

## Growth performance, nutrient utilization, haematology and serum biochemistry of African catfish (*Clarias gariepinus*) broodstock fed varying levels of *Aspilia africana* leaves-paste

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### Abstract

Dietary supplementation with phytobiotics is a promising area in fish nutrition towards promoting the growth and health status of cultured fish. This experiment was conducted to determine the effects of *Aspilia africana* leaves-paste (AAL) on the growth, haematology and serum biochemistry of *Clarias gariepinus* broodstock. Experiment was carried out in a 24 net-happa (0.6m x 1.07m x 1.2m) suspended in an earthen pond (30 m x 5 m x 1.2m). Forty-eight (7 months old fish, 24 males (0.80±0.04kg) and 24 females (0.70±0.03kg)) were stocked at two fish per net-happa under four treatments in six replicates in a completely randomized design. Four diets (40% crude protein) were formulated to contained: control (0%); AAL1 (0.5%); AAL2 (1%) and AAL3 (1.5%). Fish were fed ad libitum twice daily for 16 weeks. Data on all parameters were analyzed using ANOVA. The highest mean weight gain, MWG: 3.13±0.15kg and lowest feed conversion ratio, FCR: 1.30±0.04 were recorded in broodstock fed 1.5% AAL3. The lowest MWG (1.27±0.03kg) and highest FCR: 1.72±0.03) were obtained in broodstock fed control diet. The lowest packed cell volume: 15.83 ± 0.17 % and haemoglobin: 5.25 ± 0.1 (g/dL) were recorded in fish fed 1% and 1.5% AAL. No significant differences in the total protein and creatinine values obtained between fish fed 1% and 1.5% AAL. The study recommended the dietary inclusion of 1.5% *A. africana* leaves-paste to effectively promote growth and nutrient utilization of cultured *C. gariepinus* broodstock

**Keywords:** Phytobiotics, dietary supplementation, packed cell volume, fish nutrition.

### Introduction

Chakraborty and Hancz (2011); Citarasu (2010) have reported that plant extracts favor various activities like anti-stress, growth promotion, and appetite stimulation, enhancement of tonicity and immunostimulation, maturation of culture species, aphrodisiac and anti-pathogen properties in fish and shrimp aquaculture. These functions performed by these plants extracts are traced to the active principles such as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids or essential oils that are inherent in them. Also, it was reported by Harikrishnan *et al.* (2011) that several plant extracts have been shown to stimulate appetite and promote weight gain when

they are administered to cultured fish. Furthermore, the use of plant-based additives in aquaculture is one of the methods or ways used to improve weight gain, feed efficiency, and/or disease resistance in cultured fish (Dada, 2017). *A. africana* (Asteraceae); (Pers) C.D. Adams, a perennial herb, ligneous at the base, its fruit quadrangular akenes and leaves opposite and hairy (Eweka, 2009). Scientific reports of Kuate *et al.*, (1999) and Okoli *et al.*, (2007) suggested that different crude extracts of the plant contain specific bioactive constituents that could have different effects on its biological activities. Recent study on aqueous extract of *A. africana* has been reported to contained alkaloid, saponin and tannins

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which all possessed growth promoting ability and anti-microbial potentials (Adegbesan, 2019). Also, the leaves of *A. africana* contained high crude protein and fibre which are necessary for promoting growth in fish (Adegbesan, 2019). Broodstock nutrition is one of the most important research areas in aquaculture (Binu Varghese *et al.*, 2009). Despite the remarkable advances in the field of fish larval and juvenile nutrition, new frontiers have to be explored regarding the nutritional requirements of broodfish (Binu Varghese *et al.*, 2009). Hence, this study was aimed at determining the growth performance, nutrient utilization, haematology and serum biochemistry of *C. gariepinus* broodstock fed varying levels of *A. africana* leaves paste.

