

RESPONSE OF *Clarias gariepinus* TO *Telfairia occidentalis* and *Vernonia Amygladina* SUPPLEMENTS

Comment [p1]: RESPONSES OF *Clarias gariepinus* TO *Telfairia occidentalis* and *Vernonia Amygladina* SUPPLEMENTS. Check the spelling for *Vernonia amygladina* or *Vernonia amygdalina*...

ABSTRACT

This study examined the growth enhancing potential of *Telfairia occidentalis* and *Vernonia amygladina* leaf powder in *Clarias gariepinus* culture. The experiment was conducted at Tiddo fish farm Makurdi. One thousand juvenile catfish were obtained from the farms hatchery and allocated to five treatment groups identified as Control (0% supplement inclusion), T1 (10% fluted pumpkin leaf powder inclusion), T2 (20% fluted pumpkin leaf powder inclusion), T3 (10% bitter leaf powder inclusion) and T4 (20% bitterleaf powder inclusion). The fish were fed thrice daily for 8 weeks and water changed daily. Results obtained showed that after the treatment period, Mean Weight gain and Specific Growth Rate was not significantly different ($p > 0.05$). However, Feed Conversion Ratio and Percentage Survival rate was significantly different ($p < 0.05$). Water quality parameters such as pH, Dissolve Oxygen and Temperature was within the recommended range and not significantly affected. Both plant species feed inclusions were utilized by fish, which was observed to facilitate the growth and survival of the fish species, with high percentage survival.

Comment [p2]: Incomplete methodology, what parameters were determine and how they were determined have to be stated here briefly.

Comment [p3]: State which treatment of either plants performed better with quantification.

Comment [p4]: Aspect of conclusion/recommendation base on the outcome of the research missing.

Comment [p5]: Growth study

Keywords: Substances, Treatment, Feed Inclusion, Feed utilization, Growth, Study.

INTRODUCTION

Vernonia amygladina (family *asteraceae*) is a valuable medicinal plant that is widespread in East and West Africa (Atawodi, 2008). It is known as bitter leaf and may be used as an active anti-cancer, anti-bacteria, anti-malarial, and anti-parasitic agent (Braide, 2012). This plant contains complex active components that are pharmacologically useful and are used in the treatment of fever, hiccups, kidney problems, and stomach discomfort. Traditional medical practitioners, herbalists, and local healers in West Africa recommend aqueous *V. amygladina* for their patients. The beneficial use of *V. amygladina* in animal nutrition in Nigeria has been well documented. However, the unrefined nature of the herbal preparations, coupled with the apparent lack of specificity or precision in the application of the plant in traditional medicine could lead to over dosage of the herbal medicine, which can result in accumulation of essential and non-essential plant ingredients in the human system. The accumulation can reach a toxic level, especially in the systems of people wholly heavily on unrefined products, with severe consequences on their biochemical and genetic system.

Comment [p6]: ..who rely...

Fluted Pumpkin (*Telfairia occidentalis*) is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds. *T. occidentalis* is a member of the family Cucurbitaceae and is indigenous to southern Nigerian (Fagbemi, 2007). The fluted gourd grows in many nations of West Africa, but is mainly cultivated in Southern eastern Nigeria and it is used primarily in soups and herbal medicines (Alada, 2000). *Telfairia occidentalis* Is an important staple vegetable grown in Nigeria. The plant produces luxuriant edible green leaves, which are rich in iron and vitamins. Stems of the plants have branching, long twisting tendrils and the leaves are divided into three to five leaflets with the terminal leaflets up to 15 cm long, while the male plant is grown principally for leaves and seeds, which are important soup condition. Recent studies have shown that *Telfairia occidentalis* leaf is rich in minerals (such as iron, potassium, sodium, phosphorus, calcium and magnesium), antioxidants, vitamins (such as thiamine, riboflavin, nicotinamide and ascorbic acid, phyto-chemicals such as

