

Growth Performance of *Clarias Gariepinus* (Burchell, 1822) Fed with Varying Substitution Levels of Fish Meal with Cattle Hoof Meal

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Abstract

Fifteen weeks (105 days) feeding trial was conducted to determine growth response and economic benefit of replacing fishmeal with cattle hoof meal (CHM) in the diets of African catfish (*Clarias gariepinus*) juveniles. Five isonitrogenous diets containing 42% crude protein were formulated in which CHM replaced fishmeal at 0%, 25%, 50%, 75% and 100% (coded as D2, D3, D4, D5 and D6 respectively). A commercial feed (D1) was used as reference diet. Two hapa partitioned into six treatments of three replicates each embedded in a concrete pond of 5m by 3m by 2m was used for the experiment. The fish were fed at 3% body weight three times daily between the hour of 6.00 am and 7.00am, 2.00 pm and 3.00pm, and 10.00 pm and 11.00 pm. The feeding ration was adjusted every three weeks after weighing. The mean of the monitored physico chemical parameters of the water in the experimental tank were pH (7.12), temperature (28.25°C), oxygen (5.77mg/l), ammonia (0.04mg/l), nitrite (0.04mg/l), nitrate (0.03mg/l) and bicarbonate (5.45mg/l). The proximate composition of cattle hoofs meal were 33.75% moisture content, 66% dry matter, 58.06% crude protein, 2.75% crude fibre, 3.25% crude lipid, 0.91%

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ash content and 1.78% Nitrogen free extract. Feeding trials indicated that all fish consumed the experimental diets actively. Fish fed with the commercial feed had the highest mean weight gain (204g), followed by those fed with the experimental diet with 25% of CHM (D3) (181g). The fish fed with the control diet with no cattle hoofs meal (D2) had lower mean weight gain compared to the fish fed with 25% CHM. The fish fed with 50% CHM, 75% CHM and 100% CHM exhibited a decreasing weight gain (148.60g, 143.73g and 129.00g respectively) as the substitution level of the CHM meal increased. The fish fed with 25% cattle hoof meal (D3) had the best growth performance and feed utilization proficiency (specific growth rate 2.58, relative growth rate 1403, protein efficiency ratio 1.67 and feed conversion ratio 1.47) among the fish fed the formulated diets (D2, D3, D4, D5 and D6). There was no significant difference ($p > 0.05$) in the survival value of fish fed with all the experimental diets. The fish fed with D3 had the best net profit (₦46.53) followed by D6 (₦45.63), D1 (₦42.54), D5 (₦41.61), D4 (₦31.49) and D2 (₦20.59) in decreasing order. This study revealed that CHM can replace fishmeal at a lower cost and efficient diets for *Clarias gariepinus*. The best result was produced at the substitution level of 25% inclusion of CHM. It is recommended that CHM be included in the feed of *Clarias gariepinus* at 25% for optimum growth performance and reduced cost of production.

Keywords: Cattle hoofs meal, *Clarias gariepinus*, Growth performance, Low-cost

INTRODUCTION

It has been acknowledged that fish is one of the solutions to problem of malnutrition due to its availability, general acceptance, nutritional quality and the relatively cheap price as a source of animal protein (Agbabiaka *et al.*, 2013). As the quantity of fish catch from the wild decreases, the best alternative worldwide is aquaculture (Kiel, 2011). In 2012, aquaculture production overtook global beef production in quantity for the first time (Pucher *et al.*, 2014). Aquaculture, the farming of aquatic organisms including fish, molluscs, crustacean and aquatic plant is growing exponentially in the world today (Diana, 2009).

Recent information by FAO (2016) stated that in Africa, Egypt and South Africa are the first and second (respectively) aquaculture producing countries while Nigeria is the third, but number one in production and consumption of *Clarias gariepinus*. The farming of catfish is important to Nigeria because it is a source of income, creates employment opportunities, contributes towards Gross Domestic Product (GDP), fetches higher price than tilapia due to the fact that it can be sold live at the market (FAO, 2016).

