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Nutritional composition of categorised artificially spawned *Heterobranchus longifilis* Valenciennes, 1840 f1

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Abstract

The nutrient composition of three growth classes of eight-week old juveniles, twenty-eight week old adult; and male and female table-size *Heterobranchus longifilis* was determined to ascertain the effect of these variables on nutritional status of fish. The moisture, protein, fat, ash, fiber, nitrogen free extract and caloric values of the samples were analysed as described by the Official Methods of Analysis of the Association of Official Analytical Chemists. Whole carcass analysis amongst the juveniles revealed combined mean percentage values of 73.54 ± 1.30, 9.76 ± 0.61, 13.8 ± 1.17 for moisture, protein and fat, respectively. Proximate analysis of muscle samples from fishes representing the growth classes of 22-week *H. longifilis* F1 revealed combined mean values of 18.89 ± 1.01%, 2.34 ± 0.42% and 98.40 ± 6.48 kilocalories for protein, fat and energy respectively. Protein content in fish was unaffected by growth rate and sex. Differential growth rate was influenced by feed accessibility and utilization as evidenced by low fat in the "runs" compared to other growth classes.

Keywords: Growth classes, *Heterobranchus longifilis*, nutritional status, proximate analysis
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Introduction

Fish protein provides a good combination of amino acid which is highly suited for man's nutritional requirements (Murray and Burt, 2001). There is considerable evidence that fish and fish oils are beneficial to heart health, reduce the risk of cancer and benefit mental health (Pawlosky, 2001).

The "active" components of fish oils are exosapentaenoic acid (EPA), a polyunsaturated fatty acid and docosahexaenoic acid (DHA), are members of the omega-3 group of essential fatty acids (Pawlosky, 2001). Danish researchers reported that women who consume fish or sea food once a week during the first 16 weeks of pregnancy has 3-6 times lower risk of giving birth to a low birth weight (less than 2500 grams) or premature (born before 259 days) baby than women who never consume fish or sea food (Olsen and Secher, 2002).

Tropical freshwater fish are comparable to temperate freshwater fish as sources of polyunsaturated fatty acids among which is the omega-3 oils (Connor, 2000). Magawata and Oyelese (2000) reported that fresh *Clarias gariepinus* contained 73.32%, 16.65%, 5.38%, 2.79%, and 1.86% of moisture, protein, ash, lipid and nitrogen free extract, respectively.

This research seeks to address the paucity of information on the nutritional composition of various growth sizes and sexes of *H. longiflis*, a commonly cultured clariid fish that is very popular among fish farmers and consumers in Nigeria (Ojutiku, 2008).

Materials and Methods

Eight and later twenty-eight weeks old *H. longifilis* that had been hatched and raised in eight 5 by 2.5m rectangular concrete grow-out ponds connected to

a water recirculation system were sorted. The fish were nurtured on an artificial dry diet of 45% crude protein (Durante Superior Fish Concentrate catfish feed). Feed was administered twice daily at a rate of 5% of their biomass. The pellet size was adjusted to suit the diameter of the fish's mouth from 2 mm diet pellet size as fish grew to 3, 4.5 and 6mm, until they attained table-size.

Estimation of Percentage Shooters

The shooters were identified at the 8th and 28th week, based on a 5% randomly selected sample via a stratified sampling technique, before sorting and sale of table-size fish. At the end of the 8th week post-hatch, a juvenile was considered to be a shooter if its total length was approximately equal to or greater than 15.5cm; and a run if its total length was less than 12.5cm (Ataguba *et al*., 2009). The 28-week old fish were considered "shooters" if they weighed above 900 grams and "runs" if they weighed between 600 to 750 grams.

Biometrics and Sex Ratio

The standard length and total length of individual fish were measured with the aid of a measuring board and weight with the aid of a digital Acculab 300-electronic balance. A 10 percent sample of fish per pond, selected randomly, was used to estimate the sex ratio of the F1 generation at 28 weeks, by identifying and counting the male and female fish per sample, based on external morphology of the "genitalia"and expressed as male:female. The males were distinguished morphologically from the female by the elongated, backward-projecting papilla, while the female had an oval genital vent (Carballo *et al*., 2008).

Determination of Carcass Composition

The samples of the shooters, average-sizes and runs of 8 and 28-weeks old, and male and female table-size *H. longifilis* were eviscerated, washed and subjected to carcass analyses. The samples were analyzed for moisture, protein, fat, ash, fiber and nitrogen free extract as described by AOAC (2000), in the Food Science Laboratory, Institute of Agricultural Research, Ahmadu Bello University, Zaria.

