Tropical and Subtropical Agroecosystems

CARCASS CHARACTERISTICS, HISTOPATHOLOGY AND HEMATOLOGICAL CONDITIONS OF BROILERS FED RAW OR THERMALLY PROCESSED *Mucuna cochinchinensis*

[CARACTERÍSTICAS DE LA CANAL Y CAMBIOS HISTOPATOLÓGICOS Y HEMATOLÓGICOS EN POLLOS ALIMENTADOS CON *Mucuna cochichinensis* CRUDA O PROCESADA TÉRMICAMENTE]

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SUMMARY

The carcass characteristics, pathological changes and haematological conditions of broilers fed raw (RMD), cooked (CMD), toasted (TMD), soaked-and-boiled (SMD), and no- (NMD) Mucuna diets were investigated using 400 broiler chicks in a completely randomized design experiment. The RMD depressed ($P<0.05$) percent liver weight, haemoglobin (Hb) and red blood cell (RBC) at both starter and finisher phases, and packed cell volume (PCV) at starter phase only. The RMD also caused wild area of periportal necrosis of the liver with some mononuclear cells infiltration as well as vacuolation and degeneration of hepatocytes of the centrilobular areas. TMD depressed ($P<0.05$) percent liver weight and RBC at the finisher phase, and Hb at both starter and finisher phases. The TMD also caused distortion of the lobular architecture of the liver, vacuolation and necrosis of the hepatocytes in the periportal areas. The SMD produced mild infiltration of mononuclear cells into the perivascular and periductular spaces in the portal areas of the liver. The CMD caused similar but milder changes in the liver section as SMD.

Key words: *Mucuna cochinchinensis,* thermal processing methods, broilers, carcass characteristics, histopathology, haematological conditions.

RESUMEN

Las características de la canal, cambios patológicos y parámetros hematológicos de pollos de engorda alimentados con Mucuna cruda (RMD), cocida (CMD), tostada (TMD), remojada y cocida (SMD), y una dieta sin Mucuna (NMD) fueron analizados empleando 400 aves en un diseño completamente al azar. La dieta RMD deprimió ($P<0.05$) el peso del hígado, hemoglobina (Hb) y células rojas sanguíneas (RBC) tanto en la fase de inicio como en la fase de finalización, y el volumen de paquete celular (PCV) sólo en la fase de inicio. La dieta RMD también causó un área de necrosis periportal en el hígado con infiltración de células mononucleares, así como vacuolación y degeneración de hepatocitos del área centrilobular. La dieta TMD deprimió ($P<0.05$) el peso del hígado y RBC en la etapa de finalización, y Hb en las etapas de inicio y finalización. La dieta TMD también causó cambios en la arquitectura lobular del hígado, vacuolación y necrosis de los hepatocitos en la zona periportal. La dieta SMD produjo una leve infiltración de cellular nononucleadas en los espacios perivasculares y periductales del área portal del hígado. La dieta CMD causó cambios similares pero más leves que los observados con SMD.

Palabras clave: *Mucuna cochichinensis*, procesamiento térmico, pollos, características de la canal, histopatología, hematología.

INTRODUCTION

Raw *Mucuna cochinchinensis* extracts have been reported to be non-lethal when administered to broilers, but that some other signs of toxicity of the extracts (like dizziness and diarrheic droppings) manifested in the birds (Ukachukwu *et al*., 1999). By feeding broilers raw *Mucuna* diet for a long time chronic toxicity effects were produced in the broilers (Ukachukwu, 2000). These effects were mainly pathologic lesions of the liver and reduction of red blood cells, packed cell volume and hemoglobin. These effects were attributed to the presence of hemagglutinin and, possibly other antinutritional factors in *Mucuna* (Carew *et al.,* 2002; Ukachukwu, 2000; Ologhobo *et al*., 1993).

