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Effects of Artificial Food Additives in Vietnam Koi, *Anabas Testudineus* (Bloch, 1792) Pond Culture System.

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Abstract
To evaluate the effects of artificial food additives on feedings efficiency, growth performances, survival and production of Vietnami climbing perch (*Anabas testudineus*), A 97 days on-farm experiment was carried out. The study was conducted into three earthen ponds with two replications of each. Two different and locally available artificial food additives (Rapid Grow and Biozyme) were used in the experimentation and experimental unit were indicated as control (C), Treatment 1 (T_1 –Rapid Grow) and Treatment 2 (T_2 –Biozyme). About 500 fry (Average wt.2 g) were stocked in each experimental pond and fed four times in a day (8 a.m.11 a.m.2p.m and 5 p. m.). Highest average food conversion ratios (FCR) were found in control 1.85, 1.48 and 1.56, T_1, and T_2 respectively. Average protein efficiency ratios (PER) were 0.86±0.036, 0.91±0.044 and 0.92±0.037 in control, T_1, and T_2 respectively. The specific growth rate (SGR) was found 0.68±0.125%/d in the control, 0.83±0.242%/d in T_1 and 0.99±0.128%/d in T_2 respectively. Average daily gain (ADG) was found higher in T_2 (3.18±1.576g/d), than in T_1 (3.01±1.635 g/d) and in control (2.66±1.486 g/d). The survival rates were found 92.75%, 98.5% and 96.85% in Control, T_1 and T_2 respectively. It was observed that growth performances and feeding efficiency were in increaed in T_1 than that of T_2 and Control respectively. The best growth was found case of Vietnami koi (*Anabas testudineus*). The BCR was found better in T_2 than T_1, lowest BCR found in control. Finding suggets that artificial food additives has profound on feedings efficiency, growth performances, survival and production of Vietnami climbing perch (*Anabas testudineus*). However, more off farm and intensive trials are suggested to optimize the effect artificial food additives in growth and feeding regime for better fingerlings surval rate, production performance and profit.

Keywords: Vietnam Koi, Food Additives, growth performance and feed efficiency

1. Introduction
Aquaculture is the fastest growing food production sector in the world and provides a significant supplement and substitute to wild fish and plant [1]. Fish is the second most valuable agricultural crop in Bangladesh and its production contributes to the livelihoods and employment of millions of people [2]. The fisheries are naturally controlled by primary productivity as phytoplankton subsequently zooplankton production of aquatic bodies [3, 4, 5]. The climbing perch (*Anabas testudineus*, Bloch) is a well-known member of the Anabantoidie family which derived their name for bearing labyrinth like accessory-breathing organ. This fish species, to South and Southeast Asia including Bangladesh, Pakistan, Nepal, Srilanka, Burma, Thailand, Vietnam, Indonesia, Singapore and China, is found in fresh and brackish waters mostly in ponds, swamps and lakes of these regions [6].

Because of Koi's ability to withstand marginalized poor quality water and breathe atmospheric air, the fish still exists in the ecosystem of Bangladesh as a common species [7]. Considering the importance of this species in nutritional, economics and biodiversity point of view, it is required to develop an appropriate culture technique of *A. testudineus*. This good aquaculture technology will be helpful to meet up the dietary demand and this tasty fish will be available for the rural people of Bangladesh [8].

Among the various culture systems medicine applied in aquaculture is more suitable in context of Bangladesh. This is comparatively a new method of aquaculture, which has gained much popularity throughout the world due to a number of advantages over the conventional method of fish farming. The cost of feed is a major constrain in fish production. It minimizes the production cost and also ensures the proper utilization of the animal waste. Therefore, the present study was undertaken to observe the growth performance of Vietnami koi in medicine culture system and to compare the growth performance in normal pond at same density. Feeding enzymes to shrimps and fishes is one of the major nutritional advances in the aquaculture sector since last few years. Exogenous enzymes are now extensively used throughout the world as additives in animal diets. Also, supplementation with enzymes can help to eliminate the effects of anti-nutritional factors and improve the utilization of dietary energy and amino acids, resulting in improved performance of fish/shrimps [9, 10]. The uses of chemicals in aquaculture systems for various purposes are widely recognized. In aquaculture, as in all food production sectors, one of the external inputs required for successful crop production is chemical [11]. They are essential components in pond construction, health management, and soil and water management, enhancement of natural aquatic

Productivity, transportation of live organisms, feed formulation, manipulation and enhancement of the production [12].

