ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

SCREENING OF MICROALGAE WITH HIGH PIGMENT AND NUTRITIONAL VALUE AS A FISH FEED FOR *CATLA CATLA* (HAMILTON)

Pranita Hajong* and Papiya Ramanujam

Algal Ecology Laboratory, Department of Botany, North-Eastern Hill University, Shillong, Meghalaya, India

**Corresponding author: pranitahajong@gmail.com*

ABSTRACT

Algae are excellent source of food for fish in natural habitats due to their high protein, carbohydrate, lipid and carotenoid content. Proper selection and cultivation of dominant algal species can be utilized to improved pisciculture. In the present study, twelve selected algal species from different water bodies were cultured and screened for high pigment and nutritional value to be used as supplemented fish feed. Feeding trial with four different diets were continued for 90 days to feed *Catla catla* (Hamilton). Among the four diets, significant ($P<0.05$) increase in length (7.40 cm), body weight gain (4.90 g), specific growth rate (4.12 % day^{-1}) and low feed conversion ratio (0.26) were found in fish fed with value added algal diet, that signifies effective utilizations of feed by the fish. Similarly, the biochemical attributes like protein (12.78 %), carbohydrate (3.91 %), lipid (2.60 %), carotenoid (0.67 %), ash (6.2 %) and moisture content (70.36%) were also found to be maximum in fish fed with value added algal diet as compared to control diet. The findings of the present investigation suggested that the twelve selected algae having high pigment content and nutritional value can be used as fish feed to enhance the growth performance and nutritional value of *Catla catla*.

Keywords: Algal diet, *Catla catla*, Growth, Carotenoid, Biochemical Composition

1. INTRODUCTION

Fishes are considered as a healthy diet due to their high quality protein and very low fat content. The consumption of fish far exceeds that of other animals and this increases its demand and their limited supply, leading to price hike. Lack of proper nutrient in the fishmeal resulted in slow growth rate and decreased survival rate. Crop plants which are being used as fish feed in most of the aquaculture are deficient in minerals, protein and have low digestibility as well as lack certain amino acids [1]. To overcome this, many researchers had investigated some of the algal species which can be replaced partially or fully as a fish feed. Algae are potential source of protein, carbohydrate, lipid, vitamin and minerals. In addition, their appropriate size, high growth rate, easy availability and ease in harvest make them ideal for pisciculture [2-5]. Some of the microalgae like *Chlorella* spp., *Scenedesmus* spp., *Ankistrodesmus* spp., *Lyngbya* spp., *Chlamydomonas* spp., *Chlorococcum* spp., *Spirulina* spp. have the potential sources of protein, lipid and carbohydrate [6-9]. *Catla catla* (Hamilton) from the family Cyprinidae is one of the most commercialized carp because of its efficient surface feeding habit and fast growth rate. The color of the fish's skin is another important factor which determines its value in the market. Fish cannot synthesize their skin pigmentation therefore they have to depend upon others sources primarily on diet. Algae posses a wide range of pigments mainly carotenoid which can enhance the coloration of the skin.

Natural or artificial water body is the prime factors for both aquaculture industries as well as for cultivation of algae in large biomass. Meghalaya is one of the wettest places on the earth and aquaculture can be the key sector in this region. There is a gap in demand and supply of fish in the market, though there are fish farms run by government as well as private sectors in this region. Fishmeal is the major factor which determines the production of fish in a short span of time with proper nutrition in fish meat. Therefore, in the present study, selected microalgae with high pigment content and high nutritional value have been screened which could serve as conventional fish feed for higher and better fish production.

2. MATERIAL AND METHODS

2.1. Culture of selected algal species

Twelve selected species, eight belonging to green algae (*Chlorella vulgaris*, *Scendesmus obliquus*, *Scenedesmus*

