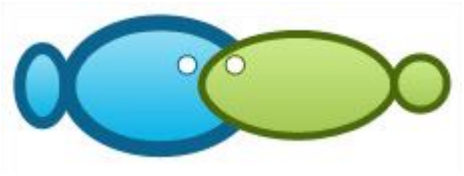

Growth performance and haematological response of *Clarias gariepinus* broodstock fed diets enriched with bitter leaf meal

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Abstract. Inclusion of bitter leaf (*Vernonia amygdalina*) meal (VALM) in the diet of broodstocks of *Clarias gariepinus* was investigated for 24 weeks. The broodfish were fed experimental/commercial diets at 0% (control), 10%, 20% and 30% inclusion levels of VALM. The control group exhibited significantly ($p > 0.05$) higher growth parameters compared to the VALM-treated groups; however, fish group fed 30% VALM-treated feed displayed the highest survival. The blood parameters generally displayed a decreasing trend with higher inclusion rate of VALM. A linear relationship was established between protein synthesis or nitrogen metabolism, *Nm* (y) and VALM-inclusion level (x) as described by the equation: $y = -27.801x + 216.46$ correlation, $r = -0.982$. Hence, the 30% VALM diet recorded the least protein synthesis resulting in elevated ammonia excretion. Using quadratic regression analysis, a 7.2% *V. amygdalina* - inclusion level in broodfish diet of *C. gariepinus* is recommended to elicit optimum growth performance and health. Intersexual variation was observed with males exhibiting significantly ($p < 0.05$) higher haemoglobin, packed cell volume and total white blood cell values than females. The blood reference values obtained for the broodfish were observed to be higher than those recorded for healthy European and African catfish species.

Key Words: compensatory growth, feed enhancers, feed utilization, steroid glycoside, tropical shrubs.

Introduction. Tilapia (*Oreochromis niloticus*) and the African catfish (*Clarias gariepinus*) are the two most farmed fish species in Nigeria and Africa. They serve as veritable sources of fish production, animal protein, economic empowerment, and employment in Nigeria and globally (Annune et al 1994). The African catfish is appreciated by farmers because of its ease of breeding; care and husbandry, rapid growth, high market value and large consumer preference for the quality of its flesh (meat), whether fresh or smoked. *C. gariepinus* enjoys a wide spectrum of farm-formulated and commercial feeds, with good conversion efficiency to utilize both artificial and natural feed components (Omitoyin 2006; Akintayo et al 2008; Fagbenro et al 2013).

The large core of catfish farmers utilizes various husbandry techniques, including indigenous techniques such as the use of non-conventional feedstuffs (NCF), some of which need testing for their suitability as sources of plant protein for aquaculture species. Many of the NCF studies have been reported for spices, soybean, cotton seed meal, groundnut meal, wheat and corn gluten, and yet many others (Madu et al 2003).

There has been an upsurge in the public space in Nigeria on derivable benefits of the use of bitters on human and animal health. A typical example of such bitters is the tropical leaf, *Vernonia amygdalina* (popularly known as bitter leaf). The plant is a shrub found in abundance in farmlands, bush fallows, forest and individual homes in the humid tropics of many parts of Africa. There are diverse medicinal uses of *V. amygdalina*. The herb is believed to have tonic, nutritional, anti-parasitic, anti-malarial, anti-tumor and anti-bacterial properties (Bonsi et al 1995). It is also used as edible vegetable in place of hops in beer production (Babalola & Okoh 2002). Its bitter taste is attributed to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides (Butter & Bailey 1973; Ologunde et al 1992) composed of sesquiterpene lactones (vernodalin and vernonioside B_1-B_3) (Koshimizu et al 1994; Al Magboul et al 1997; Lima et al 1993; Maatooq & Hoffmann 1996).

The proximate analyses of *V. amygdalina* reveal a crude protein (CP) content of 17.9% (Bonsi et al 1995) on dry matter basis which compares favourably with that of cassava leaf meal (Mecha & Adegbola 1980) and far exceeds the minimum protein requirement of ruminants, 10-12% (NRC 1983). Its crude fibre (CF) content is 15.4%, ether extract (EE) - 5.2% and ash content of 12.08% (Aduku 1999; Okoli et al 2003).

Environmentally, *V. amygdalina* has a reputation as a natural air purifier, exhaling oxygen and keeping the oxygen level in the atmosphere balanced. It is a drought and termite resistant plant, which is useful as stakes for lining out plantations and as a live fence. It plays vital role in controlling soil erosion, and in preventing floods. It also helps to improve soil fertility and rehabilitating degrading wasteland (Bonsi et al 1995)

A wide use of this leaf in animal feed stuff would lead to a reduction in the cost of production. *V. amygdalina* has been utilized for feeding small ruminant (Okoli et al 2003), as replacement in maize-based feed diet in broilers (Teguia et al 1993; Mohammed & Zakariya'u 2012), as fertility enhancer in the giant African catfish (*Heterobranchus bidorsalis*) broodstock (Francis et al 2013) and to ensure greater availability of oestrogen and androgens to the fish gonads (Eik-Mes & Hall 1965).

Blood facilitates circulation and transport of gases such as oxygen and carbon (iv) oxide between respiratory organs; hormones and nutrients from digestive track to tissues and storage organs. Blood possesses constituents which defend the body against diseases, maintains water balance in the body, transports urea and prevents excess loss of blood by clotting over an injury (Brown 1980). Hence, haematological parameters are considered patho-physiological indicators in ascertaining the health status of the fish (Svobodova et al 1991; Udoh & Udoidiong 2004). Fish blood parameters vary as a result of internal and external environmental conditions, fish size, age, diseases (Gabriel et al 2004), nutritional state, season, reproductive activity, spawning, sex and genetic variation (Larsson et al 1985), as well as diet composition (Fagbenro et al 2013), water quality (Erondu et al 1993) and starvation (Udoh & Udoidiong 2004). Normal ranges for various blood parameters in *C. gariepinus* have been reported by different investigators in relation to lacustrine habitat (Etim et al 2013), dam habitat (Adedeji & Adegbile 2011), diet composition (Omitoyin 2006; Ochang et al 2007; Akintayo et al 2008), environmental toxicants (Ololade & Ogini 2009; Stanley & Omerebele 2010), and in response to acclimation (Ezeri et al 2004; Gabriel et al 2004), but none specifically on *C. gariepinus* broodstock.

This study was undertaken to establish and provide reference information on the blood profile of male and female *C. gariepinus* broodfish in captivity, and to evaluate the effect of *V. amygdalina* as a feed additive, on its growth performance and haematological characteristics; to identify the sensitive haematological and intersexual responses of *C. gariepinus* broodfish to VALM-inclusion feed, which could serve as reference points in the effective management of broodfish of this and other related fish species in fish farms in Nigeria and elsewhere.