Comment [p7]: *Telfairia occidentalis*

Comment [p8]: Condition or ingredient..check

phenols. The leaf extract is useful in the management of cholesterolemia, liver problems and impaired defense immune systems (Eseyi *et al.*, 2005). The amino acid profile of *T. occidentalis* had also been shown to be very rich and includes alanine, aspartate, glycine, glutamine, histidine, lysine, methionine, tryptophan, cystine, leucine, arginine, serine, threonine, phenylalanine, valine, tyrosine and isoleucine (Fasuyu, 2006). Emeka and Obidoa (2009) revealed that the long term feeding of *T. occidentalis* supplemented diet caused a significant increase in weight of animals which may be due to its content of rich nutrients. The high protein content in leaves of plants such as *Telfairia occidentalis* could have supplementary effect for the daily protein requirement of the body. The symptoms of protein energy malnutrition such as Kwashiorkor and Marasmus were rarely observed among dwellers in region where adequate amount of protein is obtained from fruits/seeds and leaves of plants rich in proteins such as *T. occidentalis* (Dike,2010). Many researchers especially in the field of medical sciences have observed free radical scavenging ability and antioxidant property in *Telfairia occidentalis*. The darkish green leafy vegetable leaves of *Telfairia occidentalis* and extracts (such as aqueous and ethanol extracts) from the leaves have been found to suppress or prevent the production of free radical and scavenge already produced free radical, lower lipid preoxidation status and elevates antioxidant enzymes both in vitro and in vivo (Oboh,2007). *Clarias gariepinus* fish is a perishable commodity which spoils immediately after capture. It is generally accepted that some tropical fish species can keep for longer periods in comparison to fish from temperate or colder water (Jimoh, *et al.*, 2013). This can be attributed to differences in the bacteria growth rates in tropical fish stored in ice.

Comment [p9]: Source or citation needed here.

Comment [p10]: space

Comment [p11]: space

Statement of Research Problem

Comment [p12]: Take as different paragraph.

Comment [p13]: Delete

Clarias gariepinus is an important aquaculture fish in Africa, but its culture is being hampered and becoming unattractive due to the high cost of feed, high cost of production and scarcity, this is because most fish feed manufactured in the country rely on imported feed ingredients. Plant based supplement included in fish feed may prove effective in supporting the growth and development of fish, thereby cutting the cost of fish farming.

Comment [p14]: Merge contents with above

Comment [p15]: Delete

Justification of the Study

Aquaculture production seems to be responding to the increased fish demand and have exclusively increased the world fish production by 20 million tons. Irrespective of the achievement of aquaculture, the high cost and scarcity of desirable feeds for aquaculture enterprises in Africa has become a serious constraint in the successful operation of intensive aquaculture enterprise. Fish meal and soybean meal which are the major sources of protein are used as food, by human, it is in high demand by industries for the production of human, animal and fish feeds and this makes the products and feeds expensive. The need to reduce this cost is imperative in the aquaculture business. An inexpensive source of fish feeds will make fish farming attractive due to its profitability.

Aim of Study

Comment [p16]: Delete all the sub-headings. This is not a thesis but manuscript for publication. Use contents to summarise why the research is necessary.

To determine the response of *Clarias gariepinus* (Catfish) to various feed inclusion ratio of *Vernonia amygladina* (Bitter leaf) and *Telfairia occidentalis* (Pumpkin leaf) supplement.

Objectives of Study

1. To determine the utilization and growth rate of *C. gariepinus* to varying percentages

- of *Vernonia amygladina* dietary inclusion
2. To determine the utilization and growth rate *C. gariepinus* to varying percentages of *Telfairia occidentalis* dietary inclusion
 3. To determine the Feed Conversion Ratio and Survival Rate of *Clarias gariepinus* fed with varying percentages of dietary inclusions of *V. amygladina* and *T. occidentalis*.

Null Hypothesis

Powdered inclusion of *Vernonia amygladina* leaf has no effect on *Clarias gariepinus* juveniles.

Powdered form of *Telfairia occidentalis* leaf has no effect on *Clarias gariepinus* juveniles. *C. gariepinus* did not feed or survive on the varying dietary inclusions of *V. amygladina* and *T. occidentalis*

MATERIALS AND METHODS

Study Area

This experiment was conducted at Tiddo fish farm Makurdi, Benue State, situated in the Guinea Savannah agro-ecological zone and in the North Central geo-political zone of Nigeria.

Collection of the Experimental Fish

The juveniles of *C. gariepinus* was bought from the university farm and transferred to five 1000 litres plastic bowls at the fish farm and were allowed to stabilize for 48 hours before commencement of experiment. The fish were randomly distributed in the 5 treatments at stocking density of 200 fish per tank. The fish were fed thrice daily, in the morning, afternoon and Evening (8:00am, 2:00pm and 6:00pm respectively), and they were weighed once a week for 8 weeks, at daily feeding rate of 3% of the total body weight, adjusted in like manner as documented by (Agarwal, 2003).

