Optimum Dietary Crude Protein and Digestible Energy Requirements for fingerlings of Hybrid Clariid catfish *Heterobranchus bidorsalis♀ X Clarias gariepinus♂* in the Tropics

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Abstract: **This study was carried out to determine the optimum dietary crude protein and digestible energy requirements for the *Heterobranchus bidorsalis♀ X Clarias gariepinus♂* fingerlings. The general objective of the study was to determine the optimum protein and digestible energy levels for the Clariid catfish fingerlings, *Heterobranchus bidorsalis♀ X Clarias gariepinus♂*, using locally sourced feed inputs. *Heterobranchus bidorsalis♀ X Clarias gariepinus♂* fingerlings were reared from hatchlings to five weeks old. Sixteen practical diets were formulated and used in the feeding trials. The diets were made up of four digestible energy levels (2400, 2600, 2800 and 3000Kcal/Kg), each at four crude proteins levels (25, 30, 35 and 40%) and were fed to the fingerlings for 70days in three replicates for each treatment. Weekly data were collected based on weight gain and feed consumption. Feed and fish carcasses were analyzed for proximate composition of the fingerlings. All data collected were subjected to two-way analysis of variance (ANOVA) test at 5% probability level.**

The total weight gain of fingerlings increase in dietary protein levels at all energy levels used in the experiment. The increases were significant in all but one and which was that between 35% and 40% crude protein at the DE of 3000kg/kg diet. The highest total weight gain (148.84/4) was obtained in fish led with the diet containing 40% crude protein at digestible energy of 2400kcal/kg diet. The values obtained at 40% crude protein and DE of 2400kcal/kg diet were significantly different (P<0.05) from those obtained on all the other dietary protein and energy levels treatments.

The lower protein levels (25 and 30%) resulted in very low weight gain. The trends in effects of dietary treatments on SGR, RGR and RWG were similar to those described for weight gain. At dietary protein levels of 35% and 40%, the amounts of feed consumed were significantly higher than those recorded at dietary protein levels of 25% and 30%. At DE of 2400 and 2800kcal/kg, the amounts of feed consumed by fish fed with the 35% crude protein diets were significantly lower than those of fish fed with the diet containing 40% crude protein. However, at DE of 2600 and 3000kcal/kg. FCR values increased with increase in dietary crude protein level and decreased with increase in dietary energy level with the exception of 2800kcal/kg diet while the PER Values decreased with increase in dietary protein levels. Protein intake increased with increases in protein level while protein intake decreased with increases in caloric intake.

Keywords: **Clariid catfish, *Clarias gariepinus*, fingerlings, optimum energy, protein levels, fish diet.**

I.INTRODUCTION

Feed formulation is a central operation in intensive catfish production, ensuring that feed ingredients are economically used for optimum growth (Robinson *et al*., 2001); The crucial role of feed production cannot be over emphasized as demonstrated in several studies (NRC, 1983; Fasakin *et al*., 2003; Gabriel *et al*., 2007). However, aquaculture expansion has been a slow process as private sector fish farmers have faced major constraints including lack of seed and quality feed (Akolisa and Okonji, 2005). For any aquaculture venture to be profitable it must have a regular and adequate supply of balanced artificial diets for the culture fishes (Faturoti and Akinbote, 1986). As fish requires high quality nutritionally balanced diet for growth and attainment of market size within the shortest possible time. Therefore local production of fish feed is very pivotal to the development and sustainability of commercial aquaculture. A standard feed mill should have a mixer, pelleting machine, hammer mill, grinder and other minor accessories. Fish feed technology is one of the least developed sectors of aquaculture particularly in Africa and other developing countries of the world (FAO, 2003).

