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Histology of liver of *Claria gariepinus* Fingerlings Fed with Spent Sorghum from Locally Fermented Drink (Burukutu)

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ABSTRACT: High fiber content, increased presence of carbohydrates, anti-nutritional factors can have adverse effects on the digestive system of fish and therefore on fitness, health and production capacity. A 70-day study was conducted to evaluate the histological alteration of the liver of *Clarias gariepinus* fed with spent sorghum. Ten isonitrogenous diets were formulated. Spent sorghum was added to the diet to replace maize at graded levels; 0%, 10%, 20%, 30%, 40%, 50%, 70%, 80%, 90% and 100%. Total of 360 fish (8.330±0.00g) were randomly assigned to the five treatment diets. Each treatment contained 10 fish per bowls and each treatment was triplicated in a completely randomized design (CRD). Fish were fed twice daily at 5% body weight in equal proportions. At the end of the feeding trials, the liver of the fish were taken for histological analysis. The histological results of the liver showed that some treatments showed cytoplasm with irregular arrangement and dislocated nucleus; slight haemorrhage in the hepatic parenchyma with peribular capsule indistinct between hepatocytes and enlarged hepatocytes which is not a dietary treatment related.

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Keywords: spent Sorghum, Burukutu, liver, and *C. gariepinus.*

The continuous increase in the demand for fish has made aquaculture industry the rapid growing food producing sector in the world (FAO, 2012). Histopathology together with other methods such as: biochemical, growth, diseases diagnostic, are biomarkers used in assessing effects of both internal (feed used) and external (aquatic) environmental conditions (Rajeshkumar and Munuswamy, 2011). Histological analysis of the digestive system, such as liver and intestine are considered a good indicator of the nutritional status of fish (Caballero et al., 2003). The intestine and liver are the most crucial organs in digestion and absorption of nutrients from food, and therefore observing these organs is considered very important. The liver is considered as a good indicator of nutritional pathology due to its function in metabolizing products coming from the digestive tract. Fish liver histology is characterized by the absence of liver lobules and portal triads that are the basic morphological unit of liver structure in mammals. Although all structural elements (liver cells-hepatocytes, blood vessels, and bile ducts) are present in the liver of fish, they are differently organized compared with mammals (Božidar *et al*. 2011)

MATERIALS AND METHOD

Study Area: The experiment was conducted in Anyigba, Kogi State. Anyigba is a town in Dekina local government area of Kogi State with Headquarter in Dekina town.

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Source and Processing of Spent Maize: Spent Sorghum was sourced from Egume in Dekina Local Government and Ejegbo in Ankpa Local Government. Spent maize in fresh form contains about 80-85 % moisture, the waste was sundried for 7 days with regular turning to prevent mold. The sundried SMZ was milled and taken for proximate analysis. The milled product was then mixed with other feed ingredients (fish meal, soybean meal, corn, vitamin/mineral premix) and grinded together.

Ingredients used in the feeding trial: fishmeal, soybean meal, corn, vitamin/mineral premix, were bought from Ilorin market, Kwara State, Nigeria. These ingredients were separately milled, screened with fine mesh net to fine particle size (<250 μm).

Experimental diets: **(**Ten (10) isonitrogenous experimental diets were formulated. The diets contained progressively increasing levels of spent sorghum (SSG) from Burukutu (BKT) production and were used for the feeding trial experiment. The experimental diets were formulated to produce diets in which 0% (SSG0), 10% (SSG10), 20% (SSG20), 30% (SSG30), 40% (SSG40), 50% (SSG50) 70% (SSG70) 80% (SSG80) 90% (SSG90) and100% (SSG100) of carbohydrate from corn were replaced with that from Spent Sorghum (SSG).

Culture condition: Fish were sorted, weighed (using a sensitive weighing balance) and randomly stocked into the experimental bowls. Twelve (12) fish were stocked per bowl. They were starved overnight before commencing the feeding trials. At the end of acclimation, fish specimen in each bowl was weighed to determine their initial mean weight. Fish were offered 5% of their body weight.

Experimental set up: (10) different treatments with three replicates were used for the experiment. Total of 30 bowls containing (12) fish for each bowl, making a total of 360 fingerlings were used for the first experiment. Temperature, dissolved oxygen and pH was monitored through the 10 weeks of the experiment.