Source and preparation of *Telfairia occidentalis* and *Vernonia amygladina* powder

Telfairia occidentalis and *Vernonia amygladina* leaves were purchased from Wadata market. The leaves were washed with clean water to remove the dust and dirt. They were subsequently air dried under room temperature and devoid of sunlight for two weeks. Upon drying and when the leaves became brittle to touch, they were blended into powdered form and sieved using a 1mm mesh size sieve as documented by (Ayinla, 2007).

Feed Formulation and Pellet Preparation

All dietary ingredients were ground and mixed with vitamin-mineral premix and water was added gradually until a desirable paste-like consistency was obtained after which the mixture was pelletized through 2mm disc and sun-dried. The pelletized feeds were then stored in air tight polythene bags, until required. Diet samples were subjected to proximate analysis feed formulation (Olurin, 2006). Diet (1) was the control with no inclusion of *Telfaria occidentalis* (fluted pumpkin leaf) or *Vernonia amygladina* (bitterleaf) used during formulation, Diet (2) contained 10% inclusion of *Telfaria occidentalis* (fluted pumpkin leaf) referred to as T1, Diet (3) contained 20% inclusion of *Telfaria occidentalis* (fluted pumpkin leaf) referred to as T2, Diets (4) contained 10% inclusion of *Vernonia amygladina* (bitter leaf) referred to as T3, while Diets (5) contained 20% inclusion of *Vernonia amygladina* (bitter leaf) referred to as T4. All these inclusion at different levels was added to determine different growth rate and

Comment [p17]: Set the concluding parts of the introduction as reason why this research was conducted.

Comment [p18]: Reported by

Comment [p19]: Where?

Comment [p20]: Reported by

wellness, via the inclusions.

Comment [p21]: Delete, not necessary.

Experimental Set Up

Water was pumped from the College farm borehole into over-head tanks to settling after which water was released into the experimental units. The 5 bowls were filled with 300 liters water uniformly, and 200 *Clarias gariepinus* juvenile were randomly stocked in each treatment bowl. The experiment lasted 8 weeks (56 days). The design of the experiment was completely randomized design prior to the introduction of the composition diet. Introduction of treatment diets containing different concentration of *Telfairia Occidentalis* (Fluted pumpkin leaves) and *Vernonia amygladina* (Bitter leaves) was carried out from the 1st to the 4th treatment bowl while the first bowl was left as the control with no inclusion of *Telfairia occidentalis* (Fluted pumpkin leaves) or *Vernonia amygladina* (Bitter leaves).

Comment [p22]: settle

Gross Composition of Experimental Diet

Table1 below, shows the composition of the various inclusion levels of *V. amygladina* and *T. occidentalis* treatments.

Table1: Gross Composition of Experimental Diet

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Soya Beans	32	32	32	32	32
Fishmeal	10	10	10	10	10
GNC	36	36	36	36	36
Maize	7.5	7.5	7.5	7.5	7.5
Lysine	0.5	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5	0.5
Vitamin	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
Cassava Flour	5	8	8	8	8
Blood Meal	7	7	7	7	7
Bone Meal	0.5	0.5	0.5	0.5	0.5
Feed Inclusion	0%	10%	20%	10%	20%
Total	100	100	100	100	100

Comment [p23]: Only diet one totals to 100, other are more. Cassava flour weight should be uniform as well, that is 5g all through. Its only the inclusion levels that vary.

Mean Water Parameters

Mean Water Physico-chemical parameters of water used for the experiment across the treatments (Mean \pm SD) was taken during the experimental period and presented in Table 2

below.