Two artificial food additives (commercial brand name: Rapid

grow, Multivitamin) and commercial brand name: Biozyme, enzymes) are used with regular feed in the present experiment. The present study intended to determine the effects of artificial food additives in growth performance and feeding efficiencey and survivality of Vietnam pond in cmmerecial aqiacultnd subsequently on pond productivity.

2. Materials and methods

Study area and experimental design

The research was carried out at a commercial aquaculture farm located at Sadar Dakshin Upazilla, Comilla, Bangladesh, from May 21 to August 28, 2014 in six earthen rearing ponds with a surface area of 1.18 acre with an average depth of 1.0 meter. Three treatments with two replicas of each.

Table 1: Experimental design for evaluation of artificial food additives in Vietnami koi *A. testudenius* fingerlings

Treatment	Replication	Stocking density/decimal	Feed type	Feed frequency	Artificial food additives
Control	R_1 R_2	500 fry	Pre-Nursery, Nursery, Starter and Grower feed	Four times a day	None
Treatment 1	R_1 R_2	500 fry	Pre-Nursery, Nursery, Starter and Grower feed	Four times a day	Rapidgrow, Gasonex plus, Polgard plus, Aqua magic plus
Treatment 2	R_1 R_2	500 fry	Pre-Nursery, Nursery, Starter and Grower feed	Four times a day	Biozyme, Gasonex plus, Polgard plus, Aqua magic plus

Fry source

The fry of Vietnami koi *A. testudenius* used in this experiment were collected from a private hatchery named Abrar Agro Fisheries & Hatcheries situated at Fulpur, Mymensingh. Fish fries were carried to the study area under well oxygenated conditions and fry release between 6 am to 7 am.

Feeding Schedule and Frequency

The fish was fed four times a day. The amount of feed given was estimated by until satiation point treatment. The fish was fed four time a day (8am, 11am and 2pm; 5pm). Initially feed were given at the rate of 35% of their body weight. When the experimental fish attained the weight 200gm (average body weight), the amount of the feed gradually decreased and it was given finally at rate of 2% (BW).

Multivitamin and Enzyme mixed with feed

Multivitamin (Rapid Grow) mixes 3gm/kg feed from 2.7.14 to 28.8.14 in T_1 and Biozyme (enzyme) mixes 2gm/kg feed 2.7.14 to 28.8.14 in T_2 pond.

Chemical applied in culture pond water

Gasonex Plus, Aqua Magic Plus and Polgard plus were used in every 10 days interval in both treatment (T_1 and T_2).

Sampling & health monitoring

The growth performance was assessed by recording the rate of growth in terms of gain in length (cm) and in weight (g). Sampling was done at 7 days interval to determine, growth efficiencies (ADG, SGR) and feeding efficiency (FCR, PER). The fry were checked once a week to inspect for signs of malnutrition or disease. The total number of samples was 14 for the above mentioned variables. Weight was taken with a balance meter (CAMRY Balance – China) and length with a measuring scale. All the data recorded in a note book and spread sheet and then finally calculated the average length and weight of fishes according to treatment on each sampling day. At the end of the period all harvested live fish were counted and weighed to determine survival rate and yield Dissolved oxygen, pH, NH_4+were monitored in the culture ponds at the time of fish sampling.

Water quality parameters

To maintain water quality, water temperature, pH, Dissolved oxygen (DO) and amonnia were measured at 7 days interval. Temperature was recorded by using a Celsius thermometer. DO, pH, NH_3 were measured directly by a Wellvet DO meter -Thailand, a VET pH test kit-Thailand and WELL AMMONIUM test kit-Thailand respectively.

Data Analysis

Specific growth rate (SGR), food conversion efficiency (FCR) and protein efficiency ratio (PER) were calculated by using followling formula

SGR (% / day) = (Ln. Final body weight - Ln.Initial body weight)/days x 100; %

Weight gain = (Final body weight -Initial body weight)/ Initial body weight x 100;

FCR = Food fed (g dry weight)/ Live weight gain (g);

PER = Live weight gain (g)/ protein fed (g dry weight).

Survival rate (%) = No. of actual fish survived / No. of actual fish stocked x 100,

Cost-benefit ratio = Gross income/ Gross cost.

