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GRADED LEVEL INCLUSION OF MELON SHELL IN THE DIET OF HYBRID CATFISH (*HETEROCLARIAS*) AS ENERGY SOURCE

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ABSTRACT
Utilization of agricultural waste- melon shell by hybrid catfish heteroclarias species 3.92±0.05g was investigated in this research. Five isonitrogenous diets containing 40% crude protein with inclusion levels of melon shell meal (MSM) at 0%, 25%, 50%, 75% and 100% in replacement of maize meal was fed to heteroclarias species for 56 days. The results indicated significant differences ($P<0.05$) in the growth parameters and body compositions. Diet containing 50% melon shell meal gave a significantly high mean weight gain, specific growth rate, protein efficiency ratio, apparent protein utilization and low feed conversion ratio respectively. The experiment supported the inclusion of melon shell meal up to 50% without detrimental effects on the growth of the fish.

KEY WORDS: Melon shell meal, agricultural waste, energy source.

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INTRODUCTION

Fish farming involves raising fish commercially in tanks or enclosures usually for food. However, greatest challenges in aquaculture is in high cost of feed ingredients which makes the feed industries and farmers to compromise quality for affordability (FAO, 2008). Agricultural waste is waste produced at agricultural premises as a result of agricultural activity. Agricultural waste and by-products have been extremely employed in ruminant nutrition in many parts of the world as a substitute for concentrate feeds which are usually very expensive (Akinfemi, 2010). In Nigeria, only a few portions are used by ruminants while the largest proportion are burnt or discarded leading to environmental pollution and health hazards. Ruminants are endowed with the ability to convert low quality feed into high quality protein and utilize feeds from land not suitable for cultivation of crop, but however, the utilization of these quality crop residues are hampered by its low protein content, high fiber, low vitamins mineral and digestibility (Akinfemi, 2010).

Melon called the *colosynthiscitrulluslanatus* is a West African oil seed (Furga, 1981).Where it can still be found in the wild in a diversity of forms together with other citrullus species. In Nigeria, there are two major types, one with small fruits that are generally bitter and mainly used for their seeds, this is the probable ancestor of egusi melon. The other type has fruits that are mainly used as a source of water during periods of drought or as cooking melons. Melon seed comprises overlapping group of cultivars that yield seed or edible fruits. Most important in Africa are cultivars of which the only edible portion is the seed. The fruit pulp of these cultivars is too bitter for human consumption. In West Africa, they are called 'egusi', derived from the Yoruba language, in wolof, they are called 'beref'. In the Kalahari region, the seeds are considered a delicacy. After roasting, they are ground into a coarse, whitish meal, which is nutritious and pleasantly nutty-tasting. Melon seeds contain fairly high amount of unsaturated fatty acid and linoleic acid (Girgis and Said, 1968) suggesting a possible hypo-cholesterolic effect (lowering of blood cholesterol). The oil is used for cooking and for cosmetic purposes and is of interest to the pharmaceutical industry. The residue from oil extraction is made into balls that are fried to produce a local snack called 'robo'in Nigeria, or is used as cattle feed. The oil is used in making soaps and it is also used as substitute for coffee when roasted. The composition of dried egusi seed without shell per bag is:

water 5.1g, energy 2340kg, protein 28.3g, fat 47.4g, carbohydrate 15.3g, Ca 54mg, Fe 7.3mg, Hiiamin 0.19mg Riboflavin 0.15mg, niacin 3.55mg, folate 2mg, ascorbic acid 9.6mg (USDA, 2002).

Mellon shell is a seed casing when slightly twisted by holding the top end and bottom end between the thumb and index finger of each hand and then pulled apart to reveal the white teardrop shaped seed. Science and engineering have been involved to help in shelling the seed shell without destroying the seed itself. Melon shell is light brown in colour and it usually floats in water because it is light in weight Schippers, (2002) and Adekunle, (2008). The shell is considered as part of agricultural wastes, because it is the outer cover of the seed which is normally discarded. This experiment therefore, seeks to replace maize with melon shell as energy source in the practical diets of hybrid catfish (*Heteroclarias)*

MATERIALS AND METHOD

The experiment was carried out in the Step-B Laboratory of Water Resources, Aquaculture and Fisheries Technology of Federal University of Technology Minna, Gidan-Kwano Campus, Niger State, Nigeria.

