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Use of Fish Silage Based Blended Protein Source for Replacement of Fish Meal in Thai-Pangas Diet

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A B S T R A C T

A study was carried out to assess the impact of replacement of fish meal by fish silage based blended protein source in Thai-pangas (*Pangasianodon hypophthalamus*) diet. Five isonitrogenous experimental diets with 35% crude protein level were formulated by replacing fish meal at blended protein source consisting of one third each of fish silage, groundnut oil cake and soya bean meal The growth performance of *P. hypophthalmus* after feeding with different experimental diets was significantly different. After 90 days of feeding trial the experimental diet with 75% of the fish meal content significantly) higher growth rate of 391.64% than all other experimental diets. The, experimental diet containing 0 % fish meal and 100 % blended protein source showed significantly lower growth rate. The food conversion ratio (FCR) of the experimental diet T_3 was also significantly better than all other diets. The better growth performance of experimental this T_3 might be due to the better availability of digestible protein due to the enzymatic action during the process of silage preparation. After considering all the factors analyzed in the study it may be concluded that the cost effective diet for Thai pangus (*P. hypophthalmus*) may be formulated by replacing 75 % of the fish meal with blended protein source for better performance in terms of growth of the fish.

Introduction

Fish silage is an attractive alternative source to replace fish meal and produced from the whole fish or parts particularly the processing waste, to which acids, enzymes or lactic acid-producing bacteria are added, where the liquefaction of the mass is provoked by the action of inherent enzymes of the fish (FAO 2003).

Fish waste generated by processing and commercialization stations cause serious environmental hazards. A viable alternative would be to use the waste material in the manufacture of the fish silage, since it does not require high investments. The manufacturing of silage fish processing waste with an aim to utilize it as an aquaculture feed ingredient has been widely studied over the last few years. Many authors believe that, due

to the similarity of this protein source with the raw material, especially amino acids, such as lysine, methionine, and cystine, silage has a high potential for use in aquaculture. Its low cost, especially when compared to fish meal is also attractive (Ferraz de Arruda 2004. Goddard and Perret 2005, Vidotti *et al.*, 2003).

Fish silage a liquid product with about 80% water content there are limitations on the quantity of silage that can be added while manufacturing feed. The silage however can be blended with some low cost protein source; after which it can be used.

The stripped catfish, Thai-pangas (*Pangasianodon hypophthalmus)* is a widely cultured aquaculture species that is famous for its fast growth rate, hardy, consume different types of food and can survive in low water quality environment. Commercial culture of this fish species is fast gaining importance as a result, there is continuous increase in its production volume that has exceeded 150,000 MT (Phuong *et al.*, 2005). The EU is currently the major market for Pangasius (especially from Vietnam, which is the largest producer). New markets such as Russia, the Middle East and some Asian countries have also demonstrated a growing demand for the fish (Josupeit, 2009b). However, the cost of production of Thai-pangas utilizing conventional fish feed is not matching well with the farm gate price of the fish. Therefore, reduction of cost of feed for Pangas is the need of the hour. It is in this context; the present study has been proposed to study on the use of fish silage based blended protein source for replacement of fish meal in Thai-pangas.

Materials and Methods

The study was undertaken to evaluate the feasibility of fish silage based blended protein as a cheaper and alternative source to replace the fish meal in Thai pangus (*Pangasianodon hypophthalmus*) diet

Experimental details

Pangasianodon hypophthalmus (Thai-pangus) fingerlings were procured from a private fish farm of Chatrapur, Ganjam (Odisha). The stock was acclimatized in 6 FRP tanks of 200 l capacity under aerated conditions for 15 days. During the period of acclimatization, the fish were fed with ABIS floating fish feed at about 5percentvof their body weight twice a day. The experiment was conducted by segregating and stocking of identical size fish with an average weight of 3.6 ± 0.08 g. Five isonitrogenous experimental diets with 32percentcrude protein level were formulated namely; T_0, T_1, T_2, T_3 and T_4. The diet T_0 is the conventional fish meal based diet and served as the control diet. Other diets T_1, T_2, T_3 and T_4 had fish silage based blended protein source replacing fish meal at 25, 50, 75 and 100percent, respectively. The blended protein source was prepared taking one third each of fish silage, groundnut oil cake and soya bean meal on dry weight basis (Table 1).

