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Effect of *Spirulina* added as feed supplement on the growth performance and survival rate of *Anabas testudineus* (Bloch, 1792)

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Abstract

To experiment the effect of *Spirulina* added as feed supplement on the growth and survival rate of *Anabas testudineus*. A total of 150 fingering was collected from Bantin village, and were reared in culture tanks providing rice bran, peanut cake and fish meal in a ration of 3:2:1 for two weeks; 60 healthy fishes were selected for treatment and control. *A. testudineus* was compared its growth by using formulated diets August 2015 to December 2015. These respective treatments were Diet-A and Diet-B (5% *Spirulina*). Diet-B was incorporated as a feeding supplement while Diet-A was kept as a controlled feed with routine diet. The survival rate was 100% in all diets. The maximum growth rate was observed in Diet-B fed fish. These were found to be highly significant (p<0.05). The experiment suggested that *Spirulina* can be recommended for augment and feed utilization in intensive culture of *A. testudines*.

Keywords: Experiment, Spirulina, added feed, Anabas testudines

Introduction

Climbing perch *Anabas testudineus* is among the popular fish for consumers in Myanmar. They are important species for fisheries: commercial aquaculture, aquarium (Nelson, 1994) ^[1]. Except marine, they can survive in fresh and brackish waters. In Myanmar they are mostly found in canals, lakes, ponds, swamps, and estuaries. They also occur in medium to large rivers, brook, flooded fields and stagnant bodies including sluggish-flowering canals (Talwar and Jhingran, 1991) ^[2]. They can tolerate extremely unfavorable water conditions, and are associated mainly with turbid, stagnant waters. It is an important food fish in South East Asia, considered a tasty fish, but not of the finest quality because of being bony. In South East Asia (SEA) countries, it is sold alive, keeping it for several days in market places by keeping it moist.

Climbing perch is a potential species for breeding in culture ponds with high economic value and commonly consumed by people of all strata. The fish can sustain itself in many types of water such as pond, field, cage, etc., especially in light acid phosphate soil areas. Propagation techniques have been successfully practiced at Vietnam (Trieu and Long 2000; Yakupitiyage ctv. 1998; and Doolindachabaporn, 1994) ^[3-5], a member of ASEAN countries. Fish fingerling production from hatcheries is gradually increasing and may meet the farm demand. Maximum size attained is 25cm and Nyunt Yi (1997) ^[6] reported 25.5cm among her specimens captured in the Mandalay environs.

Most people accept that this fish is very tasty among other fresh and salt water fishes. *A. testudineus* was once available in ample supply for human consumption in fish markets of Myanmar. But they are no more so, though available in considerable numbers from creeks, paddy fields and small bodies of freshwater. Local fishermen used to release them back into water from among fish caught in the net. According to them, these fish do not bring a sizable income as they do not fetch a better price compared to other species. For the purpose of study, the specimens were collected by self as much as possible and special orders were also be made to the local fishermen.

A. *testudineus* possesses features as a potential culture species. These features include the ability to survive in any kind of freshwater, under extreme environmental conditions, and possession of an accessory air-breathing organ which enables them to survive for several days or weeks out of water.

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The climbing perch is an omnivore, feeding on macrophytic vegetation, shrimps and fish fry among others (Nelson, 1994) ^[1]. Although *Anabas testudineus* is not a very large, it is fleshy and highly esteemed as nourishing food (Nyunt Yi 1972) ^[6]. In Myanmar *A. testudineus* (Climbing perch) was a delicatessen and widely used when it was abundant.

The *A. testudineus* is indigenous to South and Southeast Asia. It is an omnivorous species (Trung, 1999)^[7]. It can thrive well in dissolved oxygen (DO) deficient waters and consumes a variety of food items such as detritus, aquatic plants, worms, crustaceans, mollusks and insects. This species has an advantage due to its air breathing ability and tolerance of adverse environmental conditions (Trieu and Long 2000)^[3].

Vonshak, 1997^[8] observed that *Spirulina* is a cyanobacterium that has been commercially cultivated for more than 10 years due to its high nutritional content; e.g. protein, amino acid, vitamin, minerals, essential fatty acid and b-carotene. *Spirulina* can be considered a nutritional supplement that has various health benefits for humans, and a feed supplement for animal having economic benefits (Watanuki *et al.*, 2006)^[9].

