



Application of Phylogenics as First Feed of Larval African Catfish *Clarias gariepinus*

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Authors' contributions

This work was carried out in collaboration between both authors. Author UDE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ASNN and UDE managed the analyses of the study. Author UDE managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Lack of fingerlings is still hampering aquaculture development in Sub-Saharan Africa. African catfish *Clarias gariepinus* do not have developed stomach at onset of exogenous feeding. Absence of fully developed digestive system at onset of larval exogenous feeding makes catfish unable to utilize dry diets. Introduction of phytogenic feeds that possess naturally occurring hormone could be helpful in enhancing catfish ability to utilize dry feeds. Consequently we made six novel diets comprising entirely of phytogenic ingredients, labeled feed 1 (F1) to feed 6 (F6). The percentage compositions of the experimental feeds were; F1, 100% lettuce seed meal; F2, 100% pawpaw leave meal; F3 100% neem seed meal; F4, (50:50) Lettuce seed meal: pawpaw leaves meal; F5, (50:50) pawpaw leaves meal:neem seed meal and F6, (50:50) Lettuce seed meal: neem seed meal. The control diet F7, was decapsulated artemia. There were three replicates aquariums per treatment diet and first feeding larvae were fed with the diets for fifteen days to examine dietary effects through larval and post larval periods. Larval African catfish were produced through artificial

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fertilization and stocked at 100 larvae 10 liters⁻¹ aquarium. The larvae fed with F1 had the highest SGR of 10.12% day⁻¹ followed by SGR of larvae fed with F5 8.27% day⁻¹ and F2, 8.02% day⁻¹. Larvae fed with F3 and F4 had similar SGR but higher than those fed with F1. The larvae fed with feed 7 and F6 had similar weight gain that was significantly better than others (P<0.05). The larvae fed with Feed F5 and F2 had similar weight gains but were better than those fed with F1, F3 and F4. Survival of larvae seems to be enhanced by inclusion of lettuce in diets of larvae Africa catfish. The survival the larvae fed with F1 was approximately 27% while survival of larvae fed with F6 was next with survival rate of 21%. The larvae fed with F3 had survival rate of 18%, which was different from F1 fed larvae. The least survival rate was noted for larvae fed with F4. Results indicate that phytogetic feeds exert significant effects on the survival, growth and weight gain of first feeding African catfish larvae. Catfish seem to benefit from natural phytogetic components of the plant diets. Feeds like lettuce seed and its combinations produced similar larval growth rate as using artemia alone.

Keywords: *Phytogenics; larval catfish; larviculture; lettuce and neem seed.*

1. INTRODUCTION

African catfish *Clarias gariepinus* is one of the most important fish in Africa. It belongs to phylum Chordata, class Actinopterygii, order Siluriformes, family Clariidae, Genus *Clarias* and Species *C. gariepinus*. Catfish is omnivorous and feed on animal and plant feed sources. Plants contain active substances which can have composite effects on feed utilization, sex determination, growth and general physiology of the fish. Plant extracts that are included in feed to enhance animal performance are called phytoegenics [1,2]. Phytoegenics may occur as having effects similar to estrogens although it may not be structurally gonadal estradiol-17 β , these groups are called phytoestrogens [3]. Phytoegenics may also occur as having similar effects as testosterone example sex reversal [4] like (diosgenin and sitosterol) in fish and other animals and these collections are together known as phytoandrogens [5]. Phytoegenic components in plants can vary in quantities and location within the roots, leaves, tubers, fruits and seeds, they can be obtained by grinding, drying or by extraction as essential oil products [1]. Processing of these plant parts would create opportunities of obtaining these hormones. Plant hormones like androgens have been identified as growth promoting in channel catfish (*Ictalurus punctatus*) [6] and bagrid catfish (*Pseudobagrus fulvidraco*; [7]. Phytoegenics improve wellbeing of fish by enablement and modulation of actions like growth [8], feed consumption, stress, immune stimulation, and anti microbial activities [9-11]. Phytoegenics are organic chemicals that can be classified based on their chemical structures into alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids (hormones) and essential oils. Essential oil are extracts from plants and odoriferous. The Essential oils like carvacrol,

limonene, thymol and anethol have been shown to improve growth and disease resistance in channel catfish [11]. The antimicrobial and inhibitory effect of essential oil has been noticed against *Vibrio harveyi*, *Aeromonas hydrophila* and *Aeromonas salmonicida* [12] and improves health status [13]. Phenols containing essential oil like carvacrol have higher toxicity on microbes than others [14,11]. Phytoegenic ingredients had been noted to increase feed intake due to positive gustatory effects like improving palatability of diet, palatability is enhanced via the essential oil and flavor [14].