The major aim of fish farming is to have optimum increase in fish production within the shortest interval at minimum cost and also provide healthy protein source with Omega-3-fatty acids as against the poly unsaturated fatty acids that are provided by meat source of protein (with the exception of white meat). The most costly resource in fish farming is the feed which consumes 65-80% of the total cost of production (FAO, 2014). Options for reducing feed costs must focus on formulation strategies to optimize nutrient utilization, particularly protein source of fish feed which is the most expensive component (FAO, 2012; Mustafa, 2021)

Fish meal is the most widely used protein source in fish feed due to its highly digestible protein, amino acids and good palatability; generally it is the conventional protein source in aquaculture feeds. It supports good fish growth because of its protein quality and palatability. Fish meal is often scarce and expensive, especially good quality brands, thereby contributing to the high cost of fish production and nutrition (Fagbenro and Davies, 2002; Jahan *et al.*, 2021). Currently, aquaculture feed use more than 20% of the world supply of fish meal, which is produced at approximately six metric tons per year. The practice of feeding cultured fish with

products emanating from finite fishery resources jeopardizes the long term sustainability of aquaculture systems (Psoufakis *et al.*, 2021).

Utilization of animal protein by-product in fish feed could be an alternative to expensive fishmeal in decreasing production cost. Several animal by-products have been used to supplement/replace fish meal in the diet of *Clarias gariepinus* at different inclusion level with each producing different results. Many studies have been conducted to determine the suitability of animal based ingredients such as poultry by product (Gomaah *et al.*, 2004), grasshopper meal (Olaleye, 2015), locust meal (Balogun, 2011), cricket meal (Taufek *et al.*, 2016), recycled dead chicken meal (Agbabiaka *et al.*, 2013), maggot meal (Ogunji *et al.*, 2011), termite meal (Sogbesan and Ugwumba 2008), Mealworm (Ng *et al.*, 2001), silkworm pupae meal (Alegbeleye *et al.*, 2012), black soldier fly meal (St-Hilaire *et al.* 2007), feather meal (Omitoyin and Faturoti 2001; Wei-kang *et al.*, 2013), to replace fish meal in the diets of different stages of African catfish. The animal by-product incorporated into the feed of African Catfish produced best result at different substitution levels depending on the nutrient composition and digestibility of the product (Agbabiaka *et al.*, 2013).

Feathers, horns and hooves are composed of keratin protein, consisting of amino acids, and were generally considered as wastes, but now, they represent a potential alternative to expensive dietary ingredients for animal feedstuffs (Jayapradha *et al.*, 2011). Horn and hoof are protein rich animal byproducts which are generated in tons in Abattoirs all over Nigeria (Qureshi *et al.*, 1962). Cattle hoof is not completely a waste product since it is often used in some cosmetic industries and production of some traditional artifacts. The utilization of hoof as a protein source for fish feed may be economical because it is widely available and relatively cheap. Therefore this study was designed to determine the growth response and economic benefit of replacing fishmeal with cattle hoof meal (CHM) in the diets of African catfish (*Clarias gariepinus*) juveniles.

MATERIALS AND METHODS

Source of Materials

A total number of 180 juveniles of *Clarias gariepinus* was procured from Arewa farm in Zaria, Nigeria and transported in 50 liter plastic container to the aquaculture unit of National Agricultural Extension and Research Liaison Services (NAERLS), Ahmadu Bello University, Zaria. The juveniles were acclimatized for two weeks within the experimental tanks. The juveniles were fed with 'Vital feed' (a commercial feed) during the two weeks period of acclimatization. Cattle hooves were obtained from the abattoir at Zango, Zaria, Kaduna State. Other feed ingredients such as fish meal, soybean meal, yellow maize, palm oil, salt, methionine, lysine, premix, were all obtained at Sabongeri market, Zaria, Nigeria.

Preparation of Cattle hoof meal and analysis

The Cattle hoofs meal was prepared by soaking the hooves in 10 % sodium carbonate for sixty hours at room temperature. Thereafter it was boiled in water for one hour and oven dried at 80°C for 48 hours (Quresh, 1962). This was done to aid the digestibility of the feed. On cooling, the hoofs were cut into pieces by using sharp kitchen knife, then pulverized into powder using pestle, mortar and a sieve and later stored in an air-tight polythene bag until required. Proximate analysis of the prepared cattle hoofs meal (Table 1) and experimental diets (Table 2) were carried out according to the Association of Official Analytical Chemist procedures (A.O.A.C., 2005) at the Animal Science laboratory, Institute of Agricultural Research (IAR), A.B.U Zaria.

