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Effects of Black Thorn, *Kaempferia galanga* Single or in Combination with Yeast Probiotic on Feed Palatability, Growth Performance and Product Quality of *Labeo rohita* Fingerling (Hamilton)

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Abstract

A 63-days indoor feeding trial was conducted to assess the growth performance of *Labeo rohita* fingerling (69.82±0.53 g) fed with three experimental diets containing black thorn, *Kaempferia galanga* as natural attractant, yeast as probiotic and their combination. Comparable feed consumption in groups fed with attractant (86.54 g) and combination of attractant and probiotic (85.17 g) compared to group fed probiotic only (74.10 g) were observed. Combined use of attractant and probiotic in the diet resulted in highest percent live weight gain (105.30%) compared to the individual inclusion of attractant (60.88%) and probiotic (72.63%). Comparison of feed conversion ratio (FCR) indicated better conversion when fed with the diet containing both the attractant and probiotic (3.69) compared to the other diets containing either probiotic (4.96) or attractant (5.20). Further, highest accretion of carcass protein was recorded in groups fed diet with combination of additives (254.79%), followed by attractant (193.03%) and probiotic (159.29%) alone. RNA/DNA ratio in both liver and muscle exhibited a significant ($P<0.05$), linear increase in groups fed diet with only probiotics followed by attractant alone, and the combination of both probiotics and attractant accordingly. Present study revealed better efficacy of feed supplemented with *Kaempferia galanga* in feed consumption, growth promotion and utilization in *L. rohita* fingerlings, as compared to that with probiotic. However, the combination of probiotics and attractant could confer the better results as compared to their individual dietary inclusion.

Keywords: Feed conversion ratio, carcass, RNA/DNA ratio.

Introduction

Indian aquaculture revolves around three major carp species. Over the decades, nutritional research has transformed the pond aquaculture practices in the country from extensive to semi-intensive and to some extent intensive. Diversification of aquaculture practices in the form of cage culture involving major carps had been attempted to improve productivity of the vast resource of reservoirs available for augmenting country's fish production. Amongst the carp the potential of *L. rohita* is compatible in polyculture system having better growth performance and wider consumer preference in Indian sub-continent. Therefore, contribution of *L. rohita* in polyculture system practiced in the country varied between 35-40%. The high growth potential of the Indian major carps has attracted the attention of several tropical South-Eastern Asian and Middle-Eastern countries. Considering its importance in the culture system, emphasis has also been given to its genetic improvement through selective breeding in India. Moreover, development of feed and feeding practices is a prerequisite for cage farming of any targeted fish species. Non-availability of suitable feed and feeding practices for cage farming of Indian major carps in reservoir is one of the constraints for its wider adoption in the country. Slow consumption of given feed in cages may incur nutrient loss through leaching in one hand and deterioration of environment on the other hand, leading to sub-optimal growth performance. Probiotics have been used in formulated diets for its role in digestion and better assimilation. Incorporation of attractants might increase feed palatability leading to faster ingestion of feed, thereby minimizing feed wastages and deterioration of water quality (Cerezuela *et al*., 2013; Jones, 1980).

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 (Shivraman, 2012). Recently, some workers in India

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have identified some of the traditionally used plant material including *Kaempferia galanga* 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 (Shivraman, 2012 Venkateshwarlu *et al*., 2009). Leaves of Marjoram found to improve growth, feed conversion, protein utilization and protein and energy digestibilities of hybrid tilapia (Guo *et al*., 2010). 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 (Guo *et al*., 2010). The dried rhizome of a perennial plant *K. galanga*, commonly known as Black thorn in English and Ekangi or Chandramukhi in Bengali, is strongly aromatic. It is abundantly found in Indonesia, Southern China, Taiwan, Cambodia and India. Considering its aromatic property, an attempt was made to evaluate its attracting role in enhancing the feed consumption or the feed palatability and growth performance of *L. rohita* fingerlings.

