PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 3 (2): 112-117, 2004

Observations on the Pathophysiology of Weaner Pigs Fed Raw and Preheated Nigerian *Mucuna pruriens* (Velvet Bean) Seeds

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Abstract: Aspects of the pathophysiological responses of weaner pigs fed raw and cracked-soaked and cooked Nigerian *M. pruriens* seed meals were investigated using twenty-five large white x land race weaner pigs with weights ranging from 17 to 22 kg in a 56 days (8 weeks) experiment. Raw Mucuna meal (RMM) and cracked soaked and cooked meal (CSCM) were incorporated into weaner pigs diets at 15% and 20, 30 and 40%levels respectively and tested against a control diet with 0% *Mucuna* meal. The results revealed that raw Nigerian *Mucuna* seeds is poisonous to pigs at 15% dietary inclusion level and significantly ($P<0.5$) affected the hematological and serum biochemical indices of the pigs. It resulted in 40% mortality among this group with dead animals manifesting lung collapse, emaciation serous atrophy of fat and enlarged urinary bladder at post-mortem. Preheated *Mucuna* seed meal, however proved safe for pigs at different dietary inclusion levels but did not improve most of the hematological and serum biochemical parameters measured. Pigs fed *Mucuna* bean diets had significantly ($P<0.05$) elevated levels of serum albumin, cholesterol, creatinine and polymorphs but decreased levels of eosinophils and monocytes when compared with the control group. At 15% dietary level of raw seed, the pigs had statistically similar total serum protein, serum calcium, K^+, HCO_3^-, total white blood cell and lymphocytes but significantly ($P<0.05$) increased hemoglobin concentration when compared with the control. Increasing the dietary levels of the preheated seeds also resulted in reduced weight gains among the pigs. This study therefore suggests that raw *Mucuna* bean is poisonous to weaner pigs but relatively safe after preheats treatment. However, changes in hematological and serum biochemical parameters indicated poor nutrient utilization and muscle damage among pigs receiving higher levels of preheated seeds.

Key words: *Mucuna*, cracked-soaked and cooked, weaner pigs, pathophysiology, serum biochemistry

Introduction

Mucuna pruriens (velvet bean) plant is a tropical legume that is little known and used for human food or animal feed in Nigeria. The use of the bean in livestock feeding is one of the best ways of exploiting its agronomic and nutritional potentials. This is because of the nitrogen fixing activity of the plant (Berhe, 2001) and the relatively high protein and energy contents of the bean (Emenalom and Udedibie, 1998; Udedibie and Carlini, 1998). Its use could also help in reducing the over-dependence on the conventional protein supplements notably soybean (*Glycine max)* and groundnut (*Archis hypogea*) and hence reduce the ever-increasing price of concentrate feeds for non-ruminant animals.

However, *Mucuna* bean like most tropical legumes contain anti-nutritional factors (Siddharaju *et al.,* 1996; Udedibie and Carlini, 1998) which affect nutrient utilization by non-ruminant animals. These anti-nutritional factors may also alter the biochemical and hematological parameters of animals fed *Mucuna* bean diets. The blood contains a myriad of metabolites and other constituents, which provide a valuable medium for clinical investigation and nutritional status of individuals hence WHO (1963) recommended the use of blood biochemical and hematological parameters in medical nutritional assessment. Dietary components have also been shown to have measurable effects on blood components (Eggum, 1970; Kerr *et al*., 1982; Church *et al*., 1984; Babatunde *et al*., 1987; Onifade and Tewe, 1993; Olorede *et al*., 1995; Iyayi and Tewe, 1998; Awosanya *et al*., 1999). Church *et al*. (1984) also reported the importance of blood chemistry profiles in relation to nutrient intake.

Esonu *et al*. (2001) reported that raw *Mucuna* bean meal had deleterious effects on the performance and blood constituents of weaner pigs. The effects of feeding cracked-soaked and cooked Nigerian *Mucuna* seeds on the hematological and serum biochemical parameters of pigs have however not been fully understood. The purpose of this paper therefore is to determine the effects of raw and cracked-soaked and cooked Nigerian *Mucuna* seeds on pathophysiological parameters such as weight gain, internal organ characteristics, hematology and serum biochemistry of large white x land race pigs raised in a humid tropical environment.