Hybrid Catfish (*Heteroclarias*) fingerlings with an average weight 3.92±0.05g were transported from T.J.FARM Ilorin Kwara state to the departmental farm and acclimatized in concrete Tank for three weeks. During the period of acclimatization they were fed on catfish commercial diet (Multifeed). At the commencement of the feeding trial which lasted for 56 days, fishes were randomly stocked in triplicate in 15 tanks of a dimension of 30 x 60 x 30cm with stocking rate of 15 fishes per tank. The tanks were filled up with freshwater at a capacity of 20 liters. The treatments were five diets at varying inclusion level of melon shell as energy replacement. The feed stuffs used for the experiment were purchased at Minna, Niger state central market and Ibadan, Oyo state these include maize, groundnut-cake, fishmeal, palm-oil, and vitamin mineral premix while melon shell was gotten from egusi mill at Bida, Niger state. The feed ingredients were milled separately and the feedstuffs were then analyzed for their crude protein, lipid, ash and fiber content according to the method of AOAC (2000). Pearsons square method of feed formulation was used to formulate the five diets with a crude protein level of 40% isonitrogenously. However, the diets contained varying inclusion levels of melon shell meal at 0%, 25%, 50%, 75% and 100% of maize replacement. The feedstuffs were mixed thoroughly with a little quantity of water to form consistent dough for each diet. The dough was then pelleted and oven dried. The proximate chemical analysis for crude-protein, ash, lipid, and fiber content of the five diets were carried out according to the analytical method of AOAC (2000). The fish were fed the test diets at 3% body weight per day. The amount of feed fed was calculated and readjusted fortnightly according to change in the body weight. The fishes were bulk weighed bi-weekly and at the end of the experiment, all fishes were weighed individually and recorded. Water exchange was done on a daily bases with the siphoning of faces and uneaten feed. The water quality parameters were monitored on a weekly bases for temperature using clinical thermometer; dissolved oxygen according to the method of Wrinker's (Lind, 1979 APHA, 1980); Hydrogen iron concentration (pH) was measured using a EIL 7045/46 pH meter in the laboratory at room temperature while conductivity was monitored using conductivity meter.

Chemical analysis

8 fishes were randomly selected and sacrificed for determination of initial carcass composition. And at the end of the feeding trial, 7 fishes from each treatment were collected for determination of final whole carcass composition. General chemical analyses were carried out on feedstuffs, diets and feacal for their proximate analysis for moisture, protein, lipid, ash and crude fibre, using standard procedures (AOAC, 2000).

Moisture

This is gravimetric measurement of moisture in the feedstuffs, diets, and carcass – expressed as a percentage of the initial sample weight. A representative sample was dried to a constant weight in an oven at 110°C. Moisture (%) was expressed as = (W1 – W2) / W1 X100

Crude Protein (CP)

This determined by the Kjeldahl method by taken 0.5g-1.0g of the sample which was digested with 20ml of concentrated sulphuric acid and a Selenium digestion tablet. Heated on a heating mantle until the solution

became clear. The ammonia in the digest were released when reacted with 10ml of 40% Sodium hydroxide during distillation which was trapped in 2% boric acid mixed with methyl red indicator. 50-75ml of distillate was collected and titrated against standard 0.1ml hydrochloric acid. A digest treated the same way was used as the blank titre. Percentage crude protein value was calculated using the titre value for the blank and test samples as follows:

$$\% \text{ crude protein} = \frac{\text{Sample titre} - \text{blank titre} \times 0.1_1 \times 0.014_2 \times 6.25_3 \times 100}{\text{Weight of sample (g)}}$$

Where,
1. Normality of hydrochloric acid
2. Molecular weight of nitrogen
3. Nitrogen factor; since protein is assumed to be 16% nitrogen

Crude Lipid
The method employed was that of solvent extraction using a Soxhlet extractor. Crude lipid in diets was determined by extraction with petroleum ether in feedstuffs and fish carcasses. The extract is collected in a cup and, when the process is completed, the solvent is evaporated and the remaining crude lipid is dried and weighed. Crude lipid was calculated using following formula:

Crude lipid (%) = (extracted lipid/ sample weight) X 100

Crude Fibre (CF)
This method depends upon digestion of moisture free and solvent extracted sample with weak acid solution and then with weak base solution. The remaining residue is ashed and the difference in weight on ashing is considered crude fibre (hydrolysis resistant organic matter). It is expressed as;

Fibre (%) = [(digested sample W1 – ashed sample W2) / sample weight] X100

Ash
Ash content was determined as total inorganic matter by incineration of the sample at 600° C.
Ash content was calculated according to the following formula; Ash (%) = (ash weight / sample weight) X 100

Nitrogen Free Extract (NFE)
Nitrogen free extract (carbohydrate) was calculated by subtracting the total percentages of moisture, crude protein, crude lipid, ash and crude fibre from 100%.

