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Influence of Extract Isolated from the Plant *Sesuvium portulacastrum* on Growth and Metabolism in Freshwater Teleost, *Labeo rohita* (Rohu)

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Fishmeal, containing high protein content is becoming very expensive and the search for locally available growth enhancing plant extracts has gained wide acceptance in the present aquaculture scenario. Crude aqueous extract of a plant *Sesuvium portulacastrum* (Linn) at 10% concentration was evaluated for its effect on growth of *Labeo rohita* by incorporation in diet. The growth performance was evaluated for a period of 90 days in three phases of 30 days duration. Parameters like length, weight, food conversion efficiency (FCE), food assimilation efficiency (FAE), specific growth rate (SGR), protein and nucleic acid contents were studied. The results indicated significant enhancement of growth in *L. rohita* fed with diet containing *S. portulacastrum* extract.

Key words: ***Sesuvium portulacastrum*, fish growth, Rohu.**

Profitable fish farming depends a lot on cheap and nutritionally balanced feeds that will enhance growth rate. Plants have always been treasures of chemicals whose properties are useful in several ways. They have the ability to combat diseases and eradicate pests. Currently, attention is focussed to examine how phyto-chemicals influence growth in animals. Unlike synthetic promoters like hormones, antibiotics, vitamins and other chemicals which have been in practice for long, natural growth promoters that are biodegradable are gaining wide acceptance, fully justifying the food safety awareness seen among the present day consumers (Vatheeswaran & Ali, 1986; Xu *et al.*, 1994; Jayaprakas & Sambhu, 1996; Sambhu & Jayaprakas, 1997a, 1997b, Lone & Matty, 1981)). This, along with many other positive factors, have persuaded aquaculturists and fish farmers to use natural growth promoters to improve fish growth and production (Kuwaye *et al.*, 1993 and Basavaraja *et al.*, 1989). Plants produce several secondary metabolites, of which steroid compounds form a major group of chemicals. It is well documented that several of these steroid metabolites mimic the action of insect hormones and therefore, are called as insect molting hormones (insect growth regulators). These compounds are also called as phytoecdysones because of their plant origin and molt inducing (growth) effects, (Bergamsco & Horn, 1983). Many plant species produce C_{27} – C_{29} ecdysteroids of a wide structural diversity (Bergamasco & Horn, 1983; Camps, 1991; Lafont, 1998). These analogues of insect molting hormone, 20- hydroxyecdysone, (20-E) are secondary metabolites which are expected to provide some protection to plants against non-adapted phytophagous insect species (Lafont *et al.*, 1991).

Phytoecdysteroids are a family of about 200 plant steroids. 20-hydroxyecdysone (20-E) is an insect molting hormone that is widely spread in various plants (Bathori *et*

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al., 2000). 20-E isolated from *Sesuvium portulacastrum* exhibited a unique stereochemical configuration (Rele *et al*, 2003). The biological activities of 20-E are demonstrated in the *Drososphila melanogaster* for ecdysteroid agonist activity (Odinokov *et al.*, 2002).

There is very little information regarding the physiological significance of the plant extracts on biological activity of fish and other aquatic animals. Kerala has a vast area of inland waters where the teleost, *Labeo rohita* commonly called rohu is cultured. In this study, an attempt is made to evaluate the growth rate in rohu with a novel herbal extract from the plant, *Sesuvium portulacastrum* (sea purslane).

Materials and Methods

The effect of a crude aqueous extract isolated from the plant *S. portulacastrum* of the family Aizoceae (Syn.Ficoideae) on the growth and body composition of the carp, *Labeo rohita* was investigated. The plant was collected from the vicinity of the atomic reactor plant, Kalpakam, Tamilnadu. *S.portulacastrum* is a perennial, succulent, trailing, herbaceous, halophytic plant with prostrate stem. It is one of the best sources of phytoecdysones (Banerji *et al.*, 1971). The crude extract was obtained form the Regional Research Laboratory (CSIR), Thiruvananthapuram.

Fish Fry (of uniform size) procured from a fish breeder at Kallikad (near Neyyar dam), Thiruvananthapuram, Kerala State, was brought to the lab and reared for four weeks in large cement tanks for acclimatization prior to the experiment. The experimental diet was prepared by spraying the crude aqueous extract at the rate of 10 ml / 100 g of the standard diet and then sun dried. The dried pellets were stored in airtight containers. The experiment was conducted for a period of 90 days by providing 10% of the crude aqueous extract of *Sesuvium portulacastrum* through a standard 40% protein feed prepared following Hardy's method (Hardy, 1980).

