ORIGINAL ARTICLE

HEMATOLOGICAL AND HISTOLOGICAL FEATURES OF THE TESTIS AND LIVER OF MALE AFRICAN CATFISH (*Clarias gariepinus*) FED RATION SUPPLEMENTED WITH *Vernonia amygdalina* Del

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ABSTRACT

The hematological and histological features of 56 male *Clarias gariepinus* fish weighing 450 ±50 g were studied after supplementing their diet with *V. amygdalina* Del leaves at four inclusion levels: 0%, 5%, 10% and 15% for 45 days. The duplicate design was used in the conduct of the experiment. Hematology, testosterone assay, condition indices, histology of the liver and testes and histomorphometry of the testes were then studied. There was no significant difference (p>0.05) in the complete blood count, testosterone concentration, gonadosomatic index and hepatosomatic index across the groups. Histology of the liver revealed greater hepatocytes vacuolation in treated groups than the control while the histology of the testes showed the control had the best histological integrity of all the groups. The 10% and 15% groups had significantly higher seminiferous tubule widths. The results of the study show that *V. amygdalina* had no significant effects on most of the studied parameters of fish and may not be steroidogenic; hence, the leaf can be included in the diet of male *C. gariepinus* at the tested levels with some degree of caution.

Keywords: *Clarias gariepinus*, hematology, testosterone, *Vernonia amygdalina*

INTRODUCTION

The general condition, including nutritional and physiological status of fish populations are usually measured using direct indices such as gonadosomatic and hepatosomatic indices (GSI and HSI) (Brown and Murphy, 2004). Hematological parameters are also of great importance for fish-farmers, serving as indicators of the physiological status (Adeyemo *et al.,* 2009). Fish reproductive biologists and catfish breeders are keenly interested in histological evaluations that are useful in providing a relatively sensitive indicator of damage (Awobajo *et al.,* 2010; Ikpegbu *et al.,* 2012)

Clarias gariepinus (Burchell 1822) commonly referred to as African catfish is a large, eel-like fish (Ikpi *et al.,* 2012) with an almost Pan-African distribution, from the Nile to West Africa and from Algeria to Southern Africa (de Graaf and Janssen, 1996). Catfish are not specific in their food requirements (Gunder, 2004) and have the ability to switch their feed (de Graaf and Janssen, 1996). The testis is the primary organ of spermatogenesis (Ikpegbu *et al.,* 2012) while the liver plays a role in digestion and acts as a storage organ for fats and glycogen (Lagler *et al.,* 1977).

Vernonia amygdalina or bitter leaf as it is popularly called is “Shuwaka” in Hausa

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language, “Onugbu” in Igbo language and “Ewuro” in Yoruba language (Oyedeji *et al.,* 2013). *V. amygdalina* has been reported to possess immunomodulatory and antioxidant properties (Omoregie *et al.,* 2009) in addition to hypoglycaemic, hypolipidaemic, antipyretic and analgesic properties (Oyedeji *et al.,* 2013). The plant contains a variety of compounds such as steroid glucosides, sesquiterpene lactones and flavonoids. These compounds are responsible for its bitter taste and bioactivities and with just a little amount of processing to remove the anti-nutritional factors, it can be said to be a healthy food (Yeap *et al.,* 2010).

A nutritionally balanced fish diet should be suitable for growth, maintenance, reproduction and health of the fish at a reasonable cost (George *et al.,* 2012) but this is not the case in African countries including Nigeria where feed cost is prohibitive (FAO, 2013a; Omitoyin, 2006). It therefore becomes very important to look into ways by which feed can be formulated and procured at minimal cost and this can be done by looking into alternative feed ingredients that can supplement or totally replace the existing ones. Plants have been receiving some attention in this regards (Amisah *et al.,* 2009) since catfish have been known to feed on large amounts of plant material (Yalin *et al.,* 2001). The accessibility of the average Nigerian to the Bitter leaf at little or no cost and the plethora of activities possessed by the plant makes it a potential alternative fish feed ingredient. Hence, this work was carried out to study the effects of *V. amygdalina* Del on some physiological indices of fish with the aim of evaluating its use as an ingredient in fish feed.

