Tropical and Subtropical Agroecosistems 4 (2004): 7 -14

Tropical and Subtropical Agroecosystems

EFFECT OF *Thevetia peruviana* SEED CAKE-BASED MEAL ON THE GROWTH, HAEMATOLOGY AND TISSUES OF RABBITS

[EFECTO DE LA PASTA DE SEMILLA DE *Thevetia peruviana* SOBRE EL CRECIMIENTO, HEMATOLOGIA Y TEJIDOS DE CONEJOS]

V.O. Taiwo[1]*, O.O. Afolabi[2] and O.A. Adegbuyi[1]

[1]Department of Veterinary Pathology, University of Ibadan, Ibadan. Nigeria;
[2]Livestock Improvement Programme, Institute of Agricultural Research & Training, Obafemi Awolowo University, Ibadan. Nigeria.
** Corresponding author*

SUMMARY

The effect of two inclusion levels of raw and processed *Thevetia peruviana* seed cake as replacement for soyabean in the diets on growth, haematology and tissue pathology of 50 New Zealand x Chinchilla crossbred rabbits was studied. Processing of the *Thevetia* seeds was carried out by deffating, oven drying and autoclaving at 120°C for 12 hours. Five groups (A, B, C, D, and E) of 10 rabbits each were allotted as follows: Group A, normal pelleted rabbit ration (controls), groups B and C, raw *T. peruviana* seed cake at 5% and 10% inclusion levels, groups D and E, processed seed cake at 5% and 10% inclusion levels, respectively. Clinical signs were observed daily and weight changes monitored once weekly during the 35-day feeding trial. Haematology and plasma protein levels were determined weekly, while rabbits that died were autopsied and tissues processed routinely for pathological examination by light microscopy.

The control rabbits (group A) gained weight progressively during the feeding trial and no mortality was recorded in this group. There was progressive weight loss and 100% mortality in rabbits fed with raw ground *Thevetia peruviana* seed cake at both 5% and 10% inclusion levels within 14 days (group C) and between 21 and 28 days (group B) of feeding trials, respectively. While no mortality was recorded in rabbits of groups D and E, they looked unthrifty and did not gain any appreciable weight. Reduction in feed intake, diarrhoea, rough, dry coat, mydriasis and hyperaesthesia were exhibited by most survivors; while muscle spasm, ataxia, paralysis of hind limbs and severe convulsions preceded death especially in rabbits of groups B and C.

Rabbits in groups B, C, D and E developed varying degrees of normocytic normochromic anaemia between 7 and 14 days, but later (28–35 days) the survivors developed hyperproteinaemia and polycythaemia indicating progressive dehydration. The anaemia and polycythaemia were more severe in groups D and E rabbits. No appreciable leucocytes changes were observed in all the test rabbits compared to the controls.

At autopsy, there was pulmonary congestion and oedema, focal or diffuse areas of hepatic and cardiac degeneration and necrosis, renal cortical degeneration and necrosis, hyaline degeneration and thickening of tunica media of blood vessels, bile duct hyperplasia, meningeal congestion, neuronal degeneration, neuronophagia, and cerebrocortical spongiosis and gliosis.

This study has revealed that raw *Thevetia* seed cake, even at as low as 5% replacement of soybean meal in the diet, is very toxic and lethal to rabbits. Processing by defatting and autoclaving appears to have removed some of the toxic and anti-nutritional factors, but could still not support productive growth. Further research is recommended to find better ways of processing, as well as determine appropriate inclusion levels of *Thevetia* seed cake that will support economic production of rabbits.

Key words: *Thevetia peruviana* seed cake, soyabean replacement, processing, rabbits, growth, toxicity.

RESUMEN

Se estudio el efecto de diferentes niveles de inclusion de pasta de *Thevetia peruviana* cruda y procesada, como reemplazo de pasta de soya en dietas y se evaluó el crecimiento, valores hemáticos y patología de tejidos de conejos New Zealand x Chinchilla. El procesamiento de las semillas de *Thevetia* consistió en eliminación de grasa, secado y autoclavado a 120 °C por 12 h. Cincuenta conejos fueron distribuidos en 5 grupos de 10 conejos cada uno: A: alimento peletizado normal para conejos (controls), grupos B y C, pasta de *T. peruviana* cruda al 5% y 10% de inclusión, grupos D y E, pasta procesada al 5% y 10% de inclusión. Se observó diariamente para signos clínicos y los cambios de peso de semanalmente durante los 35 días de la prueba. Se realizaron análisis hematológicos y de proteína plásmatica semanalmente. Se tomaron muestras de tejidos para estudios patológicos de rutina de aquellos conejos que murieron.