Local works on protein supplementation include the use of *Spirulina* as protein source in fingerling *Ctenopharyngondon idellus* (Than Than Sint, 2005) ^[10]. All these researchers found positive growth. Stimulatory effect of *Spirulina* has been reported in *Piractus branchatus* (Thida Thin *et al.*, 2005 and Phyo Phyo Aung *et al.*, 2005) ^[11, 12]

Since no work has been done on growth stimulation by incorporation of plant proteins as feed supplement in this species, the present work attempts to use plant proteins from different sources to enhance growth performance of *A. testudineus*. In the experiment, *Spirulina* was used as protein supplement. *Sprulina* is a microalgae plankton vegetable food which contains more than 60% protein. It has a high nutritive value. Ayyappan *et al* 2001 ^[13] reported that *A. testudineus* is considered as one of the potential new candidate species for aquaculture and captive breeding. Therefore, in this work this species was chosen chosen as an experimental subject to find out the effect using *Spirulna* supplement diet to enhance growth rate of *A. testudineus* and to improve survival rate as well as the efficiency of fish food.

Materials and Methods

Collections of specimens

A total of 150 young *Anabas testudineus*, measuring 4.8 to 9.8 cm standard length, was collect at the sites of capture in the Mandalay Township, brought to culture room, and leave in a brick culture tank $90 \times 120 \times 90$ cm, for two weeks to get acclimated to their new environment. During this period they were fed rice bran, peanut cake and fish meal in 3:2:1 ratio. Zero death indicates that acclimatization to the aquaria water has been achieved.

Spirulina spp was obtained from Ye-khar in Sagaing Region. It was kindly provided by Dr Min Thein Director, *Spirulina* spp Production Unit, Ministry of Industries (1), Myanmar.

Experimental design

When collected fingerlings were well acclimated to their new environment, 60 healthy looking and agile individuals were selected. Each treatment accompanied by three replicates and each group was randomly placed into glass aquarium. Each aquarium was filled with 50L of water. Each group was assigned and allocated into each aquarium according to the numbering given by random numbers of Fisher and Yates (1963)^[14].

Maintenance and feeding

Routine food which was made of rice bran, defatted peanut cake and fish meal in a ratio of 3:2:1 was provided to each group. Feeding was made twice a day, one in the morning at 7:00 am and another in the evening at 5:00pm. The food was provided to the 5% of the total body weight.

In case of Diet-A was kept as control without *Spirulina*. Diet-B, *Spirulina* was provided as a feeding supplement. It was incorporated into routine feed at 5% of each meal to the treatment groups.

Each aquarium of 75L capacity was covered with the size of 120×30 cm mosquito net to prevent the fish jumping out of water. All six aquaria were kept in the same room so as to alleviate the effects environmental variables. Water was changed every third day and the deposits of debris and their feces was checked daily and siphoned off with small caliber plastic pipe. Daily check was made for any debility of the fish, which deleterious effected on the growth performance.

Parameters employed

Monthly changes in standard lengths and body weight were taken with a standard plastic rule and a digital balance (A&D compact scale HL series), and were considered as the indexes of the growth.

Number of fish in each tank was checked every day to seen mortality and survival rate.

Calculation of growth

Survival rate and absolute growth rate were calculated following Ricker (1979)^[15].

Survival rate (%) = $100 \times (\text{final number of fish} / \text{initial number of fish})$

Absolute growth was calculated using the formula given by Ricker (1979)^[16] as:

Absolute Growth AG = Y2 - Y1 and

Absolute Growth Rate AGR =
$$100 \times \frac{Y_2 - Y_1}{t_2 - t_1}$$

Where, Y1 = initial weight, t1 = initial time Y2 = final weight, t2 = final time

The absolute growth rate is expressed as percentage per unit time.

Statistical analysis

Differences of means monthly body weight were compared statistically using Student's "t" test. The level of significance will be at 0.05 levels. Data were analyzed by one way SPSS (version 21). The parameters used were absolute growth rate (AGR) and survival rate (SR).

