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Vegetable Processing Wastes as Dietary Ingredients for the Striped Catfish *Pangasianodon hypophthalmus*: A Case Study

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Abstract

All food processing industries generate wastes of varying nature in significant quantities. Managing these wastes so as to minimise the impact on the environment is a prime concern. The concept of waste has undergone much change in recent times, with the focus being on utilising the waste materials as inputs for generation of new or reusable products. Vegetable wastes are generated in significant quantities and are easily available at minimal charge. The possibility of utilising vegetable waste as a dietary ingredient was assessed employing the striped catfish as the test species. The study was conducted over a period of 90 days. Vegetable waste was incorporated at inclusion levels of 5, 10 and 15 % in formulated diets. The three test diets were designated T_1, T_2 and T_3 respectively. A clam meal based diet devoid of vegetable waste served as the control (T_0). Feeding was done once daily @ 5% of the body weight. The water quality parameters like dissolved oxygen, water temperature pH, total alkalinity, ammonia, nitrite and nitrate as well as growth response were monitored at fortnightly intervals. The quality of water was maintained by periodic partial replenishment over the period of study. On termination of the trial, better growth response was recorded in the T_1 treatment containing 5% level of vegetable waste. The initial average weight and length of fishes in T_0, T_1, T_2 and T_3 were 6.16 g, 7.14 g, 6.22 g and 7.40 g and final average weight was recorded 17.69, 19.19, 16.79 and 13.46 respectively. Utilisation efficiency was also found to be superior in the T_1 diet as evidenced by the best food conversion ratio of 1.81. Fish fed with T_3 diet recorded the lowest Food conversion ratio of 2.35. Specific growth rate (%) was better in *Pangasianodon hypophthalmus* fingerling fed the control diet. Survival rate of the striped catfish was unaffected by vegetable waste meal supplementation. Organoleptic quality of fish was not affected adversely by vegetable waste incorporation in the diet. The results suggest the possibility of utilising vegetable waste meal in diets for the striped catfish at low levels of incorporation. Further studies on the use of ensiled vegetable waste in diets for the striped catfish are warranted.

Keywords: Vegetable processing waste, Formulated diet, *Pangasianodon hypophthalmus,* Conversion efficiency, Organoleptic quality.

Introduction

In the present day, one of the fastest growing types of aquaculture the world over is that of *Pangasianodon* species. *Pangasianodon hypophthalmus* commonly known as iridescent shark, pangas, sutchi catfish in Thailand, belongs to the family Pangasiidae[1]. The body of *P. hypophthalmus is* long and laterally flattened with no scales. Head is relatively small, mouth broad with small sharp teeth on jaw, eyes are relatively large. Two pairs of barbels are present, the upper one shorter than the lower. *P. hypophthalmus* is omnivorous, feeding on algae, higher plants, zooplankton, and insects, while larger specimens also take fruit, crustaceans and fish. Mature fish can reach a maximum standard total length of 130 cm and up to 44 kg in weight. This species is benthopelagic, typically living within the ranges of pH 6.5-7.5 and 22-26°C [2]. In the year 1997, *P. hypophthalmus* was introduced in India in the state of West Bengal from Bangladesh. Initially its farming was carried in limited area in the state of West Bengal, later on this was cultured on a large scale in the state of Andhra Pradesh too[1].

Demand for pangasiids remains stable throughout Asia. Supplies come from domestic production and through imports, both of which have been on an upward path. In India, demand for pangas catfish in the domestic market is growing. Increasing imports from Vietnam are complementing local harvests to satisfy this demand. The culture activity is spreading fast in our country and now it is not restricted to Andhra Pradesh and West Bengal, rather it has extended to other coastal areas including fishery hotspots of Western Ghats and also in northeastern parts of the country[1]. *P. hypophthalmus* is raised using improved management methods and improvised, supplementary feeds are available commercially. Because of its remarkable growth rate (almost one kg in 90 days), this fish is being cultured in many states particularly the Andhra Pradesh, West Bengal, Kerala and Orissa.

