

Effects of *Kaempferia galanga* (Ekangi) and *Spirulina* spp. on the Growth Performance and Feed utilization of Singhi, *Heteropneustes fossilis* (Bloch, 1794)

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Abstract: A 60 days indoor feeding trial was conducted to assess the growth performance and feed utilization in *Heteropneustes fossilis* fingerlings fed with three experimental diets viz. control diet, a diet with 1% *Kaempferia galanga* (Ekangi) and a diet with 1% *Spirulina* spp. in three treatments i.e. T1, T2 and T3 respectively. Proximate composition of the diets did not differ significantly in three treatments. Analysis of proximate composition of fishes revealed that moisture content was lowest in T2 whereas highest protein and fat content were found in T2 which was significantly different than T1 but not with T3. Ash content was also significantly higher in T2 than T1 and T3. Average Daily Gain (ADG) in T2 was significantly higher than T1 but did not differ with T3 whereas values of Specific Growth Rate (SGR), and Condition factor (K) were higher in T2 than both T1 and T3. Survival rate (%) in T2 was significantly higher than T1 but did not differ with T3. Better Feed Conversion Ratio (FCR) was observed in T2 which was significantly lower than T1 but did not differ significantly with T3. Protein Efficiency Ratio (PER) was significantly higher in T2 than T1 and T3. It may be suggested that addition of *Kaempferia galanga* (Ekangi) in the feed of *H. fossilis* might increase palatability and have positive effects on growth performance and feed utilization.

Keywords: *Heteropneustes fossilis*, *Kaempferia galanga* (Ekangi), *Spirulina* spp. Growth performances, Feed utilization.

Date of Submission: 25-02-2019

Date of acceptance: 11-03-2019

I. Introduction

Bangladesh holds leading position among the fish producing countries in the world. In the last ten years average growth performance of fisheries sector is 5.43%. Bangladesh ranked 5th in the world aquaculture production which actually alone contributed 56.44% of the total fish production in Bangladesh [1]. In Indian subcontinent and Southeast Asian region *Heteropneustes fossilis* (common name: Singhi) is considered as one of the most important fish species for aquaculture for its high growth rate, short life cycle, significant tolerance to environmental stress, ease of reproduction and high market demand [2,3,4]. Moreover, this fish species contain high protein, iron (226 mg/100 g), calcium associated with low fat for which it is considered as nutritionally superior fish with medicinal value [5,6,7,8].

Spirulina spp. has been one of the most widely used micro-algal species in aqua feeds due to its high contents of protein, vitamins, essential amino acids, minerals, essential fatty acids and antioxidant pigments such as carotenoids [9]. Several studies have been conducted to investigate the effects of *Spirulina* spp. on growth, nutrient utilization and immune responses of various fish species, including rainbow trout *Oncorhynchus mykiss* [10] red sea bream *Pagrus major* [11] common carp *Cyprinus carpio* [12], tilapia *Oreochromis niloticus* [13], Mekong giant catfish *Pangasianodon gigas* [14], and African sharptooth catfish *Clarias gariepinus* [15]. Recently, some workers in India have identified some of the traditionally used plant material including *Kaempferia galanga* (Ekangi) in fishing and angling in Tripura and evaluated their roles in fish feeds by conducting laboratory trial on freshwater prawn and species of Indian major carp [16]. Ekangi, tumbul, kharboj, chotokakla and latakasturi are the plant materials that have been used for alluring fish during harvest and angling by traditional fish farmers [17]. Seventeen kinds of Chinese medicinal plants including Ekangi (*K. galanga*) were tested for their role as attractant in bait for *Brachydanio rerio* with varying results [18]. The rhizomes of ekangi are strongly aromatic and rich in protein, amino acids, minerals, sugars, and lipid, also contains an essential oil, alkaloid, starch, gum and fatty matter [19].

Feed attractants were added to fish feeds to increase feed consumption and, as a consequence, increasing nutrient retention, i.e. growth. [20]. Natural attractants were traditionally used in angling and fishing in natural waters around the globe. Information on the use of such natural herbs in fish feeding is limited.

