

## Organosomatic indices and histopathological response of *Clarias gariepinus* fed Roselle (*Hibiscus sabdariffa*) meal diets

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### Abstract

In the study, the effects of different dosages of *Hibiscus sabdariffa* meal diets on the organosomatic indices and histopathology of organs ( $n=20$ ) of juvenile *Clarias gariepinus* was evaluated. The fish with initial mean weight and length:  $10.94\pm 0.02\text{g}$  and  $10.28\pm 0.04\text{cm}$  were stocked in triplicates per treatments. The fishes were fed twice daily at 5% body weight, for 12 weeks. Six isonitrogenous diets containing 40% crude protein were formulated and coded as *Hibiscus sabdariffa* meal 1 (SM1Control), *Hibiscus sabdariffa* meal 2 (HSM2), *Hibiscus sabdariffa* meal 3 (HSM3), *Hibiscus sbadariffa* meal 4 (HSM4), *Hibiscus sbadariffa* meal 5 (HSM5) and *Hibiscus sabdariffa* meal 6 (HSM6), respectively. Organosomatic indices such as cardiosomatic index (CSI), Hepatosomatic index (HIS), Renalsomatic index (RSI), Visceralsomatic index (VSI), Cerebrosomatic index (CBSI) and Fat deposit index (FDI) of the fish were measured using standard methods. Histopathological examinations of the organs were carried out following routine laboratory techniques. Data were analyzed using descriptive statistics and ANOVA at  $\alpha 0.05$ . The result revealed as significant differences in all the orgaosomatic indices except VSI within the treatments and control. 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 *H. sabdariffa* meal diets increased. The intestine showed a mild stunted gastric villi in fish fed HSM4 diet which had the highest growth data not shown. The study revealed that the consumption of unnecessarily high dose of *H. sabdariffa* for a long period should be taken with caution, despite the improved growth performance observed. Further studies on the effect of processing cum detoxification of the Roselle in fish as an animal model should be undertaken.

**Keywords:** somatic indices, organs, aquaculture, phytogetic, additive, Fish nutrition, health management

### Introduction

The consumption of botanicals (phytogetic) as complementary/alternative medicine has been encouraged because they are relatively cheap and their significant contribution to the improvement of both human and animal health in term of cure and the prevention of various human/animal disorders, in addition to the less frequent side effects reported when compared to modern medicine or synthetic drugs (Hu *et. al.*, 2003). Most of the phytogetic used can be regarded as spices.

Spices are being used as food additives since ancient times mostly for their organoleptic attributes. It is now understood that spices also exhibit several beneficial nutritional/ physiological effects in addition to enhancing taste and flavour of food (Ezekwesili *et al*, 2004). The digestive stimulant action of spices has been well recognized for a long time, and a few of them find pharmacological application against digestive disorders (Atal and Kapur, 1987). In addition to the various nutrients possessed by these spices, arrays of

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phytochemicals have also been identified in their different parts used for food items, those that have been identified include saponins, tannins, anthocyanin's, cyanogenic glycosides, flavones, and polyphenols (Wang *et al*, 2000) from *H.sabdariffa*. Furthermore, phytogenic feed additives 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 *H. sabdariffa* as dietary additive and growth promotants in *C. gariepinus* have been reported (Adewole, 2014). However, the present studies focuses on reporting the effects of feeding different concentrations of *H. sabdariffa* on the organosomatic indices and histopathological conditions of the organs of *C. gariepinus*.

### **Materials and methods**

#### ***Study area***

The study was carried out at the Department of Aquaculture and Fisheries Management's Teaching and Research Laboratory, University of Ibadan, Ibadan, Oyo State, Nigeria.