The survival rate of *P hypophthalmus* is satisfactory in culture systems, 85% up to survival rates having been reported[3]. Continued increase in price of fishmeal together with environmental concerns has forced the aquaculture sector to identify alternative protein sources for inclusion in fish feeds.

Presently, the most relevant alternatives are protein sources derived from vegetables sources. Among them, soya bean protein has become a commonly accepted fish feed ingredient and fish meal alternative[4]. In culturing fish in tanks or in ponds due attention needs to be given to nutrition and adequate feeding. If there is no utilizable feed intake by the fish, there can be no growth and death eventually results. Undernourished or malnourished fish cannot maintain health and growth, regardless of the quality of the aquatic environment. Therefore, before any attempt at fish culture, it would be wise to understand the fundamental aspects about nutrition of the cultured species.

Vegetable wastes are available in plenty in urban areas which can be exploited by using as ingredients in fish feed. India is the second major producer of fruits and vegetables in the world. It contributes 10% of world fruit production. In India, over 35 million tons of fruits and vegetables are processed annually and this results in about 10 million tons of wastes[5]. The waste from vegetables processing operation constitutes a large untapped source of energy and proteins. Most of these wastes are merely dumped in fields, which causes pollution. Possible uses of these wastes in animal feed preparation have been suggested by many workers[6]. Utilization of these huge quantities of wastes generally escapes the attention of animal nutritionists, especially in case of fish feed. Very little emphasis has been given to the use of these vegetables processing wastes, which is very cheap and easily available, but somewhat high in fibre content. Vegetable protein sources are often deficient in some essential amino acids. However, the composition of these ingredients may be improved by adding protein rich products such as silages, fishmeal or hydrolysate[7]. Herbivorous fishes can utilize these wastes more efficiently though carnivorous fish may not. Moreover, undigested or partially digested fibre can also enhance the fertility of the pond.

There is hardly any study on the utilisation of vegetable weste in feed for *P. hypophthalmus*. The present study was taken up to assess the utility of vegetable processing wastes in feed for *P. hypophthalmus* with the following broad objectives. i. To assess the utility of vegetable waste as a dietary ingredient in diets for *P. hypophthalmus.* ii. To evaluate the effect of different levels of incorporation of vegetable waste in feeds for *P. hypophthalmus.* iii. To evaluate the growth performance of the species fed vegetable waste incorporated diets. iv. To assess the utilization efficiency of the formulated diets. v. To determine the organoleptic quality of fish fed the test diets.

Materials and Methods

The present study was conducted to evaluate growth performance of *Pangasianodon hypophthalmus* on vegetable waste based diets. The study was carried out at the Kerala University of Fisheries and Ocean Studies, Panangad, Kochi. The fingerlings of *Pangasianodon hypophthalmus* were procured locally and transported to the Kerala University of Fisheries and Ocean Studies, Panangad with oxygen packing in polythene bags. The fingerlings were acclimatized to the culture conditions for a period of two weeks prior to commencement of the experiment. The fishes were kept in oval, flat bottom, fiber glass of 600 litre capacity for acclimatization. The tank was half filled with fresh water and provided with gentle aeration. The fingerlings were fed with commercially available pelleted feed. No water exchange was done during acclimatization. After 14 days the fingerlings were transferred to experimental tanks.

Experimental design and procedure: The acclimatized fingerlings of *Pangasianodon hypophthalmus* were transferred to cement tanks. Each tanks was stocked with 5 fingerlings of average size (6.5±1.25g and 7.5±1.2g). The fingerlings were fed with prepared feeds at the rate of 5% of biomass for 90 days, twice a day. Optimum water quality parameters were maintained. Sampling was done at fortnightly intervals to check the water quality parameters. At each sampling all the *Pangasianodon hypophthalmus* stocked were taken and weighed.