Some of the anti-nutritional factors of *M. cochinchinensis* are heat labile (Lloyd *et al.,* 1978; Nelson *et al.,* 1978; Church and Pond, 1974; Arnold *et*

al., 1971). To make it edible some ttreatments applicable include autoclaving, cooking or boiling, steam-heating, infra-red cooking, extrusion cooking, toasting, salt-bed roasting, radio frequency dielectric heating (Udedibie *et al.,* 1996; Integrated Farming Systems Programme (IFSP), 1988; Waldroup and Hazen, 1978; Raghavan *et al.,* 1974; Borchers *et al.,* 1972; Gustafson *et al.,* 1971).

Ukachukwu and Obioha (2000) employed three locally adaptable processing methods requiring the use of no sophisticated equipment or heavy capital investment to process *Mucuna* bean. They achieved 50-75% reduction of hemagglutinin and 40-50% reduction of trypsin inhibitors. Also, Ukachukwu and Szabo (2003) processed *Mucuna* by boiling with wood ash additive and reported reduction of L-dopa content from 6.15% (raw bean) to 2.20% (processed bean).

Ukachukwu (2000) has opined that chemical analysis alone cannot clarify issues on nutrient utilization. Therefore, selection of processing methods on the basis of chemical analysis alone can be misleading. It is therefore, necessary to verify the results of the chemical analysis by investigating the effects of feeding processed *Mucuna* bean to farm animals. This work, as a preliminary step, therefore, aims at investigating the carcass characteristics, pathological changes and haematological conditions of broilers fed diets containing raw or thermally processed *Mucuna cochinchinensis*.

MATERIALS AND METHODS

Raw, toasted, boiled and soaked-and-boiled *Mucuna* meals, processed in accordance with the recommendation of Ukachukwu and Obioha (2000), were used to formulate isocaloric and isonitrogenous diets for starter (22%CP and 2.9Mcal ME/Kg) and finisher (20%CP and 2.9Mcal ME/Kg) broiler birds and designated RMD, TMD, CMD, and SMD, respectively (Tables 1 and 2). A fifth diet that contained no seed but of the same nutritional regime as the other four above was also formulated to serve as control and designated NMD. These were fed to 400 unsexed Anak strain broiler chicks in a completely randomized design (CRD) experiment using 80 birds per dietary treatment. Each treatment was replicated four times with 20 chicks per replicate.

Table 1: Composition of five starter treatment diets containing either raw, cooked, toasted, soaked-and-boiled or no *Mucuna cochinchinensis* seed

Ingredients	Treatment diets				
	RMD	CMD	TMD	SMD	NMD
M. cochinchinensis, %	5.00	5.00	5.00	5.00	-
Maize, %	49.98	49.98	49.98	49.98	51.35
Wheat offal, %	4.00	4.00	4.00	4.00	4.00
Palm kernel cake, %	6.00	6.00	6.00	6.00	6.00
Soybean meal, %	29.27	29.27	29.27	29.27	32.90
Fish meal, %	2.00	2.00	2.00	2.00	2.00
Bone meal, %	3.00	3.00	3.00	3.00	3.00
Salt, %	0.50	0.50	0.50	0.50	0.50
Vit./Min. Premix* , %	0.25	0.25	0.25	0.25	0.25
Calculated:					
Crude protein, %	22.00	22.00	22.00	22.00	22.00
ME (Mcal/Kg)	2.90	2.90	2.90	2.90	2.90

*1Kg of premix contains: Vitamin A (5,000,000 I.U.), Vitamin D3 (1,000,000 I.U.), Vitamin E (16,000mg), Vitamin K3 (800mg),Vitamin B1 (1,200mg), Vitamin B2 (22,000mg), Niacin (22,000mg), Calcium pantothenate (4,600mg), Vitamin B6 (2,000mg), Vitamin B12 (10mg), Folic acid (400mg), Biotin (32mg), Choline chloride (200,000mg), Manganese (48,000mg), Iron (40,000mg), Zinc (32,000mg), Copper (3,400mg), Iodine (600mg), Cobalt (120mg), Selenium (48mg), Anti-oxidant (48,000mg).
RMD, TMD, CMD, SMD and NMD represent raw, toasted, boiled, soaked-and-boiled and *Mucuna*-free (control) diets

Throughout the entire experiment feed and water were offered *ad libitum* on daily basis. Weighing of the birds took place weekly. The chicks were reared in a deep litter house throughout the experiment. They were fed a common proprietary broiler starter feed (TOP Feed[Registered Trade Mark]) in their first 14 days of age. After this preliminary period the chicks were introduced to the experimental diets. The starter and finisher phases lasted for 3 and 5 weeks, respectively. Three birds per replicate were slaughtered at the end of starter and finisher phases. The blood of the sacrificed birds was used for aspects of haemotological studies including Packed Cell Volume (PCV), Red blood cells (RBC) count and Haemoglobin (Hb) determination.