At the end of acclimatization, fry of same length and weight were randomly grouped into six and recorded as three sets of two, each set representing one phase of study. Each group consisting of 20 fry in glass tanks sized (1.5 x 1.2 x 0.8m) without any soil base and kept covered by nets in the lab where sufficient light was available. The water level was maintained at 60 ± 5 cm depth with a temperature 26 ± 2°C with balanced photoperiod (12L: 12D) throughout the experiment. Feeding was done once daily at the rate of 10 % of the body weight. The quantity of feed was readjusted if necessary after sampling based on the weight of fishes at the end of 30 days each. In order to assess the feed conversion efficiency (FCE) and the feed assimilation efficiency (FAE), of the fry in a group at a time, accurately weighed amounts of respective diets were given on every fifth day of the experiment. The unconsumed feed was siphoned out 2 h after feeding. The faecal matter was siphoned out the next day prior to feeding. The unconsumed feed and the faecal matter were oven-dried at 60 ^{0}C and weighed. Having used no binder in the feed preparation, the water in the tanks were completely replaced every 24 hrs. Due to the same reason, physical parameters like oxygen, carbon dioxide and pH of the water were not analysed. At the end of 30, 60 and 90 days, representing the three phases, fish from both experiment and controls of corresponding set were sampled for their length and weight. The liver and muscle tissue were excised immediately, washed in saline, blotted and frozen for the extraction of total protein (Lowry *et al.*, 1951), DNA (Burton, 1956) and RNA (Munro & Fleck, 1966) were estimated. Chemicals used were of analytical grade procured from SRL, Bombay. Analysis of variance was studied to find out the statistical significance of growth. The specific growth rate (SGR %) was calculated using the formula, Log W2 – log W1/T2 – T1 x 100 w here W1 is the weight of the

fish at time T1 and W2 the weight at time T2. Assessments of food conversion efficiency (FCE) and feed assimilation efficiency (FAE) were done as;

$$FCE\% = \frac{\text{Wet weight gain (g)}}{\text{*Food consumed (g)}} \times 100$$

$$FAE\% = \frac{\text{Food assimilated (g)}}{\text{**Feed consumed (g)}} \times 100$$

*Food consumed was calculated subtracting the food remains after two hours of feeding from the weighed quantity of food given.

** Food assimilated was calculated subtracting the food consumed from the faecal remains.

Student's T-test was done to assess significance in physical parameters like length and weight of fish along with protein and nucleic acid content of liver and muscle.

Results and Discussion

Mean length and weight, feed conversion efficiency (FCE), food assimilation efficiency (FAE) and specific growth rates (SGR) attained by fish are shown in Table 1. In terms of length and weight, food conversion efficiency (FCE), food assimilation efficiency (FAE) and specific growth rate, the percentage increase was higher in the experimental fishes at the end of first and third phases, respectively.

The composition of protein, DNA and RNA in the liver and muscle tissue is shown in Table 2. Total protein in muscle showed significant increase during the first and third phases of study while liver protein increased significantly during the third phase. Significant increase in DNA during the third phase and decrease in RNA during the first and third phases were also recorded.

The results of the present study demonstrate the growth promoting capability of the the aqueous extract. Since muscle protein synthesis is an excellent overall measure of cell growth (Houlihan *et al.*, 1995), the significant increase ($P<0.001$) in the muscle protein at the end of the first and third phases of experiment indicate an appreciable effect of *S. portulacastrum* on fish growth.

Although there are reports of the influence of many plant extracts on higher animals like rats and mice, no study hitherto has been reported on the effect of *S. portulacastrum* extract on fish growth. The extract is confirmed to have ecdysteroids (20-hydroxyecdysone), as one of its constituents (Rele *et al.*, 2003). The 20-E in the plant

Table 1. Growth and food utilisation of *Labeo rohita* fed with *Sesuvium portulacastrum* extract added diets.

Parameter	30 days		60 days		90 days	
	Control	Experiment	Control	Experiment	Control	Experiment
Initial length, cm	6.45 ± 0.62	6.45 ± 0.62	7.5 ± 0.66	8.0 ± 0.61	8.5 ± 0.64	9.0 ± 0.62
Final length, cm	7.50 ± 0.65	8.0 ± 0.61	8.5 ± 0.69	9.0 ± 0.62	10.1 ± 0.60	11.0 ± 0.62
Length gain, cm	1.05 (16%)	1.55*** (24%)	1.0 (13%)	1.0^{NS} (12.5%)	1.6 (18.9%)	2.0 *** (22%)
Initial weight, g	3.4 ± 0.59	3.4 ± 0.59	5.0 ± 0.60	6.10 ± 0.61	6.3 ± 0.60	7.0 ± 0.62
Final weight, g	5.0 ± 0.60	6.10 ± 0.61	6.3 ± 0.60	7.35 ± 0.62	10.38 ± 0.64	12.93 ± 0.62
Weight gain, g	1.6 (47%)	2.7*** (79%)	1.3 (26%)	1.25^{NS} (20%)	4.08 (65%)	5.93*** (85%)
SGR (%)	0.56	0.86	0.33	0.27	0.72	0.89
FCE (%)	22.4	36.33 (13.93%-)	11.01	8.40 (1.61% ¯)	27.02	28.23 (1.21-)
FAE (%)	70.02	71.83 (1.83%)	72.03	70.64 (1.39%)	70.52	72.71 (2.19%)
Survival (%)	100	100	100	100	100	100

