



Organosomatic Indices and Histopathological Response of *Clarias gariepinus* Fed Black Cumin (*Nigella sativa*) Meal Diets

¹*Kela, E, ²Sogbesan, A. O., ²Michael, T. E., ³Ishaku, A. H. and ¹Kabiru M.

¹Department of Biological Sciences, Gombe State University, Gombe, Nigeria.

²Department of Fisheries, Modibbo Adama University, Yola, Nigeria.

³Department of Fisheries Technology, Yobe State College of Agriculture, Science and Technology, Gujba

Corresponding Author: estherkela2016@gmail.com

ABSTRACT

The effects of different treated black cumin (*Nigella sativa*) meal diets on the organosomatic indices and histopathological response of organs of juvenile *Clarias gariepinus* was evaluated. The fish with initial mean weight and length: 9.94 ± 0.02 g and 10.08 ± 0.04 cm were stocked in triplicates per treatments. The fishes were fed experimental diets twice daily at 5% body weight, for 12 weeks. Six isonitrogenous diets containing 40% crude protein were formulated and coded as black cumin meal 1 (BCD Control), black cumin meal 2 (BCD2), black cumin meal 3 (BCD3), black cumin meal 4 (BCD4), and black cumin meal 5 (BCD5) respectively. Organosomatic indices such as cardiosomatic index (CSI), Hepatosomatic index (HSI), and Renalsomatic index (RSI) of the fish were measured using standard methods. Histopathological examinations of the liver, kidney and heart were carried out following routine laboratory techniques. Data were analyzed using descriptive statistics and ANOVA at a 0.05 level of significance. The result revealed a significant differences in all the organosomatic indices. The histopathological examinations of the liver of *C. gariepinus* revealed a gradual changes from mild to moderate and severe diffuse hepatic vacuolar degenerations as the concentrations of black cumin meal diets increased. The study revealed that the consumption of black cumin does not have negative implication and improved growth performance of the fish.

Keywords: *Clarias gariepinus*, Black cumin meal, Histopathology, Organosomatic index, Additive

INTRODUCTION

Fish farming is often faces high feed cost, approximately 70 to 89% of total production costs (Mohammed *et al* 2017). An attempt to increase the profit is to reduce the feed cost as minimal as possible and one way to do this is to explore the sources of local feed ingredients that can be used as an alternative for fish feed. However, problem of high cost of feeding in aquaculture is further exacerbated due to the scare and expensive nature of some of the ingredients used in the formulation of fish feeds. Black cumin (*Nigella sativa*) is one of the alternative to

conventional fish feed ingredients and it is belongs to the family Ranunculaceae. Black cumin is used as dried seed in food, flavor, medicine and seed oil as in pharmaceuticals and perfumery industries. The seeds of these spices contain volatile oil, which makes the important and valuable. Black cumin seed is black in colour and generally appears similar to onion seed creating confusion. It has strong, pungent odour and aromatic, pungent taste. It can be used either whole or as ground.

Atta (2003) conducted scientific investigations on nutritional value of black cumin seeds (*Nigella sativa* L.). They have

depicted its composition moisture, oil, proteins, ash and total carbohydrates contents in the range of 3.8-7.0 percent, 22.0 to 40.35 percent, 20.85-31.2 percent, 3.7-4.7 percent and 24.9-40.0 percent respectively. Analysis of the proximate composition revealed that the spices had considerable carbohydrate and crude protein content, but low ash, fibre, moisture and fat. Furthermore, phyto-genic feed additives such as black cumin are used in animal feeds to improve performance through amelioration of feed properties, promotion of production performance and improving the quality of animal origin food/feed (Aletor and Osho, 2009). The use of black cumin as phyto-dietary additive and growth promoters in *C. gariepinus* have been reported (Adewole, 2014). However, the present studies focuses on reporting the effects of feeding different concentrations of black cumin on the organosomatic indices and histopathological conditions of the organs of *C. gariepinus*.

MATERIALS AND METHODS

Study Area

The experiment was carried out in the laboratory of Department of Biological Science, Gombe State University, Gombe. The university is located at about 37km from Gombe town on latitudes 10^o 18' 00''N to 10^o 18' 35''N and longitudes 11^o 10' 10''E to 11^o 10' 52''E.

Collection and Processing of Black Cumin

The dried black cumin seeds was purchased from Gombe old market and grinded with hammer mill machine into fine powder form. They were packed in polythene bags labeled and stored at room temperature until when needed.

