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Growth performance of Pacu (*Piaractus brachypomus*) fed with different protein sources

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Abstract
The present study was conducted to compare the performance of growth and survival of Pacu, *Piaractus brachypomus* fed on different experimental diets. Pacu (1.00 ± 0.02g) were fed with nine practically prepared diets for 90 days. Ground Nut Cake (GNC), Fish Meal (FM) and Cotton Seed Meal (CSM) were used at three different protein levels (20%, 25% and 30%), basal supplemented with rice bran and maize. The growth performance of *P. brachypomus* was found to be best when fed with diet FM25 (126.50gm) and the least with GNC20 (117.13gm). Survival rates were best in all the treatment, which showed 100% survival rate at the end of the experiment except on GNC20 and CSM20 (90%).

Keywords: *Piaractus brachypomus*, ground nut cake, fish meal, cotton seed meal, growth performance

Introduction
Protein is the main organic component of the fish tissues and at the same time, the most abundant and costly in commercial feeds. In addition to being used for growth and repair of tissues, protein is also used extensively for providing energy in routine metabolism by fish (Guillaume *et al*., 2004). The dietary crude protein requirement of fish is an important nutritional consideration because protein is often the major limiting nutrient for growth. Most of the research was focused on total dietary protein levels rather than daily requirement for protein and is the most important factor affecting growth of fish and feed cost. In general, fish require relatively high levels of protein. The demand for protein among the different groups depend upon factors such as: feeding habits, water temperature (cold or tropical), environment (marine or freshwater) and stage of physiological development. According to reports by NRC (1993) [23], for maximum growth of fish, require diets with protein level that vary between 31 and 56% NRC (1993) [23]. Protein ingested by fish is used in maintenance and growth, but when they are overfed only one part of this protein is effectively used for synthesis of new proteins and the remaining is metabolized to produce energy. When fish are fed with optimal dietary protein levels they retain a proportionally higher amount of protein in the body (growth), decreasing production costs and pollution (Thoman *et al.* 1999) [25].
Pacu, was introduced in India as an alien species during 2003 and 2004 from Bangladesh. These Pacu species are South American fishes which are native of Brazil, Peru and Venezuela. Pacu (Piaractus brachypomus) is a freshwater fish of order characiformes, family characidae and sub-family serrasalminae. The common name Pacuis generally applied to fish classified under the genera Colossoma, *Metynnis*, *Mylesinus (Mylopus)*, *Mylossoma*, *Ossubtus*, *Piaractus*, *Tometes*, *and Utiaritichthy*s. However common name for these species vary by region, in Brazil the fish is known as Pirapatinga, USA as Cachama, UK as freshwater pompano and in India especially in Tripura, Assam and West Bengal the fish is locally known as Rupchanda. Pacu, contrary to its popular image of an active carnivore is actually an herbivore / omnivore, preferentially feeding on leaves, flowers, fruits and seeds of superior plants. Pacu have also been reported to feed on zooplankton, insects, snails and decaying plants. A hardy fish, this characid combines several desirable features like fast growth, superior flesh quality, omnivorous feeding habit and compatibility, besides accepting a wide range of low cost feed. There is little information on dietary protein requirements for growth in these species and it is important that studies on its nutrition should be carried out to develop cost-effective and nutritionally balanced feed formulation for these fish. The present study was designed to study the dietary protein requirement and evaluate the effects of dietary protein level on growth performance Pacu, *Piaractus brachypomus*.

Material and Methods
Nine isoenergetic diets were formulated to contain various percentages of GNC, CSM, FM, Rice bran and maize for comparison. All diets were isonitrogenous and containe 35% protein.

Diet 1, 2, and 3 is 20%, 25% and 30% protein GNC; Diet 4,5 and 6 is 20%, 25% and 30% protein FM and Diet 7,8 and 9 is 20%, 25% and 30% protein CSM respectively. The composition and proximate analysis of the experimental diets are given in Table 1, 2 and 3 2 respectively.

Table 1: Feed formulation of the diets (Ingredients g/100g)

Diets	Ingredients						Total
	Groundnut Cake	Fish meal	Cotton seed meal	De-oiled rice bran	Maize	Vitamins & mineral mixture	
GNC20	32.36	-	-	32.82	32.82	2	100
GNC25	50	-	-	24	24	2	100
GNC30	69.54	-	-	14.23	14.23	2	100
FM20	-	20	-	39	39	2	100
FM25	-	31.5	-	33.25	33.25	2	100
FM30	-	43	-	27.5	27.5	2	100
CSM20	-	-	30.5	33.75	33.75	2	100
CSM25	-	-	48.2	24.9	24.9	2	100
CSM30	-	-	65.8	16.1	16.1	2	100