Material and Method. Forty-eight farm-raised broodfish of *C. gariepinus*, males and females (496.67±0.027 g, 250-800 g and 42.23±0.033 cm, 31.5-48 cm) were selected for this study at the Fish Hatchery of University of Uyo. The fish were acclimated for two weeks after which they were randomly assigned to four treatments.

Fresh bunches of *V. amygdalina* were sun-dried at ambient temperature (28-30^{o}C) and pulverized to fine particles, weighed and included into pelleted feed (Multi Feed®) to obtain homogenous mixture. The *V. amygdalina* leaf meal-inclusion (VALM) rations were as follows:

- treatment 1: 0 g of *V. amygdalina* leaf meal inclusion into 1 kg of pelleted feed;
- treatment 2: 100 g of *V. amygdalina* leaf meal inclusion into 1 kg of pelleted feed;
- treatment 3: 200 g of *V. amygdalina* leaf meal inclusion into 1 kg of pelleted feed;
- treatment 4: 300 g of *V. amygdalina* leaf meal inclusion into 1 kg of pelleted feed.

The experimental units for each treatment received six broodfish (three males and females each) per tarpaulin tank (2m x 1m x 1m with water depth of 0.8 m) and duplicated. Fish were fed the diets at a daily rate of 3% body weight, three times daily in three split doses for a period of 24 weeks (September, 2013 to February, 2014), i.e., six months. Growth sampling (length and weight) was carried out at four-week intervals by

individually weighing fish to the nearest 0.1 g using electronic weighing balance (Ohaus®) and measuring total length with a measuring board. Feed conversion ratio (FCR), growth rate (GR), specific growth rate (SGR), total live-weight gain (TWG), and percentage weight gain (PWG), protein-energy ratio (PER) were calculated according to Nabil et al (2010):

GR (g/day) = (final weight, W_2 – initial weight, W_1)/culture interval (day), t

$$SGR \text{ (\% body weight, } W) = (Ln\ (W_2 - Ln\ W_1/t) \times 100$$

$$FCR = \text{total weight of dry feed offered, } F\ (g)/TWG$$

$$TWG = (W_2 - W_1)/W_1 \times 100$$

$$PER = \text{wet weight gain (g)/amount of protein fed (g)}$$

Survival was determined by simple percentage.

Protein synthesis was deduced from Nitrogen metabolism (N_m) using Zeitoun et al (1973): $N_m = (0.549)(a+b)h$ where, a = initial weight of fish, b = final weight of fish, h = culture period in days. Gross feed conversion efficiency (GFCE) was calculated as the reciprocal of the feed conversion ratio (FCR) expressed as a percentage: GFCE (%) = FCR^{-1}.100 (Lovell 1989).

At the end of the 24-week feeding period four fish (two males and two females) were randomly selected from each treatment and blood samples taken from the posterior caudal vein (Brown 1980) using 2 mL heparinized syringe and 22-gauge needle. The fish blood was decanted into a plastic specimen tube containing dipotassium EDTA as an anticoagulant (Brown 1980) and analysed thereafter.

Red blood cell (RBC) and total white blood cell (WBC) counts were done using the Neubauer haemocytometer. The haematocrit or packed cell volume (PCV) and haemoglobin (Hb) concentration values were determined by the microhaematocrit capillary tube (Brown 1980) and using Lovi bond comparator (Van Lerberghe et al 1983) methods, respectively. Plasma protein was obtained using biuret method as a standard method (Annune et al 1994). Blood smears made and stained with Leishman's stain were used to determine differential WBC counts. WBC were counted until 200 WBC were enumerated in the central and peripheral areas of the blood smears, and the percentage of each WBC type (classified as Neutrophil, Eosinophil, Monocyte and Lymphocyte) were multiplied by the total WBC count to obtain absolute differential cell counts (Dacie & Lewis 2001). From these parameters, the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the data using standard formulae as in Udoh & Udoidiong (2004) as:

MCHC = Haemoglobin/PCV (g dL^{-1});

MCH = Haemoglobin x 10/RBC per liter (pg, i.e., g 100 mL^{-1})

Temperature, pH, salinity, total dissolved solids (TDS) and conductivity were measured *in situ* using Exotic II EC500 meter. Dissolved oxygen (DO) was determined using the DO meter. Nitrate (NO_3) and ammonia (NH_3) were measured using colorimetric methods of Brucein and Nessler, respectively (APHA 2005).

The data obtained from this study were subjected to statistical analyses of mean, standard error and variance by one-way analysis of variance, ANOVA, to test the effect of the dietary treatments. Duncan Multiple Range test, DMRT, was also applied to compare the means when a significant difference ($p < 0.05$) was detected by ANOVA (Zar 1984). Quadratic regression analysis was conducted to analyze the SGR of the fish in response to % VALM-inclusion in diet. The relationship between nitrogen metabolism and VALM-inclusion level (x) was described by linear regression of the form: $y = a + bx$, where y is nitrogen metabolism and x is the % VALM-inclusion in diet (Güroy et al 2012, 2013). Regression analysis was used to establish relationship between the various blood parameters of *C. gariepinus* broodstock for fish group fed the control feed (0% VALM-inclusion) and for those fed 10-30% VALM-inclusion diet. The regression coefficients (r^2) and correlation values (r) were then analyzed for statistical significance using the student t-Test at 5% level of significance. All the statistical analyses were done using SPSS program version 17 (SPSS, Richmond, VA, USA).

Results. The physicochemical parameters of culture pond water for each dietary treatment are provided in Table 1. The pH ranged from 6.87 to 7.00, conductivity (µs cm^{-1}), 155.57-459.64, TDS (mg L^{-1}), 104.69-268.56, temperature (^oC) 28, nitrate (mg L^{-1}) 2.53-20.25, ammonia (mg L^{-1}) 10.32-17.25 and DO (mg L^{-1}) 2.23-2.63. The water quality of ponds with VALM-treated feed experienced elevated values of conductivity, ammonia and temperature (Table 1) compared to the control and FAO standard.