However, few studies were conducted to utilize herbs and plant parts as feed additives to enhance growth and feed efficiency [21]. To make aquaculture profitable more number of plant based ingredients were being used for preparation of fish feed and the palatability of such feed can be increased by addition of attractants [22]. So, considering the potentiality of *K. galanga* (Ekangi) and *Spirulina spp.* as feed attractants, an attempt was made to evaluate their role in enhancing growth performance and feed consumption of *H. fossilis*.

II. Materials and Methods

2.1. Experimental procedure

2.1.1. Collection of fish

Fingerlings of *H. fossilis* were collected from Water World Motsho Hatchery, Mymensingh. Live and healthy fishes were collected in 2014 and carried in oxygenated bags with water to the laboratory. Fingerlings were kept in Circular tanks with adequate aeration and sufficient water.

2.1.2. Experimental diet formulation

The basal fish feed (Quality Feeds Limited) which served as control feed, consisted of fish meal, soybean meal, wheat flour, vegetable oil, soybean oil, salt, trash fish and vitamin mineral premix, were collected from local market near Central Veterinary hospital, Dhaka; which were then formulated to achieve other supplemented feeds. *Spirulina spp.* were collected from Fish Technology laboratory, BCSIR, Dhaka and Ekangi rhizomes were collected from Kustia district. Three experimental diets were used in this experiment to evaluate the growth performances and feed utilization on *H. fossilis* and these were Treatment-1(T1): Commercial feed without feed attractants, as control diet; Treatment-2 (T2): Control diet + 1% *K. galanga* (Ekangi) used as a feed attractant; Treatment-3(T3): Control diet + 1 % *Spirulina spp.* used as a feed attractant. Dietary ingredients of feed additives (Spirulina and Ekangi) were ground using a laboratory grinder and then separately blended with commercial feed at fixed ratio into a homogenous doughy matter by adding water, which was pelleted by pressing through a 4 mm die in a grinding machine. The pellets were then dried in the sun and then further dried in an oven and after complete drying they were stored in plastic containers at room temperature for further use.

2.1.3. Experimental design

An indoor rearing system consisting of nine glass aquaria was used for this purpose. Each aquarium supplied with almost 30L of water where *H. fossilis* were stocked. Each aquarium provided with 15 fishes and before stocking the batch weight of fishes in each tank was also recorded to the nearest gram. Tap water was the source of water supply in the aquarium and an adequate level of oxygen in each aquarium was maintained through artificial aeration by using air pump. About 30% of the water in each tank was exchanged weekly. The experiment was conducted in triplicates for each dietary treatment for 60 days.

2.1.4. Feeding trial

The experimental feeding trial was scheduled for a period of 60 days as stated earlier. The fish were fed once daily at the rate of 5% of total body weight of fish. The average mass weight of the fish from each aquarium was taken at the end of the experiment. The quantity of supplied feed was adjusted weekly according to the increase in fish body weight. At the end of the experiment, the fish were weighed and analyzed for proximate analysis.

2.1.5. Waste Water Removal

Feces in the aquaria were cleaned in every week before feeding commenced without creating any disturbances to fish.

2.1.6. Water quality management

Routine measurements for water quality (such as Temperature, DO, pH etc.) were taken on a weekly basis.

2.2. Proximate composition analysis

The proximate compositions of each of the diets were done following the AOAC method [23]. At the end of the experiment, the fish were weighed and analyzed for proximate composition following the AOAC method [23].

2.3. Calculation:

The following variables were calculated:

2.3.1. Average Daily Weight Gain

Average Daily Weight Gain (ADG) = $(W_t - W_0)/t$

2.3.2. Specific Growth Rate (SGR)

Specific growth rate (SGR) = $(\ln W_t - \ln W_0) \times 100 / t$

2.3.3. Survival rate

Survival rate = $N_t \times 100 / N_0$

2.3.4. Condition Factor

Fulton's condition factor $K = W \times 100 / L^3$

2.3.5. Feed Conversion Ratio

FCR = Feed given (dry weight) / Wet gain (wet weight)

2.3.6. Protein Efficiency Ratio

Protein Efficiency Ratio = Wet weight gain / Crude Protein in diet

Where, W = weight of fish (g), L = Length of fish (cm), W_t and W_0 were final and initial fish weights (g) respectively; N_t and N_0 were final and initial numbers of fish in each replicate respectively; t is the experimental period in days.