GNC 20, 25, 30 = Groundnut Cake diet Containing 20%, 25%, 30% protein
FM 20, 25, 30= Fishmeal diet Containing 20%, 25%, 30% protein
CSM 20, 25, 30= Cottonseed Meal diet Containing 20%, 25%, 30% protein

All ingredients were procured from local market, dried, powdered and proximate analysis was done and percentages of moisture, crude protein, ether extract and total ash were estimated according to AOAC (1990) [1]. The protein content of the feed ingredients were 55%, 38.4%, 40%, 12.5%, and 10.7% for fish meal, groundnut cake, cottonseed meal de-oiled rice bran and maize respectively. All the diets were analyzed for proximate composition. Roopchand were fed at the feeding rate of 8 - 6% body weight depending on the body weight. Fishes were fed twice a day.

Table 2: Proximate composition of the ingredients (% on dry matter basis)

Ingredients	Composition				
	Moisture	Crude Protein	Ether extract	Total ash	Acid insoluble ash
Groundnut cake	8.8	38.4	7.3	5.6	7.6
Fish meal	7.03	55	4.02	3.47	5.6
Cottonseed meal	7.69	40	16.65	3.53	4.24
De-oiled ricebran	7.7	12.5	22.5	3.9	15.8

Table 3: Proximate composition of Test Diets

Feed	Composition			
	Moisture	Crude protein	Crude fibre	Ether extract
GNC20	8.42	20	7.3	5.55
GNC25	8.31	25	7.35	5.57
GNC30	8.35	30	7.31	5.57
FM20	8.8	20	4.01	3.45
FM25	8.75	25	3.95	3.47
FM30	8.65	30	3.97	3.44
CSM20	8.91	20	16.15	3.51
CSM25	9.02	25	16.2	3.53
CSM30	8.95	30	16.23	3.51

Red-bellied pacu, *Piaractus brachypomus* (300 numbers) in fry stage were obtained from the fish farmers, Ganapavaram, Bhimavaram, West Godavari District, Andhra Pradesh and stocked in the experimental tanks for 1 week before the beginning of the experimental regime, in order to condition the fish to the laboratory system and handling procedures. The photoperiod was set at 12-h light; 12-h dark. During the acclimatization period, the fish were fed with ground nut cake and ricebran. At the start of the growth trial, uniform-sized fish (1.0±0.2 g body weight) were randomly distributed into 27 tanks, with three replicates per diet. Ten fish were stocked in each tank. Continuous aeration was provided to each tank through air stones. Water quality parameters were measured every 3 days.

For determining the weight increment, specific growth rate, survival percentage, food conversion rate, ten fishes from each aquarium were taken at every 10 days of interval and calculated using the formulae

a. Weight Gain (WG %) = Final body weight – Initial body weight ÷ Initial weight
b. Weight increment = Final body weight (g) – Initial body weight (g).
c. Specific growth rate (SGR) = ([(Ln FBW - Ln IBW) / day]÷ days) x 100
d. Survival Rate (%): (Total number of fish survived ÷ Total number of fish stocked) x 100
e. Feed Conversion Ratio (FCR) = Feed given (dry weight) (g) ÷ Body weight gain (wet weight) (g)

Statistical analysis

The data was analyzed using two-way ANOVA for completely randomized design, 9×9 factorial scheme was performed (Table 3). Means of statistically different parameters and factors were compared by Tukey's test ($P < 0.05$). Pearsons correlation coefficient (r) values were utilized to evaluate possible interactions among parameters. Data were analysed with the aid of software STATISCA version 7.0.

Results Discussion

The growth performance and weight gain of red-bellied pacu fed diets containing different percentages of GNC, FM and CSM are presented in Table 2. Among the treatments, fish fed with FM25 was highest and followed by FM30, CSM25 and CSM30. FCR ranged from 0.15 to 4.40 among the treatments. FCR significantly lower in diet fed with CSM30 and higher in FM20. The general health and appearance of the fish were good and the fish in all treatments were very active. The survival rate at the end of experiment there was 100% survival in maximum all groups except in GNC20 (90%) and CSM20 (90%).

Table 4: Mean initial body weight, weight gain, FCR, SGR, survival rate of red-bellied pacu (*Piaractus brachypomus*) fed test diets containing different protein levels

Diets	Initial weight (g)	Final weight(g)	SGR	FCR	Survival
GNC20	1.00 ±0.02	112.5 ±0.26	5.25 ±0.004	4.02 ±0.18	90%
GNC25	0.95 ±0.05	119.85 ±0.37	5.38 ±0.003	4.10 ±0.23	100%
GNC30	0.95 ±0.05	121.9 ±0.28	5.40 ±0.004	3.97 ±0.21	100%
FM20	0.95 ±0.03	119.12 ±0.51	5.37 ±0.005	4.40 ±0.34	100%
FM25	1.00 ±0.04	126.50 ±0.26	5.38 ±0.003	4.10 ±0.31	100%
FM30	0.9 ±0.05	126.01 ±0.34	5.50 ±0.004	4.23 ±0.29	100%
CSM20	0.95 ±0.06	117.23 ±0.33	5.45 ±0.004	4.08 ±0.21	90%
CSM25	0.9 ±0.05	123.52 ±0.26	5.45 ±0.004	4.42 ±0.25	100%
CSM30	1.00 ±0.04	122.52 ±0.36	5.36 ±0.004	3.89 ±0.09	100%