MATERIALS AND METHODS

Fifty-six mature *Clarias gariepinus* males (weight 450 ± 50 g) sourced from a commercial fish farm in Ibadan, Oyo State, Nigeria, were used in the study. *Vernonia amygdalina* Del (bitter Leaf) was harvested from Ibadan, Oyo State, identified and authenticated at the Forest Research Institute of Nigeria, Ibadan, Oyo State with Authentication number FHI 109940 (Ajala and Owoyemi, 2015). The leaves were processed by soaking for 24 hr in water to reduce the amount of anti-nutritional factors and, thus, making it more palatable and healthier for consumption (Ogunji *et al.,* 2005). The leaves were sundried until they were brittle and were later ground and added to the feed in graded proportions of 0 g/kg diet (the control diet), 50 g/kg diet (5% inclusion level),100 g/kg diet (10% inclusion level) and150 g/kg diet (15% inclusion level). The duplicate design was used in this study whereby the fish were grouped equally into four concrete ponds and four plastic containers at 7 fish per facility. The fish reared in the plastic containers served as the replica for those in the concrete ponds. The fish were allowed to acclimatize for two weeks before the commencement of the study. They were fed at 3% of their body weight (Omitoyin*.,* 2006) on randomly assigned 45% crude protein commercial feed supplemented with 0%, 5%, 10% and 15% processed bitter leaf for 45 days. Water quality in the facilities in the course of the experiment were dissolved oxygen, 8.7 mg/l, nitrate and ammonia levels, 2.12 mg/l and 0.43 mg/ l respectively, pH of 7.38 and temperature 25-30^{o}C (Ajala and Owoyemi, 2015) .

Laboratory procedures were carried out after feeding for 45 days. The fish were stunned by giving a hard blow to the head (Adakole, 2012).The total length of each fish was measured in centimeters using a measuring tape while body weight was measured in grams using a spring balance (Salter 12). Blood collection was done via the caudal vein using 5 ml needles and syringes for complete blood count and hormone assay. Complete

blood count of red blood cell (RBC), white blood cell (WBC) and platelet, packed cell volume (PCV), haemoglobin (Hb) and differential white blood cell was done according to the methods described by Adeyemo *et al.* (2010). The RBC, PCV and Hb values were used to calculate the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) using standard formulae. Testosterone was assayed using Enzyme-Linked Immunosorbent Assay (ELISA) (Oyedeji *et al.,* 2013).

Liver and testes were excised by making a midline incision on the ventral aspect of the fish with a knife. The organs were weighed in grams using a digital top load balance (Lark®LP502A). Thereafter, the gonadosomatic index and hepatosomatic index were calculated as the ratio of gonad weight to body weight and the ratio of liver weight to body weight respectively. The two indices were expressed in percentage (Brown and Murphy, 2004; Diyaware *et al.,* 2010). The weighed liver and testes were immediately fixed in Bouin's fluid for 72 hr after which they were dehydrated in graded ethanol and cleared in xylene. The tissues were then infiltrated with paraffin wax. Embedding, trimming and sectioning were done successively. Finally, staining was done with Haematoxylin and Eosin for light microscopy examination (Ikpegbu *et al.,* 2012). The Motic Images 2000 software was used to take the measurements of the width of the seminiferous tubule lumen and width of the interstitia of the stained testes as described by Motic China Group Co. Ltd.

The one-way analysis of variance (ANOVA) was used to compare variation across the treatment groups. P-value less than 0.05 was considered significant (Cavaco *et al.,* 2001). The results were presented as mean and standard deviation.

RESULTS

The results obtained for complete blood count (Table 1) revealed there were no significant differences (P>0.05) in the hematology of the four treatment groups. Testosterone assay revealed there was no significant difference among the four treatment groups (P>0.05) with 3.8±1.30, 3.2±0.84, 2.5±1.32 and 2.8±0.50 ng/ml for 0%, 5%, 10% and 15%, respectively. Table 2 shows the effects of treatment levels on the body and organs. There was no significant difference among the treatment groups (P>0.05) in all the parameters studied. Histomorphometry of the testes (Table 3) showed that the 10% and 15% groups had significantly higher (p<0.05) seminiferous tubule widths (cm) than other groups. The mean interstitial width (cm) of the 15% group was significantly lower (P<0.05) than the 5% group.

Figure 1 shows the liver of the various treatment groups with varying degrees of vacuolation. The hepatocytes of the liver of the 0% (control) group exhibit mild vacuolation while a mild diffuse vacuolation is seen in the hepatocytes of the 5% group. There is a moderate diffuse vacuolation of the hepatocytes of the liver of the 10% group. The liver of the 15% group depicts the presence of a moderate to marked hepatocyte vacuolation.

