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Efficacy of Almond (*Terminalia Catappa*) Leaves Extracts and Vitamin C Supplemented Diet on Growth Performance and Nutrient Utilization of *Clarias gariepinus* Juveniles

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Abstract

The efficacy of Almond Leaves (AL) extracts and Vitamin C (VIT C) as an ingredient in the diet of *Clarias gariepinus (*4.86±0.02g was evaluated over a 56-day rearing period. Five experimental diets: Control (0%), AL_2 (1%), AL_3 (2%), $VITC_4$ (250mg/kg), and $(VITC+AL)_5$ were formulated and replicated twice at 45% crude protein. Weight gain (WG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Productive Value (PPV), and Nitrogen Metabolism (NM) were measured. Data were analysed using ANOVA at P = 0.05. Results showed that the fish on VIT C and AL extract based diets had higher values in WG, SGR, FCR, PPV, and NM and they were significantly different ($p<0.05$) than those on control diet. The results suggest that inclusion of VIT C and AL leaves extracts in fish diets could positively affect growth of *C. gariepinus* juveniles.

Keywords:
Clarias gariepinus, Almond leaves, Growth, Vitamin C, Fish feeds

Introduction

Aquaculture production has increased over the years and as a result of wild fisheries approaching their biological limits, and increased world demand for cultured fish (Ayinla, 2012). Feed formulation accounts for more than 50% of the total production costs in modern intensive aquaculture (Ibrahem *et al.*, 2010). In order to optimize the fish feed, there is a need to increase feed efficiency, especially by improving the assimilation of dietary nutrients (Ibrahem *et al.*, 2010). The increasing pressure on the aquaculture to reduce or eliminate feed antibiotics as growth enhancers has initiated new research to find safe and efficient natural alternatives. This new generation of feed additives includes herbs and their essential oils and extracts (Brenes and Roura, 2010). Scientists have confirmed that the addition of plants or their extracts in the diets has a beneficial effect to improve growth parameters and protect from diseases in aquaculture (Sasmal *et al*., 2005; Johnson and Banerji, 2007).

Terminalia catappa Linn (*Combretaceae*) also known as tropical almond is a medium size deciduous medicinal plant**.** All parts of the plant contain secondary metabolites that are used in traditional medicine (Akharaiyi *et al*., 2011). The leaves of the plant are used in aquarium to lower the pH and heavy metal content of water. It has been used in this way by fish breeders for many years, and is active against some parasites and bacterial pathogens. It also helps to prevent fungus forming on the eggs of the fish (Hnawia *et al*., 2011). Vitamin C probably is the most important because it is a powerful antioxidant and immunomodulator for fishes/ shrimps. The fish body needs vitamin C (ascorbic acid or ascorbate) to remain in proper health condition. Vitamin C has various benefits to the fish body by holding cells together through collagen synthesis. Collagen is a connective tissue that holds muscles,

bones, and other tissues together. Collagen is also needed for the healing of wounds. There is however limited information on the effects of inclusion of Vitamin C and *T. catappa* leaves extracts in feed for culture of African catfish (*C. gariepinus*).

This study assessed the effect of inclusion of *T. catappa* leaves extracts and vitamin C on growth performance and nutrient utilization of African catfish (*C. gariepinus*).

Materials and Methods

Plant Preparation and Extraction

The almond (*T. catappa*) leaves were obtained in Igodan Secondary School, Okitipupa and air – dried for three weeks. The air-dried *T. catappa* leaves were ground with a hammer mill and 200g of fine powder of *T. catappa* was soaked in 1000ml of 95% ethanol for 48 hours. The plant was properly mixed with ethanol at constant interval, filtered using sterile muslin cloth after which the extract was obtained, air-dried and stored at 25^0C until required.

Experimental Procedures and Feeding Trials

Each treatment has two replicates, 20 fish per replicate with mean initial body weight of 4.86±0.02 and uniform-sized fish were selected from 460 juveniles, weighed and distributed in experimental bowl. The fish were acclimated for 14 days in the experimental bowl before the experiment. The experiment lasted for eight weeks during which the fish was fed at 3% body weight daily. Measurement of the weight changes was performed weekly and the feeding rate adjusted weekly according to the new body weight.

Experimental System and Design

The feeding trial was carried out in ten plastic experimental tanks for eight weeks in the Fisheries Laboratory, Ondo State University of Science and Technology (OSUSTECH), Okitipupa. The water level in each tank was maintained at 35 litres throughout the experimental period. Water in each tank was replaced every three days throughout the period of the experiment to maintain relatively uniform physiochemical parameters and also to prevent fouling that may resulting from feed residues. The source of water was from OSUSTECH water station and each experimental tank was well aerated using air stone and aerator pumps (Cosmos aquarium air pump, double type 3500 50 Hz, 2.5 – 3 W) as described by Lawson (1995). The dissolved oxygen content of the experimental tanks was monitored using dissolved oxygen metre (Jenway 3015pH metre, 0.01 accuracy, Genway, Staffordshire, UK). The water temperature of the experimental tanks was monitored by mercury-in-glass thermometer (producer Paragon Scientific Ltd, Birkenhead, Wirral, UK). The pH value was measured by using pH metre (Jenway 3015pH metre, 0.01 accuracy, Genway, Staffordshire, UK) after standardizing the metre.