The quantity of individual ingredients required to formulate a kg of diet was worked out using Hardy's square method to balance protein levels. Energy level was balanced by adding oil. All the ingredients were pulverized in a hammer mill pulverizer (Kohinoor make) to get the fine powder of each (Table 1). Then, all the ingredients in required quantity except vitamin and mineral mixture were hand mixed to ensure homogenous mixing followed by addition of required quantity of boiled water and hand kneaded to form thick dough. The dough thus prepared was cooked in an autoclave for 15 minutes at 15 PSI pressure to sterilize the mixture and to remove anti-nutritional factors if any. The cooking also helped in gelatinization of starch content and improved the binding capacity of the feed for

water stability. Dough was then cooled under room temperature. After proper cooling, required quantity of vitamin and mineral premix was added, mixed properly by hand kneading to prevent immobilization of vitamin and mineral premix.

The dough was then pelletized by using a hand pelletizer and oven dried for overnight at 75^0 C to in a hot air oven (MIC make).

Finally, the dried pellets were crumbled to approximate size and stored in air tight container before feeding to the experimental animals.

Method of feeding

Feeding was done at 4% of body weight initially and then the feeding was adjusted to the consumption so that there is almost nil feed is left. Daily ration was divided into two parts; one part was given at 09:00 hours and the other was given at 16:00 hours.

Physico-chemical parameters of water

Water quality of rearing water namely: temperature, pH, DO, total alkalinity and ammonia nitrogen were recorded before and after water exchange during the experimental period following standard protocol (REF).

Proximate composition of experimental diet

The proximate composition of experimental diets was done by prescribed method (AOAC, 1998)

Growth parameters

The growth parameters of the *Pangasianodon hypophthalmus* were assessed by taking their body weight with respect to the feed given at an interval of 30 days. The feeding ration was also adjusted according to the weight gain by the fishes.

Results and Discussion

The experiment was conducted to evaluate the possibility of using silage prepared out of fish dressing waste as an alternative source of protein in the diet of Thai-Pangas (*P. hypophthalmus*) and to find out its effectiveness and other possible consequences. The results of the research have been presented as tables and graph in this chapter with appropriate statistical analysis.

Physico-chemical parameters of water

Water temperature was recorded once in the early morning and again in the late afternoon once in a month.

pH

There was not much variation in pH values during the experimental period. The average pH value just before and after water exchange were recorded as 8.8 ± 0.12 and 8.2± 0.22, respectively in all the tanks.

Temperature

The average water temperatures of the experimental tanks in the morning and in the late afternoon were 26.8 ± 0.18°C and 28.6± 0.25 °C, respectively.

Dissolved oxygen

The average dissolved oxygen (DO) concentrations of the experimental tanks were recorded just before water exchange and after water exchange was 4.2± 0.14 and6.8± 0.18mg/l, respectively.

Total alkalinity

The total alkalinity was 250± 0.08and 298± 0.13 ppm, respectively before and after water exchange during the experimental period of 90 days in all the tanks.