The palatability of feed and development of gut flora is enhanced by some phytoegenics in animals [15], however in fish like sea bream the action of phytoegenics can be due to increase in enzyme action example pepsin and alkaline phosphatase [16]. Improve growth have been recorded in African catfish, by addition of phytoegenics ingredient in their diets [17]. Larval fish can be divided into three groups according to development of their alimentary tract and digestive enzyme secretion [18]. The larvae with functional stomach at first exogenous feeding are called precocial example Channel catfish, those without functional stomach at start of exogenous feeding are called altricial larvae example African catfish *C. gariepinus* [19,20]. The third groups of fish larvae are the Stomach less larvae, these do not have functional stomach all their life example carp and trout [21]. Larvae without functional stomach have low capacity for absorptive and proteolytic abilities during their first feeding [22]. Larval African catfish develops functional stomach after 5 days subject to temperature, enzyme secretion starts after 4 days [23]. Since larval African catfish does not utilize dry feed very well it may be possible that inclusion of phytoegenic component could provide modulating

factors and gut enhancers to improve digestion and growth. It is against these backdrops of benefits in utilizing phytochemicals that we designed this research. This research is aimed at elucidating the nutritional and growth beneficial effects of some crude phytochemicals made from Lettuce seeds (*Lactuca sativa*), Pawpaw seeds (*Carica papaya*), Neem seeds (*Azadirachta indica*) and their different combinations on first feeding African catfish *C. gariepinus*.

2. MATERIALS AND METHODS

2.1 Experimental Fish

This experiment was done between the months of April and May 2015. This period marks the end of dry season and onset of wet and rainy season in Nigeria. The research period was not natural reproductive season for African catfish. Breeding at this season is marked by poor ovulation and high mortality of larvae. The larvae of African catfish were obtained by artificial dry fertilization of catfish brooders maintained in the ponds of Godfrey Okoye University Enugu. The 2.3 kg female catfish was injected on the dorsal muscle with one pellet of ovopel per kg. Ovopel contains a mammalian GnRH analogue, D-Ala6, Pro9NEtmGnRH-a (18–20 µg/pellet) and water-soluble dopamine receptor antagonist – metoclopramide (8–10 mg per pellet) [24]. The ovopel used in this experiment was donated by a research associate in Hungary. The female catfish was stripped after 8 hrs of ovulation and eggs were dry fertilized with fresh semen obtained from 2.2 kg male. The weights of the egg were taken and the eggs were spread in a single layer upon a mosquito nylon mesh suspended in floating plastic frame. The plastic frame was within 2l plastic aquarium. The fertilized eggs were incubated at 28.4±0.05°C, in a six replicate 2l rectangular plastic aquarium connected to a flow through system. The incubators were subjected to 12L: 12D period and the light intensity was 6 lux. Light intensity was maintained by using black nylon screening of entire hatchery area. Photoperiod was maintained at 12D:12L at 60 lux (HD 9221 lux meter Delta OHM, Padua, Italy). The water flow rate was 8.0 ml per minute. The eggs hatched after 24 hr.

2.2 Estimation of Fertilization and Hatchability

The estimation of percentage (%) fertilization was done by counting the number of eggs that

turns white three hours post fertilization. This was [25] removed from the incubating system and divided by total number of eggs incubated. The resulting sum was multiplied with 100.

Fertilization rate= (No of eggs turning white/ Total number of incubated eggs)*100

Hatching rate= (Total number of eggs incubated – Number of unhatched eggs)/ Total number of eggs incubated*100.

Hatchlings were reared through yolk sac stage before the start of the feeding experiment. The stocking density of the larvae was 100 larvae per each of the three replicate aquariums per treatment diet. The initial weights of the larvae were taken using an electronic balance sensitive to 0.0001 g.