Feed formulation

The Cattle hoofs meal (CHM) produced was mixed with other feedstuffs (fish meal, soybean, yellow maize, methionine, lysine, bone meal, vitamin premix, salt and palm oil) to produce five isonitrogenous diets (Crude Protein 42%) where Cattle hoofs meal replaced Fish meal at 0%, 25%, 50%, 75% and 100% (Table 3). The diets were denoted as D1 (Commercial feed), D2 (0%CHM), D3 (25% CHM), D4 (50%), D5 (75% CHM), and D6 (100% CHM). The formulated practical diets were pelleted using the locally fabricated pelleting machine to produce diet of 2mm, 3mm and 4mm which were fed to the fish as they aged. Pelleted diets were sun-dried (during the mid-day at temperature of 40°C ± 3°C) for two days until crispy and then packed in an air-tight polythene bags with appropriate labeling.

Experimental Setup

The feeding and growth experiment was conducted in the Aquaculture unit of NAERLS, Ahmadu Bello University, Zaria. Two hapas (2m by 2m by 2m) partitioned into eighteen hapas of dimension 0.70 m × 0.70 m × 2m each embedded in a concrete pond of 5m by 3m by 2m were used (three hapas per experimental diet). The three hapas per experimental diet which were randomly distributed represented three replicate per experimental diet. Ten fish were stocked in each hapa in replicate, this sum up to a total of thirty fish per treatment. Catfish of uniform sizes (length and weight) were stocked in each hapa to avoid cannibalism.

Table 1 Proximate analysis of boiled cattle hoof meal

Composition	Cattle hoof meal (%)
Crude protein	58.06
Moisture	33.75
Crude lipid	3.25
Crude fibre	2.75
Ash	0.91
NFE	1.78
Dry matter	66.25

Table 2 Proximate analysis of experimental diets

Composition	D1 (CF)	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)
Crude protein	42.34	41.09	40.81	40.95	40.38	40.85
Moisture	7.86	10.84	10.36	11.37	13.05	10.27
Crude lipid	14.69	12.54	12.21	12.51	12.35	12.11
Crude fibre	2.80	2.54	2.72	2.83	3.47	1.92
Ash	7.86	10.78	8.03	7.82	7.27	6.60
NFE	25.70	22.34	25.87	24.52	23.48	28.25
Dry matter	92.23	89.16	89.64	88.63	86.95	89.93
Gross Energy	2048.91	1874.33	1916.63	1908.16	1869.86	1955.49

CF: Commercial feed; NFE: Nitrogen free extract

Table 3 Gross composition of Experimental Diets Fed to *Clarias gariepinus*

Ingredients	D1 (C F)	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)
Cattle hoof meal	-	0.00	11.73	17.60	26.39	35.19
Fish meal	-	33.00	24.75	16.50	8.25	0.00
Soybean meal	-	35.19	35.19	35.19	35.19	35.19
Yellow maize	-	19.62	19.62	19.62	19.62	19.62
Vitamin Premix	-	2.89	0.41	1.79	1.25	0.50
Methionine	-	2.00	2.00	2.00	2.00	2.00
Lysine	-	3.00	3.00	3.00	3.00	3.00
Bone Meal	-	1.00	1.00	1.00	1.00	1.00
Salt	-	0.30	0.30	0.30	0.30	0.30
Palm oil	-	3.00	2.00	3.00	3.00	3.20
TOTAL	-	100	100	100	100	100

CF: Commercial feed

Fish feeding and culture

The fish were fed thrice daily between 6.00 am-7.00 am, 2.00 pm-3.00 pm and 10.00 pm-11.00 pm (i.e eight hours interval for every meal to ensure uniformity in the feeding) at 3% body mass throughout the 105 days of the experiment. The ration was adjusted every three weeks when new mean weights of fish for the various experimental units were determined. The water in the pond was changed every week to 1.0m level of the pond in which the cages were immersed.

Physicochemical parameter of water

Water temperature and pH were measured weekly, before and after changing the water using HANNA instrument (model 98129) while dissolved oxygen (DO) was measured using DO meter (model DO-510), Ammonia and nitrate were measured using the comb II test strips.

Weighing and Measuring of Fish

The initial mean weight of fish was determined after the acclimatization period of two weeks. The mean weight per replicate was calculated after taking the bulk weight of the fish in each of the hapa. Mean weight (g) per hapa was taken every three weeks throughout the 105 days period of the experiment. Both standard length (SL) and the total length (TL) of the fish used for the experiment were recorded using a measuring board.