Fig.1 Proximate composition of experimental diets (on wet weight basis)

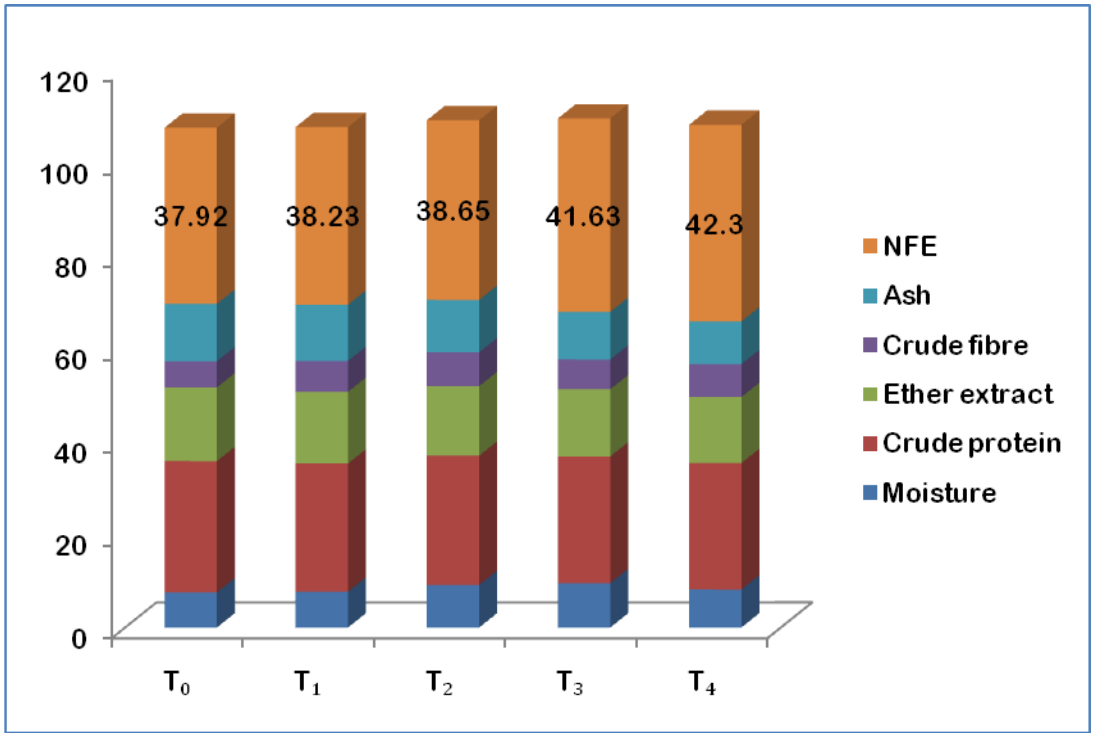


Fig.2 Proximate composition of experimental diets, T_0 (on wet weight basis)