NFE (%) = 100 – (moisture* + protein + lipid + ash + fibre**)
* In case of dry matter basis, moisture was excluded
** Fibre is included here so that NFE represents potentially available carbohydrate

Acid insoluble Ash (AIA)
For the digestibility analysis, Acid insoluble ash of the diets and feacal samples were determined according to Cockrell *et al*., (1987). Fish within each group were pooled for carcass analysis. All samples were analyzed in triplicate. The diet and the feacal samples were ashed at 600^{o}C for 6 hours. After which they were boiled with 250 ml 10% hydrochloric acid (HCl) for 5-10 minutes. The solution was filtered through ashless filter paper and thoroughly washed with hot water. The filter paper including the residue on the filter paper were then put into a dry crucible and placed in a muffle furnace at 600 ^{o}C for 2 hours. The resulting acid insoluble ash were cooled and weighed.

$$\% \text{ Acid insoluble Ash} = \frac{\text{wt. of Acid Insoluble Ash}}{\text{Wt. of sample taken}} \times \frac{100}{1}$$

Proximate Composition of Feedstuffs

Proximate composition of Feedstuffs (%)	Fishmeal	Groundnut cake	Maize meal	Melon shell meal	Palm oil/ Premix
Crude Protein	71.00	44.63	12.68	6.56	0 0
Lipid	15.03	22.00	4.00	7.00	0 0
Ash	9.83	8.93	1.50	5.00	0 0
Crude fibre	1.32	5.48	1.46	25.2	0 0
Moisture	3.40	2.64	3.27	5.20	0 0

Table 1: Formulated diets and their Proximate Composition

	DIETS				
Feedstuffs	D1 (0% MS) Control	D2 (25% MS)	D3 (50% MS)	D4 (75% MS)	D5 (100% MS)
Fishmeal (FM)	28.75	28.75	28.75	28.75	28.75
Groundnut Cake (GNC)	28.75	28.75	28.75	28.75	28.75
Maize meal (MM)	37.5	28.13	18.75	9.38	0
Melon Shell Meal (MSM)	0	9.38	18.75	28.13	37.5
Palm Oil	3	3	3	3	3
Vitamin Premix	2	2	2	2	2
Proximate composition of diets (%)					
Crude Protein (CP)	42.86	43.75	43.31	42.7	43.75
Lipid	21.00	29	12.00	15.00	22.00
Crude Fibre (CF)	1.58		11.52	17.84	
Ash	4.50	9	8.00	8.50	9.00
Moisture content	3.20	2	1.80	3.40	1.80

Biological evaluations

The following parameters were evaluated ccording to Maynard *et al.,* (1979); Bondi (1987) and Halver (1989) as described below

1. Mortality

   This measure the death rate in the fishes as expressed below

   $$\%\text{Mortality} = \frac{\text{No of dead fish}}{\text{No of fishes stocked}} \times \frac{100}{1}$$

2. Specific Growth Rate (SGR) (Brown 1957)

   This is expressed as

   $$\text{SGR} = \frac{\text{Ln MFW (Mean final weight)} - \text{Ln MIW (Mean initial weight)}}{\text{Time in days}} \times 100$$

3. Food Conversion Ratio
   This is expressed as

$$FCR = \frac{\text{Weight of food fed (Dry gram weight)}}{\text{Weight gain of fish (Wet gram weight)}}$$

4. Protein Efficiency Ratio (Osborne *et al.*, (1919)
   This measure the protein efficiency ratio

$$PER = \frac{\text{Weight gain of fish}}{\text{Protein fed}}$$

5. Apparent Net Protein Utilization (ANPU) (Bender and Miller (1953), Miller and Bender (1955)
   This is expressed as

$$\%\ ANPU = \frac{\text{Carcass protein gain (g)}}{\text{Protein fed}} \times 100$$

6. Apparent Digestibility Coefficient (ADC) (Maynard *et al.*, (1979); Bondi (1987). The Acid Insoluble Ash (AIA) was used as internal indicator according to the method of Church and Pond (1988)

$$\%ADC = 100 - \frac{(100 \times \%AIA \text{ of diet} \times \%Nutrient \text{ in faecal})}{\%AIA \text{ of faecal} \times \%Nutrient \text{ in diets}}$$

Statistical analysis

The results for the feeding trial were subjected to one way Analysis of Variance (ANOVA) to establish the significant difference among the treatments. Means were separated using Turkeys test (Steel and Torrie, 1980, Duncan, 1955). Statistical software package Minitab release 14 was used for the statistical analysis while, Microsoft excel package was used for graphical analysis.