### **Materials and method**

#### ***Experimental system***

The experiment was conducted at the Earthen Pond Unit of dimensions 30 m x 5 m x 1.2 m (LBH) of Aare Amoke Farms, Ifo Southwestern Nigeria. The study was conducted in twenty-four (24) net-happa of dimensions 0.6 m x 1.1 m x 1.2 m (LBH) suspended in the pond supplied with fresh water. Forty-eight (seven months old) table size fish (24 males, 24 females), purchased at Eja- Nla Fish Farm in Ijako, Ogun State, Nigeria, were randomly stocked to four (4) treatments in the net-happa at a stocking rate of two table size fish (one male, one female) per net-happa in six replicates.

#### ***Preparation and processing of the phytobiotic***

Fresh young leaves of *A. africana* harvested within the premises of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The plant was authenticated in the Department of Forestry and Wildlife where a voucher specimen was maintained for the plant: *A. africana* ID No UAHA/08.180001. The leaves were

obtained by harvesting the whole plants as described by Kalu *et al.* (1986). Each leaf was hand-plucked from stems and placed directly into jute-bag. The leaves were thoroughly rinsed with distilled water to remove dirt and weighed on an electronic scale, cut into smaller pieces with the aid of a knife, blended with water which was added at a ratio of 1:1 (1g of the leaves is equivalent to 1mL of water.) in a household electric blender (Century, CB-8231-M, China), and was poured in a glass container and stored in the refrigerator before incorporation into the basal diets as described by Ajibola *et al.* (2016).

#### ***Experimental diets***

Four iso-nitrogenous diets were formulated at 40% crude protein to containing three varying levels of *A. africana* leaves-paste (0.5%, 1% and 1.5%) and the control (0%).

The samples of *A. africana* leaves paste with the three levels (0.5%, 1.0%, and 1.5%) were added on top of the basal diets and thoroughly mixed with the use of a mixer. Compounded feeds was pelletized (6mm) using the pelletizing machine from the feed milling factory, dried to 10% moisture content with the use of a dryer, allowed to cool in an open-air, packed and stored in an opaque nylon bag according to the treatments. The gross composition of the experimental diets is shown in Table 1.

The Proximate analysis of the four diets formulated and the fish were carried out following the procedure as described by AOAC (2011) in SOAR research and diagnostic laboratory. Abeokuta, Ogun state, Nigeria.

#### ***Experimental procedure***

The fish were weighed in batches; one per treatment and the six replicates at the beginning of the experiment, acclimation of the fish to the experimental system for 7 days was done before the commencement of the study and fish were fed two times daily with a commercial diet (40% C.P.). Before

commencing the experiment, all the fish were starved for 24 hours to increase their appetite. Fish were fed with the diets at two feeding regimes, in the morning (08:00 - 09:00) and evening (17:00 – 18:00), *ad libitum* for 16 weeks (4months).

**Monitoring of fish growth**

Fish was weighed in each net-happa weekly using a sensitive electronic weighing scale (Mettler Toledo FB602) to monitoring the growth of fish and ensuring that the fish are consuming the feeds.

**Blood collection**

The samples of the blood were collected from the caudal fin region of the fish described by Klontz and Smith (1986) and taken to the Department of Veterinary Microbiology of College of Veterinary Medicine (COLVET), Federal University of Agriculture, Abeokuta (FUNAAB) for hematological and serum biochemistry analysis. The blood samples were analyzed according to methods adopted in fish hematology (Haghighi, 2010).

