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Utilization and growth performance of *Clarias gariepinus* fingerlings fed soybean meal-based diets supplemented with pineapple flavour

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Abstract
An experiment was conducted to investigate the nutrient utilisation and growth performance of fingerlings of *Clarias gariepinus* fingerlings fed soybean meal-based diets supplemented with pineapple flavour as feed additive. Four treatments with three replicates each at 25 fishes per tank (mean weight of 22.32 g) were fed four diets (D1-D4) including the control and supplemented with powdered pineapple flavour at 0%, 1.5%, 2.0%, and 2.5% respectively in rectangular glass tanks filled with 30 liters of water each. Feeding was done at 5% body weight on daily basis throughout the feeding trial. Weights of the fish were recorded every week following food adjustment. The water quality parameters were within the range recommended for tropical fish culture. After 56 days, no significant differences ($p<0.05$) were observed in the proximate composition of the fish fed the different diets. However, protein increased (56.66±0.6% in D2 to 57.13±0.6% in D4) and fat content (4.75±1.1% in D3 to 5.05±1.2% in D4 while ash, crude fiber and nitrogen-free extract (NFE) reduced. The mean weight gain, food intake and the specific growth rate were significantly highest ($p<0.05$) in D3 (2%). Fish fed D3 (2%) performed favourably in terms of nutrient utilisation and growth performance compared to other formulated diets with the best record of the food conversion ratio. It was recommended that powdered pineapple flavour should be included at 2.0% in the diets of *Clarias gariepinus* fingerlings.

Keywords: *Clarias gariepinus*, feed utilization, growth performance, soybean meal pineapple flavour

1. Introduction

The promise of aquaculture has long been recognised till the recent time, despite the virtual demise of the ancient fish pond system. The ancient control over water use and aquaculture represented power and provided stable food supply for the ruling class. For present political leaders, aquaculture represent an opportunity for economic expansion and diversification, yet in-spite of the past history of success and current encouragement, aquaculture has not achieved the level of success that was envisaged [1]. Aquaculture development program points out an increasing demand for aquaculture products on a worldwide basis and could become a major industry for meeting the world future protein demand of the populace [2].

Fish nutrition is recently dominated by the need to develop low fishmeal and fish oil feeds for commercial production. Demand for seafood is expected to increase by 2030 [3] and this demand can only be accomplished by increase in aquaculture production. Alternative source of protein and oil have long been used in fish feeds but the challenge remain to increase their levels in feed while maintaining fish growth, producing healthy products for human consumption and reducing the effects of aquaculture production on the aquatic environment [4, 5].

According to Miles and Chapman [6], and Hasan and New [7], feed management determines the viability of aquaculture, as food and feeding account for 40-60% of the total fish farm production. The cost of feeding in aquaculture result to loss of interest or operation at reduced capacities, which made some farmers to shift to crop production. For several years, there has been continuing interest in identifying and developing ingredients as alternatives to fish meal for use within aquaculture industry [8, 9]. Among the ingredient being investigated as alternative to fishmeal are the products derived from Soybeans (*Glycine max* L.), largely because of the security of supply, affordable price and protein/amino acid composition [4] and [10]. Soybeans protein has one of the best amino acid profiles of all protein rich plant feed stuffs for meeting the essential amino acid requirement of fish [11].

Jindal *et al.* [10] reported that fish meal provides the major portion of protein in dry commercial aquaculture feeds because fish protein is used efficiently in feed formulation. Plant materials can contain bitter-tasting compounds (anti-nutritional factors) which may reduce their palatability.

The use of feed additives in the aquaculture industry has received considerable attention in the recent time. The rationale behind their use is to preserve the nutritional characteristics of the diet or feed ingredients prior to use, enhancing ingredient dispersion, facilitate feed ingestion thereby promoting growth of the cultured fish [12]. However, feed attractants may lose their stimulatory effect over prolonged study period; therefore, studies of feed attractants are performed over short intervals [13]. Feed additives are added to fish feeds to improve their quality and performance [14].