Completely Randomized Design was employed for the studies: treatments being clam meat based control (T_0), 5% vegetable processing wastes (T_1), 10% vegetable processing wastes (T_2) and 15% vegetable processing wastes (T_3). The experimental fishes were acclimatized for 2 weeks prior to stocking. After acclimatization period the initial weight and length measurement of individual fish were recorded. The initial average weight and length of fishes in T_0, T_1, T_2 and T_3 were 6.16 g, 7.14 g, 6.22 g and 7.40 g and length 7.36 cm, 8.13 cm, 7.54 cm and 8.39 cm respectively. Each treatment group of animal was fed at the rate of 5% biomass for the experiment period of 90 days, twice a day. Water quality was maintained in the experimental tanks over the duration of the study by partial water exchange.

After 90 days, the harvesting was done after noting the final individual length and weight and survival ascertained.

Processing of feed ingredients: Feed ingredients: Four experimental diets (T_0, T_1, T_2 and T_3) were prepared for *Pangasianodon hypophthalmus* fingerling. Control diet (T_0) was prepared by using clam meal, rice bran, ground nut oil cake and vitamin-mineral mixture. Different test diets (T_1-T_3) were prepared by replacing clam meal with vegetable wastes. The ingredient proportions of the experimental diets is given Table-1. The composition of feed ingredients and formulated feed were analysed for proximate composition[8].

Processing of the feed ingredients: Various ingredients used for the feed formulation were processed as fallows.

Clam meat: Meat of the black clam *Villorita cyprinoides* purchased from the local market was washed thoroughly and then dried in sun light for two days and powdered.

Rice bran: Fresh rice bran was purchased from the local market and dried for one day in sun light and sieved.

Ground nut oil cake: Fresh ground nut oil cake was purchased from the local market and dried for one day in sun light, powdered and sieved.

Wheat flour: Fresh packed wheat flour was purchased from the local market.

Vegetable: Vegetable waste purchased from the local market, was washed thoroughly, cooked, mashed in to a paste, dried in sun light for two days, powdered and sieved for inclusion in the feed

Vitamin mineral mix: Supplevit'+-M (Sarabhai Chemicals) was used as the vitamin mineral supplement.

The dried ingredients were powdered separately in a pulverizer and sieved. The powdered ingredients were packed separately in airtight plastic bag and used for feed preparation, after analysis of proximate composition.

Table-1
Percentage proportion of ingredients of the test diets

Ingredients (%)	FEEDS			
	T_0 (0%)	T_1 (5%)	T_2 (10%)	T_3 (15%)
Vegetable waste	-	5	10	15
Clam meal	25	20	15	10
Rice bran	40	40	40	40
G.N. Oil cake	24	24	24	24
Wheat flour	10	10	10	10
Vitamin-Mineral mix	1	1	1	1
Total	100	100	100	100

Proximate composition of feed ingredient and test diets: The methods used for the analysis are shown below: Crude protein % was estimated by Micro Kjedahl's method[8]. Percentage of nitrogen obtained was multiplied by the factor 6.25 to get the crude protein content.

Crude fat % was estimated by Solvent extraction using petroleum ether (B. P 40-60°C) in a Soxhlet extraction apparatus for 6 hours.

Moisture content % was estimated by drying the sample at 105°C till a constant weight was arrived.

Ash content % was estimated by incinerating the sample at 550°C for 6 hours in a muffle furnace.

Crude fiber % was estimated by the method of Pearson.
Nitrogen free extract = % of dry matter – (% crude protein +crude fat +% ash content + % crude fibre).

Formulation and processing of experimental diets: Three test diets were formulated incorporating vegetable waste meal at 5%, 10% and 15% level of incorporation. The diets were designated T_1, T_2 and T_3 respectively. The other ingredients were clam meal, rice bran, groundnut oil cake, wheat flour and mineral mixture. The base diet without vegetable waste served as the control. Feeding was done @ 5% of the body weight daily. For the preparation of the experimental diets all ingredients were procured, powdered and sieved. Required quantities of the ingredients with the exception of vitamin mineral mix were weighed out, and mixed uniformly. The mixture was made into a dough by adding sufficient amounts of water and kneaded well. The dough was steamed for 20 minutes in a pressure cooker. The cooked dough was spread out and cooled. To the cooled dough, vitamin - mineral mix @ 1% was added and mixed thoroughly to ensure uniform distribution. The dough was pelletized using a hand pelletizer with 2mm diameter in the die. The pellets were then oven dried in a hot air oven to a moisture content of less than 10%. The dried pellets were cooled and stored in airtight containers for use.

