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Effect of Tannic Acid on Feed Intake, Survival and Growth of Striped Bass (*Morone saxatilis*) larvae

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ABSTRACT

The role of tannins (tannic acid and other derivatives) has been extensively studied in plant kingdom. Quite a few investigations have report the function of these compounds in animals specifically the fish. Current studies were aimed to find the role of tannic acid in feeding of larval striped-bass (*Morone saxatilis*). Fish was fed on live *Artemia*, encapsulated *Artemia* with and without tannic acid and encapsulated casein based diet with and without tannic acid. Live *Artemia* was also supplemented to some of the dietary treatments irrespective of the presence or absence of tannic acid. Fish raised on live food achieved significantly higher growth and survival than rest of the treatments. Presence of tannic acid in the capsule materials alone or supplemented with live *Artemia* enhanced the growth exceeding the survival of striped bass larvae to the rest of the dietary groups. Fish group raised on the diet prepared without tannic acid in the capsule material exhibited very poor response.

Key Words: Striped bass larvae; Survival; Growth; Tannic acid

INTRODUCTION

Tannins, a toxic component present dominantly in plants and vegetables, were in commercial use long before there was any clue about their natural functions. Halsam (1979) and Swain (1965) reported an interesting background regarding the use of tannins in the leather industry and also demonstrated the toxicity of the tannery effluent to the larvae of the mosquito. Negative effects of these effluents were also observed in fish whenever exposed to them. These effluents significantly decreased food transportation capabilities in *Channa straitus* and *cyprinus carpio* (Viswaranjan *et al.*, 1988). They also altered the biochemical composition of fish tissues (Katti & Sathyanesan, 1983; Ram & Sathyanesan, 1984) functioning as a generalized protein complexing agent (Rhoades & Cates, 1976; Zucker, 1983). Wheat gluten containing tannins have been used in wine fining as clarifying agent (Marchal *et al.*, 2002), to remove adhesiveness from pikeperch, *Sardar lucioperca* eggs and for fungus control in common carp eggs (Woynarovich & Horvath, 1980). However, the role of tannins at ecological level is almost certainly a mixed function involving a defense mechanism in living plant enemies (Feeny, 1976; Rhoades & Cates 1976; Bernays 1981) and a delay in decomposition of plant tissues viz. roots and leaves (Grant, 1976; Swain, 1979).

Tannins and their by-products are well known in degrading aquatic habitat, are problem for sick fish and inflict mortalities in aquatic organisms (Johnson, 2001). Surprisingly, systematic studies are lacking to trace the physiological response of the aquatic organisms to sub-lethal concentrations of the tannic acid, an important member of the tannin family. The objective of the present studies was to investigate the role of this controversial compound if incorporated in the encapsulated feeds as one of the component, for striped bass larvae.

MATERIALS AND METHODS

Experimental Design. The feeding trials were planned following the completely randomized statistical design. The treatment group (Table I) fed on live *Artemia* nauplii served as control. An 'unfed' group was included to test the possibility of entering the foreign food particles. There were three replicates in each treatment with 25 individuals in each except that of unfed that was not replicated. The fish rearing units were randomly allotted to each treatment group.

Preparation of Diets. *A.* nauplii were hatched from a single batch of reference *Artemia* cysts II (RAC II) (Bengtson *et al.*, 1985) in 500 mL separatory funnels containing sea water with pH range 7.75-8.3, under constant light and aeration. The *A. nauplii* were harvested after 36 hours and fed to the fish perusing the procedure developed by Ashraf *et al.* (1993). Encapsulation of the natural foods and artificial feeds was accomplished according to the procedures used by Leibovitz (1990) except that the albumen:alginate ratio was 1:1. Artificial diets were prepared following the methodologies developed by Ashraf, 1992 (Table II).

Chemical Analysis. Nutritional status of the diets and feed ingredients was determined by the following analytical techniques. Crude protein was determined by the Microkjeldahl method (AOAC, 1984). Moisture was determined by evaporating sample water to a constant weight in convection oven at 95°C (AOAC, 1984). Ash contents were determined after incinerating of the feed

samples at 550˚C in a muffle furnace. Crude lipids were estimated by the Bligh and Dyer (1959) Lipid Extraction Method as modified by Kates (1986) (Table III).