Some of the organs of the sacrificed birds were used for carcass characteristics and histopathlogical studies. The organs were weighed and examined grossly and histopathologically for any lesions arising from possible toxicity due to the dietary treatments. The organs involved were liver, spleen, kidney, heart and pancreas. They were preserved in formalin. The organs were fixed in 10% formal saline for a maximum of 24h, processed and infiltrated with paraffin wax. Sections (5 microns thick) were cut, stained with haematoxylin and eosin (H and E) and examined with the light microscope for pathological lesions.

Data collected on carcass characteristics and hematological conditions were subjected to analysis of variance (ANOVA) in CRD, and the Duncan's new multiple range test (DNMRT) was applied to partition means, where necessary (Gomez and Gomez, 1985; Snedecor and Cochran, 1980; Duncan, 1955), as packaged in the MSTAT-C (1993) computer software.

Table 2: Composition of five finisher treatment diets containing either raw, cooked, toasted, soaked-and-boiled or no *Mucuna cochinchinensis* seed

Ingredients	Treatment diets				
	RMD	CMD	TMD	SMD	NMD
M. cochinchinensis, %	5.00	5.00	5.00	5.00	-
Maize, %	48.71	48.71	48.71	48.71	51.04
Wheat offal, %	8.00	8.00	8.00	8.00	8.00
Palm kernel cake, %	8.00	8.00	8.00	8.00	8.00
Soybean meal, %	23.04	23.04	23.04	23.04	25.71
Fish meal, %	2.00	2.00	2.00	2.00	2.00
Bone meal, %	3.00	3.00	3.00	3.00	3.00
Oyster shell	1.50	1.50	1.50	1.50	1.50
Salt, %	0.50	0.50	0.50	0.50	0.50
Vit./Min. Premix* , %	0.25	0.25	0.25	0.25	0.25
Calculated:					
Crude protein, %	20.00	20.00	20.00	20.00	20.00
ME (Mcal/Kg)	2.90	2.90	2.90	2.90	2.90

* 1Kg of premix contains: Vitamin A (5,000,000 I.U.), Vitamin D3 (1,000,000 I.U.), Vitamin E (16,000mg), Vitamin K3 (800mg), Vitamin B1 (1,200mg), Vitamin B2 (22,000mg), Niacin (22,000mg), Calcium pantothenate (4,600mg), Vitamin B6 (2,000mg), Vitamin B12 (10mg), Folic acid (400mg), Biotin (32mg), Choline chloride (200,000mg), Manganese (48,000mg), Iron (40,000mg), Zinc (32,000mg), Copper(3,400mg), Iodine (600mg), Cobalt (120mg), Selenium (48mg), Anti-oxidant (48,000mg).
RMD, TMD, CMD, SMD and NMD represent raw, toasted, boiled, soaked-and-boiled and *Mucuna*-free (control) diets

RESULTS AND DISCUSSION

The carcass characteristics and hematological conditions of broiler chicks that fed on the experimental diets are presented in Tables 3 and 4 for starter and finisher phases, respectively, while Plates 1 – 4 depict histopathological changes observed.