Estimation of water quality parameters: Water quality parameters viz., temperature, pH, dissolved oxygen, alkalinity, ammonia, nitrate and nitrite were monitored at fortnightly intervals according to standard procedures[9].

Nutritional indices: The growth response of fish fed with different diets was monitored by noting average gain in weight, average gain in length, % Specific growth rate (SGR), Percentage survival and food conversion ratio (FCR). Fish growth and water quality parameters were monitored at fortnightly intervals.

Average gain in weight: It gives the increase in weight of the animals during the experimental period. It was calculated using the formula.
Average gain in wt. (g) = Average Final wt. (g) – Average Initial wt. (g)

Average gain in length: This gives the increase in standard length during the experimental period. It was calculated using following formula.
Average gain in length (cm) =Average Final length (cm) – Average Initial length (cm)

Specific growth rate: SGR was calculated using the following formula.

$$SGR\ (\%) = \frac{\ln (W2) - \ln (W1)}{\text{Time interval in days}} \times 100$$

Where: W1= Initial weight of animal (g), W2= Final weight of animal (g).

The calculated value gives the average percentage increase in the body weight per day over a period of 90 days.

Survival rate: $$\text{Survival } (\%) = \frac{\text{Final Number}}{\text{Initial Number}} \times 100$$

The survival rate of fishes is expressed in terms of percentage. This was calculated as follows:

Feed conversion ratio:

$$FCR = \frac{\text{Average weight of feed consumed in dry weight (g)}}{\text{Average live weight gain (g)}}$$

Organoleptic evaluation: On termination of the experiment, the harvested fish in each treatment were assessed for organoleptic quantity using Hedonic Scale (table-2). The score obtained were converted to numerical scores and the data analysed statistically. Fillets, both raw and cooked in 5-10% brine solution were assessed for sensory characters determining the influence of vegetable waste on organoleptic quantity of fish flesh.

Table-2
Sensory evaluation Score sheet

Description of quality	Marks
Dislike extremely	1
Dislike very much	2
Dislike moderately	3
Dislike slightly	4
Neither like or Dislike	5
Like slightly	6
Like moderately	7
Like very much	8
Like extremely	9

Statistical analysis: The experiment was designed in a completely randomized block design with three replications for each treatment. On termination of the experiment all surviving fishes were collected and length and weight recorded individually. The average fortnightly gain in weight and length for each fishes was subjected to a two way analysis of variance (ANOVA) with three replication. The comparisons of means were done using Duncan's multiple range tests. Different water quality parameters were subjected to Two way analysis of variance and was significant. Duncan's multiple range test was applied. The sensory evaluation of *P. hypophthalmus* fillet was subjected to nonparametric test. All statistical significance was done ($P \leq 0.05$). All statistical analysis was performed using IBM SPSS Statistics version 20.

Results and Discussion

Biochemical constituents of feed: Feed ingredients: The data pertaining to proximate composition of the various ingredients used in formulating the pelleted feeds are given in table-4.

The moisture content of the feed ingredients used *viz.* vegetable processing wastes, clam meat, rice bran, ground nut oil cake and wheat flour varied from a minimum of 7.1% in wheat flour to a maximum of 10.7% in ground nut oil cake. Clam meat had the highest percentage of crude protein (54.6%), followed by ground nut oil cake (40.5%), vegetable processing wastes (15.8%), wheat flour (16.5%). Rice bran had the minimum crude protein content of (13.9%). The proximate composition of the formulated feeds is presented in table-3. The percentage of moisture in the four feeds varied between 8.80% (diet T_1) and 10.30% (diet T_3). Lower protein level were maintained in the diets with increasing level of vegetable waste incorporation. Crude protein content ranged from 24.75% (diet T_3), 26.69% (diet T_2), 28.63% (diet T_1) and 30.58% (diet T_0). The crude fat content ranged from 4.28% (diet T_3), 4.60% (diet T_2), 4.84% (diet T_1) and 5.10% (diet T0). (Table3).

