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Histopathological changes in the organs of *Clarias gariepinus* fed processed velvet beans

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Abstract
Processed velvet beans (PVB) was substituted for soybeans diet at four inclusion levels and fed to the juveniles of *Clarias gariepinus* for 84 days after which the fish were examined for possible histopathological changes in its vital organs. Results showed that fish fed 0% (control) diet showed no pathological changes in any of the vital organs, however, fish fed PVB diets showed various histopathological changes in the brain, liver and kidney. Fish fed diet containing 10% PVB showed moderate spongiosis of the white matter in the cerebellum of the brain while those fed 20% and 30% showed marked spongiosis. The liver recorded various forms of changes in its hepatocytes in all treatments except the control. Marked congestion was observed in the kidney of fish fed diets containing 20% and 30% PVB. It was concluded that the level of processing in this study is insufficient for safe consumption by *C. gariepinus*.

Keywords: Histopathology; processed velvet beans; vital organs; *Clarias gariepinus*

1. Introduction
Feed accounts for about 70% of the total cost in aquaculture and protein is the most expensive component of aquaculture diets [1, 2]. Commercial aquatic feeds have traditionally been based on fishmeal as the main protein source because it's high protein content and balanced essential amino acid profile. Fishmeal is also a good source of essential fatty acids, digestible energy, minerals and vitamins. However, fishmeal is relatively expensive and its supply is inadequate. Aside these problems, the quality of fish meal is often compromised and do not meet the need for proper growth and development of cultured fish. Hence, there is the need to constantly source for alternative meal supplement that are cheap, locally available yet nutritionally safe
In the poultry industry, considerable efforts have been made towards sourcing for alternative locally abundant raw materials that could be used in the formulation of poultry feed. One leguminous plant that has been identified in this regard is the velvet bean, *Mucuna utilis*. The velvet bean is a tropical legume that has been used in traditional medicine and pharmacology [3]. Although, the seed of this plant is rarely consumed by humans (4Kay, 1979), it is reported to contain high levels of protein and carbohydrates along with an adequate concentration of minerals compared to the commonly consumed legumes [5, 6]. The seed is also known to have similar amino acids profile as other legumes seed such as soybeans [7, 8] opined that *Mucuna* can be a good source of essential amino acids for man and animals consumption [9]. reported that lysine, threonine, valine, isoleucine, leucine and phenylalanine in *M. utilis* is more than the recommended standard by Food and Agriculture Organization (FAO) and World Health Organization of the United Nations.
Despite the rich nutritive composition of this plant, studies have shown that the seeds contain a number of anti-nutritional factors, such as trypsin, phytate, cyanide, saponin, tannins etc. [10, 11]. The effects of these anti-nutritional factors can be completely eliminated or at least reduced by processing e. g. boiling [10]. While the seed of this plant has been used in the formulation of poultry diet, not much has been done to investigate the potential of including it in fish diets. We [12] had, in an earlier study, recommended processing of the seeds before its inclusion in fish feed this study investigates the effects of boiling the seed on the histology of vital organs of *C. gariepinus*.

2. Materials and Methods

Raw seeds of velvet beans (*Mucuna utilis*), were collected from International Livestock Research Institute (ILRI), located in International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (N07^{0}30.669; E03^{0}53. 907). The seeds were cooked in boiling water for 30 minutes with a kerosene stove after which they were quickly removed from the boiling water and fresh cold water was allowed to run over it in order to cool it down speedily. The seeds were then de-hulled with hands and sun–dried for three days. The dried seeds were then ground into meals. Four isonitrogenous (CP 40%) and isocaloric (ME 2900 kcal/kg) diets were formulated; diet 1 without velvet bean served as control (0%), diets 2, 3, 4, had the soybean component replaced progressively by cooked velvet bean at 10%, 20%, and 30% respectively. Fish were maintained on these different formulations for 84 days after which fish organs and tissues such as the brain, liver, gills and kidney from each experimental unit were removed and examined for possible changes due to the treatment effects on the fish. One fish was sacrificed from each replicate (i.e. three fish per treatment) and selected organs and tissues promptly removed. Tissue samples were processed for hematoxylin and eosin (H & E) staining following the procedures reported by [13].