**Table 1: Feed ingredients of the experimental diets (%)**

Ingredients	Control	AAL1	AAL2	AAL3
Fishmeal <sup>a</sup>	19.32	19.32	19.32	18.32
Soybean meal <sup>b</sup>	26.32	26.32	26.32	26.32
Groundnut cake <sup>c</sup>	26.32	26.32	26.32	26.32
Yellow Maize <sup>d</sup>	21.04	20.54	20.04	20.54
Vitamin Premix <sup>e</sup>	1.0	1.0	1.0	1.0
Lysine	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Vegetable Oil	4.0	4.0	4.0	4.0
Methionine <sup>f</sup>	0.5	0.5	0.5	0.5
DCP <sup>g</sup>	0.5	0.5	0.5	0.5
<i>A. africana</i> leaves paste (%)	0.0	1.0	2.0	3.0
<b>TOTAL</b>	100	100	100	100

Note

<sup>a</sup>Local fishmeal, 58% CP; <sup>b</sup>De-hulled soybean, 48% CP; <sup>c</sup>Local groundnut cake, 42% CP; <sup>d</sup>Local yellow maize, 10% CP; <sup>e</sup>Premix composition (kg- 1 of mixture): vitamins: E, 58.3 g; K3, 3.3 g; B1, 3.3 g; B2, 6.6 g; B6, 3.3 mg; B12, 10 mg; folic acid, 3.3 g; biotin, 100 mg; C, 33.3 g; nicotinic ac id, 16.6 g; pantothenic acid, 13.3 g. Minerals: Co, 170 mg; I, 248 mg (Ca(IO3)2); Mn, 10 g (MnO); Zn, 33 g (ZnO); Ca 235 g; Se 2,5 mg (Na2SeO3); Na 247,5 mg (Na2SeO3).; <sup>f</sup>Ajinomoto Eurolysine S.A.S., Paris, France; <sup>g</sup>Di calcium phosphate

AAL = *C. odorata* leaves paste

**Hematological and serum biochemistry analysis**

The parameters analyzed were the packed cell volume (PCV), hemoglobin (Hb), and red blood cell (RBC). Also, the mean cell hemoglobin (MCH), mean cell volume (MCV), and mean cell hemoglobin concentrations (MCHC) were calculated. The white blood cell (WBC) and differential count (neutrophils and lymphocytes) were analyzed as described by Dacie and Lewis (2001). Serum biochemistry parameters analyzed are total protein, albumin, and globulin, aspartate

aminotransferase (AST) and alanine aminotransferase (ALT) following standard methods (Annino, 1976)

**Data analysis**

**Analysis of fish growth performance**

Growth performance of fish were obtained following the procedures of Agbebi *et al.* (2012) in terms of final weight, mean weight gain (MWG, g), percentage weight gain (PWG, %), survival (%), specific growth rate (SGR, %/day).

**Analysis of food conversion and nutrient utilization**

Nutrient utilization parameters such as

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protein efficiency ratio (PER), feed conversion ratio (FCR), and net protein utilization (NPU) were calculated and data were obtained from them.

Primary data were obtained from the proximate analysis of feeds and fish, hematology and serum biochemistry parameters of the fish

### Statistical analysis

Primary data obtained were subjected to one-way analysis of variance (ANOVA). Tukey's Multiple Comparisons Test was used for comparison among diets means at a

significant level of 0.05. Computations were subjected to GraphPad Prism version 7.04.

## Results and discussion

### Proximate composition (%) of the experimental diets

Proximate compositions of the four diets formulated for the study is presented in Table 2. There was no significant difference ( $p > 0.05$ ) in the values obtained for crude protein and crude fibre among diets supplemented with *A. africana* leaves.

**Table 2: Proximate compositions of the experimental diets (% Dry weight)**

Proximate component (%)	Control 0%	AAL1 0.5%	AAL2 1%	AAL3 1.5%
Ash	8.13 ± 0.07 <sup>a</sup>	7.92 ± 0.24 <sup>b</sup>	7.98 ± 0.02 <sup>b</sup>	7.98 ± 0.02 <sup>b</sup>
Ether extract	5.23 ± 0.16 <sup>b</sup>	5.91 ± 0.05 <sup>b</sup>	5.17 ± 0.10 <sup>b</sup>	7.08 ± 0.04 <sup>a</sup>
Crude Fiber	11.78 ± 0.13	10.50 ± 0.29	10.81 ± 0.11	9.21 ± 0.12
Crude Protein	39.84 ± 0.12	40.80 ± 0.33	41.72 ± 0.17	40.97 ± 0.24
Moisture content	8.98 ± 0.12 <sup>b</sup>	10.82 ± 0.42 <sup>a</sup>	10.81 ± 0.03 <sup>a</sup>	10.98 ± 0.38 <sup>a</sup>
Nitrogen free extract	8.98 ± 0.12 <sup>b</sup>	10.82 ± 0.42 <sup>a</sup>	10.81 ± 0.03 <sup>a</sup>	10.98 ± 0.38 <sup>a</sup>

