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Partial replacement of fish feed with seaweed *Ulva* and its effect on growth and survival of *Catla catla* fingerlings

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ABSTRACT

Green seaweed Ulva is found in greater amounts on the southern coast of Gujarat. The availability of the nutrient profile of Ulva makes it a viable candidate for use as a fish feed ingredient. This experiment was conducted to check whether Ulva is suitable as partial fish meal on Catla. Diets were prepared by adding different percentages of Ulva (5%, 10%, 15% wet weight or dry weight). 240 Catla fingerlings were randomly distributed among the four treatments. Three replicates of each treatment were set up. To assess growth performance, fish were fed three times a day and data was gathered every 15 days. The whole experiment was conducted for 60 days. Upon completion of experiment specific growth rate, survivability, length gain, weight gain and physiochemical parameters of water were measured. The study suggests that 10% of seaweed Ulva can be incorporated as a partial feed-in diet of Catla. Maximum weight gain, length gain, and specific growth rate were observed in Treatment 2 i.e. at 10% of Ulva incorporation. While survivability had no significant difference in all treatments. From the proximate composition, the highest protein composition was observed in T2 whereas fat and moisture content were higher in T0. Ash content was substantially high in T2. Results indicate that using Seaweed Ulva at a concentration of 10% in a Catla diet can be considered safe, since there were no detrimental impacts on development or nutrient utilization.

Keywords: *Aquaculture, Fry, Fish, Feed, Seaweed, Ulva, Growth, Catla, Survival, Macroalgae*

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Introduction

Fish and aquaculture are one of the most important sources of food and nutrition. It is important key ingredient in diets of Asian people. The demand for fish is increasing continuously day to day and so is aquaculture. One of the essential things for the success of aquaculture is having balanced nutrients in the fish diet. It should be cheap as well as cost-effective. One of the alternatives is the use of seaweed as a partial replacement. In a 60-day feeding trial, the development and physical characteristics of Indian major carp, *catla*, were studied. A study was carried out to check whether *Ulva* is suitable as partial replacement of fish meal with seaweed *Ulva*. *Catla* (*Catla*) is one of the important fishes among the major Indian carps. *Catla* is considered to be a surface feeder that mainly feeds on plankton, micro algae, larvae of insects, and small pieces of rotten grass. Carps are popular farm fishes in India due to their quicker growth and higher acceptance. Because of a variety of factors, it posseses great amino acid profile, high protein level, high digestibility of nutrients, low cost, broad availability and prominent lack of anti-nutrient [8]. The use of seaweed as a fish meal. Research is going on for two decades to find an alternative material that can be used as a local ingredient [29]. Seaweed possesses the great possibility of being used as an alternative to fish-feeding ingredients because of its nutrient content and composition [20]. *Ulva* is one of the green algae that is high in protein, minerals and vitamins. It has become important macro algae in recent years and has been researched as food source for many different animals.

Material and Methods

Site of experiment: The experiment was carried out at inland fisheries, research station, at Junagadh agriculture university, Junagadh (Gujarat) for 60 days from 14 Feb 2021 to 14 April 2021. The biochemical and proximate studies were carried out in the department of Aquaculture, college of Fisheries, JAU, Veraval.

Experimental fish: Advanced fingerlings of *Catla catla* with a total length of 6.3cm (mean±se) and weighing 2.40 g were chosen for the experiment. Advance fry was acquired from ukai trust . The fish were carried to the inland fisheries research station, Junagadh agriculture university (Gujarat).

Experimental design: Fingerlings of *Catla catla* were selected at random basis and allocated among four different experimental groups . No. of replication were 3(Three), following a CRD (Completely Randomized Design).

Experimental setup: The setup of the experiment consisted of 12 plastic tank (30 L capacity). After completely cleaning the tank with potassium permanganate solution, the fresh water was used to clean it once again.

240 fishes were divided randomly into four different experimental groups each group had three replicas i.e. following factor random block design. Each tank had 20 fishes stocked in chlorine free water, throughout the experiment the water that was used from bore well i.e. ground water. In each tank the adequate level of oxygen was maintained through aeration. The air pressure was uniformly maintained in the entire tank through aeration pipe.

Rearing: To prevent the fish from jumping out, each tank was covered with a plastic cover. Before the starting of the experiment control diet was given to the fish for 10 days, no attempts were made to regulate environmental conditions. Throughout the experiment the experimental conditions were exactly same. At an interval of 15 days growth parameters like length and weight were measured. Fishes were starved for an entire night before taking body weight. water quality Parameters such as DO, alkanity, temperature and pH were evaluated weekly.

