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Substitution of Fishmeal with Astaxanthin Chlorella vulgaris on growth, survival rate and feed efficiency of Tilapia (*Oreochromis niloticus*)

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Abstract— The purpose of this study was to try the effect of the use of earthworm meal and chlorella vulgaris on the growth rate, survival rate and feed efficiency of tilapia. The research method is experimental with completely randomized design (CRD) with four treatments, one control and three replications. The treatments used were: (i) S. Vulgaris at 25%, Astaxanthin at 0.2% and fish meal by 50%, (ii), 25% fishmeal, 50% S. Vulgaris and Astaxanthin by 0,2%, (iii) 40% C. Vulgaris, 0.4 Astaxanthin and 20% fishmeal (iv) 40% C. Vulgaris, 0.4 Astaxanthin and 30% fishmeal. Analysis of the data using analysis of variance (ANOVA) and to know the difference between treatments using Duncan's Multiple Range Test. Results showed the administration of C. Vulgaris and Astaxanthin on feed rations provide a significantly different effect ($p > 0.05$).The results of this research showed that the highest feed efficiency was 29.45 % highest survival rate was 86.67 % obtained on treatment iii. The highest absolute weight and length growth was 4.96 g and 0.9 cm on treatment (ii). Water quality of red tilapia rearing media was temperature range between 25-28 °C, pH 7-8, dissolved oxygen 4-5 mg/L and ammonia 1.5 to 3.0 mg/L.

Keywords— feed, tilapia, growth rate, feed efficiency.

1. Introduction

Nevertheless, the increasing cost of feed because of the high cost of fishmeal is the major limitation in the culture of nile tilapia. The cost of fishmeal is about 80% of aquaculture industry operating costs where protein is the controlling factor, which determines the cost of fish diet [1]. However, it is not only expensive but the supply is stagnated due to over-exploitation of the natural resources and competition from humans and other livestock ventures [2]. Hence there is need to find a suitable replacement for it in order to bring down production cost. Recently, the use of microalgae is gaining the attention of several researchers as a promising alternative to fishmeal. They are used as feed for zooplankton used in aquaculture. Microalgae are rich in carotenoid pigments, essential fatty acids, essential amino acids, minerals, and vitamins for aquatic animals [3]. Several authors have reported the remarkable performance of various microalgae as a source of protein and carotenoid for shrimp [4]. Mustafa &Nakagawa [5]. Reported that fishmeal replacement with microalgae enhanced carcass quality, feed utilization, disease resistance, physiological activity, starvation tolerance, stress response, and growth (protein accretion). Chlorella as a feed supplement has beneficial effects on growth, immunity, antioxidant activity and tissue rebuilding [6]. The dietary inclusion of 5-10% C. Vulgaris protects Nile tilapia against arsenic-induced oxidative stresses and immunosuppression [7]. Supplementation of C. Vulgaris at 5% in diet of Cyprinus carpio 4 plays an important role in the stimulation of fish immune system [8]. The dietary supplementation of 10-15% C. Vulgaris enhances growth performance, catalase enzyme activity and lipid metabolism in Olive flounder [9].

Ameenat et al. [10] explored fishmeal replacement with two freshwater microalgae: Spirulina Platensis and Chlorella Vulgaris in African catfish (Clarias Gariepinus) diet. The result shows that all the diets substituted with both S. Platensis, and C. Vulgaris boosted the growth performance based on specific growth rate (SGR) and body weight gain (BDWG) when compared with the control diet. The feed conversion ratio (FCR) and protein efficiency ratio (PER) was significantly influenced by all the supplementations. The hematological analysis of the fish shows a significant increase in the value of red and white blood cells upon supplementation with microalga the white blood cell (WBC), red blood cell (RBC) increased, while total cholesterol (TCL), and Plasma glucose levels decreased significantly upon supplementation of algae. Shuang et al [11] using Chlorella vulgaris and Senedesmus obliquus as a feed replacement to Do rosophila melanogaster results showed that compared to control, the flies fed on 80% microalga (80-flies) in the total weight (w/w) had the double increased apparent step size, while both 60-flies and 80-flies exhibited longer travel distances of 27.77 ± 1.99 cm is 31.50 ± 3.70 cm, respectively. Subsequently, 40-flies exhibited less optimal growth performance with decreased body weights (0.51 ± 0.006 mg vs 0.60 ± 0.005 mg for control) and shorter mean lifespan (36 days vs 55.8 days for control. However, 20-flies showed no statistical differences in all parameters tested with respect to control flies, indicating that 20% microalgae treatment did not greatly change the primary food component such as carbohydrate which might play a critical role in fly performance. Therefore, the inclusion of 20% microalgae could be an alternative to fly standard food without compromising fly physiological performance. Astaxanthin is used as a feed additive in fish feed for the production of edible fish species in aquaculture. Here, it is used to impart red colour ("salmon colour") to the flesh of some salmonids (Salmo salar, Salmo trutta or Oncorhynchus mykiss). In some countries (e.g., Germany), rainbow trout showing a pale white flesh when grown in aquaculture without the addition of carotenoids, is marketed in its colour form known under the name of "salmon trout." Other carotenoids commonly found in fish are canthaxanthin, tunaxanthin, lutein, β-carotene, zeaxanthin, echinenone, and taraxanthin [12].

