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RESEARCH ARTICLE

EFFECTS OF GREEN MACROALGAE *ULVAFASCIATA* SUPPLEMENTED DIET ON BIOCHEMICAL COMPOSITION IN FRESH WATER FISH *LABEOROHITA* (HAMILTON)

Kiruthika, K. and *Dr. Dhanalakshmi, B.

Department of Zoology, Nirmala College for Women, Coimbatore-18

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ABSTRACT

The present study was carried out to investigate the potential of the supplementation of the green seaweed *Ulvafasciata*, as a dietary additive ingredient along with the basic feed ingredient in fish feed, to improve the fresh water fish Rohu so called *Labeorohita* (Hamilton) biochemical composition. The experiment was carried out in the PG and Research laboratory, Nirmala College for women, on *Labeorohita* of a mean length (10cm) and weight of (45±1g). Five experimental diets composed of dietary protein were prepared using dried algae meal ingredient incorporated at levels of 0% (control), (TF1) 5%, (TF2)10%, (TF3)15%, (TF4)20% and (TF5)25% of fish feed. By the end of the experiment, fish biochemical composition like protein, lipid and carbohydrate were evaluated. With increasing *Ulvafasciata* level in the fish diet protein, carbohydrate and lipid concentration increased significantly ($P < 0.05$) till (TF3)15%.The highest value was maintained at fish fed the diet containing 15% *Ulvafasciata* while the lowest was maintained at control treatment. From the present result highest significant values of protein, carbohydrate and lipid values were obtained from fish maintained at (TF1) 5%, (TF2)10%, (TF3)15% *Ulvafasciata* incorporated dietary treated fishes till 45 days which states that *Ulvafasciata* can be supplemented to *Labeorohita* diet till an optimum level of 15% which will not show any adverse effect on their survival rate.

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INTRODUCTION

Being a plant of unique structure and biochemical composition, seaweed the world's third largest aquaculture crop could be exploited for its multi-functional properties in the form of food, energy, medicine and cosmetics. Biologically, seaweeds are classified as marine macroalgae which are categorized by their pigmentation, morphology, anatomy, and nutritional composition as red (Rhodophyta), brown (Phaeophyta) or green seaweeds (Chlorophyta) (Dawczynski *et al*., 2007). Their amino acid content is well balanced and contains all or most of the essential amino acids needed for life and health. They have more than 54 trace elements required for human bodies physiological functions in quantities greatly exceeding vegetables and other land plants (Challan and Hamingway, 1966). The nutritive value of seaweeds is mainly due to their protein, polysaccharide, mineral and vitamin contents. The high levels of non-digestible polysaccharide in their cell wall make seaweeds a rich source of dietary fibre. The nutritional value of such supplement is evaluated in terms of feed given to fish and by estimating the biochemical composition in the tissues of freshwater fish rohuas effect of dietary macroalgae and adequate levels will probably vary with the species of both algae and fish. Therefore, the objective of the present work was framed to determine the optimum level of seaweeds (*Ulvafasciata*) supplemented formulated dietfor fresh water fish Rohu (*Labeorohita*) by analyzing the following parameters basic biochemical composition parameters like protein, lipid and carbohydrate.

Corresponding author: Dr. Dhanalakshmi, B.
Department of Zoology, Nirmala College for Women, Coimbatore-18

MATERIALS AND METHODS

The present study was carried out in the Department of Zoology, PG research laboratory.

Study speciescollection: The fingerings of the freshwater major carp *Labeorohita* were purchased from New Golden farm Fisheries, at the Palakkad district of Kerala in South India.

Transportation of fish: They were transported to the laboratory in polythene bags filled with oxygenated water.

Acclimatization of experimental fish: Before trial, the fingerlings were acclimatized to experimental condition for 2 weeks at temperature 28±2°C in a concrete cement tank (6' x 4' x 3'). During this period, fingerlings were fed *ad libitum* with boiled egg albumin, and commercial feed (Toiya) twice a day alternatively at 10% of body weight. Water was routinely changed every day in order to maintain a healthy environment for the fingerlings apart from providing artificial aeration. This ensures sufficient oxygen supply to the fingerlings and an environment devoid of accumulated metabolic wastes. Ten fingerlings were randomly stocked in each plastic tubs. Plastic tubs were continuously supplied oxygen through aerator (Daivo pump NS 4200) and a 12:00 hour. Individual weight of the *Labeorohita* fingerlings was determined using an electronic scale (TSI model 58) at the start of experiment and biweekly thereafter in order to monitor the weight gain.

