Journal of Aquaculture Research & Development

Blessy et al., J Aquac Res Development 2017, 8:4
DOI: 10.4172/2155-9546.1000486

Research Article Open Access

Effect of Algal Oil Incorporated Diet on Growth Biochemical and Immunological Response in Ornamental Fish *Daniorerio rerio*

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Abstract

The study was undertaken to know the effect of algal oil as one of the diet ingredients for the betterment of growth, microbial identification and immunological parameters in the fish *Daniorerio rerio.* The oil was extracted from four micro algae such as *Tetraselmis* sp., *Dunaliella* sp., *Pavlova*sp sp., and *Chaetoceros* sp., The oil obtained from the four different algae were mixed with other feeding ingredients and fed to Zebra fish *Daniorerio rerio.* The water quality parameters like temperature, pH, dissolved oxygen, ammonia (NH_3), growth parameters such as weight (absolute growth rate, specific growth rate, food conversion ratio), food consumption and food conversion efficiency were studied biochemically in *Daniorerio rerio*. Bacterial clearance was evaluated and total viable count of bacteria in different parts of the fish such as gut, gill and body surface were enumerated. Among the oil incorporated diet prepared from the four species of microalgae, *Tetraselmis* sp., had the maximum growth that ranged from 1.34 g to 2.86 g and control had the minimum growth that ranged from 1.14 g to 2.16 g. The maximum food consumption rate recorded in *Pavlova* sp., was 0.27 g. Among the total protein estimated in 1st, 5th, 10th, 15th, 20th, 25th and 30th days, the maximum protein 6.147 mg/ml was noticed in the fishes which are fed with the feed incorporated with oil obtained from *Chaetoceros* sp. Among the total lipid estimation in 1st, 5th, 10th, 15th, 20th, 25th and 30th days of feeding, the maximum lipid 6.147 mg/ml was noticed in feed prepared from *Dunaliella* sp. Among the total carbohydrate estimation in 1st, 5th, 10th, 15th, 20th, 25th and 30th days, the maximum carbohydrate 2.751 mg/ml was noticed in feed *Pavlova* sp. Among the total carotenoid estimation in 1st, 5th, 10th, 15th, 20th, 25th and 30th days of feeding, the maximum carotenoid 0.70 mg/ml was noticed in the animal they are fed with the diet incorporated with oil obtained from *Chaetoceros* sp. Among the total bacterial clearance noticed in 1, 2, 3 and 4 hours, the maximum bacterial clearance was noticed in feed having *Pavlova* sp. oil incorporation after 4 hours. It was found that the Zebra fish fed with the diet incorporated with oil from *Tetraselmis* sp., gave good growth and pigment production.

Keywords: Feed ingredients; Food conversion ratio; Food consumption; Growth parameters

Abbreviations: PUFA: Polyunsaturated Fatty Acids; EFA: Essential Fatty Acids; DHA: Docosahexaenoic acid

Introduction

Globally the ornamental fish culture is a powerful income and employment generating industry. In the aquaculture sector, ornamental fish breeding, culture and trade provide excellent opportunities as a non-food fishery activity for employment and income generation. It is environment friendly, socially acceptable and involves low investment for adopting as a small-scale enterprise with high return. The attractive colouration and quiet disposition of ornamental fish provide a source of joy and peace for people irrespective of age group [1].

Polyunsaturated Fatty Acids (PUFA) are Essential Fatty Acids (EFA), which cannot be synthesized *de novo* by fish, nor in general by all animal, and it must be supplied through the diet. The exact dietary requirement of EFA in fish requires consideration not only for the relative and absolute amounts of individual fatty acids in the fish diets, but also the fish's innate abilities to metabolize these fatty acids, whether anabolically or catabolically [2].

DHA comprises about half of the fatty acids in the brain and is associated with the additional set of health benefits established for omega-3s, notably the protection of the retina, the development of the brain and the prevention of cognitive decline. DHA and EPA (eicosapentaenoic acid) are omega-3 fatty acids found in fatty fish such as salmon, tuna and mackerel. Both fatty acids are recommended for consumption, but recommendations are higher for those who are pregnant, lactating or at risk of CHD. Wild fish obtain these omega-3 fatty acids from the marine algae on which they feed. However, these fish populations are severely declining due to overfishing. Aquaculture (fish farms) had tried to fill the gap and provide an alternative source of fish, but there are environmental concerns surrounding its practice [3].

Studies have been conducted to extract the DHA and EPA directly from the microalgae. Michael Babu et al. in 2013 had enabled the study of the fatty acid profile of algal oil and provide the algal associated bacteria living along with the culture of microalgae enhanced the PUFA level in microalgae.

A few studies have been carried out using algal oil as a feed ingredient in the pellet diet of ornamental (or) any cultivable fishes and hence this study is important.

The major objective of the present study was to test the efficiency of algal oil as a feed ingredient in the pellet feed of Zebra fish (*Daniorerio rerio)*.

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Received May 17, 2017; **Accepted** June 22, 2017; **Published** June 24, 2017

Citation: Blessy G, Ajan C, Citarasu T, Michael Babu M (2017) Effect of Algal Oil Incorporated Diet on Growth Biochemical and Immunological Response in Ornamental Fish *Daniorerio rerio*. J Aquac Res Development 8: 486. doi: 10.4172/2155-9546.1000486

Materials and Methods

Extraction of algal oil from microalgae

The algal oil was extracted by following the method described by Browne et al. [4].

Extraction of algal oil

Hexane was heated within the miscilla tank, creating vapor rising to the condenser. The hexane then condenses and was released into the extraction chamber with the algae. The hexane begins to break down the cellular wall, releasing lipids into the extraction chamber. The hexane lipid mixture then reaches a critical height level within the extraction chamber. This initializes the siphoning process. Once siphoned back into the miscilla tank, the process starts over, turning the hexane into vapor under specified temperature and pressure while retaining the algae oil within the miscilla tank for roughly 2.5 hrs, until the cellular wall has been completely broken down. The hexane/lipid mixture was then heated once more, converting the liquid hexane into vapor. The hexane vapor was run through a condenser and released into the hexane chamber. All the hexane was released from the chamber leaving only algae oil.

Collection of experimental fish

The experimental fish *Daniorerio rerio* was collected from J.J. Fish farm Puthalam in Kanyakumari district, Tamil Nadu. The fish was purchased in polythene cover and placed by water until both temperatures comes equal. The pH level of water was 7.21. The collected fishes were taken to the laboratory and stocked in recirculation water tanks (5 litre capacity) and acclimatized to the ambient laboratory condition, prior to the experiment.

