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EFFECT OF DIFFERENT PROCESSING METHODS OF *MUCUNA PRURIENS SEEDS* ON HISTOPATHOLOGICAL CHARACTERISTICS OF BROILER CHICKENS

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ABSTRACT

A four week feeding trial was conducted to determine the histological changes of livers of broiler chickens fed with differently processed Mucuna seed meal (MSM). Three hundred and fifteen five (315) five- week old Marshall Strain of broiler chickens were randomly assigned in a completely randomized design (CRD) to pens, after the birds were acclimatized for one week and balanced for weight. There were three replications of the seven treatments in which differently processed Mucuna seed meal were fed at different levels of T1 (0% MSM), T2 (5% BMSM), T3 (10% BMSM), T4 (5% soaked and fermented MSM), T5 (10% soaked and fermented MSM), T 6 (5% soaked MSM) and T7 (10% soaked MSM).

The tissues of liver were collected from two sacrificed birds per treatment at the end of the feeding trial to see the effect of feeding differently processed MSM to broiler chickens. The tissues were washed with ice-cold saline immediately after isolation and then fixed in 10% formalin solution and embedded in paraffin wax. Sections from each liver cell were made at a 3 micron thickness with a microtome and were stained with hematoxylin and eosin (H and E) for histopathological examination by light microscope. The sections were labeled as Plate 1-7. Plates 1-6 showed no degenerating pathological effects on the liver cells. However, the pathological effect of inclusion of soaked Mucuna at 10% (plate 7) showed a mild sinusoidal expansion, extensive and severe hepatocytic degeneration and vascular necrosis and the severe distension of the capillary vessels. It was concluded that boiling was most effective in detoxifying mucuna seeds as there were no serious damages done to the liver of broiler chickens fed BMSM diets at both 5% and 10% inclusion levels.

Keywords: Boiling, Soaking, Fermentation, Broiler finishers, Liver cells

INTRODUCTION

The prevalence of hunger and malnutrition in the tropical and subtropical areas of the world is well documented (FAO, 2003). In Nigeria the average daily protein intake per adult is still far less than the 35g per adult per day recommended by FAO (2007). This low level of animal protein consumption has direct influence on the general well-being and health of the Populace (Bamigbose *et al.,* 2002). As viable as the poultry industry is to ameliorating the animal protein deficiency in the third world, it has its own limitations and challenges. One of the greatest threats to poultry industry is the scarcity of feed ingredients and high cost of feeds. There is therefore the need to source for alternative but promising feedstuffs. One of such alternative feed ingredients is *mucunapruriens* (Akure, 2013). It is a widely available leguminous seed that thrive well where others fail due to excellent adaptability to extreme climatic conditions. It yields about two to four tones of seed per hecter. It has a crude protein of about 29.89% (Akure, 2013). Mucuna seed as a source of protein for monograstric animals is limited by the presence of anti-nutritional factors like trypsin inhibitors, haemaglutinins, phytic acid hydrocyanic acid and tannins (Emiola *et al.,* 2003; Akure, 2013). Removal

of these undesirable components is essential in order to improve the nutritional quality of seeds and to effectively utilize their full potential as feeds (Akinmutimi and Okwu, 2006; Akure, 2013). There are different methods of removing the anti-metabolic factors in mucuna seeds (Udensi *et al.*, 2005; Akure, 2013). One of such methods identified is fermentation (Akure *et al.*, 2020). This study therefore aimed at evaluating the histopathological characteristics of broiler finishers fed differently processed Mucuna seed meals such as Boiling, soaked and fermented and soaked.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the poultry section, Department of Livestock, Ministry of Agriculture, Mariri, in Kumbotso Local Government Area of Kano State. The area lies between latitude $11^0$55'N and longitude $8^0$36'E at an altitude of 460m above sea level with an average annual rainfall of 600-1000mm, mean temperatures of 21.21^{0}C and humidity of 52.81 %. (KNARDA, 2011).

Source and preparation of seeds

Mucunapruriens seeds used in this study were purchased from villages in Markudi local government area, Benue State of Nigeria, and subjected to three processing methods: boiled, soaked and fermented, and soaked.