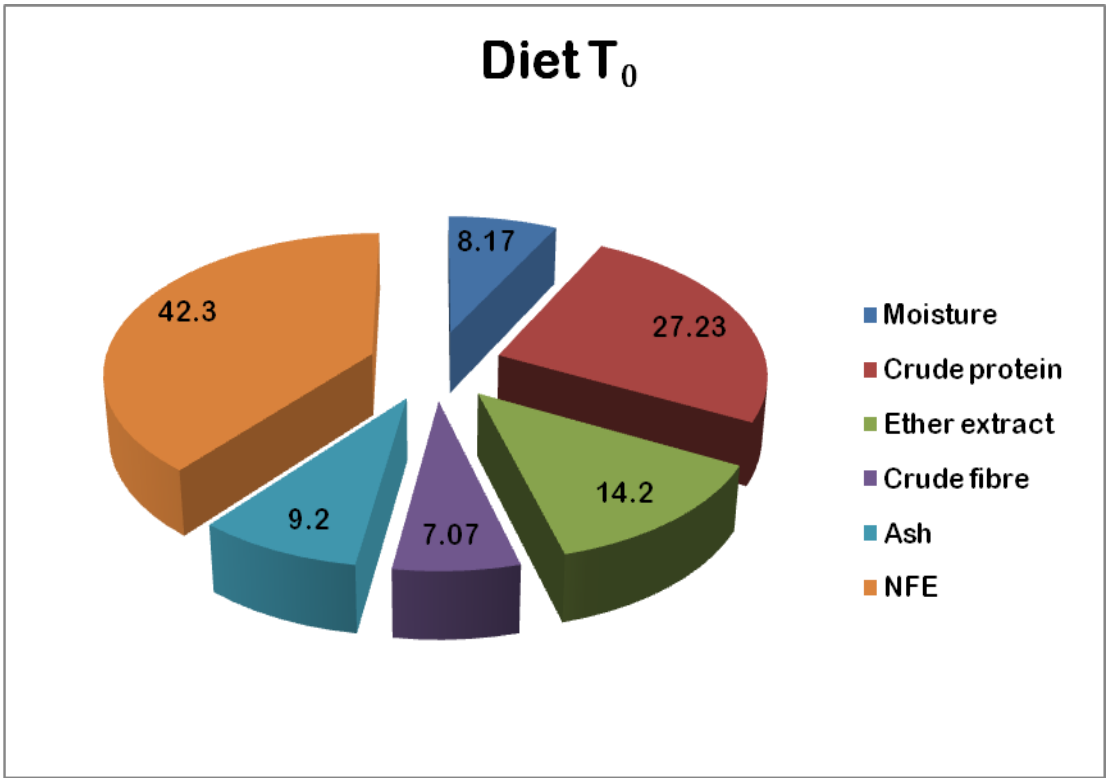
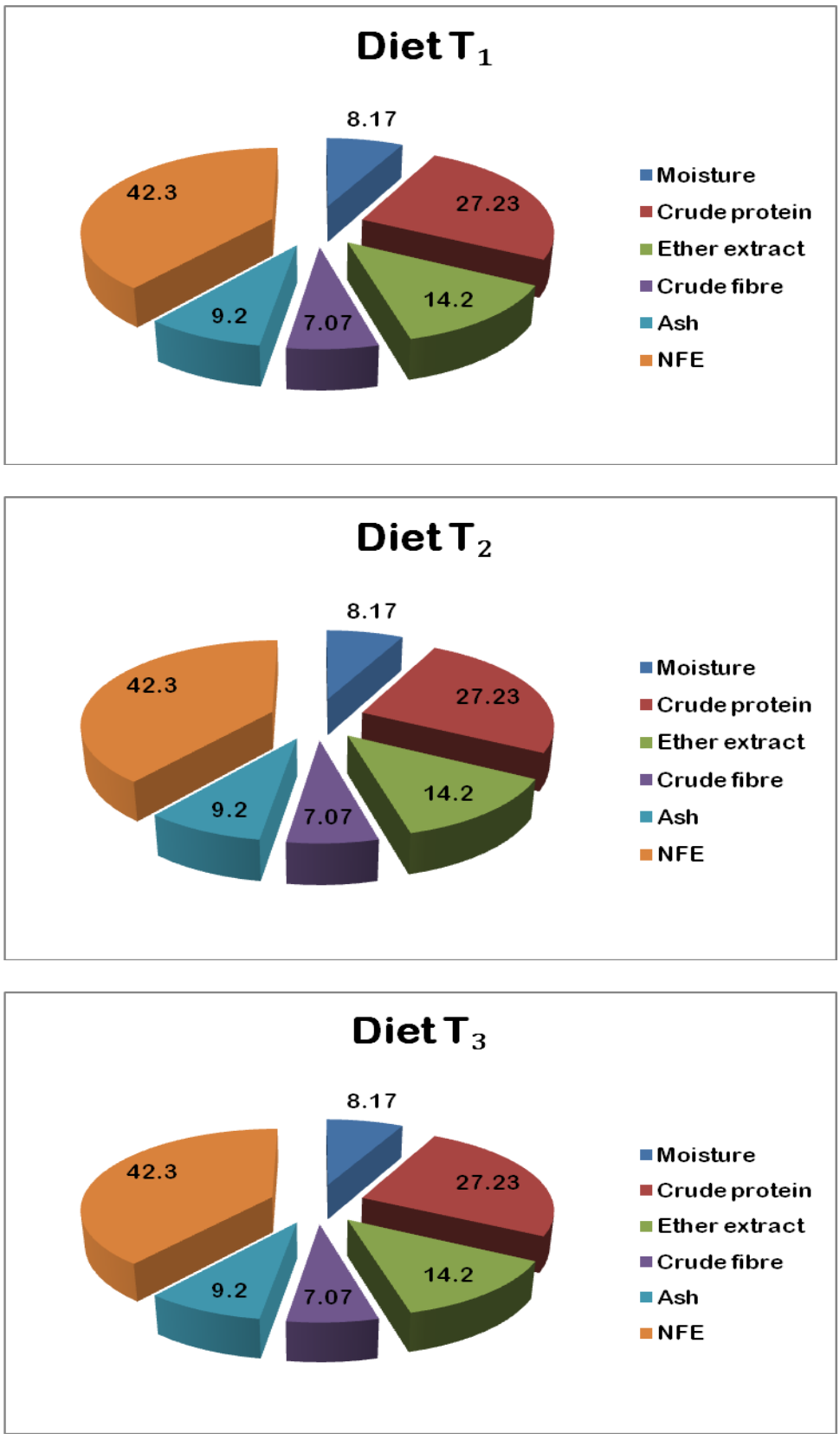
