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Effect of Detoxification on the Nutrient Content of *Thevetia peruviana* Seed Cake

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Abstract: The aim of this research was to investigate suitable detoxification method(s) for thevetia seed meal. This should lead to the production of a meal with significantly reduced cardiac glycoside content with minimal loss of nutrient. Acid hydrolysis of the defatted meal followed by alcoholic extraction of the freed aglycones with 80% aqueous alcohol gave a detoxified meal with 95% reduction in the toxic glycoside content (from 42.7 g kg^{-1} to 2.15 g kg^{-1}) and a 3.9% increase in crude protein (from 42.79 to 44.45%) but with a 48% loss of meal (100 g defatted meal giving 52 g detoxified meal), while direct alcoholic extraction of the glycoside led to a 98% reduction in the glycoside content (from 42.7 g kg^{-1} to 0.83 g kg^{-1}); a 25.3% increase in crude protein content (from 42.79 to 53.6%) and an 18% loss of material (with 100 g defatted meal giving 82 g detoxified meal). The results showed that alcoholic treatment is more effective for reducing the toxin content of thevetia meal with minimal loss of material and nutrient than acid hydrolysis.

Key words: Detoxification, toxins, cardiac glycosides, aglycones

INTRODUCTION

Many seeds that could be potential sources of protein for animal feed contain a number of toxins and antinutrients which reduce their nutritive values. The antinutrients in oilseeds are of various types and nature, and have different effects on ingestors. Generally they may be divided into a heat-labile group consisting of lectins, proteinase inhibitors and cyanogens which are sensitive to standard processing temperatures, and a heat-stable group including among others, tannins, alkaloids, glucosinolates, saponins, etc. (Liener and Kakade, 1980).

Thevetia peruviana, juss, more commonly known as yellow oleander, be-still tree, milk bush etc, belongs to the order apocynales and the family apocynaceae. It is a native of tropical America, but has naturalized in tropical and sub-tropical regions of the world, where it is usually grown as an ornamental shrub. The seeds of *T. peruviana* contain about 60-65% of oil (Ibiyemi and Faloye, 1988) and the defatted cake is 30-37% rich in protein. However, thevetia plant has remained primarily an ornamental plant because of the toxic nature of the plant. The toxins are mostly cardiac glycosides and their free aglycones. Various glycosides have been isolated from the seed, thevetin being major (Sun and Libizor, 1965). Other glycosides that have been isolated from thevetia plant include thevetoxide, theveside, neriifolin, cerberin, peruvoside, perusitin, evomonoside etc (Perez-Amador *et al*., 1994; Lang and Sun, 1965; Huang *et al*., 1966; El Tanbouly *et al*., 2000).

Several previous attempts have been made at utilizing various treated thevetia seed cake as protein source in animal feed formulation but these have not been very successful. The feed experiment results show that with dietary levels as low as 10% replacement of soybean or groundnut with thevetia seed cake, the mortality rate was high, feed consumption and utilization was low, and general performance of the birds used in the experiment was poor (Atteh *et al*., 1995). Attempts to detoxify the seed cake by heat treatment, autoclaving or fermentation were not successful either as evident by other results of feed experiments (Odetokun *et al*., 1999; Taiwo *et al*., 2004). This might imply that detoxification of the cake was not achieved by the treatments earlier mentioned, thus the need for a method or methods that will detoxify thevetia cake and effect removal of most freed aglycones. This reports two cost effective methods of detoxification, one involving the hydrolysis of the glycoside and removal of the aglycones with ethanol; while the other is a direct extraction of the glycosides with ethanol.

MATRIALS AND METHODS

Matured fruits of thevetia plant were collected from a location in Ilorin, Kwara State, Nigeria, by direct plucking of matured (black) fruits from plants and by picking those that have fallen off plants. The fruits were cracked to remove the hard pericarp and mesocarp and the soft seeds crushed into a paste. The paste was defatted first by mechanical pressing, followed by solvent extraction using pre-distilled n-hexane.

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Two different methods were employed in the detoxification experiments. The first was the solvent extraction of the defatted cake using aqueous alcohol mixture, while the second involved the hydrolysis of the cake prior to solvent extraction.

Detoxification by solvent extraction was done using a modification of the method of Finnigan and Lewis (1988). Eighty percent aq. solution of $EtOH^{-1}MeOH$ (8:2) was used to soak the defatted meal twice. A solvent to meal ratio of 10:1 was used the first time and the mixture was stirred well and left overnight. The solvent was then decanted and fresh solvent added (ratio 5:1) and this was also left overnight. The final product was then pressed free of solvent and the cake air-dried.

The hydrolysis of the cake was done using the method of Usman *et al.* (2003). 0.1M HCl was used for the hydrolysis and mixed with the defatted cake in ratio 4:1 (solvent: meal). The hydrolyzed cake was air dried before solvent extraction as described above.