RESULTS

From the results obtained (Table 2), there were significant differences ($P<0.05$) in the growth parameters among the diets fed *Heteroclarias*. Diet 1 with 0% melon shell meal and diet 3 with 50% melon shell recorded highest mean weight gain (MWG) of 6.59g and 6.51g respectively with no significant difference ($P>0.05$) between them. However, diets 2, 4 and 5 gave low mean weight gain with no significant differences ($P>0.05$) among them. The feed conversion ratio (FCR) of diets 2, 3, and 5 showed no significant difference ($P>0.05$) while diet 1 and 4 were significantly different from each other ($P<0.05$) as well as other diets. Diet 5 with 100% melon shell has the highest FCR of 1.56 followed by diets 3 and 2 which showed no significant difference ($p>0.05$). Diets 4 and 1 has the lowest FCR of 1.35 and 1.43 which were significantly different from each other ($P<0.05$). The specific growth rate (SGR) of diets 2, 3, and 5 showed no significant differences ($P>0.05$) among each other, while diets 1 and 4 varied significantly from each other ($P<0.05$) as well as other diets. Diet 1 with 0% melon shell recorded the highest SGR value of 1.67 followed by diets 3, 2 and 5 with SGR of 1.56, 1.47and 1.38 respectively which differed insignificantly ($p<0.05$) from each other. Moreover, diet 4 recorded lowest SGR value of 1.27. On the protein efficiency ratio (PER), diets 1, 2, 3 and 4 showed no significant differences ($P>0.05$) in their PER values but diet 5 showed significant difference ($P<0.05$) from other diets. Diet 5 with 100% melon shell meal and 0% maize meal recorded highest PER value of 0.7 followed by diet 1and 3 (100% maize meal, 0% melon shell, and 50% maize meal/melon shell meal respectively) with 0.15 PER value, while diet 4 which contained 75% melon shell meal/25% maize meal had lowest PER of 0.12. However, there were no significant differences ($P>0.05$) among diets 1, 2, 3 and 4. The Apparent net protein utilization (ANPU) of diets 3 and 5 showed no significantly difference ($P>0.05$) with each other while diets 1, 2, and 4 varied significantly from other diets ($P<0.05$). Diet 2 recorded the highest ANPU with 7.84 followed by diet 6 and 3 while diet 1 recorded the lowest ANPU value of 4.27.There was no record of mortality for diets 3, 4, and 5 ($P<0.05$) while diets 1and 2 had 6.67% and 2.22% mortality with significant difference ($P<0.05$). The growth response curve in figure 1 demonstrated the growth pattern of the diets which showed that diets 1 and 3 performed at almost level. Table 3 showed the body composition of the initial and final carcass. There were significant differences in the final body composition ($P< 0.05$). Diet 2 with 75% maize meal and 25% melon shell meal recorded highest crude protein (CP) of 60.30 with a significant difference from other diets ($p < 0.05$).

However, diet 1, 3, 4 and 5 showed no significant difference from each other (P>0.05). The crude lipid of diet 3 and 4 showed no significant difference (P>0.05) while diet 1, 2 and 5 were significantly different from each other (P< 0.05). Diet 1 with 100% maize meal had the highest lipid value of 27.82 followed by diet 3, 4 and 2 respectively while diet 5 recorded lowest value of 18.76.

The ash content of diet 3 and 4 showed no significant difference from each other while diets 2 and 5 showed significant differences from other diet (P< 0.05). Diets 2 and 5 recorded the highest ash content with 12.33 and 12.03 respectively, which differed significantly from other diets (p < 0.05)

Table 4, showed the apparent digestibility coefficient (%) of the diets fed to the fish which showed significant differences (P<0.05).There were no significant differences (P>0.05) among diets 1 to 5 which were significantly higher (P<0.05) than initial value. Diet 1, moreover gave high lipid digestibility (27.83%) while diet 5 exhibited low digestibility value (18.76%). The ash values were also significant (P<0.05). Diet 2 and 4 gave significantly high (P<0.05) ash values (12.32 and 12.01 respectively) which were not significantly different (P>0.05) from each other. Finally diets 4 recorded high digestibility of dry matter (4.12%) while diets 1, 2, and 3 gave low values which were not significantly different (P>0.05) from each other.