Source of the Experimental Fish

The experimental fish was procured from Mallam Inna modern Cat-fish production farm located at Wuro Juli, behind Gombe State University, Gombe.

Collection of organs and histopathological analysis

The fish (n=5) were dissected to remove the organs after the feeding trial from each treatments. The organs were weighed separately and recorded for somatic indices as adapted from Dogan and Can (2011). Each organ was dehydrated in Periodic Acid Schiff's reagent (PAS) following the method of Hughes and Perry (1976). They were cleaned, impregnated with wax and sectioned with a microtome (5m thickness). Slides were prepared at the Department of Biological Sciences, Gombe State University, Gombe, Nigeria. Histopathological examination was carried out to investigate possible abnormalities in the organs and the magnification was taken at X100 according to Roberts (1978).

RESULTS

Organosomatic Indices of Clarias gariepinus Fed BBlack Cumin Additive Meal Diet

Table 1 presents the organosomatic indices of *Clarias gariepinus* fed black cumin additive meal diets. The values showed a variation of range of fluctuation among the additive diets for kidney (80-97%), liver (1.21-1.32%), and heart (13-48%). Black cumin inclusion diet (BcD3) recorded 97% value for kidney, followed by BcD2 (94%), BcD5 (85%), BcD4 (84%), while the least value of 80% was recorded in BcD1. The value for liver had 1.32% (BcD3) as the highest percentage while the least value of 1.21% was noted in BcD1. The heart gave highest percentage of 48% in BcD5 and the least value of 13% was recorded in BcD1

Table 1: Organosomatic parameters or indices of *Clarias gariepinus* fed Black cumin meal diet

Parameter	BcD1 Control	BcD2	BcD3	BcD4	BcD5
Kidney	0.80 ± 0.10	0.94 ± 0.10	0.97 ± 0.10	0.84 ± 0.10	0.85 ± 0.10
Liver	1.21 ± 0.10	1.24 ± 0.10	1.32 ± 0.10	1.30 ± 0.10	1.14 ± 0.10
Heart	0.13 ± 0.01	0.23 ± 0.10	0.41 ± 0.10	0.23 ± 0.10	0.48 ± 0.10

Key: BcD1 = Black cumin diet 1 (control diet), BcD2 = Black cumin diet 2, BcD3 = Black cumin diet 3, BcD4 = Black cumin diet 4 and BcD5 = Black cumin diet 5

Histopathology of *Clarias gariepinus* fed Garlic Additives Diets

Plate 1a (BcD2 Liver) is a micrograph of the section of liver of *Clarias gariepinus* showing normal hepatocytes which is unexceptional. Plate 2a (BcD2 Heart) is the photomicrograph of the section of heart of *Clarias gariepinus* showing normal cardiac myocytes with few congested channels which is unremarkable. Plate 3a (BcD2 Kidney) is the photomicrograph of the section of kidney of *Clarias gariepinus* showing normal renal tubules containing lymphocytes which is unremarkable. Plate 1b (BcD3 Liver) shows photomicrograph of the section of liver which appears showing normal hepatocytes. Plate 2b (BcD3 Heart) is the photomicrograph of the section of the heart showing normal cardiac myocytes with few congested vascular channels which is Unremarkable. Plate 2c (BcD3 Kidney) display photomicrograph of the section of the kidney showing normal

renal tubules containing lymphocytes which is unremarkable.

Plate 1c (BcD4 Liver) is the photomicrograph of the section of the liver showing normal hepatocytes which is unremarkable. Plate 2c (BcD4 Heart) is the photomicrograph of the section of the heart showing normal cardiac myocytes with few congested vascular channels which is unremarkable. Plate 3c (BcD4 Kidney) is photomicrograph of the section of the kidney showing normal renal and dilated tubules containing lymphocytes which is unremarkable. Plate 1d (BcD5 Liver) is the photomicrograph of the section of the liver showing normal hepatocytes which is unremarkable. Plate 2d (BcD5 Heart) is the photomicrograph of the section of the heart showing normal cardiac myocytes with few congested vascular channels and it is unremarkable. Plate 3d (BcD5 Kidney) is the photomicrograph of the section of the kidney showing normal renal tubules containing lymphocytes and it is unremarkable.