The testes of the treatment groups are shown in Figure 2 with the testis of the 0% (control) group showing a uniform distribution of abundant spermatogenic series within the seminiferous tubule lumen whereas that of the 5% group is scantily filled uniformly with the spermatogenic series. The testis of the 10% group also displays uneven distribution of germ cells within the lumen while that of the 15% group shows sparse to moderately filled seminiferous tubules.

Table 1. Effect of treatment levels on hematology of African catfish fed *Vernonia amygdalina.*

Blood parameter	Treatment groups			
	0%	5%	10%	15%
PCV (%)	33.6±5.32[a]	37.6±1.14[a]	33.6±3.91[a]	38.2±3.50[a]
Hb (g/dl)	11.0±1.95[a]	11.8±0.27[a]	10.8±1.57[a]	12.4±1.24[a]
RBC (10^6/ml)	3.7±0.63[a]	4.4±0.41[a]	3.9±0.45[a]	4.2±0.57[a]
WBC (10^3/ml)	13.6±2.59[a]	17.6±0.80[a]	15.9±2.78[a]	16.0±2.49[a]
Platelet (10^3/ml)	174.4±19.83[a]	143.6±30.44[a]	137.2±21.67[a]	170.0±63.86[a]
Lymphocyte (%)	75.0±5.66[a]	67.4±6.35[a]	71.0±10.82[a]	68.0±5.10[a]
Hetrophil (%)	20.4±6.84[a]	27.0±5.39[a]	24.0±10.12[a]	27.5±4.65[a]
Monocyte (%)	2.6±0.55[a]	3.2±1.30[a]	3.0±0.71[a]	2.5±1.29[a]
Eosinophil (%)	2.0±1.00[a]	1.8±0.84[a]	1.8±0.84[a]	2.0±1.41[a]
MCV (fl)	90.5±0.97[a]	87.0±7.06[a]	86.3±7.19[a]	90.8±9.32[a]
MCH (pg)	29.7±0.36[a]	27.2±2.41[a]	27.7±2.59[a]	29.6±3.85[a]
MCHC (%)	32.9±0.54[a]	31.3±0.54[a]	32.0±1.20[a]	32.6±1.20[a]

[a]Mean values with the same superscript within a column are not significantly different (P>0.05).
MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin: MCHC: mean corpuscular hemoglobin concentration.

Table 2. Effect of treatment levels on body and organ parameters of African catfish fed *Vernonia amygdalina.*

Body and organ parameters	Treatment groups			
	0%	5%	10%	15%
Average weight of testes(g)	1.7±1.16[a]	2.6±2.38[a]	1.6±1.01[a]	1.7±1.07[a]
Total body length (cm)	40.1±5.62[a]	44.4±1.37[a]	41.8±2.60[a]	40.8±2.11[a]
Body weight (g)	408.0±157.07[a]	542.0±33.47	456.0±130.12[a]	412.5±73.20[a]
Liver weight (g)	7.3±2.94[a]	10.4±1.59[a]	7.5±2.51[a]	7.1±1.54[a]
GSI (%)	0.8±0.22[a]	1.0±0.49[a]	0.7±0.23[a]	0.8±0.41[a]
HSI (%)	1.8±0.30[a]	1.9±0.29[a]	1.6±0.27[a]	1.7±0.32[a]

[a] Mean values with the same superscript are not significantly different (P>0.05).
GSI – Gonadosomatic index; HSI – Hepatosomatic index.

Table 3. Effect of treatment levels on widths of the seminiferous tubule lumen and interstitia of the testis in African catfish fed *Vernonia amygdalina.*

Testes parameters	Treatment levels			
	0%	5%	10%	15%
Width of seminiferous tubule (cm)	0.59±0.13[b]	0.43±0.18[c]	0.72±0.28[a]	0.61±0.14[a]
Width of interstitia (cm)	0.01±0.01[ab]	0.02±0.02[b]	0.01±0.00[ab]	0.01±0.00[a]

[a] Mean values with the same superscript are not significantly different (P>0.05).

