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Effects of dietary inclusion of Chaya leaf (*Cnidoscolus chayamansa*) meal as feed additives in the diets of African catfish (*Clarias gariepinus*, Burchell, 1822) Juveniles

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Abstract
The dietary effects of Chaya leaf meal as feed additives in the diets of African catfish (*Clarias gariepinus*) Juveniles of mean average weight (12.5±0.03 g) were investigated for a period of ten weeks. Five experimental diets were formulated at 0% (CYMT), 0.5% (CYMA), 1.0% (CYMB), 1.5% (CYMC), and 2.0% (CYMD) inclusion level of the Chaya leaf meal (CYM). All the diets were iso-nitrogenous (40% crude protein level). A total of three hundred (300) fingerlings randomly allocated in triplicate to fifteen experimental tanks (0.53x0.37x0.25 m dimensions) in a completely randomized design (CRD) and each tank contained 20 fingerlings. Fish were fed at 5% body weight biomass to triplicate treatment. The highest mean weight gain of 48.08±4.75 g was obtained in fish fed control diet, the value was significantly (p<0.05) higher when compared with those obtained in fish fed diets containing various inclusion levels of chaya leaf meal. However, the mean weight gain, percentage weight gain and specific growth rate for all the fish fed control diet and the fish fed dietary treatments maintained similar trend. The best feed conversion ratio (FCR) of 3.27±0.21 was obtained in fish fed control diet while the poorer feed conversion ratio was observed in fish fed diet containing 0.5 to 2% inclusion levels of chaya leaf meal, the feed conversion ratio increased as the level of the leaf meal increases until inclusion level of 1.5% after which a decrease occurred at 2% inclusion level of chaya leaf meal. Similarly, the values of feed efficiency and protein efficiency ratio for all the fish fed control diet and the fish fed containing leaf meal maintained the same trend. The gross feed conversion efficiency decreased as the level of the leaf meal increases in fish fed diet containing 0.5 to 1.5% inclusion level of chaya leaf meal, but an increased was observed at 2% inclusion level of the leaf meal. Further investigation on the utilization of chaya leaf meal in fish feeding and adopting appropriate processing methods for the removal of anti-nutritional factors is desirable.

Keywords: Additives, Chaya leaf meal, *Clarias gariepinus*, growth performance, nutrient utilization

Introduction
In Nigeria, greater proportion of fish supply is from the capture fisheries, which do not meet the demand of the ever-increasing population [1]. The need to explore aquaculture for massive fish production is imperative [2]. One of the most important components of aquaculture is fish feeding [3]. Feed alone has been estimated to account for 40-70% of the cost of aquaculture operations [4, 5]. Thus, the importance of efficiency of utilization of the fish feed cannot be over emphasized.

Non-Conventional Feed Resources (NCFRs) are feeds that are not usually common in the market and are not the traditional ingredients for commercial fish feed production [6, 1]. NCFRs are credited for being non-competitive in terms of human consumption and cheaper to purchase [1]. They are mostly by-products or waste products from agriculture, farm made feeds and processing industries. They may include all types of feed stuffs from animal (silk worm, maggot, termites, earth worm, snails, tadpoles etc.), plant waste (cotton seed meal, soy bean

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meal, maize bran, rice bran, palm kernel cake, groundnut cake and brewers waste) from plants (duckweed, cajanus and Chaya) waste from animals such as animal dung, offal, visceral, feather, fish silage, bone and blood [6, 7]. All these can be recycled to improve on fish feed production if economically justified and technologically possible [6, 7].

Chaya's high nutritional value and low cost produced good economic results when included in poultry diets [8]. When added to swine diets in meal form, chaya had no effect on apparent digestibility and produces good growth [9]. It is currently used as tilapia feed in rural areas. The leaves of the Chaya plant (*C. chayamansa*) seem promising as a potential feedstuff/additive considering its impressive nutritional composition: 27–34.02% crude protein on a dry matter basis, with balanced essential and non-essential amino acid profiles suitable for both humans and animal [10, 11]. The present study aimed at investigating the potentials of using Chaya leaf meal in the diet of *Clarias gariepinus*.

Materials and Methods

Study Area

This study was carried out at the Department of Biotechnology, Nigeria institute for oceanography and marine research, Lagos state, Nigeria from February 2023 to April 2023 in a recirculatory aquarium system consisted of 15 plastic tanks.