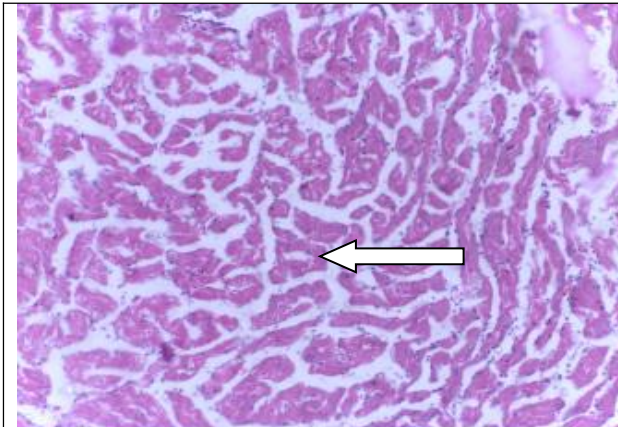


Plate 1a. BcD2 Liver Photomicrograph of the section of liver of *Clarias gariepinus* showing normal hepatocytes (unremarkable)

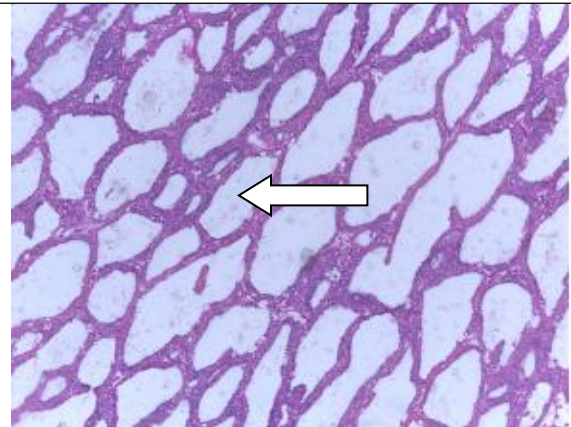


Plate 1b. BcD3 Liver Photomicrograph of the section of liver which appears showing normal hepatocytes

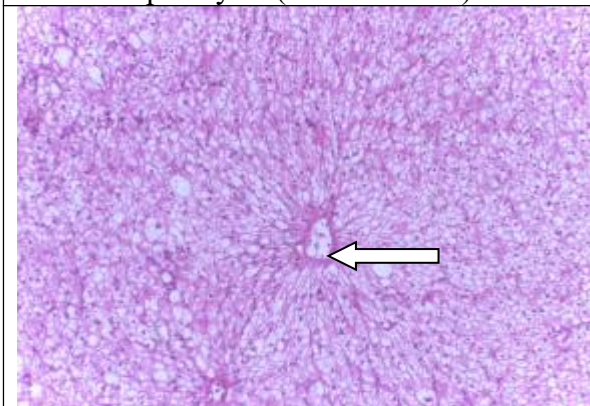


Plate 1c. BcD4 Liver: Photomicrograph of the section of the liver showing normal hepatocytes (Unremarkable)

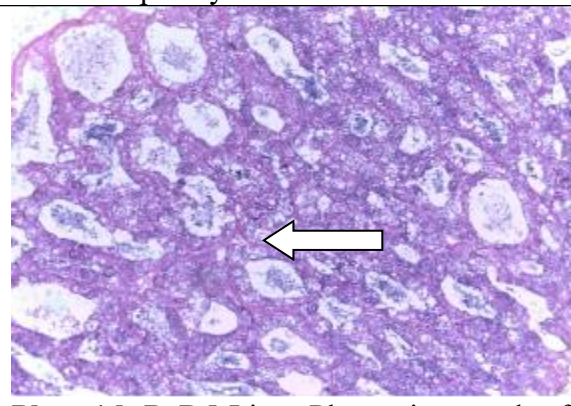


Plate 1d. BcD5 Liver Photomicrograph of the section of the liver showing normal hepatocytes (Unremarkable)

Plate 1. Histology sections of the Liver of *Clarias gariepinus* fed different Inclusions of Black cumin additive diets

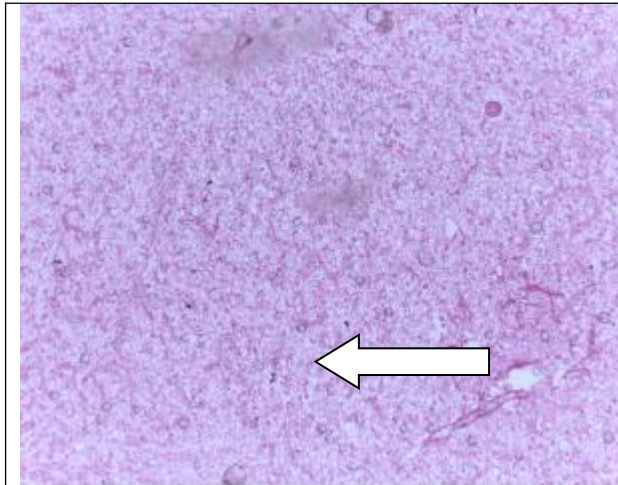


Plate 2a. BcD2 Heart Photomicrograph of the section of heart of *Clarias gariepinus* showing normal cardiac myocytes with few congested channels (unremarkable)

