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Aspergillus niger SIGNIFICANTLY IMPROVED NUTRIENT CONTENT AND QUALITY OF COCOA WASTE *(Theobroma cacao* L.)

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ABSTRACT

Experiments were carried out to study the effect of fermentation of cocoa pod waste using *Aspergillus niger* on its nutrient content and its effect on the growth of Bali cattle. Cocoa pod waste were collected from 4 difference districts; Buleleng, Jembrana, Tabanan and Badung which considered as the centers of cocoa plantation in Bali. Four different types of cocoa waste: cocoa pod, cocoa fruits bored by insect (*Conopomorpa cramerella* Snellen); rotten cocoa fruit (infected *by Phytopthora palmovera)* and cocoa fruit infected by *Helopeltis sp.* were chopped then fermented using *Aspergillus niger* for 6-7 days. After fermentation period each samples were dried under the sun for 3-4 days then ground up. All samples were analyzed for proximate analysis and for minerals as well as for theobromine and cyanide content. Result of the pooled data in this experiment shows that the protein, ash, calcium and phosphorus content were increased 38.51%, 28.5%, 1.55% and 29.0% respectively. On the contrary, crude fiber, organic matter, ether extract were decreased 21.61%, 2.48% and 31.31% respectively. Effect of fermentation using *Aspergillus niger* significantly reduced theobromine and cyanide content as much as 28.97% and 28.23% when compared to the unfermented samples. When the fermented cocoa waste was used to composed 50% of the cattle diets and fed 3 kg per head/day to Bali cattle, the daily weight gain recorded was 660 g/day versus 530 g/day for the control counterpart. It was concluded that fermentation of cocoa waste using *Aspergillus niger* significantly improved its nutrient content and quality of the feeds, and when fed to the cattle it gave 24.5% higher weight gain than their control counterpart.

Key words: Cocoa waste, *Aspergillus niger*, Fermentation, Nutrient content, *Bali cattle.*

INTRODUCTION

The main problems of animal production in Indonesia in general, and in the eastern part of Indonesia in particular, is insufficiently of both quality and quantity of available feeds for the whole year (Mastika 1996; 1997). Consequently, animal performance is poor and a high mortality rate for a new born calves (Bamualim and Wirdahayati, 2003) especially during dry season. The scarcity of the feed availability is also applied for non ruminant production consequently most of the feedstuffs are imported, causing the cost of animal production in Indonesia is very high.

These problems mentioned above leads us to a new approach of using agricultural and agro-industrial by products for animal feeds. However, the basic characters of waste in general is characterized by high fibre and low nutrient content and quality. Further some of agricultural and agro-industrial by-product waste containing toxic substances such as antitrypsin inhibitor (Scott *et al.*, 1982), tannic acid in sorghum (milo), theobromine (Devendra, 1979) which causing to some extent a depression in animal growth (Bo Gohl, 1981; Preston, 1986).

With the new approach and technology development, those constraints can be eliminated to some extent, thus the quality and availability of nutrient content of agricultural by-product can be increased to a certain stage. These methods include of using physical, chemicals or biotechnologies process or in combinations in improving the quality of the feedstuff. Among those techniques available, apparently biotechnology approach is the most acceptable due to the fact that this technique has no deleterious affect both to the human and animals, and has environmentally healthy and friendly.

The potential agricultural waste in Indonesia was reported by Lacony (1998) that cocoa pod/fresh and waste every year is about 251,927 ton/year. In Bali Province, the growth rate of cocoa plantations is increase about 11.7% per year and in 2007 was reported that the total plantations is 12,328 ha, and production of fresh fruit cocoa was estimated about 8,013.78 ton/year (Dinas Perkebunan Provinsi Bali, 2007). Ginting (2004) reported that the ratio of the cocoa seed and waste was 1: 3.4. From this figure it was calculated that the productions of cocoa waste was about 75.67% and that was 6,192.47 ton per year, which is considered as potential feedstuffs in the future.