In the recent years, probiotic occupied a centre stage because of its beneficial role in fish and shellfish culture. According to World Health Organization, probiotic are live microorganisms that when administered in adequate amounts confer a health benefit on the host (WHO, 2001) in terms of improving activity of gastro-intestinal microbiota (Pandiyan *et al*., 2013; Wang, 2007; Meena *et al*., 2014), enhanced immune status (Irianto and Austin., 2002; Salinas *et al*., 2006), disease resistance, survival, feed utilization (Mohapatra *et al*., 2012; Pandiyan *et al*., 2013; Meena *et al*., 2014), growth and the improvement of nutrition of host species by producing digestive enzymes (Wang, 2007; Mohapatra *et al*., 2012). Therefore, while developing feed for Indian major carp, *L. rohita*, for its cage culture; efforts were made to study the role of natural attractants and probiotics in their feed consumption pattern, feed utilization and growth performances. Keeping this in view, the present experiment was thus designed to investigate the effect of incorporation of the attractant *K. galanga* and probiotic, baker's yeast, alone and in combination on feed consumption and growth performance of Indian major carp rohu, *L. rohita* fingerling.

Materials and methods

Experimental Diets Formulation

Three iso-nitrogenous (25% CP) and isocaloric (3.72 Kcal/g) diets were formulated using agricultural by-products fortified with Baker's yeast as probiotic (Feed P), powdered rhizome of *K. galanga* as natural attractant (Feed A) individually at 2% incorporation level each and a third diet containing both probiotic and attractants (Feed PA) at same inclusion level. Known quantities of dry ingredients were measured using digital balance (Anamed MX-7301A ±0.001 g) which were then well mixed and steam cooked. A vitamin-mineral premix (Agrimin Forte) was added to the dough after removing from the cooker when the temperature came down to 60°C. Pellets were prepared using hand pelletizer fitted with 2 mm die. The pellets were then oven dried (60°C) and ground in a machine to obtain the final fine product. Proximate composition of feed ingredients is given in Table 1. Ingredients composition and proximate analysis of the different formulated diets are given in Table 2.

Experimental Design

The feeding trial involving rohu fingerlings were conducted in nine 55 L circular trough fitted with flow through system (water exchange rate: approx. 2 L/min) using un-chlorinated fresh tap water. After acclimatization, 35 fingerlings were stocked in each trough. The experiment was conducted in triplicates for each dietary treatment for 63 days. Average initial weight (69.82±0.53 g) of the fish was recorded prior to commencement of each experiment. 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 trough was taken at the end of the experiment. The quantity of supplied feed was adjusted weekly according to the increase in fish body weight. To determine the feed consumption, the leftover feed was collected after 4 h of each feeding and kept in a thermostat for drying. The pooled samples were weighed after oven drying. At the termination of the experiment, the fish were weighed and analyzed for carcass composition. The water quality parameters,

Table 1. Proximate composition of feed ingredients

Feed ingredients	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
Fish meal	6.12±0.14	62.42±0.54	10.75±0.25	16.8±0.24
Soybean oil cake	6.02±0.14	42.12±0.60	1.6±0.03	9.46±0.16
Mustard oil cake	13.17±0.22	33.25±0.53	6.35±0.12	7.57±0.11
Groundnut oil cake	11.45±0.19	34.45±0.43	4.67±0.08	6.46±0.12
Wheat bran	11.1±0.23	14.44±0.66	3.87±0.07	4.72±0.09
Rice bran	7.75±0.13	8.13±0.56	7.66±0.14	15.9±0.26
Black gram	7.8±0.12	12.38±0.63	1.7±0.03	17.13±0.23
Bengal gram	6.3±0.11	8.42±0.54	2.05±0.04	16.27±0.20
Green gram	8.0±0.17	12.82±0.82	2.03±0.08	3.53±0.04

Table 2. Composition and proximate composition of experimental diets (% dry matter basis)