GNC: Ground nut cake; FM: Fish meal; CSM: Cotton seed meal

Table 5: Statistical Analysis

Parameters	CV	CD
Weight Increment	2.151	1.083
Weight Gain	7.033	0.886
SGR	6.134	Non Significant
FCR	10.207	Non Significant
Survival rate	2.143	1.894

During feed formulation, the protein to lipid ratio is closely monitored especially for the carnivorous fish(barramundi), whose dietary protein requirements are comparingly higher (40 to 55%) than those for herbivorous fish (*Tilapia zilli*, 19.0%,) (Glencross, 2004) [13]. *Piaractus brachypomus* requires high quality feed requirements, there is need to balance between the dietary protein and lipid requirements of the fish because fish requires more proteins than lipids, the lipid requirements being characteristically less compared to those for terrestrial animals (Miller, 2003) [22]. Feed formulations therefore have to closely regulate proteins and lipids in fish diets to ensure that the diets produced meet the nutritional requirements of fish at an acceptable cost (Thoman *et al*., 1999) [25].

Bureau *et al*., (2002) [6] stated that data on growth show that there was a gradual increment in WG (46.5±5.0g) as dietary protein levels increased to 32% crude protein (CP). Fish fed diet beyond 36% Crude protein could not produce additional growth. There is some evidence that at very high feeding levels, protein deposition tends to level off (plateau). Fish fed diets with lower CP levels showed reduced weight gain and efficiency of feed utilization. Dietary protein is the most important factor affecting growth performance of fish and feed cost. The demand of fish protein for growth is different according to species, feeding habits, physiological and development state, conditions of culture, sources of protein, dietary energy levels and the protein: energy ratio (Lupatsch, 2001) [17]. Sipabua – Tavares *et al*., (1999) [24] indicated when fish fed with feed with two levels of crude protein (16% and 34%) for the three different densities of Red-bellied pacu, indicated that feed containing 34% level of crude protein satisfactory growth than the diet with 16% protein.

In the present study, the maximum weight gain was observed with 25% dietary protein levels. Differences in weight gain in relation to protein content in the diet was similar to those observed for different species of fishes (Meyer & Fracalossi, 2004) [20]. According to NRC (1993) [23], the average protein requirement in diets for growth of the fishes cultivate in the world may oscillate between 31-55%. In the present study also the growth was maximum at 30% dietary protein but the specific growth rate was little less when compared to 25% protein level.

Fish meal supplies the largest portion of dietary protein in fish diets (Biswas *et al*., 2007) [3]. Available data show that about 30% to 50% of fish meal (FM) can be successfully replaced in fish feeds by plant protein sources. However, the presence of anti-nutritional factors and other active biological compounds limit the use of substitutes, and toxic effects have been reported in fish (Francis *et al*., 2001; Lee *et al*., 2006) [12, 16]. Groundnut cake with crude protein content of 40-45% is a good supplement. It promotes growth and palatable to fish. Groundnut cake protein is known to be deficient in lysine and methionine and also has a limited amount of tryptophan and theanine but amino acid quality improves in artificial diets when reinforced with lysine, methionine and tryptophan (Eyo and Olatunde, 1998) [8], Groundnut is a valuable source of vitamins E, K and B. It is richest plant source of theamine (B_1) and also rich in niacin, which is low in cereal (FAO, 2000) [9]. The main constraint to its utilization is its easy contamination by toxic substances due to bad storage. The most dangerous substance is aflatoxin (FAO, 2014) [10].

The results of numerous studies evaluating cotton seed meal (CSM) in catfish, salmonid and tilapia diets indicated that between 10% and 30% of solvent extracted, 40% protein CSM can be used in aquaculture diets without growth depression. Lee *et al*., (2006) [16] summarized a series of studies in rainbow trout where CSM replaced fish meal entirely over a 3-year period without significantly impacting growth rate of rainbow trout, although diets with CSM had significantly lower protein and phosphorus assimilation. Studies on fish have focused on cottonseed meal supplementation, reporting adverse effects on blood parameters (hematocrit and haemoglobin), growth, and development (Blom *et al*., 2001) [4]. The addition of iron salts to the diet reduces gossypol toxicity (Wenegaertner, 1981; Yildirim *et al*., 2003) [29, 30]. Reports are controversial and the level of dietary iron necessary to prevent toxic effects has not been conclusively determined (Meric I. *et al*., 2011) [19]. Early studies have indicated that the amount of CSM that can be used in Nile tilapia feed depends mainly on the level of free gossypol and available lysine content of the diets.