Table 1

Mean values of physicochemical parameters of 24-week culture of *C. gariepinus* broodstock fed varying levels of *V. amygdalina* leaf meal (VALM) - inclusion feed for 24 weeks

Physicochemical parameters	*Dietary treatment (Mean ± SE)*				
	0% VALM	*10% VALM added*	*20% VALM added*	*30% VALM added*	*Standard*
Conductivity (µs cm^{-1})	382.23±92.54[a,b]	459.64±106.8[b]	209.90±81.10[a]	155.57±10.11[a]	10-1000
TDS (mg L^{-1})	229.24±62.27[a]	268.56±69.91[a]	140.14±56.15[a]	104.69±10.54[a]	500
Temperature (°C)	28.08±0.07[a]	28.19±0.07[a]	28.32±0.08[a]	28.71±0.24[b]	25-32
Nitrate (mg L^{-1})	2.77±0.53[a]	2.53±0.39[a]	2.7±10.23[a]	20.25±12.08[a]	≤ 3.0
Ammonia (mg L^{-1})[x]	10.32±1.50[a]	14.30±2.05[a,b]	17.25±2.07[b]	11.78±2.43[a,b]	≤ 0.05
Dissolved oxygen (mg L^{-1})[y]	2.60±0.16[a]	2.55±0.24[a]	2.23±0.16[a]	2.63±0.15[a]	≥ 5
pH	6.87±0.16[a]	6.66±0.09[a]	6.90±0.19[a]	7.00±0.22[a]	6.0-9.0

[a,b] denote significantly different values in a row at $p < 0.05$ by one-way ANOVA and Duncan's Multiple Range Test; [x] Values in culture pond higher than maximum allowable; [y] Values in culture pond lower than recommended level.

Growth and survival of C. gariepinus broodstock fed VALM-inclusion feed. Growth performance of *C. gariepinus* broodstock fed feeds containing different levels of VALM inclusion is indicated in Table 2 and Figure 1. The initial mean length (42.23 cm), final mean lengths (42.95-51.96 cm) and initial mean weight (496.67g) are shown. The mean final weight ranged from 596.11 g (30% VALM) to 1403.82 g (0% and 10% VALM) and was significantly lower in fish fed 30% VALM than the other diets. The highest growth rate ($p < 0.05$) was recorded in the (control) fish group fed the 0% VALM-added diet, followed by those fed the 10% and 20% VALM-added diet. Finally, the fish group fed 30% VALM-added diet recorded the least growth rate right from inception till the experiment was terminated at 24 weeks. However, the growth of broodfish fed 0%, 20% and 30% VALM diet was not significantly different compared to fish fed the 10% VALM. Quadratic regression analysis indicated that broodfish recorded maximum growth when fed 7.2 % VALM-inclusion diet (Figure 1). Weight gain tended to decrease with increasing dietary VALM-inclusion level, and fish fed 0%, 10% and 20% VALM diet exhibited significantly higher weight gain compared with fish fed the 30% VALM diet (10.13%). As expected, on the reverse, fish fed the 30% VALM diet displayed significantly higher FCR and lower GFCE% than other fish groups. However, no significant ($p > 0.05$) differences were observed in PER values of all fish groups. VALM-inclusion in the diet of *C. gariepinus* broodfish tended to decrease protein synthesis or nitrogen metabolism (*Nm*); fish fed 0% and 10% VALM diet exhibited significantly higher Nm compared with fish fed the 30% VALM diet. Nm suggests efficiency of ammonia excretion and a linear relationship exists between nitrogen metabolism (y) and VALM-inclusion level (x) described by the equation: $y = -27.801x + 216.46$, $R^2 = 0.9635$, $r = -0.982$ (Figure 2). The fish groups did not exhibit significant differences in condition factor; CF was highest for fish fed 0% VALM and least for the 30% VALM diet group. The experimental diets were all accepted by the broodfish and elicited growth.

Significantly ($p < 0.05$), the highest specific growth rate (SGR), feed conversion ratio (FCR), gross feed conversion efficiency (GFCE), protein efficiency ratio (PER) and nitrogen metabolism (N_m) were also observed in the (control) fish group fed the 0% VALM-added diet (Table 2). The highest survival (98%) was achieved in fish groups fed the 30% VALM-added diet and the lowest (92-93%) in fish groups fed the 10% and 20% VALM-added diets (Figure 3, Table 2).

Table 2

Mean values of growth and survival of African catfish broodstock fed varying levels of *V. amygdalina* leaf meal (VALM) - inclusion feed for 24 weeks (Mean± SE)

Parameters	*Dietary treatment (Mean ± SE)*			
	0% VALM	*10% VALM*	*20% VALM*	*30% VALM*
Initial mean length (cm)	42.23±0.033[a]	42.23±0.033[a]	42.23±0.033[a]	42.23±0.1[a]
Final mean length (cm)	51.96±2.482[a]	50.33±2.157[b]	48.65±2.069[b]	42.95±1.2[c]
Initial mean weight (g)	496.67±0.027[a]	496.67±0.024[a]	496.67±0.024[a]	496.67±0.02[a]
Final mean weight (g)	1403.82±102.91[a]	1403.78±82.85[a,b]	1071.6c±120.65[c]	596.11±32.28[d]
Mean weight gained (g)	568.06±202.56[a]	486.15±230.07[a,b]	302.86±162.85[b]	50.30±66.0[c]
Specific Growth Rate	0.62±0.29[a]	0.62±0.27[b]	0.46±0.13[a]	0.11±0.12[a]
Survival (%)	95.43±5.03[a,b]	92.13±12.22[b]	92.55±14.55[b]	98.61±3.41[c]
Feed Conversion Ratio	0.33±0.05[a,b]	0.31±0.03[a,b]	0.42±0.34[b]	1.93±0.22[c]
ADWG (g)	8.83±1.01[a,b]	7.93±2.00[a,b]	4.48±1.18[b]	0.71±0.02[c]
Weight gain (%)	114.38±40.79[a]	97.88±46.32[b]	60.97±32.76[b]	10.13±13.4[c]
PER	74.09[a]	74.00[a]	73.97[a]	74.52[a]
Nitrogen metabolism (N_m)	182.46±15.25[a]	167.35±14.94[a]	138.64±11.06[a,b]	99.36±4.63[b]
GFCE %	303.03 [a,b]	322.58 [a,b]	238.09[b]	51.81[c]
Condition Factor	0.72±0.82[a]	0.62±0.27[a]	0.46±0.13[a]	0.11±0.12[a]

[a,b] denote significantly different values in a row at $p < 0.05$ by one-way ANOVA and Duncan's Multiple Range Test; ADWG = Average daily weight gain (g); PER = Protein-Energy Ratio; GFCE% = Gross feed conversion efficiency.