Collection and preparation of feed: Samples of the seaweed species *Ulvafasciata* were collected personally (i.e. handpicked during low tide) and purchased from local fishermen who collected them manually from the coastal area of the Kaniyakumari, India during the month of August 2013. Collected seaweeds were identified by the algologist, CMFRI; Parangipatti, Caddalour, Chiadambarm. Collected seaweed sample was transported immediately to lab in an iced condition. The fresh seaweeds were thoroughly cleaned with running water and with distilled water to remove salt, foreign materials such as their holdfasts, epiphyte, sand, and shells and then placed in a freezer (20°C) immediately. All cleaned seaweeds were shade dried at 38±2°C in a tray dryer for twenty-four hours then pulverized into fine powder that could pass through a 0.5 mm sieve. The powder was dried again in an air oven at 60°C for three hours. The ground samples were stored in air-tight plastic containers covered with aluminum foil and stored at 20°C for further analysis. Analyses were carried out in triplicate. This species was chosen because of its abundance, distribution and also due to rich protein and vitamins.

Experimental setup: Experiment was carried out under laboratory conditions for 45 days. Fish were distributed in substrate free circular twenty plastic troughs a 100-liter capacity each at a rate of 10-fish/ plastic troughs. These tubs were supplied with de-chlorinated tap water with continuous aeration. Fish were weighed individually at the beginning and at the end of the experimental period using a digital scale with precision of 0.1 g. The natural light cycle was close to 12h light/12h dark. The tubs were divided into seven groups with three replicates per group. Water quality was monitored once in fortnight throughout the experiment. Dissolved oxygen concentration (DO), pH were determined with digital oxygen meter and pH-meter, temperature using thermometer, Carbondioxide (CO_2) and Alkalinity by titration method. The first group was fed normal diet and kept as a control. Fish in these groups were fed at a rate of 5 % of live body weight with pellet fish diet twice daily throughout the experimental period. Semi–dynamic method for removal of excreta was used every day by siphoning a portion of water from the aquarium and replacing it by an equal volume of water.

Everyday unfed feed and faeces were collected to quantify. Triplicate analyses per treatment were done for the comparison of the means.

Trialgroups: Fingerlings were fed with (5%, 10%, 15%,20% and25%) of and *Ulvafasciata* mixeddiet for 45 days. The feed trials were carried out in 5 treatments in triplicate groups.

Proximate analysis: The proximate and nutrient compositions of the powder samples were determined according to the standard method (AOAC, 2000).

Biochemical Analysis: Estimation of total protein, carbohydrate and lipid was estimated in selected experimental fish tissue following the standard procedure of Lowry *etal.,* 1951 for protein; Hedge and Hofreiter ,1962 for carbohydrate and Folche*etal*.,1957 forlipid.

Statisticalanalysis: One-way ANOVA using SPSS was used to analyze the difference between treatments and controls. Values of $p<0.05$ were considered significantly

RESULTS AND DISCUSSION

Theproximate and nutrition composition of the powdered green macroalgae sample was estimated using standard procedure and represented in the form of Table: 1 and Table: 2.

Water quality parameters of culture system: To obtain good growth in fishes under intensive culture conditions, the water quality parameters such as, pH, temperature, dissolved oxygen, total alkalinity was estimated. During the culture period of 45 days the temperature fluctuated between 22.8^0C – 24.8^0C. pH remained same i.e. 7-7.9 and free Carbon-dioxide remained between1.4-3.7 The level of dissolved oxygen remained good 4.6-6.2(mg/l) due to continuous aeration and alkalinity remained between 1.4-3.7 mg/l and 235-310 mg/l, respectively during the acute experimental period.Table-2. It was observed that all the water quality parameters were similar for all the treatments and the mean values of water quality parameters were optimum for rearing of *Labeorohita* fingerlings (Table:3) Recently, aquaculturists have been showing interest in developing technologies to induce fish growth by feeding supplementary diets enriched with various feed additives and growth promoter's. Formulation of well-balanced diet and adequate feeding are the most important requirement of successful farming. A well balanced diet should contains all the essential nutrients in the right proportion in higher production improve recovery from disease and strength to overcome the effects of environment stress.