MATERIALS AND METHODS

Cocoa waste samples.

Four cocoa waste types namely: cocoa pod waste; cocoa fruits bored by insects (*Conopomorpa cramerella* Snellen); rotten cocoa fruits (infected *by Phytopthora palmovera)*, and cocoa fruits infected by *Hellopeltis sp.*,

were used in this experiment. Those four types samples were collected from four different districts that are Buleleng, Jembrana, Tabanan and Badung districts which was considered as the cocoa plantation centers in Bali.

Aspergillus niger **Solution as fermentor.**

The seeds of *Aspergillus niger* as fermentor was bought/ provided by Balai Penelitian Pertanian (Agricultural Research and Development Office) Denpasar, Bali. The concentrated fermentor was diluted from one litre into 100-200 litres of fresh clean water and was activated for 3 days without aerator or one day with the aerator, using the method explained by Guntoro *et al.* (2002). Diluted fermentor can be sprayed and mixed into 1000-2000 kg of chopped cocoa waste.

Chopper and Grinder Machine.

To cut the cocoa waste into pieces (3-4cm), a double fractions chopper and grinder machine (sold by UD. Asoka Wijaya, Tabanan) was used and this machine has 2 ton daily capacity for chopping and 1 ton daily for grinding the dried materials. This machinery has 0.25 mm sieved holes so the final product is ready for finely ground animal feeds.

Analytical Laboratory.

After the four different samples were finely ground, each sample were sent to the analytical laboratory for proximate analysis (dry matter, protein, crude fiber, ether extract, organic material, ash, and gross energy) and minerals (Calcium and phosphorus) as well as theobromine and cyanide content of each samples. The analysis works was carried out at Udayana Analytical Laboratory Denpasar.

Animals and Feeds for Experimentation.

Ten matured Bali bulls weighed 250 to 275 kg were used in this feeding trials. Experimental animals were divided into two groups of five animal each as replication and one group were fed 3 kg commercial concentrate as control group and the other group were fed of 50% fermented cocoa waste mix with 5% fish waste, 10% ground corn, 10% copra meal, 10% rice brand, 8% pollard and 2% mineral supplement. This homemade feeds contain approximately 14-15% protein and 61% TDN. Fresh grass and water provided *ad libitum* at the afternoon. All data collected were analysis using T- test as explained by Chang (1979).

Design and Data Analyses.

The experiments were designed using pairing system/method where four different source of samples were used as replicates and fermented and nonfermented were used as treatments. In animal trials, pairing system/method was employed and the number of the animals used as replicates and fermented cocoa waste based feed and commercial feed as treatments. All data collected were analyzed using T-test as described by Chang (1979).

Preparation of the Fermentor.

All procedures in preparing fermentor following the step by step procedures explained by Guntoro *et al.* (2002). Briefly the one litre *Aspergillus niger* concentrated solution was diluted into 100-200 litres of fresh clean water. This mixture was steer using aerator for one day, or 36 hours without aerator and this mixture was ready to use as fermentor. Each of the fresh cocoa waste were chopped into 3-4 cm wide then placed in fermentation box. Every 5cm layers samples were sprayed and mixed using *Aspergillus niger* fermentor and covered using black plastic sheet and this fermentation process was maintained for 5-6 days period. After fermentation period completed, this fermented cocoa waste was dried under the sun for 3-4 days and when in was dry, all this materials was ground up to make a fermented cocoa waste into fermented cocoa waste powder.

RESULTS AND DISCUSSION

Results of the experiments shows that *Aspergillus niger* as fermenting agent has a significantly effect in increasing nutrient content of cocoa waste especially in its protein content (Tabel 1). The pooled value of protein content has increased about 38.5% from 9.72% of unfermented to 13.46% of fermented waste. This finding is agreement with the results reported by other workers (Guntoro *et al.*, 2002).