Cleaning and siphoning: The experimental tanks were washed manually, to remove the residual feces and excess feed pellet siphoning was done every day. The siphoned water was replaced with an equivalent amount of clean water. This was done for entire 60-day duration.

Experimental diets: Fish meal, GNOC (Groundnut oil cake), wheat flour, tapioca, fish oil, sunflower oil, vitamin and mineral mixture, and *Spirulina* were used as ingredients in the feed formulation (Table 1).

Proximate analysis of Ingredients: Standard method i.e., AOAC (Association of Official Analytical Communities 1995)[3] was utilized in determining the approximate composition of the ingredients. After heating for 30 minutes at 105°C and drying at 65°C, samples moisture content was determined until they reached at constant weight [6]. The semi-automatic Micro-Kjeldahl digestion and distillation apparatus was used to obtain crude protein values [14]. The Soxhlet apparatus was used to measure crude fat. A muffle furnace was used to assess the total ash content.

Formulation and preparation of feed: The experimental diet was created with a protein content of 15% and ingredients that were readily available in the region. As shown in the feed formula, the necessary quantity of ingredients was precisely weighed (Table 2). Seaweed *Ulva* was added to three experimental diets at a rate of 5%, 10%, and 15% ingredients, respectively, whereas no *Ulva* was added to the control diet. The prepared feed mixture and prepared dough were then thermally processed for 10-15 minutes at 121°C and 15 lbs pressure. After the feed mixture was steam cooked, the vitamin and mineral mixture was combined. The feed combination was pelletized using a hand pelletizer. After being spread out on a plastic sheet, the pellets were allowed to dry in the sun. The pellet feed was then packaged in clearly labeled air tight plastic jars.

Feeding: The daily ratio was given twice a day, in the morning and the evening, after being split into two equal halves, with the fish being fed at a weight of 10% of their body weighing initially.

Growth parameters: Fish were sampled at 15-day intervals to determine their body weight. The fish were starved for 24 hours before being weighed.

An electronic weighing balance was used to determine the weight.

According to the WHO, health determinants are defined as *"the circumstances in which people are born, grow, work, live and age, including the broader set of forces and systems that influence the conditions of daily life"*. They directly influence the quality of life of people and it is of utmost importance to know them in order to prevent the onset of diseases, as well as to promote different alternatives to improve lifestyles[1] and generate a sense of responsibility and self-care among one or more population groups.

Chronic Diseases and Therapeutic Adherence

Currently, Chronic Diseases present a higher risk for the population. Some of the most common are: Diabetes, Obesity, Hypertension, Renal Disease, Respiratory Tract Disease and Cancer. At this point, it is important to mention the relationship between the different behaviors that may be present in people's lifestyles and that can trigger these diseases.[2]

$$\text{Weight gain \%} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \; X\,100$$

$$\text{Specific Growth Rate (SGR \%/day)} = \frac{\text{Log W2} - \text{Log W1}}{\text{T2} - \text{T1}} X100$$

$$\text{Survival (\%)} = \frac{\text{Total no. of fish survived after rearing}}{\text{Total number of fish stocked}}$$

Where,

T_1 and T_2 are 0 and 60th days of the experiment and

W_2= weight of fish at time T_2

W_1= weight offish at time T_1

On each sampling day, water samples were obtained and analyzed for pH, temperature, and dissolved oxygen using Winkler's methods. Total alkalinity was determined using a standard process [2]. After the experiment, the approximate composition of the fish was calculated. The fish meat was properly consumed. As previously mentioned, the samples were examined for crude protein, crude fat, ash, moisture, and carbohydrate using standard methods.

Statistical analysis

The significance of differences in growth and survival rate was determined using the two-way analysis of variance (ANOVA) test, which was performed according to standard statistical methods [26].

Results and Discussion

The majority of commercial fish feed ingredients are expensive. Availability of high-quality Nutritious feed for fish farmers who practice aquaculture. The experiment aims to find out a alternative to feed containing naturally available ingredients which are widely available and cost efficient. Despite the fact that algae are easy to grow and a less expensive source of protein, fats, and other nutrients. They are not yet regarded as a major fish food. In fish diets, plant-based feeds are advantageous and less expensive [10][24][21]. The amino acid profile of these feeds is considered to be excellent [18]. In recent years, industries have evolved into plant-based Feeds, which have proven to be more successful in boosting Indian major carp growth. to create appropriate fish feed. In certain instances, aquatic and terrestrial macrophytes have been used as an unconventional source of plant proteins [11;9].