Los conejos del grupo control (grupo A) incrementaron de peso de manera durante la prueba y no registraron mortalidad. Hubo una pérdida de peso progresiva y 100% de mortalidad en un período de 14 días para el grupo C y entre 21 y 28 días para el grupo B. Aunque no se registro mortalidad en los animales de los grupos D y E los animales no ganaron peso y lucían en mal estado. Se observó reducción en el consumo de alimento, diarrea, pelaje tieso y seco y midriasis en la mayoría de los animales sobrevivientes, mientras que espasmos musculares, ataxia, parálisis de tren posterior y convulsiones severas precedieron la muerte, especialmente para aquellos animales del grupo B y C.

Conejos de los grupos B, C, D y E desarrollaron diversos grados de anemia normocítica normocrómica entre los días 7 y 14, pero posteriormente (28-35 días) los sobrevivientes desarrollaron hiperproteínemia y policitemia indicando deshidratación progresiva. La anemia y policitemia fueron más severos en los grupos D y E. Comparados con el control, no se observaron cambios apreciables en la cuenta de leucocitos.

En la necropsia se observó congestión pulmonar y edema focal o áreas de degeneración o necrosis hepática y cardiaca, degeneración y necrosis cortical renal, degeneración hialina y engrosamiento de la túnica media de los vasos sanguíneos, hiperplasia del conducto biliar, congestión meníngea, degeneración neuronal, neuronofagia y espongiosis cerebrocortical y gliosis.

Inclusión en la dieta de solo 5% de pasta cruda de *Thevetia* es tóxica y letal para conejos. Procesamiento (desengrasado y autoclavado) parece remover algunos factores tóxicos y antinutricionales, pero no lo suficiente para promover el crecimiento.

Palabras clave: *Thevetia peruviana*, procesado, conejos, crecimiento, toxicidad.

INTRODUCTION

Rabbits have been known to be a good source of animal protein, especially in developing countries like Nigeria with acute shortage of proteins of animal origin (Fadina *et* al., 1999). Rabbits, being highly prolific and relatively easier to rear compared to the other livestock, have recently attracted the patronage of homestead retired public servants and middle level livestock farmers. The major constraint is feeding, and sources of plant proteins are not only limited, they are very expensive because of competing needs of these sources by man and other livestock. Rabbits given a diet less than 12% of crude protein eat less and utilize food less (personal experience). The proteins can be of plant and animal origin (soybean, groundnut, cottonseed meal, etc). However, because of high cost of plant and animal proteins, attempts have been made to look into alternative sources of protein at a cheaper rate. The percentage of proteins in various feeds differs widely and ranges from 39% in cane molasses, 3.9% in cottonseed meal to 40% in soybean meal and peanut meal.

Steyn (1934) and Nair *et al.* (1982) reported that *Thevetia peruviana* seed contained about 35% protein and this is comparable in quality to soybean meal. *Thevetia peruviana* seed can be made into cake and used as a protein supplement, while the plant is highly nutritious and has medicinal uses (Watt and Breyer-Brandwijk, 1962; Singh *et al*., 1980; Ramirez *et al*., 1988). It is also reported that raw *Thevetia* cake is toxic to livestock and man (Nayar, 1957; Inman, 1967; Ahlawat *et al*., 1994; Eddleston *et al*., 2000), and horses (Siemens *et al*, 1995), hence needs further processing before it can effectively be used as an ingredient in livestock feeds.

Thevetia peruviana (yellow oleander) is mostly grown as ornamental tree in gardens. Currently, there is no human dietary or commercial demands for the seed, which is very cheap compared to other conventional protein concentrates. The only cost involved in *Thevetia* cake production is that of seed collection and crude fat extraction by Soxhlet method. However, it is rarely utilised either in human diets or livestock feed because of presence anti-nutritional toxic components of the seed such as cardiac glycosides, phenols, terpinoids, oxalates, phytic acid and saponins (Daniel and Sabnis, 1978; Begum *et al*., 1993; Abe *et al*., 1995; Oji and Okafor, 2000).