2.3 Experimental Feed and Feeding of Larvae

Six experimental diets were produced for this research. The feeds were labeled Feed 1 (F1) to feed 6 (F6). The control diet was Feed 7 (F7) which was decapsulated artemia. Feed production started with procurement of raw materials. For the production of feed 1 (F1), raw lettuce seed were obtained from a supplier that sells lettuce seed imported from France at New Market Enugu Nigeria. The seeds were dried to a constant weight under hot tropical sun temperature range of 28±0.4 - 31±0.6°C. Sun drying was done by spreading lettuce seed in a single layer on an aluminum tray and exposing them to direct sun rays till a constant weight was achieved. Similarly for F2 the seed of ripe pawpaw were obtained and their seeds were removed and dried whole under the sun. Sun drying was as stated for lettuce seed. Care was taken not to mix any other part of the fruit with the seeds during removal from fruit. For F3 ripe neem seed were obtained from the tree and exercised. The seed were sun dried as stated above for lettuce and the rest of the seed. The seed meals were produced by grinding the dried seeds to dust using a Phillips kitchen grinder. The grinded seed meals were stored in a zip sachet bag under refrigeration till used. Feed 4 (F4) was made up of 50% lettuce seed meal: 50% pawpaw meal. Feed 5 (F5) comprises of 50% pawpaw and 50% neemseed meal, feed 6 (F6) has 50% lettuce and 50% neemseed. Treatment diet compositions are tabulated in Table 1. The composition of feeds 1, feed 2 and feed 3 was 100% of lettuce seed meal, pawpaw

leave meal and neem seed meal respectively. The feeds were produced with only seed and pawpaw leave meal ingredients to avoid any interactions of the phytochemicals with other feed components. This was also in order to obtain sole effects of the crude phytochemical feed on the larvae without mixing it with other larval diet as a preliminary research. The feed ingredients inclusion rate was in line with previous works [5,25]. The compositions of the experimental feed are tabulated in Table 1. The control diet was decapsulated artemia.

The fish were fed four times daily with the experimental diets. Feeding was in the morning 08 hrs, 11 hrs, 15 hrs and 18 hrs daily. The tanks were cleaned on daily basis before feeds were administered. The larvae were fed till they were no more feeding to avoid over feeding.

2.4 Water Quality Maintenance and Analysis

The experimental aquariums were connected to a flow through system. Water supply was from

university overhead plastic tanks. The tank was properly washed with water and re-filled before experiment started. The aquarium water was also totally changed every morning before feeding. This was in order to remove all waste products and excess feed. The values for water parameter analyses are recorded in Table 2. The water pH was measured with a pH meter (Combo pH & EC meter, Hanna Instruments, Arizona, USA), Water turbidity was measured with a turbidity meter and reported in Nephelometric turbidity unit (NTU). Dissolved oxygen was measured using oxygen meter at the water temperature recorded, (YSI oxygen meter model 550A, YSI Inc., Yellow Springs, USA). Temperature was measured with mercury in glass thermometer and reported in Celsius scale.

2.5 Growth Parameters

The growth parameters measured were specific growth rate weight gain and survival of the larvae. The catfish larval Specific growth rate (SGR, % day⁻¹) was calculated as $100 (\ln W2 - \ln W1) \times t^{-1}$, where W1 and W2 were average

Table 1. Composition of experimental diets and proximate analyses of feeds used in the feeding of larval African catfish for larval period of 15 days

| Ingredients | Feed 1 | Feed 2 | Feed 3 | Feed 4 | Feed 5 | Feed 6 | Feed 7 |
|--------------------|--------|--------|--------|--------|--------|--------|--------|
| Lettuce seed | 100 | 0 | 0 | 50 | 0 | 50 | 0 |
| Pawpaw leave | 0 | 100 | 0 | 50 | 50 | 0 | 0 |
| Neem seed | 0 | 0 | 100 | 0 | 50 | 50 | 0 |
| Artemia | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| Proximate analysis | 452 | 546 | 240 | 514 | 512 | 394 | 53 |
| Protein | | | | | | | |
| Carbohydrate | 464.2 | 567.2 | 456.9 | 315.3 | 294.5 | 423.6 | - |
| Lipids | 210 | 83 | 162.5 | 179.3 | 128 | 216 | 11 |
| Dry matter | 95.7 | 90.14 | 91.94 | 94.87 | 92.6 | 97.3 | - |
| Fiber | 198.6 | 99.6 | 235 | 296.7 | 379.3 | 201.4 | 1 |
| Moisture | 43 | 98.6 | 80.6 | 51.3 | 74 | 92.6 | - |
| Phytic acid | 13.68 | 10.66 | 11.08 | 11.2 | 10.55 | 11.27 | - |

