Histopathological changes in liver and kidney of sharptooth catfish fed on cooked *Jatropha curcas* seedmeal based diets

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**Introduction:** Histology of the organs of fish species is important in the understanding of the pathological changes related to nutritional sources. This study evaluates the histopathological alteration in the liver and kidney of sharptooth catfish fed diets containing *Jatropha curcas* seedmeal in a 56-day feeding trial.

**Methods:** *Clarias gariepinus* fingerlings of average weight 2.61±0.02g were acclimatized for a week, and allotted into five dietary treatments; containing 0, 25, 50, 75 and 100% *Jatropha curcas* seedmeal replacement levels for soybean meal respectively. Each treatment was replicated three times with fifteen fish per replicate. Fish were fed 5% body weight on two equal proportions per day. Histological assessment was conducted using standard procedure.

**Results:** There were no lesions on the photomicrographs of the kidney of fish exposed to all the dietary treatments while moderate vacuolation of the hepatocytes was observed in the liver of fish fed diet D1-control diet. There were very prominent melanomicrophage centres in the liver of *Clarias gariepinus* fed test diet D3 with some hepatocytes which appeared vacuolated. The trend of vacuolation of hepatocytes of the liver among some treatments were not dietary related as no visible lesions were seen in the liver of fish fed test diets D2 ; D4 and D5.

**Significance:** This study established that *Jatropha curcas* based diets exert hepatoprotective effect on the liver of fish fed with the diets.

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Kumar et al. (2008) can only be removed by using organic solvent extraction method which is one of its nutritional limitations. The use of *Jatropha curcas* seedmeal in fish diets in fish feed is well documented; detoxified *Jatropha curcas* meal for rainbow trout in freshwater culture (Kumar et al., 2011b); for carp (Kumar et al., 2010a, Kumar et al., 2010b; Kumar et al., 2011a); boiled *Jatropha curcas* meal for *Clarias gariepinus* (Fakunle et al., 2013; Alatise et al., 2014); cooked *Jatropha curcas* meal for *Clarias gariepinus* (Jimoh et al., 2016; Jimoh et al., 2019) and recently toasted *Jatropha curcas* meal for *Clarias gariepinus* (Jimoh et al., 2020). There are few studies that centre majorly on histopathological observations of liver and kidney parenchymatous tissues sequel to dietary supplementation of *Jatropha curcas* in catfish diet. This study attempts to investigate the histopathological changes in the liver and kidney of the sharp-tooth catfish (*Clarias gariepinus*) fed diets containing cooked *Jatropha curcas* seedmeal. This would be of importance to the tropical fish farmer and professionals which could have a positive impact on aquaculture and agro-economics.

**Materials and methods**

**Sources and processing of ingredients**

Sample of dried *Jatropha curcas* seeds (1kg) were obtained in Osogbo, Osun state at the beginning of the rainy season. The *Jatropha curcas* seeds were de-kernelled, by cracking and removing the coat using manual method, rinsed with water and boiled for 15 minutes after which they were sundried (29-31°C) for three days and then ground in a hammer mill to produce a meal. The meal was thereafter analysed for their proximate composition (AOAC, 1990). Fish meal, soybean meal and other feedstuffs obtained from commercial sources in Nigeria were separately milled and screened to fine particles size. Samples were taken in triplicates and analysed for their proximate compositions to show compositions for standardization (AOAC, 2010) (Table 1).

**Experimental diets**

The experimental diets were formulated (Table 2) using the nutrient composition of the protein feedstuffs (Table 1). The experimental diets contained full-fat soybean meal which was replaced by cooked *Jatropha curcas* seedmeal at the rate of 0, 25, 50, 75, and 100%. The diets were isolipidic and isonitrogenous containing 40% crude protein and 12% lipid with fish meal, full fat soybean meal, fish oil, vitamin premix and starch serving as ingredients. Starch was considered binder and filler and water was added to aid binding after which it was introduced into a pelleting and mixing machine (Hobart A-200T) to obtain a wet homogenous mass and then passed through a mincer to produce 2mm size pellet which was immediately sundried at 30-32°C. After drying for three days, the diet was kept in a cool place.