Materials and Methods

Seed processing and diet formulation: Nigerian *Mucuna* bean seeds were harvested from the wild and subjected to two forms of processing. The first part of the

seeds was ground raw while the second part was cracked into pieces (2-3 parts/seed) using ASKO A11 grinding machine. The cracked seed samples were soaked in water (1kg/2l of water) for 48 hours, rinsed with tap water, cooked for 1 hour at 100°C timed from boiling, sun dried and finally ground into meal (CSCM). The two meals, raw meal (RM) and cracked-soaked and cooked meal (CSCM) were incorporated into weaner pigs diets at 15% and, 20 30 and 40% levels respectively and tested against a control (0%) diet that contained no *Mucuna* meal (Table 1).

Experimental animals and feeding trials: Twenty-five large white x land race weaner pigs with initial weights ranging from 17 to 22 kg were randomly allocated on weight basis to five groups, of five pigs each and fed diets containing the control (0%), 15% RM and 20, 30 and 40% CSCM respectively. The pigs were housed in concrete floored pens. Feed consumption was restricted to 4% of their weekly body weights. Water was however provided *ad libitum*. Starting from the second week of the feeding trial, the general conditions of the test animals and their pens were monitored. The animals were reweighed on the 56th day of the trial to determine their weight gains over the treatment period. All dead animals were necropsied. The feeding trial lasted for 56 days.

Blood collection and analysis: On the 56th day, 10ml of morning (9-10am) blood samples were collected from the anterior vena carva of 3 pigs from each of the treatment groups. Three millilitre (3ml) of each blood sample was discarded into EDTA treated bottles for hematological assay while the rest 7ml was used for standard serum preparation.

Hematological measurements were determined using the methods outlined by Schalm *et al.* (1975); Kelly (1979). Biochemical assays were carried out using standard chemical procedures: total serum protein by Biuret method (Reinhold, 1953) albumin (Doumas *et al.*, 1971), Creatinine (Boisness *et al.*, 1945), blood sugar (Toro and Ackerman, 1975), Na^+ and k^+ by flame photometry and, $HC0_3^-$ and Cl^- (Schales and Schales 1941).

Data analysis: All the data collected were subjected to analysis of variance (ANOVA). Standard error of means (Steel and Torrie, 1980) was used to identify the significant treatment mean effects ($P<0.05$).

Results

Clinical and pathophysiological observations: Two pigs having initial body weights of 17 and 18kg and receiving raw *Mucuna* meal diet died on the 12th and 32nd days of the feeding trial, respectively. The other surviving pigs in the treatment group also had mean initial body weight of 21kg. Prior to death, the affected pigs manifested severe dyspnea, polyuria, anorexia, progressive rapid emaciation, dullness and incoordination. At necropsy, gross lesions observed included emaciation, serous atrophy of fat, enlarged urinary bladder (retaining abnormal volume of urine) and collapsed flabid lungs. The affected lungs were firm to touch and consolidated, and lacked the spongy texture of normal lungs tissue. No mortality was recorded among the other treatment groups.

Weight gain: At the end of 56 days treatment, mean weight gains for the groups stood at 17, 18, 19, 15 and 14kg for control, 15% RM, 20, 30 and 40% CSCM diets, respectively (Table 2). Whereas pigs on 20% CSCM diets had a 100% increase in their body weight over the treatment period, pigs on 0 and 15% RM, and 30 and 40% CSCM had 80.95, 81.82, 78.95 and 70.73% respectively.

Hematology and serum biochemistry: The data on the hematological and serum biochemical parameters of the pigs analyzed for the treatments are presented in Table 3. There were significant ($P<0.05$) differences among the treatments in the hematological parameters measured. Pigs on 15%RM diet recorded the highest percentage hemoglobin of 65% which is significantly ($P<0.05$) different from the control (55%), 20% (45%) and 40% (50%) but similar to that of 30% (60%) CSCM diet. The white blood cell figures were similar among groups on the control and 15% RM diets but decreased significantly among the CSCM diet groups. *Mucuna* bean diets also significantly ($P<0.05$) decreased the eosinophils and monocytes values when compared with the control.