Compositions are in g kg⁻¹ dry matter, the phytic acid is in mg 100⁻¹g. Feed 7 the control diet was Coppens International bv, Helmond Netherland decapsulated artemia, a commercial catfish feed, other components of artemia are vit.C.550 mg/kg, vit E 160 mg/kg

Table 2. Water parameters of Aquariums water used for the analyzing effects phytochemical feed labeled F1 to F6 on the first feeding larvae of African catfish *Clarias gariepinus*

| Feed | Ingredient | Temp | pH | Turbidity | DO ₂ |
|------|------------------|---------|-------------------------|------------------------|-----------------------|
| F1 | Lettuce | 25±0.10 | 7.09±0.04 ^a | 2.04±0.0 ^b | 7.0±0.01 ^a |
| F2 | Pawpaw | 25±0.12 | 6.66±0.02 ^b | 4.36±0.06 ^c | 3.4±0.05 ^c |
| F3 | Neem | 25±0.04 | 6.82±0.02 ^b | 0.19±0.04 ^a | 3.8±0.04 ^c |
| F4 | Pawpaw + Lettuce | 25±0.04 | 7.16±0.09 ^a | 8.06±0.03 ^d | 5.0±0.05 ^b |
| F5 | Pawpaw + Neem | 25±0.05 | 6.93±0.02 ^{ab} | 3.28±0.10 ^c | 4.0±0.04 ^c |
| F6 | Lettuce + Neem | 25±0.03 | 6.96±0.05 ^{ab} | 2.97±0.04 ^b | 5.8±0.02 ^b |

Means not followed by same superscript are significantly different (P<0.05)

weights in g at the start and the end of the experiment and t was the length of the experiment in days [8,17]. Average weight gain was calculated from final weight – initial weight of catfish larvae / number of catfish larvae [5]. The survival of the catfish was calculated as $100 \times$ Number surviving/original number of larvae.

2.6 Experimental Procedures

The stocking density of the larval African catfish was 100 larvae aquarium⁻¹. There were three replicate aquariums per treatment feed. The treatment feed were ground to powder form and stored zip sachet bag and refrigerated to maintain quality of feed. The treatment feeds were not mixed together with other compounded feed before being given to the fish. The larvae were fed *ad libitum* and this was also adopted for feeding with artemia. In feeding the larvae about 5g of dry feed is measured from where larvae are fed and the final weight taken thereafter. However, due to size of larvae and dusty nature of feed we could not easily ascertain the quantity of feed eaten and what remained in aquarium. The rate of administration phytogetic feed to the fish was in line with similar experiments in [5] and [25]. The authors mixed 25, 50 and 75% of the phytogetic feed with compounded or commercial diets before administration to the fish. This was done for tilapia *Oreochromis mossambicus* [25] and for African catfish *C. gariepinus* [5,26]. We did not mix phytogetic feed with commercial diets to prevent nutrient interactions that could diminish effects phytogetic hormones. The larvae were weighed per replicate treatment aquarium to obtain their initial weight before the start of feeding experiment. Larval feeding was done to satiation with treatment diets. The food conversion ratio of the larvae could not be measured because it was hard to determine the feed intake given the size of the fish. The aquariums were cleaned daily with siphon to avoid stress on the fish. The larval weight was taken again after two weeks using electronic balance. Collections of water samples for physico-chemical analyses were done before cleaning the aquariums. The incubation water was discarded after hatching and hatchlings were transferred to rearing 20l rectangular aquariums where they feeding experiment was carried out.

2.7 Statistical Analyses

Randomized complete design was used in this work. The growth differences of the larvae based on feed treatment was analyzed using one-way analysis of variance. Fishers least significant

difference ($P < 0.05$), was used in separation of treatment means. The statistical package used for analyses was v18. PASW. The three replicate tanks per treatment feed were used as observational units.