Sample collection

Fresh leaves of the Chaya plant (*Cnidoscolus chayamansa*) were obtained at Badagry, Lagos, washed and dried at ambient temperature. The dried leaves were milled into fine powder using a laboratory milling machine (Inifitek, Whirlwind mill 370w, 220V), sieved and then stored in an air-tight container for analysis. Incorporation of dried Chaya leaf meal into five experimental cat fish diets (Table 1). The five formulated feeds were pelleted through a 3 mm die using a manual feed pelletizing machine (Hand-operated/6 mm die/ 4 mm die/2 mm die).

Experimental set-up and feeding trial

This experiment was carried out at the wet-laboratory facility of the Nigerian Institute for Oceanography and Marine Research (NIOMR). Five diets containing varying levels of Chaya leaf meal at 0% (CYMT), 0.5% (CYMA), 1.0% (CYMB), 1.5% (CYMC) and 2% (CYMD) were formulated (Table 1). A total of 400 fingerlings of *Clarias gariepinus* were purchased from the hatchery unit of the Nigerian Institute for Oceanography and Marine Research (NIOMR). Acclimatization of fingerlings was done for 14 days during which they were fed commercial diet prior to the commencement of the feeding trial. The fish were afterward, starved for 24 hours to remove food residues in their gut, so as to ensure accurate fish weight, increase fish appetite and prepare the gastrointestinal tract for the experimental diet. Out of 400 fingerlings purchased, 300 fishes were randomly allocated in triplicate to fifteen experimental tanks (0.53x0.37x0.25 m dimensions) in a completely randomized design (CRD) and each tank contained 20 fingerlings.

Table 1: Gross composition of experimental diets

	Chaya leaf meal inclusion levels				
Ingredients (Chaya)	**I (0%)**	**II (0.5%)**	**III (1.0%)**	**IV (1.5%)**	**V (2%)**
Fishmeal (70 CP)	20	20	20	20	20
Additive (Chaya = 28.54 CP)	0	0.5	1	1.5	2
SBM (Defatted) (43 CP)	40	40	40	40	40
Cassava (10 CP)	36.5	36	35.5	35	34.5
Vit/Min	0.5	0.5	0.5	0.5	0.5
Veg Oil	2.5	2.5	2.5	2.5	2.5
Vit C	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Proximate Analysis

Proximate analysis of Chaya leaf meal and other feed ingredients was carried out following the method of the Association of Official Analytical Chemists [12]. This was to ensure the determination of their moisture contents, crude protein, crude fibre, carbohydrate, fat and ash content. The proximate composition of chaya leaf meal is shown in Table 2

Table 2: Proximate composition of Chaya leaf meal; ± SE

Parameters	Percentage (%)
Moisture	9.40±0.28
Crude Protein	28.54±0.11
Crude Fat	3.01±0.05
Fiber	7.43±0.40
Ash	9.53±0.23
CHO	42.09±0.46
Metabolizable energy	2727.90±0.51

Experimental fish and rearing condition

Twenty experimental fish were distributed randomly into the tanks with three replicates per treatment and fed twice daily at 5% of their body weight for 10 weeks. They were fed with Coppens 45% crude protein diet (2mm size) for one week while, being acclimatized. Dead fish were removed from the tank daily and the number recorded. Five fish were randomly selected from each tank biweekly and their length and weight measured. Average and total weight per tank were computed in order to monitor their growth and also adjust the feed ration. The dissolved oxygen (DO) was maintained at 5-7 mg/l. The pH between 6.5-7.5 water quality was measured using a water quality kits (aquaculture water quality kit, LaMotte). Water temperature, DO, pH was recorded in the tanks and the sump daily, at 9.00am. The nitrite (NO-2), nitrate (NO-3) and NH3 was measured every other day (Monday, Wednesday and Friday) Table 1.

Average water temperature was 26.2±0.3 °C, dissolved oxygen ranged between 4.0±0.20 and 4.6±0.11 mgL^{-1}. The pH varied between 6.30 and 7.3, alkalinity ranged between 108 mgL^{-1} and 118 mgL^{-1} while the phosphate and nitrate varied between 0.1 mgL^{-1} and 0.6 mgL^{-1} and between 0.01 mgL^{-1} and 0.02 mgL^{-1}respectively (Table 3).

