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Research Article

ALTERNATIVE FEED FOR AQUACULTURE FROM DESQUAMATED ORGANIC MATERIAL

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ABSTRACT

Fish nutrition has advanced vividly in contemporary years with the expansion of new and balanced commercial diets that promote optimal fish growth. This research aimed to evaluate the effects of different feeds, made from desquamated organic material as the sole ingredient and were compared to the commercial feed, to observe various growth parameters of zebra fish. Juvenile zebra fish were fed with pellets, for a period of 3 successive weeks. The health of the fish and the environmental parameters were also observed as a part of the investigation. Of the different feeds given to the fish, apple peel feed can be considered as one of the substitute for cost effectiveness of the feeds.

INTRODUCTION

Good nutrition plays a noteworthy role in providing sound health for all organisms. In Pisciculture (Fish farming), nutrition has become an imperative criteria as feeds represent 40-50% of the total of fish rearing cost. However, influencing the later physiology of an organism, becomes easier when proper nutritional conditions are provided at an earlier age (Erick and Manuel, 2016).The development of new diet formulations supports the aquaculture industry as it satisfies the increasing demand for affordable and high-quality fish products (Louis *et al*, 2009). It is necessary to provide a feed with complete nutrition, by which fish grows rapidly and attains maximum weight. Zebra fish (Danio rerio) is a common model organism in fish studies including various fields like toxicology, developmental biology, neurobiology and molecular genetics; it has also been anticipated as a model organism for nutrition and growth studies, researches have been conducted on larvae (Carvalho *et al*, 2006; Onalu and Langdon, 2000; Spence and Smith, 2006)and sexually mature zebra fish. However, the knowledge available related to the nutritional requirement of the species is very little (Karga and Mandal, 2016). Variousessential (indispensable) amino acidsthat cannot be synthesized by the fish, and are necessary for growth and maintenance of fishes include; methionine, arginine, threonine, tryptophan, histidine, isoleucine, lysine, leucine, valine and phenylalanine, (Steven and Louis, 2009; Louis *et al*, 2009) each of which have their peculiar significant role. Methioninepromotes optimal growth in many cell functions (Cowey,1994); threonine, leucine, isoleucine and valine are required in high amounts for growth (Steven and Louis, 2009) whereas, tryptophan deficiency leads to scoliosis, lordosis, eye cataracts, renal calcinosis, caudal fin erosion and short gill opercula (Louis *et al*, 2009). Lysine deficiency causes erosion of the caudal fin (Cowey, 1994; Steven and Louis, 2009). Apart from amino acids and proteins, the other important parameters that direct the proper growth of fish within a suitable environment are analysis of length, weight, BMI, condition factor and survival rate. Environmental parameters which help in detecting the environmental state of the fish; are pH of water, dissolved oxygen (DO) content and biological oxygen demand (BOD) in water. The increase of pH in the water content, indicates the presence of a toxic environment for the fish. Dissolved oxygen (DO) and Biological oxygen demand (BOD) are the most important parameters in water quality assessment and they reflect the physical and biological process prevailing in water. Its presence is essential to maintain the higher forms of biological life in water.

Till date various experiments have been designed considering soya bean and cheese as an active source for protein (Manuel *et al*, 2013).A commercially available fish feed consist of soya bean meal, cod liver oil, vitamins, protein, calcium, iron, phosphorous, amino acids, fatty acids, etc. Replacement of fish meal with economically affordable ingredients of plant origin, rich in amino acids are desired because of rising cost (Higgs *et al*, 1995). Aim of the present investigation is to develop a feed as an alternative feed, to meet the affordable cost of feed.

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MATERIALS AND METHODS

Preparation of diet (pellets): For the formulation of diet, specific pulse *Vigna radiate* (Whole Green Moong), vegetables *Pisum sativum* and *Cucuis sativus* (Green peas and Cucumber) and fruit *Malus domestica* (Apple) peels, which are rich in amino acids were selected. 100gms each of these selected samples were used to prepare the pellets. The peels of fruits and vegetables were washed to remove any impurity, shade dried and ground; whereas the pulse (whole moong) were sieved to check for impurity and then ground into powder. Pellets were formed with clean, filtered water as a binding agent in the ratio of 1:2 (10gms of powder and 20ml of water), for each set.

