Biodegradation of Cyanogenic Glycoside of Cassava Leaves (*Manihot esculenta* Crantz) Via Fermentation as A Mean of Ruminant Feed Supply

CH Prayitno*, Suwarno and T Rahardjo

Faculty of Animal Science, Jenderal Soedirman University
Jln. Dr. Soeparno No. 60, Po. Box 110, Purwokerto 53123, Central Java, Indonesia
***Corresponding author email: caribu_prayitno@yahoo.co.id**

Abstract. The development of ruminants must always be followed by forage sources as its feed. The usage of agro industrial by-product like cassava leaves is one of steps that can be conducted. The purpose of this research was to study the effect of leaves-of-bitter-cassava fermentation using a mixture of *Aspergillus niger*-cattle bolus on the concentrations of HCN, crude protein, digestibilities of dry matter and organic matters. Experimental method was used in this study, using completely randomized design with six treatments namely fresh and wilted leaves of bitter cassava, added with *Aspergillus niger* and 0, 2, 4, 6, and 8% of cattle bolus, each of which was repeated four times. The results showed that the mixture of *Aspergillus niger*-cattle bolus in cassava leaves had a highly significant effect on HCN, crude protein, dry matter and organic matter digestibilities. The conclusion of this research is that fermentation of leaves of bitter cassava with 6% of *Aspergillus niger* and cattle bolus is able to degrade cyanogenic glycoside and increase digestibility.

Key Words: cassava leaves, glycoside, *Aspergillus*, cattle-bolus.

Introduction

Indonesia is a tropical country which has two seasons, dry and wet seasons, that cause difference in the quality and quantity of forages produced in the given season. Several kinds of forage species can grow in this region; however, their quality is relatively inferior compared to those from the sub-tropics.

One of the plants that are cultivated for food and feed is cassava (*Manihot esculenta* and *M. utilissima*), which represents an important plant for food and feed. The tubers are generally utilized for food, and the stems, leaves, and the peels of the tubers are generally utilized for feed. The tubers contain tapioca that is used for human needs, whereas the leaves contain high level of crude protein (20.7%, DM basis, Preston and Ly, 2003) as a protein source, and the peels of the tubers contain high level of carbohydrate, that can be used as energy source for animals.

The data from the Agricultural Department of the Republic of Indonesia (2006) shows a width of harvest area of cassava as 1,259,125.00 ha, with the greatest production of 19,507,049.00 tons, and the average production of 155.0 quintals/ha, spreads over 30 provinces in Indonesia especially Lampung, Central Java and East Java. Based on the information above, and from the data that a single plant of cassava produces tubers with an average weight of 4–5 kg and leaves with an average weight of 0.5–1.0 kg, it can be assumed that the potent production of bitter cassava leaves of 975,352.45 tons with the assumption that bitter cassava plantation area is 50% of the total cassava cultivation.

The major handicap for the usage of bitter cassava leaves for feed is their high content of sianogenic glycoside. The initial study (Caribu and Suwarno, 2006) finds out that the usage of *Aspergillus niger* as much as 6% to ferment bitter cassava leaves decreases this handicap, however, the critical point for the safety measure is not yet achieved. In this research, a combination of *Aspergillus niger* cattle bolus was studied. The objective of this research was to decrease sianogenic glycoside of bitter

cassava leaves in order to keep the safety if it is consumed by animals.

Materials and Methods

Experimental method, using Completely Randomized Design (Steel and Torrie, 1981) was used in this research. Two-stage anaerobic fermentations were conducted in 10-kg capacity poly bags. In the first-stage fermentation, there were five treatments using A. niger, each of which consisted of 5 replicates. The peel to leaf ratio was 3:1, 500 g of dry matter per experimental unit. At the 4^{th} day, the second stage fermentation was conducted by the addition of cattle bolus in accordance with the treatment. The incubation period was 6 days at room temperature. The treatments were as follows:

C0= wilted bitter cassava leaf +A. niger 6% .

C1= fresh bitter cassava leaf + A. niger 6%.