Astaxanthin, being the most important carotenoid, is available from natural sources (crustaceans, algae, and yeast), from synthetic sources, and by metabolic engineering [13] (eg, Gassel et al., 2014). Alex[14] studied was to quantify and model the combined effects of dietary concentration of astaxanthin (32.5–102.5 ppm) and time of supplementation (29–69 days) on the growth, survival, and colouration of Penaeus monodon (Black tiger prawn), using natural (Haematococcus pluvialis) and synthetic (Carophyll Pink®) astaxanthin. The astaxanthin concentration and supplementation time also had a significant effect on the colour of boiled prawns, where higher concentrations of astaxanthin for longer supplementation times resulted in improved colouration up until a plateau of 98 ppm for 66 days for natural astaxanthin, and 90 ppm for 63 days for synthetic astaxanthin. George et al [15] was studied effect of different dietary oils on the pigmenting efficacy of astaxanthin in terms of astaxanthin serum concentration, induced muscle colour, and astaxanthin muscle retention by feeding olive oil replacing fish oil to rainbow trout for 6 weeks. Astaxanthin sources were green micro-algae H. pluvialis and synthetic astaxanthin. observation in this study was that olive oil can be used up to 10% replacement of dietary fish oil for rainbow trout during the grow out period (6 weeks) without negative effects on mortality or growth. For astaxanthin content in serum and muscle of trout the effects of dietary oil source gave faint results. On one hand, fish fed diets OL displayed higher ($P>0.05$) serum astaxanthin levels than those fed diets FI. On the other hand, after 6 weeks of experiment muscle astaxanthin levels were not different ($P>0.05$) for fish fed olive or fish oil. Xiang jun et al [16] evaluated the potential of these natural microorganisms, including 75 g kg−1 S. platen (SP), 200 g kg−1 single R. palustris, photosynthetic bacteria (PB) and 200 g kg−1 effective microorganisms (EM) including, as sources of dietary pigments for colouring the skin of Showa koi (C. carpio L.). These sources were compared to a positive control of synthetic 1.5 g kg−1 astaxanthin (CR) and a negative control, a diet with no pigment added. The result shows the weight gain and feed conversion ratio were significantly affected by dietary treatment. The fish in the SP diet group had a significantly higher rate of weight gain (WGR) and specific growth rate (SGR) than the other fish groups

(P>0.05). The food conversion ratio (FCR) of the fish fed the SP diet was significantly lower than that of the other fish groups (Pb0.05). The FCR of the control fish was significantly lower than that of fish fed the PB diet or the EM diet (P>0.05). The FCR of the fish fed the CR diet was significantly lower than that of the fish fed the EM diet (P>0.05). Effects of experimental diet on the body color with intensity of color, red and yellow tonalities, and chromatic aberration for the chromatic varieties of Showa koi. All of the pigments increased the lightness (L*) of the black zones, and there was a significant difference between the SP and the control diet groups (P>0.05). The black and white chromatic aberrations (E of the black zones) of the CR and PB diet groups were significantly higher than those of the EM, SP and control diet groups (P>0.05). The CR diet groups showed a significantly lighter red zone than that of groups fed the control, PB or SP diets (P>0.05). The group fed the control diet showed weak red tonality (a*) and E of the red. Some studies have shown that Chlorella vulgaris is as efficient as synthetic pigments in the pigmentation of gilthead seabream Sparus aurata [17], koi Cyprinus carpio and goldfish Carassius auratus [18]. Pigments obtained from xanthophores [19], the carotenoid [20] were used as sources of dietary carotenoids. In practice, we find out that some ornamental fish culturists used astaxanthin to improve body color of fish such as goldfish (Carassius auratus auratus), ornamental Cichlid (Cichlidae sp.) and so on. On the other hand, we found that red color of kohaku koi had been significantly improved by photosynthetic bacteria (Rhodopseudanonas palustris) when we splashed this bacteria liquid into koi cultural ponds to improve the water quality [21]. Based on the experiment and above researches, the present study evaluated the potential of these natural astaxanthin as sources of dietary pigments for coloring the skin of tilapia. These sources were compared to a negative control, a diet with no pigment added.

2. Methods

2.1. Material and apparatus

Material: shrimp waste, sunflower seed, Methanol, NaCl 1% and Na_2SO_4 anhydrate. Apparatus: glassware, hotplate, magnetic stirrer, rotary evaporator, FTIR (Fourier Transformation Infrared) spectrophotometer and HPLC (High Performance Liquid Chromatography).

2.2. Preparation of shrimp powder

Clean shrimp waste has been processed in order to prepare shrimp waste powder assumed to be protein free. The processing of shrimp waste included 10 min. digestion of shrimp waste in water at moderate temperature. After digestion the color of shrimp waste was changed to bright red due to bonding looser between carotenoid and protein, then the protein would be denaturized, coagulated and precipitated in water. The process was followed by drying the shrimp waste at temperature lower than 60 ˚C and the dried shrimp waste grinded to yield fine powder.

2.3. Preparation Green Solvent ME-SF

ME-SF preparation was carried out in a 1 L round bottomed reactor equipped with a thermostat, mechanical stirrer, sampling outlet, and condensation system. Briefly, sunflower oil (500 g) was preheated to the set temperatures 60°C on a heating plate prior to starting the reaction. A fixed amount of freshly prepared methanol solutions of the catalysts NaOH1% in the molar ratio, 6:1 was added into the reactor, and mixed for 120 min with agitation 900 rpm. After the completion of the reaction, the reaction mixture was allowed to cool to room temperature and equilibrate, resulting in the separation of two phases. After separation of the two phases by sedimentation, the upper Me-SF phase was purified by distilling at 800 °C to remove excess methanol, followed by successive washes with distilled water and treatment with Na_2SO_4 and filtration. The lower glycerol layer was acidified with concentrated H_2SO_4 to neutralize any unreacted catalyst and

decompose the soaps formed during the trans-esterification. The resulting mixture was subjected to distillation at 800 °C under moderate vacuum to recover the excess methanol thus purifying the Lycerine. Determine the yield and test the ME-SF characteristics with ASTM D 6751 method.