Values represent means ± standard error of mean (SEM) of six replicates of the feeding trial. Values along the same row with same letter are not significantly different ( $p > 0.05$ )

Nitrogen free extract = 100- (Ash+ ether extract + crude fibre + crude protein + moisture content)

AAL: *A. africana* leaves

### Carcass compositions of experimental fish

The initial and final carcass compositions of the fish fed with varying levels of *A. africana* leaves and the control are presented in Table 3. The highest ash content, 11.72 ± 0.17% was obtained in fish

fed 1% AAL and the lowest, 4.66 ± 0.05% was obtained from fish fed the control diet. There was no significant difference ( $p > 0.05$ ) among fish fed the supplemented diets. Crude protein was highest (52.63 ± 0.31%) in fish fed 1.5% AAL and lowest (45.26 ± 0.28%) in fish fed the control diet.

**Table 3: Proximate compositions of experimental fish (% Dry weight)**

Proximate component (%)	Initial	Control 0%	AAL1 0.5%	AAL 2 1%	AAL 3 1.5%
Ash	3.45	4.66 ± 0.05 <sup>c</sup>	7.54 ± 0.15 <sup>b</sup>	11.72 ± 0.17 <sup>a</sup>	11.17 ± 0.22 <sup>a</sup>
Ether extract	2.89	9.61 ± 0.51 <sup>b</sup>	10.52 ± 0.26 <sup>a</sup>	11.86 ± 0.48 <sup>a</sup>	11.20 ± 0.60 <sup>a</sup>
Crude Protein	41.05	45.26 ± 0.28 <sup>cb</sup>	48.12 ± 0.44 <sup>b</sup>	49.38 ± 0.23 <sup>b</sup>	52.63 ± 0.31 <sup>a</sup>
Moisture content	2.55	9.39 ± 0.58 <sup>a</sup>	7.90 ± 0.23 <sup>b</sup>	8.16 ± 0.09 <sup>a</sup>	7.32 ± 0.20 <sup>b</sup>
Nitrogen free extract	50.06	31.09 ± 0.58 <sup>a</sup>	25.92 ± 0.34 <sup>a</sup>	18.56 ± 0.61 <sup>b</sup>	17.67 ± 0.75 <sup>b</sup>

Values represent means ± standard error of mean (SEM) of six replicates of the feeding trial. Values along the same row with same letter are not significantly different ( $p > 0.05$ )

Nitrogen free extract (including fibre content) = 100- (Ash+ ether extract + crude protein + moisture content)