2.4. Statistical Analysis:

Data were analyzed using one way analysis of variance (ANOVA) to determine the differences between treatment means demonstrating a significant variation of P value of <0.05 (at 95% confidence intervals) by using the statistical software package IBM SPSS Windows version 20.0 (IBM Corp.)

III. Results and Discussions

Proximate compositions of the experimental feeds are presented in table 1. No significant differences were found among moisture, protein, lipid and ash in different feeds.

Table 1: Proximate composition of the experimental diets (%)

Variables	T1	T2	T3
Moisture (%)	32 ±0.16 ^a	35.4±0.11 ^a	32.3±0.09 ^a
Protein (%)	12±0.93 ^a	10.93±1.2 ^a	11.84±1.02 ^a
Fat (%)	5±0.09 ^a	4.57±1.52 ^a	4.98±0.096 ^a
Ash (%)	18±1.25 ^a	18.44±0.088 ^a	18.62±1.31 ^a

Whole body proximate composition of *H. fossilis* fed with three experimental diets (% fresh weight basis, ± SEM) after 60 days is presented in table 2.

Table 2: Proximate composition of the *H. fossilis* under different treatments

Variables	T1	T2	T3
Moisture (%)	79.13±0.046 ^b	73.66±0.053 ^a	78.63±0.20 ^{ab}
Protein (%)	16.42±0.215 ^b	17.84±0.385 ^a	16.77±0.159 ^{ab}
Fat (%)	2.43±.045 ^b	3.03±.126 ^a	3.013±0.059 ^{ab}
Ash (%)	2.25±0.142 ^a	2.87±0.184 ^b	2.35±0.07 ^a

Analysis of proximate composition of fishes revealed that moisture content was lowest in T2 whereas highest protein and fat content were found in T2 which was significantly different than T1 but not with T3. Ash content was also significantly higher in T2 than T1 and T3. According to the size and age of the fish, proximate composition showed variations for three experimental diets. No difference in the moisture and protein content in carcasses of common carp fed on diets incorporated with up to 55% *Spirulina* powder [12]. A similar trend for fat was reported in *Cyprinus carpio*[24]. The fat content in T2 was almost same as in T3 (fish fed diet containing *Spirulina* spp.). The effects of *Spirulina* spp. on whole-body protein and lipid contents were correlated with their synthesis and accumulation rate in muscle, as well as the growth rate of the organisms [25, 26, 27].

The growth performance data are presented in table 3. Average Daily Gain (ADG) in T2 was significantly higher than T1 but did not differ with T3 whereas values of Specific Growth Rate (SGR), and Condition factor (K) were higher in T2 than both T1 and T3. Survival rate (%) in T2 was significantly higher than T1 but did not differ with T3. So, the controls in T1 might have problem with feed intake which might be

related with less palatability. The value of K is influenced by age of fish, sex, season, stage of maturation, fullness of gut, type of food consumed, amount of fat reserve and degree of muscular development. Value of condition factor was less in the current experiment compared to the study on the survival and growth of cat fish after giving selected supplemental feeds where values of condition factor were between 0.81-0.87 [28]. The average survival rate of fish was 86.67±2.23% in fish fed with control diet and 97.77±2.23% and 93.32±3.84% in fish fed diet with *K. galanga* and *Spirulina* spp. and these findings have more or less similarities with the findings [29]. However, 100% survival rate of *H. fossilis* was observed in a study with formulated pelleted feed [30]. Survival rate of red sea bream ranged from 77.8-87.8% when fed with dietary algae [31] which is more or less similar with the current findings.

Table 3. The growth performance data (mean ± SEM) of experimental fish in three treatments.