Ingredients	% inclusion in diets		
	Feed P	Feed A	Feed PA
Fish meal	5	5	5
Soybean oil cake	5	5	5
Mustard oil cake	15	15	15
Groundnut oil cake	15	15	15
Wheat bran	20	20	20
Rice bran	17	17	17
Black gram	5	5	5
Bengal gram	5	5	5
Green gram	5	5	5
Binder	5	5	5
Vitamin-Mineral Premix	2	2	2
Probiotic	2	-	2
Attractant	-	2	2
Proximate composition of diets (% dry matter basis)			
Dry matter	91.98±0.06	91.85±0.09	91.84±0.12
Crude protein	25.29±0.15	25.36±0.13	25.12±0.05
Crude lipid	2.21±0.03	2.23±0.04	2.26±0.04
Ash	14.45±0.25	14.86±0.17	14.12±0.24

viz., temperature (°C), pH, dissolved oxygen (mg/L) and specific conductivity (µs/cm) of water from each experimental set were monitored at regular intervals as per the standard methods of American Public Health Association (APHA, 1985).

Proximate and Biochemical Analysis

The proximate composition of the feed ingredients and experimental diets and protein of fish carcass were analyzed both prior to commencement and on termination of the experiment by following the standard methods of Association of Official Analytical Chemists (AOAC International, 1990). For this purpose, 12 fish from each trough were sampled at the termination of the experiment.

DNA and RNA Estimation from Tissue

Quantitative determination of nucleic acid in different fish tissues were performed by pentose analysis and were calculated as follows (Schneider, 1955).

$$\mu g\ DNA / ml = \frac{OD\ at\ 600nm}{0.019}$$

$$\mu g\ RNA / ml = \frac{(OD\ at\ 600nm + 0.08) - (\mu g\ DNA\ /ml \times 0.013)}{0.116}$$

Statistical Analysis

Statistical analysis was done by one way analysis of variance (ANOVA) using MS-Excel software. Mean difference between three treatments were tested for significance at $P<0.05$ and comparisons were made by Duncan's multiple range test (Duncan, 1955) to find out significant difference between the three treatments in respect of feed consumption, growth performance, carcass composition and general performance of the fish.

Evaluation of Growth Performance

Growth performance of the experimental diets was measured by calculating following parameters:

Live Weight Gain (%) = Final body weight-Initial body weight/Initial body weight x 100

Feed Conversion Ratio = Dry feed intake/Wet weight gain

Carcass Protein Gain (%) = Final body protein x final body weight–initial body protein x initial body weight x 100

Results

The water quality parameters recorded during the feeding trial were; temperature, 23-27°C; pH 7.6-7.9; specific conductivity 409-419 µs/cm and dissolved oxygen 5.2–7.7 mg/L within the optimum range for this fish. Results of the 63 days growth trial conducted in the indoor feed trial facility is given in Table 3. The fish fed with feed PA showed insignificant difference in feed consumption (85.17%), compared to the groups fed with other feed A (86.54%) and P (74.10 %).

Performance of diet containing various feed additives individually and in combination were measured in terms of percent live weight gain and dry weight gain, both indicated significantly ($P<0.05$) higher weight gain (105.30%) in groups fed with feed PA followed by A (72.63 %) , and P (60.88%). Gain in dry weight and carcass protein was considered as indicator which implied highest values in groups fed with feed PA (215.63-254.79%), followed by the

groups fed with feed A (168.48-193.03 %), and feed P (145.73-159.29%). Feed utilization measured in terms of FCR was lowest (3.69) in fish fed with feed PA revealing significantly ($P<0.05$) better feed conversion in this groups compared to the groups fed other diets (Feeds P and A; Table 3).