Experimental protocol and setup. Twelve day-old striped-bass larvae were transported in oxygen permeated plastic bags from the Verplanck Hatchery Hudson River, New York. They were transferred to 190 liter aquaria and acclimated to the laboratory conditions in 5‰ saline water for three days. The experimental system consisted of acrylic cylindrical fish egg hatching jars, modified after the design of Buss (1959). Fish were raised and maintained pursuing the procedures used by Ashraf *et al.* (1993). At the end of experiment, the fish were harvested, weighed. Absolute growth rate (AGR), relative growth rate (RGR) and specific growth rates (SGR) were determined by the following mathematical formulas:

$AGR = (Y_2-Y_1) \div (t_2-t_1)$

$RGR = (Y_2-Y_1) \div [Y_1(t_2-t_1)]$

$SGR = (\log_e Y_2-\log_e Y_1) \times 100 \div (t_2-t_1)$

Statistical Analysis. One Way Analysis of Variance followed by Duncan's Multiple Range Test was used to evaluate the statistical significance of the treatment differences. Differences between the treatment means were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Survival and growth. Treatment 1 exhibited the highest survival. Among the rest of the treatments, treatment 6 (Casein + Tannic Acid + Live *Artemia*) exhibited significantly higher survival (51%) than treatment 2, 3, and 7 (Table IV). Survival in these groups ranged from 6% (Casein without Tannic Acid + Live *Artemia*) to 25% (microencapsulated *Artemia* + Tannic acid). Treatments 4, 5 and 8 could not survive longer and died within the first two weeks of experiment. Fish fed on diets with tannic acid containing microcapsules showed better survival over those fabricated without tannic acid. Periodic supplementation of live *Artemia* amplified this response.

A trend similar to survival was observed in growth. The fish fed on live *A.* nauplii expressed the greatest weight increase, while larvae fed on combined diets (live *Artemia* + artificial diets with tannic acid in them) showed significantly greater gains in weight than did rest of the treatments. The treatment groups fed on diets with tannic acid containing microcapsules irrespective of the type of treatment gained significantly more weight than those fed diets with no tannic acid incorporated in the microcapsules (Table IV). This implied that tannic acid had a pivotal role in capsule fabrication and preservation. The AGR, RGR and SGR proportional to the net increments observed in individual treatment were contrary to the observations of Zucker (1983), who reported that tannic acid is a toxic and enzyme inhibitor. It strongly interacts with proteins and precipitates them. Plants use it as a protection against predators. But in the present situation tannic acid-containing diets when fed to striped bass larvae alone, made the fish live longer and when combined with live *A.* nauplii boosted its growth and survival.

Table I. Detail of Experimental Treatments

S.No.	Treatment	Tannic Acid(Y/N)
1	Live *Artemia* nauplii	N
2	Encapsulated *Artemia* nauplii	Y
3	Encapsulated *Artemia* nauplii	N
4	Encapsulated casein based diet	Y
5	Encapsulated casein based diet	N
6	Encapsulated casein based diet with live *Artemia*	Y
7	Encapsulated casein based diet with live *Artemia*	N
8	Control(unfed)	N

Table II. Ingredient composition of casein based diet for striped bass, Morone saxatilis, larvae

S.No.	Ingredient Name	% In Diet
1	Casein	60
2	Vitamix	5
3	Mineralmix	4
4	Menhaden fish oil	13
5	Fatty acyl methyl esters	4.2
6	Stay-C(Ascarbyl-pp)	0.1
7	Carboxymethylcellulose	14

Table III. Proximate composition of *Artemia* and casein based diet on dry weight basis

Nutrients	Live Encapsulated Artemia	Casein based diet
Crude protein	61	57
Ether extract	11	17
Ash	11	4
Moisture	5	5