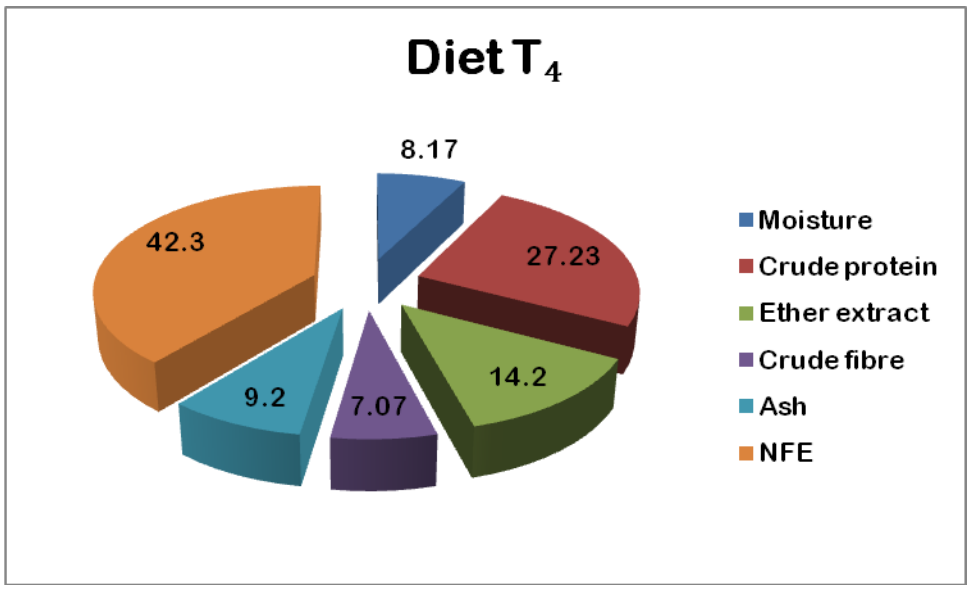