Effect of seaweed *Ulva*-supplemented diet on Growth and Survival of *Catla catla* fingerlings.

A. Effect of seaweed *Ulva* on weight gain of *C. catla*

The average wet weight (g) of *Catla* observed at periodical intervals is shown in Table-3. The final average wet weight recorded at 60 days was 4.94, 4.38, 5.21, and 4.89 g in treatment T0 (Control), T1, T2, and T3 respectively. The final wet weight gain was significantly highest in T2 (5.21 g) and lowest in T1 (4.38 g) treatments. The wet weight was found to be at par in treatments T0, T1, and T3. While a significant difference was found in treatment T2 (p 0.05). In the present study, the highest weight gain was observed in fish fed with a moderate inclusion level (i.e. 10%) of seaweed *Ulva*, while the lowest weight gain was observed at the inclusion level (i.e.5%), the highest weight gain was observed in T2 followed by T3 and T1. The findings of Valente et al. (2006) (28) are supported by the growing success of fish in this experiment. They discovered that juvenile sea bass can include up to 10% of *G. Bursa pastoris* (GP) and *U. rigida* in their diets, which makes them highly intriguing components. Growth-promoting behavior is thought to be an indirect result of polysaccharide-induced nonspecific immune stimulation. [1] conducted a study to evaluate the effect of diet i.e. *U. lactuca* on growth performance, feed utilization, and body composition of African catfish, which had 10, 20, and 30 *U. lactuca* as a meal. The overall result concluded that 10% of *U. lactuca* were ideal as a feed supplement for the catfish. In 2019, [15] investigated the effects of substituting processed seaweed (*Ulva lactuca*) for fish meal to check.growth and survival of *Catla*. It indicated that a diet of up to 15% *Ulva lactuca* produced better growth performance than the control group. It also noted an improvement in immune response, suggesting that *Ulva* has immuno-stimulatory properties. However, a higher concentration can be harmful. [16] observed a decrease in growth performance when seaweed exceeds 20% of the total diet.

B. Effect of Seaweed *Ulva* length gain of *C. catla*

Significantly, the highest length increase was observed in T2. (5.22 cm) and the lowest in T0 (control). (4.24 cm). However, the mean length of T2 and T3 was found to be at par with each other. As shown in Table 4. Similar kind of results was obtained by Patel et. al (22) In 2018, the experiment was conducted on *Labeo rohita* i.e. effect of seaweed *Ulva* as a feeding additive in the diet for the growth and survival of *Labeo rohita* fry. He incorporated the seaweed *Ulva* at 10%, 20%, and 30%. Along with the traditional feed, and concluded that 10% of *Ulva* can be used as a feed additive. He recorded the highest length gain in Treatment T1 i.e. at 10%, followed by Treatment T2 (20%), T3 (30%), and T1 (Controlled diet).

C. Effect of seaweed *Ulva* survival rate of *C. catla*

Data for the survival of *Catla* fingerling in the respective treatments are presented in Table 5. A significant difference was observed in the mean survival rate. It was observed at 96.53, 96.13, 97.67, and 95.20 percent in treatment T0 (control) to treatment T3, respectively. The highest survival rate was found in treatment-2 with 97.67%, while, lowest in T3 (95.20). However, treatments T1 and T2 were found to be at par with each other. While significant differences were observed in survival rates over the period, up to 60 days. [27] concluded that fish fed with 10% *Ulva* meal showed highest growth performance as compared to fish fed in control although there was no difference in survival rate and health of a fish. [28] Found that feeding *G. bursa pastoris* and *Ulva rigida* in juvenile sea bass improved their survival. [13] reported that 15% of *Ulva* can be supplemented with Red Tilapia, this improves its growth performance without having a negative impact on survival rate or feed efficiency. [5] stated good survival of Grass carp, *Ctenopharyngodon idella*, using *Ulva* powder, *Spyridia* powder, and *Sargassum* powder. [30] found that adding dry seaweed *Gracilaria lemaneiformis* to Teleost fish *Siganus canaliculatus* diets strengthened survival rates. [25] emphasized or focused on the immune response of carps, that is the effect of sulphated polysaccharides from *ulva* sp on various fishes and carps. They concluded that the production of immune-related enzymes increased, which increased the fish's ability to fight off pathogens and survive under stressful conditions.