dimorphus, *Chlamydomonas reinhardtii*, *Chlorococcum infusionum*, *Gloeocystis vesiculosa; Ulothrix tenuissima* and *Desmidium swartzii*) three belonging to Cyanobacteria (*Calothrix marchica*, *Anabaena variabilis* and *Leptolyngbya boryana*) and one belonging to Bacillariophycae (*Navicula veneta*) were collected from different water bodies of Garo Hills, Meghalaya. For obtaining biomass, these species were grown in the laboratory using appropriate culture medium. The modified bold basal media [10] for green algae (KH_2PO_4-1.75 g/100ml, $CaCl_2.2H_2O$-0.25 g/ 100ml, $MgSO_4.7H_2O$-0.75 g/100ml, $NaNO_3.7H_2O$-2.5 g/100ml, K_2HPO_4-0.75 g/100ml, NaCl- 0.25 g/100ml, Na_2 EDTA.$2H_2O$- 1 g/100ml, KOH-0.62 g/100ml, $FeSO_4.7H_2O$- 0.49 g/100ml, H_2SO_4-0.1 ml/100ml, H_3BO_3-1.15 g/100ml) were prepared and the pH was maintained at 6.8. The BG11 media [11] for Cyanobacteria ($NaNO_3$-1.5g /l, $K_2HPO_4.3H_2O$-0.04g/l, $MgSO_4.7H_2O$-0.075 g/l, $CaCl_2.2H_2O$-0.027 g/l, $C_6H_8O_7$- 0.006 g/ l, $C_6H_8O_7$.nFe.nH_3-0.006 g/l, EDTA Na_2Mg- 0.001g/l, Na_2Co_3-0.02 g/l) and Guillard media [12] for diatom [$Ca(NO_3)_2.4H_2O$- 0.02g/l, KH_2PO_4-0.012g/l, $MgSO_4.7H_2O$-0.025 g/l, $NaHCo_3$-0.015 g/l, EDTA Fe Na-0.002 g/l, EDTA Na_2-0.002 g/l, H_3BO_3-0.002, $MnCl_2.4H_2O$-0.001 g/l, $(NH_4)_6Mo_7O_{24}.4H_2O$-0.001g/l,$C_{63}H_{88}CoN_{14}O_{14}P$-0.0004g/l, $C_{12}H_{16}N_2O_3S$-0.0004 g/l, $NaSio_2.9H_2O$-0.057 g/l]. The light and dark period were maintained (16 hours light period and 8 hours dark period) throughout the growth period.

2.2. Methods of biochemical parameters analysis in algae and fish

The estimation of protein, carbohydrate, lipid and carotenoid from algae and fish were assessed following the standard methods [13-16].

2.2.1. Protein

10 mg of sample was taken into a test tube; 10 ml of hot TCA was added to it and left for 1 minute. It was centrifuged and the supernatant was decanted and then pellet was collected, this was dissolved in 1N NaOH (NaOH was added equal to the amount of sample in the tube) and left overnight. 0.1 ml of the above extract was taken in the test tube and to that 4.5 ml of alkaline reagent was added to dissolve the TCA precipitated proteins and then allowed to stand for 1 minute. 0.5 ml of 1N folin reagent was added and mixed uniformly. The test tube was kept at room temperature for 30 minutes and the absorbance was taken at 540 nm against the blank.

2.2.2. Carbohydrate

4 mg of anthrone reagent was added to 1 mg of sample in a tube. It was shaken thoroughly and boiled in a hot water bath for 15 minutes with marble ball at the mouth of the tube. It was cooled in running tap water and absorbance was taken at 620 nm against a blank.

2.2.3. Lipid

100 mg sample was taken in test tube and 10 ml of 0.2 M ice cold $HClO_4$ was added. The tube was thoroughly vortexed for 15 minutes, keeping intermittently on the ice bath. The test tube was centrifuged for 15 minutes and the supernatant was decanted. The procedure was repeated with another aliquot of $HClO_4$, after collecting the pellet. 10 ml of chloroform: methanol mixture was added to the pellet. It was vortexed and then allowed the pellet to stand for 5 minutes. The sample was centrifuged and the supernatant was collected. 0.2 ml of distilled water was added to the chloroform: methanol extract. The solution was mixed uniformly and centrifuged to separate the phases. The lower organic phase containing lipids was collected in a preweighed petriplate. The chloroform: methanol mixture was allowed to evaporate, after complete evaporation the plate was reweighed and the lipid present in the sample was estimated.

2.2.4. Carotenoid

10 mg of sample was taken in a test tube and to that 10 ml of 80% acetone was added and macerated using mortar and pestle. Colored homogenate was centrifuged at 2000 rpm for 20 minutes and filtrate was collected. Debris was again crushed with acetone. This procedure was repeated for three more times for complete extraction, each time the filtrate was collected in a volumetric flask. The filtrate was dried at 37°C by keeping in the dark and then it was mixed with a solvent mixture containing equal volume of 10 ml petroleum ether and 10ml of methanolic KOH. This mixture was kept in dark for 2 hours for saponification. It was further diluted with 30 ml of aqueous NaCl in a separating funnel and shaken gently, and allowed to stand for complete separation into two distinct layers. The upper yellow fraction containing carotenoid was separated with 30 ml of aqueous NaCl. The final volume of petroleum ether extract was noted and optical density was taken at 450 nm against petroleum ether as blank.