3. Results and Discussion

The histo-pathological changes in organs of the catfish fed graded levels of PVB based diets showed lesions with variable intensity as presented in Table 1 and Plates 1 - 11. The details of changes in each organ are as listed below:

3.1 Brain

The fish brain is an integral part of the nervous system and like in the human's nervous system; it coordinates all the actions of the body. Studies have shown that alterations in the structure of the brain could result in various forms of neurological disorders [14, 15]. There was no histological lesion seen in the brain of fish fed with the control diet. The brain of fish fed diets 10, 20 and 30% PVB showed between moderate and marked spongiosis of the white matter (Plates 1 – 3). This observation is similar to the findings of [16, 17] who reported vacuolar changes in the brain of C. *gariepinus* exposed to glyphosate herbicide. Spongiosis is a vacuolation of white matter in the brain [18]. The vacuolation of white matter in the current study could be ascribed to the presence of certain anti-nutritional factors in the velvet bean.

3.2 Gill

The gills of *C. gariepinus* fed control diet did not show any lesion. The gill of *C. gariepinus* fed diet containing 10% PVB severe oedema (Plates. The gill has been in constant assaults by various biotic and abiotic stressors, this may be due to its close proximity to the ambient environments. [19], for example, also reported severe vaculoation of the gills' secondary lamellae in catfish that were infected with bacteria. The gill is principally involved in gaseous exchange in fish. The marked congestion and oedema in the gills of fish fed with diets including velvet bean would mean that these fish could have some challenges with gaseous exchange since the secondary lamellae helps to improve the surface area for gaseous exchange in fish's gills [20].

3.3 Liver

The liver is probably the most important organ in fish, just as in other vertebrates. It is principally involved in digestion, biotransformation of xenobiotics [21] and also takes part in contaminant detoxification. The liver of *C. gariepinus* in this study showed varying levels of alterations of its structure (Plates 4 -7). Fish fed diets containing 10% and 30% PVB had moderate diffuse and mild diffuse vacuolation of hepatocytes of the liver respectively, those fed diets containing 10 and 20% PVB both suffered marked diffuse vacuolation and marked diffuse vacuolation of hepatocytes while marked widespread vacuolation of the hepatocytes was observed in fish fed 20% PVB. The hepatocytes are the major cells of the liver, and have been shown to be involved in all of the metabolic activities carried out by the liver. Vacuolated hepatocytes are usually accumulated with glycogen and have little or no degenerative and regenerative ability [22] and the excessive vacuolation of the hepatic cells would result in abnormal functioning of the liver cells for example immobilization of fat, which could consequently results in fatty infiltration of the hepatic parenchyma [19].

3.4 Kidney

The kidney of fish functions principally in the maintenance of constant osmotic conditions of the fish. There was no lesion observed in kidney of *C. gariepinus* fed control diet but the kidney in fish fed diets 2 and 3 suffered marked congestion (Plates 8 and 9). The marked congestion of the kidney cells would impair the maintenance of constant homeostatic conditions thus implying that fish that were fed on diet that includes PVB may have problem with maintaining constant osmoregulatory mechanism.

Table 1: Histological changes in organs of *C. gariepinus* fed PVB based diets

Organs/Tissues	Histological signs	0%	10%	20%	30%
Brain	Moderate spongiosis of the white matter	-	+	-	-
	Marked spongiosis of the white matter	-	-	+	+
Gill	Mild clumping of the secondary lamella	-	-	-	-
	Marked congestion and oedema	-	-	-	-
	Severe oedema	-	-	-	-
Liver	Mild diffuse vacuolation of hepatocytes	-	-	-	+
	Moderate diffuse vacuolation of Hepatocytes	-	+	-	-
	Marked diffuse vacuolation of hepatocytes	-	+	+	-
	Marked widespread vacuolation hepatocytes	-	-	+	+
	Focal marked centrilobular vacuolation of hepatocytes	-	-	-	-
Kidney	Marked congestion	-	+	+	-

Legends: - No visible lesions + Observed lesion.

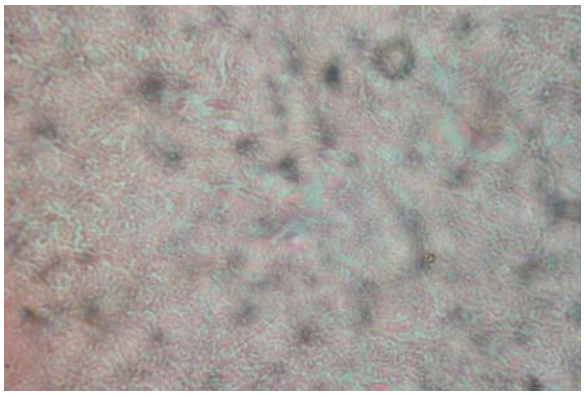

Plate 1: Photomicrograph of a section of brain of *C. gariepinus* juveniles fed 0% PVB showing normal cells