***Nutrient evaluation of thevetia cake*:** Standard methods of the Association of Official Analytical Chemists (AOAC, 1984) were used in the nutritional evaluation of crude and detoxified thevetia seed cake. Parameters tested include crude protein content, crude fat, total ash, crude fibre and moisture content. Crude protein (% total nitrogen ×6.25) was determined by the Kjeldahl method (Kjeldahl, 1883), using 2.0 g samples: Crude fat was obtained by exhaustively extracting 5.0 g of each sample in a Soxhlet apparatus using petroleum ether (boiling point range 40-60°C) as the extractant. Ash was determined by the incineration of 1.0g samples placed in a muffle furnace maintained at 550°C for 5h. Crude fibre was obtained by digesting 2.0g of sample with H_2SO_4 and NaOH and incinerating the residue in a muffle furnace maintained at 550°C for 5h. Moisture content was determined by heating 2.0 g of each sample to a constant weight in a crucible placed in an oven maintained at 105°C. Total carbohydrate was obtained by difference and the energy content calculated by multiplying the mean values of crude protein, crude fat and total carbohydrate by Atwater factors of 4, 9 and 4, respectively, taking the sum of the products and expressing the result in kilocalories per 100 g sample as reported by Onyeike and Acheru (2002). The buffer extractable protein was determined by a modification of the method of Schroeder (1982). 1g of each sample was extracted twice with 0.1 M phosphate buffer (pH 7.0-7.2) containing 5% NaCl for 1.5 h on an end-over-end shaker. The final weight: volume ratio was 1: 25. The extracts were centrifuged at 4000 g for 30 min. The combined supernatants were filtered and the protein evaluated by the biuret method and expressed relative to crude protein.

Quantitative determination of cardiac glycosides in thevetia cake: The quantity of glycosides in the raw and treated samples was evaluated using Baljet's reagent (95 mL aqueous picric acid + 5 mL 10% aqueous NaOH) as described by El-Olemy *et al.* (1994). Digitalis cardiac glycosides develop an orange-red colour with Baljet's reagent. The intensity of the colour produced is proportional to the concentration of the glycosides. This colour formation is made use of for the quantitative estimation of cardiac glycosides present in thevetia. One gram of each sample was extracted by soaking overnight with 10 mL of 70% alcohol and filtered. The extracts were then purified using lead acetate and Na_2HPO_4 solutions before the addition of freshly prepared Baljet's reagent. The intensity (absorbance) of the colour produced was then measured using a spectrophotometer at 495 nm. A blank was carried out at the same time using distilled water and Baljet's reagent. The intensity of the colour produced is proportional to the concentration of the glycoside.

RESULTS AND DISCUSSION

There is a general loss of material consequent of the detoxification process. For instance, 100 g of raw thevetia meal produced about 82 g of alcohol treated meal, while it gave about 52 g of acid treated meal. This may explain the increase in crude protein of the detoxified meals. Assuming that the materials lost are mainly non-protein materials, the final product is expected to have a higher percentage of protein than the starting material. Table 1 shows the proximate composition of thevetia seed meal. The protein content was calculated from the nitrogen content using the conventional conversion factor 6.25.

Table 1: Proximate composition (%) of raw and detoxified Thevetia Seed Meal (TSM)

Constituents	Raw TSM	Acid treated TSM	Alcohol treated TSM
Moisture	6.76±0.31	8.40±0.28	8.83±0.20
Dry matter	93.23±0.31	91.60±0.28	91.17±0.20
Total ash	6.34±0.13	4.90±0.40	7.85±0.17
Crude protein	42.79±0.46	44.45±0.05	53.60±0.22
Crude fat	4.40±0.39	4.68±0.10	4.07±0.43
Crude fibre	3.22±0.64	3.92±0.20	2.55±0.30
Total Carbohydrate	36.48±0.76	33.65±0.44	23.09±0.26
Calorific value (kCal 100 g^{-1} sample)	357	355	343

Values are means±standard deviations of triplicate determinations

Table 2: Buffer extractable protein of Thevetia Seed Meal (TSM)

	Buffer extractable protein	
Sample	% seed meal	% crude protein
Raw TSM	5.86±0.27	13.69±0.27
Acid treated TSM	2.15±0.09	4.83±0.09
Alcohol treated TSM	2.88±0.19	5.37±0.19

Values are means±standard deviations of triplicate determinations

Table 3: Cardiac glycoside content of Thevetia Seed Meal (TSM) samples (expressed as digitoxin content)

	Total cardiac glycoside	
Sample	%	g kg^{-1}
Raw TSM	4.27±0.44	42.7±0.44
Acid treated TSM	0.22±0.71	2.15±0.71
Alcohol treated TSM	0.08±0.25	0.83±0.25

Values are means±standard deviations of duplicate determinations

The two detoxification methods employed resulted in an increase in the protein content (3.9% increase in the acid detoxified and 25.3% increase in the alcohol treated meal). Moisture content followed the same trend with a 24.3% increase in the acid detoxified sample and 30.6% increase in the alcohol treated meal. The increase in the moisture content can be attributed to the water that is used in the detoxification processes. The different trend observed for the ash content with the acid treated meal having the lowest value while the alcohol treated meal has the highest ash content is interpreted to suggest that acid treatment has resulted in the loss of some mineral elements which were not lost by alcohol treatment.

The buffer extractable proteins i.e., globulin and albumin (Table 2) also showed that treatment with alcohol gave a meal with a higher protein extractability than the acid treated meal which involves heat treatment. This result corresponds with what has been established by Schwenke *et al.* (1990) for the flour of rapeseed whereby the solubility of the nitrogen in rapeseed meal decreases after soaking and alcohol/water treatment but remains higher than that of heat treated flours or protein concentrates. Rauchberger *et al.* (1979) reported that protein solubility in water at 25°C decreased from about 70% in rapeseed meal prepared from unheated seeds to about 15% following 30 min. steaming.

Table 3 shows the content of cardiac glycoside, calculated as digitoxin, in the various thevetia seed meals and the results show a marked reduction in glycoside content irrespective of detoxification method employed. Acid detoxification resulted in a 95% reduction in the cardiac glycoside content while alcohol treatment gave a 98% reduction.

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