The significant increased of protein content of cocoa waste fermented by *Aspergillus niger* (38.5%) is in contrast with the crude fibre, organic materials content of the fermented materials. The possible explanation for this, was that partly due to the development of mycelium of the Aspergillus entering the tissue of the cocoa waste. This mycellium known has rich and high digestibility for its protein content. Probably, similarly pattern has occurred in the process of making "tempeh" using yeast *(Rhizopus oligosporous)* and it was reported that the digestibility and protein content of the "tempeh" is higher than the unfermented soybean (Murata *et al.*, 1967).

Table 1 Effect of fermentation using *Aspergillus niger* on nutrient content of cocoa waste

Samples	Treatments	Nutrient content (%)						
		Dry matter (%)	Crude protein (%)	Crude fiber (%)	Ash (%)	Organic matter (%)	Gross energy (Kcal/g)	Ether extract (%)
Cocoa shell	Unfermented	88.26 ±2.00	6.86 ±1.25	27.78 ±4.64	8.87 ±0.96	91.13 ±0.96	3.83 ±0.18	4.77 ±0.63
	Fermented	88.08 ±0.87	11.44 ±1.75	25.82 ±5.08	11.12 ±0.28	88.88 ±0.28	3.57 ±0.42	5.19 ±1.21
Cocoa pod borred by *Conopomorpa cramerella* Snellen	Unfermented	90.00 ±0.80	10.13 ±0.84	19.12 ±5.37	7.41 ±0.71	92.59 ±0.71	4.60 ±0.13	20.11 ±1.48
	Fermented	88.65 ±1.31	13.56 ±0.95	13.72 ±6.38	10.24 ±0.97	89.76 ±0.97	4.34 ±0.50	11.96 ±7.24
Rotten cocoa pod infected by *Phytopthora palmovera*	Unfermented	89.23 ±0.24	10.99 ±2.32	21.35 ±4.28	8.07 ±0.94	91.93± 0.94	4.71 ±0.39	15.65 ±2.70
	Fermented	89.54 ±1.06	14.98 ±1.46	15.35 ±5.80	9.94 ±0.91	90.06 ±0.91	4.37 ±0.23	11.85 ±4.70
Cocoa pod infected by *Hellopeltis sp*	Unfermented	89.70 ±1.01	10.90 ±1.19	20.60 ±6.15	7.64 ±0.79	92.36 ±0.79	4.70 ±0.15	17.59 ±6.19
	Fermented	89.27 ±0.73	13.87 ±1.20	14.74 ±4.00	9.83 ±0.66	90.17 ±0.66	4.47 ±0.37	10.94 ±3.85
Pooled value	Unfermented	88.60 ±0.93	9.72 ±1.15	22.21 ±4.54	8.00 ±0.76	92.00 ±0.76	4.46 ±0.06	14.53 ±1.64
	Fermented	89.89 ±0.97	13.46 ±1.04	17.41 ±5.04	10.28 ±0.43	89.72 ±0.43	4.19 ±0.16	9.98 ±3.86

This significant improvement of both quality and quantity of the protein content of cocoa waste is quite important from nutritional point of view, since protein of the feedstuffs is very important in composing feed for animals, however the price of protein is very expensive. The price of the feeds presently is steadily increase, making every effort in finding out source of protein for animal will support the Indonesian Government Plan in increasing national animal production.

The effect of *Aspergillus niger* fermentation is not only increasing protein, ash and minerals content of the cocoa waste but also improving the quality of the feed through decreasing of the crude fibre and the antinutrition factors e.g; cyanide and theobromine content of the feedstuff (Table 2). The similar findings have been reported by Laconi (1982); Guntoro *et al*, (2002) that biofermentation process will reduce the antinutrition factors of cocoa shells.