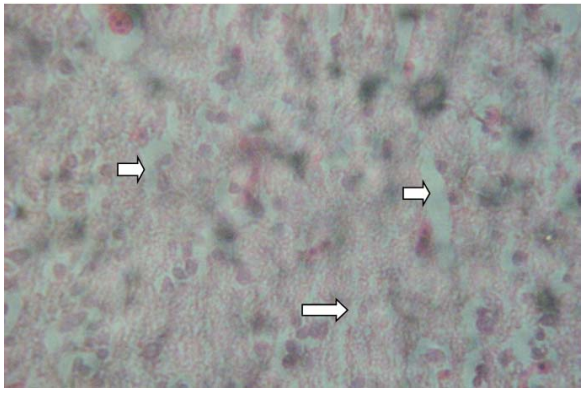

Plate 2: Photomicrograph of a section of brain of *C. gariepinus* juveniles fed 10% PVB showing moderate spongiosis of the brain cells.

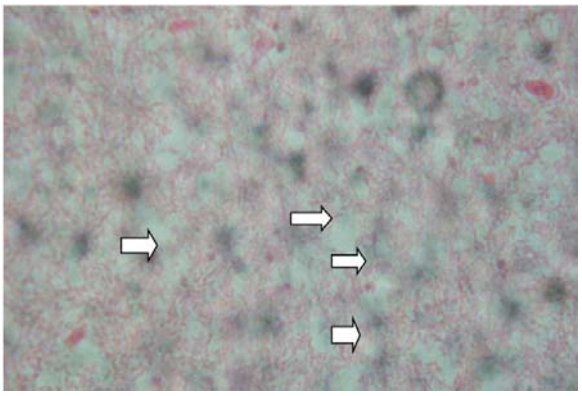

Plate 3: Photomicrograph of a section of brain of *C. gariepinus* juveniles fed 20 and 30% PVB showing marked spongiosis of the white matter

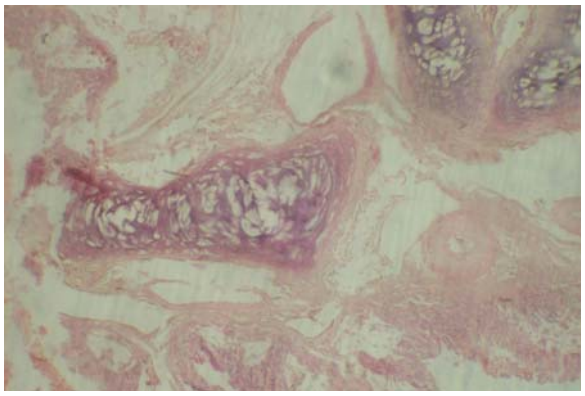

Plate 4: Photomicrograph of a section of kidney of *C. gariepinus* juveniles fed 0% PVB showing normal cells

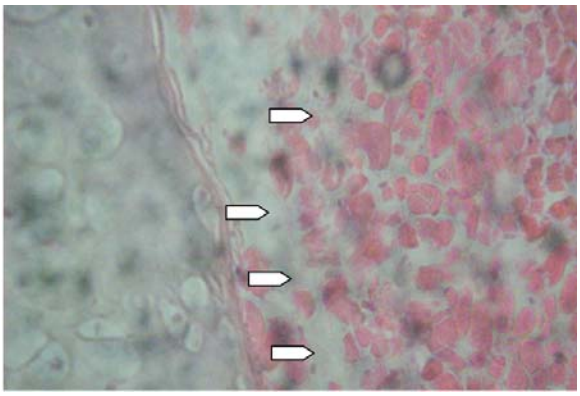

Plate 5: Photomicrograph of a section of gill of *C. gariepinus* juveniles 20% fed PVB showing severe oedema

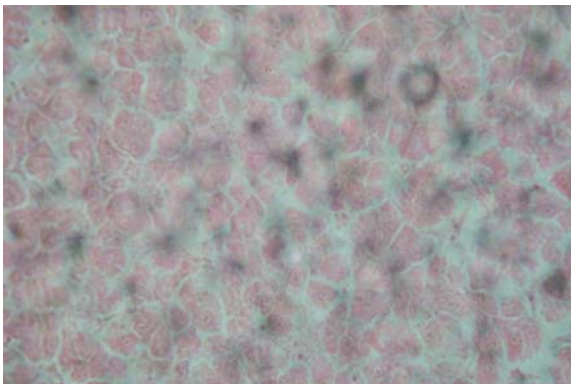

Plate 6: Photomicrograph of a section of liver of *C. gariepinus* juveniles fed PVB showing normal cells.