SGR – Specific growth rate; FCE – Food conversion efficiency; FAE – Food assimilation efficiency.
- Increase; ¯ Decrease cm – centimetre; g - gram

Table 2. Mean Protein, DNA and RNA contents in the muscle and liver of *Labeo rohita* fed with Sesuvium added diets

Parameter	30 days		60 days		90 days	
	Control	Experiment	Control	Experiment	Control	Experiment
Protein (Muscle) mg/g	101.21 ± 1.16	121.44 ± 2.06 ***	140.35 ± 2.42	97.35 ± 1.40 ***	154.05 ± 1.04	173.22 ± 1.48 ***
DNA (Muscle) mg/g	1.37 ± 0.01	1.46 ± 0.53NS	8.77 ± 0.10	6.39 ± 0.47*	1.82 ± 0.13	2.94 ± 0.19***
RNA (Muscle) mg/g	3.40 ± 0.02	3.23 ± 0.18*	1.98 ± 0.02	5.53 ± 0.05***	1.90 ± 0.05	1.41 ± 0.01 **
Protein (Liver) mg/g	73.61 ± 1.61	76.45 ± 1.47 NS	124.64 ± 1.73	79.51 ± 1.34 ***	71.95 ± 1.05	103.23 ± 1.87 ***
DNA(Liver) mg/g	6.25 ± 0.03	6.30 ± 0.05 NS	7.57 ± 0.07	6.24 ± 0.04 ***	4.61 ± 0.04	7.25 ± 0.05 ***
RNA(Liver) mg/g	7.58 ± 0.05	7.18 ± 0.02*	3.42 ± 0.02	5.53 ± 0.04 ***	4.81 ± 0.09	3.94 ± 0.01 **

Values are expressed as mean ± SEM of 20 animals (n=20). The significant Difference between the groups was analysed by the student's T test.
P values: * <0.05; ** <0.01; *** < 0.001; NS –not significant

extract resembles many other steroid hormones that are reported to promote growth by enhancing the feed conversion efficiency (FCE) in the experimental animals (Jayaprakas & Sindhu, 1996). Studies have also shown that dietary hormones have the ability to induce synthesis of nucleic acids in muscle and liver of fishes (Lone & Matty, 1981). Extracts prepared from silkworms containing ecdysteroids enhanced protein in mammals (Slama & Lafont, 1995). Specific growth rate is considered as a reliable index of growth in the evaluation of diets.

In the present study, fish experimentally fed with the extract exhibited higher feed conversion efficiency (FCE) than controls. Food conversion efficiency (FCE) can be considered as an index of the food utilization of the animal. The anabolic steroid (20-E) may have induced growth by influencing the feed conversion efficiency. Application of hormones, either alone or in combinations as feed additives is a method to improve fish growth and feed conversion efficiency in aquaculture (Jayaprakas & Sambhu, 1996). The percentage increase in food assimilation efficiency (FAE), whenever growth was reported and vice versa indicates that exogenous steroids administered along with diet may be acting by enhancing the absorption process by the intestine. Most growth promoters enhance protein synthesis by providing the carbon source for the synthesis of amino acids.

The muscle, the site where protein accumulates and the liver the chief metabolic organ, were assayed to quantify protein. In muscle and liver, protein level increased significantly in the experiment. This finding when equated with length and weight increase, leads us to believe that growth manifested by protein synthesis depends on protein synthesis. Transcription and translation being two precursor steps of protein synthesis, the quantification of DNA and RNA were also made. The fast depletion of RNA manifests increased protein synthesis.

It can be concluded that growth increase in *L. rohita* fed with *S. portulacastrum* supplemented diet is due to improved food utilisation and high protein synthesis. Increase in length and weight confirms the effect of ecdysteroiods on growth. Studies on the growth promoting potential of *S. portulacastrum* in other animals are not available for comparison. Having confirmed the extract as growth promoter, further

studies need to be done to recommend it as a safe additive to feed in fish culture.

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