Due to unfavorable physiological effects of gossypol and to a reduction in the biological availability of lysine because of the binding properties of gossypol application of higher levels of CSM in fish diets is limited (Dorsa *et al*., 1982) [7]. Cottonseed meal which generally costs less than both fish meal and SBM(Soy bean meal), would be beneficial in reducing feed costs for commercial fish farming and ensuring sustainability of the enterprises. More importantly, CSM contains high levels of proteins (Forster and Cahloun, 1995) [11]. The use of cottonseed proteins as a dietary protein has been examined for many commercial important fish species, viz., rainbow trout *Oncorhynchus mykiss*, channel catfish

Ictalurus punctatus, tilapia, parrot fish *Oplegnathus fasciatus* and sunshine bass However, the major problem associated with the use of CSM is the toxicity of free gossypol. According to Guillaume and Metailler (1999) [14] the astringent taste of saponin which is present in cotton seed meal and it high fibre content could lower feed utilization of fish. CS50 with groundnut cake and cotton seed meal substituted at 50:50% recorded the highest weight gain. The anti nutritional factors present in groundnut cake (aflatoxin) and cotton seed meal (gossypol) were well tolerable at this level when mixed at 50:50%,) can be used in fish diet without detrimental effects on growth. Toko *et al*., (2008) [27] reported that cottonseed meal could only be successfully included at 30% of the total diets without effects on fish growth performance in the diets of Juveniles African catfish.

Studies have indicated that the amount of cottonseed meal in fish fed depends on the level of free gossypol and available lysine of the diet. Iron as ferrous sulphate, has been successfully used to counteract the toxicity of free gossypol in diets of monogastric, terrestrial animals**.** High level of supplemental iron used to counteract the toxicity of gossypol may be harmful to fish because it has been suggested that a delicate balance exists between the need of iron for host defense mechanisms and the need of iron to sustain microbial growth (Magdy *et al*., 2011) [18]. In the present study of pacu's diet of cottonseed meal, adding addition of 1000mg/kg feed iron tablet powder to nullify the toxicity of free gossypol which is a toxic substance present in cottonseed meal.

Studies done by Michael *et al*., 2003 [21] revealed that Pacu exhibited good growth and feed conversion ratio with plant proteins than animal protein. Results from this study indicated that both GNC and CSM could totally replace FM inclusion rate in commercial diets for juvenile to sub-adult of pacu.

Walter *et al*., (2011) [28] concluded that feed intake was higher in fish fed with the lowest dietary protein level and gradually diminished in fish fed with 32%. Low food consumption and high weight gain obtained in fish fed with 32% CP produced a FCR of 1.10 ±0.03, significantly better than that observed in fish fed with dietary protein levels above and below this level ($P<0.05$). Consequently, these studies infers that the higher feed ingestion by *P. brachypomus* fed diets isoenergetics with low protein. In agreement with the results of the present experiment, the FCR value decreased as the dietary content of the protein increases. Gutierrez *et al*., (1996) [15] observed a decrease in the FCR value from 3.9 to 2.4 when *P. brachypomus* fed with increasing dietary protein levels from 27 to 30%. It agrees with the present study, incase of plant dietary proteins, were also observed a decrease in the FCR from 4.42 to 3.89 (g) and 4.10 to 3.97 (g) when Pacu fed with increasing dietary protein levels from 25 to 30%.

In the present study, the 25% protein diet showed remarkable growth than 20% and 30% of all the three diets i.e., fish meal, groundnut cake, cottonseed meal. It agrees with the results of Borghetti & Canzi (1993) [5] who recommended the 27% to 28% of crude protein is good for pacu growth and maintenance of adequate levels of water quality.

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Conclusion

Use of plant-based feed in aquaculture is inevitable in the near future. Increasing demand for fishmeal for various sectors like livestock and poultry in addition to aquaculture, has made this commodity more expensive. It is almost impossible to include fishmeal in aqua feeds and still be able to keep the production cost low (Baruah *et al*., 2004) [2].

In the present study, it can be concluded that for *P. brachypomus* juveniles a dietary protein level of 25% CP is the optimum level for maximum growth and Feed Conversion Ratio. An increase on dietary protein level upto 30% seems to generate less than the optimum growth on weight gain in comparison with 25% protein. It might be concluded that from the economic point of view that all the low cost oil seed cakes like cottonseed meal and groundnut oil cake which are locally available can be used in combination from as the alternative protein source for the feed of Roopchand.

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