### **Growth performance and nutrient utilization**

The growth performance and nutrient utilization of *C. gariepinus* broodstock fed *A. africana* leaves paste at three varying levels of dietary supplementation is shown in Table 4. There were significant differences ( $p < 0.05\%$ ) in the final weight (FW), weight gain (WG) and percentage weight gain (PWG) among fish fed the diets supplemented *A. africana* leaves. The highest WG,  $3.13 \pm 0.15\text{kg}$  was recorded in fish fed 1.5% AAL and the lowest ( $1.27 \pm 0.03$ ) was recorded in fish fed the control diet. Weight gain increased as the inclusion increased among fish fed the supplemented diets. There were significant improvements ( $p < 0.05$ ) in growth responses of *C. gariepinus* broodstock fed the phytobiotic. This is a first report of its kind regarding the potential of *A. africana* leaves paste as a growth promoting agent in *C. gariepinus* broodstock. This present study showed that this phytobiotic leaves paste supplemented diets enhanced and promoted the growth and nutrient utilization parameters which resulted in improved weight gain, better FCR and good specific growth rate. Highest mean weight gain recorded in broodstock fed 1.5% *A. africana* leaves in this present study inferred that the plant could promote the growth of *C. gariepinus* broodstock. This is in agreement with the report of Dada and Ikuerowo (2009) that *Garcinia kola* seed extract a phytobiotic also promoted growth when supplemented in *C. gariepinus* broodstock diet. Similar findings were reported by Turan and Akyurt (2005) who used the extract of medicinal herb *Trifolium pretense* as a growth promoting agent for *C. gariepinus*. Comparing the efficacy of this phytobiotic in rabbit nutrition, a similar result was observed in the works of Amata and Okorodudu (2016) who revealed significant improvement in weight gain of weaned

rabbit fed *T. procumbens* leaves diet. These superior performances in growth of fish fed the phytobiotic compared to the fish fed the control diet could be linked to the presence of growth promoting nutrients in the plant. The increased feed intake as the inclusion of the phytobiotic increased as observed in this study could be as a result of palatability of phytobiotic supplemented diet. The report of Amata and Okorodudu (2016) that feed intake increased as the inclusion level increased in rabbit fed *T. procumbens* diet corroborated this present study.

The highest feed conversion ratio,  $1.72 \pm 0.03$  was recorded in fish fed the control diet and the lowest,  $1.30 \pm 0.04$  was recorded in fish fed 1.5% AAL. Feed conversion ratio (FCR) is a ratio or measure of the efficiency with which fish convert the feed into the desired output i.e. flesh (Cottle and Pithford, 2014). The lower and desirable FCR observed in broodstock fed the phytobiotic supplemented diets over the fish feed control diet is similar to the report of Dada and Ikuerowo (2009) who reported similar achievement in *C. gariepinus* broodstock fed *Garcinia kola* seed extract. According to Lebas *et al.* (1986), lower conversion ratios in animals indicates that these animals are efficient users of feed, thus the lower FCR observed in this study indicated that the feeds are well utilized and efficient and thereafter converted to flesh as seen in the wet weight gain of broodstocks. There was no significant difference ( $p > 0.05$ ) in the survival rate among fish fed the dietary treatments and the control.

### **Haematological indices of fish**

The haematological indices of *C. gariepinus* broodstock fed *A. africana* leaves at three varying levels of dietary supplementation and the control are shown in Table 5. The highest packed cell volume, PCV ( $27.68 \pm 0.33\%$ ) recorded in fish fed 0.5% *A. africana* leaves diet and the lowest ( $15.83 \pm 0.17\%$ ) recorded in fish fed 1.5% *A.*

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*africana* leaves. No significant differences ( $p > 0.05$ ) in the PCV and haemoglobin values between 1% and 1.5% *A. africana* leaves diets, showing a decline in the blood parameters. This result showed that red blood cell, RBC decreased as the inclusion level increased among fish fed the phytobiotic which were not statistically different ( $p > 0.05$ ) from the fish fed control diet. RBC values appreciated from the 0.5% *A. africana* leaves diet to 1% from where it showed a decline in 1.5% *A. africana* leaves diet. In the fish fed *A. africana* leaves, white blood cell appreciated from the 0.5% *A. africana* leaves diet to 1% from where it showed a decline in 1.5% *A. africana* leaves diet. The dietary inclusion of phytobiotics in cultured fish diets have been reported to altering the haematological and biochemical parameters of the fish (Adegbesan *et al.*, 2017). Wilson and Brigstoke (1981), stated that the packed cell volume (PCV) is an indicator of blood dilution. Abnormal PCV values (15%) were recorded for 1.0% and 1.5% *A. africana*

