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Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 03

Res Jr of Agril Sci (2021) 12: 990–992

CARAS

Res. Jr. of Agril. Sci. (May-Jun 2021) 12(3): 990–992
www.rjas.org

Full Length Research Article

Cultivation of *Haematococcus pluvialis* and *Nostochopsis* for the Enhancement of Proximate Analysis of *Catla catla* (Hamilton, 1822)

Ashaq Hussain Rather*[1], Surendra Singh[2] and Sameer Choudhary[3]

Received: 12 Mar 2021 | Revised accepted: 27 May 2021 | Published online: 08 Jun 2021

ABSTRACT

In the present investigation, *H. pluvialis* and *Nostochopsis* were grown in the bold basal medium in controlled air-conditioned culture room. *Catla catla* fish weighing 40 g were randomly distributed in 2 rectangular of 20 L capacity at a density of 6 fish per tank. Tank 1 fish were given normal feed, tank 2 fish were given *H. pluvialis* and *Nostochopsis* biomass (100 mg/kg b.w.). After 24 h gut evacuation period, all fish were anaesthetized and weighed. Out of six, three samples were randomly collected and stored at -20°C, until proximate analysis and lipid extraction. In normal feed treated *Catla catla* group, lipid content was found as 82.2±1.3, in *H. pluvialis* and *Nostochopsis* biomass-based feed group, lipid content was observed as 177.6±2.1 However, protein and ash content were reported as 133.6±1.2 and 14.4±2.1 in *H. pluvialis* and *Nostochopsis* biomass-based feed group. The present study reveals that *H. pluvialis* and *Nostochopsis* biomass can be used for the different aquatic feed formulations.

***Key words*:** *Haematococcus pluvialis*, Astaxanthin, *Nostochopsis* feed, Formulations

Astaxanthin is a xanthophyll carotenoid [1]. Antioxidant activity of astaxanthin was found more than b-carotene and vitamin E [2]. *Haematococcus pluvialis*, freshwater green microalga is considered a potent producer of astaxanthin (3, 3′-dihydroxy-b, bcarotene-4, 4′-dione) [3-6]. Astaxanthin is used in shrimp farming, especially for kuruma prawn and tiger prawn, which has in high demand worldwide [7]. Fish, like other animals, cannot synthesize their own coloring pigments *de novo*, and must obtain these pigments from their diet. Pigmentation of cultured salmonids has been achieved with the inclusion of various synthetic carotenoids (β-carotene, canthaxanthin, zeaxanthin, and astaxanthin) and/or natural sources (yeast, bacteria, algae, higher plants, and crustacean meal) [8-9] in their diets. Global population increase is associated with increased demand of food. To overcome this challenge, the World Health Organization has suggested a doubling of food production by 2050. The range of non-conventional biotechnological measures involving improvement in CO_2 fixation efficiency of crop plants can be used to enhance the food productivity per hectare of agricultural land [10-11]. Several factors such as nutrient mining, water limitation, accumulation of noxious xenobiotic compounds in the soil, soil corrosion and climate change have deteriorated the quality and fertility of agricultural land [12]. In our previous study [13-14] *Haematococcus pluvialis* was cultivated under laboratory conditions and astaxanthin was extracted from the green alga *Haematococcus pluvialis*. In present investigation, efforts were made to study the application part of crude astaxanthin.

* Ashaq Hussain Rather
✉ drashaqhrather21919@gmail.com

[1-3] Algal Biotechnology Laboratory, Department of Post Graduate Studies and Research in Biological Sciences, Rani Durgavati University, Jabalpur - 482 001, Madhya Pradesh, India

MATERIALS AND METHODS

Cultivation of Haematococcus pluvialis and Nostochopsis

Haematococcus pluvialis and *Nostochopsis* were grown in the bold basal medium (BBM) [15]. For the preparation of the inoculum, the cells from the stock culture were centrifuged at 5000 rpm for 5 minutes the supernatant was discarded and the pellet was washed with the sterilized double distilled water thrice. The pellet was homogenized in 1 ml BBM and transferred aseptically in a 250 ml conical flask containing 100 ml of fresh BBM (KH_2PO_4, 17.5; $CaCl_2 \cdot 2H_2O$, 2.5; $MgSO_4 \cdot 7H_20$, 7.5; $NaNO_3$, 25; K_2HPO_4, 7.5; NaCl, 2.5; Na_2ETDA, 10; $FeSO_4 \cdot 7H_2O$, 4.98; H_3BO_3, 11.5 g/L, pH 6.8) and incubated under continuous illumination of 35 µmol m^{-2} s^{-1} at 25±2°C for 4 days. A 4-day old culture was used as an inoculum for the experiment. The experiment was performed in 250 ml conical flasks. 4-day old culture approximately 1×10^6 cells mL^{-1} was inoculated into 100 ml sterilized fresh medium in 250 ml flasks and incubated in controlled air-

conditioned culture room at 25±2°C. All the cultures were shaken thrice a day with a rotary flask shaker.

Harvest and drying of Haematococcus pluvialis and Nostochopsis

The *H. pluvialis* and *Nostochopsis* biomass were centrifuged at 5000 rpm for 5 minutes, the supernatant was discarded and the pellet was dried in the oven at 70°C [16] and packed for the further analysis.