#### ***Collection, dosages, preparation, processing of H. sabdariffa for formulation and preparation of diets, stocking and feeding***

The dried flower (calyx) of *H. sabdariffa* was purchased at Bodija Market, Ibadan Oyo State. The preparation, processing and calculation of the dosages ranged from (0.0375 to 0.60g/100g) for Roselle calyx were done as reported by Adewole (2014). The diets were later dried, labeled and coded: *Hibiscus sabdariffa* meal1 (HSM1) – *Hibiscus sabdariffa* meal 6 (HSM6) as reported by Adewole (2014). Twenty juveniles mean weight and length of 10.94±0.02g and 10.28±0.04 cm respectively were stocked and fed twice

daily at 5% body weight in triplicates for 12 weeks in a completely randomized design as reported by Adewole (2014).

#### ***Collections 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 microtone (5m thickness). Slides were prepared at the Department of Veterinary Pathology, University of Ibadan, Nigeria. Histopathological examination was carried out to investigate possible abnormalities in the organs and the magnification was taken at 100X according to Roberts (1978).

### **Results and discussion**

The Cardiosomatic indices (CSI) of the fish fed the experimental diet ranged from 0.11± 0.01% – 0.21 ± 0.02%. The highest CSI value of 0.21 ± 0.02% was from the fish fed control diet, followed closely by 0.15 ± 0.01% that was jointly from fish fed HSM4 and HSM6 diets respectively, while the lowest value for CSI was from fish fed HSM5 diet (Table 1). The HSI values varied from 0.84 ± 0.18% to 1.66 ± 9%. The highest value was from the fish fed HSM2 diet, while the lowest was from the fish fed HSM3 diet. The fish fed HSM2 diet had HIS significantly higher (P<0.05) than fish fed HSM1, HSM3 and HSM5 diets, but not significantly different from fish fed HSM4 and HSM6 diets, respectively (Table 1). The highest value (0.04 ± 0.01%) for RSI was from the fish fed HSM4 diet and the lowest value (0.21 ± 0.02%) was from the fish fed HSM4 diet. The fish fed the HSM3, HSM4 and HSM6 diets had significantly

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higher ( $p < 0.05$ ) RSI than fish fed HSM1, HSM2 and HSM5 diets respectively (Table 1). The VSI ranged from  $2.92 \pm 0.05\%$  to  $2.32 \pm 0.30\%$ . The highest value was from the fish fed HSM6 diet, followed closely by  $2.85 \pm 0.09\%$  from the fish fed HSM3 diet, while the lowest was from fish fed the control diet. The fish fed the control diet was insignificantly lower ( $p > 0.05$ ) than

the others (Table 1). The histopathological results showed that there were no visible lesion found in the heart, brain, kidney, gill of *C. gariepinus* fed graded levels of HSM diets (Table 2), but there were varied degree of histopathological alterations in the structural architecture of the liver (Plate 1a and 1b) and the intestine (Plate 2a and 2b) of fish fed the *H. sabdariffa* meal diets.

**Table 1: Organosomatic indices of *Clarias gariepinus* fed *Hibiscus sabdariffa* meal diets for 84 days in plastic tanks**

Values with different superscripts in each row are significantly different ( $P < 0.05$ ).

