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Effect of dietary raw and autoclaved Ulva supplemented meal on the growth performance of *Cirrhinus mrigala*

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Abstract

To evaluate the effect of *Ulva* supplemented meal on the growth performance on mrigal (*Cirrhinus mrigala*) after 10 days acclimatization to the new conditions. The fish were stocked at density of 10 fish per aquarium (1×1×1 feet) designated as U₀, U₁, U₂ and U₃ respectively, U₃ as control and were distributed randomly among 12 aquaria for 60 days and data were evaluated on fortnightly basis. Fish with initial body weight U₀ (63.64gm.) U₁ (63.87gm), U₂ (64.16gm) and U₃ (64.14gm). Final average body weight U₀ (146.47gm), U₁ (271.16gm), U₂ (196.14gm) and U₃ (186.14gm). Fish were fed with *Ulva* supplemented diets at 5%, 10% and 15% in U₁, U₂ and U₃ tank respectively while U₀ fish fed with a diet containing no *Ulva*. Feed pellets were given at 3% body weight per day. The water quality parameters were monitored on weekly basis interval and the ranges were temperature 27.03-28.8°C, pH 7.13-7.69, dissolved oxygen 5.06-5.5mg/l, alkalinity 50.33-64.93mg/l and ammonia 001-0.11mg/l. The result of the present study showed that, among the 3 different dietary *Ulva* levels, mrigal fed with 5% *Ulva* supplemented feed showed the best growth performance. Growth performance of fish fed with *Ulva* were evaluated on the basis of net weight gain, percent weight gain, feed conversion ratio and specific growth rate. The proximate composition was done using soxtron apparatus for crude fat, kjeltron apparatus for crude protein and by estimating ash and moisture content.

Keywords: Ulva meal, proximate composition, growth, physico-chemical parameters

1. Introduction

Nutritionally balanced fish feeds are known to generally contain soy product meal, wheat meal products and fish meal. In order to produce low-cost pelleted feeds, to become available for small scale farmers, investigations for cheaper alternative feedstuffs such as protein and energy source for fish diets have become a priority. Due to the presence of high protein content and production rate various algae are receiving attention as possible alternative protein sources for cultured fish, particularly in tropical areas, for small scale farmers [1]. Seaweeds are considered as rich source of bioactive compounds due to their ability of producing great variety of secondary metabolites characterized by a broad spectrum of biological activities against fish pathogens [2-4]. The nutritional value of such feed supplements is evaluated in terms of growth and survival, with little attention paid to other physiological merits. Although dietary algae used as feed supplements may be expected to improve growth and digestive efficiency of feed, the addition of algae in small amounts to the fish diet can produce considerable improvement of physiological condition, fish vitality, disease resistance, desired body composition and carcass quality [5].

Many kinds of macro algae or their extracts are being used or have been used as a human food in various locations around the world ^[6-8]. The amino acid composition in seaweeds was found to be 10-30% of the dry weight; and the content of vitamin A, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin C and niacin are very high ^[9]. The range of crude protein content in ulva species is between 10 and 26% of dry weight, while it may reach up to 47% of dry weight in the red seaweeds which implies their potential for human and animal nutrition ^[10]. This protein content is considered to be high quality protein, since the green algae (example *Ulva lactuca*) contain all the essential amino acids (EAA) and accounted for 42.1-48.4% of the total amino acid content ^[11]. Ulva is a good source of protein, pigments, minerals and vitamins and it is also especially rich in vitamin C ^[12-13]. *Ulva lactuca* and other species of ulva for example *Ulva rigida* among few seaweeds are potential alternative source of nutrients for aqua feeds ^[14]. Even if they are containing low protein content 5-30% of the dry weight ^[15] their advantages include high nutritional values. Ulva is a genus of algae that includes many species which is probably a considerable underestimate that look like bright green sheet and primarily

Corresponding Author: Ujjwala Upreti Gobind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India in marine environments ^[8b]. These can also be found in brackish water, particularly in estuaries. The effect of ulva species on fish growth, feed efficiency and nutrient utilization have been examined for several species, which includes black sea bream, *Acanthopagrus schlegeli* ^[5b], red sea bream, *Pagrus major* ^[16].