leave diets. A decline in the PCV and haemoglobin (Hb), values recorded for 1.5% *A. africana* leaves in this present study is similar to the findings of Ajeigbe *et al.* (2013) who observed a decline in the PCV and Hb values of rats administered with 750mg/kg aqueous leaf extract of *A. africana* administered to rats. However, the lowest haematological effect observed with the 1% / kg and 1.5% / kg of the leaves paste in comparison with the control and other test group (.05% / kg) as recorded in this present study suggests that the leaves may have haematotoxic effect, and at high concentration may induce anaemia in animals due to prolonged dietary supplementation or feeding. A previous study of Ajeigbe *et al.* (2013) on the oral administration of aqueous extract of *A. africana* in albino rat corroborated this present study on the haematotoxic effect of *A. africana* administration at high concentration. Thus this present study suggests that administration of *A. africana* above 0.5% could possibly cause anaemia in animals especially in fish.

**Table 4: Growth performance and nutrient utilization of *Clarias gariepinus* broodstock fed *Aspilia africana* leaves paste**

Parameters	Control 0%	AAL1 0.5%	AAL 2 1%	AAL 3 1.5%
Initial Weight (kg)	1.37 ± 0.05 <sup>a</sup>	1.47 ± 0.07 <sup>a</sup>	1.53 ± 0.01 <sup>a</sup>	1.67 ± 0.13 <sup>a</sup>
Final Weight (kg)	2.63 ± 0.33 <sup>c</sup>	3.60 ± 0.06 <sup>b</sup>	4.05 ± 0.16 <sup>a</sup>	4.80 ± 0.21 <sup>a</sup>
Weight Gain (kg)	1.27 ± 0.03 <sup>c</sup>	2.20 ± 0.10 <sup>b</sup>	2.52 ± 0.16 <sup>b</sup>	3.13 ± 0.15 <sup>a</sup>
Percentage Weight Gain (%)	92.86 ± 4.12 <sup>d</sup>	150.89 ± 11.61 <sup>c</sup>	165.03 ± 14.33 <sup>b</sup>	190.38 ± 17.35 <sup>a</sup>
Feed Intake (kg)	2.18 ± 0.02 <sup>c</sup>	3.35 ± 0.13 <sup>b</sup>	3.65 ± 0.13 <sup>b</sup>	4.05 ± 0.10 <sup>a</sup>
Feed Conversion Ratio	1.72 ± 0.03 <sup>a</sup>	1.48 ± 0.01 <sup>b</sup>	1.46 ± 0.05 <sup>b</sup>	1.30 ± 0.04 <sup>b</sup>
Protein Intake	0.86 ± 0.00 <sup>c</sup>	1.32 ± 0.05 <sup>b</sup>	1.59 ± 0.05 <sup>a</sup>	1.66 ± 0.09 <sup>a</sup>
Specific Growth Rate (%/day)	0.58 ± 0.03 <sup>c</sup>	0.69 ± 0.01 <sup>a</sup>	0.82 ± 0.00 <sup>a</sup>	0.83 ± 0.08 <sup>a</sup>
Protein Efficiency Ratio	1.47 ± 0.03 <sup>b</sup>	1.66 ± 0.02 <sup>a</sup>	1.61 ± 0.10 <sup>a</sup>	1.98 ± 0.11 <sup>a</sup>
Apparent Net Protein Utilization (%)	45.44 ± 5.60 <sup>c</sup>	53.28 ± 2.29 <sup>c</sup>	55.71 ± 3.95 <sup>b</sup>	69.00 ± 4.40 <sup>a</sup>
Survival Rate (%)	100.00 ± 0.00 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>

Values represent means ± standard error of mean (SEM) of six replicates of the feeding trial.