2.4. Extraction and purification of Astaxanthin

The extraction of Astaxanthin from shrimp powder used and experiments were carried out by adding ME-SF in three different ratios to shrimp powder: 4:1, 6:1, and 8:1 at temperatures of 30, 50, 70 °C. The sample was mixed by mechanical stirrer 200 rpm for 24 h. During the extraction experiments, pigmented samples were collected each 60 and30 min respectively and centrifuged at 1000 rpm for 15 min before analysis using a spectrophotometer.

2.5. FTIR spectra for Astaxanthin identification

The FTIR spectra for Astaxanthin extract from shrimp waste powder has been obtained from Perkin Elmer instrument using KBr pellets with a scanning region of 700 cm^{-1} to 4000 cm^{-1} vs. % transmission (T).

2.6. HPLC for Astaxanthin quantization

A Shimadzu HPLC conducted the quantitative analysis of astaxanthin. The condition of HPLC is as follows: (i) volume of injection: 5 µL, (ii) dilution factor: 3 times, (iii) solvent: eluent A (water-TFA 0.1%); eluent B (acetonitrile-TFA 0.1%), (iv) fluid rate: 0.2mL/min., (v) oven: 30 °C, (vi) detector: SPD-M20A and (vii) column: Xselectwaters C18 2.1 x 100 mmID. A linear calibration for quantization of astaxanthin in shrimp waste extract is conducted in this study.

2.7. Feed Formulation

Making feed starts with weighing feed ingredients according to the formulation. Feed raw materials are separated between dry ingredients such as C. vulgaris, Astaxanthin, fish meal, tilapia, tapioca flour, soy flour, fine bran, turmeric flour and vitamin mix with liquid ingredients such as soybean oil and hot water. The whole dry ingredient is stirred using a homogenizer so that a homogeneous mixture is formed. Add soybean oil and hot water, re-cook until it forms a solid, then cast in a pellet form. Then the feeds were dried at a temperature of 500-600 °C for 10 hours until the moisture content was less than 10%. The dried feed pellets were physically examined for visual appearance, such as uniformity, color and fragrance. The feed ingredients percentage composition and nutrient composition are provided in **Table 1**.

Table 1. Formulation (g kg^{-1}), of the experimental diets Feed Formulation

No	Feedstock	Control	CAF 1-0,2-2	CAF 2-0,2-1	CAF 2-0,4-1	CAF 4-0,4-3
1	Fishmeal	400	200	100	50	80
2	C.Vulgaris	0	100	200	175	240
3	Astaxanthin	0	0.2	0.2	0.4	0.4
4	Wheat bran	105	105	105	105	105
5	Rice bran	115	115	115	115	115
6	Mineral premix	15	15	15	15	15
7	Vitamin premix	25	25	25	25	25
8	Salt (Nacl)	20	20	20	20	20
9	Perch oil	60	60	60	60	60

10	Binders (Cassava)	20	20	20	20	20

C = chlorella, A = Astaxanthin, F = Fish meal

2.8 Feed and sample analysis

Proximate analyses of the diets and fish whole body were carried out in triplicate following the standard methods [22]. Dry matter was determined by oven drying at 105 °C to constant weight. For moisture analysis, known quantities of fresh tissue were taken and excess moisture was removed using filter paper and dried and the final weight was calculated with % of pre weighed sample, while ash was determined by incineration in a muffle furnace at 550 °C for 3 h. Protein was analyzed by the Kjeldahl method after acid digestion, using an automatic Kjeltec™ 8400(35) Total lipids were estimated by chloroform: methanol method, and for quantification of total CH content the thymol-sulfuric acid method was used. Amino acid composition of the experimental diets was analyzed by HPLC.

2.9 Experimental procedure

Tilapia with the length and weight range of (5.2 ±0.29) cm and (1.30 ± 0.14) g respectively were used for the feeding experiment. Before the trial, experimental fish were acclimated with a commercial diet (Hi Provit 781-2) for 3 day, and then groups of twenty fish were randomly distributed into eighteen plastic tank 40-L. Each diet was randomly assigned to three replicate groups of fish. During the 8-week feeding trial, fish were fed by hand to apparent satiation two times per day (7:00 and 17:00 h) under a natural photoperiod. During the rearing period, each tank had supplemental aeration and continuous flow of water at a rate of 3 L/min, water temperature was 28±3 °C, salinity was 4.0 ± 0.5 g/L, and pH was 6.7 ± 0.4, dissolved oxygen≥4.0 mg/L, and ammonia 1.5 to 3.0 mg/L.

2.10 Data collection and analysis

At the termination of the feeding trial, puffer fish Tilapia were not fed for 24 h, then all of the fish in each tank were weighed, measured and counted to calculate weight gain (WG), specific growth rate (SGR), feed conversation ratio (FCR), and survival rate. The growth performance was assessed using following equation (1) to (4).

$$WG = final\ wieght\ (g) - initial\ weight\ (g) \quad (1)$$

$$SGR\ (\%) = \frac{(Final-initial)weight\ (g)}{Initial\ weight\ (g)} \times 100\% \quad (2)$$

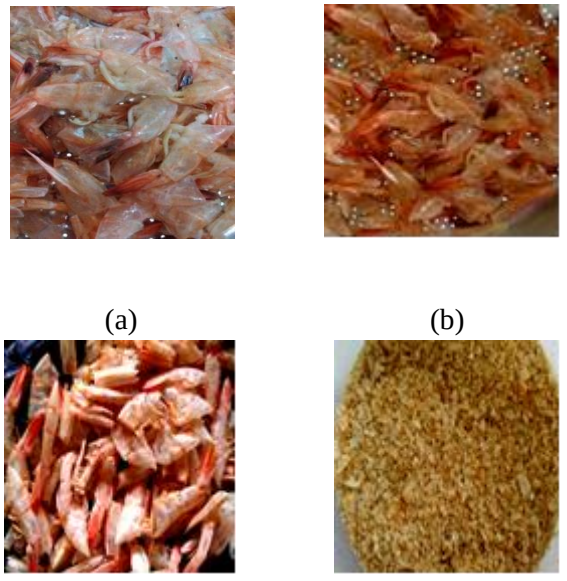
$$Survival\ rate(\%) = \frac{Final\ fish\ number}{Initial\ fish\ number} \times 100\% \quad (3)$$

$$Feed\ Conversion\ Rate = \frac{Dry\ diet\ fed}{Wet\ weight\ gain} \times 100\% \quad (4)$$

3. Results and Discussion

3.1. Shrimp waste product

Fig. 1 illustrates the production of shrimp waste after cleaning, digestion, drying and refining. The process involves with 10-minute digestion and drying at ≤ 60 °C.