Tissue Processing: Grossing: The tissues were observed and cut into small pieces of not more than 4mm thick into pre-labeled cassettes. These were further immersed in 10% formal saline for 24 hours to fix.

Tissue Processing: this is done automatically using automatic tissue processor (Leica TP 1020). The tissue were allowed to pass through various reagents including; stations 1 & 2 containing 10% formal saline, station 3 to station 7; alcohol (70%, 80%, 90%, 95%, absolute 1 & absolute 11) for the purpose of dehydration. The tissues continued to pass through station 8 and station 9 containing two changes of xylene for the purpose of clearing and finally transferred into three wax baths for infiltration/impregnation. The machine has been programmed to run for 12 hours, tissues stayed in each station for 1hour.

Embedding: each processed tissue was given a solid support medium (paraffin wax) and this is done using a semi-automatic tissue embedding center. The molten paraffin wax was dispensed into a metal mold and the tissue was buried and oriented in it, a pre labeled cassette was placed on this and was transferred to a cold plate to solidify. The tissue block formed was separated from the mold.

Microtomy: the blocks were trimmed to expose the tissue surface using a rotary microtome at 6micrometer. The surfaces were allowed to cool on ice before sectioning. The tissues were sectioned at 4 micrometer (ribbon section)

Floating; the sections were floated on water bath (Raymond lamb) set at 55^0C and these were picked using clean slides. The slides were labeled.

Drying; the slides were dried on a hotplate (Raymond lamb) set at 60^0c for 1hour.

Staining: the staining technique used was Haematoxylin and Eosin technique

Procedure for Haematoxylin and Eosin (H&E) Technique: The tissue was Dewaxed in Xylene for 15mins, then take through Absolute Alcohol, 95% and 70% Alcohol, the section was rinsed to water, stained in Harris haematoxylin for 5mins, rinsed in water, differentiated in 1% acid alcohol briefly, rinsed in water, Blued under running tap water for 10mins, Counterstained with 1% aqueous Eosin for 2min, Rinsed in water.Dehydrated in ascending grades of alcohol.Cleared in xylene and Mount in DPX. (Avwioro O.G. 2010)

RESULTS AND DISCUSSION

Photomicrograph of liver of *C. gariepinus*(x400) fed with spent Sorghum meal (SSM), stained by Haemuoxylin and Eosin (H&E). (A, B, C D, E F, I J) showing normal central vacuole without congestion with the control (CV). A, B, C, D, E F, and J showing the morphology of the hepatocytes show glycogenic and lipid-filled cytoplasm with centrally located nucleus with the control (HCN).