Preparation of experimental Diets

Seven experimental diets were formulated. The inclusion levels of processed Mucuna seed meal were as follows

T1- 0% MSM,

T 2- 5% boiled MSM,

T 3-10% boiled MSM,

T 4 -5% soaked and fermented MSM,

T 5 -10% soaked and fermented MSM,

T 6 -5% soaked MSM,

T7- 10% soaked MSM.

Birds were fed 20% CP during the finisher phase (Tables 1).

Management of experimental birds

A total of three hundred and fifteen (315) five-week old chicks were used for this study. The birds were weighed at the beginning of the experiment and allotted into pens in a completely randomized design (CRD). There were seven treatments and each treatment was replicated three times with 15 birds per pen which constituted forty-five birds per treatment. The routine management such as cleanliness in and out of the poultry house, vaccination and drug administration were carried out appropriately. Birds were supplied with experimental diets and fresh water *ad libitum* throughout the trial period.

Histopathological studies

The tissues of liver were collected from two sacrificed birds per treatment at the end of the feeding trial to see the effect of feeding differently processed MSM to broiler chickens. The sections were labeled as shown below:

Plate 1 – Photomicrograph of the liver of chicken fed 0% MSM diet

Plate 2 – Photomicrograph of the liver of chicken fed 5% boiled MSM diet

Plate 3 – Photomicrograph of the liver of chicken fed 10% boiled MSM diet

Plate 4 – Photomicrograph of the liver of chicken fed 5% soaked and fermented MSM diet

Plate 5 – Photomicrograph of the liver of chicken fed 10% soaked and fermented MSM diet

Plate 6 – Photomicrograph of the liver of chicken fed 5% soaked MSM diet

Plate 7 – Photomicrograph of the liver of chicken fed 10% soaked MSM diet

Statistical Analysis

All data obtained from the feeding trial were subjected to analysis of variance using the SAS (2002).Differences among treatment

means were separated using the Duncan's multiple range test (Duncan, 1955)

RESULTS AND DISCUSSION

The results of histology of liver cells observed in broiler chickens fed mucuna seed meal diets is as shown in Table 2. It ranges from no congestion, mild congestion, and cellular infiltration and necrosis of the cells in the liver. Plate 1 and 2 are the Photomicrograph of liver of broiler chickens fed the control and 5% BMSM diets, they show similar results where the hepatocytes, Kupfer cells and the sinusoids were normal without periportal necrosis. Plate 3 and 4 are the Photomicrograph of liver of broiler chickens fed 10% BMSM and 5%SFMSM diets, they also show similar results where the hepatocytes, Kupfer cells and the sinusoids were normal with mild periportal necrosis. Plate 5 and 6 are the Photomicrograph of livers of broiler chickens fed 10% SFMSM and 5%SMSM diets, they also show similar results where the hepatocytes and Kupfer cells had a mild degeneration, the sinusoids were normal with zonal periportal necrosis. Plate 7 is the Photomicrograph of liver of broiler chicken fed the 10% soaked MSM diet, the hepatocytes and the kupfer cells had an extensive degeneration, sinusoids were mildly degenerated with extensive zonal periportal necrosis. It could be said that the severity of histopathological changes were dependent on the level of residual anti-nutritional factors present in the differently processed mucuna seeds. The result of histology of liver cells and liver structure showed no residual effect of the anti-nutritional factors in treatments 2 to 6, as liver cells did not respond negatively or abnormally. The use of differently processed MSM at these levels had no physiological effect on tissue development and function. However the liver cells of birds on treatment 7 (10% soaked MSM) showed a condition of disturbed metabolism leading to tissue reaction/cellular infiltration. This is an indication that the processing method (soaking) for *Mucuna* seed and at 10% inclusion levels is not adequate enough to remove the anti-metabolic factors in mucuna seed to a completely safe level for the consumption of broiler chickens.