Table 3: Report on analysis of water sample

Water Quality parameter	WHO standard	Desirable range of water parameter for freshwater fish culture	Water parameters for experimental fish
Iron (mg/l)	0.3	< 0.5 mg/l	-
Carbon dioxide (mg/l)			
P^H	6-8	6.5-8.5	6.3-7.3
Total Ammonia(mg/l)	-	< 0.02 mg/l	0.01 mgL^{-1}
Nitrite(mg/l)	-	< 0.25 mg/l	0.01
Dissolved Oxygen(mg/l)	-	> 4 mg/l	4.0 - 4.6 mgL^{-1}
Alkalinity(mg/l)	200 mg/l	50-400 mg/l	108 – 118 mgL^{-1}
Hardness(mg/l)	100 mg/l	50-400 mg/l	-
Salinity(ppt)	0.1ppt	< 1.0 ppt	0.1ppt
Colour	Colourless	Colourless	-
Conductivity(ms/cm)	1.0 ms/cm	< 1500 ms/cm	-
Temperature (^{o}c)	-	24-32 °C	25-26.5^{o}C
Taste	Tasteless	Tasteless	-
Odour	Odourless	Odourless	Odourless
Nitrate (mg/l)	10	0-3 mg/l	0.01 -0.02 mgL^{-1}
Phosphate (mg/L)		0.05-0.2mg/l	0.1-0.6 mgL^{-1}
Total suspended solid (mg/L)	10 mg/l	80 mg/l or less	-
Total dissolved solid (mg/L	50 (mg/L	-	-

Fish Survival Rate

$$SR = \frac{\text{Initial number of fish stocked – mortality}}{\text{Initial number of fish}} \text{ X } 100 \text{ [13]}$$

Fish Growth indices
Fish weight

Fish weight (g) = Fish weight of the experiment

Weight gain
Mean weight gain (g) = Final mean weight (g) - Initial mean weight (g)

Percentage Weight Gain

$$PWG = \frac{\text{Final mean weight-initial mean weight}}{\text{Initial body weight}} \text{ X } 100 \text{ [14]}$$

Specific Growth Rate (SGR)

$$SGR = \frac{In_{W2}\text{-}In_{W1}}{T_{1\text{-}}} \text{ x } 100 \text{ [15]}$$

Where
In = Natural logarithm
$W_2 – W_1$ = final and initial weight of fish (g) and
$T_2 – T_1$ = period in days

Nutrient utilization indices
Feed Conversion Ratio (FCR)

$$FCR = \frac{\text{Feed fed (g)}}{\text{Weight}} \text{ [16]}$$

Feed Efficiency Ratio

$$FER = \frac{\text{Weight gain (g)}}{\text{Feed fed}} \text{ [17]}$$

Gross Feed Conversion Efficiency (GFCE)

$$GFCE = \frac{1}{FCR} \text{ X } 100 \text{ [17]}$$

Protein Efficiency Ratio (PER)

$$PER = \frac{\text{Weight Gain (g)}}{\text{Crude Protein Consumed}} \text{ [16]}$$

Apparent Net Protein Utilization (APPNPU)

$$APNPU = \frac{Nb\text{- }Na}{Ni} X\ 100 \text{ [17]}$$

Where
N_b = Body protein at end of the experiment
N_a = Body protein at the beginning
N_i = Amount of Nitrogen (protein ingested)

Statistical Analysis
The data on nutrient composition of Chaya leaf meal, growth performance, nutrient utilization indices obtained were subjected to analysis of variance (ANOVA) and the treatment means were separated using Duncan Multiple Range Test [18]. The analysis was carried out using SPSS version 20.0.

Results
Evaluation of Growth performance and Nutrient utilization indices
The biweekly changes in weight of all the experimental fish throughout the period of the experiment are shown in Table 4. The growth performance and nutrient utilization of *Clarias gariepinus* fed with chaya fortified diets are represented in Table 5 and 6 respectively. There were decreases in weights fish samples on all the diets throughout the experimental periods. The mean weekly gains of the juveniles are illustrated in Figure 1. The highest mean weight gain of 48.08±4.75 g was obtained in fish fed control diet, the value was significantly (p<0.05) higher when compared with those

obtained in fish fed diets containing various inclusion levels of chaya leaf meal. However, the mean weight gain decreased as the level of the chaya leaf meal increases. The mean weight gain, percentage weight gain and specific growth rate for all the fish fed control diet and the fish fed dietary treatments maintained similar trend. There was a reduction in the growth rate with increasing the level of the chaya leaf meal. The best feed conversion ratio (FCR) of 3.27±0.21 was obtained in fish fed control diet while the poorer feed conversion ratio was observed in fish fed diet containing 0.5 to 2% inclusion levels of chaya leaf meal, the feed conversion ratio increased as the level of the leaf meal increases until inclusion level of 1.5% after which a decrease occurred at 2% inclusion level of chaya leaf meal. Similarly, the values of feed efficiency and protein efficiency ratio for all the fish fed control diet and the fish fed containing leaf meal maintained the same trend. There was a reduction in the feed efficiency and protein efficiency ratio as the level of leaf meal increases in the diets. The gross feed conversion efficiency decreased as the level of the leaf meal increases in fish fed diet containing 0.5 to 1.5% inclusion level of chaya leaf meal, but an increased was observed at 2% inclusion level of the leaf meal.