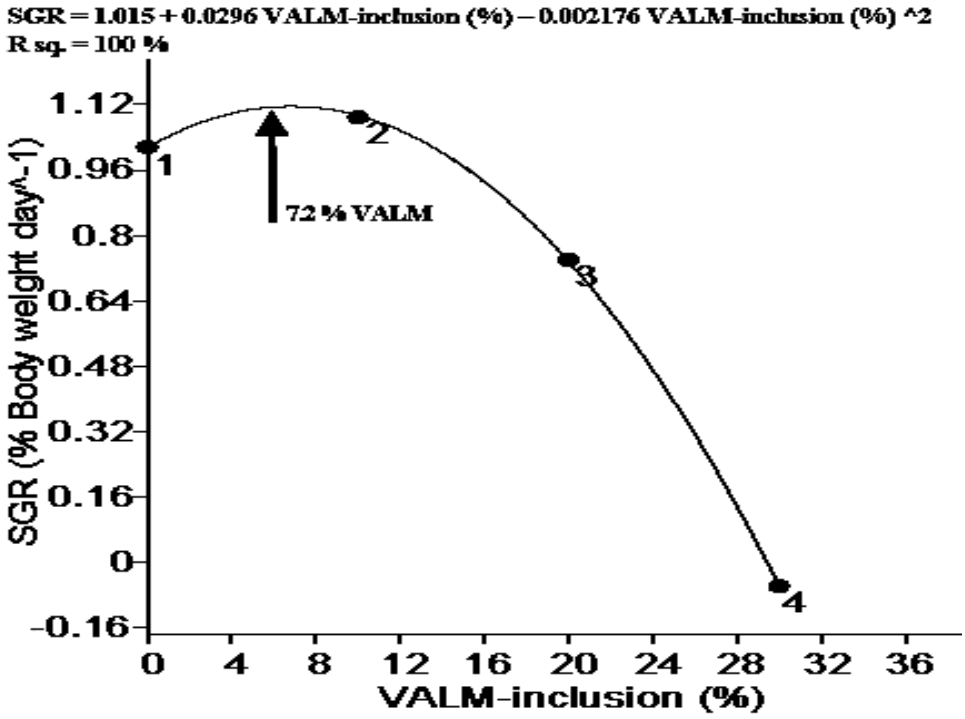

Figure 1. The quadratic relationship between specific growth rate (SGR) and VALM-inclusion level in diet of *C. gariepinus* broodstock (arrow indicates VALM-inclusion level, 7.2%, at which maximum growth was recorded; $R^2 = 0.99996$, F = 11618, p = 0.00656).

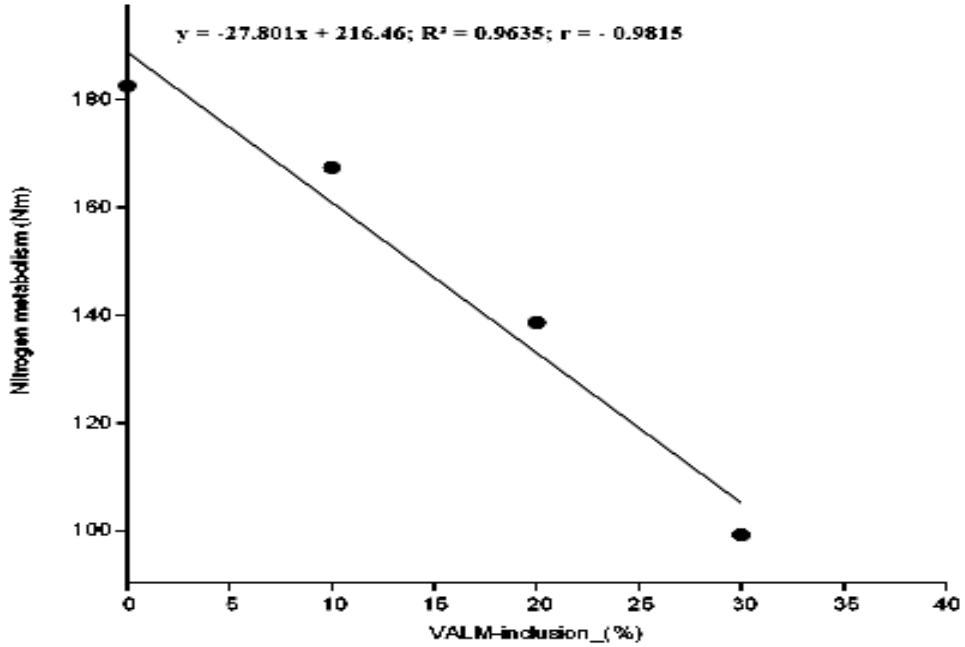

Figure 2. The linear relationship between nitrogen metabolism (y) and VALM-inclusion level (x).

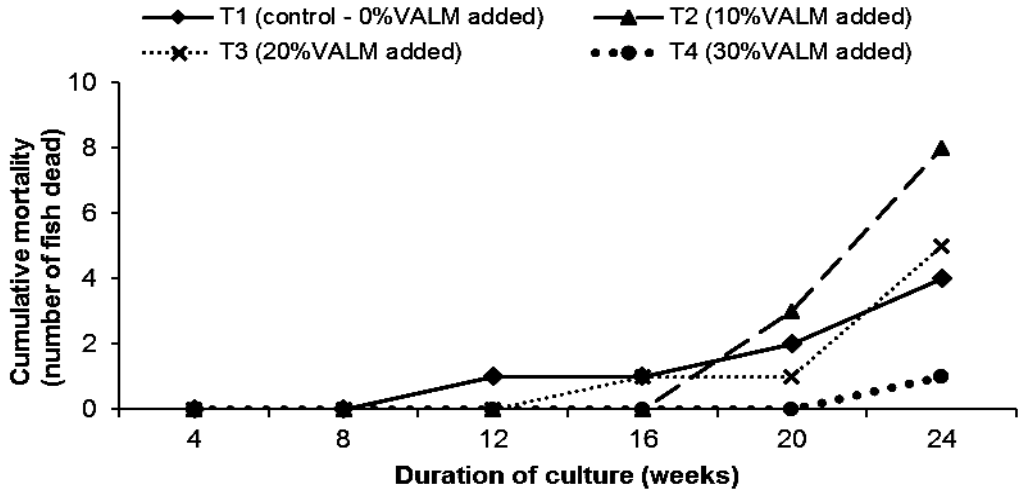

Figure 3. Number of fish death recorded among *C. gariepinus* broodstock fed with increasing inclusion rate of VALM - inclusion feed during the 24-week culture period.

ANOVA revealed significant differences ($p < 0.05$) in growth performance with culture period (Figure 4). DMRT revealed that the pooled monthly growth parameters were initially similar ($p > 0.05$) in the first three months and increasingly exhibited significant differences ($p < 0.05$) from the third to fourth month and till end of project.