Experimental protocol- 6 tank of dimensions 12*7*9(l*b*h) were taken. Zebra fish were procured from a local vendor and were set up in laboratory conditions. The selected fish had an average length of 3cms and an average weight of 1.30 grams. Fish were taken into various tanks and were subjected to various dietary treatments for a period of 3 weeks after the acclimatization period. Tanks were cleaned at an interval of 3-4 days.

After the acclimatization period, the control set continued with the standard commercial feed (Hi-Red: Feed for all fishes), whereas the rest were subjected to various dietary treatments as follow:

Groups	Diets
Control	Standard commercial feed (Hi-Red:Feed for all fishes)
Group 1	*Malus domestica* (Apple)
Group 2	*Cucuis sativus* (Cucumber)
Group 3	*Pisum sativum* (Green Peas)
Group 4	*Cucuis sativus*+ *Pisum sativum* (Cucumber + Green)
Group 5	*Vigna radiata* (Whole Green Moong)

For the experiment, the fish were divided in 6 sets as mentioned above, each containing 6 specimens and these sets were further studied in triplicate. Dosage of 2 pellets were given at an interval of every 10-11 hours. The sets were maintained for 21 days and then the fish were taken for analysis of the following parameters: Environmental parameters such as dissolved oxygen (DO), Biological oxygen demand (BOD) and pH were regularly monitored to check the quality of water and survival rate was also checked at the end of the experiment. Fish were monitored for their changes in length and weight. To check the health status of the fish, BMI and Conditioning Factor were also checked. Biochemical parameters like amino acid and protein content of the muscles were checked at the end of the experimental setup. The pellets were tested for their protein content.

Data analysis

Analysis of weight: For the analysis of weight the live fish were taken in a pre weighed beaker and measured for weight gain or loss by using the following formula (Gonzales, 2012)

Weight gain = W_f-W_i

Where W_f is the final weight and W_i is the initial weight.

Analysis of length: Increase in length were measured using a scale by placing the live fish on tile and covering it with a net. Increase in length was monitored at an interval of every 5 days. The difference was calculated and noted.

BMI: To quantify the amount of mass in the tissue BMI is used. It is derived from the mass (weight) and the height of the fish.

Conditioning Factor: Condition factor is necessary to compare the length and weight relationship of the fish. The condition of the fish based on its length, height and thickness, can be estimated by the following formula (Richter *et al*, 2000)

$K = 100 \times M/L3$

Where, K = condition factor, M = body mass, L = body length.

Amino acid estimation: After 21 days of treatment with their respective diets the muscles of the fishes of different sets were homogenized with NaCl and used for amino acid estimation by Ninhydrin method (Jayaraman, 1981).

Protein estimation of muscles and pellets: After 21 days of treatment with their respective diets, the muscles of the fishes of different sets were mixed with NaCl and further the samples were used for protein estimation using Folin lowry method. Whereas, for pellets 1gms of pellets of each set was dissolved in 10ml of water. It was mixed thoroughly and 1ml of the sample solution was taken to perform Folin-Lowry test.

Survival rate: Rate of survival was calculated using the formula (Gonzales, 2012):

Survival rate (%) = (D_f/D_i) x 100%

Where D_i represents the initial number of fishes in a tank and D_f represents the final number of fishes in a tank.

Water from different tanks were collected at the end of the experimental setup:

pH: pH was checked with the help of pH strips and pH meter.

Dissolved oxygen (DO): Dissolved oxygen was calculated on the last day, in ppm using Winkler's method (Patel *et al*, 1978).

Biological oxygen demand (BOD): BOD is the amount of oxygen dissolved in the water, which is required for breakdown of organic matter by aerobic biological organisms.

All the experiments were conducted in triplicates.