The data were analyzed through one way Analysis of Variance (ANOVA) using SPSS (ver. 12, Inc., Chicago, USA). Standard deviation in each parameter and treatment was calculated and expressed as mean ± SD. The level of significance pond was set at 5% (P>0.05)

3. Results & Discussion

Feed conversion ratio (FCR ;%)

FCR of the experimental feed were found significant better in the treatment 1 rather than other two replicas. The highest FCR in the control could be due to less utilization of feed while low FCR in the treatment 2 and 1 might be explained by better utilization. Habib *et al* (2015) [13] Found that, Feed Conversion Ratio (FCR**)** in Vietnami koi Treatment 1, Treatment 2, Treatment 3 were3.32 ± 0.164b 2.21 ± 0.160a 3.13 ± 0.193b wherein 100 fish m^3, 150 fish $m^3$and 200 fish m^3 were stocked.

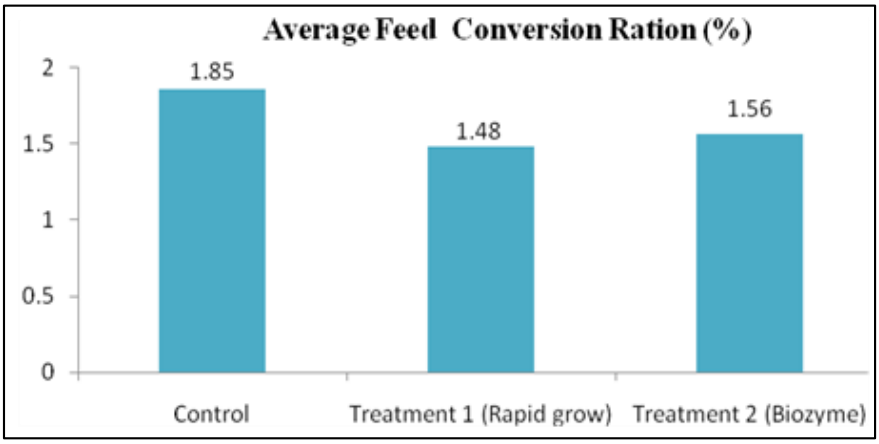

Fig 1: Feed conversion ratio (FCR) of Vietnami Koi fish (*Anabas testudineus*) treated with two different feed additives.

Protein Efficiency Ratio (PER)

Highest PER was found in the treatment 2 treated with Biozyme followed by the treatment 1 and the control. This differences in the PER could also be linked to the low utilization efficiencies of same feed due to no and presence of the different chemical (rapid grow and biozyme), Habib *et al* (2015) [13] Found that, Protein Efficiency Ratio (PER) in Vietnami koi Treatment -1, Treatment- 2, Treatment- 3 were 1.00±0.0473, 1.524±0.117and 1.073±0.121 wherein 100 fish m^{3}, 150 fish $m^{3}$and 200 fish m^{3} were stocked. Hasan (2003) [14] observed best PER in Nile tilapia *Oreochromis niloticus* when fed feed supplemented with vitamin compared to the feed without vitamin.

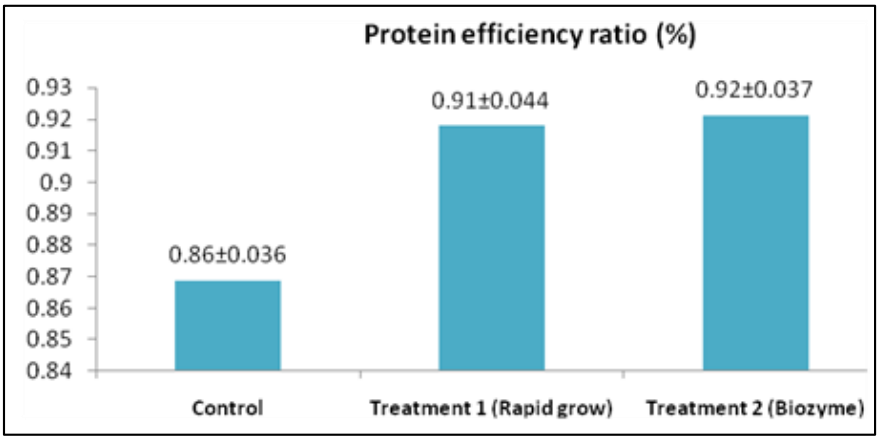

Fig 2: Protein efficiency ratio (PER,%) of Vietnami Koi fish (*Anabas testudineus*) treated with two different feed additives.