2.3. Fish experimental setup

Catla catla (Hamilton) fingerlings of same age group (0.6-0.7g $\pm$ 0.03) were collected from Digri Chiring fish seed

farm, West Garo Hills, Meghalaya, India. They were acclimatized for two weeks in the aquarium (61L x 31B x 31H cm) and fed with commercial diet. All the experimental fish were starved for 24 hours prior to feeding trial. Four experimental sets were prepared for each diet. Aeration was provided for continued supply of dissolved oxygen in the water. Water in the aquarium was changed after every seven days in order to avoid turbidity and maintained the water quality.

2.4. Experimental fish feed formulations

In the present experiment, a conventional feed composed of rice bran and mustard oil cake (2:1) was used as the control diet. For the experimental diet, dry algae were mixed in a proportion of *Chlorella vulgaris : Chlamydomonas reinhardtii : Scendesmus obliquus : Anabaena variabilis : Desmidium swartzii : Scenedesmus dimorphus : Ulothrix tenuissima : Chlorococcum infusionum : Gloeocystis vesiculosa : Navicula veneta : Leptolyngbya boryana : Calothrix marchica* (25:18:14:10:8:7:5:4:3:3:2:1) to prepare a composite mixture and 75% of this composite mixture was mixed with 25 % of control diet and designated as value added feed 1 (VAF1). Green algae were mixed in a proportion of *Chlorella vulgaris* : *Chlamydomonas reinhardtii : Scendesmus obliquus : Desmidium swartzii : Scenedesmus dimorphus : Ulothrix tenuissima : Chlorococcum infusionum : Gloeocystis vesiculosa* (25:20:15:10:9:8:7:6) to prepared a composite green algal mixture and 75% of this composite green algal mixture was mixed with 25 % of control diet and designated as value added feed 2 (VAF2). A proportion of *Anabaena variabilis : Leptolyngbya boryana : Calothrix marchica* (45:30:25) were mixed to prepared a composite of Cyanobacterial mixture and 75 % of this Cyanobacterial mixture was mixed with 25 % of control diet and designated as value added feed 3 (VAF3). All the feeds were prepared according to the 40% requirement of *Catla catla* [17]. A feeding trial of 90 days was conducted. All the experimental fish were fed with their respective diets twice a day at 3% of body weight. Set 1 was fed with control diet; set 2 was fed with VAF1 diet; set 3 fed with VAF2 diet and set 4 was fed with VAF3 diet.

2.5. Analysis of growth performance of *Catla catla*

For analysis of various parameters like body weight, specific growth rate and feed utilization, fishes were randomly selected from each experimental aquarium for estimation following standard methods [18] as follows: Body weight gain = [(Final body weight (g) - Initial body weight (g)) / Initial body weight (g)] x 100. Specific growth rate = [(In Final body weight (g) – Initial body weight (g) / number of days] x100. Feed conversion ratio = Dry feed fed (g) / Live body weight gain (g). Survival rate = 100 x (Nt/N0). Where, Nt =Final number of fish, N0= Initial number of fish [19]. Total body length was taken along the anterior-posterior body axis from mouth tip to the end of the caudal fin [20].

2.6. Analysis of biochemical parameters in experimental fish *Catla catla*

The estimation of protein, carbohydrate, lipid and carotenoid was carried out following the same standard methods as described above. In addition, the moisture and ash content were estimated by AOAC [21]. For these analyses fish were randomly collected from respective aquarium after 90 days of feeding trials.

2.6.1. Moisture content

The fresh (wet) sample was dried in an oven at 105°C temperature for 16 to 18 hours till constant weight.

$$\text{Moisture content, \%} \left(\frac{wt}{wt}\right) = \frac{\text{Wt of fresh (wet) sample} - \text{Wt of dry sample}}{\text{Wt of fresh (wet) sample}} \times 100$$

2.6.2. Ash

Crucible was first dried at 100°C for 2 hours in an oven and placed in dessicator, cooled and then recorded its weight. 2 gm of fresh sample was placed into the crucible and recorded its weight again. The sample in the crucible was placed in a furnace for 2 hours at 550°C until all the carbon get removed. The percentage of the ash content was measured by the resulting inorganic residues.

$$\text{Ash content (\%)} = \frac{\text{Wt of ash}}{\text{Wt of sample}} \times 100$$

2.7. Statistical Analysis

All the growth and biochemical data were subjected to one-way ANOVA (SPSS16.0) to measure significant differences among the control and experimental fish.