Protein – Muscle: Protein plays an important role in the maintenance of blood glucose (Jrugger *et al*., 1968) and it is fundamental and abundant biochemical constituent present in the fish body. Protein content in the muscle of the control fish were 1.94±(0.032), 2.13±(0.021),2.24±(0.036)mg/g wet tissue for 15^{th},30^{th}, and 45^{th} day respectively. Similar intervals of observation in experimental fish exhibits significance increase over the control value in 5%, 10%, 15%, 20% and 25% sea weed incorporated feed. The protein content in the TF1 fish fed with 5% *Ulvafascita* feed were 2.74 (±0.012), 2.94 (± 0026) and 3.17 (±0.019) mg/g wet tissue, in TF2 it was observed 2.78 (±0.014), 2.96(± 0.029) and 3.16 (± 0.016) in TF3 (15% sea weed in corporate feed) fish it was 2.93(±0.016), 3.07(±0.014) and 3.27(±0.018) mg/g wet tissue.

Table 1. Proximate composition of the Macroalgae (*Ulvafasciata*) supplemented experimental feed of concentrations (g/100g/dry matter)

Parameters	Control	D1 (5%)	D2 (10%)	D3 (15%)	D4 (20%)	D5 (25%)
Moisture	20.32	19.33	18.4	17.81	17.62	16.98
Total Nitrogen	4.49	5.07	5.45	5.42	5.45	5.42
Crude protein	24.8	25.4	26.5	28.9	28.7	29.1
Crude fibre	5.15	5.25	5.33	5.30	5.32	5.31
Crude fat	6.29	6.17	5.89	5.27	5.11	5.09
Ash	12.6	11.8	10.68	10.42	10.40	10.70
Crude Carbohydrate	31.3	30.2	29.6	28.45	28.12	27.34

*Each value is Mean of triplicate observations

Table 2. Nutrient composition of *Ulvafasciata* (% dry wt of sample)

Nutrients (%)	*Ulvafasciata*
Protein	16.13 ± 0.33^{a}
Lipid	1.94 ± 0.08^{a}
Ash	20.61 ± 0.54^{b}
Dietary fibre	10.85 ± 0.31^{a}
Carbohydrate	53.31 ± 2.17^{c}
Moisture	20.80 ± 1.14^{b}

*N=3 Values are expressed as Mean± Standard deviation
*Values with different superscripts letters in the same column are significantly different (P<0.05)

Table 3. Physico chemical parameter of water experimental and control tubs exposed to varying inclusion of Macroalgae supplemented diet concentration

Parameters	Treatments					
	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Temperature	23.3	22.8	24.0	24.3	24.5	24.8
Oxygen	6.2	6.1	5.7	5.4	5.0	4.6
Carbon- dioxide	1.8	1.4	2.1	2.5	3.2	3.7
pH	7.6	7.4	7.5	7.3	7.0	7.9
Alkalinity	310	287	254	235	246	294

* N=3 Values are expressed in means
* All the parameters expect pH and Temperature are expressed (mg/l); Temperature (C)

Table 4. Protein content of the muscle (mg/g/wet tissue) of the fresh water fish *Labeorohita* fed with macroalgae supplemented diet of different concentration for 45 days

S.No.	Treatment period (Days)	Experimental Feed Concentrations					
		Control	(TF1)5%	(TF2)10%	(TF3)15%	(TF4)20%	(TF5)25%
1.	15	1.95±0.032	2.74±0.012	2.78±0.014	2.93±0.016	2.37±0.018	2.39±0.026
2.	30	2.13±0.021	2.94±0.026	2.96±0.029	3.07±0.014	2.39±0.016	2.43±0.031
3.	45	2.43±0.036	3.17±0.019	3.16±0.016	3.27±0.018	2.43±0.019	2.45±0.038

*TF- Trial feed
*Each value represents the mean of 6 replications; NS-Non significant
*-Denotes decrease over the control value + Denotes % increase over the control value
*-P<0.05;**-P<0.01; ***-P<0.01 – Significant

Table 5. Lipid content of the muscle (mg/g/wet tissue) of the fresh water fish *Labeorohita* fed with Macroalgae supplemented diet of different concentration for 45 days