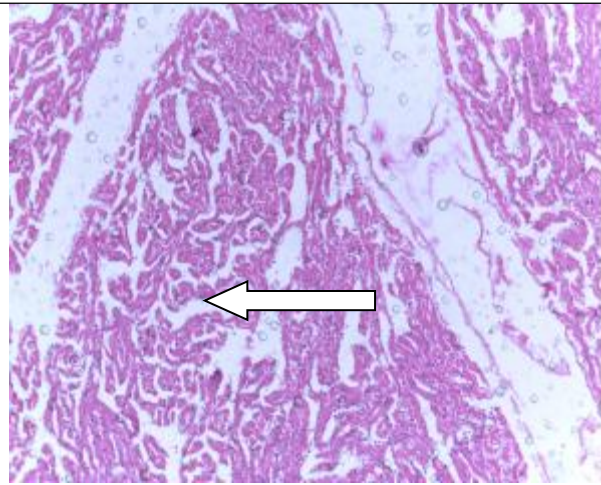


Plate 2b. BcD3 Heart Photomicrograph of the section of the heart showing normal cardiac myocytes with few congested vascular channels (Unremarkable)

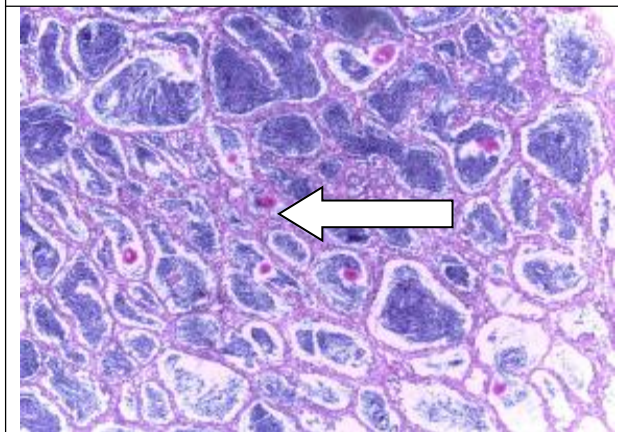


Plate 2c. BcD4 Heart Photomicrograph of the section of the heart showing normal cardiac myocytes with few congested vascular channels (Unremarkable)

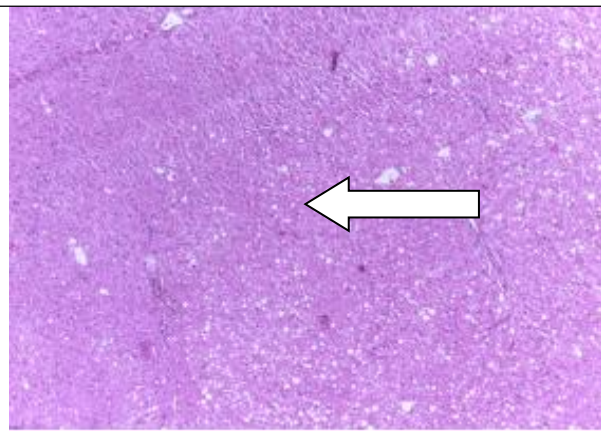


Plate 2d. BcD5 Heart Photomicrograph of the section of the heart showing normal cardiac myocytes with few congested vascular channels (Unremarkable)

Plate 2. Histology sections of the Heart of *Clarias gariepinus* fed different Inclusions of Black cumin additive diets