Specific growth rate (SGR, %/d) and average daily gains (ADG)

The highest SGR (0.99±0.128%/d) was measured in the treatment 2 while the lowest SGR (0.68±0.125%/d) was found in the control. However, the SGR (0.83±0.242%/d) observed in the treatment 1 was significantly lower than that of treatment 2 and higher that the control.

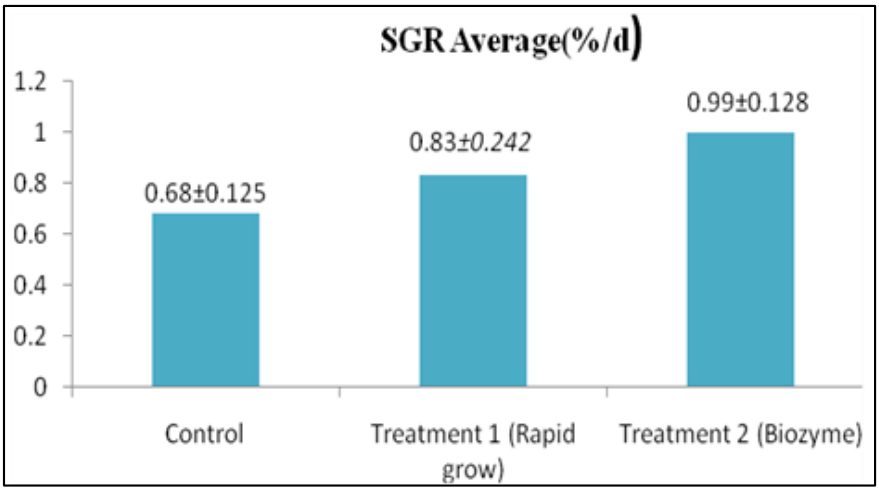

Fig 3: Specific Growth Rate Average (%/d) of Vietnami Koi fish (Anabas testudineus) treated with two different feed additives.

The higest ADG was observed on treatment 2(3.18±1.57). While lowest was observed in control (2.66±1.48).

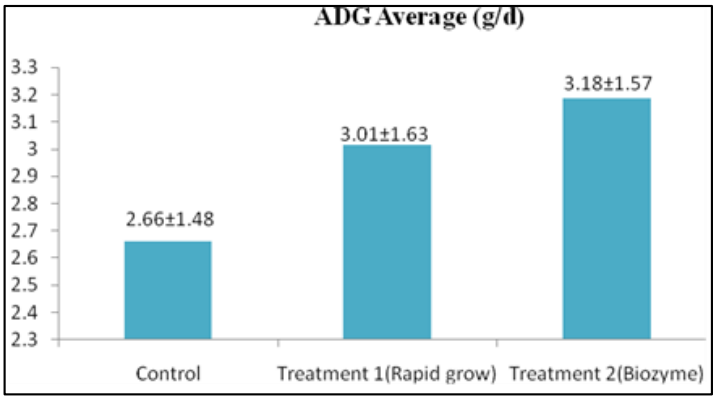

Fig 4: Effect of artificial feed additives on average daily gain (g/d) of Vietnami Koi fish (Anabas testudineus) treated with two different feed additives.

Fish Diet (Treatment 2) contain different amino acid (Methionine, Lysine), enzyme (Amylase, Protease) and bacillus spp. would be the reasons of the highest ADG and SGR in the Treatment-2. Habib *et al* (2015) [13] found that, SGR (% day 1) in Vietnami koi Treatment -1, Treatment- 2, Treatment- 3 were 3.47 ± 0.014a 3.60 ± 0.0033c 3.54 ± 0.0066b. The experimented result is in agreement with the report of Sangrattanakhul (1989) [15] reported that the ADG of *A. testudincus* fish ranging from (0.10-0.12). Mustafa *et al* (2010) [16] observed that Specific growth rate of Thai koi in T1, T2, T3 and T4 were (% day/fish) 0.98 1.43 1.30 1.26. The above finding has more or less similarities with the present experimentation.