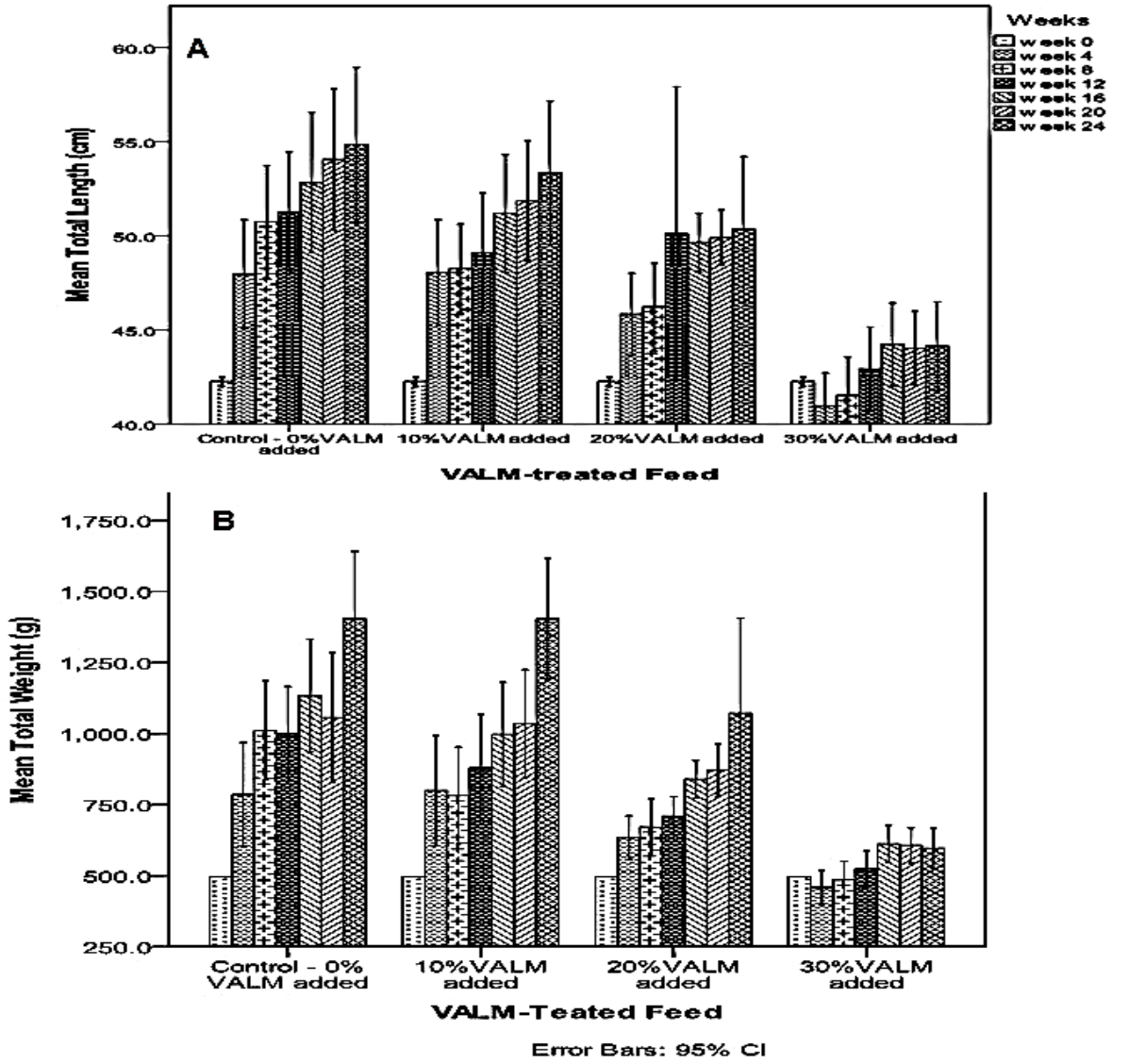

Figure 4. Temporal growth performance of *C. gariepinus* broodstock fed varying levels of *V. amygdalina* leaf meal (VALM) - inclusion feed for 24 weeks (A - total length; B - total weight).

Mean and intersexual variation in haematological values of C. gariepinus broodstock fed V. amygalina-inclusion diets. The haematological parameters of *C. gariepinus* broodstock fed *V. amygalina* - inclusion diets are shown in Table 3. Inclusion of VALM did not impart significantly ($p > 0.05$) on the blood values of the experimental fish. The blood parameters (except neutrophiles) generally displayed a decreasing trend with higher inclusion rate of VALM; while the blood eosinophile, MCHC and protein levels displayed minimal fluctuations or were not affected by with inclusion of VALM in fish diet. However, the male and female experimental fish responded differently to inclusion of VALM in fish diet indicating intersexual variation in haematological responses (Figure 5). Males exhibited significantly higher PCV, Hb and WBC values than females ($p < 0.05$), and similar values ($p > 0.05$) in other blood parameters in all diets assessed. Table 4 suggests some blood reference values for *C. gariepinus* broodstock fed with or without VALM inclusion feed for both sexes.

Table 3

Mean values of heamatological parameters of *Clarias gariepinus* broodstock fed *V. amygdalina* Leaf meal (VALM) - inclusion feed for 24 weeks

Haematological parameters	*Dietary treatment (Mean ± SE)*				
	0% VALM	*10% VALM*	*20% VALM*	*30% VALM*	*Normal range*
PCV (%)	44.00±4.38	37.25±9.38	38.25±8.42	34.50±4.37	29.10±0.30
Hb (g dL^{-1})	14.40±1.48	12.25±3.15	12.63±2.88	11.50±1.31	9.60±0.20
WBC (x 10^9 L^{-1})	743.53±157.62	725.30±152.27	703.60±158.20	880.35±71.66	920±0.20
RBC (x 10^2 L^{-1})	3.55±0.09	3.87±0.74	3.50±1.23	3.17±0.35	3.80±0.18
Neutrophile (%)	75.75±2.46	72.75±2.43	80.25±3.07	79.75±2.06	
Lymphocyte (%)	19.50±1.85	23.25±2.14	16.75±2.21	17.00±2.38	63.45±1.93
Eosinophile (%)	1.50±0.65	1.25±0.48	1.25±0.75	1.00±0.71	
Monocyte (%)	3.25±0.48	2.75±0.48	1.75±0.85	2.25±0.48	
MCHC (g 100 mL^{-1})	0.33±0.00	0.33±0.01	0.33±0.01	0.34±0.00	0.33±0.07
MCH (pg)	40.62±4.1	30.49±3.28	45.54±14.55	37.09±4.70	25.26±0.14
Protein (g dL^{-1})	5.93±0.01	5.92±0.02	5.92±0.04	5.86±0.05	9.20±0.20
RBC/WBC (x 10^{-10})	4.77	5.34	4.97	3.60	4.13

Values are mean±standard error; $p > 0.05$

Table 4

Intersexual variation in blood values of *C. gariepinus* broodstock fed with or without *V. amygdalina* leaf meal (VALM) - inclusion feed for 24 weeks