Moisture Content

Moisture (%) in the fish samples was determined by a gravimetric method (AOAC, 2000). One gram of sample was pre-weighed (W_1) in a beaker and placed in an oven (Gallenkemp Drying Oven, LCON53 CF, model 94 M003, Gen Lab. Widnes, England) at 105°C for 24hr. The sample was removed from the oven, cooled in a desiccator, and reweighed (W_2). Moisture percentage was calculated according to the formula:

$$\text{Moisture (\%)} = \frac{(W_1 - W_2)}{W_1} \times 100$$

Total Ash Content

Total ash content was determined as total inorganic matter by incineration of sample at 600°C (AOAC, 2000). One gram of sample was weighed into a pre-weighed porcelain crucible and incinerated overnight in a muffle furnace (MF-1-02, PCSIR Labs., Lahore, Pakistan) at 600°C. The crucible was removed from the muffle furnace, cooled in desiccator and weighed. Ash content was calculated according to the following formula:

$$\text{Ash (\%)} = \frac{\text{Ash weight}}{\text{Sample weight}} \times 100$$

Crude Protein Content

Crude protein was determined by the Kjeldahl method as described by AOAC (2000). 700 milligram of the sample was placed in a Kjeldahl digestion tube. 5 gram K_2SO_4, 0.5 gram $CuSO_4$ and 25 ml conc. sulphuric acid were added to the sample. The sample was digested for one hour. 20 ml deionized water was added to the sample after allowing it to cool. After adding 25 ml NaOH (40%), the sample was then distilled and the ammonia liberated was collected in boric acid and titrated with 0.1N hydrochloric acid. A blank was prepared and treated in the same manner except that the tube was free of sample. Protein percentage was calculated according to the formula:

$$\text{Crude protein (\%)} = \frac{(\text{sample titre} - \text{blank titre})}{\text{sample weight}} \times 14 \times 6.25 \times 100$$

Where, 14 is the molecular weight of nitrogen
6.25 is the nitrogen factor.

Crude Fat Content

Crude fat was estimated by employing solvent extraction using a Soxhlet extraction unit (AOAC, 2000). One gram sample was weighed into an extraction thimble and covered with absorbent cotton. 50 ml solvent (petroleum ether) was added to a pre-weighed cup. Both thimble and cup were attached to the extraction unit (PCSIR Labs. Complex, Lahore, Pakistan). The sample was subjected to extraction with solvent for 30 min followed by rinsing for 1.5 h. The solvent was evaporated from the cup to the condensing column. Extracted fat in the cup was placed in an oven at 110°C for 1 h and after cooling the crude fat was calculated using following formula:

$$\text{Crude fat (\%)} = \frac{\text{Extracted fat}}{\text{Sample weight}} \times 100$$

Crude Fibre Content

Crude fibre in the samples was determined by the method described by AOAC (2000). Defatted sample (1g) was placed in a glass crucible and attached to the extraction unit (InKjel, D-40599, behr Labor-Technik GmbH, Dusseldorf, Germany). 150 ml boiling 1.25% sulphuric acid was added. The sample was digested for 30 min and then the acid was drained and the sample washed with boiling distilled water. After this, 1.25% sodium hydroxide solution (150 ml) was added. The sample was digested for 30 min, thereafter, sodium hydroxide was drained and the sample washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was allowed to cool in a desiccator and weighed (W_1). The sample was then ashed at 550°C in a muffle furnace (MF-1-02, PCSIR Labs., Lahore, Pakistan) for 2 h, cooled in a desiccator and reweighed (W_2). Extracted fibre was expressed as percentage of the original undefatted sample and calculated according to the formula:
Digested sample (W_1) – Ashed sample (W_2)

Crude fibre (%) = Difference in sample weights × 100

Nitrogen Free Extract (NFE) Content

Nitrogen free extract (carbohydrate) in the samples was calculated by difference as described by AOAC (2000).
NFE (%) = 100 – (Crude protein + Crude fat + Total ash + Crude fibre)

Energy Calculation

The percentage calories in samples were calculated by multiplying the percentage of crude protein and carbohydrate with 4 and crude fat with 9. The values were then converted to calories per 100gm of the sample (Gul and Safdar, 2009).