DISCUSSION

Hybrid catfish (*Heteroclarias*) fed graded inclusion levels of melon shell meal exhibited utilization of the meal. Diets 3 containing 50% melon shell meal performed as good as diet 1 containing 100% maize meal. The performance was an indication of positive contribution to growth of the fish as opined by Houlihan *et al.* (2001). The growth curve showed that (Figure 1), diet 3 peaked faster than other diets, while diet 2 was the slowest in growth phase. This could be attributed to balance energy sources from maize and melon shell inclusion levels of 50% each compared with diet containing high or low inclusion levels of melon shell meal.

The growth curves from week 0-2 represented the lag growth phase while, week 6-8 represent the exponential growth phase. This exponential growth was a direct impact of the diet on the growth of the fish (Hoelihan *et al.*, 2001). The poor values observed for FCR, SGR, PER, MWG in diets 2, 4, and 5 were indication of inefficient utilization of diets. Diet 2 recorded the highest ANPU value with low FCR which implied adequate utilization of the diet. The implication of this is that, as the fishes grow bigger the rate at which the conversion of feed to flesh decreases. This is not good enough especially at fingerlings stage when the fish is still going through the lag phase. The slowdown in growth, could be attributed to the high fibre level of melon shell meal in the diets as the percentage inclusion level increases. Clarias has been reported to have poor handling of high level of fibre in its diets (Cruz, 1975). Moreover, the study revealed that, the hybrid clarias tolerated up to 50% melon shell meal despite the fibre content. This was achieved due to the high digestibility of its fibre content (Table 4).

There are also some relationships that featured in the carcass composition showed in Table 3, where the protein, lipid, Ash and moisture content showed significant difference (p<0.005) to the initial values. The performance of the diet is in agreement with the work of Jauncey (1998) who stated that carcass composition should reflect the diet. The apparent digestibility for crude protein in the present study is justified by the report of Jauncey (1998), who observed that apparent digestibility coefficient for crude protein of fish meal in carp was 88.9%. This present study showed that *Heteroclarias* catfish conveniently digest the protein in melon shell meal based diet as high as 95.11%. The digestibility of the nutrients contained in the treatments were optimal however, even at up to 100% melon shell meal inclusion level digestibility was still significant which was evident on the growth recorded. This is however, contrary to the report of FAO (1990) which stated that high fibre may reduce the digestibility of food. On the other hands, fibre content of melon shell could be established as digestible type.

Table 2: Growth Parameters of hybrid *Heteroclarias* spcies fed melon shell meal as energy source for 56 days.

Growth parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SD±
Mean Initial weight gain {MIW(g)}	4.28^{a} ±0.94	4.2^{a}1±0.78	4.90^{b}±1.57	4.70^{b}±0.63	4.47^{b}±0.21	0.94
Mean Weight gain {MWG (g)}	6.59^{a}±2.68	5.58^{b}±2.67	6.51^{a}±1.05	5.31^{b}±4.37	5.59^{b}±3.09	2.95
Feed Conversion Ratio (FCR)	1.35^{a}±0.25	1.53^{b}±0.13	1.55^{b}±0.03	1.43^{c}±0.13	1.56^{b}±0.02	0.14
Specific Growth Rate (SGR(%/day)	1.67^{a}±0.54	1.47^{a}b±0.40	1.56^{a}b±0.43	1.27^{c}±0.83	1.38^{a}b±0.83	0.58
Protein Efficiency Ratio (PER)	0.15^{a}±0.06	0.13^{a}±0.5	0.15^{a}±0.03	0.12^{a}±1.04	0.70^{b}±1.04	0.47
Apparent Net Protein utilization (ANPU %)	4.27^{b}±0.01	7.84^{a}±0.01	5.679^{b}±0.01	4.81^{bc}±0.01	6.01^{b}±0.01	0.01
% Mortality	6.67^{a}±6.66	2.22^{b}b±3.84	0.00^{c}±0.00	0.00^{c}±0.00	0.00^{c}±0.00	3.44