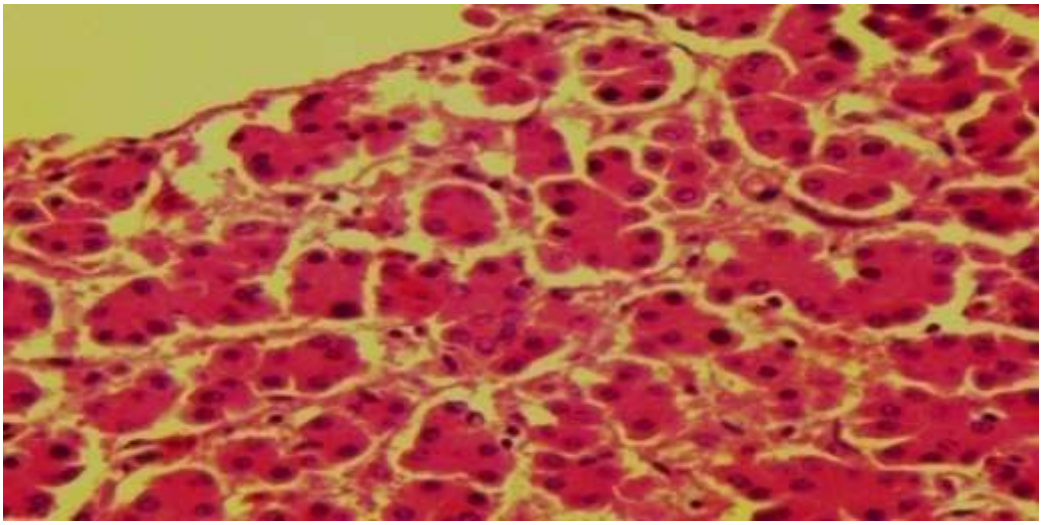


Plate 1: *Photomicrograph of a section of liver of broiler chicken fed 0%MSM diet*