The mean values of total serum protein, albumin, calcium, cholesterol, creatinine, blood sugar and electrolytes were significantly ($P<0.05$) affected by the treatment. Serum albumin, cholesterol, creatinine and blood sugar were significantly ($P<0.05$) higher in the *Mucuna* diet groups than the control. At 15% dietary inclusion of raw *Mucuna* bean, total serum protein (5.8g/100ml) and serum calcium (10.0mg/100mg) values compared statistically with those of the control group (6.5g/100ml and 9.8mg/100ml), but differed significantly ($P<0.05$) from those of the CSCM diet groups. The electrolyte values also showed significant ($P<0.05$) differences among the treatments. Except for Na^+ at 30% CSCM meal diet, pigs on 15% raw *Mucuna* meal diet had a significantly ($P<0.05$) higher Na^+ and Cl^- values than the control and other *Mucuna* bean diet groups. Pigs on 20% CSCM meal diet had a significantly ($P<0.05$) low HCO_3^- values relative to the control and the other treatments groups.

Table 1: Ingredient compositions of raw and processed *Mucuna* meal diets

Ingredients (%)	Control 0%	RM[1]15%	CSCM[2]20%	CSCM 30%	CSCM40%
Maize	55.0	47.0	44.0	38.0	30.0
Mucuna meal	-	15.0	20.0	30.0	40.0
Soybean meal	15.0	8.0	6.0	2.0	-
Brewers dried grain	5.0	5.0	5.0	5.0	5.0
Wheat offal	12.0	12.0	12.0	12.0	12.0
Palm kernel cake	5.0	5.0	5.0	5.0	5.0
Fish meal	2.0	2.0	2.0	2.0	2.0
Blood meal	2.0	2.0	2.0	2.0	2.0
Bone meal	3.0	3.0	3.0	3.0	3.0
L-lysine	0.2	0.2	0.2	0.2	0.2
L-methionine	0.2	0.2	0.2	0.2	0.2
Vit/TM premix[3]	0.3	0.3	0.3	0.3	0.3
Salt	0.3	0.3	0.3	0.3	0.3
Total	100.0	100.0	100.0	100.0	100.0
Calculated analysis					
Crude protein	19.1	19.5	19.3	19.4	19.5
Crude fibre	4.9	4.5	4.4	4.3	4.2
Ether extract	4.2	4.2	4.2	4.1	4.1
Calcium	1.2	1.4	1.4	1.5	1.5
Phosphorus	1.0	1.1	1.1	1.1	1.1
ME (Kcal/kg)	2776.5	2787.6	2791.2	2793.1	2795.5

[1]RM-raw meal. [2]CSCM-cracked-soaked and cooked meal. [3]Provided the following per kg of feed: Vit. A. 10,000 iu, Vit. D_3 2000 iu., niacin 500 iu., Vit. K. 2mg, riboflavin, 3mg, panthotenic acid, 5mg., nicotinic acid 20mg., choline 5mg., Vit. B_{12}. 0.08mg., Folic acid. 4mg., Mn., 8mg., Zn. 0.5mg., iodine, 1.0mg, iron, 20mg, Cu. 10mg., Co.125mg.

Table 2: Effect of dietary raw and processed *Mucuna* seeds on mortality and mean weight gain of treatment pigs

Group	Pretreatment Weight	Post treatment Weight	weight gain	Mortality
Control	21.00	38.00	17 (80.95)*	0 (0.0)
RM (15%)	22.00	40.00	18 (81.82)	2 (40.0)
CSCM (20%)	19.00	38.00	19 (100.0)	0 (0.0)
CSCM (30%)	19.00	34.00	15 (78.95)	0.(0.0)
CSCM (40%)	20.50	35.00	14.5(70.73)	0.(0.0)

*Percentage weight gains and mortality are given in parenthesis.

Carcass and internal organ weight characteristics: Data on dressed and internal organ weights relative to body weight of pigs are shown in Table 4. The pigs fed 30 and 40% CSCM diets had a significantly ($P<0.05$) lower carcass weights than the other treatment groups. The dressed percentage of the 20% CSCM diet group was on the other hand comparable with the control but differed significantly ($P<0.05$) from those of the other *Mucuna* diet groups. The kidney, heart and lung were significantly ($P<0.05$) decreased at 15% RM and 40% CSCM diets, respectively. The liver weights were statistically similar except for that of pigs fed 30% CSCM diets.

Discussion

M. pruriens has been reported to contain typsin inhibitors, phytates, cyanogenic glycosides, tannins, lectins and L-3,4 dihydroxyphenylalanine (L-Dopa) (Ravindran and Ravindran, 1988; Mary Josephine and Janardhanan, 1992; Siddhuraju *et al.*, 1996; Berhe, 2001). Most of these toxicants have been shown to be completely eliminated when raw seeds are subjected to 1 hour of cooking at 96°C or 30 minutes of cooking after soaking in water for 48 hours (Udedibie and Carlini, 1998). However there is very little published work on the value of both raw and processed *M. pruriens* seeds for pigs. Caution was therefore exercised on the use of the raw seeds in this study.