Mean data on the same raw carrying different superscripts differ significantly from each other ($p<0.05$)

Table 3: Body composition of *Heteroclarias* fed graded levels of melon shell meal (MSM) for 56 Days

Proximate composition (%)	Initial Body Composition (%)	Final Body Composition (%) D1	D2	D3	D4	D5	SD±
Crude protein (CP)	$56.88^{c}\pm0.01$	$58.70^{b}\pm0.01$	$60.30^{a}\pm0.01$	$59.33^{b}\pm0.01$	$58.93^{b}\pm0.01$	$59.50^{b}\pm0.01$	0.01
Crude Lipid	$21.03^{c}\pm0.06$	$27.82^{a}\pm0.01$	$22.40^{bc}\pm0.01$	$24.33^{b}\pm0.01$	$24.20^{b}\pm0.02$	$18.76^{d}\pm0.02$	0.03
Ash	$9.50^{c}\pm0.01$	$9.78^{c}\pm0.02$	$12.3^{a}\pm0.01$	$11.36^{b}\pm0.01$	$11.44^{b}\pm0.01$	$12.03^{a}\pm0.05$	0.03
Moisture	$3.60^{b}\pm0.01$	$2.82^{c}\pm0.01$	$5.01^{c}\pm0.01$	$2.56^{d}\pm0.01$	$4.12^{a}\pm0.01$	$3.33^{b}\pm0.01$	0.11

Table 4: Apparent Digestibility Co-efficient (ADC%)

% ADC	Initial	Treatments Diet 1 (0% Melon shell meal)	Diet 2 (25% Melon shell meal)	Diet 3 (50% Melon Shell Meal)	Diet 4 (75% Melon Shell Meal)	Diet 5 (100% Melon Shell Meal)
Crude protein (CP)	$56.8^{b}7\pm0.02$	$59.03^{a}\pm0.59$	$60.30^{a}\pm0.01$	$59.33^{a}\pm0.01$	$58.93^{a}\pm0.01$	$59.50^{a}\pm0.01$
Lipid	$21.33^{c}\pm0.58$	$27.83^{a}\pm0.01$	$22.40^{c}\pm0.01$	$24.33^{b}\pm0.01$	$24.20^{b}\pm0.01$	$18.76^{d}\pm0.01$
Ash	$9.50^{c}\pm0.01$	$9.79^{c}\pm0.01$	$12.32^{a}\pm0.01$	$11.36^{b}\pm0.01$	$11.43^{b}\pm0.01$	$12.01^{a}\pm0.01$
Moisture content	$3.60^{b}\pm0.01$	$2.83^{c}\pm0.01$	$3.02^{c}\pm0.01$	$2.56^{c}\pm0.01$	$4.12^{a}\pm0.01$	$3.33^{b}\pm0.01$

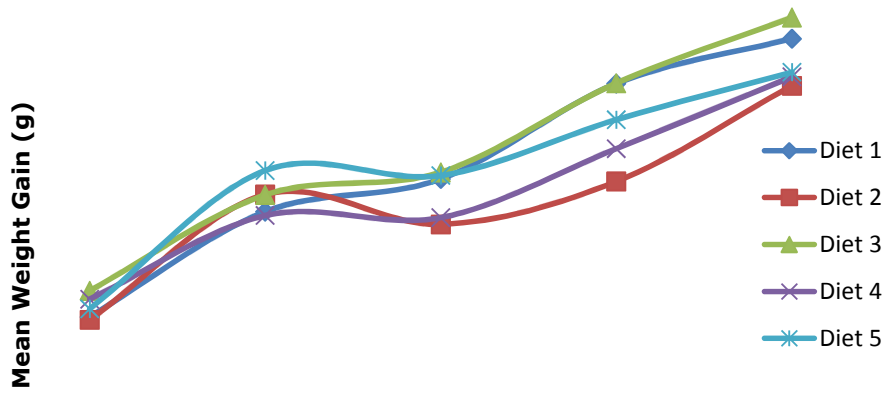

Fig. 1: Growth response of *Heteroclarias bidorsalis* fed Melon Shell Based Diets for 8 weeks

CONCLUSION

From the experiment 50% melon shell meal inclusion level in the diet of *heteroclarias* was utilized efficiently for its growth. This indicated that, melon shell meal could replace maize up to 50% in the fish feed composition without any adverse effect.

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