AFM -03

Organosomatic Indices and Histopathological Response of *Clarias gariepinus* Fed Roselle (*Hibiscus sabdariffa*) Meal Diets

A.M. Adewole
Department of Animal and Environmental Biology, AdekunleAjasin University, Akungba Akoko, Ondo State, Nigeria.
E-mail address: adewole.adeyemo@aaua.edu.ng / adewoleyemo68@gmail.com; Tel: +2347037354046

Abstract
This study evaluated the effects of different dosages of *Hibiscus sabdariffa* meal diets on the organosomatic indices and histopathology of organs (n=20) of juvenile *Clarias gariepinus* with initial mean weight and length: 10.94±0.02g and 10.28±0.04cm, that were stocked in triplicates per treatments, fed twice daily at 5% body weight, for 12 weeks. Six isonitrogenous diets containing 40% Crude Proteinwere formulated and coded as HSM1 (Control), HSM2, HSM3, HSM4, HSM5 and HSM6 respectively. Organosomatic indices such as cardiosomatic index (CSI), Hepato-somatic index (HIS), Renal somatic index (RSI), Visceral somatic index (VSI), Cerebro-somatic index 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. The result revealed asignificant differences in all the orgaosomatic indices except VSI within the treatments andcontrol. 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, phytogenic, additive

Introduction

The consumption of botanicals (phytogenics) 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 phytogenics 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 phytochemicals have also been identified in their different parts used for food items, those that have been identified include saponins, tannins, anthocyanins, cyanogenic glycosides, flavones, and polyphenols (Wang *et al,* 2000) from *H.sabdariffa*. Furthermore, phytogenic feed additives are used in animalfeeds to improve performance through amelioration offeed properties, promotion of production performanceand improving the quality of animal originfood/feed (Alector 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. The dried flower (calyx) of *Hibiscus sabdariffa* was purchased at Bodija Market, Ibadan. The preparation, processing and calculation of the dosages ranging 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: HSM1 – HSM6 as reported by (Adewole, 2014). Twenty juveniles mean weight and length of 10.94±0.02gand 10.28±0.04cm 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). 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 (5μm 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 ×400according to Roberts, (1978).

Results and discussion

The cardiosomatic indices of the fish fed the experimental diet ranged from 0.11± 0.01% – 0.21 ± 0.02%. The highest cardiosomatic index of 0.21 ± 0.02% was from the fish fed control diet, followed closely by 0.15 ± 0.01% jointly from HSM4 and HSM 6 diets, while the lowest value for cardiosomatic index was from HSM5 diet respectively (Table 1). The hepatosomatic index values varied from0.84 ± 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, had hepatosomatic index significantly higher (p<0.05) than HSM1, HSM3 and HSM5, but not significantly different from HSM4 and HSM6 diets (Table 1).The highest value (0.04 ± 0.01%) for Renal somatic indices 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 higher (p<0.05) renalsomaticindices than HSM1,HSM2 and HSM5 diets (Table 1). The visceral somatic indices ranged from to 2.92 ± 0.05% to 2.32 ± 0.30%. The highest value was from the fish fed HSM6 diet, followed closely by 2.85 ± 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).

Table 1. Organosomatic indices of *Clarias gariepinus fed Hibiscus sabdariffa* meal diets for 84 days.

PARAMETERS	HSM1	HSM2	HSM3	HSM4	HSM5	HSM6
Cardiosomatic index	0.21±0.02^{a}	0.14±0.02^{b}	0.12±0.01^{b}	0.15±0.01^{b}	0.11±0.01^{b}	0.15±0.01^{b}
Hepatosomatic index	0.87±0.19^{b}	1.66±0.09^{a}	0.84±0.18^{b}	1.10±0.41^{ab}	0.87±0.11^{b}	1.22±0.08^{ab}
Renosomatic index	0.21±0.02^{c}	0.29±0.03^{bc}	0.34±0.02^{ab}	0.4±0.01^{a}	0.28±0.01^{bc}	0.37±0.05^{ab}
Visceral somatic index	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	0.62±0.05^{a}	0.60±0.13^{ab}	0.45±0.08^{ab}	0.49±0.06^{ab}	0.35±0.03^{b}	0.43±0.07^{ab}
Fat deposit index	2.73±0.23^{a}	2.39±0.21^{a}	0.90±0.17^{cd}	1.83±0.01^{b}	0.47±0.20^{d}	1.05±0.12^{c}

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

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 than 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 cardiosomatic index when compared with the control. The result obtained in the present study differs from the reported non-significant effect of administration of aqueous and ethanolicextracts 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*usedin other studies.

Hepatosomaticindex 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 bio-indicator 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 hepatosomatic indices, 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 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 0.25m/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 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 examination of the organs of *C. gariepinus*particularlyofthe liver revealed a gradual changes from mild to moderate and severe diffuse hepatic vacuolar degenerations as the concentrations of *H. sabdariffa* meal diets increased. The liver of fish fed HSM4 meal dietwhich had the highest growth (data not shown) revealed that the liver demonstrated mild diffuse hepatic vacuolar degeneration and the intestine showed mild stuntedgastric villi. The observed changes in the liver of *C. gariepinus* fed *H. sabdariffa* are similar to the reported alteration 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 histology showed some level of atrophy suggesting indigestibility. This could be attributed to the presence of 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 cyanogenicglucosides has been reported to be an inhibitor of several enzyme catalyzed processes (Aletor, 1993).

Conclusion

Despite the improved growth performance of *H. sabdariffa*in diet of *C. gariepinus,* this study revealedthe 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 in fish production and in the quest to finding an alternative to synthetics drugs in fish health management.

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