Plants containing cardiac glycosides are usually unpalatable and are often ingested when clippings from ornamental plants are accidentally provided to animals. Other sources are when cuttings from a garden are thoughtlessly thrown into a dry lot, especially when animals are accustomed to having heaps of hay placed before them. All parts of *Thevetia peruviana* are very poisonous, especially the sap and the oily seeds (Langford and Boor, 1996). Human toxicities from have occurred after consuming teas brewed from plant parts or after consuming leaves, flowers or seed from plants containing cardiac glycosides (Langford and Boor, 1996).

In assessing the usefulness of the protein cake of *Thevetia peruviana* in poultry feeds Odetokun *et al.* (1999) reported that replacement of soybean in the diets with 10%, 20% and 30% *Thevetia peruviana* produced satisfactory survival, but 40% and above produced high mortality, great reduction in body weight, feed intake and feed conversion efficiency. The use of *Thevetia peruviana* seed in commercial feed production has not been given a serious attention in the livestock industry in Nigeria. The main aim of this experiment was to determine the suitability or otherwise of using raw and processed *Thevetia peruviana* seed cakes at 5% and 10% inclusion levels in the meal of rabbit on their haematology, plasma biochemistry and tissue pathology, in case of any mortality. It is also aimed at assessing good replacement levels of *Thevetia* seed cake in the diet of the animals. The results from this study will assist in solving some of the problems associated with conventional sources of plant proteins for livestock thus tapping this largely unused and cheap resource, thus making the more conventional plant protein sources more non-competitive and adequate for human consumption.

MATERIALS AND METHODS

Animals and Preparation of *Thevetia peruviana* Seed Cake Diets

Fifty rabbits (New Zealand x Chinchilla crossbreed), aged 4 months were used for the experimental work. They were purchased from the Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The rabbits were acclimatized to their new housing environment for one week during which they were fed with commercial pelleted livestock feed (growers' mash) and given clean drinking water *ad libitum*.

Ripe *Thevetia* seeds were harvested, their epicarps removed and kernels broken to remove the dried seed. Two experimental diets were prepared: raw *Thevetia* seed cake and processed *Thevetia* seed cake. The processing was carried out by initial sun-drying of seeds, defatting by Soxhlet extraction and finally by autoclaving at 120^{o}C for 12 hours. Both the raw and processed seeds were grounded into fine powder, pelleted and added to commercial rabbit feed as shown in Table 1.

Table 1. Composition (%) of experimental diets containing raw or processed *T. peruviana* seed cake inclusions

	Diet groups (inclusion levels as replacement of soybean meal)				
		Raw		Processed	
Ingredients	A (0%)	B (5%)	C (10%)	D (5%)	E (10%)
Maize	25.00	25.00	25.00	25.00	25.00
Maize offal	34.00	34.00	34.00	34.00	34.00
Soybean	20.00	15.00	10.00	15.00	10.00
Thevetia seed	0.00	5.00	10.00	5.00	10.00
Brewers' dried grain	18.00	18.00	18.00	18.00	18.00
$CuSO_4$	0.10	0.10	0.10	0.10	0.10
Vitamin premix	0.30	0.30	0.30	0.30	0.30
Lysine	0.10	0.10	0.10	0.10	0.10
Bone meal	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00

Feeding and Monitoring of the Rabbits

The rabbits were divided into five groups of ten each, corresponding to the five diet types (i.e. groups A, B, C, D and E). They were given the control and test diets for 5 weeks (35 days). During this period, the rabbits were monitored daily for clinical signs and mortality and weighed once weekly using a suspended spring weighing scale.

Haematology

The rabbits were mildly sedated with diethyl ether applied to the nostrils through soaked cotton wool in a conical flask and blood was collected by slightly traumatizing the medial canthus of one eye with a plain capillary tube into heparinized tubes and gently mixed to prevent clotting. The following parameters and indices were determined as described by Jain (1986): packed cell volume (PCV), haemoglobin (Hb) concentration, erythrocyte (RBC) counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total and differential leucocyte (WBC) counts, total plasma proteins, albumin and globulin levels.

Organ Pathology

Postmortem examination was performed on dead rabbits immediately after death. The internal organs were exposed by dissection, and the liver, heart, spleen, kidney, lungs, and brain were observed for gross lesions. At the end of the 35-day feeding trial, two rabbits each from groups where no mortality was recorded, i.e. groups A, D and E, were sacrificed by exsanguinations and postmortem also carried out. Small portions of each organ were preserved in 10% phosphate-buffered formalin for 24 hours. The organ sections were dehydrated with graded concentrations of xylene, embedded in paraffin, sectioned at 5µ, and stained routinely with heamatoxylin and eosin (H&E) for histopathological examination using the light microscope.