Survival rate (%)

Habib *et al* (2015) found Vietnami koi Treatment -1, Treatment- 2, Treatment- 3 were Survival rate (%) 74.33 ± 1.452b 89.66 ± 4.66a 84.33 ± 4.044. Mookerjee and Mazumdar (1946) [6] in a study with climbing perch with prepared feeds (containing 35- 45% protein) observed survival rate ranging from75%-89%. This observation is within our observed value of survival rate of *A. testudineus.* High survival rate of climbing perch was also reported by Rao (1971) [17]. Experimented highest survival rate was found in the T1 (98.5%) and followed by T 2 (96.85%) and control group respectively (92.75 %,). All three trails similar stocking densities were maintained. Multivitamins supplemented in fish diet has the capacity to increases immunity levels and let the species to live in stress free condition.

Table 2: Effect of artificial feed additives on Survival rate of Vietnami Koi fish (Anabas *testudineus)*

Control	T_1 (Rapid grow)	T_2 (Biozyme)
92.75%	98.5%	96.85%

Water Quality Parameters

Boyd (1982) [19] reported that the range of water temperature from 26.06 to 31.97°C is suitable for fish culture. According to Swingle (1967) [20], pH from 6.5 to 9.0 is suitable for pond fish culture and pH more than 9.5 is unsuitable. Mustafa *et al* (2010) [16] observed that Average temp. Of Thai koi in T_1, T_2, T_3 and T_4 were 29.6 ^{0}C, 28.8^{0}C, 29.2^{0}C, 29.8^{0}C Mustafa *et al* (2010) [16] observed that Average pH Of Thai koi in T_1, T_2, T_3 and T_4 Average 7.9 8.1 7.6 7.8 Mustafa *et al* (2010) observed that Average DO (mg/l) Of Thai koi in 5.7 6.2 5.9 5.5.

Habib *et al.* (2015) [13] found that the range of mean values of ammonia-nitrogen was 0.566 ± 0.054 and 0.694 ± 0.052 mg /l 1 in pond and cage, respectively. The highest NH_3-N value was 1.20 mg/ l in the month of September cage and lowest value was 0.20 mg l in the month of August in pond water.

Table 3: Water quality parameters on experimental ponds

Water quality parameters	Control	Treatment 1 *(Rapid Grow)*	Treatment 2 (Biozyme)
Dissolved oxygen (mg/l)	7.12	7.64	7.7
Temperature (^{0} C)	29.28	30.42	30.10
pH	7.56	7.65	7.68
Ammonia (ppm)	3.07	1.28	1.27

Cost–benefit analysis

According to the cost-benefit analysis, all three systems in the present study were economically profitable. However, ponds with the stocking density of 500 individuals per decimal were the most profitable system. In the cost-benefit analysis, all input costs were strictly considered despite the fact that, in reality, most of the small-scale fish farmers use their own resources like lands and labours etc. In this case, cash input costs would be lower, and net profit would be higher than the present analysis. BCR were found 1.32, 1.57 and 1.58 in C, T_1 and T_2 respectively.

According to Roy *et al.* (2013) [18] 550 Koi fry/dec in the T_1, 400 Koi fry/dec in the T_2 and 350 Koi fry/dec in the T_3 respectively. The same sizes fry were stocked for the research purpose BCR was calculated as the ratio of gross income to gross cost. The BCR was found in the three treatments T_1, T_2 and T_3 viz., 1.6, 1.48, and 1.51 respectively. The highest BCR was found in T1 (1.60), and the lowest one was found in T2 (1.48).

Table 4: Cost-benefit analysis of Control, T_1 and T_2

BCR	Control	T_1 (Rapid grow)	T_2 (Biozyme)
1.	1.32	1.57	1.58

4. Conclusion

The investigation reported here that supplemented multivitamin and enzyme in the fish diet would achive better production through effective growth, feeding efficiency and suitable survival rate. Increased PER, ADG and SGR has demonistrated the hypotheis that food additives help to beter

utilization of supplies feed and performed significnt production. This information might influence the Vietnami Koi fish (*A. testudineus)* farmers if demonstrated massive field trail. Further studies should be conducted on multivitamin, and growth promoter levels on fish larvae and brood stock of Koi fish. More research is needed to evaluate the involvement of combine effect on multivitamin, synthetic enzyme and bioenzyme and growth promoter to physiological function of fish. Production of adequate quality of Vietnami koi fish through application of present findings might be helpful to meet up the demand of protein as daily ration and monoculture technology of Vietnam koi can be postulated as the ideal method of choice for an eco-socio-economically sustainable koi, culture in Bangladesh.

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