Flavour can be simply defined as chemical compound that gives pleasant odour and taste. In this study, a commercial pineapple flavour was added to the feed as feed additive. This is a common strategy in other areas of animal production, where industrial flavours are used to promote intake in the earlier life stages and also to hide the unpleasant taste of vitamins, supplements or therapeuticals. Pineapple flavour is made from pineapple fruits pulp which exhibit high moisture, high sugar, soluble solid content ascorbic acid and low crude fibre. Pineapple (*Ananas comosus*) is a wonderful tropical fruit having exceptional juiciness, vibrant tropical flavour [15]. This fruit are used as supplementary nutritional fruit for good health. It is an excellent source of vitamins and minerals. Also, according to Pardo *et al.* [16], Pineapple contain 1.58% protein, 3.0% ash, 3.19% crude fat, 24.14% crude fibre and 68.79% nitrogen free extract, useful for healthy and body development.

In practice, an attractant is needed to induce fish and shrimps to consume proteins they would otherwise reject. Moreover, less feed wasted leads to an improvement in water quality and to a reduction in production costs. There is dearth of information on the use of flavour to enhance feed intake and growth response of the cultured species. This study therefore examined the impact of such feed components in the body composition and growth performance of *Clarias gariepinus* fingerlings.

2. Materials and Methods

2.1 Study site

The experiment was carried out in the Limnology Laboratory at the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure (FUTA), Ondo state, Nigeria. The university lies between Latitudes 7°17′ - 7°19′N and Longitudes 5°7′ - 5°9′E in the north-western part of Akure metropolis.

2.2 Experimental Diets

The feed ingredients used for the formulation of the diets were purchased from K2 Feed Mill, Akure, Ondo State, Nigeria. Pineapple flavour was purchased from OK Store at King's Market, Akure, Ondo State. Each feed ingredient was properly milled into fine powder, mixed and pelletized using Hobart A-200 with a 2.0 mm duel (Hobart Manufacturing Ltd, UK). The feeds were immediately dried at room temperature because of the volatility of the flavour and later broken mechanically into smaller sizes and packed in dry, airtight small container and labeled prior to use. The gross composition of the diets is presented in Table 1. There were four treatment including control. Each diet was formulated to give 40% crude protein isonitrogenously. Fish meal constitutes only 5% of the composition. The control (D1) was prepared without pineapple flavour (0%), while diets (D2-D4) were formulated with 1.5, 2.0 and 2.5% of pineapple flavour respectively.

2.3 Experimental Procedure

The experimental fish for the study, *Clarias gariepinus* were obtained from the Department of Fisheries and Aquaculture Technology Fish Farm in the Federal University of Technology, Akure (FUTA), Ondo State, Nigeria. The experiment was conducted using 12 rectangular glass aquaria (70cm x 45cm x 30cm), comprising four treatments with three replicates each. Prior to the commencement of the experiment, the fish were acclimatized for two weeks and fed within the hours of 07:00am - 08:00am with nutritionally balanced commercial diet of 40% crude protein. After the acclimation period, 25 fingerlings were stocked in each of the twelve glass aquaria tanks filled with 30 liters of water each. The fish were fed at 5% of their body weight twice daily in two equal rations for 56 days. Camry Digital Sensitive weighing balance Ek5055 max (5 kg/11 lbd = 1g/0.052) was used for fish weight measurement of the fish on weekly basis.

Table 1: Gross Composition of the Experimental Diets.