3. RESULTS

3.1 Culture Water Analyses

The quality of incubating aquarium water were as follows oxygen 5.8 ± 0.02 mg l⁻¹, pH 6.82 ± 0.02 , temperature 26.5°C and turbidity 0.19 ± 0.04 NTU. Water parameters of rearing water were different from the hatching water which was discarded after hatching. Rearing aquarium water parameters differed according to feed types.

The aquariums receiving treatment feed 1 (F1), had the lowest turbidity 2.04 ± 0.0 NTU but highest dissolved oxygen content 7.0 ± 0.01 mg ($P < 0.05$) Table 2. The larval feeds comprising of pawpaw leaves meal (F2 and F4) had the lowest dissolved oxygen F2, 3.4 ± 0.05 mg and F4 5.0 ± 0.05 mg and F5 4.0 ± 0.04 mg and were more acidic than other treatments. Conversely feeds containing lettuce meal F1, F4 and F6, seem to have significantly ($P < 0.05$) more dissolved oxygen and normal pH than the pawpaw 5.0 ± 0.05 mg l⁻¹ and neem 5.8 ± 0.02 mg l⁻¹ (Table 2).

3.2 Fertilization and Hatching Rate

The weight of eggs was 56.4 g. Fertilization rate of the catfish egg was $95 \pm 0.08\%$. The hatching of catfish egg commenced after 20 h of incubation. The hatching rate was $91 \pm 0.06\%$. We noticed that the eggs that could not hatch were double layered. Hatched larvae were healthy moved from hatching net into the bottom of incubation aquarium.

3.3 Growth and Nutritional Analyses

African catfish *Clarias gariepinus* larvae readily accepted experimental diets. The larvae grew with best specific growth rate (SGR) of $11.20 \pm 0.07\%$ day⁻¹ for the larvae fed with F7 control diet artemia. The larval catfish fed with F6 was next in SGR to those fed with F7 with SGR of $10.12 \pm 0.05\%$ day⁻¹ and this was significantly lower than the SGR of catfish fed with F7 ($P < 0.05$). The SGR of catfish fed with F6 was better than SGR of other treatment feed in the experiment ($P < 0.05$). The larvae fed with F5 grew with SGR of $8.27 \pm 0.06\%$ day⁻¹. There were

no significant differences ($P>0.05$) between the SGR of larvae fed with F5 and F2 $8.02\pm 0.03\%$ day⁻¹. Similarly there was no significant differences ($P>0.05$) between the SGR of the larval catfish fed with feeds F4 ($5.74\pm 0.03\%$ day⁻¹) and F3, $5.65\pm 0.05\%$ day⁻¹. The catfish larvae fed with F1 had the lowest specific growth rate of $5.34\pm 0.04\%$ day⁻¹. The SGR of the larval catfish are recorded in Table 3.

The average weight gain (AWG) of the larval catfish was highest for those fed with control diet F7 (artemia) and F6. There was no significant difference ($P>0.05$) between AWG of the larvae fed with either F7 or F6. Similarly feeding the catfish larvae with F5 and F2, did not produce any significant ($P>0.05$) AWG difference. The AWG of larvae fed with F7 and F6 were however higher than those of F5 and F2 ($P<0.05$). The lowest average weight gains were for those catfish fed with F1, 0.0223 ± 0.01 g, F3, 0.0233 ± 0.02 g and F4, 0.0237 ± 0.05 g with no significant differences ($P>0.05$) between them.

The survival of the larvae was very low irrespective of treatment feed. Although catfish larval survival was low, those fed with F1 survived significantly more ($26.99\pm 0.04\%$) than others ($P<0.05$). The larvae fed with F6 had survival of with $21.89\pm 0.05\%$ which was significantly lower than those fed F1 ($P<0.05$). The survival of larvae reduced further to 18.9% for those being fed with F3. Survival of F3 fed larvae was higher than those fed with pawpaw F2 ($P<0.05$). The combination of 50% pawpaw: 50% lettuce (F4) gave a survival of $16.19\pm 0.03\%$ but it was significantly lower than that of F3 fed larvae ($P<0.05$). The catfish larvae fed with F7 control diet (artemia) had low survival of 9% while those fed F5 and F2 had lowest survival of all treatment feeds. The results on the survival of the catfish larvae are recorded in Table 3.

