

Effects of *Vernonia amygdalina* Leaf Powder on Growth Performance of *Clarias gariepinus*

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Abstract

Fish is a vital high quality protein necessary for growth and development. It is affected by biological and chemical substances that pollute the body of water and affects its growth during production. This often leads to the use of different antibiotic growth promoters which have residual effects on consumers of fish while sometimes continual or sub-optimal use leads to development of antibiotics resistant pathogens. This growing concern for antibiotic growth promoters in animal nutrition has elicited the need to search for phytobiotic growth promoting agents. Bitter leaf (*Vernonia amygdalina*) has been of use as antioxidant and growth promoter in man and some animals. The study examined the growth enhancing potential of *Vernonia amygdalina* leaf powder in Catfish (*Clarias gariepinus*) production. Two hundred and forty juvenile catfish were allotted to six treatment groups identified as A, B, C, D, E and F consisting of two replicates with twenty fishes per replicate in a Completely Randomized Design (CRD). Treatment A serve as the control, B was administered 1.5ml of known antibiotics (Florphenicol) and served as positive control while treatments C, D, E and F were administered 10, 20, 30, and 40g/15litres of water respectively for 3hours after which the water was changed. The fishes were fed 3% of their biomass twice daily 800-900 h and 1500-1600h with Durante feed containing 42% crude protein for the 8 weeks experimental period. Twenty fishes were selected per treatment and blood samples were collected by caudal vessels puncture into bijoux bottles containing ethylene diamine tetracetic acid (EDTA) as anticoagulant. The result indicated that feed intake, condition factor, mean final weight, protein efficiency ratio and weight gain were significantly higher ($p < 0.05$) in treatments B, C, D, E, F than the control (A). However, feed conversion ratio, initial weight, specific growth rate and survival percentage were not significantly different ($p > 0.05$), though it numerically increase with the treatments. Red blood cell (RBC) and haemoglobin (Hb) concentration were significantly higher ($p < 0.05$) than the control, while the test substance did not significantly affect other hematological parameters measured. The water quality parameters such as pH, hardness, dissolved Oxygen and Nitrate was within the recommended range and not significantly affected. *Vernonia amygdalina* could be used in improving fish growth and the 40g/15litres of water for 3hours was the best.

Keywords: Bitter-leaf, Antibiotics, Phytobiotics, Growth-promoters

Description of Problem

Fish is a vital source of high quality protein providing approximately 16% of the animal protein consumed by the world population and its culture is one of the fastest growing sectors in the world of animal production with an annual increase of about 10% (1). A major aquaculture fish species in Africa, *Claria gariepinus*, (2) is most popular with fish farmers and consumers. *C. gariepinus* commands a very good commercial value in Nigerian markets (3). It has been noted that farming is hardly imaginable without the availability of fish seed (4). Disease caused by helminthes in livestock continues to be a major productivity constraint with various attempts at control with the use of anti-helminths and antibiotics to increase productivity (5, 6, and 42).

Vernonia amygdalina (VA) is a shrub or small tree that grows throughout tropical Africa. It is popularly called bitter leaf because of its abundant bitter principles (7). The leaves contain a considerable amount of anti-nutritional factors like high level of tannic acid and saponin (8), high cyanide (60.1mg 100-1g Dry matter, DM) and tannin content (40.6mg 100-1g DM) in young leaves than older ones (9). Proximate composition of *Vernonia amygdalina* leaf meal (VALM) shows a chemical composition of 527.83 ME kcal/ kg, 86.40% DM, 21.50% CP, 13.10% CF, 6.80% EE, 11.05% Ash, and the result on mineral composition indicate that *V. amygdalina* has 3.85% Calcium, 0.40% Magnesium, 0.03% Phosphorus, 0.006% Iron, 0.33% Potassium and 0.05% Sodium (10). Research has also shown that *V. amygdalina* have some beneficial effects in disease management of poultry (11), such as anti-coccidiosis, anti-bacterial and anti-parasitic (12, 9); as an antioxidant (8) and as a growth promoter enhancing the gastro intestinal enzymes thus increasing feed conversion efficiency (13, 41). It has also been reported to contain alkaloid,

carbohydrate, tannin, saponin, flavanoids and non cyanogenic glycosides (14) and has active antimicrobial activity (15). *V. amygdalina* is cheap and readily available in many household gardens in Nigeria. Given the antioxidant and growth promoting properties of *V. amygdalina*. The objective of the study was to determine the antioxidant/ growth promoting effect of *V. amygdalina* in comparison with a synthetic antibiotics (Florphenicol) used in Fish production.