All the data from each group of rabbits were subjected to statistical analysis using 2-way analysis of variance (ANOVA) and Duncan's multiple range test (SAS, 1987; Duncan, 1959) for the determination of differences, if any, between the test and control rabbits and within the periods of the feeding trial. Significant differences were based on 95% confidence interval or $p<0.05$ at each level.

RESULTS

Clinical Signs, Weight Changes and Mortality Patterns

The control rabbits (group A) showed no untoward clinical signs and recorded no mortality throughout the study period. However, all the test rabbits (i.e. groups B, C, D and E) showed initial anorexia within the 1st week, which changed with time. Rabbits in groups B and C showed the most severe clinical signs from 14 days onwards. These signs, which were variable and increased with time until death, include spontaneous and severe convulsions, extensor rigidity, muscle spasm, hyperpnoea, dyspnoea and mydriasis. The rabbits were usually found dead with head and neck bent backwards. Rabbits in groups D and E showed milder but more prolonged symptoms than those of groups B and C.

The results of the weight changes are shown on Table 2. The control rabbits (group A) recorded progressive weight gains from 1.7±0.1kg on day 7 to 2.4±0.1kg on day 35 of study. However, while rabbits in groups B and C recorded progressive weight losses before dying, those in groups D and E did not show any appreciable weight gain.

Table 3 shows the mortality pattern of the rabbits in each group. Mortalities were recorded only in groups B and C. All 10 (100%) rabbits in group C died within 14 days of the feeding trial, while deaths in group B occurred between 21-28 days. No mortalities were recorded in rabbits of groups D and E throughout the feeding trials.

Table 2. Weight changes in rabbits fed raw or processed *T. peruviana* seed cake based diets

Rabbit Group	Days of Feeding Trial 7	14	21	28	35
A	1.7 ± 0.1^{b}*	2.1 ± 0.1^{ab}	2.3 ± 0.1^{ab}	2.5 ± 0.1^{a}	2.7 ± 0.2^{a}
B	2.2 ± 0.1^{a}	1.6 ± 0.3^{b}	1.2 ± 0.1	Died	Died
C	1.6 ± 0.1^{a}	1.2 ± 0.2^{b}	Died	Died	Died
D	2.1 ± 0.1^{ab}	2.4 ± 0.2^{a}	2.2 ± 0.1^{ab}	2.2 ± 0.1^{ab}	2.0 ± 0.2^{ab}
E	1.9 ± 0.1^{a}	2.1 ± 0.0^{a}	1.8 ± 0.2^{a}	1.9 ± 0.1^{a}	2.1 ± 0.2^{a}

*Data expressed as mean ± standard error of mean.
Data along the same row with different superscripts differ significantly ($p<0.05$)

Table 3. Mortality pattern in rabbits fed raw or processed *T. peruviana* seed cake based diets

Rabbit Group	Days of Feeding Trial 7	14	21	28	35
A	0 (0%)*	0	0	0	0
B	0	0	4 (40%)	6 (60%)	-
C	0	10 (100%)	-	-	-
D	0	0	0	0	0
E	0	0	0	0	0

* - Number of dead rabbits (%) in each group

Haematology

The results are as shown on Table 4. While the haematological parameters and indices of control (group A) rabbits remained unchanged ($p>0.05$) throughout the feeding trial, slight normocytic normochromic anaemia ($p<0.05$) was observed in rabbits of groups B, C, D and E on days 7 and 14 of feeding. However, polycythaemia ($p<0.05$; $p<0.01$), characterized by abnormally high PCV, Hb concentration and RBC counts, was observed in all the rabbits on days 28 and 35 of the feeding trial. No significant variations were observed in both total and differential WBC counts in all the experimental rabbits throughout the study period (data not shown). Significant hyperproteinaemia ($p<0.05$), notably hyperglobulinaemia, was recorded in rabbits of groups B, C and E on most of the days of the assay, while no appreciable variations occurred in the plasma protein levels of the control rabbits throughout the feeding trial (Table 4).