**Experimental fish and system**

The experiment was conducted at the nutrition unit of the Department of Fisheries Technology, Federal College of Animal Health and Production Technology, Moor Plantation Ibadan. *Clarias gariepinus* fingerlings (n = 225; 2.61±0.02g average weight) were obtained from a reputable fish farm in Ibadan, Oyo state and transported live to the project site in an aerated bag. *Clarias gariepinus* fingerlings were acclimated for 7 days prior to the feeding trial while being fed on a commercial pelleted diet. Fingerlings (n = 15) were allotted into each of the fifteen 25-litre capacity rectangular tanks containing 20 litres of water. Experimental diets were assigned randomly to the tanks with three replicates per treatment. Fish in each tank were fed 5% body weight per day in two equal proportions between 9.00 – 10.00am and 5.00 – 6.00 pm for 56 days.

**Histological examination of test organ**

At the end of the experiment, three fish per treatment were sampled for histological analysis. The test organisms were anaesthetized using 100 mg/l clove oil and were cut open to excise the kidney and liver. The organs were fixed in 10% formalin for three days after which the tissue was dehydrated in graded levels of 50%, 70%, 90% and 100% alcohol for 3 days, to allow paraffin wax to penetrate the tissue during embedding. The tissues were then embedded in melted wax and sectioned into thin sections (5-7μm), by means of a rotatory microtome and each section was cleared by placing it in warm water (38°C), where it was picked with clean slide and oven-dried at 58°C for 30 minutes to melt the wax. The slide containing sectioned tissue was cleared using xylene and graded levels of 50%, 70%, 90%, 95% and 100% alcohol for two minutes each and stained with Harris hematoxylin-eosin (H&E) stain following the methods of Bancroft and Cook, 1994). The stained slides were observed under a light microscope at varying X400 magnification, sections were examined and photographed using an Olympus BH2 microscope fitted with photographic attachment (Olympus C35 AD4), a camera (Olympus C40 AB-US) and an automatic light exposure unit (Olympus PM CS5P). The interpretation of the micrographs was done at Department of Veterinary Anatomy, University Ibadan, Nigeria.

**Table 1. Proximate composition of feed ingredients**

| Parameters       | Fish meal | Soybean meal | *JSM  | Corn Meal |
|------------------|-----------|--------------|-------|-----------|
| Moisture         | 9.75      | 10.70        | 3.90  | 10.48     |
| Crude protein    | 72.4      | 38.74        | 30.34 | 9.87      |
| Crude lipid      | 10.45     | 16.68        | 45.89 | 4.28      |
| Crude fiber      | -         | 5.10         | 8.41  | 5.78      |
| Ash              | 8.32      | 4.48         | 4.42  | 6.73      |
| NFE              | 24.30     | 7.04         | 52.35 |           |

*Jatropha curcas* seedmeal
Table 2. Gross composition of experimental diets (g/100g dry matter) containing Jatropha curcas seedmeal fed to Clarias gariepinus

| Feed ingredients | D1     | D2     | D3     | D4     | D5     |
|------------------|--------|--------|--------|--------|--------|
| Fishmeal         | 33.33  | 33.33  | 33.33  | 33.33  | 33.33  |
| Yellow maize     | 10.00  | 10.00  | 10.00  | 10.00  | 10.00  |
| Full fat JSM     | -      | 12.36  | 24.72  | 37.08  | 49.44  |
| Full fat SBM     | 50.00  | 37.50  | 25.00  | 12.50  | -      |
| Fish premix      | 5.00   | 5.00   | 5.00   | 5.00   | 5.00   |
| Starch           | 1.67   | 1.81   | 1.95   | 2.09   | 2.23   |
| Total            | 100    | 100    | 100    | 100    | 100    |

D – Diet; JSM – Jatropha curcas seedmeal, SBM – Soybean meal
Each 1Kg Premix Contains:: Vitamin A; 4000000 IU, Vitamin D3; 8000000 IU, Vitamin E; 40000 IU, Vitamin K3; 1600mg, Vitamin B1; 4000mg, Vitamin B2; 3000mg, Vitamin B6; 3800mg, Vitamin B12; 3mcg, Nicotinic acid; 18000mg, Pantothenic acid; 8000mg, Folic acid; 800mg, Biotin; 500mcg, Cholin chloride; 120000mg, Iron; 8000mg, Copper; 800mg, Manganese; 6000mg, Zinc; 8000mg, Iodine; 400mg, Selenium; 400mg, Vitamin C(coated); 40mg, Inositol; 60000mg, Cobalt; 10000mg, Lysine; 150mg, Methionine; 10000mg, Anti-oxidant; 25000mg.