Results

Nutrient Profile of Juvenile *Heterobranchus longifilis*

Whole carcass analysis of the juveniles revealed mean percentage values of 73.54 ± 1.30, 9.76 ± 0.61, 13.8 ± 1.17, 0.30 ± 1.17, 1.16 ± 0.36, 1.47 ± 0.21 and 168.03 ± 10.10 for moisture, protein, fat, fibre, carbohydrate, ash and energy value respectively. Analysis of variance for mean values across the three growth classes showed significant differences ($P \leq 0.05$) for most of the parameters with the exception of moisture and ash. Fat was lowest (12.49%) in the "runs", while the difference observed for the "shooters" (14.93%) and "averages" (14.01%) were not significant ($P > 0.05$). Energy value was highest for the shooters (178.78 kilocalories), % fibre was highest for the runs (0.50) and decreased significantly ($P \leq 0.05$) from the "shooters" to the "runs" (Table 1).

Nutrient Profile of 28-week *Heterobranchus longifilis* F1

Proximate analysis of muscle samples from fishes representing the growth classes of 28-week *H. longifilis* F1 revealed mean values of 18.89 ± 1.01%, 2.34 ± 0.42% and 98.40 ± 6.48 kilocalories per 100g for protein, fat and energy respectively. An analysis of variance showed significant difference ($P \leq 0.05$) across the growth classes with respect to all the parameters except moisture and protein. The "shooters" had significantly

higher ($P \leq 0.05$) values for fat and energy compared to the other growth classes (Table 1). Correlation analysis ($P \leq 0.05$) revealed high positive correlation between protein and energy value (r = 0.94), fat and energy value (r = 0.86), fibre and carbohydrate (r = 0.89). High negative correlation was observed between fat and carbohydrate (r = -0.88), moisture and energy value (r = -0.76), moisture and protein (r = -0.87), fat and fibre (r = -0.74) across the groups (Table 2).

Nutrient Profile of Male and Female Table-size *Heterobranchus longifilis* F1

Proximate analysis of muscle samples from table-size 28-week *H. longifilis* F1 male and female revealed significantly higher ($P \leq 0.05$) values for male fish samples with respect to fat (3.82 ± 0.03), fibre (0.15 ± 0.02), carbohydrate (1.62 ± 0.26) and energy value (115.29 ± 0.99) than their female counterparts (Table 3).

Table 1: Nutrient profile of Juvenile* and 28-week old# *Heterobranchus longifilis* F1

Parameter (%)	"Shooters"	"Averages"	"Runs"	Mean	Standard Deviation
Moisture	*73.32[a]	74.26[a]	74.05[a]	73.54	1.30
	#77.06[a]	76.06[a]	77.74[a]	76.95	1.32
Protein	*10.23[a]	9.02[a]	10.05[a]	9.76	0.61
	#18.45[a]	19.75[a]	18.46[a]	18.89	1.01
Fat	*14.93[a]	14.01[a]	12.49[b]	13.8	1.17
	#2.83[a]	2.26[b]	1.92[c]	2.34	0.42
Fibre	*0.10[c]	0.30[b]	0.45[a]	0.30	1.17
	#0.10[a]	0.05[b]	0.04[b]	0.06	0.01
Carbohydrate	*0.88[b]	1.07[ab]	1.52[a]	1.16	0.36
	#0.90[a]	0.22[b]	0.40[b]	0.51	0.02
Ash	*1.56[a]	1.44[a]	1.41[a]	1.47	0.21
	#1.79[a]	1.80[a]	1.09[b]	1.56	0.01
Energy Value (Kilocalories/100g)	*178.78[a]	166.41[b]	158.90[b]	168.03	10.10
	#105.23[a]	94.20[b]	95.78[b]	98.40	6.48

Means with the same superscripts across a row are not significantly different ($P > 0.05$)
N.B: * Mean of triplicate determination pooled from 5 juveniles, # Mean of triplicate determination pooled from 5 fish

Table 2: Correlation between Nutrient Indices of 28-Week *Heterobranchus longifilis* F1

Parameters	Means	Std. Dev.	Moisture	Protein	Fat	Fibre	Carbo-hydrate	Ash	Energy Value (Kcal)
Moisture	76.9555	1.1301	1.0000						
Protein	18.8866	1.0119	-0.8658	1.0000					
Fat	2.3366	0.4219	-0.4667	0.6606	1.0000				
Fibre	0.0677	0.0233	-0.1075	-0.2041	-0.7360	1.0000			
Carbo-hydrate	0.5055	0.3206	0.2590	-0.4236	-0.8768	0.8889	1.0000		
Ash	1.5611	0.4020	-0.5131	0.3690	0.1549	0.3267	0.1841	1.0000	
Energy Value (Kcal/100g)	98.4033	6.4781	-0.7593	0.9412	0.8628	-0.4179	-0.6165	0.3304	1.0000

N.B: Mean of triplicate determination pooled from 5 fish
* Significance difference at 95% confidence interval

Table 3: Comparative Nutrient Profile of Female and Male Table-size *Heterobranchus longifilis* F1