According to Aremu (2006), Protein comprises about 70% of the dry weight of fish muscle. A continual supply of protein is needed throughout life for maintenance and growth. At maintenance level, the fish requires for replacement of worn-out tissues and proteinous products such as internal epithelial cells; Enzymes and hormones, which are vital for the proper function of the body, and are recycled quite rapidly. Also, Carbohydrate is one of the most important parts of fish diet (Robinson *et al.,* 2006). Carbohydrates are the main form of energy stored in seeds, roots and tubers. Carbohydrates have several functions in animals. Although catfish do not have a specific need for carbohydrates in their diet (Raj *et al*., 2008), catfish feeds contain considerable amounts of carbohydrates that are supplied from grains or grain by-products that are rich in starch. Starch is not only an inexpensive energy source but also aids in feed manufacture. Normally catfish feed contains 25 % or more soluble carbohydrates plus 3 to 6 % less soluble carbohydrates that are in general present as crude fiber (Hogendoorn *et al*., 1983). African catfish cannot digest crude fiber well, so it should be kept at as low a level as possible.

II. MATERIALS AND METHODS

This study was conducted in the Experimental fish farm of the Department of Fisheries, University of Benin, Benin City, Nigeria to ascertain the optimum protein and digestible energy levels for *Heterobranchus bidorsalis♀ X Clarias gariepinus♂* fingerlings

Experimental Diets

Sixteen (16) diets were prepared for the feeding trials. The diets were formulated containing four digestible energy (DE) levels of 2400, 2600, 2800 and 3000Kcal/kg, each at four (4) crude protein levels of 25, 30, 35 and 40%. The layout of the dietary treatment is shown in Table 1. Each diet constituted a treatment. The detail of nutrient composition of feedstuffs of experimental diets and proximate analysis is shown in Table 2. The levels of feed ingredients used to formulate the diets were manipulated to obtain the desired levels of DE and CP. Calculation of the DE levels of the diets were based on the cumulative of DE of the ingredients as recommended for channel catfish by Lovell (1984). For the crude protein, lysine and methionine, the various recommended by New (1987) were used. These values are shown Table 2.

In preparing the diets, ingredients were milled, mixed and prepared as described by Martinez-Palacios *et al*, (1996). The milled ingredients were sieved through standard sieve Nos. 16 and 20 (maximum of 1.19mm). The homogenous feed mixes were processed into pellets or granules (2 mm) with gelatinized corn starch component as the binder. After preparation, pelleted diets were oven-dried at 70^0c for 24 hours. Feed samples were stored in polythene bags in cupboard at laboratory temperature. Dried granules of feed samples were taken for proximate analysis. All ingredients were locally sourced for the trial conducted.

Table 1:

Digestible Energy (DE Kcal/Kg)	Diets (% Crude protein)			
	25%	30%	35%	40%
2400	2400(1)	2400(5)	2400(9)	2400(13)
2600	2600(2)	2600(6)	2600(10)	2600(14)
2800	2800(3)	2800(7)	2800(11)	2800(15)
3000	3000(4)	3000(8)	3000(12)	3000(16)

NB: Numbers in parenthesis represent the various treatment codes.

Table 2: Ingredient composition and proximate Analysis of Experimental Diets (%)