Parameters	Treatments fed to <i>Clarias gariepinus</i> for 84 days					
	<i>Hibiscus sabdariffa</i> meal1 (HSM1)	<i>Hibiscus sabdariffa</i> meal2 (HSM2)	<i>Hibiscus sabdariffa</i> meal3 (HSM3)	<i>Hibiscus sabdariffa</i> meal4 (HSM4)	<i>Hibiscus sabdariffa</i> meal5 (HSM5)	<i>Hibiscus sabdariffa</i> meal 6 (HSM6)
Cardiosomatic index (CSI)	0.21±0.02 <sup>a</sup>	0.14±0.02 <sup>b</sup>	0.12±0.01 <sup>b</sup>	0.15±0.01 <sup>b</sup>	0.11±0.01 <sup>b</sup>	0.15±0.01 <sup>b</sup>
Hepatosomatic index (HIS)	0.87±0.19 <sup>b</sup>	1.66±0.09 <sup>a</sup>	0.84±0.18 <sup>b</sup>	1.10±0.41 <sup>ab</sup>	0.87±0.11 <sup>b</sup>	1.22±0.08 <sup>ab</sup>
Renosomatic index (RSI)	0.21±0.02 <sup>c</sup>	0.29±0.03 <sup>bc</sup>	0.34±0.02 <sup>ab</sup>	0.40±0.01 <sup>a</sup>	0.28±0.01 <sup>bc</sup>	0.37±0.05 <sup>ab</sup>
Visceral somatic index (VSI)	2.32±0.30	2.61±0.31	2.85±0.09	2.56±0.23	2.39±0.26	2.92±0.05
Brain somatic index (BSI)	0.62±0.05 <sup>a</sup>	0.60±0.13 <sup>ab</sup>	0.45±0.08 <sup>ab</sup>	0.49±0.06 <sup>ab</sup>	0.35±0.03 <sup>b</sup>	0.43±0.07 <sup>ab</sup>
Fat deposit index (FDI)	2.73±0.23 <sup>a</sup>	2.39±0.21 <sup>a</sup>	0.90±0.17 <sup>cd</sup>	1.83±0.01 <sup>b</sup>	0.47±0.20 <sup>d</sup>	1.05±0.12 <sup>c</sup>

**Table 2. Histopathology of organs from *Clarias gariepinus* fed *Hibiscus sabdariffa* meal diets for 84 days in plastic tanks**

Organs	Treatments fed to <i>Clarias gariepinus</i> for 84 days					
	<i>Hibiscus sabdariffa</i> meal1 (HSM1)	<i>Hibiscus sabdariffa</i> meal 2 (HSM2)	<i>Hibiscus sabdariffa</i> meal 3 (HSM3)	<i>Hibiscus sabdariffa</i> meal 4 (HSM4)	<i>Hibiscus sabdariffa</i> meal 5 (HSM5)	<i>Hibiscus sabdariffa</i> meal 6 (HSM6)
Brain	NVL	NVL	NVL	NVL	NVL	NVL
Gill	NVL	NVL	NVL	NVL	NVL	NVL
Heart	NVL	NVL	NVL	NVL	NVL	NVL
Liver	NVL	large loci of vacuolation	diffused vacuolation (moderate)	mild diffused vacuolation	severe vacuolation (diffused)	severe vacuolation
Intestine/ Stomach	NVL	NVL	NVL	slightly shortened gastric villi	short villi	short villi
Kidney	NVL	NVL	NVL	NVL	NVL	NVL

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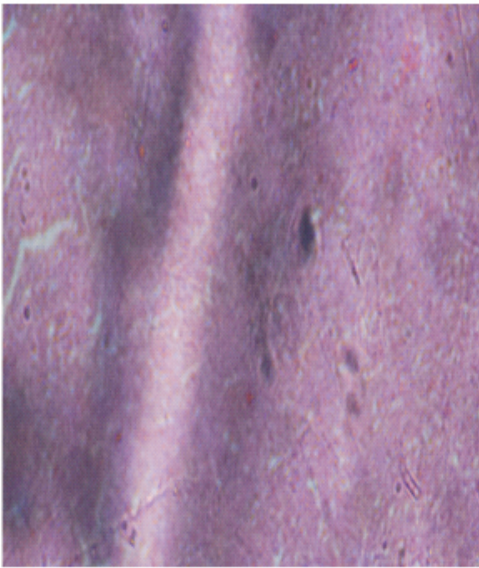


Plate:1a.Photomicrograph of the transverse section of the liver of *Clarias gariepinus* fed *Hibiscus sabdariffa* meal (HSM1) diet, showing no visible lesion. H & E. X 100.