Statistical analysis: All data observed during the experiment were completely on random basis. Data obtained for all the parameters were subjected to one way ANOVA to test the level of significance. The difference was considered to be significant at $P<0.05$. Data sets showing significant difference were subjected to post hoc unpaired T test, to check the difference of each group with control. The data sets were further checked with Bonferroni correction. All the statistical analysis were performed using Microsoft excel 2013.

RESULTS

After completion of the 21 days of experimental setup; carried out for various parameters, the results were recorded. For the same, a mean was calculated and further statistical analysis were carried out

Table 1 Demographic parameters

Demographic parameters (mean ± standard deviation)				
Feeds	**Weight (grams)**	**Length (cms)**	**Bmi**	**Condition factor**
Control	0.54± 0.007	0.58 ±0.011	0.092± 0.014	27.056± 5.046
Group 1	0.068± 0.002	0.63 ± 0.017	0.107± 0.00	27.28± 1.564
Group 2	0.055 ± 0.005	0.54 ± 0.017	0.101 ± 0.012	35.28±6.561
Group 3	0.025 ± 0.002	0.34 ± 0.036	0.076 ±0.014	69.925 ± 11.030
Group 4	0.044 ± 0.002	0.42 ± 0.025	0.104± 0.011	56.503±15.853
Group 5	0.057 ± 0.005	0.58± 0.026	0.098 ±0.012	29.81 ± 6.508

Table 2 Environmental Parameters

Environmental Parameters (Mean ± Standard Deviation)				
FEEDS	**pH**	**D.O (ppm)**	**B.O.D (ppm)**	**Survival Rate (%)**
Control	5.52± 0.01	15.14±0.005	0.713± 0.005	83.33 ± 0.01
Group 1	5.42± 0	14.54±0.023	1.33± 0.023	83.33± 0
Group 2	6.60±0.005	11.51±0.015	4.346 ±0.015	60 ± 0.01
Group 3	6.64±0.005	11.71±0.011	4.133± 0.011	50 ± 0.07
Group 4	6.11 ± 0.01	17.57± 0	-1.71± 0.00	60 ± 0.01
Group 5	5.65 ± 0	16.96±0005	-1.11 ±0.005	66.66± 0

Table 3 Biochemical parameters

Biochemical Parameters (Mean ± Standard Deviation)			
FEEDS	**Amino acid (mg)**	**Protein estimation-muscles (mg)**	**Protein estimation – pellets(mg)**
Control	0.38± 0.015	0.11± 0.003	0.41± 0.01
Group 1	0.626± 0.005	0.077± 0.002	0.31± 0.005
Group 2	0.25± 0.01	0.027 ±0.0017	0.06± 0.00
Group 3	0.616± 0.02	0.05± 0.005	0.025± 0.001
Group 4	0.526± 0.01	0.041± 0.004	0.04± 0.00
Group 5	0.45± 0.02	0.061± 0.001	0.51± 0.01

DISCUSSION

The main aim of this study was to provide indispensable amino acids to the fish, crucial for their growth and development. Amino acids play a dominant role as the building blocks and as intermediates in metabolism, which further help to maintain health and vitality (Zygmunt *et al*, 2009). The composition of various combinations of amino acids formed by linkages, gives each protein its individual characteristic of either being an immunoglobulin, hormone or an enzyme (Craig and Helfrich, 2009; Delbert, 2010). Proteins are digested in fish in their gastrointestinal tract in a similar manner like any other monogastric animal, which involves the secretion of pepsin (proteolytic enzyme) and hydrochloric acid (Delbert, 2010, Conceicao *et al*, 2010). However, comparing the results of all the parameters in the current study performed, apple peel feed can be considered as superlative over rest of the formulated diets. As observed in the results obtained in Table 3, there is a drastic decrease in the amount of proteins in fish muscle compared to the amino acid content. In general, amino acids are utilized by the body for 3 main factors - 1) Protein turnover, 2) Oxidation of amino acids and 3) formation of N- compounds from amino acids (Cowey, 1994). The high amount of amino acid content in the muscles, indicates that the amino acids have not been completely utilized by the fish. The probable reasons could be the extreme high amount of amino acid in the pellets or the short duration of the study. The proportion of the protein content in the pellets and the amino acids in the muscles are proportional in all the pellets. However, amongst the selected peels, apple peel and green pea pellets have been proven to have the maximum amount of amino acid content. The data subjected to ANOVA showed a statistical difference for all the 3 biochemical parameters. Post hoc test showed a significant difference between all the groups when compared with control for protein estimation in pellets and muscles. For amino acid estimation, onlygroup 5 showed a significant difference from control.