Ingredient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Maize	29.79	27.29	24.79	22.29	18.29	19.20	19.79	17.79	24.44	22.94	20.44	17.44	3.44	6.14	13.44	10.94
Fishmeal	7.00	7.00	7.00	7.00	14.50	14.50	14.50	14.50	21.40	21.40	21.40	21.40	26.40	26.40	26.40	26.40
Soybean meal	16.77	16.77	16.77	16.77	18.77	18.77	18.77	18.77	20.20	20.20	20.20	20.20	23.20	24.20	24.20	24.20
Brewers yeast	12.77	12.77	12.77	12.77	20.20	17.86	14.77	14.77	16.40	16.40	16.40	16.40	27.40	25.40	18.10	18.40
Wheat bran	27.58	27.58	27.58	27.58	23.60	23.08	22.58	22.58	14.10	14.10	14.10	14.10	16.10	14.40	14.40	14.10
Soybean oil	2.63	5.13	7.63	10.13	1.18	3.13	6.13	8.13	0.00	1.50	4.00	7.00	0.00	0.00	0.00	2.50
Bonemeal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vit. Premix	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin E	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Analyses																
DE calculated	2400	2600	2800	3000	2400	2600	2800	3000	2400	2600	2800	3000	2400	2600	2800	3000
CP calculated	25.0	25.0	25.0	25.0	30.0	30.0	30.0	30.0	35.0	35.0	35.0	35.0	40.0	40.0	40.0	40.0
CP Analysed	24.92	24.68	24.44	24.20	32.51	31.54	31.54	30.02	35.54	35.33	35.15	34.86	43.47	43.10	40.54	40.38
Moisture (%)	8.08	8.14	8.05	8.31	8.16	8.09	8.06	9.06	9.07	8.82	8.91	8.69	8.71	8.84	9.01	9.02
Lipid (%)	3.51	6.03	8.09	11.01	2.05	3.56	6.01	8.57	1.59	3.41	4.91	7.53	2.05	2.31	2.45	4.70
Crude fibre(%)	7.69	7.81	7.07	7.41	7.43	7.38	7.49	7.47	5.64	5.61	5.60	6.01	5.07	4.91	4.03	4.01
Ash (%)	8.01	8.03	8.41	8.50	8.09	8.61	8.19	8.08	7.72	7.69	7.71	7.81	7.70	7.57	7.49	7.53
Lysine calculated	5.68	6.54	6.54	6.51	7.83	7.90	9.30	8.41	7.49	7.46	7.43	7.37	8.30	8.65	8.30	6.17
Methionine calculated	2.76	3.40	3.40	3.50	3.77	3.76	5.33	4.02	3.59	3.54	5.52	4.25	3.83	4.20	4.20	3.58

There were four trials, one trial for each type of feed. Glass tank was used for the trials. Each tank was connected to a central aerator. Water supplied by the university of Benin Campus domestic water services was maintained at 35 litre mark/level throughout the experiment. Fingerings were fed test diets twice daily during daylight (9:30 am and 4:00pm). At each time of feeding, animals were fed to satiation i.e. hand fed access to food, during which diet was provided in small amount at a time, so that the fish will eat nearly all the diet offered. Water temperature was measured twice daily during feeding. Dissolved oxygen (DO) was measured once a week using Winkler's method. Daily observations were made to detect any abnormality and fish mortality. Unconsumed diets and faecal wastes were removed by siphoning daily. Each trial lasted 70days. Weight of fish per treatment and per replicate was recorded weekly. Weight of food consumed by fish was also recorded weekly for each replicate. In order to obtain the weights of the fish, fish were batch weighted in a dish containing pre-weighed water.

Carcass Analysis

All the diets and carcasses were subjected to proximate analysis at the end of the trials. Crude protein (N X 6.25) was determined by the micro-kjeldahl method and crude fibre (CF) was by the system based on acid-alkaline digestion. Lipids, ash and moisture were determined using standard methods in triplicate.

Growth and Nutrient Utilization indices

Weights of fish and feed consumption were obtained at weekly intervals. From the fish weights and feed consumption, the following were determined:

Weight gain = $W_1 - W_0$ (g)

Relative Weight Gain (RWG%)=$(W_1 - W_0) / W_0 \times 100$ (%)

Specific Growth Rate (SGR %)= $\{(\ln W_1 - \ln W_0)/ T\} \times 100$ (%/week)

Where;

W_0: mean initial weight (g)

W_1: mean final weight (g)

T: time in 7 days between weightings

Feed conversion ratio (FCR) = feed intake (g) / wet weight gain (g)

Protein efficiency ratio (PER) = weight gain (g) / protein intake (g)

Net protein utilization (NPU) = $\{(BP_1 - BP_0)/ CP\} \times 100$

Where;

BP_0: Initial body protein content (g)

BP_1: Final body protein content (g)

CP: Protein intake (g)

Statistical Analysis

At the end of the experiments, recorded data were subjected to two-way ANOVA test using a Genstat software eight edition, 2005 package for statistical problems. All the means we're compared at 5% level of probability with Duncan multiple range tests. Similarly, responsiveness of fingerlings to treatments was evaluated.