All the fish had size ranges from 3.5 cm to 5 cm. Water exchange was done in every alternative day. The oxygen level maintained was 5.3 mg/l to 6.2 mg/l.

Taxonomy of fish

Kingdom - Animalia

Phylum - Chordata

Class - Actinoptrygii

Order - Cypriniformes

Family - Cyprinidae

Genus - Danio

Species - rerio

Feeding ingredients

The following feed ingredients were used for the preparation of control and experimental diets [5] (Supplementary Table 1).

Fish meal: Fish meal was obtained from freshly dried and powdered anchovies. Fish meal carries large quantities of energy per unit weight and is an excellent source of protein, lipids (oils), minerals and vitamins. Fishmeal contains certain compounds that make the feed more acceptable and agreeable to the taste. This property allows for the feed to be ingested rapidly, and will reduce nutrient leaching.

Wheat flour: It contains more protein and lysine but a similar energy value to that of corn. Wheat has a tendency to flour and form small, fine particles. While wheat improves pellet quality, the non-starch polysaccharide decreases. For the present study Wheat flour obtained from the commercial market was used as an ingredient in fish feed preparation.

Rice bran: Rice bran is a good source of energy and is also a good source of starch, phosphorous, potassium, manganese and zinc, niacin, pantothenic acid and biotin. Ground whole rice flour is widely used in fish feeds. Gelatinization improved water stability. For the present study, rice bran obtained from the commercial market was used as an ingredient in fish feed preparation.

Topioca powder: Tapioca powder purchased from the commercial market was used as the ingredient as well as binder.

Chicken intestine: Chicken intestines were collected in raw from local market, washed with tap water and converted into meal after sun drying and grinding.

Vitamins and minerals: Vitamins are chemically diverse group of organic substances. It was used for the maintenance of normal metabolic and physiological functions, resulting in increase of growth and high survival rate of organisms.

Minerals are the important constituents of the structural compounds of tissues and skeleton in the regulations of osmotic pressure, nerve impulse transmission and in muscle contraction.

Collection of marine microalgae

The marine microalgae such as *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp. and *Pavlova* sp were obtained from the Planktology division of Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam, Tamil Nadu, India. The collected algal samples were then brought to the laboratory for stock culture and mass culture studies.

Stock culture maintenance of microalgae

The collected algal cells were multiplied in 5 ml test tube and then transferred to the test tubes and conical flasks. The culture media used in the stock culture was Walne's medium [6]. The stock culture was provided with 2000 lux fluorescent light and no aeration.

Tank preparation for mass culture of marine microalgae

The FRP tanks of two hundred and fifty liters capacity were used for mass culturing of algae. The tanks were rinsed with soap water and washed thoroughly with the tap water. The sea water enriched with Walne's medium was filled in the tank. Then, 10% to 20% of the inoculum of growing phase was added in to the respective mass culture tanks. Finally, the culture tanks were placed at the direct sunlight with continuous aeration. The growth rates of the algae were measured in every 6 hour interval by taking sample from the mass culture tank and counted the cells by using improved Neubauer chamber (haemocytometer).

Algal oil

It was obtained from the microalgae named *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp. and *Pavlova* sp. This alga was isolated, mass cultured, harvested and made in to powder. The powder was used as the raw material for the oil extraction following the method described by Michael Babu et al. The oil possesses high HUFA and PUFA and also contains carotenoid and some minerals.

Control diet

The control diet was also prepared with all the above ingredients except algal oil.

Preparation of feeds

Fish feeds should have adequate energy for body maintenance and growth. It was contributed by three major nutrients, namely protein, fat and carbohydrate. The feeds should have vitamin and minerals to meet their deficiencies. Such attractants and flavors are needed to fish for quick consumption and effective utilization of feed.

The feed ingredients were weighed and mixed well in a container by adding sufficient quantity of distilled water and then the ingredients were made into dough. The dough was then placed in a container and boiled in a pressure cooker for 20 minutes. After boiling, the dough was taken out of the container and then vitamins and minerals mixture, and gelatin was added to the dough and mixed well.

The dough was then allowed to pass through a pelletizer having perforation diameter of 1.5 mm in the diet. Then the control as well as the experimental diets was dried in duration of 15 hours. Then the dried pellets were collected and stored in air tight plastic container.

Altogether, five diets were prepared, a control diet (C) and four experimental diets having 5 ml oil obtained from *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp. and *Pavlova* sp. In control diet, there was no algal oil.

Water quality parameters

The water quality parameters were monitored and maintained at an optimum level during the entire experimental duration. Water samples were collected from respective tanks and analyzed. Temperature, pH, dissolved oxygen and ammonia [7] in culture tanks were measured and recorded.

Growth parameters

To find out the growth, the weight of the animal in each tank was measured once in 5 days. Weight was measured by using balance with least disturbance to the fish on wet weight basis.

Production (growth)

$$\text{Production}(g) = \text{final wet weight} - \text{initial wet weight}$$

Food consumption

$$\text{Food consumption}(\text{g dry weight}) = \text{Food provided} - \text{Unfed remains}$$

Food Conversion Efficiency (FCE)

$$\text{FCE} = \frac{\text{Wet weight of fish produced (g)}}{\text{Dry weight of the feed given (g)}} \times 100$$

Absolute Growth Rate (AGR)

$$\text{AGR (g / body wt / day)} = \frac{\text{Final body weight - Initial body weight}}{\text{Total number of days}}$$

Specific Growth Rate (SGR)

$$\text{SGR (\%)} = \frac{\text{In final wet weight (g) - In initial wet weight (g)}}{\text{Experimental period (days)}} \times 100$$

Food Conversion Ratio (FCR)

$$\text{FCR} = \frac{\text{Total amount of feed given (dry weight, g)}}{\text{Total production of fish (wet weight, g)}}$$

Biochemical analysis

The protein [8], carbohydrate [9], lipid [10] and carotenoid composition of fish were estimated.