The main objectives of this study were mainly to produce cost effective, cheap and efficient feed for better growth of carps. Since carps readily accept artificial pelleted feeds under any culture conditions, therefore, *Cirhinus mrigala* belonging to the same group was selected to study the performance of feed developed in the study. Thus, the present study aimed to determine the chemical composition of *Ulva spp.*, collected from the coastal region of Okha, Gujarat in rainy and summer seasons in order to gain extensive information about their nutritional value.

2. Materials and Methods

The experiment was conducted for six months including pilot test and actual test (60 days)

2.1 Experimental laboratory

The experiment was conducted in the wet lab of Doon (PG) College of Agriculture and Allied Sciences. The experiment was conducted in the glass aquariums of size $1\times1\times1$ feet. Glass experiments were filled with 50 liters of fresh water.

2.2 Experimental animal

Cirrhinus mrigala, mrigal, fingerlings used in this experiment were collected from the pond area of Doon P.G. College of Agriculture and Allied Sciences, Dehradun, through netting operation.

2.3 Experimental procedure

2.3.1 Acclimatization of experimental fish

The fishes were disinfected with 5ppm KMnO₄ solution for 5 minutes and transferred to regular tank of 100 L capacity filled with aged filter tap water. The dead and weak fishes were removed immediately and the healthy ones were stocked @ 10 no. /tank. The fishes were fed twice daily with commercial feed during acclimatization. Continuous aeration was provided. All the fishes were maintained in such conditions for at least 10 days prior to experimentation. The wastes and faecal matter were siphoned out every day.

2.3.2 Preparation of experimental diet

Three ulva meal based experimental diets were formulated containing 5%, 10% and 15% incorporation levels, respectively, by substituting wheat flour on equal weight basis. A fourth diet which has no ulva supplementation serve as the control (U_0) diet. Diets were formulated to contain 28% total protein using rice bran, mustard oil cake, soya bean powder and wheat flour as the feed ingredients. After thoroughly mixing the dry ingredients and vitamin mix, distilled water was added after which the experimental diets were pelleted with the help of a laboratory pelleting machine and dried at 40 $\,^{0}$ C in a fan assisted drying cabinet. Experimental feeds were stored in an air tight container to avoid any type of microbial contamination.

Sr. No.	Treatment	Ulva (%)
1	U_0	Control
2	U_1	05
3	U_2	10
4	U_3	15

2.3.3 Physico-chemical parameters

Water quality parameters *viz*. dissolved oxygen, temperature, pH, total alkalinity and ammonia nitrogen were recorded during the experimental period. The pH and temperature were measured using digital pH meter (Hanna, Portugal) and mercury thermometer respectively. Dissolved oxygen and alkalinity were estimated using titration method. Continuous mild aeration was provided to ensure sufficient dissolved oxygen. About 20% of water in the tanks was exchanged daily by siphoning and refilling. The feed remnant, faecal matter etc. were removed during the water exchange. The animals were closely examined for health and to assess their number during morning, noon, evening and night.

2.4 Proximate composition

The experimental diets for the experimental fish were analyzed for the proximate composition as per standard methods [27] of AOAC (2000).

2.4.1 Crude protein

Three grams of sample is taken in digestion tube. Digestion system is preheated up to 250°C. 3 gm. Of catalyst mixture is added, and finally 10ml conc. H₂SO₄ is added. Tubes are loaded in the digestion block along with manifolds. Switch on the scrubber system. Ensure any frothing of sample, if sample behavior is normal then rise the temperature to 420°C. Leave the tubes in the block for 1 hour, afterwards ensure the colour of the sample turned to bluish green then remove the tubes. These digestion tubes are loaded in the distillation system. Take 25ml of 4% boric acid in a 250 ml conical flask in the receiver end. After distillation collected ammonia is titrated with 0.01N HCL or 0.02 H₂SO₄, colour changes from bluish green to pink.