Parameters	T1	T2	T3
Initial body weight (g)	5.23±0.64	4.84±0.75	6.64±0.96
Final body weight (g)	10.54±.93	11.96±.13	13.11±0.94
Initial length (cm)	10.23±.47	10.2±0.91	11.76±0.27
Final length (cm)	13.02±.21	14.96±.86	15±0.36
Weight gain (g)	5.31±.31	7.12±.41	6.47±0.02
Percentage of Weight gain (%)	103.11±7.57	150.17±19.037	101.33±13.67
Average daily weight gain (ADG)	0.088±0.001 ^a	0.12±0.01 ^b	0.11±0.00 ^{ab}
Specific growth rate (%)	1.75±0.07 ^a	1.54±0.12 ^b	1.58±0.12 ^a
Survival rate (%)	86.67±0.00 ^a	97.77±2.23 ^b	95.54±2.22 ^{ab}
Condition factor (K)	0.36±0.032 ^a	0.46±0.015 ^b	0.38±0.017 ^a

Table 4. Feed utilization data (mean± SEM) of *H. fossilis* fed in three treatments.

Items	T1	T2	T3
Feed conversion ratio (FCR)	1.78±0.067 ^a	1.43±0.102 ^b	1.65±0.046 ^{ab}
Protein efficiency ratio (PER)	0.89±0.064 ^a	1.043±0.230 ^b	0.86±0.118 ^a

Feed utilization data are presented in table 4. Better Feed Conversion Ratio (FCR) was observed in T2 which was significantly lower than T1 but did not differ significantly with T3. Protein Efficiency Ratio (PER) was significantly higher in T2 than T1 and T3. It may be suggested that addition of *K. galanga* (Ekangi) in the feed of *H. fossilis* might increase palatability and have positive effects on growth performance and feed utilization. These results might be possibly due to the improved feed intake and nutrient digestibility. The fish groups fed with two experimental diets (diet I and diet III) showed a higher feed intake rate than control diet during the experimental periods. This might be due to the attractive color, racy flavor and good nutrient composition of the experimental diets. Feed supplemented with *Spirulina* powder improved the feed conversion ratio and growth rates for striped jack [32]. A 3% supplementation of *Spirulina* meal in moist pellets reconfirmed the efficacy of *Spirulina* in improving the growth performances and feed utilization of feed in fish [33]. Rainbow trout fed with experimental diet consisting of *Spirulina* spp. showed higher growth (final weight, condition factor, weight gain, SGR, average daily growth) when compared to rainbow trout fed with a standard trout diet but that was not statistically significantly different [34]. Interestingly that increasing level of *Spirulina* in diet provided better growth comparing to the other commercial feeds [35]. However juvenile tilapia fed solely on the alga show a lower feed efficiency and protein efficiency ratio than commercial-diet-fed tilapia [13]. The net weight gain and feed intake were significantly higher and FCR was significantly lower in *O. pabda* fed with *K. galanga* mixed feed although survival (%) did not differ significantly [36]. Significant weight gain, daily growth rate, specific growth rate, relative growth rate, and feed conversion ratio were observed in common carp fingerlings when diet with *Spirulina* spp. at the rate of 5 gm/kg was administered [37]. Combined use of ekangi and probiotic in the diet resulted in highest percent live weight gain, highest accretion of carcass protein and lower FCR compared to the individual inclusion of ekangi and probiotic [38].

IV. Conclusion

From the observations in the experiment, it might be concluded that addition of both *K. galanga* (Ekangi) and *Spirulina* spp. in the feed of *H. fossilis* might have better effects in growth performances and feed utilization. Both of the feed attractants might be used in other fish feeds to evaluate the efficiency. Current study was completely laboratory based, so future field studies are necessary to determine the original effect of *K. galanga* (Ekangi) and *Spirulina* spp.

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Shawkat Ali. "Effects of Kaempferia galanga (Ekangi) and Spirulina spp. on the Growth Performances and Feed utilization of Singhi, *Heteropneustes fossilis* (Bloch, 1794)." IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 12.3 (2019): PP- 67-71.