	Treatments (D1 – D4)			
Ingredients	**D1 (0%)**	**D2 (1.5%)**	**D3 (2.0%)**	**D4 (2.5%)**
Fish Meal (65%)	5	5	5	5
Soya Bean Meal (45%)	34.77	35.01	35.08	35.15
Ground nut cakes (50%)	34.77	35.01	35.08	35.15
Wheat offal (15%)	11.73	10.74	10.42	10.10
Yellow maize (10%)	11.73	10.74	10.42	10.10
Mineral/Vitamin Premix	1.0	1.0	1.0	1.0
Cod liver oil	0.5	0.5	0.5	0.5
Salt	0.3	0.3	0.3	0.3
Binders	0.2	0.2	0.2	0.2
Powdered Pineapple flavor	0.0	1.5	2.0	2.5
Total	100.00	100.00	100.00	100.00
Proximate composition of the diets (%)				
Moisture content	10.2	11.57	11.71	11.05
Crude Protein	40.0	40.0	39.9	40.1
Crude Fiber	6.70	6.29	6.33	6.93
Ether Extract (fat)	11.13	12.17	11.67	13.33
Ash content	10.67	10.80	11.23	9.23
Nitrogen-free Extract	27.70	24.36	23.26	22.62

2.4 Water Quality Parameters

The water used for the experiment was being changed on every other day to avoid accumulation of nitrogenous waste in the tanks. Water quality parameters such as dissolved oxygen was measured using DO_2 meter, pH using pH meter, temperature was measured using mercury in glass thermometer, Ultra meter 6P II water quality test meter (Cole Palmer, USA) for conductivity and hydrometer (Fisher Scientific, UK) for measuring total hardness. Temperature and pH were measured daily while other parameters were determined on weekly basis.

2.5 Proximate analysis, growth performance and nutrient utilization: The proximate analysis of the experimental diets, fish sample before and after the experiment was carried out using AOAC methods [17]. The following growth parameters were estimated according to the method used by Fasakin *et al.* [18] and Ogunji *et al.* [19].

2.5.1 Weight Gain (WG)
$W_2 - W_1$. Where W_1 the initial weight of fish at the beginning of the experiment and W_2 the final weight of fish at the expiration of the experiment.

2.5.2 Specific Growth Rate
This was calculated from the following relationship:
SGR = $\ln W_2 - \ln W_1$ x 100/days, Where W_1 = initial weight of fish, W_2 = final weight of fish, ln = Natural logarithm to base e, T_2-T_1= experimental period in days.

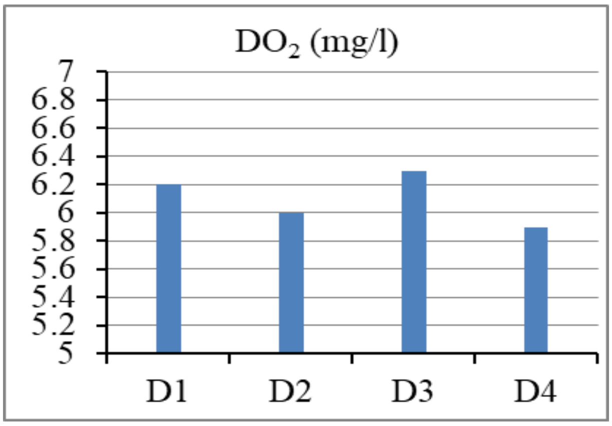
2.5.3 Total Feed Intake
This is the summation of the amount of feed per days of the whole experimental period. It was estimated from 5% of the treatment weight adjustment.

2.5.4 Food Conversion Ratio (FCR)
The FCR was calculated according to Boyd [20] from the relationship between food intake and weight gain by fish.

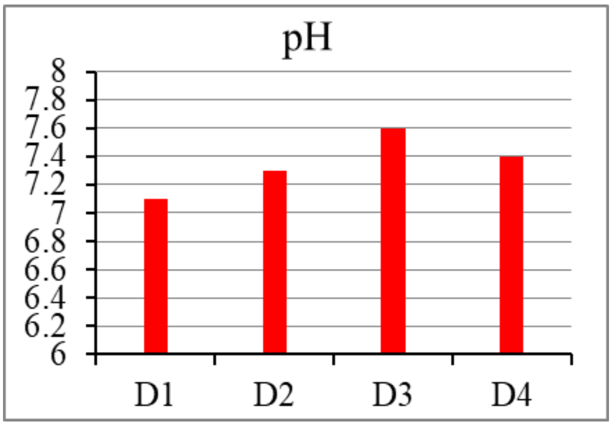
$$FCR = \frac{\text{Feed Intake}}{\text{Weight Gain}}$$

2.5.5 Statistical Analysis
Data collected from the experiment were subjected to normality test and analysis of variance (ANOVA) and the differences among means were tested for significance ($p<0.05$) using SAS statistical package (Version 2.6).