In the animals trial, a mixture of 50% of fermented cocoa waste and other feedstuffs as a completed diet and fed 3 kg perday as concentrate (14.5-15.0% crude protein and 61% Total Digestible Nutrient) to the Bali cattle. Another control groups were fed 3 kg of commercial feed per day as control counterpart. The results shows that Bali cattle fed 50% mixture of fermented cocoa waste gained 660g/day vs 550g/day for their control counterpart (Table 3). The similar finding was reported by Mastika (2006) when Bali cattle were fed 2 kg of fermented cocoa shell as pure concentrate. This higher daily weight gain is probably due to the higher quality of protein content of the fermented cocoa waste diets. As mentioned by Murata *et al.* (1967) that "tempeh" (cooked soybean fermented *by Rhizopus sp)* has higher and better protein quality than the unfermented soybean. Probably, similar action has accured in cocoa waste fermented by *Aspergillus niger.*

Table 2. Effect of fermentation using *Aspergillus niger* on mineral and anti nutrition factor of cocoa waste

Samples	Treatments	Mineral (%)		Anti nutrition factors	
		Ca	P	HCN (ppm)	Theobromine (%)
Cocoa shell	Unfermented	2,86 ±0,61	0,22 ±0,12	132,98 ±13,90	5,11±1,88
	Fermented	2,98 ±0,63	0,27 ±0,05	91,13 ±27,54	3,46±0,74
Cocoa pod borred by *Conopomorpa cramerella*	Unfermented	2,47± 0,15	0,29± 0,02	178,07± 45,47	3,99±0,09
	Fermented	2,83 ±0,24	0,43 ±0,13	159,98 ±24,49	3,46±0,52
Rotten cocoa pod infected by *Phytopthora palmovera*	Unfermented	2,66 ±0,35	0,37± 0,05	138,78 ±82,27	5,77±1,48
	Fermented	2,52 ±0,14	0,45± 0,02	82,22 ±15,45	3,60±0,27
Cocoa pod infected by *Hellopeltis sp*	Unfermented	2,31 ±0,31	0,35 ±0,05	144,05 ±26,93	5,14±1,99
	Fermented	2,15± 0,17	0,44 ±0,10	92,88 ± 4,43	3,69±0,60
Polled value	Unfermented	2,58 ±0,24	0,31 ±0,04	148,47 ±18,73	5,00±0,87
	Fermented	2,62 ±0,24	0,40 ±0,06	106,55 ± 5,73	3,55±0,34

Table 3 The growth performance of Bali cattle when fed concentrate containing 50% of fermented cocoa waste for 90 days)

No	Parameters	Bali cattle fed 3kg concentrate containing 50% of fermented cocoa waste	Bali cattle fed 3kg of commercial feed
1	Initial body weight (kg)	256.25±8.17	259±9.7
2	Final body weight (kg)	315.65±12.12	307.20±12.6
3	Weight gain (kg)	59.40±7.7	48.20±6.19
4	Daily gain (kg/day)	0.660±0.20	0.530±0.24
5	Feed consumtion (kg/head/day)	5.50	5.75
	Concentrate (kg/head/day)	2.53	2.70
	Grass (kg/head/day)	2.97	3.05
6	Feed Conversion ratio (FCR)	8.92	9.80

The results of this study suggested that fermentation of cocoa waste using *Aspergillus niger* significantly improved its nutrient content and quality of feed, and when fed to the Bali cattle, its gave 24.5% higher daily weight gain compared to their control counterpart. The higher daily weight gain of Bali cattle fed home made feed containing 50% fermented cocoa waste due to the fact that the home made feed contained higher protein (15.7% CP) compared to the commercial diet which contained only 7.5% CP (Lab. analysis). The similar pattern of growth rate was reported by Mastika (2003) that Bali cattle fed good quality concentrate (18.34% CP, 72.5% TDN) gained 852 g/day vs 606 g/day when fed rice bran as concentrate. The results of this experiment therefore suggest that feeding a higher and better quality feed could significantly improve the growth rate of Bali cattle.

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