Materials and Methods

Preparation of *Vernonia amygdalina* powder

The experiment was conducted at the small animal unit of the Biotechnology Laboratory of the Faculty of Agriculture, University of Ilorin, Nigeria. Fresh leaves of *Vernonia amygdalina* was obtained from the University garden, washed, air-dried for four days and ground into powder using a food blender (Starlite, Model No: SL-999 CHINA).

Sourcing of Juvenile catfish (*Clarias gariepinus*)

The juvenile catfish was purchased at Madester farms in Offa, Kwara State, Nigeria. A total of 2400 Juvenile catfishes were used for this experiment. Two hundred juvenile fishes were stocked into twelve 600 litre plastic tanks. The total and average initial weights of the fish were 9800.0g and $4.08 \pm 0.1g$, respectively. They were randomly assigned to the treatments tanks and allowed a 72hours period to acclimatize during which they were given a 2mm size commercial feed (Durante^R) twice daily and their water changed daily for the first two weeks and subsequently after three days to disallow pollution by *V. amygdalina* leaf powder.

Experimental design

The catfish were randomly assigned to six antibiotics/phytobiotics treatments A, B, C, D, E and F. The treatments were the control A

(0ml), B (150ml Florphenicol), C (100g *Vernonia amygdalina* powder), D (200g *Vernonia amygdalina* powder), E (300g *Vernonia amygdalina* powder) and F (400g *Vernonia amygdalina* powder). The *Vernonia amygdalina* powder were dissolved in 150ml distilled water prior to application on a weekly basis and are left in water for 3 hours after which the fish tank water was changed. The fishes were fed 3% of their body weight twice daily at 8.00-9.00 h and 15.00-16.00h. Durante feed of 2mm size containing 42% crude protein was purchased to feed the catfish for the experimental period that lasted for 8 weeks period. Water quality was closely monitored and water was changed daily for the first two weeks and later every three days.

Data collection

The growth parameters were calculated following the method described by Bagenal (16).

Data on the following performance indices was collected on a weekly basis. The weight gain was obtained by subtracting final average weight from the initial average weight. The specific growth rate (SGR) was obtained by using this formula -

$$SGR = \frac{100 \log \text{final body weight} - \log \text{initial body weight over duration of expt}}{\text{duration of expt}}$$

$$\text{Feed conversion ratio} = \frac{\text{Feed consumed}}{\text{Mean weight gain by fish}}$$

$$\text{Protein efficiency ratio} = \frac{\text{Weight gain by fish}}{\text{Protein intake}}$$

$$\text{Mean weight gain (MWG)} = (W. Sub 2) - (W. Sub 1)$$

where W. Sub. 2 is initial weight (g) of fish and W. Sub. 1 is the final weight (g) of fish.

$$\text{Condition factor (K)} = \frac{100W}{L^3}$$

where W is the final mean body weight (g) and L is the mean standard length (cm)

$$\text{Survival rate (SR)} = \frac{\text{Initial number of fish stocked} - \text{Mortality}}{\text{Initial number of fish}} \times 100$$

The haematological indices: Twenty fishes were selected per treatment and blood samples were collected by caudal vessels puncture into ethylene diamine tetracetic acid (EDTA) bottles for the determination of the packed cell volume, haemoglobin, red blood cell, white blood cell, lymphocytes, neutrophils, eosinophils, and basophils following standard methods as described by Rainza-paiva et al., (17).

Mean erythrocyte haemoglobin concentration (MEHC), mean erythrocyte haemoglobin (MEH) and mean erythrocyte volume (MEV) were calculated from the equations given by Blaxhall and Daisley (18)

$$\text{MEHC (\%)} = \frac{\text{Haemoglobin, g\%}}{\text{Haematocrit, \%}} \times 100$$

$$\text{MEH (g)} = \frac{\text{Haemoglobin, g\%}}{\text{Erythrocyte count, per/L}} \times 10$$

$$\text{MEV (\mu 3)} = \frac{\text{Haematocrit, \%}}{\text{Erythrocyte count, per/L}} \times 10$$

Water quality test

Water quality parameters were measured on weekly basis. Water quality parameter measured includes water pH, water temperature, nitrite, total hardness and dissolved oxygen. Water pH was determined using pH micrometer. Total hardness was determined using direct titration method in which 100ml of the water sample was buffered into 5ml of Ammoniacal buffer which was then titrated against 0.01M EDTA. Hardness was determined using 0.01M EDTA, buffer Erichrome and dissolved oxygen was

determined by pipetting 20ml of the sample water into the biochemical oxygen demand bottle of MnCl solution, follow by 5ml of alkaline iodide solution, 10ml of HCl solution added. The precipitate was titrated with 0.05m to a colourless solution at the end point according to the methods of Bagenal, Gabriel and Izevbigie (16, 25 and 32).