Nucleic acid make up of cell as DNA and their expression counterpart as RNA could indicate the cellular growth, thereby their ratios in liver and muscle of *L. rohita* fingerlings in different treatment groups can provide estimate for growth status at cellular level. There was no significant effect ($P>0.05$) of feeds P, A and PA on DNA levels in liver and muscle (Table 4). This may be due to inherent nature of DNA, which remains almost unchanged in variable environmental and nutritional conditions. The DNA content of liver was marginally higher as compared to muscle DNA content irrespective of all feed groups. The DNA content for muscle was observed to be similar (0.21 µg/ml) for fish fed with all type of feeds while DNA content in liver was highest (1.68 µg/ml) for fish fed with diet A, followed by diet P (1.66 µg/ml) and diet PA(1.65 µg/ml). However, a positive correlation between RNA levels and growth was observed in liver ($R^2 = 0.98$) and muscle ($R^2 = 0.99$). Unlike DNA, RNA keeps on changing in face of different nutritional and environmental variables. RNA content of fish fed with group PA, A and P was 0.77, 0.38 and 0.22 µg/ml respectively. The highest RNA content (1.62 µg/ml) was observed in liver of fish fed with diet PA followed by Diet A (1.26 µg/ml) and diet P (0.94 µg/ml). RNA/DNA ratio was significantly ($P<0.05$) higher in the groups fed with feed PA. The RNA/DNA ratio linearly increased in muscle ($y = 0.875x + 0.392$, $R^2 = 0.99$) and liver ($y = 0.218x + 0.467$, $R^2 = 0.98$) when arranged in order of feed P, feed A and feed PA. For all the diets, RNA/ DNA ratio was higher in muscle than liver. Similarly the ratio were in the order of PA>A> P for both muscle and liver (Table 4).

Discussion

In the present experiment, the beneficial role of feed additives in *L. rohita* fingerling diet was elucidated. The probiotic, baker's yeast was used as a growth promoter in the present study. The beneficial effects and responses application of probiotic in aquaculture in order to promote growth and disease control in form of alternate to antimicrobial compounds has become a common practice (Irianto and Austin, 2002; Jones, 1980; Wang, 2007), has reviewed by Meena *et al.* (2014). However, growth performance of fish group fed with diet A was found to be better than the group fed with feed P alone. This might be attributed to the fact that *K. galanga* resulted in enhanced palatability and hence better feed consumption in the group fed with feed A that resulted to better growth in the *L. rohita* when fed on this diet. In the present study, the growth performance of the fish fed with feed PA, differed significantly than those fed with the feeds A and P alone. However, insignificant difference was noted in feed consumption between the group fed with feeds A and PA. This phenomenon might be due to the addition of attractant in this diet that resulted to almost same feed consumption in both the treatments. The corollary of the observation might be found in the earlier studies (Guo *et al*., 2010). On the other hand, comparatively

Table 3. Growth performance, feed consumption pattern and protein accretion in experimental fish

Parameters	Feed P	Feed A	Feed PA
Initial weight (g)	69.82±0.53^b	73.27±0.60^a	65.26±0.52^c
Final weight (g)	112.33±1.59^c	126.49±1.64^b	133.98±1.68^a
Live weight gain (%)	60.88±1.05^c	72.63±0.82^b	105.30±0.95^a
Dry weight gain (%)	145.73±1.41^c	168.48±2.23^b	215.63±1.94^a
Feed consumption (g)	74.10±0.51^b	86.54±0.54^a	85.17±0.52^a
Feed conversion ratio	4.96±0.08^b	5.20±0.05^c	3.69±0.02^a
Carcass protein gain (%)	159.29±1.30^c	193.03±2.24^b	254.79±4.19^a

Different superscripts in the same row signify statistical differences at 5% level of probability, ($P<0.05$) (mean±SE) (n = 6).

Table 4. DNA and RNA levels in tissues

Parameters	Diets		
	Feed P	Feed A	Feed PA
Muscle DNA(µg/ml)	0.21±0.02	0.21±0.02	0.22±0.02
Liver DNA (µg/ml)	1.66±0.02	1.68±0.02	1.65±0.03
Muscle RNA (µg/ml)	0.22 ±0.01 ^{c}	0.38 ±0.02 ^{b}	0.77 ±0.03 ^{a}
Liver RNA (µg/ml)	0.94 ±0.05 ^{c}	1.26 ±0.04 ^{b}	1.62 ±0.03 ^{a}
Muscle (RNA/ DNA) Ratio	1.04 ±0.06 ^{c}	1.80 ±0.03 ^{b}	3.5 ±0.03 ^{a}
Liver (RNA/ DNA) Ratio	0.56 ±0.09 ^{c}	0.75 ±0.07 ^{b}	0.98 ±0.04 ^{a}