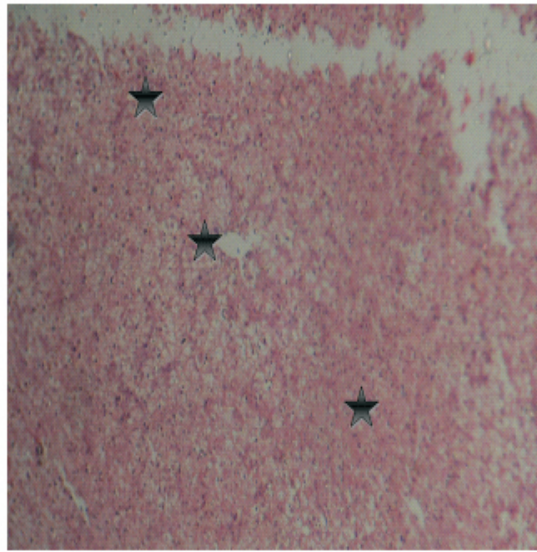


Plate:1b.Photomicrograph of the transverse section of the liver of *Clarias gariepinus* fed *Hibiscus sabdariffa* meal (HSM4) diet. Stars show liver large loci of hepatic vacuolation degeneration. H & E. X 100.

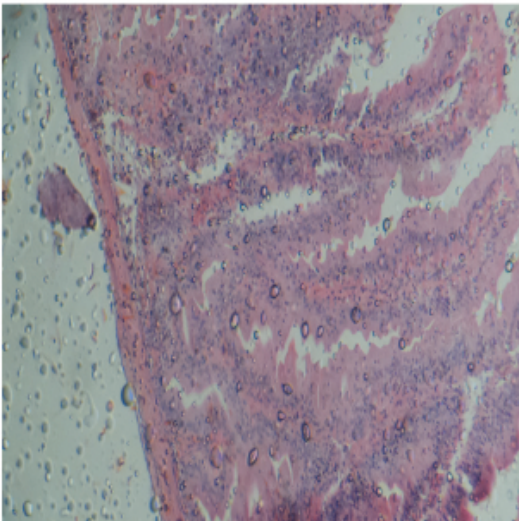


Plate2a:Photomicrograph of the transverse section of the stomach/intestine of *Clarias gariepinus* fed *Vernonia amygdalina* meal HSM1 diet showing no visible lesion. H & E. X 100.

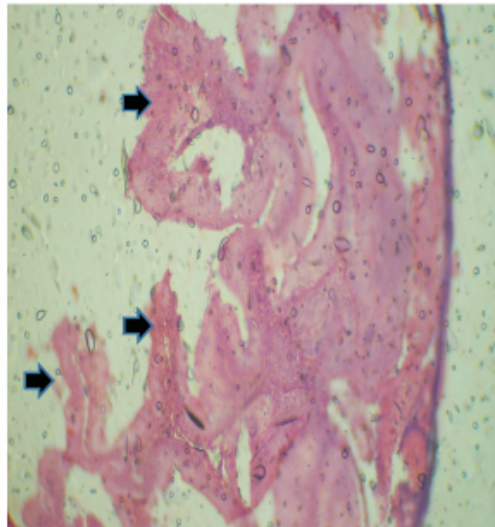


Plate 2b:Photomicrograph of the transverse section of the stomach/intestine of *Clarias gariepinus* fed HSM4 diet. Arrows show slightly shortened gastric villi. H & E. X 100.

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 (Guillo and Hinton, 2008). It can also be used as indices of changes in nutritional and energy status (Maxwell and Dutta 2005). 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 (Omotosho, 2017). The assumption that is generally made with these indices is that lower than