Table 4: Mean (±) Biweekly increase weights for ten weeks.

Treatments	Mean Biweekly weights increase					
	Week 0	**Week 2**	**Week 4**	**Week 6**	**Week 8**	**Week 10**
I	16.14±0.02	21.90±0.03	38.98±0.02	54.43±0.02	78.19±0.03	90.57±0.04
II	16.65±0.12	26.40±0.11	32.70±0.08	50.18±0.04	60.84±0.06	67.72±0.05
III	15.97±0.02	23.48±0.02	32.68±0.11	38.72±0.08	40.05±0.04	45.58±0.03
IV	16.55±0.08	26.40±0.12	36.74±0.02	41.45±0.14	44.79±0.04	43.84±0.02
V	15.84±0.11	26.18±0.02	34.21±0.04	40.01±0.10	40.97±0.05	43.28±0.04

KEYS:

I- Chaya leaf meal (CYM) treatment I (0% CYMT, Control no CYM)
II- Chaya leaf meal treatment II (0.5% CYMA, CYM inclusion)
III- treatment III (1.0% CYMB, CYM inclusion)
IV- treatment IV (1.5% CYMC, CYM inclusion)
V- treatment V (2.0% CYMD, CYM inclusion)

Table 5: Growth performance of *Clarias gariepinus* fed Chaya leaf meal

Parameters		Diets/treatment			
	I (0%)	**II (0.5%)**	**III (1.0%)**	**IV (1.5%)**	**V (2%)**
Survival rate	78.33±10.13^{a}	76.66±4.40^{ab}	51.66±4.40^{c}	58.33±8.81^{bc}	53.33±6.66^{bc}
Initial Mean body weight (g)	16.14±0.31	16.65±0.14	15.97±0.34	16.55±0.20	15.84±0 .21
Final mean body weight (g)	64.22±4.53^{a}	46.16±2.70^{b}	37.86±3.15^{bc}	33.61±1.94^{c}	34.96±2.42^{c}
Mean weight gain (g)	48.08±4.75^{a}	29.51±2.81^{b}	21.89±2.82^{bc}	17.05±1.76^{c}	19.12±2.25^{bc}
Percentage weight gain (%)	298.85±34.50^{a}	177.50±18.22^{b}	136.47±14.53^{bc}	102.83±9.79^{c}	120.42±13.10^{bc}
Specific growth rate	5.51±0.14^{a}	4.82±0.13^{b}	4.38±0.17^{bc}	4.03±0.15^{c}	4.19±0.18^{c}
Condition factor (K)	0.61±0.04	0.73±0.14	0.74±0.13	0.62±0.02	0.66±0.06

Means in rows having same letter are not significantly different (p>0.05)

Table 6: Nutrient utilization of *Clarias gariepinus* fed Chaya leaf meal

Parameters		Diets/treatment			
	I (0%)	**II (0.5%)**	**III (1.0%)**	**IV (1.5%)**	**V (2%)**
Feed fed	155.33±6.19^{a}	133.75±1.96^{b}	102.31±4.49^{c}	107.49±1.47^{c}	104.73±2.55^{c}
Feed conversion Ratio	3.27±0.21^{b}	4.62±0.49^{ab}	4.84±0.69^{ab}	6.46±0.79^{a}	5.63±0.68^{a}
feed efficiency	0.31±0.02^{a}	0.22±0.02^{b}	0.21±0.03^{b}	0.16±0.01^{b}	0.18±0.02^{b}
Protein efficiency ratio	0 .88±0.06^{a}	0 .63±0.06^{b}	0.62±0.09^{b}	0.08±0.04^{b}	0.52±0.05^{b}
Gross feed conversion efficiency	30.84±2.08^{a}	22.14±2.44^{b}	21.62±3.47^{b}	15.90±1.80^{b}	18.23±2.04^{b}

Means in rows having same letter are not significantly different (p>0.05)