4. DISCUSSION

The performances of the fish on the diets seem to be due to more than just nutritional factors. Water parameter result of the culture system seems to have extended effects on the survival of the larvae cultured in them. The very low turbidity of lettuce based diets could be a considerable factor to the dissolved oxygen availability in the culture system. Low DO₂ negatively affects the growth rate of the fish causing high biological oxygen demand. Consequently F1 had higher survival over the rearing periods than other treatments. Similarly survivals reduced with reduction in DO₂ in the treatments. Although African catfish have accessory airbreathing organs they are fully developed during the whole larval stage [27].

The results of these experiments demonstrate that phytochemicals have effects on the African catfish. The direct use of phytochemical feed meals instead of mixing it with conventional feed before feeding the catfish larvae, precluded uncertainties on their nutrient interaction, nutrient utilization and digestion. Combination of phytochemical feeds with commercial diets could result in improper mixing, digestive degradation and differential feed uptake by fish associated with mixing with other feeds [28]. Lettuce seed meal is known to be high in phytic acid, this could have reduced growth rate but not survival of catfish in this study. However, the comparable growth rate of larvae fed with F6 was impressive and suggests good utilization of the plant ingredient. The utilization could have been enhanced by the hormones present in the feed that could have contributed to ingredient assimilation and feed conversion ratio. It had been noticed that phytochemical hormones like androgens were effective in promoting growth of

Table 3. Growth performances and survival of African catfish fed phytochemical feeds F1- F6 for larval period of 15 days

| Feed | Content | AWG | SGR% | %Survival | % Phytic acid |
|------|------------------|--------------------|-------------------|-------------------|-------------------------|
| F 1 | Lettuce | 0.0223 ± 0.01^c | 5.34 ± 0.04^e | 26.99 ± 0.04^a | $13.68\pm 0.06/100$ mg |
| F 2 | Pawpaw | 0.0333 ± 0.04^b | 8.02 ± 0.03^c | 2.67 ± 0.01^e | 10.66 ± 0.04 mg/100g |
| F 3 | Neem | 0.0233 ± 0.02^c | 5.65 ± 0.05^d | 18.89 ± 0.05^c | 11.08 ± 0.03 mg/100g |
| F 4 | Pawpaw : Lettuce | 0.0237 ± 0.05^c | 5.74 ± 0.03^d | 16.19 ± 0.03^d | 11.20 ± 0.02 mg/100g |
| F 5 | Pawpaw : Neem | 0.0347 ± 0.06^b | 8.27 ± 0.06^c | 3.83 ± 0.06^e | 10.55 ± 0.06 mg/100g |
| F 6 | Lettuce : Neem | 0.0457 ± 0.05^a | 10.12 ± 0.05^b | 21.89 ± 0.05^b | 11.27 ± 0.04 mg/100g |
| F 7 | Deacp. Artemia | 0.0537 ± 0.01^a | 11.20 ± 0.07^a | 9.00 ± 0.02^d | N/A |

Values not followed by same superscript are statistically different ($P<0.05$). AWG stands for average weight gain while SGR stands for specific growth rate. N/A means not applicable

bagrid catfish *Pseudobagrus fulvidraco*; [26], Channel catfish *Ictalurus punctatus* [6], and common carp *Cyprinus carpio* [28]. Phytoestrogens has also been found to improve the growth rate of African catfish *C. gariepinus* [17].

The growth rate of catfish larvae fed with F6 and F1 shows the beneficial effects of combining lettuce with neem seed meal. The SGR of catfish larvae that was fed with neem seed diet (F3) was $5.65 \pm 0.05\% \text{ day}^{-1}$ while that of lettuce diet F1 was $5.34 \pm 0.04\% \text{ day}^{-1}$ SGR. This represent about 50% lower SGR than their combination in F6 $10.12 \pm 0.05\% \text{ day}^{-1}$. Similarly there is high SGR of the catfish fed with pawpaw leaves F2. The growth of larvae fed with pawpaw leave meal could have been due to the protein content of the diet. The protein content of F2 was 546g kg which was highest among all other treatment feed. It could be that the improved growth rate of catfish larvae fed with F5 and F2 was due to enhanced higher protein content of feed. The combination of pawpaw leaves and neem seed meal improved the growth rate of the catfish larvae up to SGR $8.27 \pm 0.06\% \text{ day}^{-1}$ but not when not when mixed with lettuce seed meal F4, SGR $5.74 \pm 0.03\% \text{ day}^{-1}$, the reason is unclear but could be due to the nutrient interactions and the phytic acids composition of the lettuce. Despite the higher SGR of pawpaw fed catfish, survival was lowest in all pawpaw diets. The reason is not very clear, but could be due to higher acidity (pH) and lower water DO_2 of all pawpaw based diets. The results present evidence that inclusion of lettuce meal to all other phytogetic feed improved the larval survival. Feed 2 fed larvae had lowest survival but of $2.67 \pm 0.01\%$ while inclusion of lettuce with pawpaw in F4 increased survival to $16.19 \pm 0.03\%$. Similarly, neem seed meal diet F3 had survival of $18.89 \pm 0.05\%$ while inclusion of 50% lettuce meal to 50% neem diet F6 increased survival to $21.89 \pm 0.05\%$. The reason for the survival enhancement of lettuce meal is not very clear. The phytochemical composition of lettuce seed meal seems to be at least partly responsible.