Feed Ingredients and Diet Formulation

Fish feed ingredients (fish meal, soya bean, wheat bran and yellow maize) were analysed for proximate composition before the formulation. The value obtained was used to formulate 40% crude protein diets using Pearson's square method to determine individual ingredient contribution at 45% crude protein per g / 100g diet. Each ingredient was weighed using sensitive weighing balance (OHAUSLS model 2000) and dry ingredients were mixed thoroughly in a mixer (ASEFAC, model 1989). Almond leaves extracts and vitamin C were added as feed additive in the study as a partial replacement for wheat bran. Each diet mixture was treated separately. Water was added and the resulting dough was pelleted through a 1/4mm die mincer of Hobart A-200T pelleting machine (Hobart GmbH, Rben-Bosch, Offenbug, Germany) to form a noodle like strand which was mechanically broken into suitable sizes for the *C. gariepinus*

juveniles. The pelleted diets were sun dried and stored in airtight containers at room temperature until required (Table 1).

Table 1: Gross and proximate composition of experimental diets (g/100g)

Ingredients/Parameters	Control	AL_2	AL_3	VIT C_4	(VIT C + $AL)_5$
Fish meal	18.80	18.80	18.80	18.80	18.80
Soy bean	40.59	40.59	40.59	40.59	40.59
Yellow maize	16.31	16.31	16.31	16.31	16.31
Wheat bran	16.31	15.31	14.31	14.31	14.31
Starch	1.00	1.00	1.00	1.00	1.00
Cod liver oil	3.00	3.00	3.00	3.00	3.00
DCP	2.00	2.00	2.00	2.00	2.00
Vitamin premix	2.00	2.00	2.00	2.00	2.00
Almond leaf	-	1.00	2.00	-	1.00
Vitamin C	-	-	-	2.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Moisture (%)	7.00 ± 0.02^a	8.00 ± 0.02^e	7.18 ± 0.02^b	7.25 ± 0.01^c	7.50 ± 0.02^d
Crude protein (%)	44.85 ± 0.02^a	44.79 ± 0.07^a	44.80 ± 0.05^a	44.79 ± 0.08^a	44.88 ± 0.01^a
Ether extract (%)	16.20 ± 0.01^d	15.52 ± 0.05^a	15.80 ± 0.01^b	16.64 ± 0.05^e	16.00 ± 0.02^c
Ash (%)	15.52 ± 0.05^c	14.70 ± 0.07^a	15.00 ± 0.03^b	15.98 ± 0.00^d	16.20 ± 0.04^e
NFE (%)	24.55 ± 0.23^a	19.69 ± 0.20^d	22.60 ± 0.08^c	21.89 ± 0.02^b	19.88 ± 0.01^a

AL = Almond leaves, VIT C = Vitamin C, DCP = Dicalcium phosphate, the mean values in each rows with similar superscript were not significantly different (P˃ 0.05)

Biological Evaluation

Fish were evaluated as follows: weight gain = final body weight - initial body weight; weight gain (%) = 100 (final body weight - initial body weight)/initial body weight; specific growth rate (SGR) = 100 (loge final body weight - loge initial body weight)/time (days); feed conversion ratio (FCR) = dry weight of feed fed (g)/fish weight gain (g); protein efficiency ratio (PER) = wet body weight gain (g)/crude protein fed; protein productive value (PPV) = 100(final fish body protein - initial body protein)/crude protein intake; survival (%) = 100 (initial number of fish stocked - mortality)/initial number of fish stocked, protein intake = (feed intake × percent protein in diet)/100 and Nitrogen metabolism = (0.549)(a + b)h/ 2 Where, a= initial mean weight of fish; b= final mean weight of fish; h= experimental periods in days.

Analytical and Statistical Analysis

Experimental diets and fish carcasses were analysed for proximate composition before and after the experiment according to the methods of Association of Official Analytical Chemists [AOAC] (2005). Growth performance and nutrient utilization indices resulting from the experiment were subjected to one-way analysis of variance (ANOVA) and Duncan multiple range test was used to compare differences among individual means at P = 0.05.