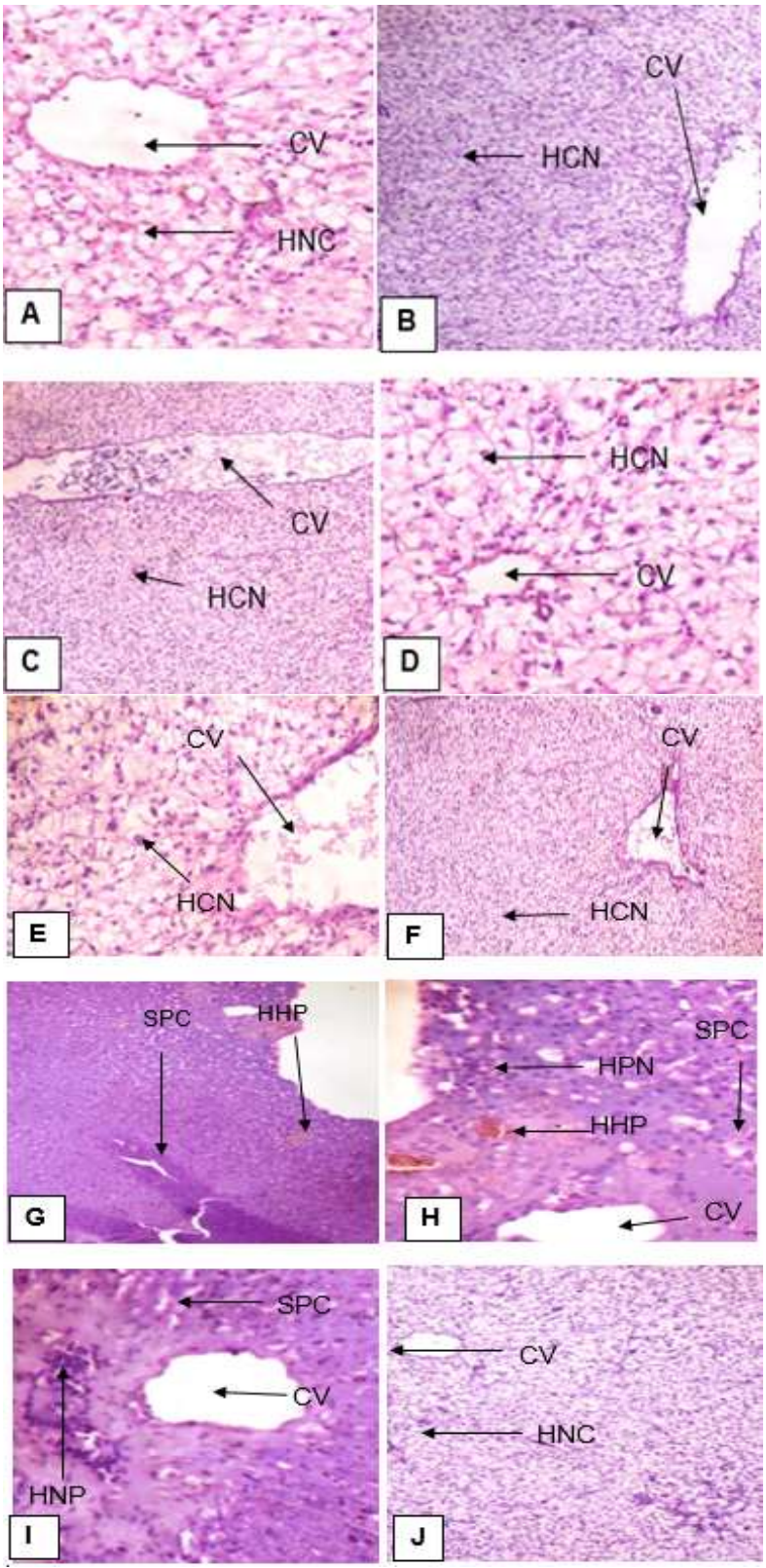

Fig. 1: Photomicrograph of Liver *Clarias gariepinus*
A = control diets (0% of SSM), B = 10%SSM, C = 20%SSM, D = 30%SSM, E = 40%SSM, F = 50%SSM, G = 70%SSM, H = 80%SSM, I = 90%SSM, J = 100%SSM

H showing cytoplasm with the irregular arrangement and dislocated nucleus (HPN), I, showing enlarged hepatocytes with pyknotic nucleus (HPN), G and H showing slide hemorrhage in the hepatic parenchyma (HHP). I showing sinusoid with particular capsule indistinct between hepatocytes (SPC). In general, there is normal architecture, normal cell, and normal parenchymal moderate fat. *Normal histology:* The histology of the liver, of *Clarias gariepinus* fed diet containing varying levels of spent Sorghum, seemed not to be altered the histology of the liver of the fish. Evaluation of histological structure of digestive organs in fish fed new ingredients provide valuable information about digestive capacity and potential health effects of new diets (Caballero *et al*., 2003) .The Results from the photomicrograph of liver shows there is normal architecture of the liver cells. Although, the histological results of the liver showed that some treatments showed cytoplasm with irregular arrangement and dislocated nucleus; slight haemorrhage in the hepatic parenchyma with peribular capsule indistinct between hepatocytes and enlarged hepatocytes which is not a dietary treatment related. The same observation was by Olukunle (2011) and Jimoh (2012). The enlarged hepatocyte of the liver may be attributed to high lipid content of the organ traceable to high lipid in the diet. This finding agreed with that of Adeboyejo *et al*., (2012) who in his study exposed *clarias gariepinus* to industrial effluents. The liver of the exposed organisms revealed different levels of vacuolated cells with increasing concentration, which indicates fatty degeneration of hepatocytes. Furthermore, there were distortions in the arrangements of the livers cell and cellular necrosis, severity also varied with increasing concentration of the effluent.

Conclusions: Spent Sorghum from locally fermented drink (Burukutu) can be utilize for fish feed in *clarias gariepinus* in requirement for expensive corn without adverse effect on the organs of the fish such as liver. Fish farmers can engaged the use of spent Sorghum as a source of carbohydrate in their feed for *Clarias garepinus* to reduce the cost of fish production and to increase their profit margin.

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