Haematological parameters	*Dietary treatment (Mean ± SE)*				
	0% VALM		*VALM- Inclusion Feed*		*Mean*
	Male	*Female*	*Male*	*Female*	
PCV (%)	47.00±9.00[a] (38.00-56.00)	41±4.00[b] (37.00-45.00)	44.83±3.84[a] (28.00-54.00)	28.50±5.54[b] (13-43.00)	38.50±3.26 (13.00-56.00)
Hb (g dL^{-1})	15.5±3.00[a] (12.5-18.5)	13.3±1.30[b] (12-14.6)	14.92±1.25[a] (9.5-18.0)	9.33±1.84[b] (4.00-9.00)	12.69±1.09 (4.00-18.5)
WBC (x 10^9 L^{-1})	872.75±72.35[a] (800.4-945.10)	614.3±332.3[b] (282-946.6)	904.00±44.32[a] (752.0-1062.4)	635.50±121.01[b] (229.6-926.2)	763.19±65.02[b] (229.2-1062.4)
RBC (x 10^2 L^{-1})	3.57±0.05 (3.52-3.62)	3.52±0.20 (3.32-3.72)	4.05±0.67 (1.81-6.03)	2.97±0.58 (1.03-6.03)	3.52±0.34 (1.03-6.03)
Neutrophile (%)	75.50±4.5 (71.00-81.00)	76.00±4.0 (72.00-80.00)	77.67±3.35 (68.00-88.00)	77.50±1.12 (74.00-82.00)	77.13±1.38 (68.0-88.0)
Lymphocyte (%)	20.50±3.50 (17.00-24.00)	18.50±2.50 (16.00-21.00)	18.67±2.76 (10.00-26.00)	19.33±1.41 (14.00-24.00)	19.13±1.18 (10.0-26.0)
Eosinophile (%)	0.50±0.50 (0.00-1.00)	2.50±0.50 (2.00-3.00)	1.50±0.50 (0.00-3.00)	0.83±0.48 (0.00-3.00)	1.25±0.30 (0.0-3.0)
Monocyte (%)	3.00±1.00 (3.00-4.00)	3.00±1.00 (2.00-4.00)	2.17±0.65 (0.00-4.00)	2.33±0.33 (1.00-3.00)	2.50±.30 (0.0-4.0)
MCHC (g 100 mL^{-1})	0.33±0.00 (0.33-0.33)	0.32±0.00 (0.32-0.32)	0.33±0.00 (0.33-0.34)	0.33±0.01 (0.31-0.35)	0.33±0.00 (0.31-0.35)
MCH (pg)	43.54±9.01 (34.53-52.55)	37.69±1.55 (36.14-39.24)	42.72±9.32 (25.7-88.39)	32.70±4.00 (24.19-49.82)	38.43±3.0 (24.19 - 88.39)
Protein (g dL^{-1})	5.95±0.02 (5.93-5.96)	5.91±0.02 (5.89-5.93)	5.87±0.03 (5.76-5.94)	5.93±0.03 (5.84-6.01)	5.91±0.02 (5.76-6.01)

[a,b] denote significantly different values in a row at $p < 0.05$ by one-way ANOVA and Duncan's Multiple Range Test; Values are mean ± standard error (and minimum to maximum values in parenthesis).

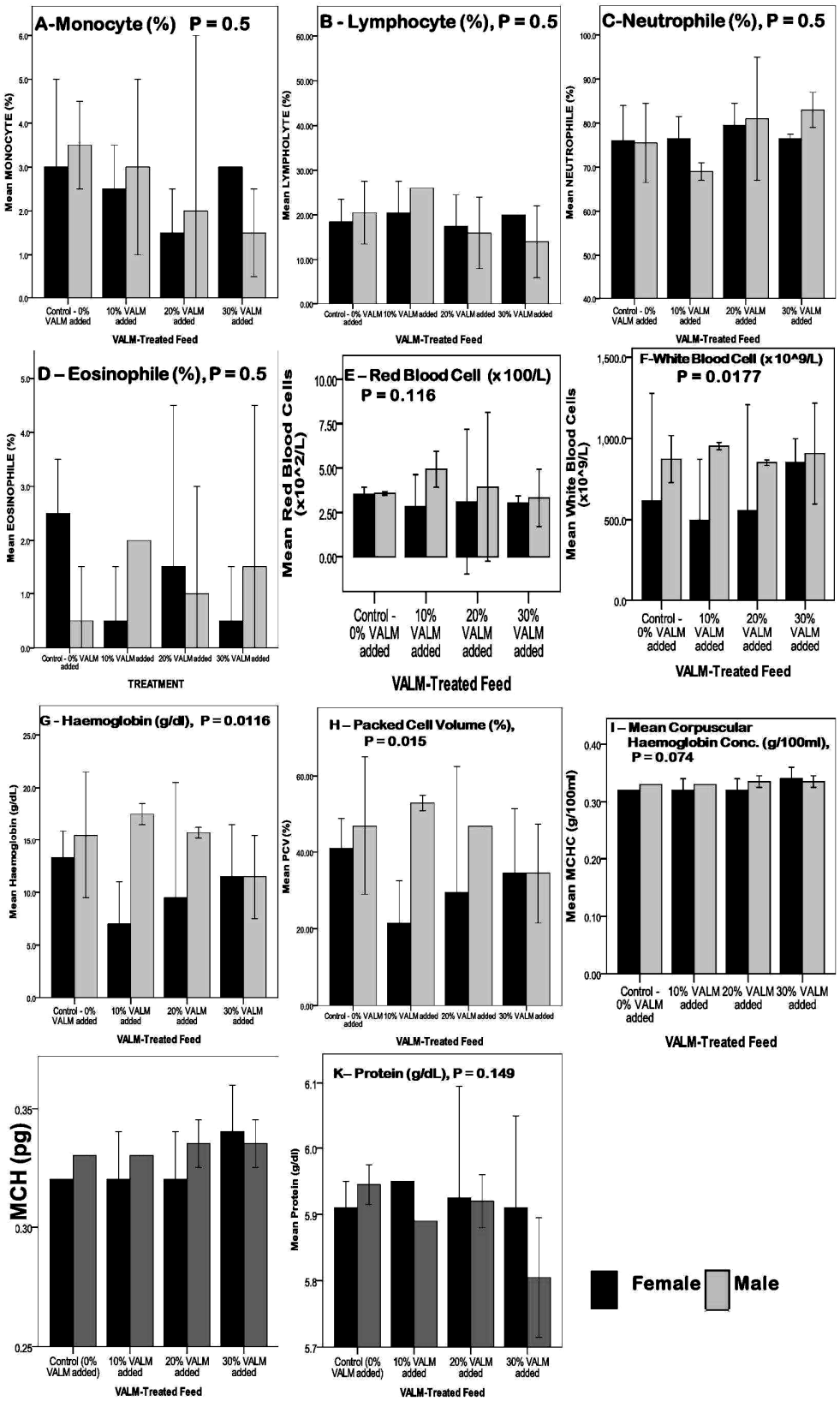

Figure 5. Haematological profiles (A-K) of male and female *Clarias gariepinus* broodstock fed varying levels of *Vernonia amygdalina* leaf meal (VALM) – inclusion feed for 24 weeks