Growth performance indices

Growth performance indices of the fish were determined using the following parameters after 105 days of the feeding trial.

Mean weight gain (g) (MWG)

$$MWG = W_{t2} - W_{t1} \quad (\text{Adikwu, 2003})$$

Feed Conversion Ratio (g) (FCR)

$$FCR = \frac{\text{Weight of feed given (g)}}{\text{Fish weight gain (g)}} \quad (\text{Adikwu, 2003})$$

Specific Growth Rate

$$\text{Specific Growth Rate (SGR)} = \frac{\text{Loge } w_2 - \text{Loge } w_1}{t} \times 100 \quad (\text{Hepher, 1988})$$

Relative Growth Rate (RGR)

$$\text{Relative Growth Rate (RGR) (\%)} = \frac{(W_f - W_i) \times 100}{W_i} \quad (\text{Wannigama et al., 1985})$$

Protein Efficiency Ratio

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Fish Weight Gain (g)}}{\text{Protein intake (g)}} \quad (\text{Wilson, 2002})$$

Condition Factor (K)

$$K = \frac{100 \times W}{L^3} \quad (\text{Wilson, 2002})$$

Survival Rate (%)

$$\text{Survival Rate (SR) (\%)} = \frac{\text{Initial number of fish stocked} - \text{mortality}}{\text{Initial number of fish stocked}} \times 100 \quad (\text{Akinwole et al., 2006})$$

Production Index

$$PI = \frac{\text{Survival rate} \times \text{final weight (g)} - \text{initial weight (g)}}{\text{Duration of rearing period (days)}} \quad (\text{Mohanty, 2004})$$

Economic Analysis

The economic analysis of the production of *Clarias gariepinus* in this experiment was conducted to assess the cost effectiveness of diets used in the feeding trial. Only the cost of feed and juvenile were used in the calculation.

Economic Weight Gain

$$\text{Economic Weight Gain (EWG)} = \frac{\text{Cost of feed (N)}}{\text{Weight gain (g)}} \quad (\text{Akinwole et al., 2006})$$

Profit index

$$PI = \frac{\text{Weight of fish crop}}{\text{Cost of feed}} \quad (\text{Aderolu et al., 2010})$$

Incidence cost

$$(IC) = \frac{\text{Cost of feed}}{\text{weight of fish produced}} \quad (\text{Aderolu et al., 2010})$$

Net profit

$$(NP) = \text{sales} - \text{expenditure} \quad (\text{Aderolu et al., 2010})$$

Benefit cost ratio

$$(BCR) = \frac{\text{total sales}}{\text{total expenditure}} \quad (\text{Mazid et al., 1997})$$

Total cost

$$(TC) = \text{fixed cost (FC)} + \text{total variable cost (TVC)} \quad (\text{Sogbesan and Ugwumba, 2006})$$

Data Analysis

Mean values of the water quality parameters measured was calculated. Mean values of the growth measurements and cost benefits were subjected to one way Analysis of variance (ANOVA) and tested for significant difference at 5% level of significance. Differences between means were separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The results of the nutrients analysis showed that cattle hoofs meal (CHM) contained 58.06% crude protein, 3.25% lipid, while values of 0.91%, 2.75% and 1.78% were recorded for ash, crude fibre and Nitrogen free extract respectively. The high crude protein of cattle hoofs meal has been proposed to be a good alternate to fish protein (68.68%) in the production of fish feed (FAO, 2006). However, it may require further processing such as fermentation which may make or break down the keratin (protein) into its constituents (amino acids).

Clarias gariepinus fed varying inclusion levels of cattle hoofs meal feed showed a positive response to the feed (Table 4). Fish fed the commercial feed had the best mean weight gain (204g) followed by the fish fed the experimental diet which contained 25% of Cattle feed meal (181g). The fish fed with the control diet which had no cattle hoofs meal (D2) had lower mean weight gain compare to the fish fed 25% cattle hoof meal. The higher growth performance observed in the group containing 25% cattle hoofs meal might be due to the synergistic effect of combining two biological compounds (i.e fish and cattle hoof) to have superior effect than when applied individually (Ugwumba *et al.*, 2001). It has been suggested that combination of protein sources is better than single protein source in the diet of fish (Ugwumba *et al.*, 2001; Sogbesan *et al.*, 2005; Sogbesan and Ugwumba, 2006). Though the fish fed with the commercial feed had the best weight gain, there was no significant difference with these 0% and 25% CHM (176.6g and 181.00g respectively). The fish fed 50%, 75% and 100% CHM exhibited a decreasing mean weight gain (148.60g, 143.73g and 129.00g respectively) as the substitution level of the cattle hoof meal increases. The feed intake and protein intake by the fish decreased with increasing substitution level, though the crude proteins of the experimental diets were not significantly different, as the difference was within the acceptable range of ± 2.00 (Agboola, 2004). This finding could be due to effective digestibility of the cattle hoof and fish meal, rather than the amino acids only (Massumotu *et al.*, 1996).