Table2: Mean water Quality Parameters

Parameter	Control	T1 (PL 10%)	T2 (PL 20%)	T3 (BL10%)	T4 (BL20%)	Normal Range
Temperature(°C)	27.20± 0.10	27.00 ± 0.00	26.80 ± 0.12	27.40 ± 0.03	27.60 ± 0.14	25- 31
pH	7.0 ± 0.00	7.4 ± 0.03	7.6 ± 0.04	8.0 ± 0.10	7.4 ± 0.10	6.5- 8.5
D.O(mg/l)	5.82 ± 0.20	5.6 ± 0.10	6.22 ± 0.11	5.7 ± 0.30	6.7 ± 0.10	5.00

Comment [p24]: mg/L

Comment [p25]: This table should move to results

Determination of Growth Performance

Data on the following performance indices were collected on a weekly basis. Initial mean weight of the fish was determined using sensitive weighing balance after which regular sampling for mean weight changes were taken at two weeks intervals for 8weeks. The data collected was processed for growth assessment and nutrient utilization using the following formulae by Jobling (1983), as described below.

Comment [p26]: Type and model should be stated

Mean Weight Gain (MWG)

The weight gain of fish in each treatment group were taken. All fish per treatment were individually weighed on a sensitive weighing balance and the respective means were recorded.

$$MWG = W_f - W_i$$

Comment [p27]: Indicate the unit of measurement

Where:

W_f = final mean weight

W_i = initial mean weight

Percentage Mean Weight Gain (PMWG)

$$PMWG = \frac{W_f - W_i}{W_i} \times 100$$

Comment [p28]: ditto

Where:

W_f = final mean weight

W_i = initial mean weight

Specific Growth Rate:

The SGR was calculated using the formula below:

$$SGR = \frac{\ln [W_f - W_i]}{T} \times 100$$

Comment [p29]: ditto

Where:

W_f = final mean weight

W_i = initial mean weight T = culture period (day)

Feed Conversion Ratio (FCR)

This is the amount of unit weight of food that the fish were able to convert into unit muscle.

$$FCR = \frac{\text{Feed Intake}}{\text{Total Weight Gain}}$$

Comment [p30]: ditto

Survival Rate (SR)

$$\text{Survival Rate (\%)} = \frac{\text{No. of Fish at the end of Experiment}}{\text{Initial No. of Fish Stocked}} \times 100$$

Comment [p31]: Separate the words

Data Analysis

All data collected during experimental period was subjected to two-way analysis of variance (Two-Way ANOVA) using SPSS version 26. Duncan's Multiple Range Test was employed to reveal significant differences among the treatment means.

Comment [p32]: To separate the mean where significant

RESULTS

Percentage Mean Weight Gain, Specific Growth Rate, Food Conversion Rate, and Percentage Survival Rate of *Clarias gariepinus* fed with *Telfairia Occidentalis* and *Vernonia Amygladina* leaf Powder was determined from data obtained, recorded, and computed.

The Specific Growth Rate (SGR) was highest (7.9) in T2 (20% Pumpkin powder) and T3 (10% Bitter Leaf Powder) and lowest (7.3) in T0 (Control) with significant difference (p<0.05).

Comment [p33]: unit

Comment [p34]: no need for this again..you have already indicated what treatments were.

Comment [p35]: ditto

The Percentage Mean Weight Gain (PMWG) was significantly different across treatments, and highest (96%) in T3 (10% Bitter Leaf Powder) and lowest (65%) in T0 (Control). There was no significant difference (p<0.05) between T2 and T3.

Comment [p36]: ditto

The Feed Conversion Ratio showed that *Clarias gariepinus* utilized feed fed across the treatments fed with a significant difference (p<0.005)(Table2). T0 at 1.4, and T1 to T4 at 1.0.

Comment [p37]: Recast. Not clear_be specific.

The highest survival rate recorded was 100% for treatment3 (10%bitterleaf), 95% for treatment 1 (10% pumpkin leaf), 90% for both treatment 2 (20% pumpkin leaf) and treatment

4 (20% bitterleaf) and 85% for treatment 1 (control).

Comment [p38]: Just T3, T1, T2 or T4 is sufficient.

Comment [p39]: Un-italicize. Use *C. gariepinus*.