All the blood parameters showed significant correlation ($p < 0.05$) with at least one other blood variable. Regression analysis established 21 significant relationships (Table 5) between the various blood parameters of *C. gariepinus* broodstock for fish group fed the control feed (0% VALM-inclusion) and for those fed 10-30% VALM-inclusion diet. The Hb

vs PCV and LYMPHOC vs NEUTRO showed significant relationships in both control fish and fish group fed VALM-diet. Four other significant relationships: RBC vs MCH, MONOC vs WBC, MCH vs PCV and MCH vs Hb were established in the control fish group and 15 in the fish group fed VALM-diet. This indicates VALM-diet impact some distortions in blood dynamics of fish. RBC were found to correlate with three variables vis-à-vis PCV ($p = 0.011$), WBC ($p = 0.017$), and Hb ($p = 0.017$) in VALM-fed fish only. PCV correlated with five variables vis-à-vis Hb ($p = 0.001$) in both fish groups; MCH ($p = 0.026$) in control fish group and with WBC ($p = 0.002$), RBC ($p = 0.011$), Eosinophile ($p = 0.017$) in VALM-fed fish (Table 5).

Table 5

Probability levels of correlation relationships between the blood parameters of *C. gariepinus* broodstock fed the control and those fed VALM-inclusion diets

Paired blood parameters	*Probability levels (P)*	
	Control	*VALM-feed*
Hb vs PCV	0.001	0.000
WBC vs PCV	0.709	0.002
WBC vs Hb	0.739	0.001
RBC vs PCV	0.777	0.011
RBC vs Hb	0.782	0.012
RBC vs WBC	0.302	0.017
RBC vs MCH	0.003	-0.443
LYMPHOC vs NEUTRO	0.039	0.000
EOSINO vs PCV	0.263	0.017
EOSINO vs Hb	0.240	0.019
EOSINO vs WBC	0.908	0.034
MONOC vs WBC	0.043	0.740
MONOC vs NEUTRO	0.099	0.009
MCHC vs WBC	0.527	0.005
MCH vs PCV	0.026	0.381
MCH vs Hb	0.023	0.347
PROTEIN vs WBC	0.098	0.048
PROTEIN vs MCHC	0.296	0.019

Labels of parameters are as identified in the text.

Discussion. The water quality of the culture ponds (Table 1) were generally within the tolerance ranges for this species (Tucker 1998; Boyd 1990) with a few exceptions. The mean DO of 2-3 mg L^{-1} was below the optimum of ≥ 5 mg L^{-1} probably owing to poor water circulation and low DO of water source (bore hole). In addition, the ammonia level of culture ponds was higher than the optimum (≤ 0.05 mg L^{-1}) in all feed treatments, including control. This was more noticed in fish group fed 10% and 20% VALM inclusion. This could partially be related to the high crude protein content of the feed (45%) in which most of its nitrogen is excreted as ammonia.

The highest growth (final weight gained), weight gained % and specific growth rate (%) were obtained from fish fed 0% VALM-inclusion diet whereas the poorest growth performance was obtained from fish fed 30% VALM-inclusion diet. Therefore, the remedial inclusion of VALM is recommended at the range of 0 to 10%. Based on empirical observation and quadratic regression analysis of SGR, 7.2% VALM-inclusion is recommended since that level elicited optimum growth performance. The results indicate that this low-inclusion of VALM could be beneficial to the growth performance, haematological characteristics, health and survival of *C. gariepinus* broodfish; higher levels could negatively impact growth, blood dynamics and well-being of broodfish. The enhanced survival in fish group fed VALM-inclusion feed may be attributed to its steroid

glycoside content (Butter & Bailey 1973; Jikasa et al 1992, 1993). There was also an inverse relationship in that fish fed excess (30%) VALM-inclusion feed, which performed significantly higher in survival and FCR, also displayed significantly lower growth parameters (weight gain, final mean length and GFCE%) against all other treatment levels. These differences may be related to the bitter taste and low palatability of the VALM-enriched feed as attested to by Onwuka et al (1989). Figure 4 clearly shows that an excessive (≥ 30%) VALM-inclusion in broodfish diet could also pose metabolic and digestive problems as similarly recorded by Ezenwanne & Ucheya (2012) in hepatic enzymes of rabbits infused with aqueous leaf extract of *V. amygdalina*. The 30% VALM-inclusion diet was initially characterized by low acceptance resulting in reduced intake and weight loss, and consequently lower growth performance. However, the group later recovered with compensatory growth attaining about 39% final mean weight compared to the control.

The metabolic machinery of fish is capable of metabolizing large quantities of protein to serve as sources for both energy and growth (Lovell 1989); but fish are more efficient in protein metabolism than in glucose for energy. The end product of the catabolic protein digestion process is ammonia. The energetic cost of this process is also usually low for the fish (which primarily excretes ammonia by diffusion through the gills), unlike in homeothermic land animals like mammals and birds which in addition form urea, uric acid or other nitrogen compounds at high energy costs; which are excreted through the kidney tissue and expelled in urine. Excretion of ammonia is found to be high when protein synthesis is low (Engin & Carter 2001, 2005). Therefore, increased ammonia content in the culture ponds could be an indicator of increased ammonia excretion by fish as a result of reduced protein synthesis with increasing dietary inclusion level of VALM. In this study, this is expressed as lower nitrogen metabolism (*Nm*) and growth; *Nm* reduced with increasing level of VALM in diet (Table 2); the highest *Nm* was observed in the 0% VALM diet which was not significantly different from fish group fed 10% VALM; the *Nm* of the 10% and 20% VALM diets were also not significantly different from each other, while the 30% VALM diet recorded the least *Nm*, and was significantly lower compared to all diets assessed. Güroy et al (2012, 2013) noted that reduced growth performance owing to reduced FCR (and amino acid imbalances), resulted in elevated total ammonia-nitrogen (TAN) excretion levels in fish; which in this study is expressed as depressed *Nm*. It is noteworthy that excess nitrogen/ammonia from fish farm effluents is a major water quality concern of fish farmers, hence, fish feeds enriched with VALM need to be handled with effective feeding and waste management practices to protect downstream water quality and avoid accelerated eutrophication (nutrient enrichment) of surface waters.