Statistical Analysis

The experiment followed a completely randomized design (CRD). The data obtained were analyzed by the Generalized Linear Model procedures of SAS Version 9.2 software (19). Means differences were considered significant at $p < 0.05$ and separated by Duncan multiple range test (20).

Results and Discussion

The total and average weight of the experimental fish at the end of the experiment was 25.43kg and 10.60 \pm 0.8g respectively. There were significant differences ($p < 0.05$) in the growth parameters among the treatments (Table 1). The feed intake, condition factor, mean final weight, protein efficiency ratio and weight gain were significantly ($p < 0.05$) higher in treatments B, C, D, E, F than the control (A). However, feed conversion ratio, initial weight, specific growth rate and survival percentage were not significantly different ($p > 0.05$), though it numerically increase with the treatments. The potency of *Vernonia amygdalina* powder treated samples could have resulted from various phytochemicals present in the leaves. (21) reported the presence of potent antioxidants such as ascorbic acid and carotenoids. Stigmastane-type saponins such as vernoniosides A1, A2, A3, A4, B2, B3 (22), C, D and E (15) have been reported, with the A-series responsible for the bitter taste. Other phytochemicals reported in the leaves include sesquiterpene lactones such as vernolide, vernodalol (8), vernolepin, vernodalin and hydroxyvernolide

(15), flavonoids- luteolin, luteolin 7-O- β -glucuroniside and luteolin 7-O- β -glucoside (23), terpenes, coumarins, phenolic acids, lignans, xanthenes and anthraquinones (24) and other bioactive peptides called edotides (25). These phytochemicals which may work singly or synergistically are believed to be responsible for the plethora of bio-activities possessed by the plant. Stimulation of higher growth rates promote better feed efficiency and carcass quality. The protein efficiency ratio (PER) of a diet is its growth promoting value and is a good indication of the quality of the feed and response of the animals to it (26). There is a direct relationship between growth rate and productive life; the higher growth rates promote better feed efficiency and carcass quality. The PER was observed to significantly increase within the *V. amygdalina* treated groups when compared with the control. *Vernonia amygdalina* and the antibiotics significantly increased the protein efficiency ratio, feed intake and weight gain of the fish in comparison with the control. This is in agreement with the work of various researchers that the introduction of *Nigella sativa*, *Hibiscus sabdariffa*, *Allium sativa* and Chloramphenicol to *O. niloticus*, *C. garipepinus*, broiler chickens and cockerel chickens respectively caused increased feed intake and protein efficiency ratio (27, 28, 29). Though this was contrary to the report of (11) that supplementation of *V. amygdalina* leaf meal cause a significantly decrease feed intake and growth rate in birds which might be as a result of depressed feed intake at high inclusion level because of the bitter taste.

The water quality parameters as seen in Table 2 were closely monitored and within the standard required for fish farming by the Food and Agriculture Organization (30). This also agrees with the work of (31) on the ecotoxicological effects of pharmaceuticals (Antibiotics and Antiparasiticides) on the environment.

Haematological parameters (Table 3) are routinely used for the evaluation of physiological environment/husbandry stressors in fishes (17). Good pond management is an effective way of reducing stress in fish culture. The change in blood characteristics of *C. gariepinus* is often caused by stress due to exposure to environmental pollutants, and pathogens in capture fisheries (32, 33). It is also necessary in monitoring feed toxicity especially with feed constituents that affect the formation of blood in culture fisheries (34). All physiological parameters measured were within the recommended physiological ranges reported for *C. gariepinus* (35, 36, 37). There was significant increase in haemoglobin (Hb) across the treatments with the control having the lowest and the antibiotics treatment recording the highest, though they were all within the recommended range. The Hb range in the *V. amygdalina* treatment were quite high and can be related to large anaerobic metabolism capacity of the fish. Phenolic compounds exert their growth promoting

effects by three main processes, which include one or more transition metal-chelating activities, singlet-oxygen quenching capacity and free radical scavenging activity (38, 39). The presence of antioxidants such as ascorbic and carotenoids in the leaf powder help the fish to withstand stress and sustain its growth capacity (40).