The Clinical signs and pathology manifested by some of the pigs receiving the seeds have been reported in pigs fed untreated cotton seed cakes (Aiello and Mays, 1998; Hunter, 1994). It appears that the two major pathological effects of the raw seeds were alveolar collapse in the lungs and general water imbalance (homeostasis) leading probably to accumulation of sodium and loss of potassium from the body. These clinical effects seem to manifest more in smaller pigs (averaging 17-18kg) than bigger ones (21kg), indicating that body weight may be

Table 3: Blood constituents in pigs fed raw and processed *Mucuna* meal diet

Parameter	Dietary Levels					
	Control (10%)	RMM (15%)	CSCM (20%)	CSCM (30%)	CSCM (40%)	SEM
Hematological Traits						
Hemoglobin (g/100ml)	55.0^{bc}	65.0^{a}	45.0^{d}	60.0^{ab}	50.0^{cd}	3.5
White blood cells (x103/L)	45^{b}	5.1^{b}	3.8^{c}	3.8^{c}	3.9^{c}	0.3
Polymorphs (%)	35.0^{c}	$41.o^{b}$	39.0^{bc}	48.0^{a}	46.0^{a}	2.4
Lymplrocytes (%)	58.0^{a}	56.0^{a}	60.0^{a}	49.0^{b}	51.0^{b}	2.1
Eosinophils (%)	5.0^{a}	2.0^{b}	0.0^{c}	2.0^{b}	3.0^{b}	0.8
Monocytes (%)	2.0^{a}	1.0^{b}	1.0^{b}	1.0^{b}	0.0^{c}	0.3
Basophlis (%)	0.0	0.0	0.0	0.0		0.0
Biochemical Indices.						
Total serum protein (g/100ml)	6.5^{cd}	5.8^{d}	7.2^{bc}	7.5^{ab}	8.2^{a}	0.4
Serum albumin (g/100ml)	2.5^{c}	3.2^{b}	4.1^{a}	3.3^{b}	3.5^{ab}	0.3
Serum calcium (mg/100ml)	9.8^{a}	10.0^{a}	8.8^{b}	7.8^{c}	7.9^{bc}	0.5
Cholesterol (mg/100ml)	109.0^{c}	121.0^{b}	130.0^{ab}	138.0^{a}	130.0^{ab}	5.0
Creatinine (g/100ml)	0.6^{c}	0.9^{ab}	0.8^{b}	0.9^{ab}	1.0^{a}	0.1
Blood sugar (mg/100ml)	40.0^{c}	45.0^{b}	43.0^{ab}	50.0^{a}	52.0^{a}	2.2
Na^{+} 9 mmol/l)	138.0^{c}	140.0^{ab}	139.0^{bc}	141.0^{a}	139.0^{bc}	0.5
K^{+} (mmol/l)	4.1^{ab}	3.9^{b}	3.9^{b}	4.2^{a}	3.9^{b}	0.1
Cl^{-} (mmol/l)	98.0^{b}	100.0^{a}	100.0^{a}	101.0^{a}	100.0^{a}	0.5
$HC0_3^{-}$ (mmol/l)	22.0^{b}	22.0^{b}	21.0^{c}	23.0^{a}	22.0^{b}	0.3

abcd: means within a row with different superscripts differ significantly (P< 0.05).

Table 4: Effect of dietary raw and processed *Mucuna* seed on carcass and organ weights characteristics of treatment pigs

Parameter	Control (0%)	RMM (15%)	CSCM (20%)	CSCM (30%)	CSCM (40%)	SEM
Carcass weight (kg)	25.0^{a}	24.0^{a}	25.0^{a}	20.0^{b}	21.0^{ab}	1.54
Dressed percentage (%)	65.8^{a}	60.0^{b}	65.8^{a}	58.8^{b}	60.0^{b}	2.16
Liver (%)	5.6^{b}	5.8^{b}	5.7^{b}	6.5^{a}	5.7^{b}	0.17
Heart (%)	1.9^{a}	1.0^{c}	1.7^{ab}	1.4^{abc}	1.2^{bc}	0.17
Kidney (%)	1.2^{b}	1.0^{c}	1.1^{bc}	1.3^{a}	1.0^{c}	0.07
Lungs (%)	3.1^{ab}	2.2^{b}	3.8^{a}	4.3^{a}	2.5^{b}	0.40

abc. Means within a row with different superscripts differ significantly (P<0.05).