Results

The fish were acclimated to the new environment after two weeks of introduction. Since the nutrition was the only factor in this work, all the environmental variables will have the same effect on the treated and the control.

No mortality occurred throughout the experimental period both in the treated and the control. In this experiment the survival rate (100%) was derived satisfaction from treated and controlled. Monthly mean standard length and body weight in fish given *Spirulina* supplement feed and control are given (Table.1). No significance differences were observed in the initial weight of treated and controlled. The treated, fish attained final mean length of 7.23 cm and body weight of 12.3 Journal of Entomology and Zoology Studies

g by feeding fed *Spirulina* supplement. The controlled group was found to be at the final mean length of 6.88 cm and body weight of 11.09 g (Table 2 and Figure 1).

During the experimental period (from April 2006 to March 2007) for the treated, it attained final mean length of 7.62cm and body weight of 13.50g by being fed *Spirulina* supplement. The controlled group was found to be at the final mean length of 7.25cm and body weight of 11.50g.

Significant differences were observed among two kinds of feeds within the study period. Those were in weight (t = 5.463, *P*<0-001) at the end of the experiment.

According to absolute growth rate (AGR), *Spirulina* supplement fed fingerlings has 0.81% (AGR), and the controlled has the role of 0.47% AGR (Table 1).

The maximum growth rate was observed in *Spirulina* –fed fishes in comparison with the controlled which was statistically very highly significant (p<0.001) (Table 2).

 Table 1: Monthly changes standard length and mean body weight in

 Spirulina and control in Anabas testudineus (April 2005 to March 2006)

Month	Spirulina		Control	
	SL (cm)	BW (gm)	SL (cm)	BW (gm)
April	6.64	10.93 ± 4.48	6.53	10.01 ± 3.64
May	6.76	11.36 ± 4.47	6.58	10.30 ± 3.64
June	6.86	11.64 ± 4.53	6.64	10.54 ± 3.67
July	7.00	12.08 ±4.72	6.76	10.78 ± 3.62
August	7.13	12.42 ±4.71	6.82	11.01 ± 3.59
September	7.23	12.53 ± 4.71	6.88	11.09 ± 3.60
October	7.32	12.72 ±4.71	6.98	11.19 ± 3.61
November	7.42	12.80 ± 4.72	7.08	11.26 ± 3.64
December	7.50	12.99 ± 4.70	7.13	11.30 ± 3.60
January	7.53	13.10 ± 4.72	7.19	11.36 ± 3.61
February	7.57	13.28 ± 4.79	7.23	11.43 ± 3.61
March	7.62	13.50 ± 4.75	7.25	11.50 ± 3.61
Mean	7.22	12.44	6.92	10.98

Table 2: Monthly Changes in the absolute growth rates in the Spirulina, and control in Anabas testudineus (April to March)

	Spirulina		Control	
	B.W (g)	AGR%	B.W (g)	AGR%
April 1	10.93		10.01	
May 2	11.36	1.48	10.30	1.03
June 3	11.64	0.97	10.54	0.83
July 4	12.08	1.52	10.78	0.83
August 5	12.42	1.17	11.01	0.79
September 6	12.53	0.38	11.09	0.28
October 7	12.72	0.60	11.19	0.34
November 8	12.80	0.28	11.26	0.24
December 9	12.99	0.66	11.30	0.14
January 10	13.10	0.38	11.36	0.21
February 11	13.28	0.62	11.43	0.24
March 12	13.50	0.76	11.50	0.24
Mean	12.44	0.81	10.98	0.47



Fig 1: Monthly changes in mean body weight in the *Spirulina* and control in *A. testudineus*



Fig 2: Monthly changes in the AGR% in Spirulina and control in the A. testudineus

Discussion

Discussion is made on the basis of increase in body weight taken every month in *Anabas testudineus* fed *Spirulina*, supplemented feed and controlled feed for 12 months.

No mortality occurred throughout the 12 months period in treated and control. Therefore, the survival rates of entire experiment were 100% for two groups. Sangrattanakhul

(1989) ^[17] reported that high survival rate of Climbing perch by culturing in garden ditches with different protein level diets. Two weeks of acclimation to new environmental for these fishes prior to experiments could tentatively be taken as sufficient duration.