Organ Pathology

Gross lesions observed in self-dead rabbits include rough hair coat, sunken eyes, emaciation, dehydration and pasted hindquarters. Other lesions include pulmonary and meningeal congestion; the livers were pale and friable and had focal areas of necrosis on the surfaces. The cortex of the right kidney of one rabbit in group B was rough and had focal depressed areas. Pale areas of necrosis were observed on the myocardia of two rabbits in group C, while currant jelly blood clots were found in the heart chambers of all dead rabbits. Apart from varying degrees of dehydration and ruffled coats, no gross lesions were observed in any of the sacrificed rabbits. Table 5 shows the qualitative and semi-quantitative grading of organ histologic lesions based on presence or absence, spread, severity and consistency of occurrence per rabbit that died or were sacrificed in each group. These lesions include pulmonary congestion and oedema, focal or diffuse areas of hepatic and cardiac degeneration and necrosis, renal cortical degeneration and necrosis, hyaline degeneration and thickening of tunica media of blood vessels, bile duct hyperplasia, meningeal congestion, neuronal degeneration, neuronophagia, and cerebrocortical spongiosis and gliosis. Splenic lesions consist mainly of hyalinized blood vessels and lymphoid depopulation.

DISCUSSION

This work studied the effects of different inclusion levels of raw and processed *Thevetia peruviana* seed cake in the diets on performance, haematology and tissue pathology of rabbits. *Thevetia* seed was processed by deffating using diethyl ether, oven drying and autoclaving at 120°C for 6 hours, ground and pelleted. Both raw and processed *Thevetia* seed cakes were included in the diets of the rabbits at 5% and 10% replacement of soybean meal.

No mortality and no physiological changes were observed among the control rabbits, and they gained weight progressively during the period. Rabbits fed with raw *Thevetia peruviana* seed cake at 5% and 10% inclusion levels showed progressive weight loss and died within 14 days (10% inclusion level) and 21-28 days (5% inclusion level). This is in line with previous reports (Oji *et al*., 1993). It was reported that *Thevetia* seeds are unpalatable and their chewing caused slight numbling sensation, a feeling of heat in the mouth, tingling of the tongue and dryness of throat (Begum *et al*., 1993; Space *et al*., 2003), hence this may have resulted in low feed consumption and the eventual weight loss observed.

Erythrocytic values revealed slight normocytic normochromic anaemia from day 7 to 14 of feeding in all the test rabbits. From 21 days onwards, polycythaemia, characterised by abnormally high PCV, Hb concentration and RBC counts were observed on day 28 and 35 of the feeding trail. This could be attributed to cardiac glycerides (thevetins A, and B) which are toxic agents that have direct stimulant actions on the smooth muscles of the intestine and blood vessel walls (Watt and Breyer-Brandwijk, 1962) leading to gastrointestinal tract irritation, hypermotility, diarrhoea, rapid fluid loss, dehydration, and subsequent haemoconcentration. The increased total protein levels at these periods can also be due to the haemocentration. The slight normocytic normochromic anaemia could also be caused by direct damage to hepatocytes and the kidney. The toxic principles and anti-nutritive factors (cyanogenic glycosides) in the plant are potent inhibitors of cytochrome oxidase thereby causing tissue anoxia and necrosis. Haemopoiesis may also have been affected by impairment of erythropoietin production by the liver and kidneys (Jain, 1986), as well as bone marrow hypoplasia, with stem cells injury by the glycosides in *T. peruviana* seed. The fact that the total and differential white blood cells were normal showed that the rabbits did not have any secondary systemic bacterial or viral infections.

Dehydration may result from metabolic disturbances (acid/base imbalance and metabolic acidosis) or the fact the animal may be too weak to get to source of water. It could also be due to diarrhoea, gastrointestinal fluid loss and decrease in plasma volume, hypovolaemic shock, intravascular coagulation and tissue damage through anoxic anoxia. A consistent finding in most of the experimental rabbits was hyalinization and thickening of blood vascular walls, especially arteries in the spleen, heart and kidney. This could have resulted in degeneration and necrosis observed in the organs (Jubb *et al*., 1995). The rabbits exhibited nervous system symptoms such as convulsion, muscle spasm, paralysis of hind limbs, mydriasis, ataxia, and coma before death. These can be attributed to meningeal, congestion and neuronal necrosis due to anoxia, with resultant gliosis and spongiosis in grey and white matter, which were evident at histology.