(a) (b)

(c) (d)

Fig 1. Shrimp waste products after (a) cleaning, (b) digestion, (c) drying and (d) refining

3.2 Preparation Me-SF

The methanol/oil molar ratio 6:1 was varied in three experiments to determine ME-SF production. In all experiments, 600 rpm agitation intensity, 60 °C reaction temperature, and 1.0% NaOH concentration were employed. The trans-esterification was proceeded to more than 76% completion after 20 min. After 110 min, the reaction was completed up to 94.8%.

3.3 Extraction Astaxanthin

3.3.1 Effect of the temperature

The effect of temperature on the Astaxanthin with ME-SF/shrimp powder 4:1 content extracted by ME SF is shown in **Table 2**. Astaxanthin content was increased from 38 % to 62% by increasing the temperature from 40 to 80 °C. Increasing the temperature resulted in lower oil viscosity [23] which enhances the diffusivity coefficient and consequently the rate of the mass transfer of Astaxanthin form shrimp waste to SF [24].

Table 2. Astaxanthin content at various temperature

No	Temperature	Astaxanthin (%)
1	30	38
2	50	61
3	70	62

3.3.2 Effect of ratio of green solvent to shrimp waste

The effect of the ratio factor on the Astaxhantin content extracted by SF was significant but with a lower statistical impact compared to the temperature influence **Table 3**. In addition, increasing the ratio of SF to shrimp powder had a positive effect on the extraction time especially at higher temperature.

Table 3. Astaxanthin content at various ratio of ME-SF to shrimp powder

No	Ratio of SF/ shrimp powder	Astaxanthin (%)
1	4:1	58

2	6:1	65
3	8:1	86

3.3.3 Effect of time extraction

In addition, the results of the analysis also show that with increasing time, Astanxanthin content decreases as shown in **Table 4**.

Table 4. Astaxanthin content at various time extraction

No	Time (min)	Astaxanthin (mg/kg)
1	60	13
2	120	31
3	180	36
4	240	32
5	300	28

3.4. Infrared analysis

Fig. 2 shows the FTIR spectra of free Astaxanthin [25] and Astaxanthin exctract from shrimp powder for extract with green solvent ME-SF and 900 rpm stirrer of rotary evaporator. In relation to the chemical structure of Astaxanthin as shown in **Fig. 3**, the FTIR spectra is characterized to certain functional groups such as carbonyl (C=O), hydroxyl (-OH), methyl aromatic and conjugated double bond -C=C-C=C-. According to Coates[26], the FTIR band of 3360 cm^{-1} of Astaxanthin extract (**Fig. 2b**) is due to the stretching vibration of hydrogen bonding between solvent and -OH group in Astaxanthin extract, this situation is in agreement with the stretching vibration of –OH due to hydrogen bonding in free Astaxanthin as shown in Fig. 2a. The FTIR band of 3360 cm^{-1} of Astaxanthin extract (**Fig. 2b**) is might be due to the presence of water residue pertained to the process of Astaxanthin related to extraction and purification of shrimp powder.

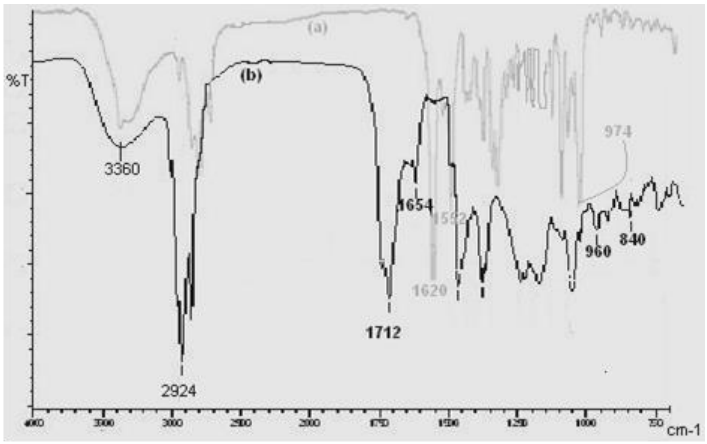


Fig 2. Infrared spectra of (a) astaxanthin (Yuan et al., 2012) and (b) astaxanthin extract from shrimp powder using SE MPF green solvent