Fig.3 Proximate composition of experimental diets, T_1 (on wet weight basis)





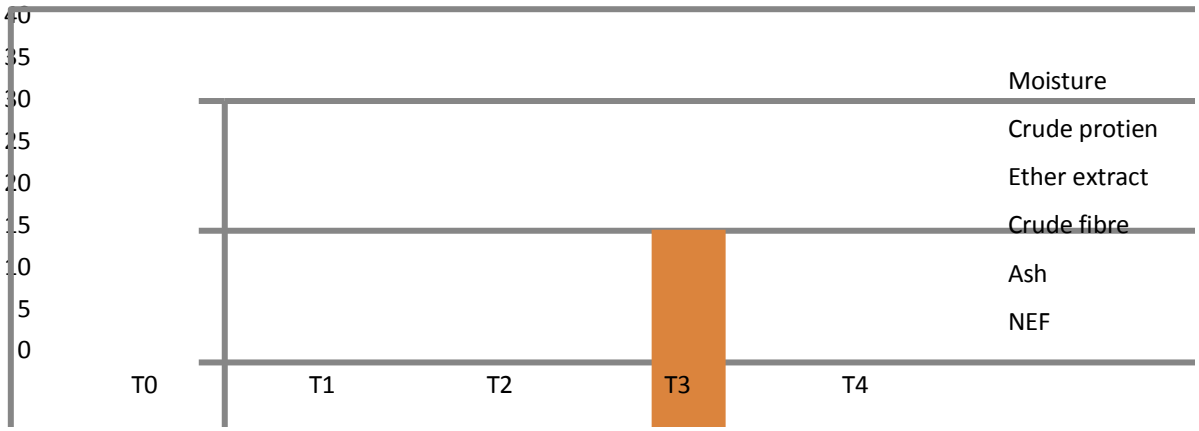


Table.1 Ingredient composition (g/kg dry matter basis) of experimental diets

Ingredients	Experimental Diets				
	T_0	T_1	T_2	T_3	T_4
Fish meal	360	270	180	90	0
Blended protein source	0	100	200	310	410
Groundnut oil cake	90	90	90	70	70
Mustard oil cake	90	90	80	60	50
Soya bean oil cake	60	60	70	90	90
De-oiled rice bran	260	250	240	240	250
Lipid (Fish oil : Sunflower Oil:: 1:1)	80	80	80	80	80
Vitamin & Mineral mixture *	30	30	30	30	30
Corn Flour	30	30	30	30	20

Table.2 Proximate composition of experimental diets

Experimental Diet	Moisture	Total dry matter	Parameters (as % of dry matter)						Gross energy (KJ/100g)	P/E ratio (g Protein/KJ)
			Crude protein	Ether extract	Crude fibre	Ash	NFE	Total organic matter		
T_0	7.55	92.45	28.25	15.86	5.63	12.34	37.92	87.66	1702.96	60.28
T_1	7.70	92.3	27.60	15.42	6.64	12.11	38.23	87.89	1680.70	60.89
T_2	9.15	90.85	27.82	14.98	7.27	11.28	38.65	88.72	1674.80	60.20
T_3	9.55	90.45	27.25	14.48	6.39	10.25	41.63	89.75	1696.19	62.25
T_4	8.17	91.83	27.23	14.20	7.07	9.20	42.30	90.8	1696.49	62.30

Table.3 Proximate composition of experimental diets (on wet weight basis)

	Experimental Diet	Moisture	Crude protein	Ether extract	Crude fibre	Ash	NFE
	T_0	7.55	26.12	14.66	5.20	11.39	35.06
	T_1	7.70	25.47	14.32	6.13	11.18	35.29
	T_2	9.15	25.27	13.61	6.60	10.25	35.11
	T_3	9.55	24.65	13.10	5.78	9.20	37.65
	T_4	8.17	25.01	13.04	6.49	8.45	38.84

Table.4 Growth performance of the experimental animals

Treatment	Replications	Initial weight (g)		Weight after 30 days (g)		Weight after 60 days (g)		Weight after 90 days (g)		Survival (%)
T_0	T_0 A	3.61		7.50		11.59		15.11		95.74
	T_0 B	3.60		7.47		11.62		14.90		
T_1	T_1 A	3.60		7.46		11.60		15.31		94.29
	T_1 B	3.59		6.89		10.69		14.99		
T_2	T_2 A	3.58		7.40		11.64		15.88		93.33
	T_2 B	3.61		8.45		13.80		18.31		
T_3	T_3 A	3.62		8.47		13.71		18.02		96.19
	T_3 B	3.58		7.84		12.35		17.38		
T_4	T_4 A	3.59		5.44		8.55		12.29		94.76
	T_4 B	3.62		6.46		9.58		13.22		