Total viable count

The fish samples such as gut, gill, body surface and water sample were dissected out under aseptic conditions and samples was ground well using ethanol. To enumerate the bacterial load present in the fish sample, 1 ml of sample was taken and serially diluted (10^{-1} to 10^{-6}). From each dilution 0.1 ml of sample was taken and pour plated on nutrient agar. After this the plates were incubated at 37°C for 24 hours in an incubator and the total number of individual viable colonies was counted using cubic colony counter.

$$\text{CFU / ml} = \frac{\text{Average of CFU counted}}{\text{Volume of inoculums}} \times \text{dilution factor}$$

Bacterial clearance

To determine how rapidly bacteria are cleared from blood, fish were injected intraperitoneally near the caudal region with 0.1 units *Vibrio harveyi* suspension containing 1000 CFU/ml The *Vibrio harveyi* was obtained from Centre for Marine Science and Technology Microbiology Laboratory. After 1, 2, 3 and 4 hours, a 100 µl of blood was drawn using insulin syringe with sterile saline. Triplicates TCBS agar plates were prepared. The samples were immediately mixed with TCBS agar poured in to petridish and incubated at 37°C for 24 hours. Number of bacterial colonies per plates were counted and divided by the volume of blood extracted, to determine the number of colony forming units in milliliter of blood. Control was also maintained, but injected with only sterile saline without *Vibrio harveyi.*

Results

Water quality parameters

In the present study, water quality parameters were maintained at an optimum level in control (C) and experimental tanks during the culture period. The water quality parameters recorded in the culture tanks are given in Table 1.

In control tank (C), the range of temperature and pH recorded during the experimentation were ranged from 32°C to 34°C and 7.08 to 7.32 respectively. The dissolved oxygen content was ranged from 4.564 mg/l to 6.893 mg/l during the experimentation. The ammonia content of the water sample also varied between 0.623 µg/l and 0.837 µg/l during the culture period.

In experimental tank (*Tetraselmis* sp. oil incorporated diet fed group), the temperature and pH value recorded during the experiment ranged from 32°C to 34°C and 7.10 to 7.36 respectively. The dissolved oxygen content ranged from 4.345 mg/l to 6.861 mg/l during the experimentation. The ammonia content of the water sample also varied between 0.521 µg/l and 0.674 µg/l during the culture period.

In *Dunaliella* sp. oil group, the temperature and pH recorded during the experimentation ranged from 32°C to 34°C and 7.25 to 7.86 respectively. The dissolved oxygen ranged from 3.429 mg/l to 5.589 mg/l during the experimentation. The ammonia of the water also varied between 0.428 µg/l and 0.738 µg/l during culture period.

In *Chaetoceros* sp. oil group, the temperature and pH recorded during the experimentation ranged from 32°C to 34°C and 7.21 to 7.39 respectively. The dissolved oxygen ranged from 3.176 mg/l to 6.783 mg/l during the experimentation. The ammonia content of the water also varied between 0.378 µg/l and 0.842 µg/l during culture period.

In *Pavlova* sp. oil group, the temperature and pH recorded during the experimentation ranged from 32°C to 34°C and 7.14 to 7.33

Feed Type	Parameters	Water quality parameters of fish in five days intervals						
		1th day	5th day	10th day	15th day	20th day	25th day	30th day
Control feed	Temperature (°C)	34 ± 0.00	33 ± 0.00	32 ± 0.00	34 ± 0.00	32 ± 0.00	33 ± 0.00	32 ± 0.00
	pH	7.13 ± 0.13	7.32 ± 0.67	7.21 ± 0.05	7.24 ± 0.30	7.08 ± 0.08	7.21 ± 0.23	7.25 ± 0.67
	Dissolved O_2 (mg/ml)	6.761 ± 0.61	4.564 ± 0.015	6.893 ± 0.18	5.823 ± 0.31	5.213 ± 0.25	5.145 ± 0.21	4.976 ± 0.082
	Ammonia (mg/ml)	0.623 ± 0.03	0.642 ± 0.06	0.665 ± 0.08	0.687±0.14	0.723 ± 0.56	0.765 ± 0.54	0.837 ± 0.18
Tetraselmis sp.	Temperature (°C)	34 ± 0.00	33 ± 0.00	32 ± 0.00	34 ± 0.00	32 ± 0.00	33 ± 0.00	32 ± 0.00
	pH	7.23 ± 0.45	7.23 ± 0.07	7.34 ± 0.07	7.28 ± 0.46	7.36 ± 0.18	7.10 ± 0.16	7.32 ± 0.04
	Dissolved O_2 (mg/ml)	5.216 ± 0.13	6.861 ± 0.018	6.253 ± 0.14	5.341 ± 0.013	4.345 ± 0.16	4.717 ± 0.023	4.453 ± 0.051
	Ammonia (mg/ml)	0.576 ± 0.07	0.585 ± 0.09	0.545 ± 0.07	0.521 ± 0.18	0.538 ± 0.42	0.574 ± 0.19	0.674 ± 0.53
Dunaliella sp.	Temperature (°C)	34 ± 0.00	33 ± 0.00	32 ± 0.00	34 ± 0.00	32 ± 0.00	33 ± 0.00	32 ± 0.00
	pH	7.35 ± 0.08	7.45 ± 0.14	7.28 ± 0.09	7.86 ± 0.08	7.36 ± 0.08	7.25 ± 0.06	7.46 ± 0.08
	Dissolved O_2 (mg/ml)	5.561 ± 0.87	4.784 ± 0.09	4.853 ± 0.018	5.589 ± 0.021	4.537 ± 0.028	3.429 ± 0.023	3.827 ± 0.53
	Ammonia (mg/ml)	0.528 ± 0.05	0.428 ± 0.08	0.628 ± 0.09	0.687 ± 0.09	0.684 ± 0.08	0.738 ± 0.07	0.529 ± 0.09
Chaetoceros sp.	Temperature (°C)	34 ± 0.00	33 ± 0.00	32 ± 0.00	34 ± 0.00	32 ± 0.00	33 ± 0.00	32 ± 0.00
	pH	7.35 ± 0.07	7.27 ± 0.18	7.29 ± 0.08	7.21 ± 0.10	7.31 ± 0.09	7.35 ± 0.19	7.39 ± 0.08
	Dissolved O_2 (mg/ml)	4.780 ± 0.03	3.176 ± 0.016	6.783 ± 0.017	5.572 ± 0.08	5.131 ± 0.029	4.272 ± 0.25	3.761 ± 0.057
	Ammonia (mg/ml)	0.378 ± 0.09	0.387 ± 0.08	0.465 ± 0.08	0.598 ± 0.09	0.654 ± 0.07	0.842 ± 0.08	0.678 ± 0.867
Pavlova sp.	Temperature	34 ± 0.00	33 ± 0.00	32 ± 0.00	34 ± 0.00	32 ± 0.00	33 ± 0.00	32 ± 0.00
	pH	7.24 ± 0.09	7.14 ± 0.72	7.17 ± 0.14	7.22 ± 0.16	7.27 ± 0.15	7.28 ± 0.18	7.33 ± 0.09
	Dissolved O_2	4.239 ± 0.06	4.157 ± 0.019	5.782 ± 0.018	5.514 ± 0.12	5.324 ± 0.027	4.256 ± 0.28	3.345 ± 0.058
	Ammonia	0.325 ± 0.12	0.376 ± 0.56	0.451 ± 0.19	0.557 ± 0.08	0.678 ± 0.28	0.748 ± 0.12	0.645 ± 0.89

Table 1: Water quality parameters of fish when feeding different diet in different days of culture.