III. RESULT

The total weight gain of fingerlings increase in dietary protein levels at all energy levels used in the experiment. The increases were significant in all but one and which was that between 35% and 40% crude protein at the DE of 3000kg/kg diet. The highest total weight gain (148.84/4) was obtained in fish led with the diet containing 40% crude protein at digestible energy of 2400kcal/kg diet. The values obtained at 40% crude protein and DE of 2400kcal/kg diet were significantly different ($P<0.05$) from those obtained on all the other dietary protein and energy levels treatments. The lower protein levels (25 and 30%) resulted in very low weight gain. The trends in effects of dietary treatments on SGR, RGR and RWG were similar to those described for weight gain.

At each DE level, the differences in feed intake by fish fed diets containing 25 and 30%o CP was not significant. Feed intakes on both protein levels were very low. At dietary protein levels of 35% and 40%, the amounts of feed consumed were significantly higher than those recorded at dietary protein levels of 25% and 30%. At DE of 2400 and 2800kcal/kg, the amounts of feed consumed by fish fed with the 35% crude protein diets were significantly lower than those of fish fed with the diet containing 40% crude protein. However, at DE of 2600 and 3000kcal/ke. the amounts of feed consumed by fish fed with the 35 and 40% crude protein were not significantly different ($P>0.05$). Generally, beyond 2600kcal/kg diet, feed intake reduced significantly. Specifically, feed intake reduced when the DE levels was increased from 2400 to 2600Kcal/kg at crude protein levels of 25% and 30% but increased at crude protein levels of 35 and 40%.

Feed conversion ratios (FCR) values were significantly affected by treatments. When the FCR values were summarized on the basis of protein levels only or DE levels only, the FCR values obtained were significantly affected dietary crude protein and DE levels. FCR values increased with increase in dietary crude protein level and decreased with increase in dietary energy level with the exception of 2800kcal/kg diet.

The PER Values decreased with increase in dietary protein levels. Protein intake increased with increases in protein level while protein intake decreased with increases in caloric intake. The differences in dietary crude protein or energy levels did not significantly affect the carcass composition. The initial and final carcass compositions were not significantly different ($P>0.05$).

Table 3: Effect of dietary protein and energy levels on growth performance and feed utilization by *Heterobranchus bidorsalis*♀ *X Clarias gariepinus*♂fingerlings

Parameter	Protein (%)					DE Kcal/kg				
	25	30	35	40	SEM	2400	2600	2800	3000	SEM
Total Weight gain	50.58^{d}	61.97^{c}	111.93^{b}	131.60^{a}	0.88	88.43^{b}	84.33^{d}	86.12^{c}	97.20^{a}	0.84
Relative weight gain	786^{c}	1001^{c}	1686^{b}	2116^{a}	111.41	1348^{NS}	1390^{NS}	1386^{NS}	1465^{NS}	113.30
Absolute growth rate (g/fish/day)	0.18^{d}	0.22^{c}	0.40^{b}	0.47^{a}	0.01	0.30^{c}	0.30^{c}	0.31^{c}	0.35^{a}	0.01
Specific growth rate (SGR)	1.36^{d}	1.48^{c}	1.82^{b}	1.92^{a}	0.04	1.64^{b}	1.63^{b}	1.59^{d}	1.71^{a}	0.03
Feed intake (g)	52.50^{c}	65.40^{c}	141.90^{b}	178.80^{a}	8.87	113.40^{a}	125.40^{a}	87.60^{b}	107.89^{b}	8.85
Feed conversion ratio (FCR)	1.04^{a}	1.06^{b}	1.27^{d}	1.36^{d}	0.02	1.28^{a}	1.46^{d}	1.02^{a}	1.11^{b}	0.01
Crude protein intake (CP) (g)	13.13^{d}	19.62^{c}	49.67^{b}	71.52^{a}	0.46	44.70^{b}	45.36^{a}	31.74^{c}	24.39^{d}	0.43
Protein efficiency ratio (PER)	3.85^{a}	3.16^{a}	2.25^{c}	1.84^{d}	0.14	1.98^{d}	1.86^{c}	2.68^{b}	3.98^{a}	0.11
Net protein utilization (NPU) (%)	48.74^{b}	43.42^{b}	32.43^{c}	28.78^{c}	11.50	27.85^{c}	26.33^{bc}	38.47^{b}	54.40^{a}	11.03