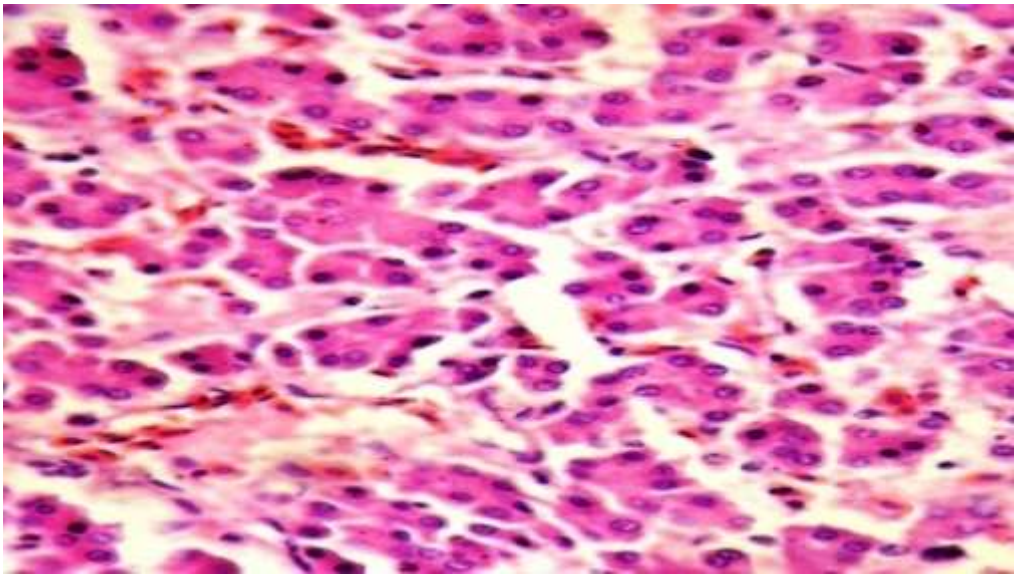


Plate2: *Photomicrograph of liver of broiler chicken fed 5% boiled MSM diet*

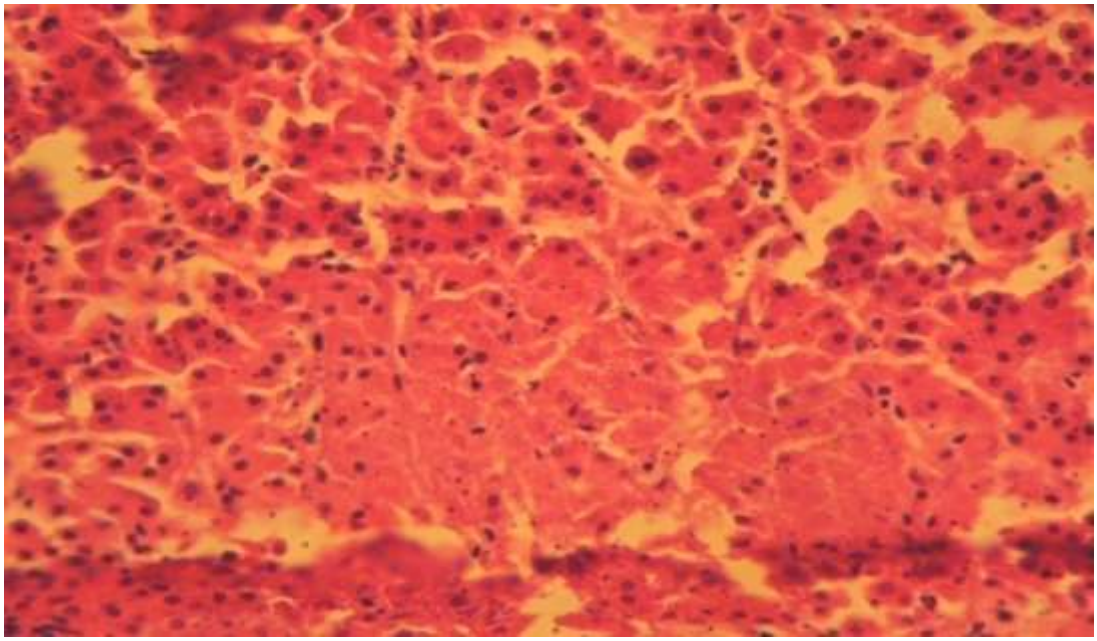


Plate 3: *Photomicrograph of a section of liver of broiler chicken fed 10% boiled MSM diet*

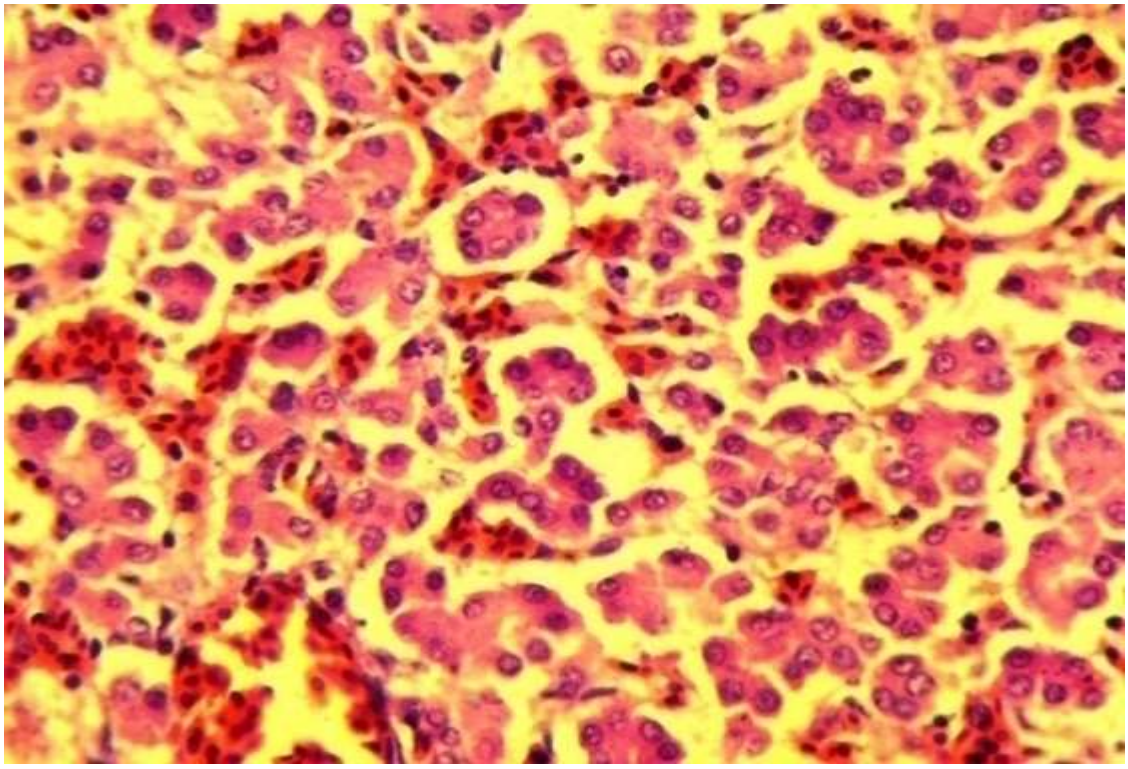


Plate 4: *Photomicrograph of a section of liver of broiler chicken fed 5% fermented MSM diet*