Chemicals

All the media chemicals used for the experiments were analytical grade, obtained from companies- Sisco Research Laboratories Pvt. Ltd., Mumbai; HiMedia Laboratories Pvt. Ltd., Mumbai; Loba Chemie Pvt. Ltd., Mumbai. Qualigens Fine Chemicals, Mumbai and Sigma-Aldrich Chemicals, USA. The feed was obtained from the golden feed, New Delhi.

Experimental design for the fish feed treatments and proximate analysis

All experiments were approved by the institutional animal ethics committee (IAEC). *Catla catla* fish weighing 40 g were used for the study obtained from a local hatchery Jabalpur. The fish were acclimated to the laboratory conditions for 2 weeks by feeding commercially available normal fish feed (Table 1) After this conditioning period, fish (average body weight, 40g) were randomly distributed into 2 rectangular of 20 L capacity at a density of 6 fish per tank. Tank 1 fish were given a normal diet, tank 2 were given *H. pluvialis* biomass and Nostochopsis (100 mg/kg b.w) (Table 2). Each experimental diet was fed to 3 replicate groups of fish twice per day for 4 weeks. Freshwater was supplied at a flow rate of 1 L/min in the re-circulating system and aeration was continuously provided in each tank. The photoperiod was left at the natural condition, and the average water temperature during the trial was 30±2°C. After a 24 h gut evacuation period all fish were anaesthetized and weighed. Out of six, three samples were randomly collected and stored at -20°C, until proximate analysis and lipid extraction [17].

Table 1 Composition of normal feed for the treatments of *Catla catla*

S. No.	Ingredients
1	Soybean
2	Rice Bran
3	Wheat bran
4	Wheat flour
5	Vitamin- Mineral mix*

*Vitamin D Complex, Vitamin E, Vitamin K, Vitamin B Complex, Mn, Fe, Mg, Zn, Cu, Co, Ca

Table 2 Formulation of *H. pluvialis* and *Nostochopsis* based feed for the treatments of *Catla catla*

S. No.	Treatments
1	Normal fish feed
2	*H. pluvialis* and *Nostochopsis* biomass

RESULTS AND DISCUSSION

Haematococcus pluvialis and *Nostochopsis* were grown in the bold basal medium for 30 days in incubated in controlled air-conditioned culture room (Fig 1).

Fig 1 Cultivation of *H. pluvialis and Nostochopsis* in controlled air-conditioned culture room

Drying of H. pluvialis and Nostochopsis

After the harvest, *H. pluvialis* and *Nostochopsis* biomass was dried in hot air oven for 8 h and packed in polybags.

Effect of Haematococcus pluvialis and Nostochopsis biomass on the proximate analysis of Catla catla fish

The parameters determined for proximate analysis include proteins, lipid and ash content. In normal feed treated *Catla catla* fish group, lipid content was found as 82.2±1.3. In *Haematococcus pluvialis* and *Nostochopsis* biomass-based feed group, lipid content was observed as 177.6±2.1. However, protein and ash content were reported as 133.6±1.2, 98.6±1.4 and 14.4±2.1, 7.2±2.2 in *Haematococcus pluvialis* biomass-based group and normal feed group respectively (Table 3).

Table 3 Effect of different feed treatments on the proximate analysis of the whole body of *Catla catla* fish (mg/kg)

S. No.	Treatments	Lipid	Protein	Ash
2	*H. pluvialis* and Nostochopsis feed	177.6±2.1	133.6±1.2	14.4±2.1
3	Normal feed	82.2±1.3	98.6±1.4	7.2±2.2

In this study, we evaluated that the inclusion of *H. pluvialis* and *Nostochopsis* biomass as a feed for the *Catla catla* at levels of 100mg/kg caused a significant effect on proximate analysis. According to previous studies testing the inclusion of 5% of *Ulva* spp. meal in feed for carnivorous fish like European sea bass (*Dicentrarchus labrax*) [18] and rainbow trout [19] reported no effect on growth however some algal feed on *Nile tilapia* [20-21), common carp (*Cyprinus carpio*) [22) gilthead sea bream (*Sparus aurata*) [23] reported significant improvement in growth performances, feed efficiency, nutrient utilization and body composition. In our present study consumption of *H. pluvialis* and *Nostochopsis*

increased the lipid as well as protein content of the *Catla catla*. For the point of view of consumers *Catla catla* showed an umpteen attractive pigmentation (data not mentioned). A critical shortcoming of the crop plant proteins commonly used in fish feeds is that they are deficient in certain amino acids such as lysine, methionine, threonine, and tryptophan [24], whereas analyses of the amino acid content of numerous algae have found that although there is significant variation, they generally contain all the essential amino acids. For example, surveys of 19 tropical seaweeds [25] Analyses of microalgae have found similar high contents of essential amino acids, as exemplified by a comprehensive study of 40 species of microalgae from seven algal classes that found that, all species had a similar amino acid composition, and were rich in the essential amino acids [26].

CONCLUSION

The present study reveals that *H. pluvialis* and *Nostochopsis* biomass can be used for the different aquatic feed formulations.

Conflict of interest

We declare that we have no conflict of interest.

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