Carcass characteristics

Significant differences were observed only in the liver out of all the organs examined at both starter and finisher phases. Percent weight of liver was significantly ($P<0.05$) depressed in birds on raw *Mucuna* diet than those on heat processed *Mucuna* and control diets. However, at the starter phase the depression was not significant when compared with the toasted *Mucuna* diet. But at the finisher phase the toasted *Mucuna* diet significantly ($P<0.05$) depressed percent liver weight. The whole scenario suggests that liver may be a target organ of the toxic factor(s) of *Mucuna cochinchinensis*. Liver has been reported to be a target organ of haemagglutinins (Ikegwuonu and Bassir, 1976, Stead *et al.,* 1966; Salgakar and Sohonie, 1965). The non-significant difference between raw and toasted *Mucuna* diets at starter phase, and the significant differences between toasted and other heat-treated *Mucuna* diets at finisher phase suggest that haemagglutinin could be the implicated factor. In an earlier work Ukachukwu and Obioha (1997) reported that raw *M. cochinchinensis* contains high level of haemagglutinin (4267HU/g). In another related work still, Ukachukwu and Obioha (2000) applied same processing methods as in this work/study and reported significant reduction of the haemagglutinin in boiled (1067HU/g) and soaked-and-boiled (1067HU/g) samples but not in toasted sample (2133HU/g).

Haematological conditions

Raw *Mucuna* diet had reduction (P<0.05) effect on packed cell volume (PCV), haemoglobin (Hb) and red blood cells (RBC) at starter phase. At the finisher phase, the haematological conditions showed significant (P<0.05) depression of only Hb and RBC of the blood of birds on raw *Mucuna* diet. Also, significant differences were observed among the processed *Mucuna* diets. Levels of Hb (at both starter and finisher phases) and RBC (at finisher phase) were lower (P<0.05) in toasted *Mucuna* diet-fed birds than in the birds fed boiled, soaked-and-boiled *Mucuna* and the control diets. This further confirms the earlier suggestion that haemagglutinin is the implicated toxic component in this experiment and hence, in the feeding of *Mucuna cochinchinensis* to broiler chicken. Ologhobo *et al.* (1993) associated reduction in the values of RBC and Hb with the direct involvement of haemagglutinin when they included protein fractions prepared from lima bean in broiler starter diets.

Histopathology

Histopathological changes were observed consistently in liver sections of broilers fed diet containing *Mucuna* beans. The degree of these changes was variable in the treatment groups. Liver sections of broilers fed diet containing no *Mucuna* (NMD) beans were devoid of histopathological changes (Plate 1). Liver sections of broiler fed diet containing soaked-and-boiled *Mucuna* beans (SMD) had mild infiltration of mononuclear cells into the perivascular and periductular spaces in the portal areas (Plate 3). These same changes were observed in the liver sections of broilers that were fed boiled *Mucuna* diet (CMD), though the condition was milder. Feeding of toasted *Mucuna* diet (TMD) to broilers caused distortion of the lobular architecture of the liver, vacuolation and necrosis of hepatocytes in the periportal areas (Plate 4). Diets containing raw *Mucuna* (RMD) caused wide area of periportal necrosis with some mononuclear cells infiltration, while the centrilobular areas showed vacuolation and degeneration of hepatocytes (Plate 2).

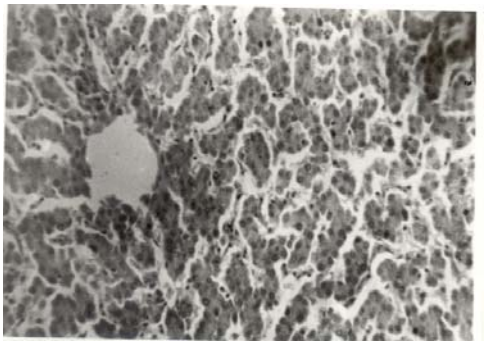

Plate 1: Liver section of broiler fed diet containing no Mucuna (NMD). Note normal lobular architecture, hepatocytes and sinusoids. (H and E stain, x320.)

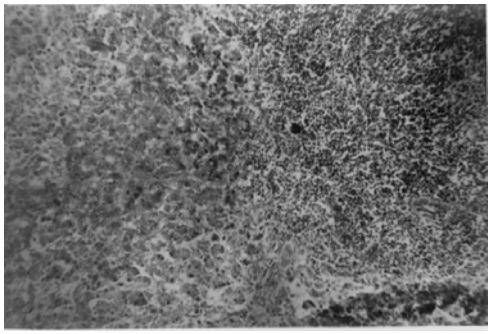

Plate 2: Liver section of broiler fed diet containing raw Mucuna (RMD). Note wild area of periportal hepatocytes necrosis (right) and centrilobular zone of hepatocytes degeneration (left). (H and E stain, x320).