3. RESULTS AND DISCUSSION

3.1. The biochemical composition of algal species used as fish feed

Algal species are well known for their high content of protein, lipid, carbohydrate and carotenoid which make them valuable for pisciculture industry [22-26]. The algal species with their respective maximum nutrient content used in the present study as fish feed are as follows: *Chlorella vulgaris* with 57.72%, *Chlamydomonas reinhardtii* with 54.99% and *Scenedesmus obliquus* with 52.01% have higher protein content, whereas *Scenedesmus dimorphus*

with 50% and *Anabaena variabilis* with 27.45% showed higher carbohydrate content. Lipid content was high in *Chlorella vulgaris* with 21.65% and *Leptolyngbya boryana* with 16.81 % as compared to other algal species. Carotenoid was higher in *Anabaena variabilis* with 0.21% followed by *Scenedesmus obliquus* with 0.14% and *Chlorococcum infusionum* with 0.10% as compared to other algal species (Table 1).

Table 1: Biochemical composition of algal species used as a fish feed

Algal species	Protein %	Carbohydrate %	Lipid %	Carotenoid %
Chlorella vulgaris	57.72±0.38	14.89±0.06	21.65±0.24	0.06±0.01
Scendesmus obliquus	52.01±0.24	15.08±0.04	12.9±0.11	0.14±0.01
Scenedesmus dimorphus	26.12±0.05	50.77±0.01	8.40±0.08	0.03±0.007
Chlamydomonas reinhardtii	54.99±0.08	6.5±0.12	19.00±0.01	0.03±0.06
Ulothrix tenuissima	22.60±0.06	12.24±0.02	7.9±0.08	0.02±0.05
Chlorococcum infusionum	20.74±0.04	9.75±0.01	12.56±0.02	0.10±0.1
Gloeocystis vesiculosa	18.75±0.004	8.27±0.005	2.00±0.03	0.05±0.02
Desmidium swartzii	49.00±0.2	11.32±0.06	1.80±0.04	0.02±0.07
Calothrix marchica	2.60±0.02	16.86±0.01	10.5±0.04	0.05±0.006
Anabaena variabilis	47.20±0.13	27.45±0.01	8.54±0.04	0.21±0.03
Leptolyngbya boryana	4.03±0.24	17.8±0.06	16.81±0.02	0.03±0.06
Navicula veneta	17.69±0.01	10.35±0.03	8.80±0.09	0.08±0.005

Table 2: Survival rate, body length, body weight, specific growth rate and feed conversion ratio of *Catla catla* fed with control diet and value added feed (VAF1, VAF2 and VAF3)

Duration	Control diet	VAF1 diet	VAF2 diet	VAF3 diet	P-value
Survival rate %					
30 days	86.2±0.31	87.09±0.29	86.1±0.41	86.0±0.34	0.5
60 days	90.35±0.23	94.33±0.38	92.2±0.17	90.4±0.5	0.001
90 days	92.16±0.11	96.12±0.33	95.23±0.56	95.0±0.41	0.02
Body Length (cm)					
30 days	3.2 ± 0.06	4.6±0.12	4.03±0.09	4.1±0.1	0.02
60 days	4.65 ±0.05	5.8±0.04	5.1±0.08	5.2±0.09	0.001
90 days	5.27± 0.14	7.4±0.15	6.3±0.34	6.2±0.13	0.002
Body weight gain (g)					
30 days	0.96±0.01	1.84±0.02	1.22±0.06	1.39±0.01	0.004
60 days	1.74±0.05	2.95±0.12	2.32±0.08	2.43±0.02	0.006
90 days	3.17±0.06	4.90±0.01	3.80±0.03	3.65±0.05	0.001
Specific growth rate (% day^{-1})					
30 days	1.08±0.05	2.01±0.07	1.45±0.07	1.34±0.24	0.01
60 days	1.96±0.02	2.69±0.2	2.52±0.23	3.06±0.26	0.01
90 days	2.87±0.18	4.12±0.12	3.61±0.1	3.26±0.39	0.04
Feed Conversion ratio					
30 days	0.78±0.03	0.68±0.01	0.71±0.04	0.70±0.01	0.8
60 days	0.65±0.01	0.44±0.02	0.52±0.03	0.55±0.06	0.04
90 days	0.40±0.05	0.26±0.01	0.31±0.01	0.35±0.02	0.03

Significant different at (P<0.05)

3.2. The growth performance of *Catla catla*

The growth performance of *Catla catla* was higher when fed with value added feed than control diet. On exposing the fishes for 90 days with four different diets, a significant difference was observed in all the growth parameters (Table 2).