Table 3: Feed Utilization and Survival of *Clarias fingerlings* in Relation to Varying Percentages of *V. amygladina* and *T. occidentalis* leaf powder inclusions

Parameters	Control	T1 (10% Pumpkin leaf)	T2 (20% Pumpkin leaf)	T3 (10% Bitter Leaf)	T4 (20% Bitter Leaf)
No.of fish Stocked	200.0	200.0	200.0	200.0	200.0
Mean Initial Weight (g)	4.0	3.9	3.9	3.9	3.9
Mean Initial Length (cm)	5.0	5.3	4.9	5.2	5.3
Mean Final Weight (g)	65.0 ^b	80.0 ^a	87.0 ^a	91.0 ^a	80.0 ^a
Mean Final Length (cm)	27.3 ^c	30.2 ^a	26.8 ^b	30.4 ^a	26.8 ^b
SGR	7.3 ^c	7.7 ^b	7.9 ^a	7.9 ^a	7.7 ^b
PMWG (%)	93.8 ^c	95.1 ^b	95.5 ^a	95.7 ^a	95.1 ^b
FCR	1.4 ^a	1.0 ^b	1.0 ^b	1.0 ^b	1.0 ^b
PSR (%)	90.0 ^c	95.0 ^b	90.0 ^c	100.0 ^a	85.0 ^d

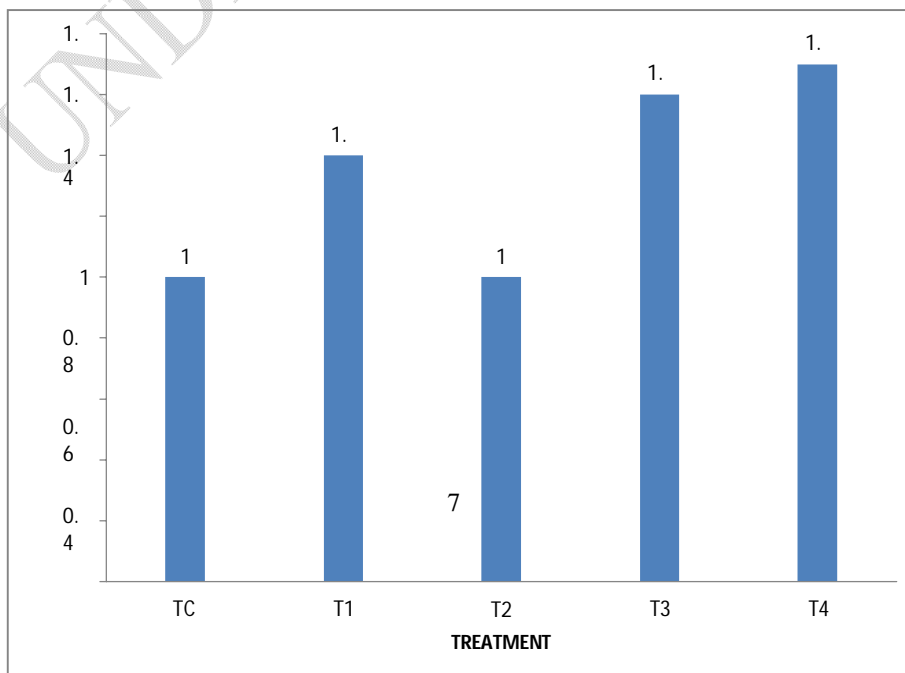
Comment [p40]: See comments above and correct. Use the details as part of the legend below the table.

*MWG: Mean Weight Gain, SGR: Specific Growth Rate, PMWG: Percentage Mean Weight Gain, FCR: Feed Conversion Ratio, and PSR: Percentage Survival Rate

*Mean Values with the same superscript along rows are not significantly different ($p > 0.005$)

*Mean Values with Different superscripts along rows are significantly different ($P > 0.005$)

Figure 1: Mean Weight Gain



T0= Control
T1 = 10% fluted pumpkin leaf
T2 = 20% fluted pumpkin leaf
T3=10%bitterleaf
T4=20%bitterleaf

Comment [p41]: Title should be below the figure. Vertical and horizontal axes should have labels; key to the side as a guide to your reader.

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Growth Performance of *Clarias gariepinus* juveniles in Relation to Treatments with Pumpkin leaf and Bitterleaf Powder

There was general growth and improvement in weight of fish across the treatments. Mean Initial Weight was 3.92grams and Mean Final Weight was 7.92grams at the end of the experiment. Mean Initial Length was 5.14 cm and Mean Final Length was 9.74 cm at the end of the experiment. When fish are fed consistently, fish utilizes feed and grows. Growth can be isometric or allometric however. The growth of fish at all stages in its life history is largely regulated by a number of factors, such as food type, food intake, feeding rate, feeding frequency and its ability to absorb nutrients (Gelineau *et al.*, 1998; Akbulut *et al.*, 2013). There was no weight loss across the treatments from the weight of fish from start of experiment till the final weight of fish at the end of the experiment.