Values along the same row with same letter are not significantly different ( $p > 0.05$ )

**Table 5: Hematological parameters of *Clarias gariepinus* broodstock fed *Aspilia africana* leaves**

Parameters	Control	AAL1	AAL 2	AAL 3
	0%	0.5%	1%	1.5%
PCV (%)	24.67 ± 0.33 <sup>b</sup>	27.68 ± 0.33 <sup>a</sup>	15.83 ± 0.17 <sup>c</sup>	15.83 ± 0.17 <sup>c</sup>
Hb (g/dl)	8.40 ± 0.06 <sup>b</sup>	9.70 ± 0.17 <sup>a</sup>	5.25 ± 0.14 <sup>c</sup>	5.25 ± 0.14 <sup>c</sup>
RBC (x10 <sup>6</sup> /l)	1.04 ± 0.01 <sup>a</sup>	1.17 ± 0.01 <sup>a</sup>	1.19 ± 0.01 <sup>a</sup>	0.63 ± 0.02 <sup>b</sup>
MCH (pg)	8.08 ± 0.01 <sup>a</sup>	7.86 ± 0.03 <sup>a</sup>	8.17 ± 0.09 <sup>a</sup>	8.37 ± 0.04 <sup>a</sup>
MCHC (g/dl)	34.07 ± 0.68 <sup>a</sup>	35.75 ± 0.65 <sup>a</sup>	35.08 ± 1.03 <sup>a</sup>	33.86 ± 0.30 <sup>a</sup>
MCV (fl)	23.73 ± 0.43 <sup>a</sup>	23.08 ± 0.22 <sup>a</sup>	23.32 ± 0.43 <sup>a</sup>	24.70 ± 0.13 <sup>a</sup>
WBC (x10 <sup>3</sup> )	5.35 ± 0.03 <sup>b</sup>	5.95 ± 0.03 <sup>b</sup>	7.30 ± 0.06 <sup>a</sup>	3.20 ± 0.12 <sup>c</sup>
NEUT (%)	29.50 ± 0.29 <sup>c</sup>	35.50 ± 0.29 <sup>b</sup>	52.50 ± 0.29 <sup>a</sup>	34.50 ± 0.29 <sup>b</sup>
LYM (%)	67.50 ± 0.29 <sup>a</sup>	60.00 ± 0.58 <sup>b</sup>	58.50 ± 0.29 <sup>c</sup>	63.50 ± 0.29 <sup>b</sup>
EOS (%)	3.00±0.00 <sup>a</sup>	2.67±0.33 <sup>b</sup>	2.67±0.33 <sup>b</sup>	3.00±0.00 <sup>a</sup>
MON (%)	0.67±0.33 <sup>a</sup>	0.67±0.33 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.67±0.33 <sup>a</sup>
BAS (%)	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

Values represent means ± standard error of mean (SEM) of six replicates of the feeding trial. Values along the same row with same letter are not significantly different ( $p > 0.05$ ); PCV, packed cell volume, Hb, haemoglobin, RBC, red blood cell, MCH, mean cell haemoglobin, MCV, mean cell volume, WBC, white blood cell, NEUT, neutrophil, LYM, lymphocyte, EOS, eosinophil, MON, monocyte, BAS, basophil

### ***Serum biochemistry of fish***

The serum biochemistry of *C. gariepinus* broodstock fed *A. africana* leaves at three varying levels of dietary supplementation and the control are shown in Table 6. This result showed that total protein decreased as the inclusion level of *A. africana* leaves increased. Globulin decreased as the inclusion level increased among fish fed *A. africana* leaves diets. These reductions in the total protein and glucose showed that *africana* leaves reduced stressful condition in fish as their elevation indicated a stressed condition in fish. Analysis of serum biochemical constituents' level have revealed valuable information in detecting and diagnosis of metabolic disturbances and diseases in fishes (Ferrari *et al.*, 2007). The ranges of serum biochemistry vary from specie to specie and could be affected by many environmental factors such as water temperature, seasonal pattern, food etc (Ferrari *et al.*, 2007). An increased blood protein level recorded in the fish fed control diet could be probably due to structural liver alteration reducing amino transferase activity, with reduced de-amination capacity and impaired control of fluid