Table.5 Parameters to analyze growth performance of experimental animals

Parameters	T_0			T_1			T_2			T_3			T_4		
	T_0 A	T_0 B	Average	T_1 A	T_1 B	Average	T_2 A	T_2 B	Average	T_3 A	T_3 B	Average	T_4 A	T_4 B	Average
Initial weight (g)	3.61	3.60	3.61	3.60	3.59	3.60	3.58	3.61	3.6	3.62	3.58	3.6	3.59	3.62	3.61
Final weight (g)	15.11	14.90	15.01	15.31	14.99	15.15	15.88	18.31	17.10	18.02	17.38	17.7	12.29	13.22	12.76
Weight gain (g)	11.52	11.30	11.40^{b}	11.70	11.40	11.55^{b}	12.30	14.70	13.5^{ab}	14.40	13.80	14.10^{a}	8.70	9.60	9.15^{c}
Percentage weight gain (%)	318.56	313.89	316.23^{b}	324.10	317.55	320.83^{b}	343.58	407.20	374.9^{ab}	397.80	385.47	391.64^{a}	242.34	265.19	253.77^{c}
Daily weight gain (g)	0.128	0.126	0.127	0.13	0.127	0.129	0.137	0.163	0.15	0.16	0.153	0.157	0.097	0.107	0.102
SGR (%) *	1.59	1.57	1.58^{b}	1.61	1.59	1.60^{b}	1.66	1.80	1.73^{a}	1.78	1.76	1.77^{a}	1.38	1.44	1.41^{c}
Total feed fed (g)	21.62	21.69	21.66^{b}	21.53	21.32	21.43^{b}	22.39	25.87	24.13^{a}	24.77	24.43	24.6^{a}	19.31	20.83	20.07^{c}
FCR *	1.88	1.92	1.9^{b}	1.84	1.87	1.86^{bc}	1.83	1.76	1.80^{cd}	1.72	1.77	1.76^{d}	2.22	2.17	2.20^{a}
FER *	0.53	0.52	5.56	0.54	0.27	0.41	0.55	0.57	0.56	0.58	0.56	0.57	0.45	0.46	0.455
Protein Fed (g)	6.11	6.13	6.12	5.94	5.88	5.91	6.23	7.20	6.7	6.75	6.66	6.71	5.26	5.67	5.47
PER *	1.15	1.13	1.14^{b}	1.17	1.14	1.155^{b}	1.23	1.47^{a}	1.35	1.44	1.38	1.41^{a}	0.87	0.96	0.92^{c}

* NB: SGR = Specific growth rate; FCR = Food conversion ratio; FER = Food efficiency ratio; PER = Protein efficiency ratio.

Ammonia – N

The ammonia - N content of all the experimental tanks were recorded and it was found to be 0.5± 0.19 and 0.2± 0.17 ppm before and after water exchange, respectively.

Growth parameters

The survibility (%) of *P. hypophthalmus* fed with different experimental diets varied y between 93.33 to 96.19% (Table).

The weight gain was varied significantly among different treatments at the end of the experimental period and was among the treatments the weight gain was significantly higher in T_3 thanin other treatments.

The weight gain percentage of the experimental animal's o vary significantly) among different treatment groups at the end of the experimental period. Among the treatments the weight gain in T_4 was significantly higher than other treatments. Almost, similar trend was also in case of SGR. Highest SGR was recorded in T (1.77 %) and the lowest in T_4 (1.41%).

The lowest percent FCR was recorded in T_3 (1.76) and the highest was in T_4 (2.20). The feed efficiency ratio (FER) values for different treatments varied y between 0.41 to 0.57with non-significant differences. The average PER value varied significant from0.92 (T_4) to 1.41(T_3).