C2= fresh bitter cassava leaf + A. niger 6% + cattle bolus 2%.

C3 = fresh bitter cassava leaf + A. niger 6% + cattle bolus 4%.

C4 = fresh bitter cassava leaf + A. niger 6% + cattle bolus 6%

C5 = fresh bitter cassava leaf + A. niger 6% + cattle bolus 8%.

The measurement of HCN concentration and crude protein as follow AOAC methods. In vitro bath fermentation were carried out with rumen fluid from fistulated Onggole cross breed. The rumen fluid was withdrawn before the morning feeding and was squeezed through four layers of surgical gauze into an Erlenmeyer flask under continuous flushing with CO_2, and efforts were made to maintain the temperature at 38 to 39^0C. The substrate (feed) was milled to pass through 1 mm sieve and 300 mg was weighed in 100-ml glass syringes. After mixing, 30 ml of diluted rumen fluid was anaerobically transferred to glass syringe containing 300 mg of each substrate. Each glass syringe was sealed with syringe cap.

The data of the fermentation process were analysed using anova (Steel and Torrie, 1981). Contrast orthogonal test was used if there was any significant/highly significant effect of treatments.

Results and Discussion

Six-day fermentation process resulted in a relatively good-quality product, as marked with no clumps in the materials of leaf-peel, no fungi detected, and no effluent produced by squeezing the product. The general conditions of the products were written in Table 1.

The smell of alcohol in treatments C0, C1, and C4 indicated that there was an activity of yeast that grew during the fermentation process, converting sugars into alcohol. The colors of fermented materials were generally green chocolatish, a sign of the damage of carotene. This damage was assumed to be caused by enzymatic reactions in the materials and by heat emerging during the fermentation process. The initial pH in treatment C1, and C2 was 7, and those in the other treatments were 6.8, and down into 5.13 (treatment C4) up to 5.58 (treatment C2) at the end of the fermentation process. This was in line with the findings of Suwarno et al. (1999) that the pH of legume (alfalfa) silage were around 5. However, these values were higher than those of grass silage with the pH ranges of 4.2 to 4.8 (Walton, 1983, Umana et al., 1991) The fermentation temperatures ranged from 26 to 28^0C, with the lowest in treatment C_0, 26.5^0C. The range in the temperature was still in a tolerable range for *A. niger* to grow, namely $20–45^0$C.

The effect of treatment on HCN concentration

The concentrations of HCN of leaf-peel of bitter cassava fermented with *A. niger* among treatments showed highly significant differences in the decrease of HCN concentration ($P<0.01$), especially for the levels of 2, 4, 6, and 8% of bolus additions. The

highest percentage of HCN reduction was found out for the group of treatments with bolus additions (Table 2).

Table 2 showed that wilting process (24 hours) decreased cyanoacid as much as 34.3% (197.8 vs 130 ppm), however the concentration after fermentation with *A. niger* was 11.74 ppm a concentration that was not save yet for the consumption by animal.

The lowest HCN reduction occurred in treatment C0, 9.95%, and the highest was found in treatment C5 (fresh bitter-cassava leaf + *A. niger* + cattle bolus) 63.96%. Single step fermentation with *A. niger* (Caribu and Suwarno, 2006) was only able to decrease HCN concentration as much as 53.9%. The decrease in HCN concentrations in this study was assumed to be caused by the ability of *A. niger* and cattle bolus to convert syanogenic glycoside (HCN) by linamarin hydrolysis, thus evaporating the toxic agent, cyanide, to the air. In the fermentation process, complex compounds are converted into simpler compounds, in this case, syanide-glycoside bound is loosen or separated, converted into glucoses, acetone, and syanide. A. niger has capability to neutralize cyanogenic glycoside by the occurrence of extra cellular enzymes. states The cytochrom p-450 is a bio-catalytic enzyme that is able to catalyze linamarine glycoside and lotaustralin biosynthesis in cassava (Anderson et al. 2000; Grunhert et al. 1994, Rojas and Romeu, 1996; Edijala et al. 1999) . Harris and Shearer (2003) stated that HCN in animal feeds at level of 100 to 150 ppm is possibly hazardous and at level of higher than 150 ppm is dangerous for animal. Kobawila et al. (2005) reported that cassava is classified according to the cyanohydric acid content into 3 categorie : Very toxic variety with more than 100 mg HCN/kg pulp, moderate toxic variety with 50-100 mg HCN/kg of pulp, and not toxic variety with less than 50 mg HCN/kg of pulp. Kavana et al. (2005), Borin et al. (2005), Fasoyi, 2005b reported that ensiling has been as an effective way of reducing the content of cyanide (HCN) which is a poisonous agent for livestock contained in cassava leaves. Cattle bolus was assumed to cause the decreases in HCN concentration in this study. Hobson (1988) reports that there are some different species of bacteria, protozoa, and fungi in cattle bolus that are able to hydrolyze cyanogenic glycoside bound. The C3 treatment was only able to reduce HCN concentration as much as 58.13%, whereas C4 treatment was able to reduce HCN concentration up to 59.64%.