Remarkable= Abnormalities seen

Unremarkable= No abnormality seen

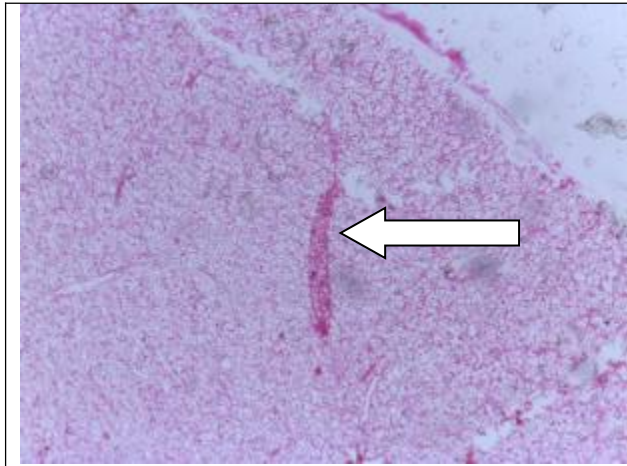


Plate 3a. BcD2 Kidney Photomicrograph of the section of kidney of *Clarias gariepinus* showing normal renal tubules containing lymphocytes (unremarkable)

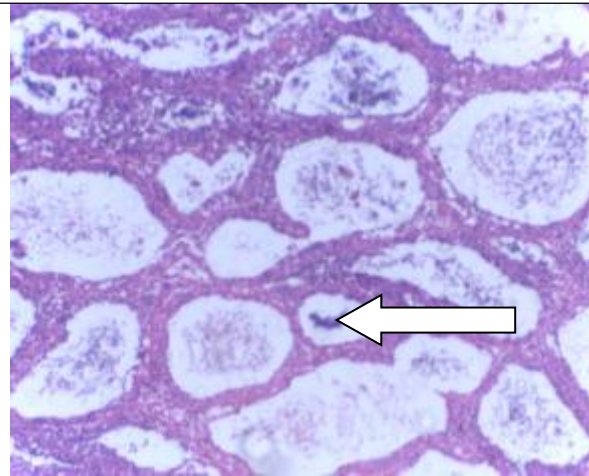


Plate 3b. BcD3 Kidney Photomicrograph of the section of the kidney showing normal renal tubules containing lymphocytes (Unremarkable)

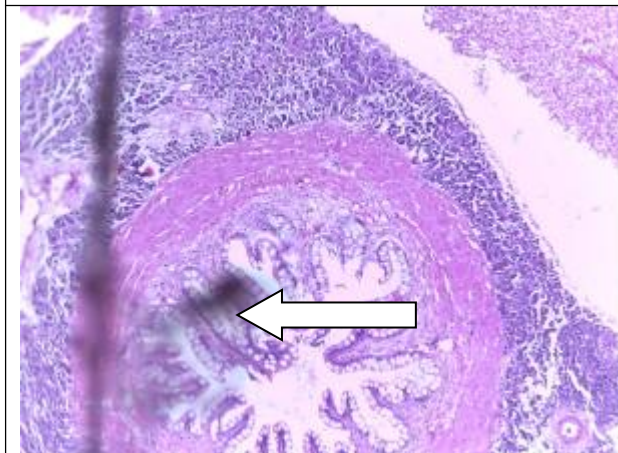


Plate 3c. BcD4 Kidney Photomicrograph of the section of the kidney showing normal renal and dilated tubules containing lymphocytes (Unremarkable)

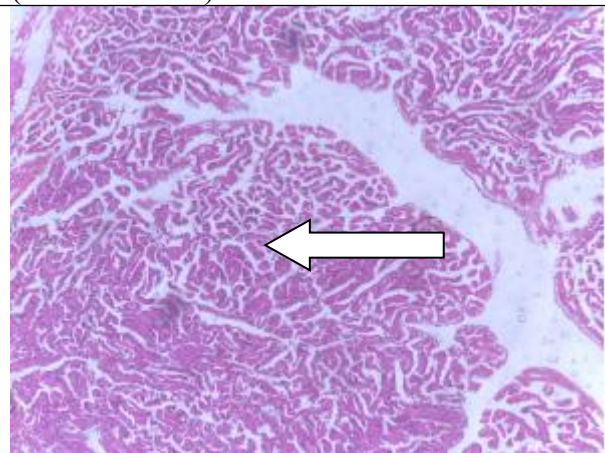


Plate 3d. BcD5 Kidney Photomicrograph of the section of the kidney showing normal renal tubules containing lymphocytes (Unremarkable)

Plate 3. Histology sections of the Heart of *Clarias gariepinus* fed different Inclusions of Black cumin additive diets

Remarkable= Abnormalities seen

Unremarkable= No abnormality seen

DISCUSSION

This study revealed that black cumin has an effect on organosomatic indices of *Clarias gariepinus* this study is in line with Guillo and Hinton, (2008) who reported that organosomatic indices can be described as the ratios of organs to body weight; measured

organs in relation to body mass can be directly linked to toxic effects of chemicals on target organ. It can also be used as indices of changes in nutritional and energy status as reported by Maxwell and Dutta (2005). The study also agreed with Omotosho (2017) who reported that, Stressors evoke non-specific responses in fish which enable the fish to cope

with the disturbance and maintenance of its homeostatic state. If severe or long lasting, the response then becomes mal-adaptive and threatens the fish health and wellbeing/death. This study also agreed with the assumption that, these indices is lower than normal values indicates a diversion of energy away from organ or tissue to combat stressor (Barton, 2002).