Table 4. Haematology of control rabbits and those given raw or processed *Thevetia peruviana* seed cake based diets

Rabbit groups	PCV (%)	Hb conc. (g/dl)	RBC counts ($x10^6 \mu l$)	MCV (fl)	MCHC (%)	Total protein (g/dl)	Globulin (g/dl)
A							
Day 7	38.0 ± 2.1^{c}*	11.9 ± 0.8^{e}	6.4 ± 0.3^{e}	59.1 ± 1.6^{b}	31.4 ± 0.5^{c}	7.9 ± 0.3^{b}	3.1 ± 0.5^{d}
14	39.3 ± 2.3^{c}	12.3 ± 1.0^{d}	6.7 ± 0.5^{e}	59.4 ± 1.6^{b}	31.1 ± 1.0^{c}	6.2 ± 0.7^{b}	3.4 ± 1.6^{c}
21	47.7 ± 1.5^{ab}	16.0 ± 1.0^{c}	7.6 ± 0.3^{c}	63.1 ± 1.9^{b}	32.6 ± 0.2^{b}	6.3 ± 1.1^{b}	3.7 ± 1.5^{c}
28	52.7 ± 0.7^{ab}	16.6 ± 0.3^{b}	8.9 ± 2.9^{a}	59.4 ± 0.9^{b}	31.6 ± 0.2^{c}	7.6 ± 0.2^{b}	4.5 ± 0.3^{c}
35	54.0 ± 7.4^{a}	16.9 ± 2.2^{a}	8.5 ± 0.9^{b}	63.1 ± 2.4^{b}	31.4 ± 0.5^{c}	6.4 ± 0.8^{b}	5.2 ± 0.5^{b}
B							
Day 7	37.7 ± 0.9^{c}	11.6 ± 0.4^{e}	6.4 ± 0.3^{e}	59.3 ± 3.2^{b}	30.7 ± 0.6^{d}	9.7 ± 1.1^{a}	7.0 ± 0.5^{a}
14	36.3 ± 1.8^{d}	11.7 ± 0.6^{e}	6.1 ± 0.2^{e}	59.8 ± 1.6^{b}	32.2 ± 1.0^{c}	9.7 ± 0.7^{a}	6.9 ± 0.9^{a}
21	46.5 ± 3.5^{ab}	16.3 ± 0.9^{b}	7.6 ± 0.6^{c}	60.1 ± 4.0^{b}	35.1 ± 1.7^{a}	8.9 ± 0.1^{a}	6.0 ± 1.1^{a}
28	Died	Died	Died	Died	Died	Died	Died
35	Died	Died	Died	Died	Died	Died	Died
C							
Day 7	39.0 ± 1.6^{c}	11.3 ± 0.4^{e}	6.6 ± 0.1^{e}	58.8 ± 0.7^{b}	29.0 ± 0.9^{e}	9.2 ± 0.7^{a}	6.0 ± 0.7^{a}
14	36.0 ± 1.0^{d}	11.0 ± 0.3^{e}	6.4 ± 0.3^{e}	57.0 ± 1.6^{b}	30.6 ± 0.2^{d}	8.8 ± 0.8^{a}	6.4 ± 0.3^{a}
21	Died	Died	Died	Died	Died	Died	Died
28	Died	Died	Died	Died	Died	Died	Died
35	Died	Died	Died	Died	Died	Died	Died
D							
Day 7	34.0 ± 3.8^{d}	10.5 ± 1.4^{f}	6.3 ± 0.4^{e}	54.8 ± 8.0^{c}	30.6 ± 0.9^{d}	7.5 ± 1.2^{b}	3.4 ± 1.4^{c}
14	33.0 ± 3.1^{e}	10.3 ± 1.0^{f}	5.7 ± 0.2^{f}	57.6 ± 4.9^{b}	31.3 ± 0.5^{c}	7.1 ± 0.6^{b}	3.6 ± 0.8^{c}
21	42.3 ± 2.2^{b}	14.1 ± 1.3^{c}	7.0 ± 0.3^{d}	59.9 ± 0.6^{b}	33.2 ± 1.4^{b}	7.3 ± 1.1^{b}	5.6 ± 0.8^{b}
28	49.3 ± 1.9^{ab}	15.4 ± 0.4^{c}	8.2 ± 0.4^{b}	60.5 ± 0.7^{b}	31.3 ± 0.3^{c}	7.4 ± 0.2^{b}	5.3 ± 0.2^{b}
35	46.7 ± 1.2^{ab}	13.5 ± 6.7^{d}	7.6 ± 8.9^{c}	61.3 ± 1.3^{b}	29.0 ± 0.6^{e}	6.9 ± 1.3^{b}	4.0 ± 0.6^{c}
E							
Day 7	41.0 ± 2.1^{b}	12.2 ± 0.8^{d}	6.4 ± 0.4^{e}	64.7 ± 3.1^{q}	29.6 ± 0.7^{e}	8.0 ± 0.7^{a}	5.2 ± 0.6^{b}
14	37.0 ± 1.2^{d}	11.8 ± 0.3^{e}	6.0 ± 0.2^{f}	62.0 ± 1.5^{b}	32.0 ± 0.7^{c}	8.1 ± 0.5^{a}	5.2 ± 0.2^{b}
21	41.3 ± 0.9^{b}	12.7 ± 0.7^{d}	6.9 ± 0.1^{d}	59.9 ± 0.7^{b}	30.8 ± 2.2^{d}	8.6 ± 0.6^{a}	3.1 ± 0.5^{d}
28	46.3 ± 3.5^{ab}	14.5 ± 1.2^{c}	7.7 ± 0.6^{c}	60.3 ± 0.9^{b}	31.2 ± 0.6^{c}	7.6 ± 0.1^{b}	4.8 ± 0.1^{c}
35	43.3 ± 3.4^{b}	13.9 ± 1.8^{c}	7.4 ± 0.6^{c}	58.4 ± 0.5^{b}	31.9 ± 1.6^{c}	6.9 ± 1.6^{b}	3.8 ± 0.4^{c}