Comment [p42]: delete

The Percentage Mean Weight Gain (PMWG) was significantly different across treatments, and highest (96%) in T2 and T3 (20% *T. occidentalis* and 10% Bitter Leaf Powder) and lowest (65%) in T0 (Control). There was no significant difference ($p < 0.05$) between T2 and T3. 20% *T. occidentalis* leaf powder inclusion and 10% *V. amygladina* leaf inclusion had a better result ($p < 0.05$) as compared with the other treatments. This indicates that the higher the percentage inclusion of *T. Occidentalis* supplements, the better the feed assimilation by the fish. *T. occidentalis* is not as toxic as *V. amygladina* leaf in water. However, *V. amygladina* has anti-bacterial and anti-parasitic properties (Tadesse *et al.*, 1993 and Gbolade *et al.*, 2003); has an anti-oxidant (Erasto *et al.*, 2006) and as a growth promoter enhancing the gastro intestinal enzymes thus increasing feed conversion efficiency (Huffman *et al.*, and , Opatá *et al.*, 2006). The more the percentage inclusion of *V. amygladina* in feed, the faster the pond water gets toxic. At 10% Pumpkin and 10% Bitter leaf (T1 and T3 respectively), Pumpkin leaf showed only a slight percentage difference (95.1% and 95.7% respectively). This indicates that both supplements (especially Pumpkin leaves) can enhance growth rates, this agrees with Yin *et al.*, (2006) that said the use of plant based additives in aquaculture is one of the methods used to improve weight gain, feed efficiency, and/or disease resistance. According to Fasuyi and Nonyerem (2007), *T. occidentalis* leaf contains ingredients such as

Comment [p43]: take note of previous comments and correct. Too much repetition of result in discussion is not good enough.

Comment [p44]: This probably indicates....avoid absoluteness.

bioflavonoid, an active chemical, plant promoter, which promotes growth in birds, and may have similar effects in fish. Aqueous *T. occidentalis* is also reported to increase haematological parameters (Alada, 2000). The result from this experiment had T2 (10% *T. occidentalis*) and T3 20% *V. amygdalina*) with highest PMWG at 96%. This agrees with the findings of Adekunle (2016) that showed better growth rate and weight gain with treatment with *T. occidentalis* inclusions in feed. However, the higher the concentration, the higher the tendency of leading to accumulation of toxins in fish and acute water toxicity (most liable to Bitterleaf toxin accumulation). The unrefined nature of the herbal preparations, coupled with the apparent lack of specificity or precision in the application of the plant in traditional medicine could lead to over dosage of the herbal medicine, which can result in accumulation of essential and non-essential plant ingredients in the human system. The accumulation can reach a toxic level, especially in the systems of people who rely heavily on unrefined herbal products, with severe consequences on their biochemical and genetic systems (Okwuzu, 2017).

The Specific Growth Rate (SGR) was highest (7.9) in T2 (20% Pumpkin powder) and T3 (10% Bitter Leaf Powder) and lowest (7.3) in T0 (Control) with significant difference ($p < 0.05$). Most treatments with the leaf powder inclusions had a higher SGR than the Control treatment (T0). T2 at 20% *T. occidentalis* leaf powder inclusions had better SGR as well as 10% *V. amygdalina*. This is a pointer to the fact from recent studies that have shown that *T. occidentalis* leaf powder is rich in minerals, antioxidants, vitamins as compared to *V. amygdalina* that has more of antibiotic, antiparasitic and disease management tendencies (Ekeocha 2011). Hence, *T. occidentalis* possesses a higher tendency to utilize feed and growth, while *V. amygdalina* inclusion had a higher disease resistant rate tendencies.

Comment [p45]: delete

Comment [p46]: See 44 above.

Results obtained show a significant difference in the Feed Conversion Ratio of *Clarias gariepinus* juvenile fed with bitterleaf and pumpkin leaf powder ($p > 0.05$). The Feed Conversion Ratio showed that *Clarias gariepinus* utilized feed fed across the treatments fed, with a significant difference ($p < 0.005$) across treatments T0 at 1.4, and T1 to T4 at 1.0. All treatments (T1 to T4) with the feed inclusions had a better FCR as compared to the Control (T0), with a significant difference ($p > 0.05$) and this agrees with the findings of Adekunle (2017) that showed better FCR for treatments of feed with additive inclusions as compared with the control treatment.