Ethical statement
The regulations and guidelines of Federal College of Animal Health and Production Technology, Ibadan, Nigeria for the care and use of laboratory animals were followed during transportation of fish and associated care of fish at the beginning, during and termination of the experiment. Animal Research: Reporting of In-vivo Experiments (ARRIVE) guidelines were followed in reporting this study.

Statistical analysis
Data obtained from proximate analysis were expressed as mean ± standard deviation (SD) and subjected to one-way analysis of variance (ANOVA). Duncan multiple range was used to separate the means where significant variation (p < 0.05) were recorded.

Results and discussion
Proximate composition of the experimental diets
The proximate composition of experimental diets fed to Clarias gariepinus is shown in table 3. There was no significant difference (p > 0.05) in the crude protein, lipid and Nitrogen Free Extract showing that the various diets prepared were isonitrogenous, isocalorific and isolipidic. All the fish responded well to the dietary treatments given to them. The protein and lipid requirement of Clarias gariepinus was met by the quantity provided in the diets. Uys and Hecht (1985) reported that the best growth rate and feed conversion efficiency in juvenile and sub-adult Clarias gariepinus are achieved with diets containing 29-42% crude protein and optimum liquid content of 10-11%.

Histological changes in the liver and kidney of Clarias gariepinus fed diets containing Jatropha curcas seedmeal
Figure 1 shows the photomicrograph of kidney of Clarias gariepinus fed diet D1, no lesion was observed. The photomicrographs showing kidney of fish fed test diets D2 to D5 are as presented in Figures 1b to 1e respectively. There was also no lesion in each of the micrographs; this implies that there was no toxicity that could be caused by feeding Jatropha curcas seedmeal on the kidney of Clarias gariepinus for the time the fish were fed the ingredient. The xenobiotic compounds in the dietary plant ingredients were completely detoxified by the liver. Our reports agree with the findings of Ali et al. (2018) who reported no lesion between kidney micrographs of fish fed up to 1% Biogen and 3% sodium butyrate and those fed control diet. Figure 2 shows the photomicrograph of the liver of fish fed control diets D1. There was a moderate vacuolation of the hepatocytes of the liver of fish fed diet D1 – control diet. There are very prominent melanomicrophage centres and some hepatocytes appear vacuolated in the liver of Clarias gariepinus fed test diet D3. The liver histology of fish groups fed control diet-D1 and test diet-D3 showed an intermediate liver condition categorised as grade 2 liver using the assessment of Martínez-Llorens et al. (2012). The liver is pathologically affected because it is the principal metabolic and detoxifying organ (Bernet et al., 1999; Bernet et al., 2004). This study showed that there was a marked vacuolation of hepatocytes of the liver in some treatments which were not dietary related because no reason could be used to explain why the vacuolation of some hepatocytes observed in the liver of Clarias gariepinus fed test diet D3 which is 50% replacement with cooked Jatropha curcas while at lower (25%) and higher (75, 100%) replacement no lesion was seen. The anti-nutrients present in plant feed ingredients could plausibly be the cause of pathological observations on the liver of fish fed Jatropha curcas based diets (Francis et al., 2001). Our observation is in consonance with the report of Pereira et al. (2002) that liver histology of the control group contained a lot of lipids and had larger number of vacuolated hepatocytes than test diets fed groups linking it to a higher glyco- gen accumulation. Valente et al. (2011) made similar observation when Senegalese sole was fed diets containing mixtures of different plant proteins.