*Data expressed as mean ± standard error of mean.
Data along the same column with different superscripts differ significantly ($p<0.05$)

Rabbits fed with processed *Thevetia peruviana* seed at 5% and 10% inclusion levels did not die, but they were unthrifty, and did not gain weight during the period. The lack of mortality could be attributed to some degree of detoxification as a result of processing of the seeds. The unthriftiness and lack of weight gain could have been due to reduced feed consumption due to the unpalatable nature and bitter taste of the seeds (Beggum *et al*., 1993).

It is suggested that mollases be added to the diets incorporating *Thevetia* seed cake in order to improve its palatability and hence, feed intake. More works need to be carried out on *Thevetia peruviana* seed processing methods and the finding of appropriate inclusion levels of the cake in order to find a cheap substitute for the highly priced and often scarce plant and animal protein sources in both human and animal foods.

Table 5. Pathology scores of rabbits given raw or processed *Thevetia peruviana* seed cake based diets

Organ/Tissue Lesions	Group A	Group B	Group C	Group D	Group E
Lungs					
Pulmonary congestion and oedema	-	-	++	+	-
Thick and hyalinized vascular walls	-	±	+	±	+
Thick peribronchiolar cuffs	±	+	±	+	+
Liver					
Sinusoidal congestion	±	+	++	±	+
Hepatic degeneration and necrosis	-	+	+++	±	+
Periportal mononuclear cell aggregations	-	+	++	+	+
Bile duct hyperplasia	-	++	+	++	+
Periportal fibrosis	-	±	+	+	++
Heart					
Thick and hyalinized vascular walls	-	++	+	+	++
Hyaline degeneration and necrosis of myocardium	-	+	++	+	+
Mononuclear cellular infiltration	-	±	+	+	+
Kidney					
Vascular congestion	±	+	++	+	++
Glomerular and tubular degeneration and necrosis	-	++	++	+	++
Thick and hyalinized vascular walls	-	++	++	++	+++
Interstitial mononuclear cell infiltration	±	+	+	+	++
Fibrosis	-	±	-	+	++
Brain					
Meningeal congestion	±	+	+	±	+
Neuronal degeneration and gliosis	-	+	++	+	+
Spongiosis	-	±	+	±	+
Spleen					
Thick and hyalinized vascular walls	-	++	++	++	++
Lymphoid depopulation	±	+	±	+	+
Haemosiderosis	±	±	-	±	±

Key to scores:-
- - = No lesions observed
- ± = Mild, focal lesions
- + = Mild, multifocal lesions
- ++ = Moderately severe diffuse lesions
- +++ = Very severe diffuse lesions

CONCLUSIONS

In conclusion, this work has shown that *Thevetia peruviana* seed cake should not be fed raw to rabbits because it is highly toxic. Death may have occurred due to the main actions of thevetins A and B (cardiac glycosides) and other toxins on heart, blood vessels, and the liver with resultant heart failure and general tissue anoxia, hepatic damage and renal failure secondary to cardiogenic shock. *Thevetia peruviana* seed cake needs adequate processing to remove the toxic agents and other anti-nutritive factors. After being processed, it can be used as a protein supplement in rabbits and other livestock feed diets, thereby reducing cost of meat animal production and competition with grains human for consumption.

REFERENCES

Abe, F., Iwase, Y., Yamauchi, T. and Nohara, T. 1995. Flavonol sinapol glycosides from leaves of *Thevetia peruviana*. Phytochemistry, 40:577-581.