The effect of treatment on crude protein concentration

There was a highly significant effect of A. niger-cattle bolus treatments ($P<0.01$) on crude protein concentration of the fermented bitter cassava leaf. The increase in CP content was assumed to be caused by two factors, namely contributed by *A. niger* and microbial biomass CP of cattle bolus.

The initial CP contents in fresh and wilted bitter cassava leaves were 27.8 and 28.8%, respectively, whereas those for the fermented product ranged from 27.89–34.23% (Table 2). The highest increase in CP content occurred in C3 treatment (cassava leaf + *A. niger* +Cattle bolus, 4%), as much as 18.56%. It was assumed that in treatment C3, there was a balance between the availability of precursors for N synthesis with the rate of microbial protein synthesis. Wobeto et al. (2006) state that cassava leaf meal at 12 months old higherd levels of crude protein, carotene, iron, phosphorus, and sulfur and from the 17 old plants contained the hihhest vitamin C, zinc and calcium levels. Nhi et al. (2003); Fasuyi and Aletor (2005) state that, althought cassava leaf meal (CLM) has high concentration of protein protein, however the CLM can not be used as a single protein source for animal, due to its limiting amino acids of methionine and

Table 1. General condition of the fermentation product

Treatment	Color	pH	Smell	Fungi	Temperature (°C)	Effluent
C0	Green, chocolatish	5.40	++	None	26.50	None
C1	Green, chocolatish	5.42	++	None	28.00	None
C2	Green, chocolatish	5.58	+	None	28.00	None
C3	Green, chocolatish	5.38	+	None	27.50	None
C4	Green, chocolatish	5.13	++	None	26.75	None
C5	Green, chocolatish	5.2	+	None	27.00	None

C0= wilted bitter cassava leaf +A. niger 6%; C1= fresh bitter cassava leaf + A. niger 6%; C2= fresh bitter cassava leaf + A. niger 6% + cattle bolus 2%; C3 = fresh bitter cassava leaf + A. niger 6% + cattle bolus 4%; C4 = fresh bitter cassava leaf + A. niger 6% + cattle bolus 6%; C5 = fresh bitter cassava leaf + A. niger 6% + cattle bolus 8%; ++ = smell of alcohol; + = a little smell of alcohol; - = the absence of fungi and effluent.

Table 2. The average values of initial HCN in fresh and wilted cassava leaf, HCN in fermented cassava leaf and reduction in HCN concentrations

Treatment	Initial HCN (ppm)	HCN in fermented cassava leaf (ppm)	CP (%)	DMD (%)	OMD (%)
C0	130,75±3.51	117,74±2.54^{d}	32.484^{b}	41.33^{c}	53.46^{c}
C1	197.80±2.07	100,80±2.31^{c}	33.688^{b}	26.67^{a}	38.80^{a}
C2	197.80±2.07	79,83±2.61^{b}	27.891^{a}	35.64^{b}	47.88^{b}
C3	197.80±2.07	82,82±2.01^{b}	34.234^{b}	42.49^{c}	56.59^{c}
C4	197.80±2.07	71,31±2.55^{a}	32.156^{b}	34.62^{b}	45.08^{b}
C5	197.80±2.07	71,29±2.61^{a}	32.266^{b}	29.34^{a}	42.31^{a}