Since each glass aquarium of 10 fingerlings was filled with 50L water, the effective volume for individual fingerling

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would be 5L (50L/10) compared to 2L used by Kay Thi Myint (2002) ^[18] who reported incidence of mortality, though of low rate. The larger effective volume could also have contributed to a better survival.

In this experiment, the initial mean length and weight for the treated were 6.64cm and 10.93g respectively for the *Spirulina* –fed, 6.53cm and 10-01g for the controlled group.

From the April 2006 to March 2007, during twelve months experiment, the initial mean length and weight were 6.54cm and 10.93g respectively for the *Spirulina*-fed and 6.53 cm and 10.01g for the control group res. The final mean length mean length and weight were 7.62cm and 13.50g in the *Spirulina* – fed and 7.25cm and 11.50g for the controlled group.

The higher level of AGR is shown in *Spirulina* controlled feed in May. In June, *Spirulina* fed showed the ascended rate than that of controlled group. *Spirulina* fed ascends greatly than that of controlled group in July. The pattern appearance growth in August and October were similar in showing high AGR of *Spirulina* and control. The declimb pattern of AGR were shown by *Spirulina* and control in September and November. The high level of AGR was shown in *Spirulina* that of the controlled in December and March. In January and February are similar in showing high AGR of *Spirulina*, than that of Controlled group. Replacement of oil cakes, such as defatted peanut meals (Higgs *et al.*, 1983) ^[19] by rapeseed has been successfully made on the Juvenile Chinock Salmon in Canada.

The boom and slump pattern is similar to those of the treated fish groups. This pattern of growth was also seen fingering Clarias gariepinus (Thida Thin, 2005) ^[10] and Puntius gonionotus (Su Thwe Khaing, 2005) [20]. The difference between the AGR of Spirulina treated and that of the controlled is 0.34% (0.81% - 0.47%) in favour of the former. In mean body weight comparison, Spirulina treated and controlled group is highly significant (P<0.001). Spirulina supplement feed is the better of two AGR value and statistical analysis on the mean body weight. The Spirulina, exerted its growth effect in 12 months time by weight gain and the length improvement in the last month of treatment. The higher protein content of Spirulina would have contributed to such a significant increase in weight. Spirulina and Nostoc have enhanced the growth performance in Ctenopharyngodon idellus (Than Than Sint, 2005) [10]. The difference of protein content and essential amino acid could be a factor in growth promotion. Spirulina, contain chemical composition and amino acid.

Anabas testudineus is omnivores. It has three pyloric caecae which present a large number of microbes. These microbes could digest the fibre which is cellulose (Brown 1957)^[21]. In this investigation, *Spirulina* supplemented contains 30.96% protein level than that of controlled group (28.69% protein level). In this experiment, the best result in treatment using *Spirulina* supplement pellet (Protein 30.96% appendix VI c) is in agreement with the report of Yakupitiyage *et al* (1999)^[22] in which be estimated required dietary protein level for Climbing perch ranged from 25–35%. Doolgindachabaporn (1994)^[23] also recommended that the feed containing 30.6% protein as the best formula in term of growth and survival for *Anabas*.

In current study, the best growth rate was observed by 5% *Spirulina* with routine diet in a 5% per total body weight fed twice a day. At the end of experiment, *Spirulina* pellet exerts the best growth promotor of controlled pellet. It contains 30.96% protein level and its digestive rate is 95.1%. It is

multicellular with a thin, weak membrane and thus it has good digestive absorption characteristics (Nakamura, 1982)^[24].

Conclusion

It is concluded that, this experiment is made on the basis of increase in body weight taken every month in *Anabas testudineus* fed *Spirulina*, supplemented feed and controlled feed for 12 months. The survival rates of entire experiment were 100% for two groups. In mean body weight comparison, *Spirulina* treated and controlled group is highly significant (P<0.001). At the end of experiment, the best growth rate was observed by 5% *Spirulina* with routine diet. *Spirulina* pellets contain 30.96% protein level more than that of controlled group (28.69% protein level). Thus, *Spirulina* pellet exerts the best growth promoter of controlled group (28.69% protein level).

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