Water stability of feeds: Test for water stability of different test diets was done for varying periods such as 1 hours, 2 hours, 4 hours and 6 hours. The results are given in Table-4. Maximum and minimum water stability of feed was recorded in treatments T_3 and T_0 respectively (table-4.)

Water quality parameters: Range of values recorded for different water quality parameters were water temperature (25-27.5°C), water pH (6.5-7.6), dissolved oxygen (4.6-6.5 ppm), total alkalinity (96-110 ppm), ammonia (0.00-0.5 ppm), nitrate (0.00-5ppm) and nitrite (0.00-2ppm).

Utilisation of test diets: The mean weight gain of *Pangasianodon hypophthalmus* in the four treatments T_0, T_1, T_2 and T_3 was found to be 17.69g, 19.19g, 16.79g and 13.46g respectively. The highest average live weight gain was found to be obtained in treatment T_1 with 5% vegetable waste based diets and lowest was observed in treatment T_3 with 15% vegetable wastes. The average gain in length of *P. hypophthalmus* fishes in the four treatments T_0, T_1, T_2 and T_3 was found to be 7.28cm, 7.85cm, 7.17cm and 6.18cm respectively. The highest average gain in length was obtained in treatment T_1 with 5% vegetable wastes and lowest observed in treatment T_3 with 15% vegetable wastes. At the end of the feeding trial, the highest weight gain of

Pangasius *hypophthalmus* was found in treatment T_1 (5% vegetable waste) followed by T_0 (control diet), T_2 (10% vegetables waste) and lastly T_3 (15 % vegetables waste). Sunitha M. and Rao D.G.[10] had reported better weight gain in *Tilipia mossambica* when fed with blue green algae (*Chlorella, Anabaena, Oscillatoria, Nostoc*) grown with the support of mango waste Hung L.T. et. al.[11]. had also reported that pangascatfish has been demonstrated to having a capacity for utilising plant feedstuff carbohydrates for energy. Therefore, it can be concluded that vegetable wastes have considerable potential for partial replacement with fish meal as supplementary feed ingredients in sustainable aquaculture of *Pangasianodon hypophthalmus.*

Feed is the single largest item of expenditure to the farmers, accounting for 79 to 92% of the total production cost in striped catfish farming[12-14]. In general, two types of feeds are used for striped catfish, wet farm made feeds and pelleted feeds, and these differ in formulation and quality[14]. According to Hung LT. et. al.[15], the traditional feeding of small scale catfish farming is largely based on trash fish (marine origin) constituting approximately 50-70% of feed formulations. *Pangas* catfish has been demonstrated to have a capacity for utilising plant feedstuff carbohydrates for energy, but little research has been performed on these fish species with regard to alternative dietary protein source selection[11]. Using plantbased proteins in aquaculture feeds requires that the ingredients possess certain nutritional characteristics, such as low levels of fibre, starch and antinutritional compounds. They must also have a relatively high protein content, favourable amino acid profile, high nutrient digestibility and reasonable palatability[16]. A number of previous studies discuss the suitability of plant protein feeds and/or local agricultural byproducts as an alternative protein source in fish feeds[17-22].

The maximum SGR was recorded in T_0 control diet and minimum in T_3 i.e., 15% vegetable waste. The average SGR values in the four treatments T_0, T_1, T_2 and T_3 were found to be 1.14, 1.08, 1.10 and 0.81 % respectively. The minimum value was 0.81 in T_3 with diet 15% vegetable waste and the maximum value of specific growth rate was (1.14) with diet clam meal based diet To respectively. This is comparable with the findings of Sunitha M.[10]. They reported better weight gain in *Tilipia mossambica* when fed with blue green algae (*Chlorella, Anabaena, Oscillatoria, Nostoc*) grown with the support of mango waste. 100% survival of pangas was recorded from all treatments, with no mortality in any of the treatments. The mean FCR values in the four treatments T_0, T_1, T_2 and T_3 were found to be 1.99, 1.81, 2.23 and 2.35 % respectively. The best FCR was thus obtained in treatment T_1.