Conclusion and Application

1. *Vernonia amygdalina* leaf powder and the antibiotics were more effective than the untreated control in enhancing growth capacity, although *V. amygdalina* was not significantly effective than the antibiotics used.
2. The inclusion up to the 400g/150litres of water can be used to replace synthetic growth promoter in fish culturing without any deleterious effect.
3. More studies should be done with increase doses and the mechanism of its effect in the fish body.

Table 1: Effect of *Vernonia amygdalina* leaf powder on growth performance of *Clarias gariepinus*

Parameters	A	B	C	D	E	F	± SEM
FI/g	0.91 ^b	1.21 ^a	1.13 ^a	1.10 ^a	1.11 ^a	1.09 ^a	0.05
FCR/g	0.05	0.05	0.05	0.05	0.05	0.05	0.80
PER	8.63 ^b	12.25 ^a	10.63 ^a	10.69 ^a	10.74 ^a	10.72 ^a	0.74
K	0.87 ^b	1.54 ^a	1.27 ^a	1.29 ^a	1.27 ^a	1.28 ^a	0.19
MIW/g	8.28	8.30	8.00	7.83	8.30	8.03	0.40
MFW/g	18.15 ^b	25.13 ^a	21.84 ^a	21.97 ^a	22.05 ^a	22.03 ^a	2.07
WG/g	13.75 ^b	16.83 ^a	13.84 ^a	14.15 ^a	13.75 ^a	14.00 ^a	2.05
SGR/g	0.61	1.39	0.78	0.80	0.78	0.85	0.32
Survival percentage	96.00	98.00	98.00	98.00	98.00	98.00	

Where FI – feed intake, FCR – feed conversion ratio, PER – protein efficiency ratio, K - condition factor, MIW – mean initial weight, MFW – mean final weight, WG – weight gain, and SGR – specific growth rate. ^{a, b} – means having different superscript along the same row are significantly different (p<0.05)

Table 2: Effect of *Vernonia amygdalina* leaf powder on water quality parameters of *Clarias gariepinus* rearing tank

Parameters	A	B	C	D	E	F	± SEM
Hardness (mg/L)	55.10	54.70	54.30	51.90	57.30	54.70	0.09
Dissolved O ₂ (mg/L)	14.80	17.60	18.80	16.60	15.80	17.60	0.77
Nitrate (mg/L)	11.60	14.10	11.10	15.50	16.30	14.10	0.25
pH	6.30	7.30	6.80	6.60	6.90	7.30	0.29
Temperature, °C	26.00	26.00	26.00	26.00	26.00	26.00	0.08

Table 3: Effect of *Vernonia amygdalina* leaf powder on haematology indices of *Clarias gariepinus*

Parameters	A	B	C	D	E	F	± SEM
PCV (%)	24.50	27.50	26.50	28.50	30.00	28.00	0.30
Hb (g/dL)	6.00 ^b	10.00 ^a	8.05 ^a	6.50 ^a	7.75 ^a	9.85 ^a	0.46
RBC (g/dL)	3.28 ^a	3.39 ^a	4.62 ^a	2.80 ^{ab}	3.32 ^{ab}	1.32 ^b	0.36
MCH(pg)	21.95	21.10	23.30	19.95	20.00	22.70	0.56
MCHC (%)	34.80	34.74	36.95	33.90	30.70	33.20	1.29
MCV (fl)	55.00	61.00	56.00	59.00	61.00	58.00	1.28
WBC (10 ⁹ /L)	14.70	17.75	19.80	23.20	11.18	13.30	1.72
Lymphocytes (%)	64.00	65.00	63.00	72.00	69.00	71.00	1.85
Neutrophils (%)	30.50	33.00	34.00	23.00	28.50	33.00	2.10
Eosinophils (%)	1.00	1.50	0.50	2.00	1.50	1.00	0.23
Basophils (%)	0	0	0	0	0	0.50	0.12

Where PCV – Packed Cell Volume, Hb – Haemoglobin, RBC – Red Blood Cell, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentration, MCV – Mean Corpuscular Volume, and WBC – White Blood Cell;

^{a, b} – means having different superscript along the same row are significantly different (p<0.05)

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