balance (Ashwood and Burtis 1996; Coz-Rakovac *et al.*, 2005). A decrease in total protein level among fish fed the phytobiotics is similar to the findings of Olusegun and Adedayo (2014) who revealed similar decrease in protein of *C. gariepinus* juvenile when exposed to different concentration of fresh root-bark cold water extract of *Plumbago zeylanica*. This was corroborated by the work of Yadav *et al.* (2003) who observed similar decrease in protein when fishes were exposed to stem-bark extract of *Croton tiglium*. The protein serves as the energy sources of fish during chronic period of stress, thus animals exposed to some concentration of toxicant experienced the greater stress during the process of detoxification of a particular toxicant on the metabolic level of animal (Yadav *et al.*, 2003). There was no significant difference in the aspartate aminotransferase (AST) among fish fed the *A. africana* leaves diets. Alanine aminotransferase (ALT) decreased as the inclusion level increased among fish fed *A. africana* leaves. There were no significant differences in the creatinine level among the five dietary treatments. Creatinine could

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be used to evaluate the functional capacity of the nephron of the kidneys, an increased in creatinine level is an indication of defective functional state (Pfaller and Gstraunthaler, 1998) but this present study

revealed no significant differences in the creatinine level of the fish across all dietary treatments. This is an indication of the normal physiological state of the fish kidney.

**Table 6: Serum biochemistry parameters of *Clarias gariepinus* broodstock fed *Aspilia Africana* leaves**

Parameters	Control 0%	AAL1 0.5%	AAL 2 1%	AAL 3 1.5%
Total Protein (g/dl)	9.75 ± 0.06 <sup>a</sup>	9.10 ± 0.06 <sup>a</sup>	8.20 ± 0.06 <sup>b</sup>	7.80 ± 0.06 <sup>b</sup>
ALB (g/dl)	2.05 ± 0.03 <sup>a</sup>	2.25 ± 0.03 <sup>a</sup>	1.83 ± 0.02 <sup>b</sup>	2.30 ± 0.06 <sup>a</sup>
GLO (g/dl)	7.75 ± 0.03 <sup>a</sup>	6.80 ± 0.00 <sup>b</sup>	6.35 ± 0.03 <sup>b</sup>	5.50 ± 0.06 <sup>c</sup>
AST (iu/l)	26.85 ± 0.09 <sup>b</sup>	25.35 ± 0.14 <sup>c</sup>	24.80 ± 0.20 <sup>c</sup>	28.20 ± 1.77 <sup>a</sup>
ALT (iu/l)	21.40 ± 0.06 <sup>d</sup>	24.10 ± 0.25 <sup>b</sup>	24.60 ± 0.17 <sup>b</sup>	22.90 ± 0.06 <sup>c</sup>
Creatinine (mg/dL)	0.45 ± 0.03 <sup>a</sup>	0.53 ± 0.02 <sup>a</sup>	0.55 ± 0.03 <sup>a</sup>	0.33 ± 0.06 <sup>a</sup>

Values represent means ± standard error of mean (SEM) of six replicates of the feeding trial. Values along the same row with same letter are not significantly different ( $p > 0.05$ )

ALB, albumin, GLO, globulin, AST, aspartate aminotransferase, ALT, alanine aminotransferase

### Conclusion

This present study revealed that dietary supplementation of *A. africana* leaves for *C. gariepinus* broodstock is encouraged to improving the growth performance, nutrient utilization and survival of the fish. Hence, it could be used as a growth promoter in catfish grow-out nutrition to broodstock up to inclusion level of 1.5% / kg of feed based on the values obtained for the growth and nutrient utilization parameters. Based on the values obtained for the haematological parameter in the fish supplemented with *A. africana* leaves, it is suggested that higher inclusion level of the leaves could alter the health status fish based on the results obtained for the PCV, Hb and RBC. Though these do not affect the fish as this study recorded no mortality thus having 100% fish survival. Based on the result obtained from the serum biochemistry of the fish, *A. africana* leaves could reduce stressful conditions in fish.

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