International Journal of Multidisciplinary Research and Growth Evaluation.

Hematococcus pluvialis: Richest source of natural carotenoid to be used as a fish feed additive

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Article Info

ISSN (online): 2582-7138
Volume: 04
Issue: 02
March-April 2023
Received: 12-02-2023;
Accepted: 06-03-2023
Page No: 156-159

Abstract
A month long observation was conducted to study the stages of *Hematococcus pluvilais* in pond water. The study was conducted in the month of June. After collecting naturally occurring *H. Pluvialis* from fish farm at Faculty of Veterinary sciences, Shuhama. The study involved observation of different stages of *H.pluvialis* under controlled conditions. The temperature during days was noted to be around 18-20 degree Celsius during morning hours and 24-27degree in afternoon. The algae were easily harvested during morning hours when the inlet of pond was closed. It was seen that under anoxic conditions, the algal mass turned red in color while as when the water was allowed to flow through inlet the algae changed color to green. The harvested algae were bought to lab conditions to observe the cells under microscope. Fine, motile, red colored and rod shaped algae were seen in abundant. The red color owed to astaxanthin that it produces under stressed conditions. *H.pluvialis* could be used as fish feed additive particularly in ornamental fish feed to improve its coloration.

Keywords: Hematococcus pluvialis, fish, feed, astaxanthin, colour

Introduction

The need and appetite for aquaculture and aquaculture products is increasing due to its contribution towards health benefits and livelihood development. With growing aquaculture, new trends emerge in market to satisfy need of consumers. In developing a sustainable aquaculture nutrition plays an important role. Fish nutrition is also advancing and has described the potential use of various ingredients to develop a balanced diet. The trend has shifted more towards nutracueticals**.** Microalgae are being studied as a potential feed ingredient, nutraceutical, and medicinal during this time. Aquaculture uses of H. pluvialis as a source of pigment are justified by the fact that it is one of the richest sources of natural astaxanthin. A highly prized red carotenoid with antioxidant characteristics, astaxanthin is widely used in the food, feed, and cosmetics industries. Astaxanthin (3,3′-dihydroxy-ß-carotene-4,4′-dione) is a bright red secondary carotenoid from the same family as lycopene, lutein, and β-carotene, synthesized *de novo* by some microalgae, plants, yeast, bacteria, and present in many of our favored seafood including salmon, trout, red sea bream, shrimp, lobster, and fish eggs (Higuera-Ciapara *et al*., 2006; Ranga Rao *et al*., 2014. Additionally, it has anti-inflammatory and anti-tumor properties. Aquaculture uses astaxanthin widely as a feed additive to enhance colour and function as an antioxidant. Salmon, trout, and shrimp can now use astaxanthin as a feed addition since it increases growth, feed efficiency, disease resistance, and embryonic survival rates. According to studies, the aquaculture sector currently uses astaxanthin to the tune of $300 million annually. Carotenoids must be received through food because fish cannot produce them on their own.
Hematococcus pluvialis is spherical, biflagellate freshwater microalgae known as one of the richest source of astaxanthin. These are widely distributed in nature and have almost 50 known strains. It is also known as *Haematococcus lacustris* or *Sphaerella lacustris*. Haematococcus was first described by J. Von Flotow in 1844 and later in 1899 Tracy Elliot Hazen extensively presented its biology and life cycle (Hazen, 1899; Leonardi *et al*., 2011). The life cycle can be split into two phases, the motile phase and the non-motile phase.

As a result, the cells are also categorised as being either motile or non-motile, as well as being either aplanospores or zoospores. The algae reproduce in an asexual manner. Vegetative reproduction happens via direct cell division during the motile phase and by cell budding during the non-motile phase. Stress, on the other hand, causes the rapidly expanding motile cells to stop moving and change into larger, immobile cells with thick cell walls.

About site

The algae were harvested from concrete ponds at fish farm, Faculty of Veterinary science, Shuhama in district Ganderbal. It is located at latitude 35 degree 30‟N, longitude 75 degree 15É and at an altitude of 1700 to 4000 meters above msl. The pond is cemented having an inlet and outlet with aquatic vegetation. The temperature during morning hours ranged from 18-20 degree Celsius and during afternoon it increased to 25-27 degree Celsius. The inlet is closed during evening and opened in morning hours.

How to harvest?

The alga appears as a red colored mat on surface of water. The easiest method employed to collect the algae was by superficial collection from surface using a jar. Care was taken not to disturb the water body. The algae were found in high density near inlet. The algae were collected during morning hours around 9-10 a.m.

Identification

The harvested algae were bought to division of fish nutrition and biochemistry to observe the cells. The jar was shaken so that algae get dispersed uniformly in every unit of water. A temporary slide was made. The algal water was filled in centrifuge tubes of 15ml and centrifuged at 3000rpm for 10min. The supernatant was discarded and the pellet was carefully placed on slide and observed under microscope at 10x and 40x. Pear shaped, motile and red colored cells were seen.