Results

Proximate Composition of the Experimental Diets

The highest value (8.00±0.02%) for moisture was obtained in AL_2 (1%) and least (7.00 ± 0.02%) was obtained in control, highest value (44.88±0.01%) for crude protein was obtained in $(VITC+AL)_5$ and least (44.79 ± 0.07%) was obtained in AL_2 (1%). The Ether extract range from 15.52 ± 0.05% to 16.64 ± 0.05% in VIT C_4 and AL_2, respectively. The highest value for ash was (16.20 ± 0.04%) and least value was (14.70 ± 0.07%), obtained in $(VIT C+AL)_5$ and AL_2 (1%) respectively; for Nitrogen free extract, highest value (24.55 ± 0.23%) and lowest value (19.69 ± 0.04%) was obtained in control and AL_2 (1%) respectively (see Table 1).

Proximate Composition of Fish before and after the Experiment

The proximate composition of experimental fish after the experiment showed an increase in the values ($P< 0.05$) of moisture, crude protein, ash and Nitrogen Free Extract (NFE) obtained in AL and VIT C supplemented diets compared to the value obtained in the control and before the experiment (Table 2).

Table 2: Proximate composition of fish before and after the experiment

	Before	Control	AL_2 (1%)	AL_3 (2%)	VIT C_4 (250mg/kg)	$(VIT C + AL)_5$
Moisture	78.02±0.05^a	67.00±0.00^b	70.10±0.02^f	69.85±0.02^d	67.55±0.05^e	72.50±0.15^c
Crude protein	48.04±0.07^a	59.05±0.33^b	69.89±0.07^c	72.80±0.15^e	74.81±0.08^f	71.97±0.03^d
Ether extracts	3.05±0.02^a	5.01±0.05^e	2.92±0.07^a	3.60±0.05^b	3.88±0.00^c	4.40±0.05^d
Ash	8.85±0.01^a	10.92±0.02^b	15.15±0.02^c	19.10±0.00^e	18.66±0.00^d	18.99±0.07^e
NFE	25.30±0.00^a	40.22±0.03^f	6.54±0.40^a	9.78±0.01^c	10.55±0.18^d	8.12±0.07^b

Keys: AL = Almond leaves, VIT C = Vitamin C, the above values are mean of duplicate data, mean values in each rows with similar superscript are not significantly different ($P\geq 0.05$).

Water Quality Parameters of Experimental Tank

The values obtained in the physico- chemical parameters was relatively closed ($P< 0.05$) within the treatments (Table 3). The highest temperature value of 26.71^{0}C was obtained in AL_2 and lowest of 25.99^{0}C was obtained in VIT C_4 while the highest Hydrogen ion concentration (PH) of 7.72 was obtained in $(VITC+AL)_5$ and lowest of 7.53 was obtained in VIT C_4

Table 3: Water quality parameters of experimental tank

Treatment	Parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Mean
Control	Temp (^{0}C)	25.07±0.05	26.80±0.05	27.90±0.06	25.70±0.20	26.07±0.04	26.80±0.05	27.20±0.01	26.57±0.05	26.51±0.02
	pH	7.20±0.01	7.74±0.44	7.83±0.26	7.25±0.03	8.14±0.01	7.74±0.44	7.75±0.28	7.71±0.08	7.67±0.01
AL 2 (1%)	Temp (^{0}C)	26.07±0.04	26.80±0.05	26.80±0.05	27.90±0.06	27.00±0.00	26.80±0.05	25.70±0.20	26.58±0.10	26.71±0.04
	pH	8.14±0.01	7.20±0.01	7.74±0.44	7.83±0.26	8.10±0.05	7.20±0.01	7.25±0.03	7.59±0.01	7.63±0.09
AL 3 (2%)	Temp (^{0}C)	27.20±0.01	26.32±0.80	27.00±0.00	26.07±0.04	25.70±0.20	26.32±0.80	26.80±0.05	26.55±0.03	26.50±0.07
	pH	7.75±0.28	7.78±0.44	8.10±0.05	8.14±0.01	7.25±0.03	7.78±0.44	7.20±0.01	7.52±0.01	7.69±0.05
VIT C 4 (250mg/kg)	Temp (^{0}C)	26.80±0.05	26.07±0.04	25.70±0.20	25.07±0.05	25.70±0.20	25.60±0.20	26.32±0.80	26.65±0.15	25.99±0.08
	pH	7.74±0.44	8.14±0.01	7.25±0.03	7.20±0.01	7.25±0.03	7.35±0.03	7.78±0.44	7.50±0.11	7.53±0.04
(VIT C + AL) 5 (2%)	Temp (^{0}C)	25.70±0.20	26.80±0.05	26.32±0.80	27.90±0.06	26.32±0.80	26.87±0.00	26.32±0.10	26.58±0.03	26.60±0.10
	pH	7.25±0.03	7.74±0.04	7.78±0.02	7.83±0.26	7.78±0.14	7.88±0.01	7.78±0.00	7.75±0.05	7.72±0.05

Key: AL = Almond leaves, Vit C = Vitamin C, Temp= Temperature, PH= Hydrogen ion concentration, the above values are mean of duplicate data, mean values in each rows with similar superscript were not significantly different ($P\geq 0.05$).