Days	Fish weight during five days intervals (g)				
	Control	*Tetraselmis* sp.	*Dunaliella*sp.	*Chaetoceros* sp.	*Pavlova* sp.
1	1.14 ± 0.19	1.34 ± 0.18	1.63 ± 0.17	1.54 ± 0.23	1.79 ± 0.32
5	1.25 ± 0.21	1.52 ± 0.14	1.84 ± 0.21	1.68 ± 0.28	1.81 ± 0.28
10	1.31 ± 0.28	1.81 ± 0.19	1.97 ± 0.38	1.94 ± 0.42	2.05 ± 0.15
15	1.58 ± 0.29	1.92 ± 0.17	2.15 ± 0.37	2.02 ± 0.34	2.25 ± 0.27
20	1.83 ± 0.18	2.23 ± 0.21	2.39 ± 0.28	2.16 ± 0.43	2.46 ± 0.42
25	1.91 ± 0.34	2.48 ± 0.20	2.57 ± 0.35	2.27 ± 0.21	2.68 ± 0.38
30	2.16 ± 0.26	2.86 ± 0.28	2.82 ± 0.14	2.38 ± 0.46	2.84 ± 0.51
The feed prepared from different algal oil significantly (P<0.01) enhanced the growth					

Table 2: Effect of different types of algal oil diet on fish weight during different days of culture.

Growth parameters	Fish growth parameter during five days of intervals (g)				
	Control	*Tetraselmis* sp.	*Dunaliella* sp.	*Chaetoceros* sp.	*Pavlova* sp.
Production growth (g)	0.31 ± 0.08	0.38 ± 0.027	0.46 ± 0.09	0.37 ± 0.051	0.52 ± 0.32
Food consumption (g/day)	0.19 ± 0.17	0.21 ± 0.08	0.23 ± 0.09	0.26 ± 0.08	0.27 ± 0.41
FCE (%)	4.12	5.34	12.38	5.26	13.78
AGR (g)	0.008 ± 0.002	0.011 ± 0.002	0.018 ± 0.008	0.014 ± 0.004	0.020 ± 0.007
SGR (%)	0.98 ± 0.34	1.03 ± 0.05	1.86 ± 0.17	1.64 ± 0.28	1.94 ± 0.04
FCR (%)	5:1	5:3	5:2	5:3	5:4
The feed prepared from different algal oil significantly (P<0.01) enhanced the growth					

Table 3: Effect of different types of algal oil diet on growth parameters during different days after feeding.

respectively. The dissolved oxygen content ranged from 3.345 mg/l to 5.782 mg/l during the experimentation. The ammonia content of the water also varied between 0.325 µg/l to 0.748 µg/l during culture period (Table 1).

Growth parameters

In all the experimental diet and control feed groups, minimum fish weight was 2.16 g during the experiment. In *Tetraselmis* sp., the maximum fish weight was 2.86 g during the experimentation (Table 2).

The maximum growth recorded in *Pavlov*a sp., oil fed animal was 0.52 g. The food consumption rate recorded in control was 0.19 g and in *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp. and *Pavlova* sp., oil incorporated diet fed animals were 0.21, 0.23, 0.26 and 0.27 respectively. The FCE value recorded in control was 4.12% and in *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp. and *Pavlova* sp., oil incorporated diet fed animals were 5.34%, 12.38%, 5.26% and 13.78% respectively. The AGR value recorded in control was 0.008 g and in *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp. and *Pavlova* sp. were 0.011 g, 0.018 g, 0.014 g and 0.020 g respectively. The SGR value recorded in control was 0.98% and in *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., and *Pavlova* sp. were 1.03%, 1.86%, 1.64% and 1.94% respectively. The FCR value recorded in control was 5:1 and in *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp. and *Pavlova* sp. were 5:3, 5:2, 5:3 and 5:4 respectively (Table 3). The growth was significant increased by the (P<0.05) incorporation of algal oil in Zebra fish.

Protein estimation of fish

Total protein estimated in the first day of animal fed with diet made

from algal oil of *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control diets were 3.192 mg/ml, 3.285 mg/ml, 3.294 mg/ml, 3.367 mg/ml and 3.513 mg/ml respectively.

Total protein estimated in the animal fed with diet made from algal oil of diet *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 5th day were 3.738 mg/ml, 3.973 mg/ml, 3.728 mg/ml, 3.812 mg/ml and 3.823 mg/ml respectively.

Total protein estimated in the animal fed with diet made from algal oil of diet *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 10th day were 3.948 mg/ml, 4.365 mg/ml, 4.676 mg/ml, 4.286 mg/ml and 3.918 mg/ml respectively.

Total protein estimated in the animal fed with diet made from algal oil of diet *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 15th day were 4.287 mg/ml, 4.843 mg/ml, 5.317 mg/ml, 4.723 mg/ml and 4.128 mg/ml respectively.

Total protein estimated in the animal fed with diet made from algal oil of diet *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 20th day were 4.638 mg/ml, 5.156 mg/ml, 5.567 mg/ml, 5.241 mg/ml and 4.320 mg/ml respectively.

Total protein estimated in the animal fed with diet made from algal oil of diet *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 25th day were 4.719 mg/ml, 5.487 mg/ml, 5.850 mg/ml, 5.579 mg/ml and 4.532 mg/ml respectively.

Total protein estimated in the animal fed with diet made from algal oil of diet *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 30th day were 4.913 mg/ml, 5.852 mg/ml, 6.147 mg/ml, 5.714 mg/ml and 4.756 mg/ml respectively.

Among the total protein estimated in 1st, 5th, 10th, 15th, 20th, 25th and 30th days, the maximum protein 6.147 mg/ml was noticed in the animals with the diet prepared using algal oil obtained from feed *Chaetoceros* sp., (Table 4). The growth due to algal oil feed was highly significant (P<0.05) in different microalgaloil feed of Zebra fish.