	Male	Female	t-value	Df	P	Male Std.Dev.	Female Std.Dev.	F-ratio – Variances	P - Variances
Moisture	76.5667	77.1966	-0.8199	4	0.4582	1.1154	0.7259	2.3610	0.5950
Protein	18.5867	18.4800	0.7162	4	0.5134	0.0642	0.2498	15.0967	0.1242
Fat	3.8200	2.2833	62.1612	4	0.0000*	0.0300	0.0305	1.03704	0.9818
Fibre	0.1533	0.0533	8.0178	4	0.0013*	0.0208	0.0057	13.0000	0.1428
Carbo-hydrate	1.6233	0.6400	6.5202	4	0.0028*	0.2581	0.0400	41.6458	0.0468
Ash	1.8967	1.9000	-0.0857	4	0.9357	0.0450	0.0500	1.2295	0.8970
Energy Value (Kcal/100g.)	115.2867	97.2266	16.5651	4	0.0000*	0.9947	1.6051	2.6039	0.5549

Note: Significant at ($p<0.05$)

Discussion

The significantly lower value ($P \leq 0.05$) obtained for fat in the "runs" compared to other growth classes probably links the differential growth rates to feed availability or uptake by individual fish. In this study this probably implies that the "shooters" fed more frequently than the other growth classes since there was no variable introduced in the experimental design in this respect. Variation in size has been attributed to genotypic differences or inadequate food supply (Hecht and Appelbaum, 1988), the latter likely being the case in this study. Low body lipid content of fish is directly related to feeding at less than optimum feed application rate (Bureau *et al*., 2006) and declined feeding frequency (Dwyer *et al*., 2002).

Similar protein percentage across the growth classes indicates that feed availability does not significantly affect the protein content of *H. longifilis* juveniles. Similarly, Marimuthu *et al*. (2011) reported that feed application rate did not affect the protein content of African catfish fingerlings.

The nutrient profile of 28-week *H. longifilis* muscle in this study deviates markedly in terms of percentage crude protein (59.8 ± 0.5), lipid (25.1 ± 0.8), ash (11.0 ± 0.4) and gross energy (23.1 ± 0.3 kilo Joules/100g) from the report of Koumi *et al.* (2011) in *H. longifilis* of final body weight 133.2 ± 52.7g following a 180-day diet experiment. The differences may be attributed to the fact that whole carcass (minus viscera) was used by Koumi *et al*. (2011). The percentage moisture (76.96 ± 1.13) obtained in this study is similar to the value of 75.7 ± 0.8 reported by Koumi *et al*. (2011). The proximate composition of fish muscle obtained in this study compares favourably with the crude protein (16.3%), crude fat (5.4%), moisture (77.3%) and ash (1.1%) reported by Robinson *et al*. (2001) as nutrient characteristic of muscle tissue for market–size farm-raised channel catfish (*Ictalurus punctatus*).

The lack of significant difference ($P>0.05$) across the growth classes in 28-week old *H. longifilis* with respect to protein further buttress the previous findings with respect to juvenile *H. longifilis* Fl in this study that feed availability does not affect the protein content of *H. longifilis* F1. The significantly high values for fat and energy observed in the "shooters" suggest that this class of fishes were "aggressive" feeders, with better feeding ability and hence feeding optimally (Dwyer *et al*., 2002; Bureau *et al*., 2006).

The high positive correlation between fat, protein and energy values could be attributed to the fact that protein and fat contents of fishes are often relatively high and constitute about 70% of the indices used in the calculation of energy value of feed or food. It could also be hinged to the theory that unlike many other vertebrates, fish generally rely more on lipids than on carbohydrates for their energy requirements as they are generally not able to utilize carbohydrate efficiently (*http://cdserver2.ry.ac.za/cd/ctfish/catfish/cat61a.htm/1/2012*).

The absence of any significant difference ($P>0.05$) in the ash content of female and male fish implies that the feed used was not deficient in phosphorus and other elements; most of the ash in fish meal is made up of calcium and phosphorus (Sugiura *et al*., 2000).

Conclusions

Protein content in fish is unaffected by growth rate and sex. Differential growth rate between and F1 generation is influenced by feed accessibility and utilization as evidenced by low fat in the "runs" compared to other growth classes. The "shooters" have more available energy for metabolic activities which enhances faster growth as observed in their higher caloric values. From the study it could be suggested that duosex systems could be maintained for the production of *H. longifilis* F1, as growth rate is not affected by sex. The preference of "shooters" or weightier fish amongst fish of the same age for dietary purposes with a view to harnessing the benefits of fish oil.

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