The FTIR band of 2924 cm^{-1} of astaxanthin extract indicates stretching vibration of methyl (-CH3) aromatic [26] whereas it experiences a slight hypsochromic shift from that of free astaxanthin [25] implies a shift to shorter wavelength probably due to the presence of other remaining constituents in the astaxanthin extract. The presence of remaining constituents caused the stretching vibration of methyl (-CH3) aromatic of the astaxanthin extract more rigid and therefore, it needs more energy to vibrate. With regard to the FTIR band

of 1712 cm^{-1} of the astaxanthin extract (**Fig. 2b**) showing the stretching vibration of carbonyl (C=O), whereas it experiences a hypsochromic effect meaning a shift to shorter wavelength compared to that of free astaxanthin of 1620 cm^{-1} (**Fig. 2a**). The presence of remaining constituents in Astaxanthin extract may inhibit the vibration movement of carbonyl group that it needs more energy to vibrate the carbonyl group. A similar phenomenon in relation to hypochromic shift is shown by the FTIR band of 1654 cm^{-1} of astaxanthin extract compared to that of 1552 cm^{-1} of free astaxanthin is due to the presence of remaining constituents in the extract retarding the stretching vibration of C-C in aromatic group. On the other hand, a bathochromic shift is shown for the stretching vibration of C-C in conjugated double bonds (960 cm^{-1} and 840 cm^{-1}) of the extract compared to that of free astaxanthin (974 cm^{-1}). In this case, the presence of remaining constituents seems to lose the conjugated double bonds of Astaxanthin extract shown on **Fig 3**.

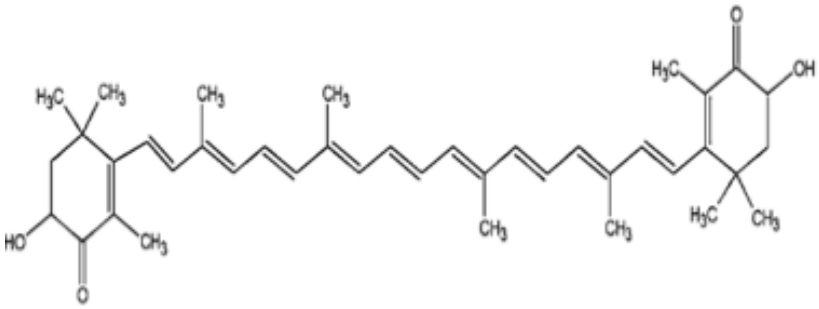


Fig. 3. Chemical structure of Astaxanthin

3.5 HPLC analysis

Semi-quantitative determination of astaxanthin extract from shrimp waste using acetone is on the basis of calibration method (**Fig. 4**) with its linear regression of y = 138.5x – 34 (R^2 = 0.988) yielding a concentration of Astaxanthin of 7.5 ppm in the extract from shrimp waste applying maceration method. Cuan & Turner [27] reported astaxanthin concentration of 24 ppm extracted from shrimp waste applying pressurized liquid extraction (PLE) whereas Sanchez et al. [28] reported a recovery of astaxanthin by 39% from Brazilian shrimp using supercritical CO_2 extraction. Low yield of Astaxanthin extract obtained by this study is due to several factors particularly pertained to number of extractions resulting constituent loss on the way of processing and number of chemicals used causing serious interference.

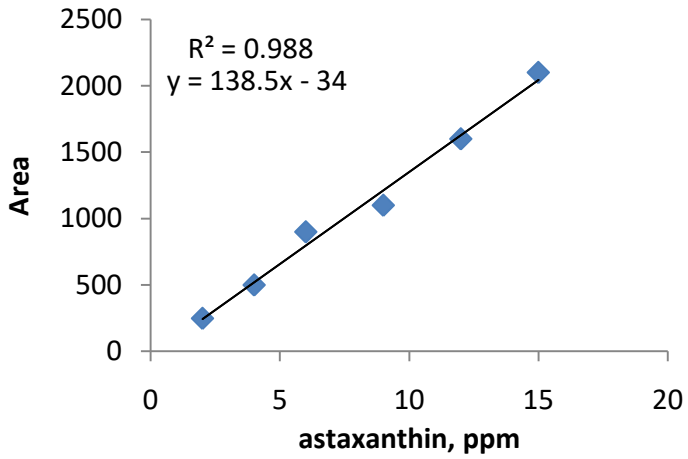


Fig. 4. Linear test of HPLC method on Astaxanthin extraction from shrimp waste applying green solvent ME-SF (λ = 460 nm)

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On the basis of absorbance measurement of Astaxanthin at given wavelength range (445-475 nm) vs. retention time (min.), it is found the 460 nm performs the highest maximal peak of Astaxanthin at retention time of 13.62 min. (**Fig. 5**).

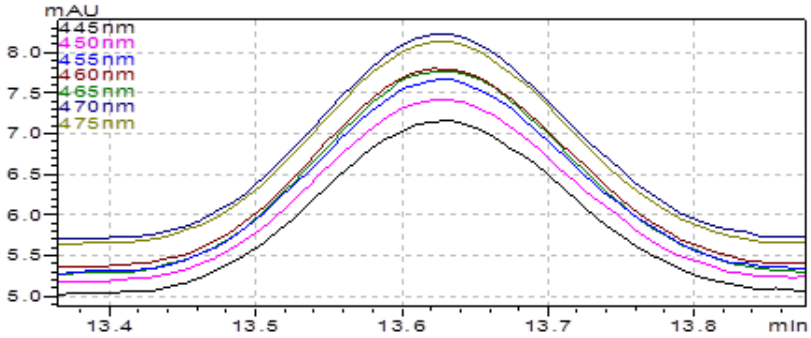
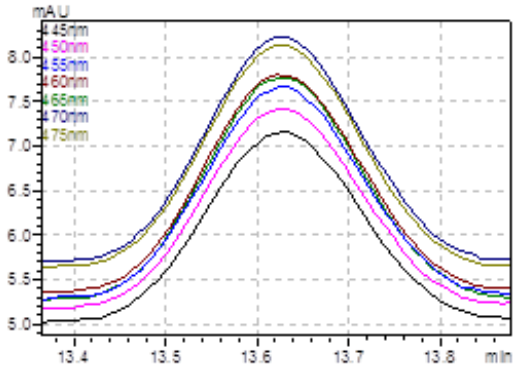
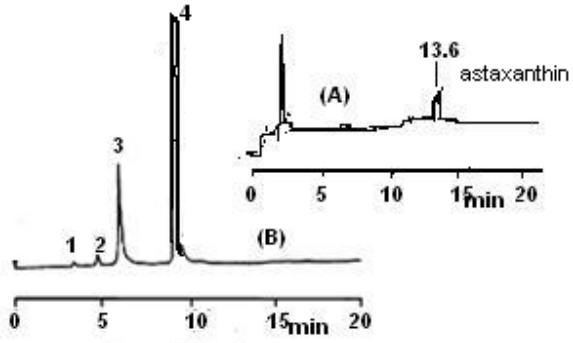