The survival rate was higher in VAF1 diet with 96.12% followed by VAF2 diet with 95.23 %, VAF3 diet with 95.0 % and control diet with 92.16 %. When fed with VAF1 there was an increase in length with 7.40 cm, body weight gain with 4.90 g and Specific growth rate with 4.12 % day^{-1} as compared to control diet. Feed conversion ratio was low (0.26 %) in fish fed with VAF1

which could be due to higher ash content in algae because higher the ash content in a diet lower the feed conversion ratio [27] and result in higher weight gain. The improvement in growth performance in the fish fed with VAF1 diet could be due to the addition of different algae with high protein, carbohydrate and minerals contents thus meeting the requirements for a balanced diet, resulting in better growth [28]. Algal feed have been reported to have better digestibility which in turn result in faster growth rate. Partial replacement of fish diets with microalgae resulted excellent growth and survival rate in many commercial carp [29, 30].

3.3. The biochemical composition of *Catla catla* fed with experimental diets

3.3.1. Protein

There was significant ($P<0.05$) increase in protein content in all the fishes fed with value added feed as compared to control diet (Fig 1). The protein content in fish fed with VAF1 was 12.78 % followed by VAF2 with 9.2 %, VAF3 with 7.34 % and control diet with 4.24 %. The increase in protein content in fish fed with VAF1 could be due to the feed containing algae from single cell green algae, filament algae, Cyanobacteria and diatoms as compared to other diet. *Chlorella* spp., *Scenedesmus* spp., and *Spirulina* spp., have been reported to have protein content and used as energy rich feed for fish fingerling [31, 32].

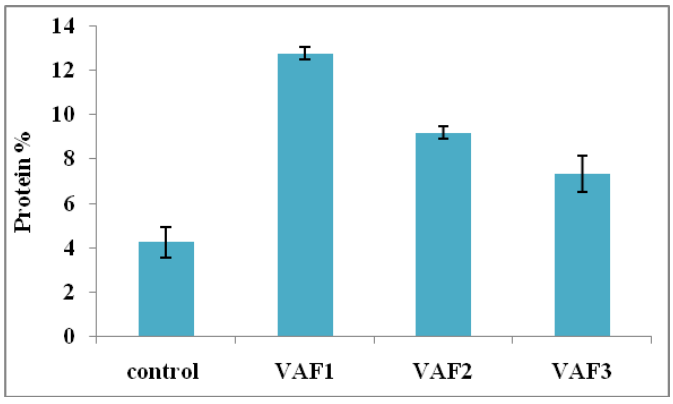

Fig. 1: Protein content in fish fed with four experimental diets

3.3.2. Carbohydrate

There was significant ($P<0.05$) increased in carbohydrate content in all the fish fed with value added feed as compared to control diet (Fig 2). The carbohydrate content in fish fed with VAF1 was 3.91 % followed by VAF2 with 2.86 %, VAF3 with 2.67 % and control diet with 2.02 %. *Scenedesmus dimorphus* and *Anabaena variabilis* are excellent source of carbohydrate which could be the reason for increased carbohydrate content in the fish fed with VAF1 which was composed of green algae and cyanobacteria that act as excellence source of carbohydrate. Carbohydrate content in *Labeo gonius* was higher when fed with mixed algal diets containing some members of Chlorophyceae, Cyanobacteria and Bacilariophyceae [33].