The study was introduced highlighting the importance of the research need on the topic and explaining the main objectives of the investigation. The information on the nutrient requirement of fish in general and that of protein in particular have been reviewed in detail. Besides, various efforts undertaken to find a suitable alternative to fish meal in general and study on fish silage in particular have also been reviewed. Five isonitrogenous experimental diets (viz., T_0 , T_1 , T_2 , T_3 and T_4) with 35% crude protein level were formulated by replacing fish meal at 0%, 25%, 50%, 75% and 100% by a blended protein source consisting of one third each of fish silage, groundnut oil cake (GNOC) and soya bean meal (SBM). Besides fish meal and blended protein source as above; GNOC, SBM and mustard oil cake (MOC) were the other protein source. Besides, DORB was added as source of carbohydrate, Vita-best as the source of lipid, corn flour as binder and vitamin mineral mixture for fortification of the feed. The feeds were fed to Thai-pangas (*Pangasianodon hypophthalamus*) fingerlings reared under laboratory condition at about 4.0% of their body weight per day for 90 days. Water quality parameters and proximate composition of experimental diets were analysed following standard protocol. The growth performance of the experimental diets was analysed taking standard parameters. The water quality parameters like DO, pH, temperature, total alkalinity and ammonia – nitrogen of the experimental tanks remained within the ideal range for fish culture throughout the experimental period. The experimental diets (T_0 , T_1 , T_2 , T_3 and T_4) had an average crude protein percentage of ranging from 28.25 % to 27.23 %, average ether extract ranging from 15.86 % to 14.20%, NFE content ranging from 37.92 % to 42.30%, crude fibre content ranging from 5.63 % to 7.07 % and total ash from 12.34 % to 9.20 %.The gross energy (KJ/100g) for the experimental diets (T_0 , T_1 , T_2 , T_3 and T_4) was estimated to be 1702.96, 2510.02, 2510.96**,** 2499.80 and 2506.24, respectively. Accordingly, the P/E ratio of the experimental diets (T_0 , T_1 , T_2 , T_3 and T_4) was estimated to be 60.28, 90.94, 90.26, 91.74 and 92.04 respectively. The growth parameters like average weight gain (g), specific growth rate (SGR) (%), food efficiency ratio (FER), food conversion ratio (FCR) and protein

efficiency ratio (PER) were recorded treatment wise. The growth performance of *Pangasianodon hypophthalmus* after feeding with different experimental diets was significantly different. After 90 days of culture the average weight gain percentage were 316.23%, 320.83%, 374.91%, 391.64% and 253.77%, respectively for T_0 , T_1 , T_2 , T_3 and T_4 . The experimental diet T_3 with 75% of the fish meal content replaced showed significantly ($p < 0.05$) higher growth rate, even significantly better than reference diet T_0 containing 100 % fish meal and 0 % blended protein source. On the other hand, experimental diet T_4 containing 0 % fish meal and 100 % blended protein source showed significantly ($p < 0.05$) lower growth rate. The food conversion ratio (FCR) of 1.9 (T_0), 1.86 (T_1), 1.80 (T_2), 1.76 (T_3) and 2.20 (T_4) were recorded, among which T_3 was significantly ($p < 0.05$) better. Similarly, the protein efficiency ratio (PER) of 1.14 (T_0), 1.155 (T_1), 1.35 (T_2), 1.41 (T_3) and 0.92 (T_4) was recorded, among which T_3 was significantly ($p < 0.05$) better. Analysis of the various parameters shows that the silage based blended protein source can replace the fish meal upto 75 % in the Thai pangus (*P. hypophthalmus*) feed. After considering all the factors analysed in the study it may be concluded that the cost effective diet for *P. hypophthalmus* may be formulated like T_3 , replacing 75 % of the fish meal with blended protein source for better performance in terms of growth of the fish.

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