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Enzyamtic and Oven-drying Method of Processing Rubber Seeds for Animal Feed and the Evaluation of the Toxicity of Such Feed in Rats

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Abstract: The hydrolytic activity of endogenous β-glucosidase of Hevea brasiliensis towards the cyanogenic glycosides of rubber seeds as a means of processing was investigated followed by the study of acute toxicity of the enzymatically and oven dried processed feeds from these seeds towards rats. Crushing/ grating of the rubber seeds and allowing interaction of the endogenous β-glucosidase with the cyanogenic glcosides content for a period of 60 min resulted in about 90-95% hydrolysis of the cyanogenic glycoside by the β-glucosidase. Oven drying of the crushed seeds after 60mins at a temperature of 60°C for 180min gave rise to about 81% reduction in the total cyanogens content. Ingestion of feed compounded from the oven dried seed containing 9.25 mg $CN^{-1}Kg^{-1}$ did not produce any sign of acute toxic effects in rats after 72 h. Elevation in blood glucose and Thiocyanate was observed but the activities of aspartate and alanine amino transferases and alkaline phosphatase did not show any significant change ($p < 0.05$) compared to those of control. The same was true of the total protein and serum albumin levels of the animals.

Key words: Enzymatic, oven drying, processing, H.brasiliensis, toxicity, rats

INTRODUCTION

The importance of increased livestock production in developing countries like Nigeria cannot be overemphasized. The human need for foods with high biological values such as meat and eggs and the possibility of covering them in this part of the world has become so necessary with increasing population and disease conditions. In spite of the great demand for protein of high biological value for human consumption, pigs and poultry production in the tropics (developing countries) is scanty and inefficient as a rule.

The number of cattle, sheep, goats and chicken found in developing countries in 1991 far out numbered those in developed countries but the quantity of meat and milk produced in developed countries was few higher than that produced in developing countries. The underlying reason for this situation is the system of management and feeding strategies in the developing countries[1].

The use of unconventional feeds has been advanced as one of the ways to improve animal production in the tropics[1]. Of particular interest among these unconventional feeds such as cassava peels, sorghum spent grams, wheat offals and others is the rubber seed meal. That rubber seeds could be used for feeding animals including monogastric species have been reported[2-4]. That rubber seed cake has nutritive values and is being used in cattle and poultry feeds has been reported[5]. However, the occurrence of cycnogenic glycosides, linamarin and lotaustralin in this plant (H. brasiliensis) appearns to limit the utilization for animal nutrition. This is because enzymatic hydrolysis of the glycosides by endogenous β-glucosidase, linamarase, occur when the plant tissue is damaged either mechanically or otherwise, thereby liberating HCN which is a deadly poison. Despite this constraint, some millers for livestock feed in Nigeria have started including rubber seed and other cyanogenic plant materials in their various feeds prepared fir domestic animals[6].

A wide variety of different methods of processing the rubber seeds to reduce their content of cyanogenic glycosides and hence their toxicity are known. These methods comprise of different combinations of drying, soaking, boiling and fermentation of whole seeds; all of these processes reduce the total cyanide content of the seeds. The most effective treatments for reducing for reducing the cyanide content was reported to be 20 h of soaking in water combined with 1hrs of cooking[7]. However, a simpler and more effective treatment could be achieved using enzymatic hydrolysis. In view of this, it was thought worthwhile to evaluate the use of endogenous β-glucosidase together with over drying in the detoxification of cyanogenic glycosides as a means of processing these seeds for animal feed.The possible acute effect of such processed feed toward animals will also be investigated.

MATERIALS AND METHODS

The Hevea brasiliensis seeds used in this experiment were collected from the Rubber Plantation of Michael Okpara University of Agriculture Umudike, Nigeria.

Treatment of seeds: Fresh seeds were used because the seeds deteriorate very rapidly after falling on the ground, due to moisture content. Shelled seeds were macerated/grated and the endogenous β-glucosidase was allowed to interact with the cyanogenic glycosides for 60mins. The cyanogenic glycosides content of the seeds was determined immediately after maceration and at an interval or 10 mins for 60 mins according to the method of[8]. This was followed by oven drying the mash at 60°C for 180 mins. The total cyanogen content of the mash was determined at the end of 180 mins. Cyanide release from the rubber seed by exogenous β-glucosidase was also carried out. This was to monitor the extent of hydrolysis of the cyanogenic glycoside content of the seeds by the endogenous enzyme. The seeds were first extracted in orthophosphoric acid (0.1 M) solution and then incubated with linamarase from cassava with activity of 3 units mL^{-1}. The cyanide released was then determined according to the method of Egan *et al*[8].

Animal experiment: The Hevea brasiliensis seeds processed as described above were used to formulate feed for rats. The composition of the feed was 76% rubber seed, 10% protein supplement in the form of defated soy bean, 4% vitamin mixture, 4% oil 4% bannana flavour and 2% salt mixture.

Animal treatment: 12 albino rat weighing 120 g on the average were used for the feeding experiment. The rats were allowed to acclimatize to the laboratory environment for about 7days. This was followed by starving them for 24 h to ensure that most of the cyanogens in their blood and urine if any, were eliminated. The animals were grouped into 4 with group 1 as the control. Groups 2,3 and 4 were fed rubber seed meal for 24, 48 and 72 h, respectively. The animals were sacrificed after each time interval and their blood and urine collected for cyanide, thiocyanate and liver enzyme determinations.