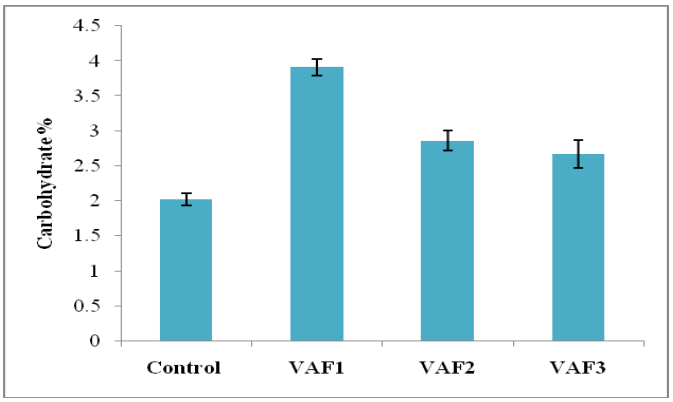

Fig. 2: Carbohydrate content in fish fed with four experimental diets

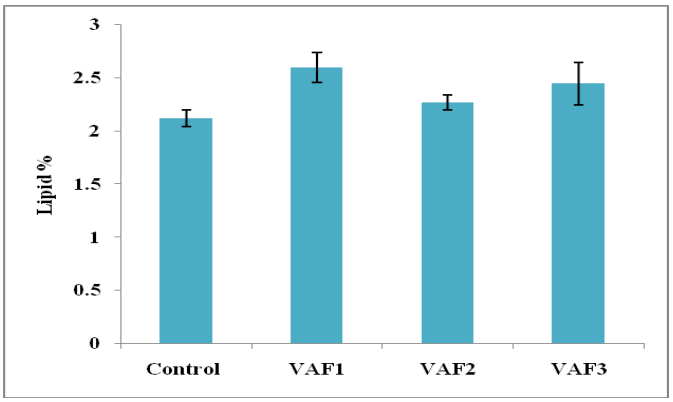

Fig. 3: Lipid content in fish fed with four experimental diets

3.3.3. Lipid

There was increased in lipid content in all the fish fed with value added feed as compared to control diet (Fig 3). The lipid content in fish fed with VAF1 was 2.6 % followed by VAF3 with 2.45 %, VAF2 with 2.27 %, and control diet with 2.12 %. The increased lipid content in the fish fed with VAF1 and VAF3 as compared to the other two diets (VAF2 and control diet) could be due to the presence of algal genera which are rich source of lipid like *Chlorella vulgaris*, *Chlamydomonas reinhardtii, Leptolyngbya boryana and Navicula veneta. Navicula* spp., and *Spirulina* spp., is excellent source of lipid and when

used as fish fed improved growth and lipid content in the experimental fish [34, 35].

3.3.4. Carotenoid

There was significant ($P<0.05$) increase in carotenoid content in the skin of all the fish fed with value added feed as compared to control diet (Fig 4). The carotenoid content in fish fed with VAF1 was 0.67 % followed by VAF2 with 0.23 %, VAF3 with 0.12 %, and control diet with 0.02 %. Algae are excellent source of pigments especially caroteniods which could enhance the coloration better than the synthetic coloring agent, therefore addition of green algae, blue green algae and diatom had improved the carotenoid content in fish skin when fed with VAF1. Increased pigmentation of fish skin mostly depends on a combination of algal diet [36, 37]. Increased carotenoid content in prawn was obtained when fed with mixed algal diet [38].