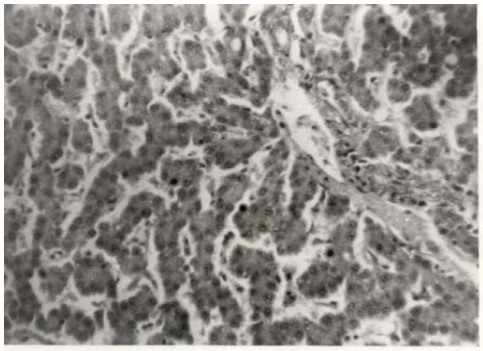

Plate 3: Liver section of broiler fed diet containing soaked-and-boiled Mucuna (SMD). Note very few mononuclear cells around vessels in the portal area. (H and E stain, x320).

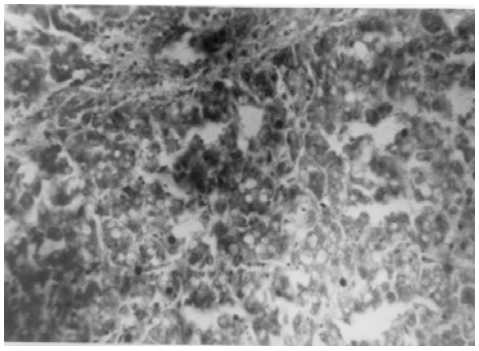

Plate 4: Liver section of broiler fed diet containing toasted Mucuna (TMD). Note distortion of lobular architecture, cytoplasmic vacuolation of hepatocytes and some mitotic nuclei of hepatocytes. (H and E stain, x320).

The severity of the lesions in the portal areas in all *Mucuna* treatment groups is related to its anatomical role as a focus of blood supply to the lobule through the hepatic portal vein from the gastro-intestinal tract, and the hepatic artery from the aorta. Consequently, ingested toxic materials getting into the liver have contact first with hepatocytes in these areas. In line with the important function of the liver as an organ of detoxification, hepatocytes in the periportal areas suffer most from toxic insults. In this situation, toxic substances present in the *Mucuna* beans must have been responsible for the histopatholgical changes found in the liver sections. Also, earlier workers have associated both zonal necrosis and complete damage of liver to the effects of haemagglutinins (Ukachukwu, 2000; Ikegwuonu and Bassir, 1976; Stead *et al*., 1966; Salgakar and Sohonie, 1965). Carew (2002) had reported alanine aminotransferase (ALT) increased in chicks fed raw *Mucuna*. ALT is a cytoplasmic enzyme whose increase in the blood plasma frequently signals either liver or muscle damage (Lumeij, 1997).

Table 3: Effect of raw or processed *Mucuna cochinchinensis* on the carcass characteristics and hematological conditions of starter broilers

	Treatment					
	RMD	TMD	CMD	SMD	NMD	SEM
Dressed, %,	55.86 (68.49)	56.54 (69.60)	56.79 (69.98)	56.51 (69.56)	56.40 (69.39)	0.49^{ns}
Kidney, %	5.69 (0.97)	5.87 (1.04)	5.29 (0.87)	5.29 (0.82)	5.88 (1.04)	0.21^{ns}
Spleen, %	1.92 (0.11)	2.14 (0.14)	2.02 (0.13)	2.21 (0.15)	2.80 (0.18)	0.13^{ns}
Liver, %	10.09 (2.66^{b})	10.46 (2.84^{ab})	9.88 (3.18^{a})	9.41 (3.30^{a})	10.34 (3.22^{a})	0.22*
Pancreas, %	3.57 (0.40)	3.53 (0.38)	3.45 (0.37)	3.35 (0.34)	3.42 (0.38)	0.18^{ns}
Heart, %	4.48 (0.61)	4.27 (0.56)	4.08 (0.51)	4.33 (0.57)	4.34 (0.57)	0.09^{ns}
Lungs, %	4.75 (0.69)	4.96 (0.75)	4.53 (0.62)	4.29 (0.56)	4.61 (0.65)	0.17^{ns}
PCV, %	32.11 (32.10^{c})	33.21 (33.68^{b})	34.91 (35.41^{a})	33.36 (35.67^{a})	33.20 (36.27^{a})	0.26*
Hb, g/100ml	2.25^{c}	10.23^{b}	11.18^{a}	11.43^{a}	11.83^{a}	0.27*
RBC($x10^{3}$)	186^{b}	269^{a}	281^{a}	282^{a}	284^{a}	6.01*