In this study, the Hepatosomatic index (HSI) of the liver is a considerable potential tool used by fish biologists to assess the toxicity situation of the exposure of fish to any toxicant as well as a management tool for evaluating growth or health status of various fish species in different environments (Hoque *et al.*, 1998). HSI is also a useful biomarker to detect the hazardous effects of the environmental stressors (Pait and Nelson, 2003). The increase in HSI value in an ideal environment is related to normal liver growth but, in cases of pollution, liver enlargement is associated with hyperplasia (Hoque *et al.*, 1998). The higher values of hepatosomatic index (HSI) observed in the fish fed 0%, 25%, 50%, 75% and 100% inclusion level additive based diets could be attributed to higher feed take at these inclusion levels and indicated normal liver growth resulting from dietary treatment. Enlargement of organs, such as liver, kidney and heart, has been associated with dietary factors especially if such diets contain toxins, anti-nutrients or heavy metals (Adejinmi, 2000).

Abdel-Hameid (2007) reported elevated HSI values for *Oreochromis aureus* juveniles due to phenol intoxication and stated that the observed hepatomegaly might partially reflect the enhancement of the liver size due to destructive changes. In the same vein, Figueiredo-Fernandes *et al.* (2006) also obtained increased values of HSI in male and female tilapias, *Oreochromis niloticus*, exposed to paraquat. However, the

progressively reduced HSI values could be linked with lesser feed intake which was probably associated with the presence of anti-nutrients due to plant based feedstuff. Akerman *et al.* (2003) also found a decrease in HSI values after nine weeks in rainbow trout, *Oncorhynchus mykiss*, injected with paraquat.

In this study, histopathological examination of the liver, kidney and heart were carried out because of their physiological importance during absorption and metabolism of nutrients (Roberts, 1989). Evaluation of histological structure of digestive organs in fish fed new dietary ingredients provides valuable information about their digestive capacity as well as potential health effects of such new diets (Diaz *et al.*, 2006). Addition of different inclusion levels in the diets has resulted in varying degrees of histopathological changes in the liver cells (hepatocytes), kidney, and heart of *C. gariepinus*. Such changes included mild/moderate diffuse vacuolations, periportal congestion, central venous congestion, mild periportal vacuolar degeneration, severe fatty infiltration, extensive hepatic degeneration and overlapping of liver tissue.

The observations closely support the finding of Hlophe and Moyo (2014) who, in a related feeding trial, observed that *C. gariepinus* fed high moringa leaf meal inclusion levels (>50%) showed an increase in the number of degraded hepatocytes with irregularly shaped cells, small dark pyknotic nuclei, poor fatty deposition and isolated necrosis. The present observations also agree with those of Uwachukwu *et al.* (2003) who reported that diets containing raw beans caused extensive periportal necrosis with some mononuclear cell infiltration in the livers of broilers while the centrilobular areas showed vacuolation and degeneration of hepatocytes. Vacuolated hepatocytes are usually accumulated with glycogen and have little or no degenerative

and regenerative ability (Nayak *et al.*, 1996) and the excessive vacuolation of the liver cells would result in abnormal functioning of such liver cells, for instance, accumulation and immobilization of fat, which could consequently result in fatty infiltration of the hepatic parenchyma (Adeyemo, 2005).

CONCLUSION

Despite the improved growth performance of black cumin in diet of *C. gariepinus*, this study revealed the usefulness of monitoring the effect of different types of fish feed (quantitatively and qualitatively) in order to determine positive and/or/negative impacts' on the morphological changes of fish organs. This is very essential in the assessment of the suitability of new or novel feed substances in fish production and in the quest to finding an alternative to synthetic drugs in fish health management. It is recommended that its consumption must be done with caution to avoid deleterious effects on the organs, while, large doses especially for longer durations should be avoided. Also, that further processing of the calyx may reduce the toxicological effects of the various phytochemical components found in black cumin.

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