3. Results and Discussion
3.1 Water Quality Parameters
The mean results of water quality parameters for all the treatments throughout the duration of the experiment are presented in Fig. 1(a-e). The dissolved oxygen ranged between 6.0 – 6.3 mg/L in D2 (1.5%) and D4 (2.5%) respectively. The pH values were between 7.1 and 7.6, temperature was between 24.6 and 25.5 ºC during the study. Conductivity ranged between 73µg and 79µg. the total hardness was observed to range between 45.2 and 50.0 mg/L. The quality of water in any ecosystem provides significant information about the available resources for supporting life in that ecosystem. A good water condition is a necessity for the survival and growth of fish since the entire life process of the fish wholly dependent on the quality of its environment [20, 42]. Water quality management is a key ingredient in a successful fish culture practice [23, 24] because, water quality determine the success or failure of an aquaculture operation. This is in agreement with the report of Wurts and Durbow [25] and Bhatnagar and Devi [24] for good fish culture. The water parameters were within the range recommended for fresh water fish culture [26, 27, 28, 29].



a



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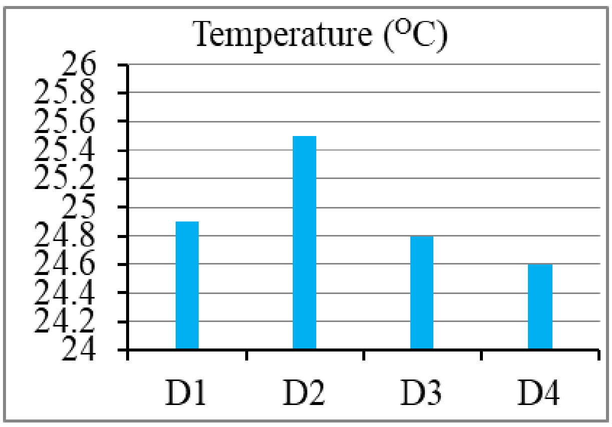

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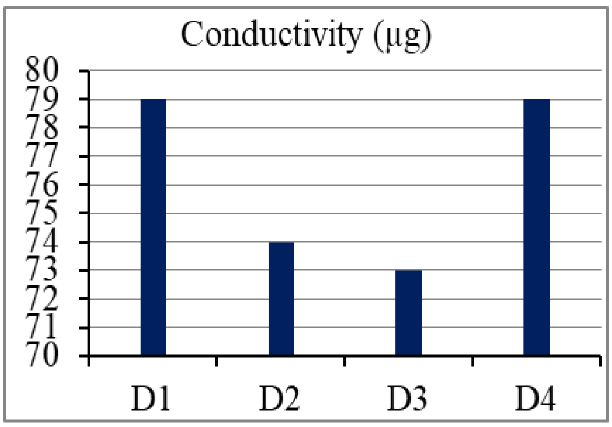

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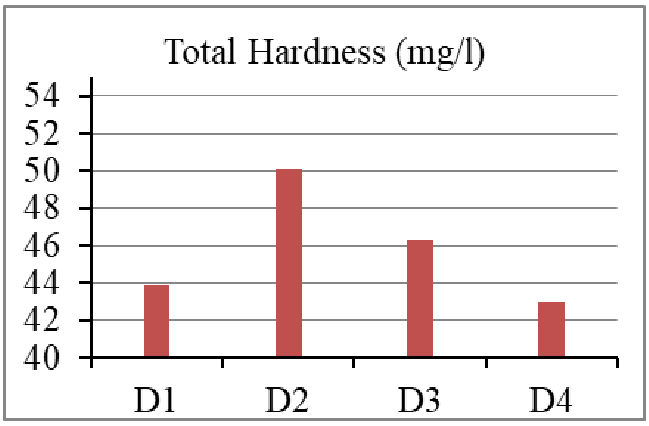