Comment [p47]: deete

Survival rate recorded showed minimal or inconsequential loss to *Clarias gariepinus* population across the treatments, and this indicates that the fish species can survive well when fed with calculated amounts of both *Vernonia amygdalina* and *Telfairia occidentalis* inclusions. The highest percentage survival rate recorded was 100% for treatment 3 (10% bitter leaf), and lowest (85%) for T4.

During the period of the experiment, Temperature and pH of experimental water fall within the normal range for aquaculture, however the data collected for Dissolved Oxygen was slightly above the normal range. This could be a factor that enhanced the survival rate of the fish as Dissolved Oxygen is a vital component required for their survival. The physico chemical parameters of the experimental water across treatments was within tolerable range for fish culture, as described by Onyia *et al.*, (2015).

Comment [p48]: the normal..

Conclusion

V. amygdalina and *T. occidentalis* powder supplements showed support for fish growth. Based on this research, both plant supplements can be used for growth development as they

Comment [p49]: Improved performance....recast

Comment [p50]: More conclusion needed here with more salient parts of your results with facts.

contain a healthy amount of vitamins and minerals and are not of harm to the fish species as shown by their survival rate.

Comment [p51]: Take as recommendation base on the outcome of the experiment.

Recommendation

Further research on these plant species used can be conducted to ascertain the actual amount of mineral components present. In addition, after growing to table size, proximate analysis of the fish species should be done for the control as well as the various treatment groups.

A taste survey analysis of the table size fish can be done on randomly selected individuals to ascertain if there is a difference in taste between the control and the treatment groups of the fish samples. Histological analysis can be done on the fish species to determine the effect of the supplement on the internal organs of the fish.

REFERENCES

Adekunle A. D (2017). Use of fluted pumpkin (*Telfairia occidentalis*) leaf powder as feed additive in African catfish (*Clarias gariepinus*) fingerlings. *Journal of Applied Animal Research*, 45:1, 566-569. DOI 10.1080/09712119.2016.1233108

Agarwal,V.P.(2003).Recent trends in aquaculture. *Agarwal printers,1174,P*, Sharma Road Mecrut. 550-556.

Alada A R A (2000), "The haematological effects of *Telfairia occidentalis* diet preparation", *African Journal of Biotechnol.*, Vol. 5, pp. 49-53.

Akbulut, B., Feledi, T., Lengyel, S. and Ronyai, A.(2013). Effect of feeding rate on growth performance, food utilization and meat yield of starlet, *Acipenserruthenus* (Linne,1758). *Journal of Fisher Scientific*,7, 216-224.

Atawodi, S., Mende, P., Pfundstein, R., & Spiegelhalter,B. (2008). Nitron stable amines and nitros amide formation in natural stimulant, *Colaacuminate*.

Comment [p52]: Incomplete

Ayinla, O. A (2007). Analysis of feeds and fertilizers for sustainable aquaculture development in Nigeria. In M.R. Hassan, T. Hecht, S.S. De Silvia and A.G.J. Tacon (eds). Study and analysis of feeds and fertilizers aquaculture development. FAO fisheries technical paper No.497. Rome FAO, 453-470.

Braide, W., Odiong, I. J. & Orarus, S. (2012). Phito- chemical and anti-bacterial properties of the seed of watermelon (*Citrillusanatus*). *Prime Journal of Microbiology Research*,2(3):99-104.

Dike, M.C. 2010. Proximate, phytochemical and nutrient compositions of some fruits, seeds and leaves of some plant species at Umudike, Nigeria. *Journal of Agriculture and Biological Science*; 5: 7-16.

Ekeocha, A.H. (2011): Response of Broiler starters fed rations Supplemented with *Vernonia amygladina* leaf meal (VALM) (Bitter leaf). *Metabolism and Nutrition Posters. Poultry Science* 90 (E-suppl 1)

Emeka, E.J.; Iweala, O. and Obidoa, O. (2009) Effect of long term consumption of a diet supplemented with leaves of *Gongronemalatifolium* benthon some biochemical and