The fish fed with the commercial diet had the highest specific growth rate (SGR), relative growth rate (RGR) and protein efficiency ratio (PER) and lowest feed conversion ratio (Table 5). This means that the commercial feed produced the best result in comparison to the experimental diets. This could be due to the fact that commercial feeds were produced with conventional protein sources with more crude protein percentage and more digestibility coefficient. Among the fish fed the experimental feeds, the fish fed with 25% cattle hoof meal (D3) produced the best SGR, RGR, PER and FCR. The higher the SGR and the lower the FCR value, the better the feed quality (Omeru and Solomon, 2014). Similarly, Adikwu (2003) also reported that the lower the FCR the better the feed utilization by the fish. The values of protein efficiency ratio in this study and in all the experimental feed were above the value one (1.00). This is a demonstration of higher feed quality in terms of the amino acids. DeSilva and Anderson (1995) reported that protein efficiency ratio is a measurement of how well the protein sources in a diet could provide the essential amino acids requirement of the fish fed.

Survival rate was higher in treatment D1 (96.67%) and D2 (93.33%) while the lowest survival rate was recorded in D3 and D6 (80% each). This may be due to stress during handling as most of it occurred after weekly sampling. The high survival rate (80% and above) in the whole

treatments may be associated with favorably high water quality of the experimental tanks and possibly, the well processed and preserved feeds administered to the fish.

The triweekly weight increase of the experimental fish fed diets with varying inclusion levels of the cattle hoofs meal in Fig. 1 shows that there was a uniform growth pattern of fish fed 0% 25%, 50%, 75% and 100% CHM for the first three weeks while fish fed diets containing CF displayed the fastest growth pattern. Fish fed the commercial feed showed a remarkable increase in weight above the other treatments from the first week to the end of the feeding period. This was followed by fish fed diet 0% and 25% while fish fed diet 50-100% lagged behind after the third week. Fish fed diet 100% CHM displayed slowest growth rate after the first three weeks to the end of the feeding trial.

The physico chemical parameters of water (Table 6) in experimental tanks were optimum for the culture of *Clarias gariepinus*. Catfish require optimum temperature of 23-32°C, dissolve oxygen of $\geq 5\text{mg/l}$, pH of 6-9, Ammonia of $< 8.8\text{mg/l}$, Nitrite of $< 0.06\text{mg/l}$ and bicarbonate of 5mg/l (FAO, 2006; Momoh and Solomon, 2017). There was no significant difference in the physico chemical parameters of the tank across the treatments because the six treatments were held in a large concrete pond but partitioned with two hapas into six treatments of three replicates. The optimum physico chemical parameters of water in this study may have contributed to the growth rate and survival percentages in all treatments.

The commercial feed (D1) had the highest cost of feed per Kg, feeding cost and total cost while diet that contained 100% cattle hoofs meal (D6) had the least cost of feed/kg, feeding cost and total expenditure/cost (Table 7). This implies that D6 is the cheapest feed among the experimental feeds while the commercial feed is the most expensive. The commercial feed had the highest total sales because the fish had the highest mean weight gain, while D6 with least mean weight gain had the lowest total sales. Next to the commercial feed is the D3 which had the second highest mean weight gain and the second best total sales value; D3 had the best net profit (₦46.53) among the whole treatments while D2 had the least net profit (₦20.59). The possibility of obtaining a significantly higher value of net profit at 25% substitution level above those of the control and higher substitution levels clearly indicates that more profit would be generated from fish fed D3.