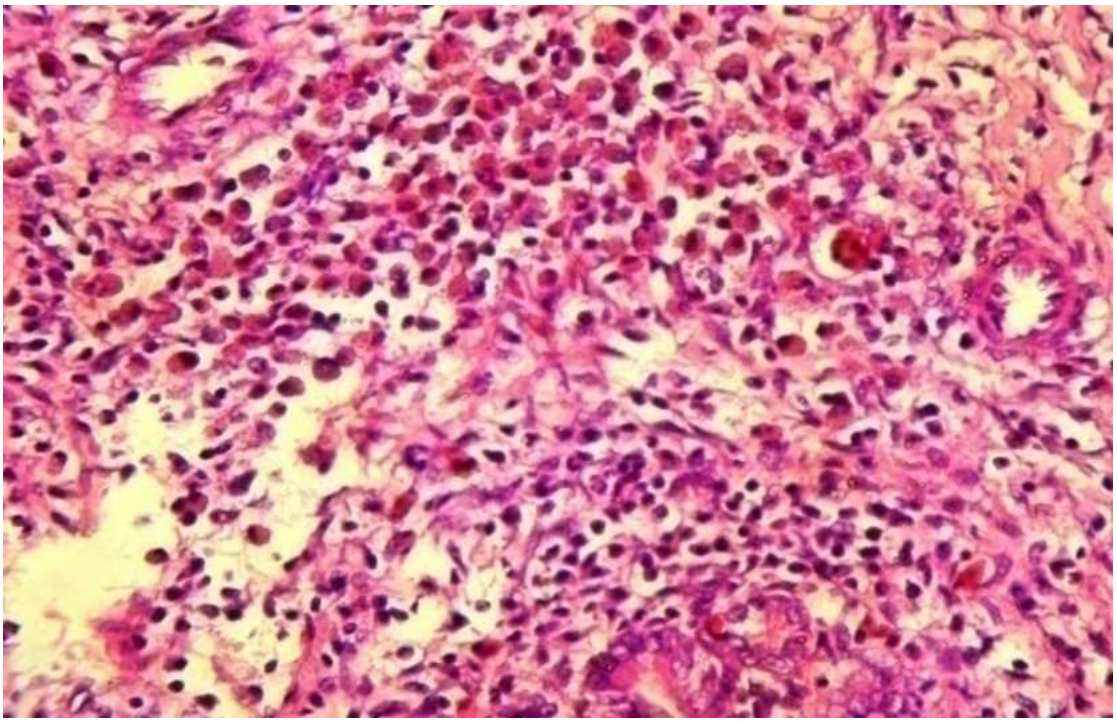


Plate 5: *Photomicrograph of a section of liver of broiler chicken fed 10% fermented MSM diet*