Values bearing different superscript at the same column differ significantly (P<0.05).
C0= wilted bitter cassava leaf +A. niger 6%; C1= fresh bitter cassava leaf + A. niger 6%; C2= fresh bitter cassava leaf + A. niger 6% + cattle bolus 2%; C3 = fresh bitter cassava leaf + A. niger 6% + cattle bolus 4%; C4 = fresh bitter cassava leaf + A. niger 6% + cattle bolus 6%; C5 = fresh bitter cassava leaf + A. niger 6% + cattle bolus 8%.

thyptophan (Fasuyi, 2005a). It is reported that digestibility of N in CLM ranged from 59.34 to 69.90%, with its biological value of 47.44 to 50,33% and net protein utilization of 27.70 to 33.39% with this potential, CLM is able to substitute fish meal up to 70% for broiler chicken starter periods.

The effect of treatment on dry matter digestibility

The capability of a feedstuff to prepare nutrients for rumen micro organisms and the host can be accessed from its dry matter and organic matter digestibilities. The digestibility can illustrate the characteristics of carbohydrates of the feedstuff. Digestibility can be determined from the materials that are undigested in the gastrointestinal tract or from the feed residue that stays in the rumen (Orskov, 1992). The average of in-vitro dry matter digestibility in this study showed a value of 38.245%, ranged from 26.958 to 46.723% (Table 2).

Table 2 showed that the addition of *A. niger* cattle bolus mixture for cassava leaf fermentation (C2, C3, C4) resulted in higher dry matter digestibility relative to that of fermented cassava leaf without cattle bolus (C1). This was as expected, because cattle bolus contains microorganisms that can degrade feed more effectively.

The effect of treatment on organic matter digestibility

Organic matters, the greatest components of dry matter, represent a total percentages of CP, CF, and carbohydrates that can be degraded during digestion process, and represent a total available nutrient. Carbohydrates are the principle part of organic matters in plants with the greatest amount, 50–70% of total organic matters (Tillman et al*.,* 1991).

Table 2 showed that the addition of cattle bolus as the source of micro organisms in the fermentation process caused the treatments of C2, C3, C4, and C5 had higher values of organic matter digestibility compared to that of C1 (without bolus). This pattern was similar with that of dry matter digestibility. It was assumed that the similarity in nutrient content made possible that the organic matter digestibility followed the dry matter digestibility. Fasuyi and Aletor (2005) who conducted the study of cassava evaluation as an alternative protein found out the results of apparent N digestibility of 37.00-50.08%, real N digestibility of 59.34-65.90%, with the biological value of 48.50 to 52.04%.

Conclusions

The usage of 6% of *A. niger* and cattle bolus to ferment bitter cassava leaves can decrease cyanogenic glycoside (HCN) and improve nutrient digestibility and it is save to be consumed by animals.

Acknowledgement

Appreciations were forwarded to those who participated in this research: Apriliani Fajar, Istiyana, Nining, and Dali Kurnia.

References

Anderson MD, PK Busk, Ib Svendsen and BL Moller. 2000. Cytochromes P-450 from cassava (*Manihot esculenta Crantz*) catalyzing the first steps in the biosysthesis of the cyanogenic glucosides linamarin and lotaustralin. The J. of Biol. Chemist. 275(3):1966-1975.

Borin K, Lindberg JE and RB Ogle. 2005. Effect of variety and preservation method of cassava leaves on diet digestibility by indigenous and improved pigs. Anim. Sci. 80(3):319-324

Caribu HD and Suwarno. 2006. Fermentability of post detoxified bitter cassava peel-leaf silage monoculture with Lactobaccilus plantarum monoculture. Proceedings of the 4th International Seminar on Animal Tropical Production (ISTAP). Jogjakarta.