Nitrogen% =
$$\frac{14 \times Normality \text{ of acid} \times Acid \text{ titrant value} \times 100}{\text{sample weight} \times 1000}$$

Protein % = Nitrogen% × Power factor

2.4.2 Crude fat

Three grams of dried sample was put in a sachet of filter paper (Whatman no.40) and its weight recorded. The sample was then extracted with ether at 60-80 °C in Soxhlet apparatus. The extraction was continued for at least six hours at condensation rate of 20-30 drops per minute. After extraction, sample was dried for 30 minutes at 100 °C, cooled and final weight was recorded. The difference in weight of sample before and after extraction indicated the total organic solvent soluble lipids. The fat content of the sample was expressed in percent of dried sample as follows:

$$Fat (\%) = \frac{Weight of fat(g)}{Weight of sample(g)} \times 100$$

2.4.3 Moisture

Two grams of sample was taken in a weighed porcelain crucible and kept in a preheated oven at 85 ± 2 °C for 24 hours. The crucible then transferred to a desiccator cooled and weighed again. The moisture in content was calculated as follows:

$$Moisture(\%) = \frac{Initial\ weight\ (g) - Final\ weight\ (g)}{Inital\ weight\ (g)} \times 100$$

2.4.4 Ash

Five grams of dried and powdered sample was taken in silica crucible and incinerated in a furnace preheated to 550 °C for four hours. The crucible containing fully burnt material was transferred to desiccators, cooled and weighed. The difference between the initial and final weight was noted. The ash was expressed in percent as follows:

$$Ash(\%) = \frac{Final\ weight\ of\ ash}{Weight\ of\ sample\ (g)} \times 100$$

2.5 Growth parameters of fish

Growth parameters *viz* survival percentage, net weight gain, percent gain in weight, feed conversion ratio and specific growth rate were determined every fortnightly and cumulatively.

2.5.1 Net weight gain

Net weight gain (g) = Final weight (g) – Initial weight (g)

2.5.2 Per cent weight gain

$$\text{Per cent gain in weight} = \frac{\text{Final weight (g)-Initial weight(g)}}{\text{Initial weight}} \times 100$$

2.5.3 Feed conversion ratio

$$FCR = \frac{\text{Weight of food given(g)}}{\text{Weight gain of fish(g)}}$$

2.5.4 Specific growth rate

$$SGR (\%) = \frac{\log W2 - \log W1}{D} \times 100$$

Where,

W1 is initial weight of fish in grams W2 is final weight of fish in grams.

2.6 Statistical analysis

The data recorded for evaluation of different treatments were statistically analyzed using standard procedure for analysis of variance of completely randomized design (CRD) in order to test the significance of experimental results. The significant difference between treatments was tested following Duncan's New Multiple Range test.

3. Result & Discussion

In the present study, the effect of seaweeds (*Ulva lactuca*) on growth performance and proximate composition *C. mrigala* were evaluated. Application of seaweeds at different supplementation levels in carp diet has significant impact on growth, feed conversion ratio (FCR), specific growth rate (SGR) etc. However, no significant effect of seaweed inclusion levels on water quality were seen. The detailed observations recorded for water quality, fish growth, growth indicators etc. are presented and described below.

Table 1: Range and value of selected water quality parameters during the experimental period (± standard error)

Donomotona	Treatments					
Parameters	$\mathbf{U_0}$	$\mathbf{U_0}$ $\mathbf{U_1}$		\mathbf{U}_3		
PH	07.34-07.69 07.50±0.03	07.34-07.48 07.42±0.015	07.13-07.42 07.26±0.029	07.13-07.28 07.23±0.017		
Temperature	27.70-28.71 28.15±0.13	28.00-28.80 28.38±0.09	27.10-28.36 27.58±0.14	27.03-28.33 27.87±0.16		
Dissolved oxygen	05.1±05.23 05.14±0.014	05.20-05.50 05.32±0.04	05.06-05.26 05.20±0.02	05.10-05.26 05.18±0.02		
Alkalinity	56.00-60.00 58.33±0.46	60.33-64.93 62.48±0.448	56.33-59.00 58.005±0.31	50.33-54.00 51.95±0.38		
Ammonia-nitrogen	0.01-0.03 0.02±0.002	0.01-0.03 0.02±0.001	0.06-0.11 0.07±0.0059	0.008-0.11 0.09±0.005		