Lipid estimation of fish

Total lipid estimated in the first day of the animal fed with diet made from algal oil of *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control diet were 2.247 mg/ml, 2.076 mg/ml, 2.175 mg/ml, 2.268 mg/ml and 2.027 mg/ml respectively.

Total lipid estimated in the animal fed with diet made from algal oil of diet *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 5th day were 2.416 mg/ml, 2.314 mg/ml, 2.258 mg/ml, 2.382 mg/ml and 2.218 mg/ml respectively.

Total lipid estimated in the animal fed with diet made from algal oil of diet *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 10th day were 2.627 mg/ml, 2.516 mg/ml, 2.573 mg/ml, 2.576 mg/ml and 2.472 mg/ml respectively.

Total lipid estimated in the animal fed with diet made from algal oil of diet *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 15th day were 2.768 mg/ml, 2.824 mg/ml, 2.876 mg/ml, 2.741 mg/ml and 2.521 mg/ml respectively.

Total lipid estimated in the animal fed with diet made from algal oil of diet *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 20th day were 2.827 mg/ml, 2.976 mg/ml, 2.902 mg/ml, 2.934 mg/ml and 2.589 mg/ml respectively.

Total lipid estimated in the animal fed with diet made from algal oil of diet *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 25th day were 2.958 mg/ml, 3.016 mg/ml, 2.980 mg/ml, 3.047 mg/ml and 2.628 mg/ml respectively.

Total lipid estimated in the animal fed with diet made from algal oil of diet *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 30th day were 2.982 mg/ml, 3.176 mg/ml, 3.065 mg/ml, 3.123 mg/ml and 2.761 mg/ml respectively.

Among the total lipid estimated in 1st, 5th, 10th, 15th, 20th, 25th and 30th days, the maximum lipid 6.147 mg/ml was noticed in the animals fed with the diet prepared using algal oil obtained from *Dunaliella* sp., (Table 5). The growth of algal oil feed was highly significant (P<0.05) different microalgal oil feed of Zebra fish.

Days	Protein Estimation of five days of intervals (mg/dl)				
	Control	*Tetraselmis* sp.	*Dunaliella* sp.	*Chaetocero* ssp.	*Pavlova* sp.
1	3.513 ± 0.283	3.192 ± 0.189	3.285 ± 0.307	3.294 ± 0.193	3.367 ± 0.461
5	3.823 ± 0.184	3.738 ± 0.286	3.973 ± 0.735	3.728 ± 0.264	3.812 ± 0.587
10	3.918 ± 0.175	3.948 ± 0.321	4.365 ± 0.581	4.676 ± 0.391	4.286 ± 0.619
15	4.128 ± 0.063	4.287 ± 0.257	4.843 ± 0.839	5.317 ± 0.427	4.723 ± 0.783
20	4.320 ± 0.271	4.638 ± 0.981	5.156 ± 0.684	5.567 ± 0.371	5.241 ± 0.516
25	4.532 ± 0.056	4.719 ± 0.381	5.487 ± 0.421	5.850 ± 0.331	5.579 ± 0.718
30	4.756 ± 0.139	4.913 ± 0.273	5.852 ± 0.417	6.147 ± 0.428	5.714 ± 0.672
The feed prepared from different algal oil significantly (P<0.01) enhanced the body protein.					

Table 4: Effect of different types of algal oil feed on total body protein during different days of culture.

Days	Lipid Estimation of five days of intervals (mg/dl)				
	Control	*Tetraselmis* sp.	*Dunaliella* sp.	*Chaetoceros* sp.	*Pavlova* sp.
1	2.027 ± 0.084	2.247 ± 0.068	2.076 ± 0.348	2.175 ± 0.281	2.268 ± 0.238
5	2.218 ± 0.093	2.416 ± 0.238	2.314 ± 0.183	2.258 ± 0.183	2.382 ± 0.176
10	2.472 ± 0.217	2.627 ± 0.163	2.516 ± 0.194	2.573 ± 0.249	2.576 ± 0.347
15	2.521 ± 0.035	2.768 ± 0.023	2.824 ± 0.098	2.876 ± 0.351	2.741 ± 0.150
20	2.589 ± 0.024	2.827 ± 0.034	2.976 ± 0.283	2.902 ± 0.278	2.934 ± 0.261
25	2.628 ± 0.134	2.958 ± 0.021	3.016 ± 0.094	2.980 ± 0.162	3.047 ± 0.374
30	2.761 ± 0.192	2.982 ± 0.026	3.176 ± 0.254	3.065 ± 0.078	3.123 ± 0.456
The feed prepared from different algal oil significantly (P<0.01) enhanced body lipid.					

Table 5: Effect of different types of algal oil feed on total body lipid during different days of culture.

Days	Carbohydrate level in different intervals (mg/dl)				
	Control	*Tetraselmis* sp.	*Dunaliella* sp.	*Chaetoceros* sp.	*Pavlova* sp.
1	1.623 ± 0.085	1.818 ± 0.194	1.712 ± 0.027	1.763 ± 0.049	1.787 ± 0.037
5	1.835 ± 0.091	1.924 ± 0.045	1.839 ± 0.043	1.806 ± 0.036	1.826 ± 0.089
10	1.914 ± 0.039	2.250± 0.021	2.357 ± 0.091	1.939 ± 0.023	1.982 ± 0.054
15	1.720 ± 0.028	2.471 ± 0.094	2.418 ± 0.089	2.384 ± 0.035	2.178 ± 0.085
20	1.038 ± 0.037	2.543 ± 0.098	2.564 ± 0.035	2.585 ± 0.042	2.365 ± 0.057
25	1.145 ± 0.138	2.589 ± 0.029	2.575 ± 0.028	2.607 ± 0.039	2.578 ± 0.078
30	1.229 ± 0.085	2.638 ± 0.021	2.581 ± 0.087	2.728 ± 0.067	2.751 ± 0.084
The feed prepared from different algal oil significantly (P<0.01) enhanced the carbohydrates.					

Table 6: Effect of different types of algal oil diet on total body carbohydrate during different days of culture.