The effects of phytoGENICS incorporated into animal feeds can be affected by its mode of preparation. Compounds like volatile essential oils could be volatile reducing their effects [29]. PhytoGENICS can also be absorbed into other feed components during compounding reducing the role [30]. The effects of the feed may be associated organoleptic properties and lipid content of the feed. Plant ingredients can

improve feed intake in catfish due to their organoleptic qualities [31]. PhytoGENICS have been noted to enhance higher feed efficiency in rainbow trout fed diets with carvacrol and thymol [32]. There had also been improved performances of African catfish *C. gariepinus* fed 75 mg kg^{-1} diet red clover [5]. There could be growth promoting agents in the ingredients but their combination also amplified the effect. In previous research growth-promoting effects of *Tribulus terrestris* extract a phytoandrogen had been demonstrated on Cichlid (*Cichlasoma nigrofasciatum*) [33] and guppy (*Poecilia reticulata*) [34]. The high survival of catfish larvae fed with F1 could be due to the nutritional value of the feed. The protein content of F1, was high and the energy content was highest than all other feeds 210 g kg^{-1} . Lettuce has high amount of carotene, Vitamin C and Vitamin E showing remarkable noticeable antioxidant properties [35]. Lettuce seed meal contains triterpenoids, saponins and simple phenols, these are phytoGENICS that possesses antinociceptive and anti-inflammatory effects [36] Lettuce also contain japonica A, isoquercitrin and caffeic acid [36]. In a previous research it was noted that addition of lettuce to diets of rats increased their weight [37]. The phytochemical nature of lettuce could have been reason for reduction in acidity of pawpaw when mixed as ingredient with lettuce in F4. Pawpaw meal had been noted to be high in acidity 47.12 and peroxide value 48.60 [38]. In this experiment it seems the anti oxidizing agents inherent in lettuce meal neutralized the oxidizing agents of pawpaw meal thereby enhancing survivability of the catfish larvae.

The high larval specific growth rate when fed with artemia could have been due the animal protein and lipid contents of artemia and the ability of fish to utilize them more than plant feed. Conversely, the poor survival of the larvae fed with F7 control could be due to the extended larviculture period in this experiment. The larvae survived very well at the initial first week but mortality set in as experimental days increased. Usually artemia as a live diet is used for the first 4-5 days after which dry diets can be introduced. Artemia is not normally used after initially weaning period, but had to be for purpose of this experiment. The use of artemia contributes to total proteolytic enzyme activity of the larvae [21]. These natural enzymes coupled with high vit. Cand E and the fatty acids in artemia could have been responsible for initial high SGR of catfish larvae fed with artemia. However prolonged usage of artemia le bottom d to poor nutrition and

lower survival since catfish larvae needed more nutrients and energy than could be supplied by artemia alone (F7).

5. CONCLUSION

These results suggest that use of phytogenic feeds have impact on the growth and development of African catfish larvae. This preliminary experiment implies that inclusion of the phytogenics in rearing of the larvae could be beneficial and cost saving. Most phytogenics are used for sex reversals neglecting the growth and nutritional potentials. A combination or inclusion of complimentary phytogenics feeds in larviculture of first feeding African catfish can help in their utilization of dry diets and fast growth. Use of phytogenics can as well be helpful in combating outbreaks of disease that usually causes mass mortality in hatcheries. More researches should be done on the nutritional values and purification of these crude phytogenic feeds.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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