Within protein or energy levels, values in a column with similar superscripts are not significantly different ($P>0.05$)

Table 4: Effect of varying dietary levels of protein and energy on the growth performance and feed utilization by *Heterobranchus bidorsalis*♀ *X Clarias gariepinus*♂ fingerlings

Dietary Treatment		TWG	RGR	AGR	RWG	SGR	FI	FCR	PI	PER	NPU
A	2400Kcal/kg										
	25% protein	52.70^{ij}	0.10^{g}	0.19^{ij}	664^{f}	1.40^{f}	57.60^{d}	1.09^{c}	13.18^{d}	3.99^{f}	53.50^{a}
	30% protein	56.10i	0.12^{efg}	0.20^{h}	855^{ef}	1.40^{f}	59.40^{d}	1.06^{c}	16.83^{c}	3.33^{g}	44.98^{a}
	35% protein	96.10^{f}	0.22^{bcde}	0.34^{e}	1541^{cd}	1.76^{d}	162.60^{bc}	1.81^{h}	33.64^{a}	2.26^{i}	40.81^{b}
	40%protein	148.84^{a}	0.35^{a}	0.53^{a}	2334^{a}	2.01^{a}	174.00^{a}	1.17^{g}	59.54^{a}	2.50^{i}	36.86^{b}
B	2600Kcal/kg										
	25% protein	49.88^{i}	0.12^{fg}	0.18^{j}	805^{ef}	1.39^{f}	58.10^{de}	1.16^{d}	14.53^{f}	4.00^{a}	53.00^{a}
	30% protein	53.25^{ij}	0.12^{efg}	0.19^{hi}	851^{ef}	1.40^{f}	57.50^{de}	1.07^{c}	15.98^{e}	3.33^{b}	4.49^{a}
	35% protein	96.42^{f}	0.24^{bcd}	0.34^{c}	1677^{cd}	1.79^{cd}	19.90^{ab}	2.03^{h}	33.75^{a}	2.86^{i}	40.92^{b}
	40%protein	137.77^{b}	0.31^{ab}	0.49^{b}	2219^{ab}	1.94^{a}	230.10^{a}	1.67^{h}	55.11^{a}	2.50^{i}	36.58^{a}
C	2800Kcal/kg										
	25% protein	44.08^{k}	0.108^{fg}	0.157^{k}	754^{ef}	1.19^{g}	58.40^{de}	1.32^{e}	11.02^{f}	4.00^{c}	52.90^{a}
	30% protein	61.61^{h}	0.14^{defg}	0.22^{g}	1011^{ef}	1.49^{f}	64.90^{e}	1.05^{b}	18.47^{g}	3.34^{c}	46.18^{a}
	35% protein	116.05^{e}	0.21^{bcdef}	0.41^{d}	1750^{cd}	1.80^{cd}	103.80^{c}	1.69^{f}	40.82^{b}	2.90^{g}	41.88^{a}
	40%protein	122.72^{c}	0.28^{abc}	0.44^{c}	2029^{abc}	1.87^{bc}	183.91^{b}	1.88^{g}	49.090^{a}	2.50^{i}	36.28^{b}
D	3000Kcal/kg										
	25% protein	55.65^{i}	0.13^{efg}	0.20^{h}	922^{ef}	1.44^{f}	55.90^{e}	1.00^{a}	13.91^{i}	4.00^{a}	54.49^{a}
	30% protein	76.25^{g}	0.18^{cdefg}	0.27^{f}	1279de	1.63^{e}	69.80^{d}	0.92^{a}	22.87^{h}	3.33^{b}	47.14^{a}
	35% protein	109.16^{b}	0.31^{ab}	0.50^{b}	1774^{bcd}	1.93^{a}	106.0^{c}	0.97^{cd}	38.21^{b}	2.86^{f}	41.19^{a}
	40%protein	117.09^{d}	0.24^{bcd}	0.42^{d}	1883^{abc}	1.84^{bcd}	127.02^{c}	1.09^{c}	46.84^{a}	2.50^{h}	36.42^{b}
	SEM	1.67	0.03	0.01	22.70	0.05	5.89	0.09	0.26	0.22	22.10