D. Effect of seaweed *Ulva* on Specific Growth Rate of *C. catla*

The specific growth rate (SGR) of *Catla* fingerlings in different treatments is given in Table-6. The highest SGR was found in T2 diet treatment (9.03 ± 0.30), followed by T0 (8.76 ± 0.10), T3 (8.01 ± 0.09) and T1 (5.97 ± 0.12). A significant difference ($p<0.05$) was observed in % SGR among all the treatment diets; T2 was found to be higher as compared to the other treatments. [1] experimented using *Ulva Lactuca* as a feed supplement for the African catfish. And found the highest SGR in D1 i.e. in a controlled diet followed by D2 (10%), D3 (30%), and D4 (40%). [12] found higher SGR in Ulva 3%, followed by 2% and 1% in a controlled condition. Bindu and Sobha (4) (2005) stated that for Grass Carp, *Ctenopharyngodon idella*, *Ulva*, *Spyridia*, and *Sargassum* powders can be used to obtain good SGR. [23] concluded that 8% of *Ulva reticulata* can be included as an ingredient of goldfish. They found the highest SGR in 8% of *Ulva* i.e.(14.09±0.65), followed by 6% (13.24±0.35), 4% (11.66 ± 1.06), 2% (11.54 ± 1.82) and in a controlled diet i.e. (7.53±0.55). In 2022, [17] conducted an experiment in which oven-dried *Ulva lactuca* was added to a diet of *Catla*. It was found that a 12% inclusion level offered the best feed efficiency and growth performance.

E. Effect of seaweed Ulva on proximate composition of fish carcass

The protein content was significantly highest (11.86 %) in T2 compared to other treatments. The fat content was significantly highest (2.49 %) in T0 as compared to T2. The ash content was significantly highest (3.84) in T2 as compared to control. The moisture content was found to be highest. (75.47 %) in T0 as compared to other treatments. As shown in table 7.

F. Physico chemical parameters of water

Water quality refers to the level of excellence that a particular body of water possesses for the growth of desired aquatic organisms that accomplish high survival, development, and reproduction. Maintaining good water quality is essential for fish survival as well as optimal development. In the present investigation, important parameters such as DO (4.0 TO 5.8 PPM), Alkanity (59 to 85 Ppm), pH (8.5), hardness (80 to 130 ppm), and total dissolved solids (601 to 672 ppm) were observed during the experiment. The parameters for water quality in the experiment are given in Table 8.

Table 1: Proximate composition of ingredients used for preparation of treatment diets (%)

Ingredients	Crude protein (%)	Crude Fat (%)	Ash (%)	Moisture (%)
Fish meal	51	5.07	7.97	6.3
GNOC	38.15	8.07	8.78	5.9
Wheat flour	8.7	1.99	1.7	11.57
Tapioca	0.19	0.11	0.06	10.6
Seaweed *ulva*	0	5	10	15

Table 2: Formulation of experimental diets (g)

Ingredients	Treatment			
	T0	T1	T2	T3
Fish meal	171.55	171.55	171.55	171.55
GNOC	230.25	230.25	230.25	230.25
Tapioca	24.1	24.1	24.1	24.1
Wheat flour	24.1	24.1	24.1	24.1
Fish oil	20	20	20	20
Sunflower oil	20	20	20	20
Vitamin mix	10	10	10	10
Sea weed	0	5	10	15

Table 3: Effect of seaweed Ulva on the weight of Catla catla fingerlings in different treatments (in gm)

Treatment (*Ulva*)	P1 (0 Days)	P2 (15 Days)	P3 (30 Days)	P4 (45 Days)	P5 (60 Days)	Mean
T0 (C)	2.19	3.17	4.22	6.37	8.76	4.94
T1 (5 %)	2.40	3.40	4.61	5.53	5.97	4.38
T2 (10 %)	2.19	3.73	4.70	6.40	9.03	5.21
T3 (15 %)	2.19	3.46	4.45	6.34	8.01	4.89
Mean	2.24	3.44	4.50	6.16	7.94	
	S.Em ±	**CD@5%**				
Treatment	0.049	0.141				
Period	0.055	0.157				
Treatment X Period	0.11	0.314				
C.V.%	**3.92 %**					

Table: 4: Effect of seaweed Ulva on length of Catla catla fingerlings in various treatments (in cm)