S.No.	Treatment period (Days)	Experimental Feed Concentrations					
		Control	(TF1)5%	(TF2)10%	(TF3)15%	(TF4)20%	(TF5)25%
1.	15	1.21±0.022	1.18±0.019	1.36±0.017	2.39±0.031	1.56±0.026	1.51±0.033
2.	30	1.32±0.026	1.34±0.036	1.84±0.022	2.93±0.012	1.97±0.025	1.79±0.029
3.	45	1.63±0.026	1.89±0.031	2.25±0.028	3.05±0.031	2.64±0.028	2.45±0.048

*TF- Trial feed
*Each value represents the mean of 6 replications; NS-Non significant
*-Denotes decrease over the control value + Denotes % increase over the control value
*-P<0.05;**-P<0.01; ***-P<0.01 – Significant

Table 6. Carbohydrate content of the muscle (mg/g/wet tissue) of the fresh water fish *Labeorohita* fed with Macroalgae supplemented diet of different concentration for 45 days

S.No	Treatment period (Days)	Experimental Feed Concentrations					
		Control	(TF1)5%	(TF2)10%	(TF3)15%	(TF4)20%	(TF5)25%
1.	15	1.71±0.016	1.73±0.035	1.82±0.220	1.89±0.023	1.80±0.019	1.79±0.021
2.	30	1.83±0.014	1.89±0.031	1.96±0.026	2.03±0.031	1.89±0.018	1.84±0.032
3.	45	1.96±0.031	2.15±0.023	2.19±0.023	2.23±0.024	2.04±0.037	1.98±0.036

*TF- Trial feed
*Each value represents the mean of 6 replications; NS-Non significant
*-Denotes decrease over the control value + Denotes % increase over the control value
*-P<0.05;**-P<0.01; ***-P<0.01 – Significant.

In experimental fish fed with 20% (TF4) and 25% (TF5)seaweed incorporated feed the protein content was observed to be 2.39 (±0.026) and 2.37 (±0.018) after 15 days and 2.43 (±0.031) and 2.39 (±0.016)mg/g after 30 days while 2.45(±0.038) and 2.43 (±0.019) mg/g (Table 4). The concentration of protein was found to be high in the muscle of the experimental fish when compared to the protein in control fish which may be due to the protein in control fish which may be due to the presence of high content of aminoacids which could have been induced by the experimental fish (Sornaray and Ranjith Singh, 2005). With respect to their high protein level and their amino acid composition, the green seaweed *Ulvafasciata* appears to be interesting potential source of food proteins. The development of novel food such as functional foods could be a new possibility for the use of seaweeds, especially for the protein-rich species, in human nutrition. The use of algae with high protein levels in the production of foods shows positive effect concerning the use of seaweed in fish feed (Satoh and Kashara, 1987; Mustafa and Nakagawa, 1995). It was found that algal fed fish had increased body weight and muscle protein deposition. According to Hurrell and Finot (1985), one major factor that influences protein digestibility is the presence of phenolic compounds. Oxidized phenolic compounds may react with amino acids andproteins, inhibiting the activity of proteolytic enzymes (Milic, Stojanovic, Vucurevic and Turcic, 1986). The ability of phenolic compounds to form insoluble complexes with protein interferes with the utilization of dietaryproteins, thus lowering their nutritional value (Shahidi and Naczk, 1995). Parsons *et al.,* 1961 found that protein was principal organic constituent of the algal meal which enhances the growth performance.