e

Fig 1: The mean water quality parameters

3.2 Proximate composition of the experimental diets

The results of the proximate composition of the experimental diets are presented in Table 1. The mean percentage crude protein value was 40%. Ayinla [30] stated that crude protein level between 35-40% is good for raising African catfish to Juvenile stage. Percentage ash content was lowest in D4 (9.23%) and highest in D3 (11.23%). The percentage crude fibre was lowest in the D2 (6.29%) and highest in D4 (6.93%). The moisture content lowest value was recorded in the Control D1 (10.20%) and highest in D3 (11.71%). The lowest value of lipids (11.13%) was recorded in the control diet and highest value (13.33%) recorded in D4. The NFE was equally lowest in D4 (22.62%) and highest in Control (D1) (27.70%).

3.3 Proximate Composition of the Experimental Fish

The carcass composition of the fish before and after the experiment is presented in Table 2.

There were no significant differences (p<0.05) observed in the proximate composition of the fish among the treatments after the experiment. Though, slight differences occurred in the values obtained. The results showed that there were increases in the values of protein and fat contents after the experiment in all the dietary treatments with pineapple flavor additive. As the crude protein and fat increases, so also the values obtained for fibre, ash and carbohydrate reduced after the experiment. The crude protein of fish ranged between 56.60±0.6 in fish fed D2 and 57.13±0.5 in the control (D1) above the crude protein before the experiment (56.33±0.1). The low values of fat obtained in the experiment were compensated by high moisture content. This is similar to the report of Tawfik [31], who reported low and varying level of fat relative to the moisture content. The ash and nitrogen free extracts of the fish fed D2 (1.5%) were similar to that of the fish before the experiment. The protein contents were high (average of 56.98) compare to the report of Manthey-Karl [32]; 18.9% to 20.3% for turbot in North East Atlantic, and Akpambang [33]; 21.28 -21.65% for some tropical fishes in Nigeria.

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

	Before	After the experiment			
Parameter (%)		**D1 (0%)**	**D2 (1.5%)**	**D3 (2.0%)**	**D4 (2.5%)**
Dry matter	88.17±0.0	88.00±0.1	88.00±0.3	87.57±0.2	87.74±0.1
Moisture content	11.83±0.0	12.00±0.1	12.00±0.3	12.43±0.2	12.26±0.1
Crude protein	56.33±0.1	57.13±0.5	56.60±0.6	57.06±0.4	57.13±0.6
Fat	4.72±0.2	4.97±0.9	4.80±1.0	4. 75±1.1	5.05±1.2
Crude fibre	2.44±0.3	2.32±0.6	2.28±0.8	2.29±0.9	2.28±1.0
Ash	15.52±0.2	14.62±0.7	15.18±0.6	14.9±0.5	14.69±0.4
Carbohydrate	9.16±0.1	8.96±0.4	9.10±0.7	8.48±0.3	8.59±1.0

Note: Values are presented as Mean±SD. There were no significant differences (p>0.05) in the values obtained for the parameters tested.