Fish fed D6 had the highest profit index (PI) and benefit cost ratio (BCR) and lowest economic weight gain (EWG) and incidence cost (IC). The higher the profit index and benefit cost ratio, the lower the economic weight gain and incidence cost, the better the feed economically (Umar *et al.*, 2016). As a rule of thumb, project with benefit ratio greater than one, equal to one, or less than one indicate profit break even or loss respectively (Olagunju *et al.*, 2007). Since the experimental feeds produced BCR greater than one, it implies that the experimental feeds are good but some are better than the other. In this regard D6 is good economically but the farmer will have to wait for a longer time before the fish could get to table size because it produced the least weight gain in comparison to other experimental diets. Fish in group D3 is the second best when ranking is based on the economic weight gain, profit index, incidence cost, and benefit cost ratio. Production estimate based on the gross and net yield for growth are the basis for estimating the economic revenue from the fish culture operation (Umar *et al.*, 2016).

In this study, the high net profit, low EWG and good profit index for fish fed 25% CHM could be attributed to good feed quality, acceptance of feed by fish and suitable water quality, which resulted in better growth performance and high survival rate. Conclusively, among the experimental feeds, D3 is the best feed because the fish fed in this group have high feed

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utilization efficiency, digestibility coefficient, carcass composition and the best economic value. Agbabiaka *et al.* (2013) reported that some non-conventional feed resources have better economic value than the costly feeds which contained orthodox feedstuffs. From the result of this study, it is suggested that cattle hoofs meal at 25% is suitable for optimum growth of *Clarias gariepinus* and so be incorporated in fish feed as an alternative to expensive protein sources.

Table 4 Growth performance of *Clarias gariepinus* fed experimental diets

Parameter	D1 (Commercial feed)	D2 (0%CHM)	D3 (25%CHM)	D4 (50%CHM)	D5 (75%CHM)	D6 (100%CHM)	
Initial Weight(g)	13.07±0.17 ^a	13.2±0.31 ^a	12.9±0.42 ^a	12.83±0.35 ^a	13.03±0.29 ^a	13.13±0.34 ^a	
Final weight(g)	217.00±10.33 ^a	189.80±4.90 ^{abc}	193.9±7.37 ^{ab}	161.43±8.05 ^{bcd}	156.77±4.5 ^{cd}	142.13±20.01 ^d	
Weight Gain(g)	204.20±10.23 ^a	176.6±5.02 ^{abc}	181.00±7.10 ^{ab}	148.60±8.31 ^{bcd}	143.73±4.79 ^{cd}	129.00±20.02 ^d	
% Weight Gain	1562±68.7 ^a	1340±59.2 ^{abc}	1403±43.55 ^{ab}	1162±98.9 ^{bcd}	1105±61.20 ^{cd}	983±158.06 ^d	
Initial Total Length (cm)	13.07±0.07 ^a	13.07±1.33 ^a	13.13±0.09 ^a	12.97±0.12 ^a	13.07±0.15 ^a	13.00±0.15 ^a	
Final Total Length (cm)	31.00±0.29 ^a	30.13±0.09 ^{ab}	30.43±0.19 ^{ab}	28.83±0.60 ^{abc}	28.17±0.60 ^{bc}	26.67±1.69 ^c	
Total Length Gain (cm)	17.93±0.30 ^a	17.07±0.22 ^{ab}	17.30±0.12 ^{ab}	15.87±0.62 ^{abc}	15.10±0.74 ^{bc}	13.67±1.72 ^c	
% Total Length Gain	135.98±1.24 ^a	130.67±3.03 ^a	131.73±0.74 ^a	122.41±5.14 ^{ab}	115.71±6.86 ^{ab}	105.23±13.66 ^b	
Initial Standard Length (cm)	11.53±0.03 ^a	11.40±0.1 ^a	11.40±0.06 ^a	11.33±0.09 ^a	11.47±0.09 ^a	11.37±0.09 ^a	
Final Standard Length(cm)	27.63±0.45 ^a	27.07±0.07 ^{ab}	27.50±0.20 ^{ab}	25.50±0.76 ^{abc}	24.83±0.73 ^{bc}	23.67±1.67 ^c	
Standard Length Gain (cm)	16.10±0.47 ^a	15.67±0.17 ^a	16.10±0.15 ^a	14.17±0.77 ^{ab}	13.37±0.77 ^{ab}	12.30±1.65 ^b	
% Standard Length Gain	139.62±4.40 ^a	137.47±2.69 ^a	141.22±0.92 ^a	125.03±6.96 ^{ab}	116.58±6.17 ^{ab}	108.19±14.40 ^b	
Condition Factor	0.73±0.02 ^a	0.69±0.01 ^a	0.68±0.01 ^a	0.67±0.01 ^a	0.70±0.03 ^a	0.75±0.04 ^a	