Growth Performances and Nutrients utilization of Clarias gariepinus fed Vitamin C, Cashew Leaf and Almond Leaf Extract

There was no significant difference (P> 0.05) in the mean initial body weight, final body weight, body weight gain, specific growth rate, food conversion ratio, protein efficiency ratio, nitrogen metabolism, and survival rate, while there was significant difference (P< 0.05) in protein productive value, protein intake, percentage body weight of fish fed experimental diets (Table 4).

Table 4: Growth performances and nutrients utilization of *Clarias gariepinus* fed vitamin C and almond leaf extract

Parameters	Control	AL_2 (1%)	AL_3 (2%)	VIT C_4 (250mg/kg)	(VIT C + AL) $_5$ (2%)
Initial body weight(g)	4.86±0.001^{a}	4.85±0.001^{a}	4.86±0.001^{a}	4.85±0.001^{a}	4.86±0.001^{a}
Final body weight(g)	7.63±0.27^{a}	7.94±0.20^{a}	7.82±0.47^{a}	7.19±0.09^{a}	7.58±0.05^{a}
Body weight gain (g)	2.78±0.43^{a}	3.09±0.20^{a}	2.97±0.48^{a}	2.34±0.10^{a}	2.72±0.05^{a}
Body weight gain (%)	57.15±0.70^{a}	63.61±0.02^{a}	61.08±0.85^{a}	48.15±2.06^{a}	56.03±1.09^{a}
Food conversion ratio	4.32±0.67^{a}	3.66±0.24^{a}	3.40±0.55^{a}	4.29±0.18^{a}	4.25±0.08^{a}
Protein efficiency ratio	0.06±0.01^{a}	0.07±0.01^{a}	0.07±0.01^{a}	0.06±0.01^{a}	0.06±0.00^{a}
Protein productive value	24.55±0.57^{a}	48.77±0.40^{c}	55.27±0.10^{e}	59.77±0.11^{f}	53.32±0.23^{d}
Protein intake (g)	209.77±0.10^{i}	201.25±0.32^{g}	175.64±0.20^{d}	178.82±0.32^{e}	207.55±0.05^{gh}
Survival rate (%)	77.50±2.50^{a}	77.50±2.50^{a}	80.00±5.00^{a}	85.00±0.00^{a}	85.00±0.00^{a}
Nitrogen metabolism	167.93±5.85^{a}	171.97±2.63^{a}	170.49±6.26^{a}	161.88±1.01^{a}	167.19±0.54^{a}
Specific growth rate	0.40±0.05^{ab}	0.43±0.02^{ab}	0.42±0.05^{ab}	0.35±0.02^{ab}	0.39±0.00^{ab}

Key: AL = Almond leaves,Vit C = Vitamin C, Temp= Temperature, PH= Hydrogen ion concentration, the above values are mean of duplicate data, mean values in each rows with similar superscript were not significantly different (P≥0.05).

Discussion

The results of proximate composition of experimental diets obtained in this study corroborated with the findings of Robinson *et al*., (2001) who reported that the inclusion of 45% crude protein in fish diets as well as the use of standard formulation and processing techniques resulted in production of diets with reduced moisture content, relatively high nutrients and highly durable diets which enhanced better palatability and growth in fish.

Physico- chemical parameters such as temperature and pH obtained during the study were within the acceptable limit for *C. gariepinus* culture. The results obtained in this study fell within the ranges of 4-32°C for temperature and 6.5- 9 for pH reported by Ndimele and Owodeinde (2012) as the best for tropical fishes. The experimental fish within all the treatments showed great increase in weight, which indicated that the fishes were able to convert feed protein to extra muscles as weight gain and species growth rate are usually considered as the most important measurement of productivity of diets as shown in the result which are in accordance with the findings of Bello *et al*., (2012). Reasons for the results obtained could be that the fish having higher values in the respective parameters measured used the available protein and other nutrients in the diet efficiently than others which led to increase in weight and some other measured parameters such as percentage weight gain, protein efficiency ratio, specific growth rate, protein productive value, nitrogen metabolism and this findings concured with that of Farahi *et al*. (2012) whose fish fed experimental diets performed better than the control in the dietary supplementation (of lemon balm and *Aloe vera*) fed to Rainbow trout.

Conclusion

It can be concluded from the results of the study that vitamin C and almond leaves extracts had nutritional properties that enhanced growth of *C. gariepinus* juveniles and the inclusion of almond leaves extracts (2%) may significantly enhance productivity in aquaculture industry.

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