normal values indicates a diversion of energy away from organ or tissue to combat stressor (Barton, 2002). Exposures of the fish to the graded levels of HSM's diets produce a significant decrease in the CSI when compared with the control. The result obtained in the present study differed from the reported non-significant effect of administration of aqueous and ethanolic extracts of *H. sabdariffa* in albino rats. The disparities obtained may be due to the species of animal models, concentrations of the extracts used, the method of extraction /solvent used, method of administration and the varieties of *H. sabdariffa* used in other studies (Ojokoh, 2006; Adewole, 2014). Furthermore, the results obtained in this study showed that there were significant differences ( $p < 0.05$ ) in most of the organosomatic indices measured except the VSI that was non –significantly different ( $p > 0.05$ ). This is contrary to the summations of Ebenebe *et al.* (2018) and Esiegwu *et al.* (2013) that reported that there were no treatment effects on the weight of the liver, lungs, and hearts of rabbits fed with *Garcinia kola* seed meal. However, Ebenebe *et al.* (2018), observed that there were significant differences ( $p < 0.05$ ) in the carcass and kidney weight of rabbits, but these indices followed no specific pattern that could be attributed to the effect of the bitter kola. HSI is one of the most investigated biomarker due to important role of liver in detoxification of pollutants. While Dogan and Can (2011) observed that organosomatic index is an appropriate bioindicator for endocrine disruption in fish consequent of chemical exposure. The results of this study showed that, a statistically significant difference ( $p < 0.05$ ) was noted in the HSI, but did not follow a definite pattern and less consistent. The decreased in the weight of liver suggests a decrease in the production of endoplasmic reticulum for protein

synthesis in liver tissue under toxicant exposure (Bennet and Wolke, 2004). Liver weight reduction could also be as a result of decreased lipid storage (Gabriel *et al.*, 2010). Similar slight decrease in weight of liver was observed as the concentration of cypemethrin increased to  $0.25\text{m/L}^{-1}$  (Ariweriokuma *et al.*, 2011). The liver enlargement (raised HSI) observed in some diets of HSMs can be associated with increased detoxification enzyme activity, and this was similarly reported by Gabriel *et al.* (2010) in catfish hybrid exposed to bleached kraft mill effluents. Also, Katalary and Pariak (2004) observed that acute toxicity varies widely among fish species, depending on the concentrations of chemical, age of fish and exposure period. Acute and sub-acute toxicity profiles of different extracts of parts of *H. sabdariffa* have been reported widely in literature (Ali *et al.*, 2005) with conflicting reports. Therefore, the histopathological examinations of the organs of *C. gariepinus* particularly of the liver revealed a gradual changes from mild to moderate and severe diffused hepatic vacuolar degenerations as the concentrations of *H. sabdariffa* meal diets increased ( $> \text{HSM4}$  diet). The liver of fish fed HSM4 meal diet which had the highest growth (data not shown) (Adewole, 2014) revealed that the liver demonstrated mild diffuse hepatic vacuolar degeneration and the intestine showed mild stunted gastric villi. The observed changes in the liver of *C. gariepinus* fed *H. sabdariffa* are similar to the reported alterations in the liver morphology of rat fed calyx diet (calyx and fermented with wood ash) by Ojokoh (2006). The results suggested that Roselle calyx may cause fatty infiltration which could possibly lead to liver damage, if not properly detoxified. Similarly, the intestinal histopathology showed some level of atrophy suggesting indigestibility. This could be attributed to the presence of

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tannins, which has been described as phenolic compounds whose degree of hydroxylation and molecular size are sufficient to form complexes with protein in the calyces of *H. sabdariffa* (Goldstein and Swain, 1963) and cyanogenic glucosides has been reported to be an inhibitor of several enzyme catalyzed processes (Aletor, 1993).

The interest in the use of functional food as dietary supplements/additives in nutritional studies can make positive alterations, as it has immense health benefits (Ajibola, 2015; Steinberg *et al.*, 1996). However, the medicinal values of *H. sabdariffa* have captured research interest recently, but, some of its anti-nutritional properties may cause some histopathological alterations. This observation from this study is similar to findings of (Braide, 1990; Braide and Grill, 1990, Esiegwu *et al.*, 2013 and Ebenebe *et al.*, 2018) that dried powdered of *G. kola* seed meals fed to rat, laying hens and rabbits caused some alterations in their livers and kidneys, respectively.

### **Conclusion**

Despite the improved growth performance of *H. sabdariffa* 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/chemical substances in fish production and in the quest to finding an alternative to synthetic drugs in fish health management. It recommended that its consumptions 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 *Hibiscus sabdariffa*.

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