RMD, TMD, CMD, SMD and NMD represent raw, toasted, boiled, soaked-and-boiled and *Mucuna*-free (control) diets

Note: Figures in parentheses are the untransformed or original figures while the ones outside the parentheses are the transformed figures using the Arc Sine Transformation method.

Table 4. Effect of Toasting, Boiling and Soaking-and-boiling of *Mucuna cochinchinensis* on the carcass characteristics and hematological conditions of finisher broilers

	Treatment					
	RMD	TMD	CMD	SMD	NMD	SEM
Dressed wt,%	54.94 (66.96)	55.18 (67.42)	55.73 (68.31)	55.61 (68.08)	55.61 (68.08)	0.44^{ns}
Kidney, %	5.41 (0.89)	5.35 (0.87)	5.29 (0.85)	5.32 (0.86)	5.29 (0.85)	0.13^{ns}
Spleen, %	2.69 (0.22)	2.63 (0.21)	2.56 (0.20)	2.56 (0.20)	2.50 (0.19)	0.12^{ns}
Liver, %	8.60 (2.24^{c})	9.33 (2.63^{b})	9.66 (2.82^{a})	9.64 (2.81^{a})	9.68 (2.83^{a})	0.06*
Pancreas, %	3.29 (0.33)	3.19 (0.31)	3.03 (0.28)	3.03 (0.28)	2.98 (0.27)	0.10^{ns}
Heart, %	4.40 (0.59)	4.40 (0.59)	4.29 (0.56)	4.29 (0.56)	4.29 (0.56)	0.04^{ns}
Lungs, %	4.83 (0.71)	4.80 (0.70)	4.69 (0.67)	4.69 (0.67)	4.69 (0.67)	0.09^{ns}
PCV, %	34.10(31.44)	36.51 (35.40)	38.28 (38.38)	38.14 (38.13)	39.12 (39.83)	0.49^{ns}
Hb, g/100ml	9.81^{c}	11.09^{b}	12.83^{a}	12.83^{a}	12.94^{a}	0.21*
RBC($x10^{3}$)	169^{c}	287^{b}	293^{a}	294^{a}	297^{a}	6.33*

Means on the same row not followed by the same superscript are significantly different from each other at * ($P<0.05$) or ** ($P<0.01$); ns= Not significant.

RMD, TMD, CMD, SMD and NMD represent raw, toasted, boiled, soaked-and-boiled and *Mucuna*-free (control) diets

Note: Figures in parentheses are the untransformed or original figures while the ones outside the parentheses are the transformed figures using the Arc Sine Transformation method.

Perhaps, the most convincing evidence of the involvement of haemagglutinin and of liver as a target organ is the histopathological changes observed. The lesions were most severe in the liver sections of broiler fed raw *Mucuna* diet. The severity reduced a little in liver sections of broiler fed toasted *Mucuna* diet. The lesions were mildest in liver sections of broilers fed boiled *Mucuna* or soaked-and-boiled *Mucuna* diets and entirely non-existent in liver sections of broilers that consumed *Mucuna*-free diet. The degree of changes could be related to levels of haemagglutinin in the variously processed *Mucuna* meals (Ukachukwu and Obioha, 2000).

CONCLUSION

Prolonged feeding of raw *Mucuna* or improperly processed *Mucuna* has adverse effect on the carcass characteristics and aspects of hematological conditions. It also induces pathological changes in the liver of broilers. Boiling and soaking-and-boiling are better processing methods than toasting of *Mucuna* for inclusion in broiler diets, and will produce minor effect on the carcass characteristics of birds and on hematological conditions and pathological changes.

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