Fig. 5. Absorbance (mAU) vs. retention time (min.) of Astaxanthin at given range of wavelength (445-475 nm) used for HPLC measurement

Fig. 6a shows the HPLC profile of astaxanthin extract from shrimp waste executed at 13.6 min. using maceration method at 45 °C and 200 rpm stirrer in this study. On the other hand, Rao et al.[28] presented a good performance of chromatogram of astaxanthin as the major constituent and some minor components such as astacene, apoastaxanthin and dehydro astaxanthin, which are clearly separated in that high resolution chromatogram (**Fig. 6b**).



(a)



(b)

Fig. 6. HPLC profiles: (a) Astaxanthin extract from shrimp waste with green solvent ME-SF, (b) astaxanthin with some minor impurities, i.e. (1) Astacene, (2) Apoastaxanthin, (3) Dehydro astaxanthin and (4) Astaxanthin [28]

3.6 Proximate Analysis

Proximate composition (g 100 g^{-1} dry matter basic of feed experimental diets, is presented in **Table 5**. The present study demonstrated the potential of C. Vulgaris and Astaxantin powder for inclusion in commercial Nile tilapia feeds, as well as being of immediate importance for feed production. In the present study, we were able to replace up to 80% fish meal with C. vulgaris and Astaxanthin powder in the diet of Nile tilapia formulated to contain 38-46% protein. These findings are similar to those which demonstrate that growth of Nile tilapia was not depressed when 20–30% dietary soybean meal was replaced with Azolla africana [30], roquette seed meal [31], Cassia fistula mea [32], pigeon pea, Cajanus cajan [33], or Ulva meal [34].

Table 5. Proximate composition (%) of the experimental diets

No	Feedstock	Control	CAF 1-0,2-2	CAF 2-0,2-1	CAF 2-0,4-1	CAF 4-0,4-3
1	Dry matter	90.3	90.6	92.7	94.6	95.3
2	Protein	41.8	38.0	46.12	45.7	46
3	Lipid	10.5	11.0	13.1	12.0	11.7
4	Ash	6.2	6.1	6.4	6.2	6.3
5	Fibre	5.8	5.8	5.8	5.8	5.8

3.7 Growth performance

Results of growth performance and feed utilization upon 10-week feeding are shown in **Table 6**. The growth rates and feed conversion ratios of red hybrid tilapia fingerlings fed diets with 0%, 4%, 8% and 12% of cod liver oil, substituted against corn oil (Table 2), showed no significant differences ($P > 0.05$) (Table 3). At the end of the 10-week feeding trial, the range of weight gain fish was 42.4-46.1 g and the range of feed conversion ratios was 1.56-1.70. It was observed, however, that specific growth rate and overall weight gain decreased slightly as dietary fish oil increased. A single mortality occurred during the feeding trial.

Table 6. Growth performance and feed utilization after 10 weeks

Parameter	Control	CAF 1-0,2-2	CAF 2-0,2-1	CAF 2-0,4-1	CAF 4-0,4-3
IBW	5.14± 0.3	5.23± 0.3	5.23± 0.3	5.23± 0.3	5.14± 0.3
FBW	9.34± 0.4	12.14± 0.3	17.06± 0.3	22.12± 0.3	19.22± 0.3
WG	4.20±0.3	6.91± 0.3	11.83± 0.3	16.89± 0.3	12,08± 0.3
SGR	2.04±0.01	2.92±0.01	3.40± 0.2	5.03± 0.1	4.08± 0.2
SR	27.14±0.3	43.0±0.3	51.4± 0.3	65,14± 0.2	58.14± 0.3
FCR	2.14±0.1	1.42±0.1	1.0± 0.2	0.92± 0.1	1.08± 0.2
EC	83.47±0.4	87.62±0.3	87.65± 0.3	88.91± 0.3	85.14± 0.2

3.8 Water Quality

The results of water physics-chemical measurements during the study are presented in **Table 7.** The concentration of NH_3 in the five systems, including the control treatment was high in the beginning of the experiment and maintained a decreasing trend in the first 20 days. This can be explained by the slow growth of the nitrifying bacteria. Probably, there were not enough nitrifying bacteria to carry out the nitrification

process efficiently [28]. At this early stage, nitrifying bacteria would multiply, and the accumulation of NH_3 is caused by the presence of nitrogen in the system introduced by waste from the fish as well as nutrient leaching from the feed. Occurrence of NH_3 in the water column could trigger the ammonia oxidizing bacteria to turn it into NO_2 while the nitrite oxidizing bacteria, especially Nicrobacter spp. and Nitrospira spp. degrade it to nitrate.