Days	Carotenoid Estimation of five days of intervals (mg/dl)				
	Control	*Tetraselmis* sp.	*Dunaliella* sp.	*Chaetoceros* sp.	*Pavlova sp.*
1	0.54 ± 0.16	0.52 ± 0.16	0.53 ± 0.08	0.62 ± 0.19	0.50 ± 0.21
5	0.55 ± 0.23	0.53 ± 0.05	0.56 ± 0.19	0.65 ± 0.18	0.52 ± 0.16
10	0.53 ± 0.18	0.56 ± 0.19	0.59 ± 0.18	0.67 ± 0.16	0.56 ± 0.23
15	0.51 ± 0.09	0.60 ± 0.16	0.59 ± 0.19	0.68 ± 0.18	0.58 ± 0.02
20	0.53 ± 0.18	0.68 ± 0.18	0.61 ± 0.12	0.69 ± 0.13	0.60 ± 0.24
25	0.52 ± 0.29	0.69 ± 0.17	0.63 ± 0.17	0.71 ± 0.06	0.61 ± 0.15
30	0.53 ± 0.12	0.70 ± 0.34	0.65 ± 0.16	0.70 ± 0.1	0.63 ± 0.18
The feed prepared from different algal oil significantly (P<0.01) enhanced the carotenoid.					

Table 7: Effect of different algal oil diet on total carotenoid in different days of culture.

Carbohydrate estimation of fish

Total carbohydrate estimated in the first day of the animal fed with diet made from algal oil obtained from *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp., and control diet were 1.818 mg/ml, 1.712 mg/ml, 1.763 mg/ml, 1.787 mg/ml and 1.623 mg/ml respectively.

Total carbohydrate estimated in the animal fed with diet made from algal oil obtained from *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp., and control in 5th day were 1.924 mg/ml, 1.839 mg/ml, 1.806 mg/ml, 1.826 mg/ml and 1.835 mg/ml respectively.

Total carbohydrate estimated in the animal fed with diet made from algal oil obtained from *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 10th day were 2.250 mg/ml, 2.357 mg/ml, 1.939 mg/ml, 1.982 mg/ml and 1.914 mg/ml respectively.

Total carbohydrate estimated in the animal fed with diet made from algal oil obtained from *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 15th day were 2.471 mg/ml, 2.418 mg/ml, 2.384 mg/ml, 2.178 mg/ml and 1.720 mg/ml respectively.

Total carbohydrate estimated in the animal fed with diet made from algal oil obtained from *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 20th day were 2.543 mg/ml, 2.564 mg/ml, 2.585 mg/ml, 2.365 mg/ml and 1.038 mg/ml respectively.

Total carbohydrate estimated in the animal fed with diet made from algal oil obtained from *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 25th day were 2.589 mg/ml, 2.575 mg/ml, 2.607 mg/ml, 2.578 mg/ml and 1.145 mg/ml respectively.

Total carbohydrate estimated in the animal fed with diet made from algal oil obtained from *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. and control in 30th day were 2.638 mg/ml, 2.581 mg/ml, 2.728 mg/ml, 2.751 mg/ml and 1.229 mg/ml respectively.

Among the total carbohydrate estimated in 1st, 5th, 10th, 15th, 20th, 25th and 30th days, the maximum carbohydrate 2.751 mg/ml was noticed in the animals with the diet prepared using algal oil obtained from feed *Pavlova* sp., (Table 6). The growth of algal oil feed was highly significant (P<0.05) different micro algal oil feed of Zebra fish.

Carotenoid estimation

The initial carotenoid level in the control fish was 0.54 µg/g. After 5th, 10th, 15th, 20th, 25th and 30th days, the carotenoids level estimated were 0.55 µg/g, 0.53 µg/g, 0.51 µg/g, 0.53 µg/g, 0.52 µg/g and 0.53 µg/g respectively.

The initial carotenoid level in the fish fed with feed prepared from *Tetraselmis* oil was 0.52 µg/g. After 5th, 10th, 15th, 20th, 25th and 30th days, the carotenoids level estimated were 0.53 µg/g, 0.56 µg/g, 0.60 µg/g, 0.68 µg/g, 0.69 µg/g and 0.70 µg/g respectively.

The initial carotenoid level in the fish fed with feed prepared from *Dunaliella* oil was 0.53 µg/g. After 5th, 10th, 15th, 20th, 25th and 30th days, the carotenoids level estimated were 0.56 µg/g, 0.59 µg/g, 0.59 µg/g, 0.61 µg/g, 0.63 µg/g and 0.65 µg/g respectively.

The initial carotenoid level in the fish fed with feed prepared from *Chaetoceros* oil was 0.62 µg/g. After 5th, 10th, 15th, 20th, 25th and 30th days, the carotenoids level estimated were 0.65 µg/g, 0.67 µg/g, 0.68 µg/g, 0.69 µg/g, 0.71 µg/g and 0.70 µg/g respectively.

The initial carotenoid level in the fish fed with feed prepared from *Pavlova* oil was 0.50 µg/g. After 5th, 10th, 15th, 20th, 25th and 30th days, the carotenoids level estimated were 0.52 µg/g, 0.56 µg/g, 0.58 µg/g, 0.60 µg/g, 0.61 µg/g and 0.63 µg/g respectively.

Among the total carotenoid estimated in 1st, 5th, 10th, 15th, 20th, 25th and 30th days, the maximum carotenoid 0.70 mg/dl was noticed in feed prepared from oil obtained from *Chaetoceros* sp. (Table 7).

Bacterial count

The initial bacterial count in the gut of control was TNTC, water sample was TNTC gut sample was 267 × 10^{-5}, gill was TNTC and body surface was TNTC, whereas in *Tetraselmis* oil diet fed animal, the number of bacterial colony in water sample was TNTC, gut sample was TNTC, gill sample was TNTC and body surface was 245 × 10^{-5}. In the *Dunaliella* sp. the bacterial count in water sample was TNTC, gut was TNTC, gill was TNTC and body surface was 218 × 10^{-5}. In the *Chaetoceros* sp., the bacterial count in water sample was TNTC, gut

was TNTC, gill was TNTC and body surface 225 × 10^{-5}. In the *Pavlova* sp., the bacterial count in water sample TNTC, gut was TNTC, gill was TNTC and body surface 226 × 10^{-5}.

After 30 days, the minimum bacterial count was found in fishes fed with diet prepared from *Pavlova* oil (water sample 112 × 10^{-5}, gut 64 × 10^{-5}, and gill 62 × 10^{-5} and body surface 76 × 10^{-5}). The bacterial count in fish fed with oil from *Chaetoceros* sp., was 124, 72, 68 and 81 × 10^{-5} in water sample, gut, gill and body surface respectively. In *Dunaliella* oil diets, 81, 108, 76 and 51 × 10^{-5} water sample, gut, gill and body surface respectively. In *Tetraselmis* oil diet, fed animal group, were found in water sample, gut, gill and body surface 138 CFU/ml, 136 CFU/ml, 107 CFU/ml and 96 × 10^{-5} CFU/ml (Table 8).