Biometric analysis of the fish were performed at an interval of every 5 days and the average was calculated and tabulated (table 1). The maximum increase in weight was observed in the fish, fed with Apple pellets (0.068 ± 0.002) and the minimum was found in Peas pellets (0.025 ± 0.002). As per data obtained from Gonzales John M (2012), the average weight gain of fish (90 days post fertilization) should range from 0.149 ± 0.023 to 0.275 ± 0.023. Another important aspect of biometrics includes the length, maximum increase in length was observed in the fish fed with Apple pellets (0.63 ± 0.017) and the minimum was found in Peas pellets (0.34 ± 0.036). Christian Lawrence *et al* (2012), put forth that the average increase in the length of the fish (150 days post fertilization) ranged from 3.15±0.06 to 4.32±0.08. In the case of the present study the gain in weight and length followed the similar pattern of increase in length and weight post treatment of 21 days. This also suggest that the increase in the desired value needs the treatment for the said period. This increase was post the dietary treatment with apple feed. Whereas the other group showed less increase in demographic parameters studied. Post hoc analysis in length showed a significant difference in group 3 and 4 when compared to control, whereas in weight analysis only group 3 showed a significant difference from the control.

BMI and Conditioning factor was calculated to check the health of the fish. The table 1 shows that there is increase in the Conditioning factor of the fish fed with the apple feed as compared to other groups diet suggesting that the fish were healthy (Richter *et al*, 2000). BMI showed no significant difference in ANOVA. Conditioning factor showed a significant difference in ANOVA, but Post hoc analysis showed no significant differences.

In the present study we also monitored the quality of water in terms of pH, DO and BOD as the dietary treatment being organic could disturb the quality of water. All the discussed environmental factors showed a significant difference in ANOVA (*P* is 0.05), when treated with post hoc all the groups showed a significant difference with control for all parameters. The pH of all the water samples were in the range of 5.00 – 7.0, indicating that the organic diet did not alter the pH of water and the excreta of the fish did not contain high amount ammonia, making the environment suitable and non-toxic for the survival of fish. The maximum amount of DO was measured in the water of Peas + cucumber sample (17.57ppm) and the minimum was found in Cucumber water sample (11.51 ppm) both the range for the survival of the fish. BOD of the fish tanks which were fed with the diet 3 and 4 was found to be relatively higher suggesting the increase in organic load in water and also the feed not being consumed by the fish. This was also observed when weight and length of the fish were monitored for these two diets with least gain in the weight.

The maximum survival rate was observed in Apple feed (83.33 %) and the minimum was found in Peas feed (50%) significantly (*P*=0.05), futher supporting our findings that due to increase in organic content in water. Post hoc results showed

that all the groups except group 1 showed a significant difference.

CONCLUSION

Comparing the length and the weight of the fish there was a good gain found in the fish fed with apple peel. Since weight is one of the important factors that can be considered to see the development of the fish, apple peel diet can be an alternative feed for fish with some standardization. It can be also supported with our results in terms of environmental parameters which suggest that there is no alteration in pH value of water making the environment favorable for the survival of fish.

Further studies could be done to see the relative development of the vital organs which may aid to make the fish a better research model. With the other prescribed formulations such as soyameal, shrimps, worms etc., synergistic combinations of desquamated material can be tried and standardized to be used as fish feed with relatively better nutritive value**.**

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