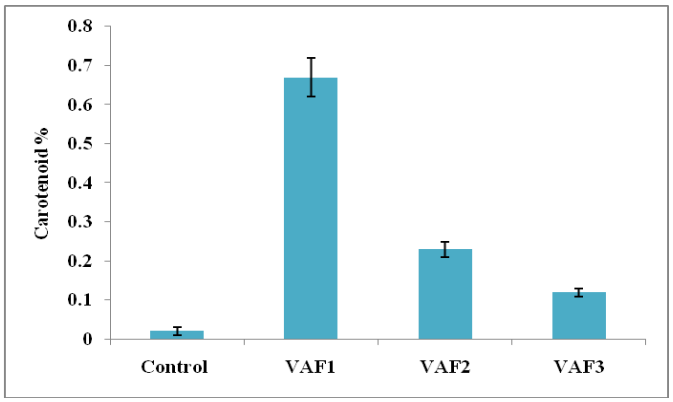

Fig 4: Carotenoid content in fish fed with four experimental diets

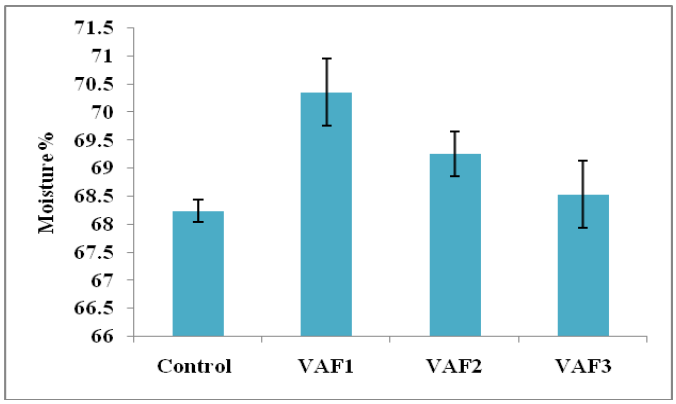

Fig 5: Moisture content in fish fed with four experimental diets

3.3.5. Moisture

There was increased moisture content in all the fish fed with value added feed as compared to control diets (Fig 5). The moisture content in fish fed with VAF1 was 70.36 % followed by VAF2 with 69.26 %, VAF3 with 68.54 %, and control diet with 68.24 %. Increased moisture was obtained in Nile Tilapia when fed with red algae [39]. Different combination of algae (diatom and green algae) and soybean resulted in increased moisture content of Nile tilapia [40].

3.3.6. Ash

There was an increase in ash content in all the fish fed with value added feed as compared to control diet (Fig 6). The ash content in fish fed with VAF1 was 6.2 % followed by VAF2 with 5.55 %, VAF3 with 4.4 %, and control diet with 3.0 %. Fish showed positive response to algal diet as ash content increase compared to control diet. Microalgae are the source of minerals and vitamins [41] and using them as a fish feed lead to increase in ash content in the fish [42].

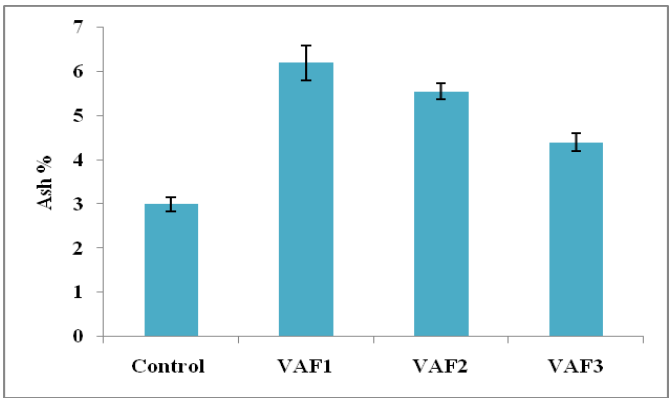

Fig 6: Ash content in fish fed with four experimental diets

4. CONCLUSION

Algae are a rich source of protein, carbohydrate, lipid, minerals and pigment which make them beneficial for pisciculture industry. Fish diet containing mixture of algae serves as a balanced diet and improves the fish growth rate and nutritional value in addition to enhancing skin coloration. Therefore, we suggest that fish farmers can use locally available algae as supplemented fish feed in pisciculture to achieve high nutritive value of fish with faster growth rate.

5. ACKNOWLEDGEMENT

The authors would like to thank to the Fishery department, Government of Meghalaya for providing fish fingerling and North-Eastern Hill University for providing all the laboratory facilities. The first author is

thankful to UGC, New Delhi and Minister of Tribal Affair, Government of India for financial support.