Means with same superscripts along row were not significantly different (p≥0.05)
CHM: Cattle hoofs meal

Table 5 Growth performance and Nutrient Utilization of *Clarias gariepinus* Fed experimental Diets

Parameter	D1 (Commercial feed)	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)	
Specific growth rate (g)	2.68±0.04 ^a	2.54±0.04 ^{ab}	2.58±0.03 ^{ab}	2.41±0.07 ^{bc}	2.37±0.05 ^{bc}	2.25±0.13 ^c	
Relative growth rate (%)	1562±68.7 ^a	1340±59.2 ^{abc}	1403±43.55 ^{ab}	1162±98.9 ^{bcd}	1105±61.20 ^{cd}	983±158.06 ^d	
Survival rate (%)	96.67±3.33 ^a	93.33±3.33 ^a	80.00±5.77 ^a	90.00±5.77 ^a	90.00±5.77 ^a	80.00±15.28 ^a	
Feed conversion ratio (g)	1.31±0.07 ^b	1.51±0.04 ^{ab}	1.47±0.06 ^{ab}	1.65±0.09 ^{ab}	1.69±0.06 ^{ab}	1.85±0.27 ^a	
Protein efficiency ratio (g)	1.81±0.09 ^a	1.62±0.05 ^{ab}	1.67±0.06 ^{ab}	1.49±0.08 ^{ab}	1.49±0.06 ^{ab}	1.38±0.22 ^b	
Feed Intake (g)	266.69	264.36	264.97	244.20	242.20	228.45	
Protein Intake (g)	112.91	108.63	108.13	100.00	97.80	93.32	

Means with same superscripts along row were not significantly different (p≥0.05)

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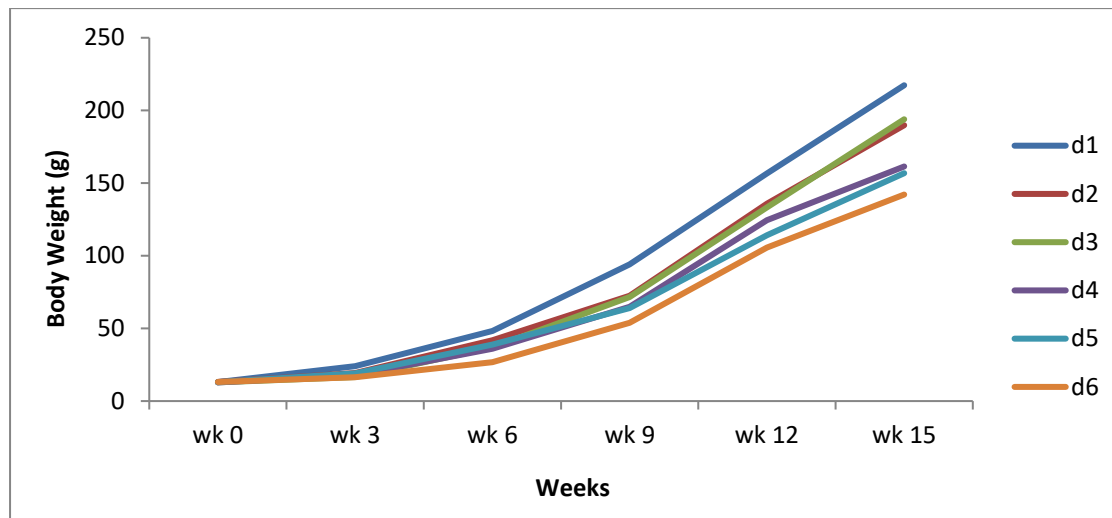


Figure 1. Triweekly growth pattern of *Clarias gariepinus* fed graded levels of Cattle hoof meal