The presence of numerous vacuolation in the liver as observed in this study seems to be pathognomonic lesion of fish exposed to high dietary lipid intake. Gatta et al. (2011) explained that high vacuolation of the hepatocytes is physiologic response to dietary lipid intake. Olukunle (2011) also made similar observation when evaluating the effect of different dietary oil sources on growth performance and nutrient utilization of Clarias gariepinus. Jimoh et al. (2015) observed vacuolation in the liver of fish fed diet containing Chrysophyllum albidum seedmeal. Mérida et al. (2010) and (Pereira et al. 2002) reported vacuolation in the liver of sharpnout sea bream (Diplodus puntazzo) when fed sunflower meal and the liver of rainbow trout (Oncorhynchus mykiss) fed brassica-by-products respectively. Vacuolation of the liver is probably induced by the phobol esters and other secondary metabolites in Jatropha curcas causing oxidative stress and compromise the integrity of the membrane of the hepatocyte.
cytes or causing lipid peroxidation. *Jatropha curcas* seedmeal has high content of fat (Kumar et al., 2011a, 2012) which could be as high as 58% lipid (Martinez-Herrera et al., 2006) with the unsaturated fatty acids and saturated fat-

| Parameters    | D1       | D2       | D3       | D4       | D5       |
|---------------|----------|----------|----------|----------|----------|
| Moisture      | 9.76±0.26| 9.88±0.62| 9.70±0.47| 9.99±0.72| 9.61±1.39|
| Crude Protein | 40.20±0.20| 40.18±0.19| 40.23±0.34| 40.13±0.19| 40.10±0.13|
| Crude Lipid   | 12.03±0.05| 12.04±0.07| 12.02±0.09| 12.12±0.18| 12.19±0.13|
| Crude Fiber   | 5.06±0.08c| 5.10±0.09bc| 5.43±0.29b| 5.83±0.11a| 5.99±0.24a|
| Ash           | 4.24±0.25 | 4.19±0.57 | 4.21±0.21 | 4.12±0.07 | 4.22±0.19 |
| NFE           | 28.72±0.26| 26.00±0.77| 26.41±0.74| 27.80±0.82| 27.89±1.14|

Means on the same row without superscript are significantly different from each other (p < 0.05)

![Figure 1](image1.png)

**Figure 1.** Photomicrographs (5µm, ×400 (a,d,e) ×100 (b,c), H&E stained) of the kidney of *Clarias gariepinus* fed:
(a) diet D1: no visible lesion seen
(b) diet D2: no visible lesion seen
(c) diet D3: no visible lesion seen
(d) diet D4: no visible lesion seen
(e) diet D5: no visible lesion seen
ty acids being in a ratio of 3:1 (Joshi et al., 2013). Nonetheless, no visible lesion was seen in the liver of fish fed test diets D2 (Figure 2b); D4 (Figure 2d) and D5 (Figure 2e). It appears from the micrographs that a form of hepatoprotective effect in *Jatropha curcas* seedmeal is conferred on the liver of fish fed the test diets. Natural products from *Jatropha curcas* exhibit an extensive variety of pharmacological activities, such as antioxidant, antiviral, antimicrobial hepatoprotective and anticancer effects (Abdelgadir and Van Staden, 2013). Jatropha seeds possess secondary metabolites such as alkaloids, lignans and phenolic compounds (Makkar and Becker, 2009; Devappa et al., 2010a; Devappa et al., 2010b). Phenolic compounds can constrain reactive oxygen species (ROS) and other oxygen radical generation in cells.
and tissues by conjugating the double bonds (Van Wyk, 2015; van Wyk and Wink, 2015; van Wyk and Wink, 2018) and can act synergistically together with flavonoids to offer enhanced protection to cells and tissues (Alok et al., 2014). The effects of these bioactive compounds in *Jatropha curcas* seedmeal could therefore be the cause of hepatoprotection of the livers of fish fed *Jatropha curcas* based diets. Hoseinifar et al. (2020) reported that antioxidant effects of bioactive components in plant sources could improve cell protection. Balaji et al. (2009) reported that oral administration of methanolic extract from leaves of *Jatropha curcas* to rat reduced lymphocytic infiltrations, hepatic necrosis and incidence of liver lesions induced by aflatoxin B1. Pertino et al. (2007) reported jatrophonol A and B in *Jatropha* seed have gastro-protective activity by reducing the HCl/ethanol-induced gastric lesions in mice by 54% at an oral dose of 100 mg kg⁻¹ and by 65% at 60 mg kg⁻¹ respectively.

**Conclusion**

No visible lesion observed in the liver micrographs of the fish fed *Jatropha curcas* based diets showed that a form of hepatoprotective effect in *Jatropha curcas* seedmeal was conferred on the liver of the fish fed with the test diets.

**Conflict of interest**

Authors declare no conflict of interest.

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