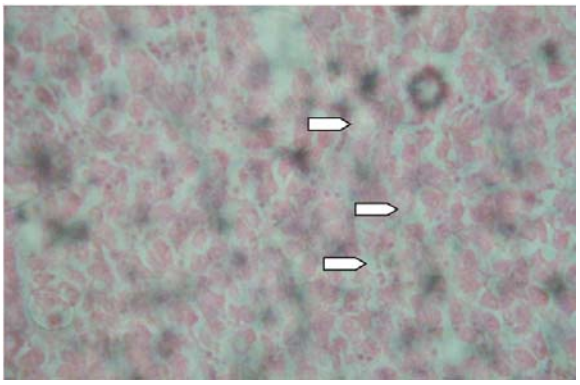

Plate 7: Photomicrograph of a section of liver of *C. gariepinus* juveniles fed 20% PVB showing marked diffuse vacuolation of hepatocytes

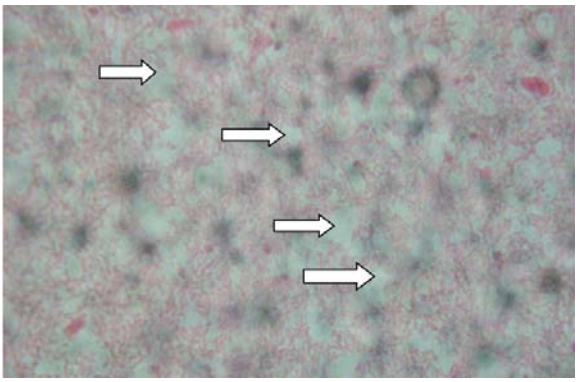

Plate 8: Photomicrograph of a section of liver fed 20% PVB showing marked diffuse vacuolation of hepatocytes

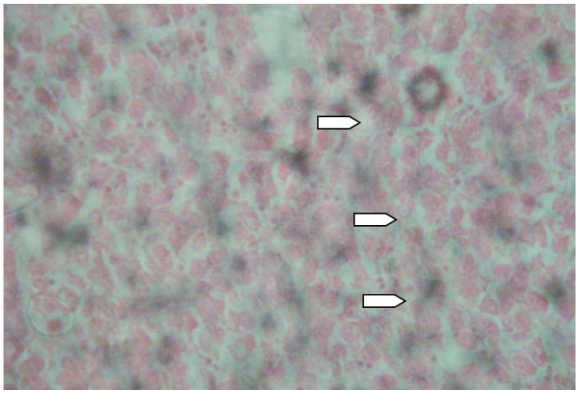

Plate 9: Photomicrograph of a section of liver fed 30% PVB showing mild diffuse vacuolation of hepatocytes

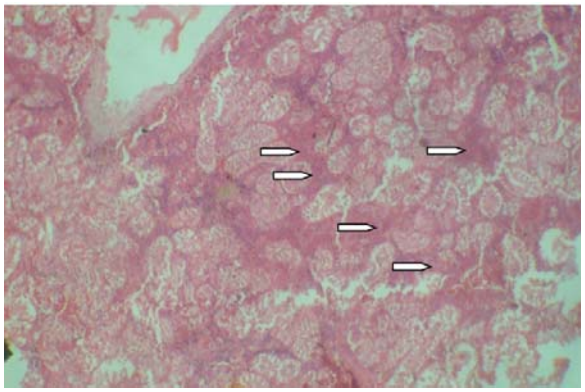

Plate 10: Photomicrograph of a section of kidney fed 10 and 20% PVB showing marked congestion.

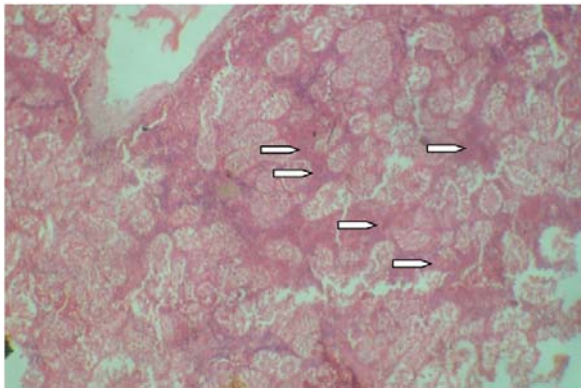

Plate 11: Photomicrograph of a section of kidney of fish fed 10 and 20% PVB showing marked congestion.

4. Conclusion

The results of this study showed the potential for disruption of normal physiological activities in *C. gariepinus* that were fed with diets that includes PVB. Although, there was an improvement in the results obtained in the current study over that of the raw seeds [12], the level of processing adopted in this study has not completely detoxified the anti-nutrients in this legume for safe consumption by *C. gariepinus*. It is therefore concluded that further processing methods should be explored before the inclusion of velvet bean in fish diets.

5. Acknowledgements

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