Table 7. Water quality after 10 weeks

Feed	pH	DO (mg/L)	NH_3 (mg/L)	NO_2^- (mg/L)	NO_3^- (mg/L)
Control	6.8-7.4	3.73-4.93	0.029-0.012	0.61± 0.3	1.16
CAF 1-0,2-2	6.8-7.4	3.73-4.93	0.023-0.01	0.42± 0.3	0.89 ± 0.37
CAF 2-0,2-1	6.8-7.4	3.73-4.93	0.027-0.007	0.50± 0.3	1.4 ± 0.39
CEF 2-0,4-1	6.8-7.4	3.73-4.93	0.026-0.007	0.53± 0.1	1.03 ± 0.32
CEF 4-0,4-3	6.8-7.4	3.73-4.93	0.027-0.009	0.59± 0.2	1.17 ± 0.32

Low nitrogen concentration in the water may be due to removal of most of the ammonia, nitrite and nitrate by an active microbial population. NO_2^- is a transition ion during the nitrification process and exhibits lower toxicity than NH_3. The concentration of NO_2^- was maintained below 2 mg/L and it decreased throughout the experiment because of the abundance of ammonia oxidizing bacteria responsible for the rate-limiting step of nitrification. The pH value during the research is also still in the optimal range, the pH value that can interfere with fish life is a pH that is too low (very acidic) and a pH that is too high (very basic), most fish can adapt well to aquatic environments that have a pH range between of 5-9. Overall, it was found that the physical-chemical nature of water in the research media could support the survival of the fish being kept and there was no significant difference between the physical-chemical values of the aquatic fish that were given chlorella and Astaxanthin with those that were only given chlorella.

4. Conclusion

Based on the results of the study, it can be concluded that the highest feed efficiency was 29.45 % highest survival rate was 86.67 % obtained on treatment iii. The highest absolute weight and length growth was 4.96 g and 0.9 cm on treatment (ii). Water quality of red tilapia rearing media was temperature range between 25-28 °C, pH 7-8, dissolved oxygen 4-5 mg/L and ammonia 1.5 to 3.0 mg/L.

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6. References

[1] Shepherd, C., & Jackson, A. (2013). Global fishmeal and fish-oil supply: inputs, outputs and markets. Journal of fish biology, 83(4), 1046-10

[2] Jabir, M., Razak, S., & Vikineswary, S. 2012. Chemical Composition and Nutrient Digestibility of Super Worm Meal in Red Tilapia Juvenile. Pakistan Veterinary Journal, 32:

[3] Augustin, J.M., Kuzina, V., Andersen, S.B., & Bak, S. 2011. Molecular activities, biosynthesis and evolution of triterpenoid saponins. Phytochemistry, 72: 435-457.

[4] Patnaik, S., Samocha, T.M., Davis, D.A., Bullis, R.A., Browdy, C.L., 2006. The use of HUFA rich algal meals in diets for Litopenaeus vannamei. Aquac. Nutr. 12: 395-401.

[5] Mustafa, M.G., Nakagawa, H., 1995. A review: dietary benefits of algae as an additive in fish feed. Isr. J. Aquacult. Bamidgeh. 47: 155–162.

[6] Guzman S, Gato A, Calleja JM.2991. Anti-inflammatory, analgesic and free radical scavenging activities of the marine microalgae Chlorella stigmatophora and Phaeodactylum tricornutum. Phytother Res. 2001 38 15:224-30.

[7] Zahran E, Risha E. Modulatory role of dietary Chlorella vulgaris powder against arsenic-induced immunotoxicity and oxidative stress in Nile tilapia (Oreochromis niloticus). Fish Shellfish Immunol. 2014 3 41:654-62. 4

[8] Khani M, Soltani M, Shamsaie Mehrjan M, Foroudi F, Ghaeni M. 2017.The effects of Chlorella vulgaris supplementation on growth performance, blood characteristics, and digestive enzymes in Koi (Cyprinus carpio). Iranian Journal of Fisheries Sciences: 16:832-43.

[9] Rahimnejad S, Lee S-M, Park H-G, Choi J.2017. Effects of Dietary Inclusion of Chlorella vulgaris on Growth, Blood Biochemical Parameters, and Antioxidant Enzyme Activity in Olive Flounder, Paralichthys olivaceus. Journal of the World Aquaculture Society. 48:103-12

[10] Ameenat Abiodun Raji, Peter Adeniyi Alaba, Indatu Yusuf, Noor Hidayati Abu Bakar, Nor Hidayah Mohd. Taufek, Hasniyati Muin, Zazali Alias, Pozi Milow, Shaharudin Abdul Razak .2018, Fishmeal replacement with Spirulina Platensis and Chlorella vulgaris in African catfish (Clarias gariepinus) diet: Effect on antioxidant enzyme activities and haematological parameters. Research in Veterinary Science 119: 67-75

[11] Shuang Qiu, Shuying Wang, Chengfeng Xiao, Shijian Ge.2019. Assessment of microalgae as a new feeding additive for fruit fly Drosophila melanogaster. Science of the Total Environment 667: 455-463

[12] Garcia-Chavarria, M., Lara-Flores, M., 2013. The use of carotenoid in aquaculture. Research Journal of Fisheries and Hydrobiology 8: 38-49.

[13] Gassel, S., Breitenbach, J., Sandmann, G., 2014. Genetic engineering of the complete carotenoid pathway towards enhanced astaxanthin formation in Xanthophyllomyces dendrorhous starting from a high-yield mutant. Applied Microbiology and. Biotechnology 98: 345-350.

[14] Alex Angella , Rocky de Nysa , Arnold Mangottb , Matthew J. Vucko.2018.The effects of concentration and supplementation time of natural and synthetic sources of astaxanthin on the colouration of the prawn Penaeus monodon. Algal Research 35 :577-585

[15] Georges Choubert, Maria Manuela Mendes-Pinto, Rui Morais. 2016. Pigmenting efficacy of astaxanthin fed to rainbow trout Oncorhynchus mykiss: Effect of dietary astaxanthin and lipid sources. Aquaculture 257: 429-436

[16] Xiangjun Sun, Yu Chang, Yuantu Ye, Zhihong Ma, Yongjun Liang, Tieliang Li, Na Jiang, Wei Xing, Lin Luo.2012 The effect of dietary pigments on the coloration of Japanese ornamental carp (koi, Cyprinus carpio L.) Aquaculture 342: 62-68

[17] Gouveia, L., Choubert, G., Gomes, E., Pereira, N., Santinha, J., Empis, J., 2002. Colouringation of gilthead seabream, Sparus aurata (Lin 1875), using Chlorella vulgaris microalga. Aquaculture Research 33: 1-7.