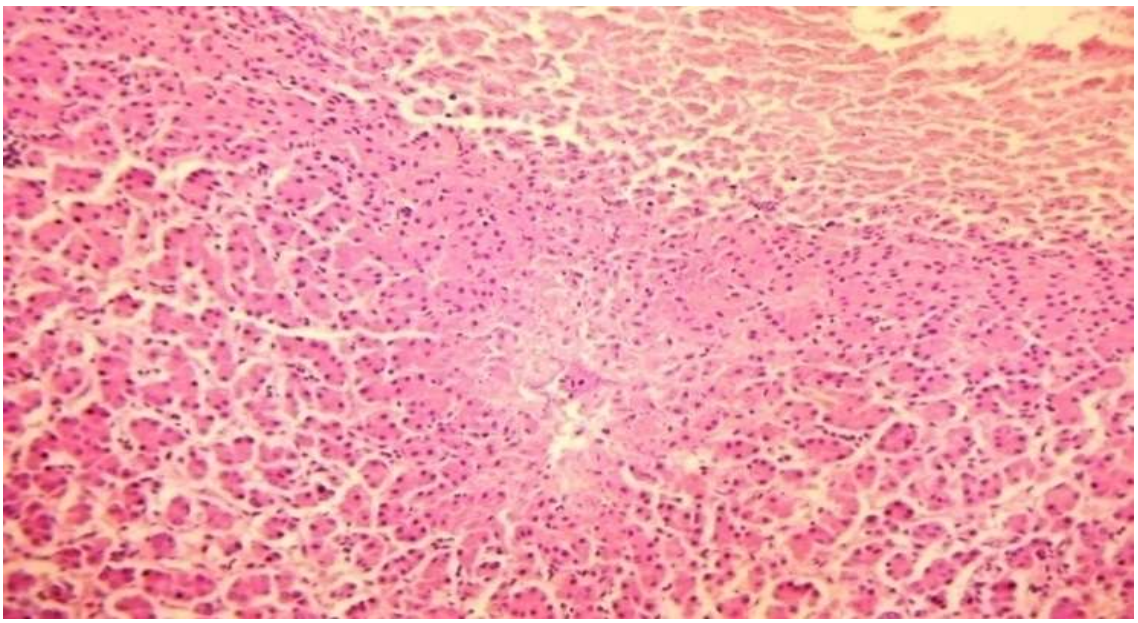


***Plate* 6:** *Photomicrograph of a section of liver of broiler chicken fed 5% soaked MSM diet*

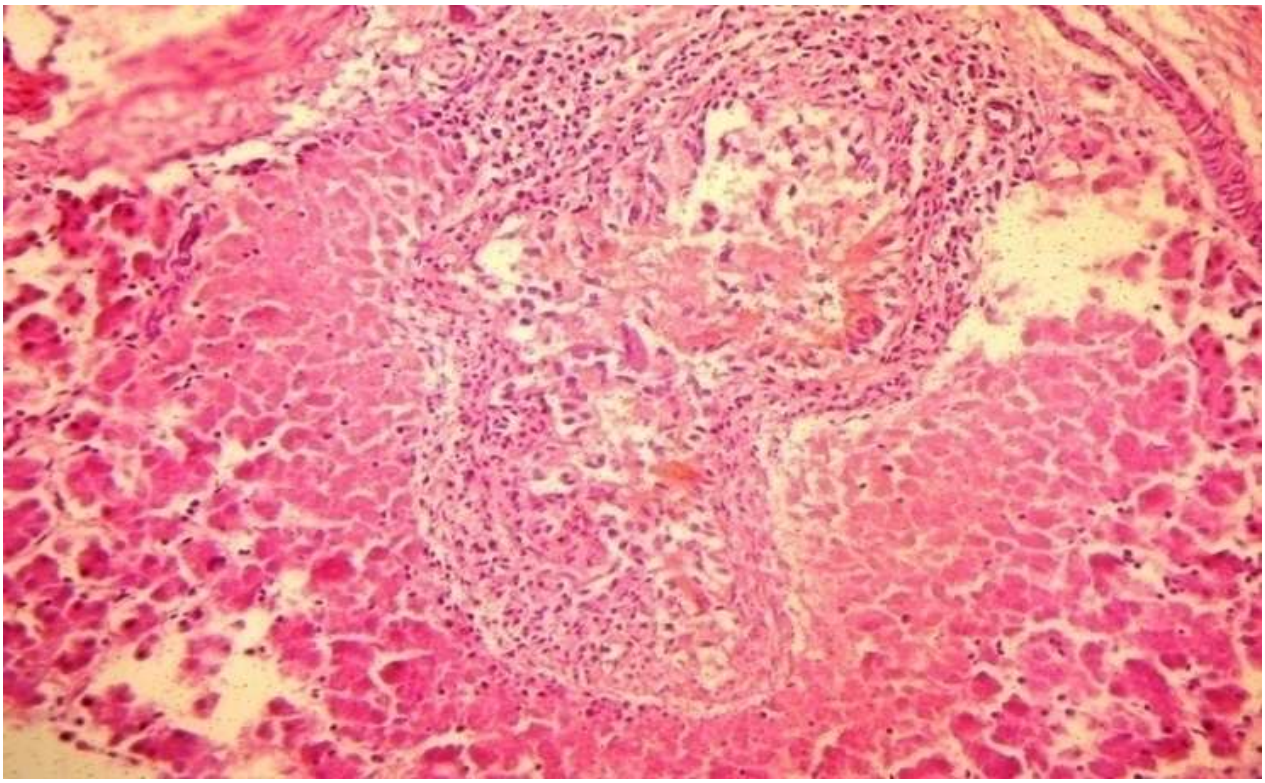


***Plate* 7:** *Photomicrograph of a section of liver of a broiler chicken fed 10% soaked MSM diet*

Table: 1 Composition of finisher diet containing differently processed *Mucuna* seed meal

Ingredients	**Levels of MSM**						
	0MSM	**BMSM**		**SFMSM**		**SMSM**	
		5%	**10%**	**5%**	**10%**	**5%**	**10%**
Maize	55.00	55.00	56.00	57.00	54.00	58.00	55.00
Mucuna	0.00	5.00	10.00	5.00	10.00	5.00	10.00
Ground nut cake	25.00	20.00	14.00	18.00	16.00	17.00	15.00
Soya bean meal	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Fishmeal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lime stone	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
M/offal	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Common Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vit/premixs	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis							
ME: (Kcal/kg)	3017	3038	3100	3028	3030	3015	3000
Crude protein (%)	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Crude fiber (%)	3.45	3.71	4,03	3.69	3.99	3.67	3.96
Ether Extract %	6.21	6.15	6.12	6.20	6.23	6.15	6.12
Ash (%)	0.64	0.64	0.64	0.64	0.63	0.63	0.63
Calcium (%)	1.36	1.37	1.38	1.38	1.39	1.38	1.40
Av. Phosphorus (%)	0.59	0.60	0.61	0.60	0.62	0.61	0.62
Lysine (%)	2. 30	2.29	2.28	2.28	2.26	2.28	2.25
Methionine (%)	0.27	0.27	0.27	0.27	0.26	0.26	0.25
Methio. + Cyst. (%)	0.75	0.74	0.74	0.74	0.74	0.73	0.73
Cost /kg diet (₦)	71.00	70.00	69.25	68.75	68.88	67.00	66.85