Ahlawat, S.K., Aggarwal, A.K. and Wadhwa, S. 1994. Rare poisoning with *Cerbera thevetia* (Yellow oleander): A report of three cases. Tropical Doctor, 24:37-38.

Begum, S., Abii, O., Siddiqui, B.S. and Siddiqui, S. 1993. Constituents of the leaves of *Thevetia neriifolia.* Journal of Natural Products, 56:613-617.

Daniel, M. and Sabnis, S.D. 1978. Chemotaxonomical studies on *Apocynaceae*. Indian Journal of Experimental Biology, 16:512-513.

Duncan, R.M. 1959. Multiple range and multiple F-tests. *Biometrics* 11: 1-42.

Eddleston, M., Ariaratnam, C.A., Ssjostrom, L., Javalath, S., Rajakanthan, K., Rajapakse, S., Colbert, D., Meyer, W.P., Perera, G., Attapattu, S., Kularatne, S.A.M., Sheriff, M.R. and Warrell, D.A. 2000. Acute yellow oleander (*Thevetia peruviana*) poisoning: Cardiac arrhythmias, electrolyte disturbances, and serum cardiac glycoside concentrations on presentation to hospital. Heart, 83:301-306.

Fadina, O.O., Taiwo, V.O. and Ogunsanmi, A.O. 1999. The effects of single and repetitive oral administration of common pesticides and alcohol on rabbits. Tropical Veterinarian, 17:97-106.

Inman, N. 1967. Notes on some poisonous plant of Guam. Micronesica, 3:55-62.

Jain, N.C. 1986. Schalm's Veterinary Haematology (Jain, N.C., ed) 4th edition, Lea and Ferbiger, Philadelphia.

Jubb, K.V.F., Kennedy, P.C. and Palmer, N. 1995. Pathology of Domestic Animals. 3rd edition, Academic Press Inc., New York.

Langford, S.D. and Boor, P.J. 1996. Oleander toxicity: an examination of human and animal toxic exposures. Toxicology, 109:1-13.

Nair, A.G.R., Gunasegaran, R., and Joshi, B.S. 1982. Chemical investigation of certain South Indian plants. Indian Journal of Chemistry, 21b:979-980.

Nayar, S.L. 1957. Poisonous seeds of India. Part II. Journal of Bombay Natural Historical Society, 52:1-18.

Odetokun, S.M., Akindumila, F. and Ibukun, E.O. 1999. Assessment of protein cake of bush milk flower (*Thevetia peruviana*) in poultry feed. Rivista Italiana delle Sostanze Grasse, 76(5):233 –235.

Oji, O. and Okafor, O.E. 2000. Toxicological studies on stem, bark, leaf and seed kernel of yellow oleander (*Thevetia peruviana*). Phytotherapy Research, 14:133-135.

Oji, O., Madubuike, F.N., and Nwalozie, M.C. 1993. Mortality in rats following dietery inclusion of *Thevetia neriifolia* seeds. Fitoterapia, 64:137-139.

Ramirez, V.R., Mostacero, L.J., Garcia, A.E., Mejia, C.F., Pelaez, P.F., Medina, C.D. and Miranda, C.H. 1988. Vegetales empleados en medicina tradicional Norperuana. Banco Agrario del Peru & National University of Trujillo, Trujillo, Peru, June, 1988: 54pp.

SAS 1987. *Statistical Analysis System*s. Users' Guide, Ver. 6.03, SAS Institute Inc., Cary, North Carolina, USA.

Siemens L.M. Galey, B., and Johnson, A. 1995. The clinical, cardiac and pathophysiological effects of oleander toxicity in horses. Proceedings of the University of California Davies, VMTH House Officer Seminar Day, March 1995.

Singh,V.P., Sharma, S.K. and Khare, V.S. 1980. Medicinal plants from Ujjain District Madhya Prades - Part II. Indian Drugs and Pharmaceutical Industry, 5:7-12.

Space, J.C., Waterhouse, B.M., Miles, J.E., Tiobech, J. and Rengulbai, K. 2003. Report to the Republic of Palau on invasive plant species of environmental concern. USDA Forest Service, Honolulu, 179pp.

Steyn, D.G. 1934. The Toxicology of Plants in South Africa. IDC, Johannesburg.

Watt, J.M. and Breyer-Brandwijk, M.G. 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. Edinburgh & London, pp. 107–109.

Submitted September 22, 2003 – Accepted March 14, 2004