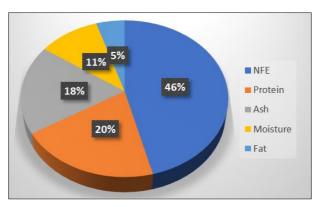
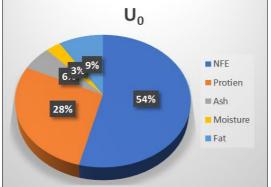
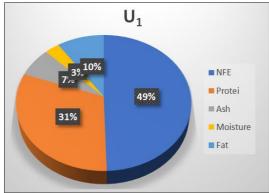
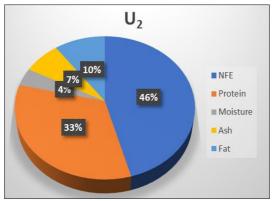


Fig 1: Proximate composition of Ulva lactuca







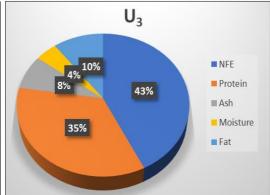


Fig 2: Proximate composition of experimental diets (Dry weight basis)

Table 2: Fortnightly net weight gain (gm.) of *Cirrhinus mrigala* fed with ulva supplemented diet

Treatment	Days			
Treatment	0-15	15-30	30-45	45-60
U_0	14.70a	20.70 ^a	27.10 ^a	20.10 ^a
U ₁	38.70 ^c	65.30 ^d	73.31 ^d	30.00 ^d
U_2	31.30 ^b	24.70 ^c	55.30°	20.78 ^b
U_3	30.98 ^b	22.92 ^b	45.10 ^b	23.08 ^c
Standard error	2.64	5.55	5.04	1.181

Mean in the same column sharing same superscript are not significantly different

Table 3: Fortnightly percent weight gain of *Cirrhinus mrigala* fed with ulva supplemented diet

Tucotmont		Days				
Treatment	0-15	15-30	30-45	45-60		
U_0	23.09a	26.42 ^b	27.36a	15.93 ^d		
U_1	56.19°	63.66 ^c	43.67°	12.43 ^b		
U_2	48.78 ^b	25.87 ^b	46.02 ^d	11.84a		
U ₃	48.28 ^b	24.09a	38.20 ^b	14.14 ^c		
Standard error	4.12	4.98	2.17	0.48		

Mean in the same column sharing same superscript are not significantly different

Table 4: Fortnightly feed conversion ratio of *Cirrhinus mrigala* fed with ulva supplemented diet

Treatment		Days				
1 reatment	0-15	15-30	30-45	45-60		
U_0	01.94 ^c	01.70 ^b	01.64 ^d	02.96a		
U_1	0.74a	0.70^{a}	01.02°	03.61 ^b		
U_2	0.91 ^b	01.72 ^b	0.97 ^b	03.84 ^b		
U ₃	0.92 ^b	01.86 ^c	0.87a	03.17a		
Standard error	0.14	0.13	13.57	0.11		

Mean in the same column sharing same superscript are not significantly different

Table 5: Fortnightly specific growth rate of *Cirrhinus mrigala* fed with ulva supplemented diet

T4	Days			
Treatment	0-15	15-30	30-45	45-60
U_0	0.60^{a}	0.68 ^c	0.70^{a}	0.43 ^d
U ₁	01.37 ^c	01.42 ^d	01.05 ^c	0.34 ^b
U_2	01.15 ^b	0.66 ^b	01.09 ^d	0.32^{a}
U ₃	01.14 ^b	0.62a	0.94 ^b	0.38 ^c
Standard error	0.08	0.10	0.04	0.01