It appears that tannic acid had direct effect on proteins present in the diet. Possibly, it enhanced the protein and finally capsule stability during manufacturing, handling and storage. However, the binding capability of these components is directly related to decrease in pH of the environment and concentration of the reacting substances. These acids lower the pH, absorb harmful chemicals, inhibit bacteria (cyanobacteria) and create soothing and calm environment fairly benign for fish. These studies are in close agreement with our observations because the stability of capsules is more prominent at lower pH (3-4) and loses its strength with the rise in pH (Van Sumere *et al.*, 1975). It seems that this complex was easily digestible by the larval gut due to higher pH. Live *A. nauplii* encapsulated by this methodology, gave excellent survival when fed to *Menidia beryllina* larvae (Leibovitz, 1990). Possibly the capsule stability guaranteed the safe delivery of the nutrients to the target organs and higher pH in the larval gut (about 6-7) destabilized the complex and released the nutrients. These nutrients may have supported better growth and survival of fish (Table V).

Previous studies have demonstrated that the toxic effects of tannic acid are less noticeable in fish compared to other vertebrates and Lepidoptera (Chang & Fuller, 1964; Feeny, 1968). Fish given a tannic acid-containing diet (75

Table IV. Final wet body weight (mg) and survival (%) of striped bass larvae. Data are presented as mean ± SE. Initial mean wet weight of the larvae was 2.6 mg. Values in a column followed by the same letter are not significantly different from each other at p<0.5.

Treatment no.	Final weight(mg)	Net increment(mg)	Survival(%)
1	64±17[a]	61.4	79±6[a]
2	16±7[b]	13.4	25±0[b]
3	11±3[c]	8.4	6±3[d]
4	-	-	-
5	-	-	-
6	26±9[d]	24.3	51±13[e]
7	26±4[d]	23.8	6±1[d]6±1[d]
8	-	-	-

Table V. Absolute growth rate(AGR), Relative growth rate(RGR) and specific growth rate(SGR) of the fry reared during these studies.

Treatment no.	Agr	Rgr	Sgr(%)
1	2.19	0.84	4.8
2	0.48	0.18	2.5
3	0.3	0.1	1.8
4	-	-	-
5	-	-	-
6	0.85	0.33	3.4
7	0.84	0.32	3.3
8	-	-	-

ppm) increased protein content in liver, gills and muscles significantly greater than the control fish. Carbohydrates were depleted and there was a substantial rise in lipid levels (Viswaranjan *et al.*, 1988). Somanath (1991) while working with *Labeo rohita* on the other hand, observed the decrease in liver, brain and muscle lipids. Working on the same fish Mukhopadhyay and Ray (1999) reported lower apparent protein digestibility with the rise in dietary tannic acid concentrations. Current studies support the former and contradict the latter findings. Storage of macromolecules in the body entails higher metabolic rate (biosynthesis), which in turn demands higher nutrients input from outside sources. Possibly, a sharp jump in metabolic activity enhanced the fish feed ingestion rate and the characteristic color of the particles imparted by tannic acid ameliorated this response (Masterson & Garling, 1986) by enticing the larvae. However, higher concentration of tannic acid can be harmful to fish, which also reduce the clarity of water (Aquascape, 2005). Effect of coloration was seen in *Artemia* capsules, which showed persistent superiority in growth and survival over rest of the treatment groups where live *Artemia* was not supplemented or perhaps its capsules retained some live components during processing and supported growth and survival of fish that demand further investigation (Leibovitz, 1990).

In contrast to the tannic acid capsules, those fabricated without tannic acid, lost their integrity and clustered. Their decomposition (Grant, 1976; Swain, 1979) started much earlier than normally expected and gave off foul odor irrespective of the type of storage. Bigger particles and bad taste could be plausible reasons for poor performance. Our observations agree with the findings of Ramanathan and Das (1992) who confirmed this phenolic compound an anti-oxidant, anti-bacterial and detoxifying agent. The water or diet remnants were not analyzed. It is not sure whether the nutrients were leached out in the water before taken up by the larvae. However, the possible reasons of the death of the larval fish exclusively raised on artificial diets are not clear. Further studies are needed to investigate the possible physiological alterations made by tannic acid that made the fish to grow better.

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