a factor in the ability of pigs to overcome the poisonous effects of raw *Mucuna* bean. This is further supported by the fact that the 17kg pig died on the 12^{th} day of the experiment while the 18kg pig died on the 22^{nd} day. The facts that mortality was recorded only in the RMM diet group also tended to confirm the report in EDN (1997) that raw *M. pruriens* seeds is poisonous to pigs at any dietary level. After 56 days of treatment, the surviving pigs on the raw Mucuna bean diet had gained weight, although one of them had started to exhibit early signs of poisoning. These signs however, receded few days after the pigs were placed on conventional ration.

The result for body weight gain across treatment groups shows that higher dietary inclusion levels resulted in decreased weight gain. This is probably due to the effects of incomplete elimination of toxic factors such as L-Dopa, lectins and tannins (Siddhuraju *et al.*, 1996; Udedibie *et al.*, 1994). Amaefule and Obioha (2001) and Iheukwumere and Okoli (2002) have also reported the effects of nutrient imbalance and poor metabolism on monogastric animals fed high levels of unconventional feed ingredients.

The white blood cell values of pigs fed the control and *Mucuna* bean diets were generally lower than the normal average 10-20x10^3/l reported for pigs (Pagot, 1992). Red blood corpuscle (erythrocytes) and white corpuscle (leukocytes) numbers of animals vary between species, individuals, sex, environment and clinical condition of the animal. Parasitism and under-feeding are also causes of anemia which could manifested as reduction of the red blood cell count or hemoglobin content of each red blood cell (Aiello and Mays, 1998). The variation in whites blood cells and hemoglobin contents of the pigs on the different *Mucuna* diets could therefore be attributed to nutrient imbalance and/or poor nutrient utilization.

The variations in the total serum protein, albumin and creatinine values recorded in this study could be viewed with earlier reports of the protein retained in animals (Akintola and Abiola, 1999). Iyayi and Tewe (1998); Awosanya *et al.* (1999) reported the dependence of blood protein and creatinine on the quality and quantity of dietary protein. Feeding raw or processed *Mucuna* bean to pigs may have caused tissue damage resulting in significantly ($P<0.05$) elevated levels of serum creatinine levels. According to Bell *et al.* (1992) the major source of creatinine in the blood of animals is the muscle when wasting occurs and creatinine phosphate is catabolized. Poor nutrient utilization and/or absorption in pigs fed raw or CSCM diets, could be due to anti nutritional factors still present in the seeds. This may have caused the variations observed in serum biochemical parameters and the suspected muscle tissue damage. The results of serum electrolytes tended to show that both raw and CSCM diets had effect on kidney function and electrolyte balance. Processing may have affected calcium up take from the intestine, probably due to the formation of unabsorbable calcium chalates. This may have led to the lower serum calcium recorded in pigs fed CSCM diets as serum calcium is dependent on intestinal calcium absorption and bone resorption (Aiello and Mays, 1998).

The reduced carcass weights with increasing dietary inclusion of processed *Mucuna* meal could be attributed to anti-nutritional problems, which increased with quantity. The highest dressed percentage was however obtained from animals receiving 20% CSCM diet indicating that this might be the optimal inclusion level of the *Mucuna* seeds at the present level of processing. The percentage weights of heart, kidney and lungs obtained at high dietary levels of CSCM are comparable to the figures recorded for the 15% RMM diets which is known to contain more of the anti-nutritional factors. This again probably indicates incomplete elimination of the toxicants in the meal.

Conclusion: It could be stated that even though preheated *Mucuna* seed might have eliminated most of the poisonous effects of the raw seed, it did not improve many of the physiological, biochemical and hematological parameters measured. The relatively poor weight gain by pigs receiving increasing levels of the processed seeds suggests incomplete detoxification of the anti-nutritional factors present in the seed by the crack-soak and cook method. Further detailed research on the pathophysiology of pigs fed *ad libitum* on the same dietary levels of raw and processed *Mucuna* beans might be fruitful in determining the actual differences in the structural, functional and chemical parameters of pigs fed *Mucuna* bean diets.

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