Mean in the same column sharing same superscript are not significantly different

Table 6: Net weight gain of *Cirrhinus mrigala* fed with ulva supplemented meal

Treatment	0-15	0-30	0-45	0-60
U_0	14.70a	35.40a	62.50 ^a	82.60a
U_1	38.70 ^c	104.00 ^d	177.31 ^d	207.31 ^d
U_2	31.30 ^b	56.00°	111.30 ^c	132.11 ^c
U_3	30.97 ^b	53.90 ^b	98.99 ^b	122.08 ^b
Standard error	2.64	7.64	12.50	13.60

Mean in the same column sharing same superscript are not significantly different

Table 7: Percent weight gain of *Cirrhinus mrigala* fed with ulva supplemented meal

Treatment	0-15	0-30	0-45	0-60
U_0	23.09a	55.61a	98.19a	129.78a
U_1	60.59 ^c	162.82 ^d	277.61 ^d	324.58 ^d
U_2	48.77 ^b	87.27 ^c	173.45°	205.89 ^c
U_3	48.27 ^b	83.99 ^b	154.28 ^b	190.25 ^b
Standard error	4.12	11.97	19.57	21.29

Mean in the same column sharing same superscript are not significantly different

Table 8: Feed conversion ratio of *Cirrhinus mrigala* fed with ulva supplemented meal

Treatment	0-15	0-30	0-45	0-60
U_0	01.94 ^c	01.80 ^d	01.73 ^d	01.99 ^d
U_1	0.74 ^a	0.71a	0.84a	01.24a
U_2	0.91 ^b	01.28 ^b	01.12 ^b	01.54 ^b
U ₃	0.92 ^b	01.32 ^c	01.26 ^c	01.62 ^c
Standard error	0.14	0.11	0.09	0.09

Mean in the same column sharing same superscript are not significantly different

Table 9: Specific growth rate of *Cirrhinus mrigala* fed with ulva supplemented meal

Treatment	0-15	0-30	0-45	0-60
U_0	0.60^{a}	0.64a	0.66^{a}	0.601a
U_1	01.37 ^c	01.37 ^c	01.28 ^d	01.04 ^d
U_2	01.15 ^b	0.90^{b}	0.97 ^c	0.80^{c}
U_3	01.12 ^b	0.88^{b}	0.90^{b}	0.77^{b}
Standard error	0.08	0.08	0.06	0.04

Mean in the same column sharing same superscript are not significantly different

Natural resources to be used as feed ingredients or additives are needed to be investigated for developing inexpensive feeds in the world. Macro and micro algae, seaweed and other alternative aquatic plants have been under research and used for decades in aquaculture. Ulva meal is one of the most studied and promising one in this sense. This study was carried out to evaluate the effect of dietary raw and

autoclaved ulva supplemented meal on the growth performance on Cirrhinus mrigala. It could be seen from the result of the present study that the growth rate was seen best in the fish fed with 5% ulva supplemented meal. Statistical analysis of growth rate in all treatments was carried out in fortnights. There was no significant effect of treatments on weight of Cirrhinus mrigala fish in first fortnight. But from second fortnight or third week considerable differences in weight increment were noticed among treatments. This revealed that there is effect of different dietary levels of ulva on the fish growth. The proximate composition of ulva supplemented diets shows minor difference among treatments. In the present study, the diets with crude protein content of 28.00 to 34.60% were fed to fishes [17] The optimum protein requirement for tilapia fingerlings has been suggested between 29.48 to 29.68% [18] The ash content of experimental diets in present study was 6.0-7.8%.

3.1 Growth parameters: In the present study, weight gain, percent weight gain, specific growth rate and food conversion ratio were found significantly different (p<0.05). This could be due to supplementation of seaweed incorporated diets. Ulva incorporated diet showed better growth performance as compared to control. Still the highest growth rate was achieved in U₁ treatment which has 5% content of seaweed powder. The common carp fingerling fed with 5% Ulva inclusion meal achieved the best growth performance [19] The seaweed diet fed Labeo rohita fingerlings, especially with ulva based diets showed comparatively higher growth and weight increment than Spyridina isignis and Sargassum wightii [20] Fish fed with Ulva rigida or Cystosiera barbata in small percentages could be used in Nile tilapia, Oreochromis niloticus diets, comparatively fish fed with 15% ulva diet exhibited lowest weight gain, Guroy et al., (2007) [21].