Table 6 Water quality parameters of the Experimental Tank

Parameter	D1 (Commercial feed)	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)
Dissolved oxygen (mg/l)	5.77±0.15 ^a	5.77±0.15 ^a	5.77±0.15 ^a	5.77±0.15 ^a	5.77±0.15 ^a	5.77±0.15 ^a
Temperature (°C)	28.25±0.36 ^a	28.25±0.36 ^a	28.25±0.36 ^a	28.25±0.36 ^a	28.25±0.36 ^a	28.25±0.36 ^a
pH	7.12±0.58 ^a	7.12±0.58 ^a	7.12±0.58 ^a	7.12±0.58 ^a	7.12±0.58 ^a	7.12±0.58 ^a
Ammonia (mg/l)	0.04±0.003 ^a	0.04±0.003 ^a	0.04±0.003 ^a	0.04±0.003 ^a	0.04±0.003 ^a	0.04±0.003 ^a
Nitrite (mg/l)	0.04±0.003 ^a	0.04±0.003 ^a	0.04±0.003 ^a	0.04±0.003 ^a	0.04±0.003 ^a	0.04±0.003 ^a
Nitrate (mg/l)	0.03±0.001 ^a	0.03±0.001 ^a	0.03±0.001 ^a	0.03±0.001 ^a	0.03±0.001 ^a	0.03±0.001 ^a
Bicarbonate (mg/l)	5.45±0.66 ^a	5.45±0.66 ^a	5.45±0.66 ^a	5.45±0.66 ^a	5.45±0.66 ^a	5.45±0.66 ^a

Means with same superscripts along row were not significantly different ($p \geq 0.05$)

Table 7 Cost and Benefit analysis of the Experimental Diets

Parameter	D1 (CF)	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)
Cost of Juvenile(₺)	35	35	35	35	35	35
Cost of Feed/Kg(₺)	480	435.89	350.90	322.70	266.24	207.54
Mean feed Intake (g)	266.67	264.36	264.97	244.20	242.20	228.45
Feeding cost (₺)	128.00	115.23	92.98	78.80	64.48	47.29
Total cost (₺)	153.00	150.23	127.98	113.80	99.48	82.29
Cost of fish/kg (₺)	900	900	900	900	900	900
Feed Intake (g)	266.69	264.36	264.97	244.20	242.20	228.45
Protein Intake (g)	112.91	108.63	108.13	100.00	97.80	93.32
Economic Weight Gain (g)	0.63±0.04 ^{ab}	0.65±0.02 ^a	0.52±0.02 ^c	0.53±0.03 ^{bc}	0.45±0.01 ^{cd}	0.38±0.06 ^d
Profit Index (₺)	1.71±0.08 ^c	1.65±0.04 ^c	2.09±0.08 ^{bc}	2.05±0.10 ^{bc}	2.43±0.07 ^b	3.01±0.42 ^a
Total Sales (₺)	195.54±9.30 ^a	170.82±4.41 ^{ab}	174.51±6.63 ^{ab}	145.29±7.05 ^{bc}	141.09±4.05 ^c	127.92±18.00 ^c
Incident cost (₺)	0.59±0.03 ^a	0.61±0.02 ^a	0.48±0.02 ^b	0.49±0.03 ^b	0.41±0.01 ^{bc}	0.34±0.05 ^c
Benefit Cost Ratio	1.28±0.06 ^{ab}	1.14±0.03 ^b	1.37±0.05 ^{ab}	1.28±0.06 ^{ab}	1.42±0.04 ^{ab}	1.55±0.22 ^a
Net Profit (₺)	42.54±9.3 ^a	20.59±4.41 ^a	46.53±6.63 ^a	31.49±7.25 ^a	41.61±4.05 ^a	45.63±18.01 ^a

CF: Commercial feed; Means with same superscripts along row were not significantly different ($p \geq 0.05$)

CONCLUSION

Substitution of fish meal with Cattle hoof meal at 25% substitution level produced the best growth performance and feed utilization (Weight gain 181g). Though the cattle hoof meal can be included up to 100%, since the fish showed good appetite for all the treatment diets, growth performance of *Clarias gariepinus* decreased with increase in substitution level of fish meal with cattle hoof meal in the diet. Inclusion of cattle hoof meal in the diet does not have detrimental effect on *Clarias gariepinus* as revealed by the survival rate. Also, substitution of fish meal with cattle hoof meal at 25% produced fish with the best mean net profit (#46.53) which reduced price of fish by 36.34% thereby enhancing profit margin.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the entire staff of the Department of Biology, Ahmadu Bello University, Zaria. Similarly, we appreciate the efforts of all the staff of the aquaculture unit of National Agricultural Extension and Research Liaison Services (NAERLS), Ahmadu Bello University, Zaria, Kaduna, which ensured success of the research.

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