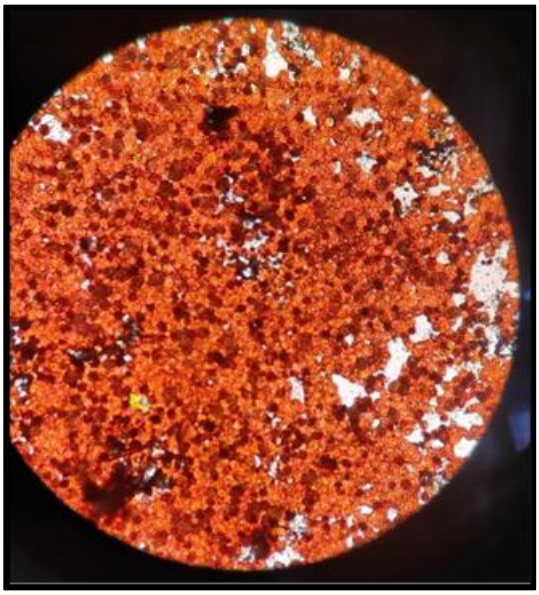

Fig 1: *Haematococcus pluvialis* under microscope

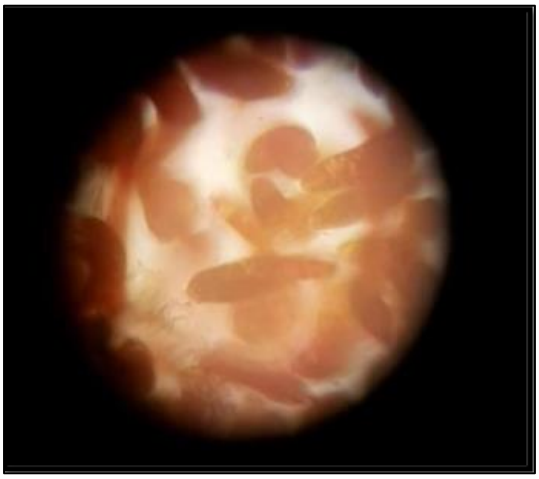

Fig 2: *Haematococcus pluvialis* under 100 X

Astaxanthin in *H. pluvialis*

Haematococcus, having dry weight astaxanthin content of 1.5–3.0%, has prospective uses as a source of pigment in aquaculture, poultry feeds, and the global nutraceutical sector (R. T. Lorenz 2000). Under stressed conditions, the fast-growing motile cells lose their motility and get transformed into larger, non-motile send thick cell walls. The non-motile cells release new cells at slow rate with formation of sporangia under unfavorable conditions. The Sporangia releases 2,4,8 aplanospores and at this point they start accumulating carotenoids in form of droplets in cytoplasm. The carotenoid content of *H. pluvialis* also depends on these factors particularly the nutrients nitrogen and phosphorus. The accumulation of astaxanthin is affected by both environmental factors such as light (Saha *et al*., 2013; Park *et al*., 2014); temperature (Yoo *et al*., 2012); pH (Hata *et al*., 2001); salt concentration (Kobayashi *et al*., 1993); and nutritional stresses (Boussiba *et al*., 1999; Chekanov *et al*., 2014), as well as various plant hormones and their derivatives (Yu *et al*., 2015). Astaxanthin is considered as “super anti-oxidant” which possesses one of the strongest known antioxidant effects (Mahfuzur *et al*, 2016). Its unique structure allows it to span biological membranes and act as an antioxidant by reducing and stabilizing free radicals (Hussein *et al*., 2006; Liu and Osawa, 2007; Ranga Rao *et al*., 2010).

Table 1

Composition content (% of DW)	Green stage	Red stage
Proteins	29–45	17–25
Lipids (% of total)	20–25	32–37
Neutral lipids	59	51.9–53.5
Phospholipids	23.7	20.6–21.1
Glycolipids	11.5	25.7–26.5
Carbohydrates	15–17	36–40
Carotenoids (% of total)	0.5	2–5
Neoxanthin	8.3	n.d
Violaxanthin	12.5	n.d
β -carotene	16.7	1.0
Lutein	56.3	0.5
Zeaxanthin	6.3	n.d
Astaxanthin (including esters)	n.d	81.2
Adonixanthin	n.d	0.4
Adonirubin	n.d	0.6
Canthaxanthin	n.d	5.1
Echinenone	n.d	0.2
Chlorophylls	1.5–2	0

Adapted from Grewe and Griehl (2012). n.d., no data.

As feed additive

Carotenoids are widely used in aquaculture for their antioxidant properties and color enhancement. Various researches have been conducted on potential use of *H.pluvialis* as source of astaxanthin. It has been reported that use of *H.pluvialis* in meal of goldfish enhanced the color and health of fish. It is also seen that *H.pluvialis* increase the weight of fish and PER. It has also been observed that use of *H.pluvialis* helped to replace 12.5% fish meal in shrimp and also lower FCR and promoted specific growth rate. *H. pluvialis* has been already approved as a color additive in salmon feeds and as a dietary-supplement ingredient for human consumption in the USA, Japan, and several European countries (Yuan *et al*., 2011). It has also been studied that *H. pluvialis* especially in dose of 3 g kg^{-1} feed administration effectively enhances the antioxidant system and some biochemical parameters in rainbow trout. It can be used as an extract to enhance flesh color of various economical species. Astaxanthin belongs to xanthophyll type and it has a potent antioxidant to prevent the intracellular oxidative stress (Capelli and Cysewski, 2013), proved in different tests to be the strongest (Ranga Rao *et al*., 2014). Natural astaxanthin is more protective due to its safe and higher antioxidant properties which makes useful nutraceutical product for humans also (Ranga Rao *et al*., 2014).

Conclusion

Thus keeping I view the benefits of astaxanthin, H.pluvialis can be used widely as a source in aquaculture feed. It has high amount of astaxanthin produced under stressed conditions. The algae can be grown under controlled conditions or can be harvested from natural water bodies.

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