3.4 Nutrients utilization and growth performance

The results of the nutrients utilization and growth parameters of the fish fed with experimental diets at different inclusion level of powdered pineapple flavour are presented in Table 3. The result showed there were significant differences (p<0.05) in the mean weight gain, feed intake, specific growth rate and the food conversion ration among the fish fed the diets. Fish fed D3 (2%) had the highest mean weight gain (164.8±3.4) followed by the fish fed D4 (2.5% pineapple flavor inclusion (145.8±4.4). The lowest value of the mean weight gain was recorded in the control diet. This difference can be attributed to the feed intake of the fish which is quite low in the control diet. It can be inferred that pineapple flavour aid more ingestion and assimilation of the feed. The result is similar to that obtained when fish were fed diets formulated with vanilla flavor. It was observed that the feed intake reduced when the level of pineapple inclusion was increase beyond 2.0%. The specific growth rate (SGR) of the fish administered with the various diets was highly significant (p<0.05) in the fish fed D3 (1.13±0.02) followed by those fed D4 (1.03±0.02), D2 (0.98±0.01) and D1 (0.85±0.02) of pineapple flavour respectively. Fish fed D2 produced the best food conversion ration of the feed into flesh. This indicates that fish was able to convert the food consumed into flesh better with 2.0% inclusion of pineapple flavour.

Table 3: Growth performance of *Clarias gariepinus* fed experimental diets.

Parameters	D 1 (0%)	D 2 (1.5%)	D 3 (2.0%)	D 4 (2.5%)
Initial Weight (g)	557.7±0.2[a]	558.1±0.2[a]	558.1±0.3[a]	558.2±0.2[a]
Final Weight (g)	898.0±3.3[d]	966.6±1.7[c]	1052.5±3.6[a]	995.7±4.5[b]
Weight Gain (g)	340.3±3.5[d]	408.5±1.6[c]	494.4±3.4 [a]	437.5±4.4[b]
Mean Weight Gain (g)	113.4±3.5[d]	136.2±1.6[c]	164.8±3.4[a]	145.8±4.4[b]
% Mean Weight Gain	20.33[c]	24.40[b]	29.53[a]	29.52[a]
Feed Intake	420±2.5[d]	450±1.9[b]	460±3.2[a]	430±2.7[c]
Specific Growth Rate	0.85±0.02[d]	0.98±0.01[c]	1.13±0.02[a]	1.03±0.02[b]
Food Conversion Ratio	1.27±0.3[a]	1.10±0.1[b]	0.93±0.1[d]	0.98±0.1[c]

Note: Values are presented as Mean±SD. Value across the same row with the same superscript are not significantly different at $p<0.05$.

This result is in line with the report of Ajiboye *et al.* [12] that addition of trace amount of feed stimulants or attractants promote feed intake, supply additional nutrients for protein and energy metabolism thereby enhancing growth performance and ingestion rates of farmed species in aquaculture. Previous studies have been reported on the application of different feed additives; probiotics in the diet of *Oncorhynchus mykiss* [34], mannan oligosaccharide (MOS) based nutritional supplements in aqua feed for Gilthead sea bream (*Sparus aurata*) [35]; European sea bass – *Dicentrachus labrax* [36]; attractants in diet for carnivorous largemouth (*Micropterus salmoides*) [37], herbal growth promoter feed additive in the diet of Tilapia (*Oreochromis niloticus*) [38], *Moringa oleifera* in the diet of Hybrid catfish [39]; *Clarias gariepinus* [40]. However none of these studies have considered the application of flavour or flavour material as feed attractant or stimulant for aquaculture species as justified in this present study. It was reported that a healthy ripe pineapple fruit can supply about 16.2% of daily requirement for vitamin C, which is the body's primary water soluble antioxidant against free radical that attack and damage normal cells [41].

4. Conclusion and Recommendation

The result of the 56 days feeding trial revealed that the dietary powdered pineapple flavour level required for maximum growth of *Clarias gariepinus* was 2.0% flavour inclusion in soya bean based diet. Experiment with soybean based diet supplement with 2.0% flavour is therefore recommended for fish farmer for optimum growth of *Clarias gariepinus*. As plant materials are part of the diet of several catfish and other culture species, it would be worth-while to investigate the importance of other water soluble plant related compounds as attractants and feeding stimulants. Using more than one attractants will help the olfactory sense of the fish and likely be attracted to consume more food and thereby improve the growth rate of fish as the end product of all aquaculture production.

5. Disclosure Statement

No conflict of interest exists among the authors.

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