Within protein or energy levels, values in a column with similar superscripts are not significantly different ($P>0.05$)

TWG	-	Total Weight gain	FI	-	Feed intake (g)
AGR	-	Absolute growth rate (g/fish/day)	FCR	-	Feed conversion ratio (FCR)
RWG	-	Relative weight gain	CP	-	Crude protein intake (CP) (g)
SGR	-	Specific growth rate (SGR)	PER	-	Protein efficiency ratio (PER)
RGR	-	Relative growth rate (RGR) (g/day)	NPU	-	Net protein utilization (NPU) (%)

IV. DISCUSSION

For the reciprocal cross, *Heterobranchus bidorsalis*♀ X *Clarias gariepinus*♂, the highest weight gain, SGR and AGR Were obtained on the 40% crude protein diet at all energy levels (2400, 2600, 2800 KcalDE/kg) tested but one (3000 kcal DE/kg). Thus 40% CP level found optimum in these studies could be considered optimum crude protein level for the reciprocal crosses. The levels of energy recommended for fish are lower than those recommended for poultry and pigs (land animal) in Nigeria (Babatunde et al., 1972, Olomu, 2011). Possible reasons, for this include the fact that fish exert relatively less energy to maintain position and movement in water than terrestrial mammals and birds (Tucker, 1969). The amount of energy required for protein synthesis is much less for fish than for poultry, pigs and rabbit (Lovell, 1989) and the fact that fish do not have to maintain a constant body temperature and so do not need much heat energy which serves the purpose of maintaining body temperature in warm blooded animals because fish body temperature corresponds to the environmental water temperature (Piper et al., 1989). Another reason is that less energy 1s lost in protein catabolism and excretion of nitrogenous wastes in fish since fish excrete most of their nitrogenous wastes as ammonia instead urea or uric acid (Goldstein and Forster, 1970). As a result of the relatively higher Crude protein requirements and lower requirements for fish the calories/protein of 6.00 to 7.43 is much lower than 9.3 to 16.25 for poultry and 14.6 to 19.30 for pigs (Olomu, 2011).

The linear increase in SGR with increase in dietary crude protein (CP) level up to the required level of CP (35 and 40%) is in agreement with the findings of Madu et al. (1992) with *Clarias anguillaris* and Dada *et al.* (2001) with *Heterobranchus bidorsalis*, who reported linear increase in crude protein level up to 40% and decline in SGR thereafter. A linear relationship between optimum crude protein requirement and SGR was reported by Tacon and Cowey (1985). In all cases except 3000kcal/kg diet for the crosses, SGR decreased with increase in dietary energy level. However, when the crude protein levels were considered alongside the DE levels, the linear relationship between SGR and CP levels or between SGR and DE to the relationship between dietary levels were not clear cut. This may possibly be due to the relationship between dietary levels were CP and DE and is a further support of the view that the effects of CP on performance of fish should not be considered independent of each other. More studies need to be conducted to elucidate the effects of diet energy level or calorie/protein ratios on growth indices.

The general trend of decreasing PER and NPU with increasing dietary crude protein is in agreement with previous findings with other fish species (Cowey *et al.,* 19974, Murray *et al.*, 1977, Davis and Stickney, 1978; Jauncey, 1982; Martinez-Palacios *et al.*, 1986). Considering the DE level alone, PER and NPU increased with increase in dietary DE. The probable explanation is that as the energy level in the diet increases, less or no protein is diverted for use as energy. Protein is thus available for use for growth purposes and this means higher protein efficiency in promoting weight gain. However, when the crude protein levels were considered alongside the DE levels, the linear relationship between PER/NPU and CP level or between PER/NPU and dietary DE level were not precise. More studies are required to further elucidate this relationship by considering both protein and energy levels or the calorie/protein ratios. The result of the present study suggests that PER and NPU are probably not sensitive enough for determining the optimum level of crude protein in fish diets.

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