Bacterial clearance

The initial bacterial clearance in the control after 1 hour was 15 CFU/ml where as in *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp. and *Pavlova* sp. were 18 CFU/ml, 17 CFU/ml, 17 CFU/ml and 16 CFU/ml respectively.

The bacterial clearance in the control after 2 hours was 12 CFU/ml whereas in *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., and *Pavlova* sp. were 11 CFU/ml, 10 CFU/ml, 12 CFU/ml and 9 CFU/ml respectively.

The bacterial clearance in the control after 3 hours was 7 CFU/ml where as in *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., and *Pavlova* sp. were 5 CFU/ml, 5 CFU/ml, 7 CFU/ml and 4 CFU/ml respectively.

The bacterial clearance in the control after 4 hours was 4 CFU/ml where as in *Tetraselmis* sp., *Dunaliella* sp., *Chaetoceros* sp., *Pavlova* sp. were 0 CFU/ml, 0 CFU/ml, 1 CFU/ml and 0 CFU/ml respectively.

Among the total bacterial clearance noticed in 1, 2, 3 and 4 hours, the minimum bacterial clearance CFU/ml was noticed in feed type *Pavlova* sp., after 4 hours (Table 9). The growth of algal oil feed was highly significant (P<0.05) different microalgal oil feed of bacterial clearance of Zebra fish.

Discussion

This work was carried out to find out the efficiency of the artificial diet prepared with algal oil on protein, lipid, carbohydrate, growth and immunological parameters in Zebra fish and to study the effect feed in changing the water quality parameters such as temperature, pH, dissolved oxygen and ammonia. The results obtained from the present study were compared with control to find out the efficiency of the experimental diet.

Artificial diet plays a major role in grow out culture system of fin and shell fishes. The efficiency of any feed can be determined by its FCR. When a feed is considered to be a best, it should have more self-life, more FCR, easy digestibility and more over it should not spoil the culture environment.

In our experiment, five diets were prepared and tested for pH, oxygen and ammonia in the culture water. Among the five diets tested, the level of ammonia in the culture water was minimum in *Pavlova* sp. oil mixing diet. It shows the fact that the particular diet did not spoil

Feed Type	Parameters	Bacterial count of fish in five days intervals (× 10^{-5})						
		1	5	10	15	20	25	30
Control feed	Water	TNTC	251 ± 4.73	194 ± 5.85	174 ±7.76	153 ± 6.82	135 ± 8.51	114 ± 5.61
	Gut	267 ± 2.48	292 ± 4.67	247 ± 8.28	238 ± 4.29	210 ± 6.13	195 ± 6.67	178 ± 7.30
	Gill	TNTC	139 ± 4.17	168 ± 6.17	135 ± 3.62	113 ± 8.78	97 ± 3.68	82 ± 1.83
	Body surface	TNTC	278 ± 7.18	274 ± 1.49	239 ± 8.65	212 ± 6.67	198 ± 4.56	145 ± 5.78
Tetraselmis sp.	Water	TNTC	TNTC	263 ± 4.76	214 ± 7.84	198 ± 5.28	174 ± 3.15	138 ± 3.49
	Gut	TNTC	245 ± 6.18	237 ± 5.63	216 ± 6.65	192 ± 4.56	145 ± 4.69	136 ± 4.71
	Gill	TNTC	187 ± 4.74	187 ± 2.98	167 ± 4.71	149 ± 3.12	123 ± 7.67	107 ± 3.65
	Body surface	245 ± 4.89	254 ± 8.87	216 ± 8.46	146 ± 4.74	123 ± 9.67	108 ± 7.15	96 ± 4.84
Dunaliella sp.	Water	TNTC	TNTC	179 ± 5.71	148 ± 4.76	124 ± 4.61	101 ±7.57	81 ± 4.85
	Gut	TNTC	251 ± 2.67	191 ± 6.37	172 ± 4.37	154 ± 8.45	124 ± 5.68	108 ± 3.49
	Gill	TNTC	185 ± 1.41	145 ± 3.74	137 ± 4.15	116 ± 4.56	91 ± 4.60	76 ± 5.38
	Body surface	218 ± 5.18	187 ± 5.54	123 ± 4.17	96 ± 7.56	78 ± 3.18	67 ± 4.72	51 ± 3.67
Chaetoceros sp.	Water	TNTC	268 ± 7.93	215 ± 8.04	193 ± 5.34	176 ± 4.76	141 ± 3.63	124 ± 4.62
	Gut	TNTC	136 ± 6.19	117 ± 4.65	106 ± 5.53	97 ± 4.52	81 ± 4.78	72 ± 2.65
	Gill	TNTC	148 ± 2.94	128 ± 8.28	108 ± 4.18	90 ± 7.34	76 ± 2.86	68 ± 2.50
	Body surface	225 ± 4.85	197 ± 7.34	167 ± 4.78	145 ± 3.67	126 ± 6.61	105 ± 8.71	81 ± 7.76
Pavlova sp.	Water	TNTC	231 ± 7.56	212 ± 8.67	175 ± 5.73	158 ± 4.78	134 ± 3.61	112 ± 4.78
	Gut	TNTC	123 ± 6.45	113 ± 4.34	108 ± 5.82	85 ± 4.34	76 ± 4.90	64 ± 2.62
	Gill	TNTC	135 ± 4.27	126 ± 8.67	101 ± 4.13	87 ± 7.37	78 ± 2.81	62 ± 2.48
	Body surface	226 ± 4.14	162 ± 7.38	156 ± 4.65	138 ± 3.18	112 ± 6.78	97 ± 8.89	76 ± 7.72

Table 8: Effect of different type of diet on Bacterial count during different days of culture.

Hours	Bacterial Clearance of Fish in 1 hour interval (100 µl)				
	Control	*Tetraselmis* sp.(I)	*Dunaliella* sp.(II)	*Chaetoceros* sp.(III)	*Pavlova* sp.(IV)
1	15.25 ± 0.75	18.20 ± 0.48	17.65 ± 0.82	17.52 ± 0.12	16.64 ± 0.42
2	12.54 ± 0.43	11.52 ± 0.14	10.94 ± 0.81	12.50 ± 0.65	9.54 ± 0.32
3	10.86 ± 0.60	5.34 ± 0.16	5.46 ± 0.8	7.34 ± 0.40	4.20 ± 0.80
4	7.42 ± 0.21	0 ± 0.50	0 ± 0.0	1 ± 0.4	0 ± 0.0
The diets prepared from oil obtained from different species of algae significantly (P<0.05) influenced in the bacterial clearance in different time intervals than the control.					