- histological parameters in male Albino rats. *Journal of Biological Science.*, 9(8): 859-865.
- Erasto, P., Grierson, D.S. and Afolayan, A.J. (2006). Bioactive Sesquiterpene lactones from the leaves of *Vernonia amygladina*. *Journal of Ethnopharmacology* 106: 117 – 120.
- Eseyin, O.A., Igboasoiki, A.C., Oforah, E., Mbagwutt, Umoh, E., Ekpe, J.F. 2005. Studies of the effects of alcohol extract of *Telfaria occidentalis* on alloxan-induced diabetic rats. *Global Journal of Pure and Applied Science*; 11:85-87.
- Fagbemi, T. N. (2007). Effects of processing on the nutritional composition of fleted pumpkin (*Telfaira occidentalis*) seed flour. *Nigerian Food Journal*, 2(25):1-22.
- Fasuyi A O and Nonyerem A D (2006). “Nutritional potentials of some tropical vegetable leaf meals: chemical characteristics and functional properties”, *African. Journal of Biotechnology.*, Vol. 5, pp. 49-53.
- Gbolade A.A. (2009): Analysis of antidiabetic plants in selected districts of Lagos State, Nigeria. *Records of Natural Products.* 3(4):187 – 192.
- Gelineau, A., Corraze, G. and Boujar, T.(1998). Effects of restricted ration, time-restricted access and reward level on voluntary food intake, growth and growth heterogeneity of rainbow trout (*Oncorhynchus mykiss*) fed on demand with self-feeders. *Aquaculture*, 167, 247–258.
- Huffman, M.A., Koshimizu K. and Ohigashi H. (1996): Ethnobotany and Zoopharmacognosy of *Vernonia amygladina*, a medicinal plant used by humans and Chimpanzees in the wild. In: *Compositae: Biology and Utilization*, Hind D.J.N., P. Caligari and S.A.L.Smith (Eds). Royal Botanic Gardens, Kew, p351 – 360.
- Jimoh W.A., Aderolu A.Z., Ayeloja A.A. Shodamola M.O., (2013). Replacement Value of Soybean meal with Luffa Cylindrica in diet of *Clarias gariepinus* fingerlings. *International Journal of Applied Agriculture. Apic Res.*, 9 (1-2):98-105.
- Jobling, M. (1983). A short review and critique of methodology used in fish growth and nutrition studies. *Journal of Fish Biology*, 23, 686–703.
- Oboh, G., & Rocha, J. B. T. (2007). Distribution and antioxidant activity of polyphenols in ripe and unripe tree pepper (*Capsicum pubescens*). *Journal of Food Biochemistry*, 31(4), 456–473.
- Okwuzu J.O, Odeiga P, Otubanjo OA, Ezechi O.C. (2017). Cytotoxicity testing of aqueous extract of bitter leaf (*Vernonia amygladina Del.*) and sniper 1000EC (2,3 dichlorovinyl dimethyl phosphate) using the *Aliumcepa* test. *Afri Health Sci.* 2017;17(1): 147-153. <https://dx.doi.org/10.4314/ahs.v17i1.19>
- Olurin, K. B., Olojo, E. A. A. & Olukoya, O. A. (2006). Growth of African catfish *Clarias gariepinus* Fingerlings fed different levels of Cassava. *World Journal of Zoology*, 4(1): 54-56.

Comment [p53]: Not correctly cited in the text..check

Onyia, L. U., Ladu, B. M. B. and Olufeagba, S. (2010). Evaluation of hatchability, survival and growth of *Clarias anguillaris*. *World Journal of Biotechnology.*, 11:1662-1667.

Comment [p54]: ditto

Opata, M. and Izevbigie, E. B. (2006): Aqueous *Vernonia amygladina* Extracts Alter MCF-7 Cell Membrane Permeability and Efflux. *International Journal of Environmental Research and Public Health.* 3(2): 174-179.

Tadesse, A., Gebre-Hiwot, A., Asres, K., Djote, M. and Frommel, D. (1993): The *in vitro* activity of *Vernonia amygladina* on *Leishmaniaaethiopica*. *Ethiopian Medicinal Journal* 31: 183 – 189.

Yin G., Jeney G., Xu P., Jun X., Jeney Z. (2006). Effect of two Chinese herbs (*Asragalus radix* and *Scutellaria radix*) on non specific immune response of tilapia (*Oreochromis niloticus*). *Aquaculture.* 253:39-47.

Comment [p55]: Check for consistency throughout especially punctuation of authors and initials.

UNDER PEER REVIEW