[18] Gouveia, L., Rema, P., Pereira, O., Empis, J., 2003. Colouring ornamental fish (Cyprinus carpio and Carassius auratus) with microalgal biomass. Aquaculture Nutrition 9:123-129.

[19] Kanta Mizusawa, Yutaka Yamamura, Satoshi Kasagi, José Miguel Cerdá-Reverter, Akiyoshi Takahash.2017. Data on the density of xanthophores in a whole scale of goldfish acclimated to white or black background color. Data in Brief 14 :724-729

[20] Sun, X.J., Li, T.L., Jiang, N., Ma, Z.H., Luo, L., 2010. The effect of nature pigments on the coloration of Japanese ornamental carp (Cyprinus carpio L.) cultured in pond. Feed Industry 31 (8), 19–20

[21] AOAC (2005). Association of Official Analytical Chemists (AOAC). (19th ed.). Hoboken, NJ, USA: John Wiley & Sons, Inc.

[22] Troell, M., Robertson-Andersson, D., Anderson, R.J., Bolton, J.J., Maneveldt, G., Halling, C., Probyn, T., 2006. Abalone farming in South Africa: An overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. Aquaculture 257: 266-281.

[23] Bird, B., Stewart, W., Lightfoot, E., 2006. Transport Phenomena, second ed. John Wiley & Sons Inc.

[24] Yuan C, Jin Z, Xu X. 2012. Inclusion complex of astaxanthin with hydroxypropyl-β-cyclodextrin: UV, FTIR, 1HNMR and molecular modeling studies, Carbohydrate Polymers, 89: 492-496.

[25] Coates J. 2000. Interpretation of infrared spectra, A practical approach, in Encyclopedia of analytical chemistry by Meyers RA (Ed), pp. 10815-10837, John Wiley & Sons Ltd. Chichester.

[26] Quan C & Turner C. 2009. Extraxtion of astaxanthin from shrimp waste using pressurized hot ethanol, Chromatographia.70: 247.

[27] Sanchez AP, Martinez HA, Paviani LC, Cabral FA. 2010. Supercritical CO2 extraction of lipids and astaxanthin from Brazilian redspotted shrimp waste, The Journal of Supercritical Fluids, 56: 164-173.

[28] Rao RN, Alvi SN, Rao BN. 2005. Preparative isolation and characterization of some minor impurities of astaxathin by high performance liquid chromatography, Journal of Chromatography A, 1076: 189-192.

[29] Fasakin, E.A., Balogun, A.M., Fagbenro, O.A., 2001. Evaluation of sun dried Azolla Africana, and duckweed, Spirodela polyrrhiza in practical diets for Nile tilapia, Oreochromis niloticus fingerlings. J. Appl. Aquac. 11: 83–92.

[30] Soliman, A.K., 2000. Partial and complete replacement of soybean meal by roquette (Eruca sativa) seed meal in diets of Nile tilapia, Oreochromis niloticus. In: Fitzsimmons, K.K., Filho, J.C. (Eds.), Proceeding of Fifth International Symposium on Tilapia in Aquaculture (ISTA V), Rio de Janeiro, Brazil, pp. 209–214.

[31] Adebayo, O.T., Fagbenro, O.A., Jegede, T., 2004. Evaluation of Cassia fistula meal as a replacement for soybean meal in practical diets of Oreochromis niloticus fingerlings. Aquac. Nutr. 10: 99-104.

[32] Obasa, S.O., Dada, A.A., Alegbeleye, W.O., 2006. Evaluation of pigeon pea (Cajanus cajan) as a substitute for soya bean meal in the diet of Nile tilapia (Oreochromis niloticus) fingerlings. Afr. J. Aquac. 23: 51-59.

[33] Azaza, M.S., Mensi, F., Ksouri, J., Dhraïef, M.N., Abdelmouleh, A., Brini, B., Kraïem, M.M., 2008. Growth of Nile tilapia (Oreochromis niloticus L.) fed with diets containing graded levels of green algae ulva meal (Ulva rigida) reared in geothermal waters of southern Tunisia. J. Appl. Ichthyol. 24 :202-207

[34] Hendrawati, T. Y., Aristio, L., Hasyim, U. H., Nugrahani, R. A., & Ramadhan, A. I. (2018). The Effects of Adding Aloe Chinensis Baker Gel Extract on the Formulation of Lotion as a Cosmetic Product. ARPN Journal of Engineering and Applied Sciences 13 (23), 9291-9297

[35] Hendrawati, T. Y., Nugrahani, R. A., Utomo, S., & Ramadhan, A. I. (2006). Effect of Evaporation Time on Physical and Chemical Properties of Aloe Vera Gel Extract (Aloe Chinensis Baker). ARPN Journal of Engineering and Applied Sciences 13 (24), 9676-9678

[36] Sari, A. M., & Syamsudin, A. B. (2019). Effect of tofu waste flour composition to hedonic test level and proximate test of Bread. In IOP Conference Series: Materials Science and Engineering (Vol. 674, No. 1, p. 012012). IOP Publishing.

[37] Sari, A. M., Hendrawati, T. Y., & Erdawati. (2018, December). The effect of zinc phosphate for enhanced chlorophyl and caretenoid production by cultivation of algae using Tofu wastewater. In AIP Conference Proceedings (Vol. 2049, No. 1, p. 020071). AIP Publishing LLC.