Likewise, the seaweed diet fed Labeo rohita fingerlings, especially with ulva based diets showed comparatively higher growth and weight increment than Spyridina isignis and Sargassum wightii [20] low level of 4% ulva meal at all dietary lipid levels could be used in juvenile gilthead seabream without any adverse effects on growth and feed utilization, Emre et al., (2013) [22] and the feeding Nile tilapia, Oreochromis niloticus juvenile with 5% Ulva inclusion meal at both dietary lipid levels significantly improves growth performance, feed efficiency, nutrient utilization and body composition, clearly supports the present work done [23] Similarly [19], stated that fish of U₅ treatment had FCR superior to U_{10} and U_{15} groups and the algal supplementation especially at 5% of *Ulva lactuca* level to Nile tilapia, Oreochromis niloticus, fingerlings may improve growth parameters and carcass composition without adverse effects on blood metabolites and liver activity is momentous to the present study [18]. It stated that fish fed with 5% Ulva supplemented diets had higher growth parameters of final body weight and SGR in comparison to other experimental diet groups, also such fish had best survival rate of 100%, and hence both of these justify the results of current study. The weight increment of fish that is notably dependent on low levels of ulva supplemented meal was documented for several species such as common carp Cyprinus carpio [19], juvenile Nile tilapia *Oreochromis niloticus*, [21] Nile tilapia juvenile [23] juvenile gilthead seabream *Sparus aurata* [22] and fingerlings Nile tilapia *Oreochromis niloticus* [18]. There has been considerably higher amount of work done on fish species based on their ability of utilizing algal meals, aquatic and terrestrial macrophytes in raw, fermented, ensilaged,

autoclaved or any other form in the diet [24] successfully incorporated nymphea in the diet of common carp, Cyprinus carpio, fingerling which resulted best growth parameters for the test diet with 400g/kg nymphea meal inclusion. Incorporated Ulva lactuca in the diet of the African cat fish, Clarieus gariepinus concluded that fish fed with 10% ulva diet sand control diet showed better growth performance than those fed with 20% and 30% levels [26]. Included Cynodon dactylon in the diet of fish Catla catla at different levels, which revealed that that 5% inclusion of C. dactylon mixed diet improves the growth performance, feed efficiency, body composition, digestive enzyme and protease activity in C. catla [27]. The inclusion of three seaweeds Gracilaria bursapastoris (GP), Ulva rigida (UR) and Gracilaria cornea (GC) as dietary ingredients on the performance, nutrient utilisation and body composition of European sea bass juveniles [14] The results suggested that the inclusion of G. pursa-pastoris (GP), Ulva rigida (UR) can be considered up to 10% as dietary ingredient for sea bass juveniles, as its no negative consequences were seen. Whereas G. cornea should be limited to 5% of the diet. Two different algae based value added feeds (one containing Spirulina platensis and Enteromorpha intestinalis and the other with Phormidium valderianum and Catenella repens) against conventional (rice bran and mustard oil cake taken in 2:1 ratio, used as control) for fingerlings of Indian major carp rohu, (Labeo rohita), The study suggested that the value-added algal feed 2 was more suitable as compared to the other two for Indian major carp L. rohita, fingerling as evident from the growth performances. While the control diet resulted in high body lipid deposition leading to poor growth of fish, the similar results were observed in present study [28].

At the end of the day, the success of including increased dietary algae species depends on the fish species and the form in which the algae are delivered. This study is an attempt to evaluated the inclusion of ulva meal in *Cirrhinus mrigala* diet. It can be assumed that product quality of fish in terms of higher weight gain could capture attention of fish producers by inclusion of ulva meal to a significant level of 5%, which showed the best growth parameters, however 10% and 15% levels of ulva in the carp diet can also be incorporated for good growth. Hence, the present study suggests that *Ulva lactuca* is a rising seaweed in terms of feed ingredient which could be used in *Cirrhinus mrigala* feed.

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