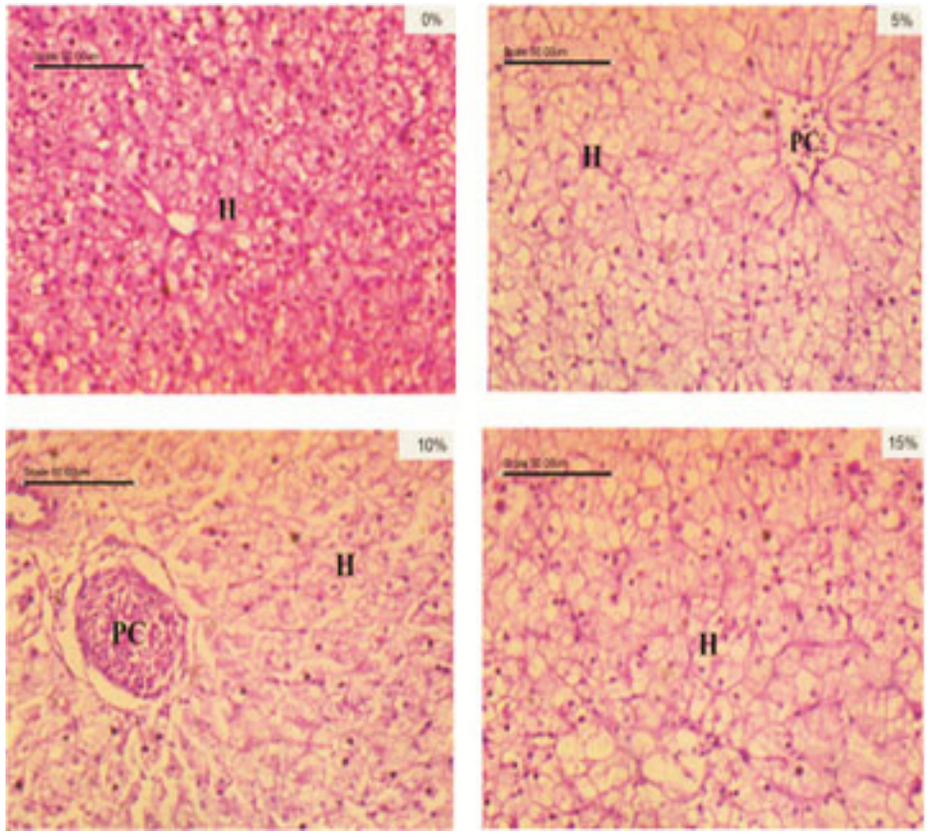

Figure 1: Liver of fish treated at 0% level showing a mild vacuolation of the hepatocytes (H) (H&E). Liver of fish treated at 5% level showing the portal canal (PC) and a mild diffuse vacuolation of the hepatocytes (H) (H&E). Liver of fish treated at 10% level showing the portal canal (PC) and a moderate diffuse vacuolation of the hepatocytes (H) (H&E). Note that the portal canal is congested. This is probably due to plane of sectioning and unconnected with the experiment Liver of fish treated at 15% level showing a marked vacuolation of the hepatocytes (H).