Treatment (*Ulva*)	P1 (0 Days)	P2 (15 Days)	P3 (30 Days)	P4 (45 Days)	P5 (60 Days)	Mean
T0 (C)	3.10	3.71	3.57	4.79	6.01	4.24
T1 (5 %)	3.21	3.54	4.28	5.21	6.23	4.50
T2 (10 %)	3.27	4.12	5.28	6.24	7.16	5.22
T3 (15 %)	3.26	4.20	5.27	6.07	6.51	5.06
Mean	3.21	3.89	4.60	5.58	6.48	
	S.Em ±	**CD@5%**				
Treatment	0.04	0.114				
Period	0.04	0.127				
Treatment X Period	0.089	0.254				
C.V.%	**3.23 %**					

Table 5: Effect of seaweed Ulva on survival rate of Catla catla fingerlings in different treatments (%)

Treatment	P1 (0 Days)	P2 (15 Days)	P3 (30 Days)	P4 (45 Days)	P5 (60 Days)	Mean
T0 (C)	100.00	100.00	98.33	96.00	94.00	96.53
T1 (5 %)	100.00	100.00	95.67	93.33	91.67	96.13
T2 (10 %)	100.00	100.00	95.00	95.00	92.67	97.67
T3 (15 %)	100.00	100.00	95.00	92.33	88.67	95.20
Mean	100.00	100.00	96.00	94.17	91.75	
	S.Em ±	**CD@5%**				
Treatment	0.333	0.953				
Period	0.373	1.065				
Treatment X Period	0.745	**2.13**				
C.V.%		**1.34 %**				

Table 6: Effect of seaweed Ulva on SGR % of C.Catla fingerlings in different treatments

Treatment	SGR %
T0 (C)	8.76 ± 0.10
T1 (5 %)	5.97 ± 0.12
T2 (10 %)	9.03 ± 0.30
T3 (15 %)	8.01 ± 0.09
S.Em ±	0.102
C.D. at 5 %	0.334
C.V. %	2.23

Table 7: Proximate analysis of fish carcass

Treatment	Protein %	Fat %	Ash %	Moisture %
T0 (C)	11.33	2.49	3.26	75.47
T1 (5 %)	11.36	2.37	3.47	73.69
T2 (10 %)	11.86	2.13	3.84	73.18
T3 (15 %)	11.42	2.26	3.06	71.96

Table 8: Physio-chemical parameters of the water used during the study period

Periods	Treatments	Do_2	Hardness	Alkalinity	pH	TDS
P_1	T_0	4.8-5.6	80-100	76-78	8.5	601-603
	T_1	4.8-5.6	80-100	76-78	8.5	601-603
	T_2	4.8-5.6	80-90	76-78	8.5	600-602
	T_3	4.8-5.6	80-100	76-78	8.5	603-605
	T_4	4.2-5.6	80-100	76-78	8.5	601-604
P_2	T_0	4.4-5.6	90-120	69-79	8.5	613-620
	T_1	4.0-5.6	90-120	59-70	8.5	612-618
	T_2	4.4-5.6	90-120	69-79	8.5	613-620
	T_3	4.0-5.6	80-120	65-78	8.5	615-621
	T_4	4.4-5.6	80-100	65-78	8.5	616-622
P_3	T_0	4.2-5.8	80-130	65-70	8.5	625-630
	T_1	4.2-5.8	90-130	61-75	8.5	618-628
	T_2	4.8-5.6	80-130	61-79	8.5	621-626
	T_3	4.2-5.8	80-130	65-70	8.5	625-630
	T_4	4.6-5.6	80-130	61-85	8.5	631-633
P_4	T_0	4.4-5.2	90-120	69-85	8.5	637-641
	T_1	4.2-5.2	100-130	61-75	8.5	634-636
	T_2	4.4-5.2	90-120	69-85	8.5	637-641
	T_3	4.2-5.6	90-110	65-70	8.5	639-643
	T_4	4.2-4.8	90-120	61-85	8.5	644-647
P_5	T_0	4.4-5.2	90-120	69-85	8.5	655-661
	T_1	4.2-5.2	100-130	61-85	8.5	650-653
	T_2	4.4-5.2	90-120	69-85	8.5	655-661
	T_3	4.2-5.2	90-110	65-85	8.5	663-665
	T_4	4.2-4.8	90-120	61-85	8.5	669-672