Edijala JK, PN Okob and R Anigoro. 1999. Chemical essay of cyanide levels of short–time fermented cassava product in the Abraka area of Delta State, Nigeria. Food Chemist. 64:107–110.

Grunhert C, B Biehl and D Selmar. 1994. Compartmentation of cyanogenic glucocides and their degrading enzymes. Plants. 195: 6–42.

Harris B and Shearer JK. 2003. Nitrate, Prussic Acid (HCN) and Grass Tetany Problems in cattle feeding. Animal Science Departement, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Retrieved February 19, 2008. http:/ edis.ifas.ufl.edu (accessed: December 1, 2010).

Fasuyi AO and VA Aletor. 2005. Protein replacement value of cassava (*Manihot esculenta*) in broiler starter effect on performance, muscle growth, haemology and seryum metabolites. International J. Poult. Sci. 4(5):339-349.

Fasoyi AO. 2005a. Nutritional evaluation of cassava (*Manihot esculenta*, Crantz) leaf protein concentrates (CLPC) as alternative protein sources in rat assay. Pakistan J. Nut. 4(1):50-56.

Fasoyi AO. 2005b. Nutrient composition and processing effects on cassava leaf (*Manihot esculenta*, Crantz) antinutrients. Pakistan J. Nut. 4(1):37-42.

Hobson, P.N.1988. The Rumen Microbial Ecosystem. Elsevier Applied Science.

Kavana PY, Mtunda, Abass A and Rweyendera. 2005. Promotion of cassava leaf silage utilization for smallholder dairy production in Eastern coast of Tanzania. Livestock Research for Rural Development. Vol.17. Number 14. Retrieved January 19, 2008 http:/www.lrrd.org (accessed: December 1, 2010).

Kobawila, S.C., D. Louembe, S. Keleke, J. Hounhouigan, and C. Gamba. 2005. Reduction of the cyanide content during fermentation of cassava roots and leaves to producse bikedi and ntoba mbodi, two food product from Congo. Africal J. Biotech. 4(7).689-696.

Nhi DL, MV Sanh and LV Ly. 2003. Suplementing cassava root meal and processed cassava leaves to diets based on forage maize or natural grasses and rice straw for growing male swamp buffaloes. http://www.mekarn.org/ saec03 (accessed: December 1, 2010).

Orskov ER. 1992. Protein Nutrition in Ruminants. Academic Press.

Preston TR and J Ly. 2003. The use of Ensiled Cassava Leaves in Diets for Growing Pigs–2. The Influence of Type of Palm Oil and Cassava Leaf Maturity on Digestibility and N balance of Growing Pigs Chay. University of Tropical Agricultur Foundation. Camboja.

Rojas A and A Romeu. 1996. A. Sequence analysis of the β-glukosidase sub- family B. FEBS Letters 378 (1):93-97.

Suwarno, Wittenberg KM and WP McCaughhey. 1999. Comparative characteristics during wilting for alfalfa conditioned by macaration or by a conventional roller-conditioner. Can. J. Anim. Sci. 79:509-519.

Umana R, CR Staples, DB Bates, CJ Wilcox and WC Mahanna, 1991. Effect of microbial inoculation and (or) sugarcane molasses on the fermentation aerobic stability and digestibility of bermudagrass ensiled at two moisture contents. J. Anim. Sci., 69:4588–4601.

Walton PD. 1983. The Production and Management of Cultivated Forage. Pp. 215–228. Reston publishing Co. Inc. Reston, Virginia.

Wobeto C, AD Correa, CMP deAbreu. 2006. Nutrient in the Cassava (*Manihot esculenta* Crntz) leaf meal at three ages of the plant. Clenc. Tecnol. Aliment., Campinan. 26(4):865-869.

Tillman AD, H Hartadi, S Reksohadiprodjo, S Prawirobisono and Lebdosobodjo. 1975. Fundamental of Animal Nutrition. Gajahmada University Press. Faculty of Animal Science, Gadjahmada University. Yogyakarta.