Table-3
Proximate composition of the feed ingredients and experimental diets

Parameter / Ingredients	Crude protein %	Crude fat %	Moisture %	Crude fiber %	Ash %	Nitrogen free extract %
Vegetable wastes	15.7±0.8	3.1±0.2	10.1±0.8	9.4±0.8	10.2±0.3	50.9±0.9
Clam meal	54.6±0.7	8.9±0.4	8.9±0.3	4.0±0.5	6.4±0.5	17.2±1.0
Rice bran	13.9±0.5	1.8±0.1	8.7±0.2	32.5±0.6	12.2±0.6	41.8±0.4
Ground nut oil cake	40.5±0.4	7.6±0.0	10.7±0.5	8.1±0.6	6.2±0.3	26.8±0.7
Wheat flour	16.5±0.2	4.1±0.1	7.1±0.8	10.5±0.9	11.0±0.4	50.7±0.8
Control diet T_0	30.9±0.7	5.1±0.1	9.5±0.6	12.4±0.5	9.0±0.8	33.4±0.9
Test diet T_1	28.6±0.2	4.8±0.8	8.8±0.6	12.5±0.5	9.1±0.4	36.0±0.9
Test diet T2	26.7±0.7	4.6±0.1	8.9±0.9	12.7±0.6	9.3±0.6	37.8±0.7
Test diet T3	24.7±0.8	4.3±0.8	10.3±0.9	13.0±0.7	9.5±0.7	38.2±0.5

Each value is mean ± SD of triplicate observations

Table-4
Percentage water stability of test diets

Diet	1 hours	2 hours	4 hours	6 hours
T0	94.00	91.20	89.00	87.34
T1	94.89	93.12	91.23	89.10
T2	95.00	93.50	91.87	89.20
T3	94.80	93.12	91.32	89.45

Table-5
Growth performance (mean ±SE) of pangas fed different test diets

Treatments	Experimental groups			
	T_0	T_1	T_2	T_3
Initial length (cm)	7.36±0.65	8.13±0.07	7.54±0.38	8.39±0.21
Final length (cm)	14.64±0.36	15.98±0.13	14.71±0.51	14.57±0.24
Length gain (cm)	7.28±0.62	7.85±0.05	7.17±0.14	6.18±0.43
Initial weight (g)	6.16± 0.20	7.14±0.08	6.22±0.24	7.40±0.26
Final weight (g)	23.85± 0.39	26.33±0.12	23.01±0.06	20.87±0.19
Weight gain (g)	17.69±0.56	19.19±0.07	16.79±0.29	13.46±0.08
% SGR	1.14±0.05	1.08±0.01	1.10±0.04	0.81±0.03
FCR	1.99±0.14	1.81±0.36	2.23±0.16	2.35±0.31
Survival (%)	100	100	100	100

Organoleptic quality: In the present study, sensory evaluation of raw and cooked pangas fillet with respect to colour, odour , texture and taste remained non-significant ($p>0.05$) in all treatments. Overall quality of flesh of the fish grown on vegetable waste based diet was superior. Similar results were also obtained by Nandeesha N.C. et. al.[23]. Based on mean panel score it could be observed that diet 5% vegetable waste led to comparatively better flesh quality in both raw and cooked pangas but lower sensory quality was observed in fish fed 15% vegetable waste raw and cooked fish. Statistical analysis indicated that there was no adverse effect of dietary vegetable meal supplementation on flesh quality.

Conclusion

From the present study, it can be concluded that the large quantity of vegetable processing wastes can be used in feeds for pangas at lower level of inclusion. Ensiling of vegetable waste might enable efficient utilisation at higher level of incorporation also studies in this direction is warranted.

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