6. REFERENCES

1. Li P, Mai K, Trushenski J, Wu G. *Amino acids*, 2009; **37:**43-53.
2. Sen Roy S, Chaudhuri A, Mukherjee S, Chaudhuri SH, Pal R. *J. Algal Biomass Utln*, 2011; **2:**10-20.
3. Roychoudhury P, Mukherjee M. *Indian J Geo-Mar Sci*, 2013; **42:**647-652.
4. Mishra K, Patra SK, Samantaray K. *Journal of fisheries sciences.com*, 2017; **11:**05-16.
5. Hemaiswarya S, Raja R, Kumar RR. *World J Microbiol Biotechnol*, 2011; **27:**1737-1746.
6. Um BH, Kim YS. *Journal of Industrial and Engineering Chemistry*, 2009; **15:**1-7.
7. Yoo C, Jun SY, Lee JY, Ahn CY, Oh HM. *Bioresource Technology*, 2010; **101:**571-574.
8. Lum KK, Kim J, Lei XG. *J Anim Sci Biotechnol*, 2013; **4:**53.
9. Abdelkhalek EI, Mohamed B, Mohammed AM, Lotfi A. *Mediterr Mar Sci*, 2016; **17:**323-332.
10. Stein JR. Handbook of Phycological methods. Cambridge University Press; 1980.
11. Stanier RY, Kunisawa R, Mandel M, Cohen-bazire G. *Bacteriol Rev*, 1971; **35:**171-205.
12. Guillard RRL. Culture of phytoplankton for feeding marine invertebrates in "Culture of Marine Invertebrate Animals." (Ed.) Smith, W.L .and Chanley, M.H. Plenum Press, New York, USA; 1975.
13. Lowery OH, Roserbrough NJ, Farr AL, Randall RJ. *J. Biol. Chem*, 1951; **193:**265-275.
14. Roe JH. *J. Biol. Chem*, 1955; **212:**335-343.
15. Dittmer JC, Well MA. *Methods Enzymol*, 1969; **14:**482-530.
16. Jensen A. In Handbook of Phycological Methods. Physiological and Biochemical Methods. (Ed.) Hellebust, J.H. Craigie, J.S., Cambridge University Press, Cambridge, United Kingdom; 1978.
17. Fitzsimmons K. *Proceedings of the Fourth International Symposium on Tilapia in Aquaculture*, 1997; **106:**9-12.
18. Sidduraju P, Becker K. *Aquaculture*, 2003; **34:**487-500.
19. Aliyu-Paiko M, Hashim R, Shu-Chien AC. *Aquac Nutr*, 2010; **16:**466-474.
20. Azaza MS, Mensi F, Ksouri J, Dhraief MN, Brini B, Abdelmouleh A, Kraiem MM. *J. Appl. Ichthyol*, 2008; **24:**202-207.
21. AOAC. Official Methods of Analysis of AOAC international Horwitz W. 17th edition. Arlington, Virginia. USA; 1990.
22. Renaud SM, Parry DL, Thinh LV. *Journal of Applied phycology,* 1994; **6:**337-345.
23. Venkataraman LV, Becker EW. Biotechnology and utilization of algae. The Indian experience. Mysore. Central Food Technological Research Institute, 1985; 25.
24. Chakraborty S, Santra SC. *Indian J. Mar. Sci,* 2008; **37:**329-332.
25. Khatoon N, Sengupta P, Homechaudhuri S, Pal R. *Pro. zool. Soc,* 2010; **63:**109-114.
26. Jha NG, Dar AB, Jha T, Sarma D, Quresh TA. *Indian J. Anim. Nutr*, 2013; **30:**404-409.
27. Shearer KD, Maagec A, Opstvedtd J, Mundheimd H. *Aquaculture*, 1992; **106:**345-355.
28. Guroy DB, Guroy DL, Merrifield S, Ergun AA, Tekinay, Yigit M. *J. Anim Physiol Anim Nutr*, 2011; **95(3):**320-327.
29. Singh PK, Gaur SR, Chari MS. *Journal of Fish Aquaculture Science*, 2006; **1:**10-16.
30. Badaway TM, Ibrahim EM, Zeinhom MM. *International Symposium on Tilapia in Aquaculture*, 2008.
31. Lavens P, Sorgeloos P. *Aquaculture*, 2000; **181:**397-403.
32. Raymundo A, Gouveida L, Batista AP, Empis J, Sousa I. *Food Res Int*, 2005; **38:**961-965.
33. Shylla O, Ramanujam P. *J. Algal Biomass Utln*, 2017; **8:**13-22.
34. Mustafa MG, Umino T, Nakagawa H. *J APPL ICHTHYOL*, 1994; **10:**141-145.
35. Sen Roy S, Barman N, Pal R. *Journal of the Botanical Society of Bengal*, 2009; **63:**47-51.
36. Dufosse L, Galaup P, Yaron A, Arad SM. Blance P, Murthy KNC, Ravishankar GA. *Trends in Food Science and Technology*, 2005; **16:**389-406.
37. Mukherjee S, Parial D, Khatoon N, Chaudhuri A, Sen Roy S, Homechaudhuri S, Pal R. *J. Algal Biomass Utln*, 2011; **2:**1-9.
38. Khatoon N, Chattopadhyay P, Mukhopadhyay A, Mukhopadhyay M, Pal R. *Fishing Chimes*, 2009; **28:**44-47.
39. Younis ESM, AI-Quffail AS, AI-Asgah NA, Abdel-Warth AWA, AI-Hafedh YS. *Saudi J. Biol. Sci*, 2018; **25:**198-203.
40. Garcia-Ortega A, Martinez-Steele L, Gonsalves D, Wall MM, Sarnoski PJ. *Journal of Aquaculture Engineering and Fisheries Research,* 2015; **1:**144-154.
41. Brown MR, Farmer CA. *J. Appl. Phycol*, 1994; **6:**61-65.
42. Kiron V, Phromkunthong W, Huntley M, Archibald I, Scheemaker DG. *Aquac.Nutr*, 2012; **18:**521-531.