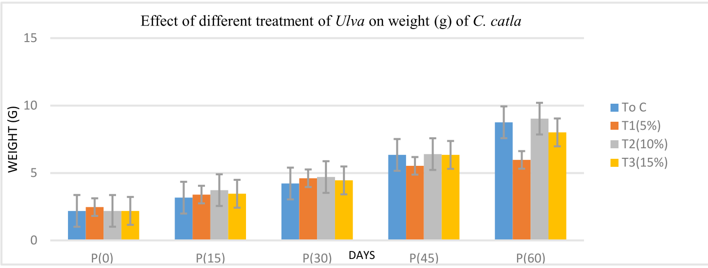

Fig.1- Effect of different treatment of Ulva on weight (g) of C. catla fingerlings

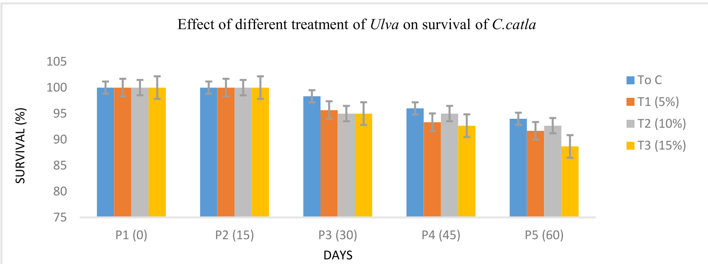

Fig.2 Effect of different treatment of Ulva on Survival of C. catla fingerling

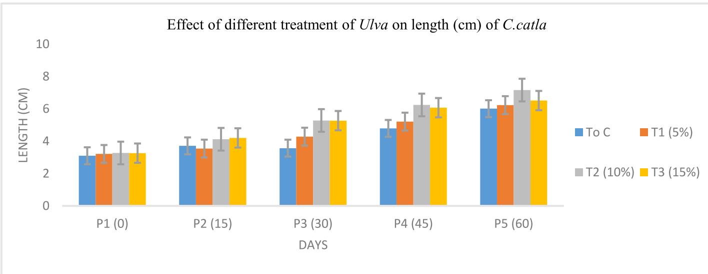

Fig.3 Effect of different treatment of Ulva on Length (cm) of C. catla fingerling

Conclusion

The four are nitrogenous experimental diets consisting of a control diet (100% basal feed) and 5%, 10% and 15%) *Ulva* incorporated diets were prepared. The diets were formulated to provide 35 % crude protein on a dry-weight basis. 20 fish of homogenous size and weight were kept in each Aquarium of

20-liter capacity. A total of 12 aquaria were maintained for the experiment. The fish were fed for 60 days to assess the effect of *Ulva* incorporation on growth, survival, feed utilization, and Carcass composition of *C.catla.* Based on the results obtained from the experiment conducted, it was found that higher growth, SGR, length, and survival were obtained by feeding the fish with a diet containing 10% *Ulva* [7]. In order to assess how various *ulva lactuca* concentrations affected the performance and antioxidant capacity of Nile tilapia, he prepared diets containing 5%, 10%, and 15% macroalgae meal. He concluded that adding 5% macroalgae to the tilapia diet enhanced antioxidant capacity under stress. The majority of materials for commercial fish feed are expensive, and their unavailability makes it difficult for fish farmers to adopt aquaculture practices. Freshwater micro algae like *ulva* contains up to 19% digestible protein. It can act as an effective fish feed ingredient. Although it also increases the immunity. The mixture of various algal proteins may enhance the growth if combined in a right ratio. In the carp diet, it was proposed that using multiple protein sources together was more beneficial than using just one. [19] also provided a clear explanation of *Ulva*'s potential as a sustainable food supply. In significant Indian carp farming, the other locally accessible algae genera can be combined with the control diet to provide an equivalent or better outcome, which could eventually have a high economic value. In a variety of fish species, supplementation with macro- and microalgal meals has been shown to improve feed utilization, lipid metabolism, body composition, disease resistance, and carcass quality, while also enhancing growth performance. The present study demonstrates that *Catla catla* fry exhibit improved growth when fed diets containing marine algae, particularly *Ulva*, which provides a valuable source of protein, carbohydrates, and lipids. The superior growth and feed utilization observed in this study highlight the potential of *Ulva* as a cost-effective dietary ingredient in aquaculture. Our findings suggest that dietary inclusion of *Ulva* at a 10% level supports growth and feed efficiency without any adverse effects, making it a promising alternative to conventional feed ingredients. Furthermore, incorporating *Ulva* could reduce dependence on fishmeal, offering both economic and ecological advantages. Nevertheless, further research through long-term

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