A1 (5%)	19,821	19,667	18,018	19,169
		A2 (10%)	19,223	19,117	19,038	19,126
		A3 (15%)	20,274	20,385	20,013	20,224
Mean			19,773	19,723	19,023	
5	Crude Fat	A1 (5%)	25,783	18,986	25,691	23,487
		A2 (10%)	26,033	19,648	22,988	22,890
		A3 (15%)	25,812	20,690	23,861	23,454
Mean			25,876	19,775	24,180	
6	Matter Ekstrak Non Nitrogen	A1 (5%)	45,603	38,653	43,709	42,655
		A2 (10%)	45,256	38,765	42,027	42,016
		A3 (15%)	46,086	41,075	43,874	43,679
	Mean		45,649	39,498	43,203	
Characteristics of Rumen Fluid						
1	NH_3	A1 (5%)	19,975	17,520	17,438	18,311
		A2 (10%)	18,555	16,820	17,953	17,776
		A3 (15%)	19,750	17,053	19,047	18,617
Mean			19,4267[a]	17,1311[b]	18,1461[ab]	
2	VFA	A1 (5%)	84,667	101,000	83,667	89,778
		A2 (10%)	85,333	85,667	85,333	85,444
		A3 (15%)	87,000	88,000	87,333	87,444
	Mean		85,667	91,556	85,444	

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3	pH	A1 (5%)	6,540	6,673	6,630	6,614
		A2 (10%)	6,623	6,543	6,490	6,552
		A3 (15%)	6,553	6,630	6,500	6,561
Mean			6,572	6,616	6,540	

The average dry matter obtained from this study that the concentration of microorganisms showed 15% with 2 days fermentation time was the result of the highest and lowest results in treatment concentration of 10% with 6 days fermentation.

This was presumably because the microorganisms that grow and develop microorganisms derived from substart itself, to grow and develop needed nutrients that were easily digested meal so that substances contained in sustrat would be used by the microbe itself. It was in accordance with the opinion In the fermentation process had also generated products that were not useful for animals such as CH4, ammonia and nitrate. The effort to increase the energy efficiency of feed had been done continuously.

Principally feed processing waste was an attempt to lower lignin content. The decrease of lignin content would be a positive influence on the increase obtained feed stuffs. Preston and Leng (1987) Stated that the deficiencies of nutrient needed by rumen microbes would reduce the biomass and consequently decrease the digestibility of feed mainly feed the fiber. It is required pretreatment of the fibrous material to improve obtained high potential of fiber. Sa'id (1996) added a useful pretreatment to increase the rate of hydrolysis lignosellulasa material. Lignin could not be digested by the rumen microbes and was a limiting factor fiber feed utilization, especially agricultural wastes.

This was in accordance with the opinion of Tillman et al (1989) observed that the Decree was a factor of chemical components which was the biggest influence on digestibility, generally the higher crude fiber the lower digestibility and rate of degradation of food in the rumen. Added by Maynard et al (1979) suggested that the digestibility of crude fiber was affected by the starch, protein and the number of microorganisms in the rumen

Characteristics of Rumen Fluid

The average content of NH3 fermentation of cocoa leaves ranged from 16.820 to 19.975 mg / 100ml, VFA 83.667 to 101.000 mM, pH 6.490 - 6,673. The Statistical test results analysis of variance showed that the concentration factor, the interaction of both factors showed no effect (P> 0.05) on the content of NH3, VFA, pH cocoa leaf. While the factors influencing the fermentation time had highly significant (P <0.01) the content of NH3 but not significantly different to the VFA and pH of cocoa leaves. It was suspected that the old fermnetasi greatly affects the concentration of ammonia generated during the fermentation of food stuffs on rumen. The concentration of ammonia in the rumen also determined microbial metabolism which in turn would affect the result of fermentation of organic material feed (Hermanto 2011). The concentration of ammonia nitrogen source describing the speed of digestion. Ammonia concentration was determined by the level of protein feed consumed, the degree degradabilitasnya, long in the rumen and rumen pH. According Sutardi (1979) levels of ammonia necessary to support the growth of rumen microbes between 4-12 mM.

IV. CONCLUSION

1. The Consentration of indigenus microorganisms by 10% with 4 days of fermentation time could increase the protein content of coarse, rough fat, VFA and lower the Dry Material Content, Crude Fiber, NH3 Cacao Leaves
2. Concentration of Microorganisms indigenus 10% with 4 days fermentation time tends to reduce protein digestibility, rude fat, crude fiber, BETN, NH3, VFA cacao leaves but increased in concentration to 15% on day 6 fermentation time.

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