Different superscripts in the same row signify statistical differences at 5% level of probability, ($P<0.05$) (mean ± SE) (n = 6).

low feed consumption was recorded in groups fed with feed P indicating the beneficial role of inclusion of *K. galanga* in the diet of *L. rohita* in inducing feed consumption. As growth is the function of feed consumption, digestion and assimilation, the reduced feed consumption in group fed with feed P had a negative impact on its growth performance.

The study revealed that the feed consumption pattern in two fish groups, the feeds PA and A were almost similar. The presence of feed attractant in both the diet exerted similar influence on feed consumption pattern but differed significantly ($P<0.05$) for the groups than that of the fish group fed feed P only. It might be due to the inclusion of the attractant, *K. galanga* in the experimental diets which could have stimulated the olfactory responses due to the presence of strong aromatic and campherous compound in it. The same had been reported in earlier study that feed additives mediated olfactory and gustatory responses had fascinating influence on fish growth, feed consumption and angling (Ajiboye *et al*., 2012; Kasumyan and Døving, 2003; Venkateshwarlu, *et al*., 2009).

Comparatively better feed conversion observed in groups fed with feed PA might be due to synergistic effects of the two additives incorporated in this diet. The attractant present in the diet accomplished a better feed consumption which was better digested and assimilated due to the presence of probiotic (Meena *et al*., 2014; Denev *et al*., 2009; Irianto and Austin, 2002).

In addition, growth of fish is the result of nutrient accretion in general and protein in particular. The highest protein accretion in group fed with feed PA may be attributed to the better feed consumption and utilization due to the combined use of additives (De *et al*., 2014; Denev *et al*., 2009; Pandiyan *et al*., 2013).

Conventional parameters like weight gain, protein accretion are mostly used to assess the efficacy of supplementing various feed additives. However, underlying mechanism at cellular level can be added by generating data on advance indicator like RNA and DNA fraction of the tissue and their ratios. Therefore, growth performances, feed conversion ratio (FCR) and protein accretion were validated with the RNA and DNA ratio which is the measure of protein synthesis at cellular level in muscle and liver tissues of *L. rohita* fingerlings. The nucleic acid indices as RNA/DNA ratio has proved to be a reliable estimate of real time growth, nutritional status and protein synthesizing potential of a cell (Meena *et al*., 2013; Shivraman *et al*., 2012). Present study showed highest RNA/DNA values in feed PA fed groups which was solely the result of better growth rate in fish attributed to the growth promoting effects of feed PA, which was significantly ($P<0.05$), differed from their individual inclusion. Kumar *et al.* (2006) has suggested that dietary inclusion of non-gelatinized corn with amylase has increased the muscle's size which was validated with increase in RNA content, DNA content, protein content and protein /DNA ratio of the muscle . This study also signifies the common assumption that the protein synthesis is a function of RNA synthesis in the respective muscles. Recently Kumar *et al.* (2010) also reported that dietary supplementation of gelatinized and non-gelatinized starch has changed RNA content of the different tissues of the *L. Rohita* due to variable fraction of crude protein in the two starch diets. Study also revealed that the gelatinized starch could increase the muscle protein contents as compared to non-gelatinized starch which might be attributed to elevated RNA content of the respective muscles. The present experiment thus highlighted the importance of *K. galanga* incorporation in the diet as a natural attractant which could enhance feed consumption vis-à-vis enhanced growth performance. Combined use of attractant and probiotic further enhanced the efficiency of the diet in terms of feed conversion, protein gain and thus true growth. The findings of the present experiment would be valuable input in formulating feed for cage culture of carp in general and *L. rohita* in particular, in reservoirs. However, further studies are warranted to understand the role of *K. galanga* as growth enhancer besides its significance as natural attractant in fish feed.

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