Table 9: Efficiency of different types of algal oil diet on bacterial clearance.

the water quality. The nitrogen compounds such as ammonia dissolve in the fish rearing tank is the most important deteriorating chemicals in larval rearing system which affects the health of fish. Most of the nitrogen compounds enter the culture tank in the form of nitrogenous wastes of fish. Generally, ammonia is found in the water either as toxic unionized (NH_3) form or in non-toxic ionized form (NH_4). Ammonia containing nitrogenous organic matter is directly or indirectly toxic to many species of aquatic animals [11].

In this present study four types of diet were prepared with algal oil to study the effect on growth, food consumption, FCE, AGR, SGR and FCT. Among the 5 diets tested, the diet (II) had the maximum growth, food consumption, FCE, FCR, AGR and SGR. It shows that the particular combination of diet is most suitable for rearing Zebra fish. In this combination, the protein, lipid and carbohydrate were 6.147, 3.176 and 2.751 respectively, and in control the level of the said biochemical combination were protein 4.756, lipid 2.761 and carbohydrate 1.229.

Conclusion

From the above result, it can be concluded that the level of protein and lipid present in *Chaetoceros* oil was higher than the control. This may be the reason that the *Chaetoceros* oil only enhanced growth, food consumption, FCE, FCR, AGR and SGR. In the present study, it is clearly evident that Zebra fish showed increase in growth, SGR, FCR and protein, when *Chaetoceros* oil which is in agreement with the previous reports [12,13].

Protein is the main constituent of the fish body and so a sufficient dietary supply is needed for optimum growth. Protein is the most expensive macronutrient in fish diet [14]. So, the amount of protein in the diet should be enough for the fish growth whereas the excess protein in fish diets may be wasteful and cause diets to be unnecessarily expensive [15].

The protein compound of feed is responsible for its high cost [16] and most especially fishmeal [17]. Thus, efficient transformation of protein into tissue protein for growth is of immense significance [18]. Furthermore, metabolization of protein by fish should be directed towards body protein synthesis rather than energy supply [19,20]. Growth rates of fish may be highly variable and, in many cases, appear to be limited by food availability, quality and quantity of dietary non-protein to protein nutrients. In hybrid tilapia (*Oreochromis niloticus x O. aureus*), optimum dietary lipid for maximum growth has been reported to be about 12% [21]. However, Tilapia has been reported to utilize vegetable oil that is high in omega 6 (n-6) fatty acids better than fish oil that is rich in omega 3 (n-3) fatty acids for maximum growth [17]. Although the available dietary energy plays an important role in determining body lipid deposition, the dietary lipid content is regarded as the most important factor influencing carcass lipid in fish [2,22]. An increase in dietary lipid level elevates the body lipid level in *O. niloticus*. The increase in carcass lipids with increasing dietary lipids and the consequent reduction in carcass proteins have been reported for most species investigated [23-25].

In order to evaluate the quality of experimental diet in the culture system of Zebra fish, the bacterial growth was analyzed from the first day to 30th day of culture water, gut, gill and body surface of fish. Among the food diets tested for the bacterial growth on different parts as well as in water, the diet (IV) was found to be more effective in reducing the bacterial count in culture water, gut, gill and body surface of the animal. The fecal matter released by the animal after feeding the diet may promote the growth of bacteria. In our experiment, the diet (IV) had a less number of bacteria. The reason may be that the biochemical components like protein, lipid and carbohydrate present in this type of diet might have been almost fully absorbed in to the animal than the other type of diets. This is the reason why the bacterial load is less in this type of diet than the other type.

The microbiology of fish skin and gastro intestinal tract has been subjected to many researches. The diet plays a major role in the existence of bacterial population in different organs of fishes. Fish can spoil from both outer surface and inner surfaces as fish stomach contain digested and partially digested food which can pass into the intestine. After fish is being caught and killed the immune system collapses and bacteria are allowed to proliferate freely on the skin surface and the stomach. The walls of intestines do break down sufficiently for bacteria to move into the flesh through the muscle fiber. It has been suggested that intestinal microflora is the causative agent for food spoilage [26]. Fish take a large number of bacteria into their gut from water sediment and food [27]. It has been well known that both fresh and brackish water fishes can harbor human pathogenic bacteria particularly the coliform group [28]. Fecal coliform in fish demonstrates the level of pollution in their environment because coliform are not named flora of bacteria in fish [29].

The colouring pigments by carotenoid play a major role in the development of colour in ornamental fishes. The diet prepared with algal oil claimed to the best producer of carotenoids because it enhanced the colouration in ornamental fishes.

In the present study, the diet (IV) offered the maximum carotenoid production in ornamental fish than the other three types of diets. The effect of the diet on production of a carotenoid had been declining from the first day to the 30th day of culture. The reason may be that the animals might have been under feeding or the water quality might not have been maintained well from first to the 30th day.

Pigments are responsible for the wide spectrum of colours in fishes which is an essential prerequisite for the quality as they fetch higher price in the commercial market. As fishes cannot synthesize their own colouring pigments *de novo*, the colouring agents which are synthesized by some plants, algae and microorganisms, need to be incorporated in their diet [30,31]. Varieties of colouring agents are used in aqua industry to impart colour for the muscle and skin of fishes. Thus, pigmentation is an important criterion for fishes, since their colour affect commercial acceptability.

One of the greatest challenges in the ornamental fish industry is appearance of the accurate natural colour of the fish in the captive environment. Various products have been introduced to alleviate this problem, but none has performed so effectively and consistently as carotenoid pigment. Varieties of carotenoids pigments are used in fish diet for colouring enhancement. The most promising carotenoids proved to be successful in enhancing colour is astaxanthin that shows marked improvement in colour on most species of brightly coloured ornamental fishes like Tetras, Cichlids, Gouramis, Goldfish, Koi, Danios and many other species [32].

Effect of the diet may also indirectly have determined by its effect in clearing or reducing the pathogenic bacteria injected in to its body if the diet is considered to be more efficient, then it should control the injected pathogenic bacteria in short time than the animal fed with other type of diet. In our experiment, the diet (IV) was found to be more effective than the other three diets. In the diet (IV) fed animal, the pathogenic bacteria injected were completely cleared or destroyed by the immune system of the fishes within 2 hours after 24 hours incubation. It shows that the diet (IV) fed animal was found to be healthier to fight against the invaded pathogens.

Acknowledgment

Authors are thankful to the UGC SAP for the financed support to conduct this study.

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