Carbohydrate–Muscle: Carbohydrate constitutes only a minor percentage of total biochemical composition. Carbohydrates are one of the major energy sources in diets besides proteins and lipids and are in important energy store in plants (Webster and Lim, 2002). They can be found in abundance in many plant based ingredients and have a molecular structure based upon carbon hydrogen and oxygen (Shiau, 1997). In the present study carbohydrate content in the muscle of the control fish were 1.71(±0.016), 1.83(±0.44), 1.96(±0.031) mg/g wet tissue for 15^{th},30^{th}, and 45^{th} day respectively. Similar intervals of observation in experimental fish exhibits significance increase over the control value in 5%, 10%, 15%, 20% and 25% seaweed incorporated feed. The carbohydrate content in the TF1 fish fed with 5% *Ulvafasciata* feed were 1.73 (±0.035) ,1.89 (± 0.031) and 2.15(±0.023) mg/g wet tissue, in TF2 it was observed 1.82 (±0.220), 1.96(± 0.026) and 2.19 (± 0.023) in TF3 (15%) sea weed in corporate feed) fish it was 1.89(±0.023), 2.03(±0.031) and 2.23(±0.024) mg/g wet tissue. In experimental fish fed with 20% (TF4) and 25% (TF5) sea weed incorporated feed the carbohydrate content was observed to be 1.80 (±0.019) and 1.89 (±0.018) after 15 days and 2.04 (±0.037) and 1.79 (±0.021) mg/g after 30 days while 1.84(±0.032) and 1.98(±0.036)mg/g (Table:6).It was observed that fishes fed with *Ulvafasciata* supplemented displayed higher glycogen content and deposition in muscle. As the levels of protein in the diet increased beyond the maximum requirement, the excess was stored as fat or as carbohydrate Cowey and Sargent, 1979. Carbohydrates serve as an inexpensive energy source in fish diets. Starches, sugars and fiber are the main forms of carbohydrates Organisms differ in their ability to use carbohydrates as an energy source.

Lipid – Muscle: Lipid content in the muscle of the control fish were 1.12(±0.022), 1.32(±0.026), 1.63(±0.026)mg/g wet tissue for 15^{th},30^{th}, and 45^{th} day respectively. Similar intervals of observation in experimental fish exhibits significance increase over the control value in 5%, 10%, 15%, 20% and 25% sea weed incorporated feed. The lipid content in the TF1 fish fed with 5% *Ulvafascita* feed were 1.18(±0.019) ,1.34(±0.036) and 1.89(±0.031)mg/g wet tissue, in TF2 it was observed 1.36 (±0.017), 1.84(±0.022) and 2.25 (± 0.028) in TF3 (15% sea weed in corporate feed) fish it was 2.39(±0.031) , 2.93(±0.012) and 3.05(±0.031) mg/g wet tissue. In experimental fish fed with 20% (TF4) and 25% (TF5)sea weed incorporated feed the protein content was observed to be 1.56 (±0.026) and 1.97 (±0.015) after 15 days and 2.64 (±0.028) and 1.51(±0.033)mg/g after 30 days while 1.79(±0.029) and 2.45 (±0.048) mg/g (Table 5). Lipids which exist in poly unsaturated fatty acids is known to play a significant role n the development of cancer, aging, diabetes mellitus (Mayes, 1995). Lipids play an important role in fish nutrition for the provision of both energy and essential fatty acids (Sargent *et al*., 1989). Fat served as food reserves along with protein. It is generally known that fat content in animal is subjected to periodic fluctuation. Temperature of external medium influences the fat content in some fishes (Johnstene, 1997).Lipids, or fats, are a group of organic compounds that include free fatty acids, phospholipids, triglycerides, oils, waxes and sterols. Lipids function as an important energy source for fish. Fatty acids (i.e) lipids causes' negative health impact on human, but poly unsaturated fatty acids (PUFA) which is present in fishes have positive effects on human health. Thus dried and green macroalgae *Ulvafasciata* examined in this work have high level of protein contents, carbohydrates contents and relatively high levels of lipid contents. Moreover with the obtained results, it could be concluded that macroalgae*Ulvafasciata* can be supplemented to Rohu (*Labeorohita*) diet at optimum level of 15% to improve growth performance without any adverse effect on feed efficiency or survival rate. By virtue of present investigations it is suggested that the efficacy of the algal protein incorporated diets was higher than the controlled one in terms of biochemical performance of fishes. The green algal diet if fed for a longer period to the experimental fishes like *Labeorohita* until they gain marketable size it would not only give us high value protein but also provide essential nutrients to fulfill our dietary demands. It is further emphasized that indigenous algae should be used in combination with the controlled diet to achieve better results in commercial farming of valuable Indian fresh water major carp *Labeorohita,* (Rohu). This will not only minimize the expenditure that is being incurred on importing expensive exotic algae but also provide excellent source of food supplementation to traditional feeds. Hence diet supplementation needs to be considered for successful and sustainable aquaculture of economically important Indian major carps.

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