Assay for cyanide and thiocyanate: The cyanide and thiocyanate of blood and urine were measured using the methods of, respectively.

Enzyme assay: The assay for some plasma enzymes as indicator of changes in liver, kidney and muscle cell damage resulting from cyanide poisoning was carried out. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were as recommended by Reitman and Reitrnan *et al*[9] alkaline phosphatase as described by[10]. Glucose was also determined using glucose oxidase method[11], total protein by Biuret method as described by Layne and serum albumin by the method of Doumas *et al*[12].

RESULTS AND DISCUSSION

From the data reported in Table 1, the results show that the endogenous β-glucosidase of the seeds has high hydrolytic activity toward the cycnogenic glucosides. About 90-95% of the total cyanogenic glycoides of seeds were hydrolyzed within 60minites as measured by the release of HCN. The cyanide release by the endogenous β-glucosidase showed higher degree of hydrolytic activity when compared to that of cassava linamarase under the assay conditions.

The implication of the high hydrolytic activity of the seed β-ghcosidase is that it could be used during processing of rubber seeds for animal feed or industry. This could be achieved by crushing/grating the seeds and allowing the endogenous enzyme to hydrolyze the cyanogeic glucosides content for a period of 60mins or more. This will result in the breakdown of most of the cyanogenic glycosides to give hydrocyanic acid and other non-glucosidic cyanide that could be easily eliminated form the preparation.

Oven drying of the mashed seeds at 60°C for a period of 30-180 mins resulted in about 81% total cyanogen removal (Table 2). The high reduction in cyanide levels of the marshed seeds resulted from the release of endogenous β-glucosidase enzyme, which hydrolyzed the cyanogenic glycoside content to give volatile hydrocyanic acid. H. brasiliensis linamarase has been reported to be active even at 80°C[13]. Thus, marshing the seeds of this plant, allowing the interaction of its cyanogenic glycosides content with endogenous β-glucosidase, followed by oven drying resulted in dried mash with very low level of residual cyanide.The benefits of processing these seeds into animal feed cannot be overemphasized. Rubber Seed Meal (RSM)

Table 1: Total cyanide release from Hevea brasiliensis seed by endogenous and exogenous β-glucosidases

	Cyanide production mg $100g^{-1}$	
Incubation time (min)	Endogenous β -glucosidase	Exogenous β -glucosidase
10	68-90	9.12
20	158.80	15.05
30	215.03	18.71
40	270.86	22.18
50	275.62	47.52
60	353.42	63.52

Total cyanide content of fresh seed was determined to be 391.6 $mg100^{-1}g$

Table 2: Percentage cyanide removal during oven drying of marshed rubber seeds at 60°C

Time (min)	% cyanide removal
30	36.89
60	45.86
90	50.32
120	62.18
150	77.38
180	81.27

Table 3: Biochemical effects on rats fed with rubber seed meal for 72 h

Blood parameter	T_{24}	T_{48}	T_{72}	C72
Total protein (mg dL^{-1})	5.23	5.48	5.39	5.7
Serum Albumin (g dL^{-1})	3.9	3.8	3.9	3.8
Glucose (mg/dl)	56.00	60.00	67.00	48.00
ALT (iu L^{-1})	21.00	24.00	21.00	22.00
AST (iu L^{-1})	67.00	65.00	62.00	66.00
Alkaline phosphatase (iu L^{-1})	30.00	33.00	30.00	30.00
Serum thiocyanate	9.12	11.16	12.13	7.30

could be used successfully for broilers depending on the source of animal protein[14]. The seeds have been reported to be a good source of energy, calcium, valine, isolecicine, phenylalanine and tyrosine[7]. Ravindran[15] has demonstrated that it is possible to formulate layer diets using rubber seed meal and achieve acceptable production and lower food cost under small farm conditions in tropical countries. In spite of these and other reports of successful use of the seeds in animal feeding, arguments still persist in scientific circles as to the safety of rubber seed meal for sustainable livestock production. Reduction in growth, response particularly in broilers and growers less than 3 months of age when rubber seed meal was supplemented for up to 30%[14] , lowered egg production, thickness, hatchibility of incubated eggs and weight of chicks hatched out[15] are among the report against the use of rubber seeds in animal production especially as constituent in breeders rations. These effects may be attributed to amino acid imbalance[14] of RSM that lowers the biological value of protein and the presence of an artificial factor which has not been identified yet Rajaguru *et al.*[15].

It is worthy to note that deleterious factors such as cyanogens when present in animal feed could result in growth depression and hamper hatchibility of incubated eggs[13]. In addition, they result in depletion of amino acids of the body when ingested. This is because sulphur-containing amino acids are needed for cyanide detoxification resulting in a condition akin to amino acid imbalance and hence reduced protein synthesis. Thus, proper processing, of these seeds to reduce the total cyanogens content will result in improved performance when used as animal feed.

Ingestion of feed compounded from the oven dried mashed seeds containing 9.25 mg CN^{-1} Kg^{-1} by rats resulted in non-significant ($p<0.05$) changes in the blood total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase activities; (Table 3) indicating that such feed may not have acute toxic effects on animals. Therefore the use of rubber seed meal as animal feed should be encouraged and the use of its endogenous enzyme for processing during feed formulation should be imbibed.

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