*****Biomix Premix supplied per kg of diet: Vit. A, 10000 i.u; vit.D_3, 2000 i.u; Vit.E, 23mg; Vit. k, 2mg vit. B1(Thiamine), 1.8mg; vit.B2(Riboflavin), 5.5mg; vit.B6(Pyridoxine), 3.0mg; vit. B12, 0.015mg; pantothenic acid, 7.5mg; Folic acid, 0.75mg; Niacin,27.5mg; Biotin,0.06mg; Choline chloride, 300mg; Cobalt, 0.2mg;Copper, 3mg; Iodine,1mg; Iron, 20mg; manganese,40mg; Selenium, 0.2mg ; Zinc, 30mg; Antioxidant, 1.25mgMSM; Mucuna Seed Meal, GNC; Groundnut Cake, SBM; Soya bean meal cake; i.u; international unit; M.E.; Metabolisable Energy.

Table 2 Effect of Processing and *Mucuna* Levels on Liver Cells

	Treatments						
	Control	BMSM		SFMSM		SMSM	
Parameters	**0%**	**5%**	**10%**	**5%**	**10%**	**5%**	**10%**
Liver structure	Normal	Normal	Normal	Normal	Mild alteration	Mild alteration	Extensive alteration
Hepatocytes	Normal	Normal	Normal	Normal	Mild degeneration	Mild degeneration	Extensive degeneration
Sinusoids	Normal	Normal	Normal	Normal	Normal	Normal	Mild expansion
Kupfer cells	Normal	Normal	Normal	Normal	Mild degeneration	Mild degeneration	Zonal degeneration
Periportal Necrosis	none	none	Mild	Mild	Zonal	Zonal	Extensive

CONCLUSION

It could be concluded from this study that feeding differently processed MSM diets to broiler chickens caused varying degrees of histopathological changes in the liver of broiler chicken. However, Boiling was most effective in detoxifying mucuna seeds as no damage was done to the liver of chicken placed on BMSM diets both at 5% and 10% inclusion.

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