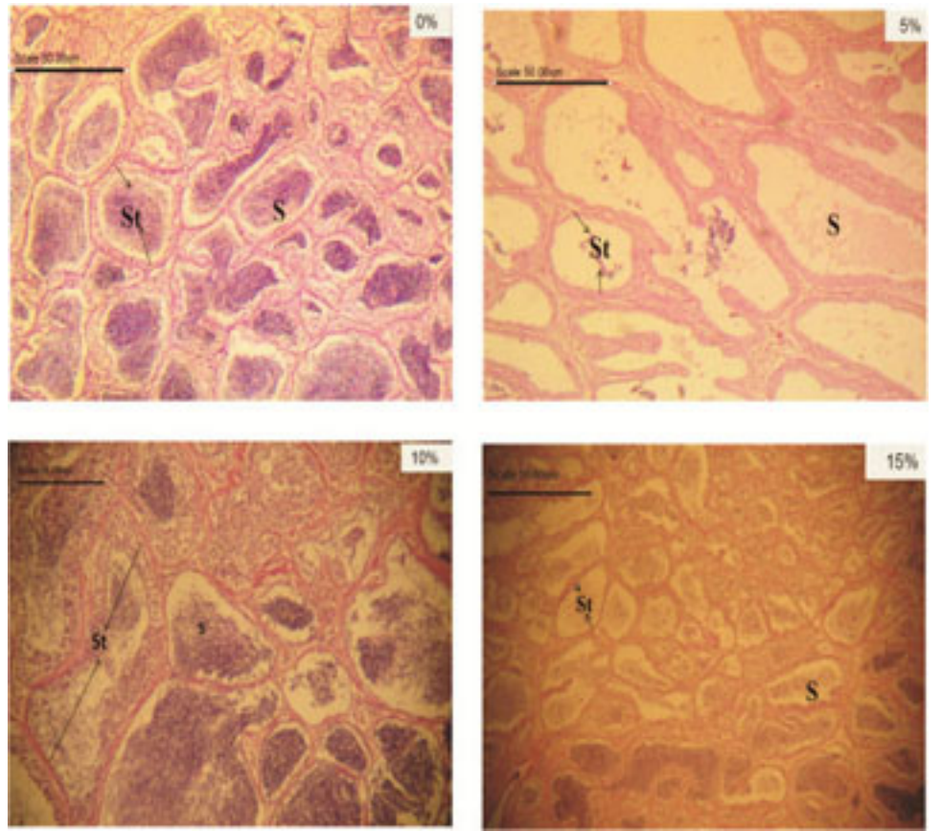

Figure 2: Testis of the 0% (control) group showing a uniform distribution of abundant spermatogenic series (S) within the seminiferous tubule lumen (St) (H&E). Testis of the 5% group showing the seminiferous tubule lumen (St) uniformly scantily filled with spermatogenic series (S) (H&E). Testis of the 10% group showing uneven distribution of spermatogenic series (S) within the seminiferous tubule lumen (St) (H&E). Testis of the 15% group showing sparse to moderate distribution of spermatogenic series (S) within the seminiferous tubules (H&E).

DISCUSSION

A general healthy status was reflected in all the groups as shown by the hematology result. This is suggestive of the fact that the plant possesses immunoprotective properties and its presence in the feed was not distressful to the fish. The effect on haematology may be due to the presence of antioxidants, vitamins A and C in the leaf which ameliorate stress. It could also be due to the method of processing causing a reduction in saponin content of the leaf.

The study showed a reduced testosterone level in treated groups. This suggests that *V. amygdalina* may be inhibitory to the production of testosterone; thus, delaying attainment of maturation in young pre-pubertal fish. This disagrees with the findings of

Nyina-Wamwiza *et al.* (2012) who recorded increasing testosterone levels with increasing proportion of local vegetable ingredient in the feed of African catfish.

There was no significant difference in the weight of the testes in all the groups. This agrees with the report of Adeparusi *et al.* (2010) that feeding of *Kigelia Africana* to male *C. gariepinus* did not cause a significant difference in the weight of testes among the groups. Subsequent to the absence of any effect of the leaf on the weight of the testes, the gonadosomatic index (GSI) of all the groups was also unaffected. This is in agreement with the report of Orlu and Ogbalu (2011) that there was no significant difference in the GSI of *C. gariepinus* treated with *Lepidagathis alopecuroides* (Vahl) from those of the control group. It also agrees with the report of Nyina-Wamwiza *et al.* (2012) that replacement of fishmeal with plant ingredients did not affect the GSI neither in males, nor in females. In all the groups, inclusion of *V. amygdalina* in the feed was found not to be harmful as observed in the weight of the liver and hepatosomatic index of the fish (HSI). The observations in the GSI and HSI indicate that these two indices especially in the male *C. gariepinus* are affected by factors other than feed. This lends credence to the FAO (2013b) report that GSI in males did not change in response to body weight.

The study showed that the control and groups treated with high concentration of *V. amygdalina* had the widest seminiferous tubules implying an increased spermatogenic activity within their testes. The control group had abundantly-filled testis in contrast to those of the treated groups. This could have been a result of reduced testosterone levels in the treated groups, suggesting that the supplementation of male *C. gariepinus* feed with *V. amygdalina* may not adequately support steroidogenesis. This is in agreement with the report of Jegede and Fagbenro (2008) that there was destruction of the testes in tilapia fed dietary neem leaf.

Histological integrity of the liver was not compromised with increasing inclusion levels. The moderate to marked vacuolation observed in the liver of the treated groups is suggestive of increased storage of glycogen in the liver. The antioxidants present in the bitter leaf are responsible for its hypoglycaemic property.

The acceptance of the feed by the fish irrespective of inclusion levels is an indicator of the palatability of bitter leaf after adequate processing. The non-significant effects of the leaf on most of the parameters, except for the histomorphometry of the testes at the studied inclusion levels, makes its use safe as a feed supplement in the feed of male *C. gariepinus* but caution should be applied when feeding to pre-pubertal ones because of its seeming non-androgenic nature.

Further studies should be carried out on the African catfish using specific compounds from the bitter leaf. Genetic engineering should also be carried out on the plant to make it safer for use in all developmental stages of fish.

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