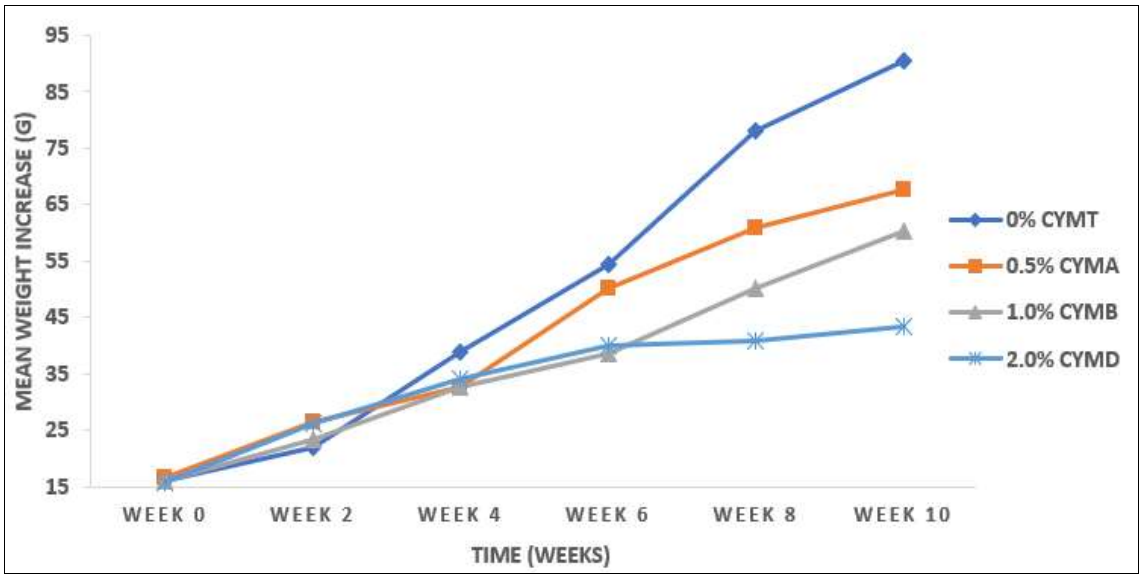

Fig 1: Mean weekly weight increase of *Clarias gariepinus* fed with Chaya leaf (*Cnidoscolus chayamansa*) fortified diets.

Discussion

The results obtained in this study indicate that the growth and nutrient utilization response of *C. gariepinus* fingerlings were influenced by levels of chaya leaf meal inclusion in the diets. The control diet was most preferred, resulting in more superior growth performance and diet utilization which could be due to the maximum portion of fishmeal it contained. The significant variation (p<0.05) in growth response and nutrient utilization among the test diets was probably an indication that those parameters were largely influenced by the different inclusion levels of chaya leaf meal. Also, the statistically significant drop (p<0.05) in final body weight of fish fed diet with higher chaya leaf meal inclusion levels can be related to the reduced feed intake as was observed during the study. This may not be unconnected with the high fiber content of Chaya leaves, as well as the presence of anti-nutritional compounds [19, 20]. The effect of these substances can alter the nutrient balance of the diet, reduce the palatability of the feed, disturb the digestive process, decrease growth and feed efficiency [21]. It is important to note that most studies using leaf meals/plant-based meals have revealed similar results to what we obtained, largely due to the afore-mentioned factors, with recommendations for lower inclusion levels [22]. Our work corroborates the findings of [23] who reported that high concentration of DCLM (dried chaya leaf meal) in chicken diets didn't help them grow faster (due to the high fiber content of DCLM which makes it difficult to digest and use); but had improved effect on other measured parameters [24, 25] reported a reduction impact on growth efficiency of cultured fish at higher replacement level of fish meal with Moringa leaf meal [26] fed Chaya leaf meal to *O. niloticus* and recorded low growth response in spite of high survival rate [27] who used bitter leaf as a feed additive for catfish brood stock, reported growth decline at higher inclusion levels, attributable to the bitter taste and low palatability of their test diet. On the other hand, [28] reported a linear improvement in growth performance of pullet chicks fed a mixture of chaya, garlic and ginger [29] showed that *Morinda lucida* (Oruwo) leaf inclusion in fish feeds enhanced growth of *Clarias gariepinus*. Also, in their study using medicinal plant additives in *C. gariepinus* feed, [30] reported enhanced growth and survival of cultured *C. gariepinus*. Dietary supplementation with fluted pumpkin (*T. occidentalis)* leaf powder in the diet of *C. gariepinus* fingerlings also resulted in their improved growth, feed utilization and survival [31].

Conclusion

Based on this work, moderate inclusion levels (0.5% - 1%) of Chaya leaf meal would be sufficient to harness its beneficial effects for the wellbeing of cultured *C. gariepinus* fingerlings. Also, improved methods of processing of the leaf to increase its bioavailability in the feed are required.

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Competing Interest

We hereby declare that the disclosed information is correct and that no conflict of interest is known.

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