The constituents of blood are affected by the quality, quantity and toxicity of the food taken by the animal and can be used to assess both the pathological and nutritional status of individual animal. The fish fed the VALM diet displayed individual differences in haematological parameters which were not significant ($p > 0.05$) compared to the control, indicating tolerance and maintenance of the general well-being (condition factor) and health status of the fish (Table 3). However, the total white blood cell increased ($p > 0.05$) with increasing inclusion levels of *V. amygdalina* leaf meal which is probably the response mechanism to its significantly higher survival ($p < 0.05$) compared to the control group. This agrees with the result of Owen & Amakiri (2011) who obtained significantly higher ($p < 0.05$) WBC, neutrophiles and lymphocytes in the VALM-treated groups as compared to the control groups. The higher the white blood cell especially lymphocytes and other phagocytes, the better the ability of the animal to perform well under very stressful conditions and to fight diseases; but a low value suggests susceptibility to infection. Olabatoke & Oloniruha (2009) reported that *V. amygdalina* is efficient in reducing infections. Udoh (2004, unpublished) documented that 20% *V. amygdalina* leaf meal supplementation in diet of poultry layers significantly ($p < 0.5$) elevated the metabolic hormonal level, growth performance and reproductive indices of the layers compared to other inclusion levels.

Intersexual variations observed in this study indicated males exhibited higher values in almost all haematological parameters compared to females; similar to the

observations of Cech & Wohlschlag (1981), and Orun et al (2003). The higher haematological values in favour of male may be attributed to their higher physiological agility compared to the female.

The intersexual variation ($p < 0.05$) in PCV, Hb and WBC (Table 4, Figure 5) further suggested that these parameters could be sensitive indicators which could serve as reference points in the effective management of *C. gariepinus* broodfish when fed VALM-inclusion feed. Owen & Amakiri (2011) obtained significantly ($p < 0.5$) higher Hb, RBC, PCV and total proteins in diet containing 0% *V. amygdalina* leaf meal than those of various levels of *V. amygdalina* leaf meal inclusions. Decrease in total protein is an indication of reduction in protein quality of the feedstuff (Nwajo 2005). The reduction in the number of red blood cell, haemoglobin and packed cell volume causes anaemia (Gabriel et al 2010) and suggests the destruction (lysis) of erythrocytes or inhibition of erythropoiesis by the active ingredients in the VALM such as haemaglutinin which has an adverse effect on blood formation similar to the actions of other toxicants (Brown 1980). It has also been established that high packed cell volume (PCV) and high haemoglobin content (Hb) are associated with high feed conversion efficiency (Mitruka & Rawsley 1997) as reflected in this study.

Normal ranges for various blood parameters in *C. gariepinus* have been established by different investigators. These analyses and indices serve as predictive tools to provide reliable information on any disorder and chronic stress status before they are present in a clinical setting. Exogenous factors, such as management, diseases and basic ecological factors, such as feeding regime and stocking density, all have direct influences on certain blood parameters (Gabriel et al 2004, 2010). Aydin et al (1998) recorded the normal ranges for blood constituents of the European catfish (*Silurus glanis*) under natural condition as: haematocrit, 27.0-31.0%; haemoglobin concentration, 8.22-9.38 (g dL^{-1}), RBC count (0.85-1.3 x 10^6 mm^{-3}), WBC count 10.0-24.0 (10^3 mm^{-3}), platelets, 0.0-0.4 (10^3 mm^{-3}), MCV, 231.5-289.2 fl, MCH, 70.5-107.2 (pg), MCHC, 30.0-33.0 (g dL^{-1}), and total protein, 3.40-5.93 (g dL^{-1}). For healthy African catfish (*Clarias gariepinus*) the same authors recorded haematocrit (24.0-43.0%), haemoglobin (7.38-12.43 g dL^{-1}) RBC count (1.03-1.61 x 10^6 mm^{-3}) WBC count (21.0-54.0 x 10^3 mm^{-3}), platelets (0.0-5.0x10^3 mm^3); and MCV (188.3-303.7 fl), MCH (74.50-92.40 pg), MCHC (28.0-37.0), and total protein (3.30-3.79). This study contributes to the knowledge on the haematological profiles for both adult male and female broodfish of *C. gariepinus* (as haematocrit, 37.00-56.00%; haemoglobin concentration, 12.00-18.5 g dL^{-1}, RBC count, 3.32-3.72 (x 10^2 L^{-1}), WBC count 282-946.6 (x 10^9 L^{-1}), neutrophile 71.00-81.00%, lymphocyte, 16.00-24.00%, eosinophile, 0.00-3.00%, monocyte, 2.00-4.00%, MCH, 34.53-52.55 pg, MCHC, 0.32-0.33 g 100 mL^{-1}, and total protein, 5.89-5.96 g dL^{-1} as in Table 4). The values were however, higher than those recorded for healthy European and African catfish species (Aydin et al 1998); which may be attributed to factors such as age, size, sample size, season, spawning, environmental conditions, nutritional state, among others, of fish sampled. Generally, RBC counts are quite stable and fish body tend to maintain it through various physiological mechanisms of compensation. Hence, where there is reduction below the normal range of value of the erythrocyte numbers, RBC, there is correspondent reduction in haemoglobin values per cell (Larsson et al 1984). Haemoglobin concentrations reflect the supply of an organism with oxygen and the organism itself tries to maintain them as much stable as possible; including through stress-related release of RBCs from the spleen and hypoxia, induced by presence of toxicants (Shah 2006) or environmental stress, e.g., starvation (Udoh & Udoidiong 2004).

This study, hence, observed that the blood parameters showed significant correlation ($p < 0.05$) with one another. Particularly, relationships involving Hb, PCV, RBC, and MCH. Satheeshkumar et al (2012) also obtained significant interrelationships in the haematological parameters (RBC/WBC, MCV and MCH) of teleost fishes of different feeding behaviour with RBC/WBC level increasing due to the decrease in WBC. This study recorded decreasing RBC/WBC Ratio with increasing VALM-inclusion level (Table 3).

Results of this study also indicated VALM-diet impact some distortions in blood dynamics of broodfish. Though Etim et al (2013) recommend the use of plasma protein and haematocrit values as the major and reliable indicators of assessing health status of

C. gariepinus; in this study, haemoglobin level in addition to plasma protein and haematocrit values should be used as the most important haematological parameters for rapid assessment of the health status of *C. gariepinus* broodfish under culture (the normal ranges are as earlier highlighted).

Conclusions. Generally, the results suggest better utilization of feeds by *C. gariepinus* broodfish in the control group (0% VALM). However, low inclusion level (7.2%) of *V. amygdalina* in broodfish diet is beneficial to the growth performance, survival and haematological parameters, hence health and well-being of the broodfish which are often kept in captivity for two to three years for breeding purposes. It is also needful to improve the processing methods to eliminate the anti-nutritional factors in *V. amygdalina* diets for fish.

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