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Effect of Algal Astaxanthin Powder Supplementation on Growth Performance, Hematological and Biochemical Parameters in Common carp, *Cyprinus carpio* L.

Nasreen M. Abdulrahman

College of Veterinary Medicine, University of Sulaimani, Sulaimaniya, Iraq

*Corresponding author: Nasreen.abdulrahman@univsul.edu.iq

Abstract: This experiment was carried out to evaluate the effect of adding algal astaxanthin in diet on hematological, biochemical parameters, growth performance and chemical composition of common carp. The study was assigned to depend on using natural products as back to nature in fish feeding. T1: Diet without any addition, T2: adding 2.5 g astaxanthin/kg diet, T3: adding 5 g astaxanthin/kg diet and T4: adding 7.5 g astaxanthin/kg diet. Significant differences were observed as astaxanthin added to fish diet in each of weight gain, daily, relative and specific weight gain. The control group was significantly higher in feed conversion ratio (FCR). Treatment with 7.5 g astaxanthin/kg diet had significantly higher effect on each of hemoglobin (Hb), RBC count, hematocrit (HCT), while the control without any astaxanthin showed significantly higher values in each of mean corpuscular volume (MCV), platelets (PLT), procalcitonin (PCT) and mean platelets volume (MPV). No significant differences occurred in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). WBC differential count was significantly higher in adding of 7.5 g astaxanthin, in monocytes account the 5 g astaxanthin was significantly higher and the granulocytes account was higher in each of 5 g and 7.5 g astaxanthin. Algal astaxanthin enhanced the immunity indicators in mean of blood biochemical parameters such as blood sugar, creatine, alanine aminotransferase activity (ALT), aspartate aminotransferase activity (AST), total proteins (TP), albumin (ALB) and globulin (Gl) with increasing astaxanthin levels in fish diet. Astaxanthin showed a positive effect on lipid profile as it decreased each of cholesterol, low density lipoprotein (LDL) and very low density lipoprotein (VLDL), and increased the high density lipoprotein (HDL), without significant differences in triglyceride levels. The relation between fish weight and length and the condition factor according to the total length was significantly higher in each of 5 and 7.5 g astaxanthin. According to standard length and forked length, the control, 5 and 7.5 g astaxanthin were significantly higher. The adding of astaxanthin increased the chemical composition of carp meat in the percentage of each protein, lipids, ash and moisture.

Keywords: Algal astaxanthin, Growth performance, Feed utilization, Blood parameters, Blood biochemical parameters, *Cyprinus carpio*

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Introduction

Numerous studies have shown that astaxanthin has potential health-promoting effects in the prevention and treatment of various diseases, such as cancers, chronic inflammatory diseases, metabolic syndrome, diabetes, diabetic nephropathy, cardiovascular diseases, gastrointestinal diseases, liver diseases, neurodegenerative diseases, eye diseases, skin diseases, exercise-induced fatigue and male infertility as reviewed by Yuan et al. (2010). It has been proven in over 65 clinical studies, featured in over 300 peer-reviewed publications. Among the most clinically substantiated main benefits for human health are those for "eye fatigue relieve" (Yamashita, 2009), "skin aging defence" and anti-photoaging (Tominaga et al., 2012) and "muscle resilience" (Earnest et al., 2011; Yamashita, 2011).

Astaxanthin is used in feed, pharmaceutical, nutraceutical and cosmetic applications (Spolaore et al., 2006). It is the main carotenoid pigment found in aquatic animals and it is present in many of our favourite seafoods including salmons, trout, red seabreams, shrimps, lobsters and fish eggs. It is also present in birds such as flamingos and quails. In many of the aquatic animals in which it is found, astaxanthin has several essential biological functions including protection against oxidation of essential polyunsaturated fatty acids, protection against UV light effects, immune response, pigmentation, communication, reproductive behaviour and improved reproduction (Guerin et al., 2003).

Some microorganisms are rich in astaxanthin; the chlorophyte alga *Haematococcus pluvialis* is believed to accumulate the highest levels of astaxanthin in nature (Orosa et al., 2001). Commercially, grown *H. pluvialis* can accumulate astaxanthin. Astaxanthin is closely related to other well-known carotenoids, such as b-carotene, zeaxanthin and lutein, thus they share many of the metabolic and physiological functions attributed to carotenoids (Orosa et al., 2001). Free astaxanthin is particularly sensitive to oxidation. In nature, it is found either conjugated to proteins, such as in salmon muscle or lobster exoskeleton, or esterified with one or two fatty acids, which stabilize the molecule (Orosa et al., 2001).

Astaxanthin cannot be synthesized by animals and must be acquired from the diet. Although mammals and most fishes are unable to convert other dietary carotenoids into astaxanthin, crustaceans (such as shrimps) and some fish species including koi carp have a limited capacity to convert closely related dietary carotenoids into astaxanthin, although they benefit from being directly fed astaxanthin (Amar et al., 2001). Mammals lack the ability to synthesize astaxanthin or to convert dietary astaxanthin into vitamin A, unlike b-carotene, astaxanthin which has no pro-vitamin A activity in these animals (Amar et al., 2001).

Based on the benefits of astaxanthin, the present experiment was designed to assess the effects of this substance in performance (growth performance and feeding utilization), haematological, biochemical parameters and chemical composition (proximate composition and organoleptic evaluation) of the common carp *Cyprinus carpio* L.

Materials and Methods

Experimental Fishes: The experiment was carried out for 84 days on 72 common carps. Fishes were brought from a commercial fish farm in Daquq, Kirkuk province, Iraq. Fish average weight varied between 59.2 to 66.4 g. Fishes were distributed among experimental plastic tanks with a mean initial weight of fish in each tank as biomass 306-309 g. Laboratory pre-acclimation and feeding with commercial pellets (their chemical composition are seen in Table 1) were for 21 days prior to the real feeding trial. The astaxanthin powder was obtained from Maple Lifesciences Astaxanthin PE Company, India.

Experimental System: Twelve plastic tanks (70 L water) were used in this trial for four treatments (replicates). Chinese's air compressors (Hailea ACO-318) were used for continuous aeration. The replicates were randomly selected to reduce differences among treatments. Remained feeds and feces were removed daily by siphoning method. The experimental trial represented four treatments with three replicates; each with six fishes per replicate as follows:

T1: Diet without any addition, T2: adding 2.5 g astaxanthin/kg diet, T3: adding 5 g astaxanthin/kg diet and T4: adding 7.5 g astaxanthin/kg diet.

Table 1: Chemical composition of the different ingredients in fish diet according to NRC (1993).

Ingredients	Crude protein (%)	Crude fat (%)	Dry matter (%)	Crude fiber (%)	Energy Kcal/kg
Animal protein concentrate	40	5	92.9	2.2	2107
Yellow corn	8.9	3.6	89	2.2	3400
Soybean meal	48	1.1	89	7	2230
Barley	11	1.9	89	5.5	2640
Wheat bran	15.7	4	89	11	1300

Calculated chemical composition	
Crud protein	26.93
Total carbohydrates	39.51
Fat	5.53
Ash	6.19
Gross energy (kcal/kg feed)	2302.9

Diet Formulation: Experimental diets contain standard ingredients found in Sulaimani city markets, enriched with the used levels of astaxanthin, preparing five different diets each contain the wanted level of astaxanthin as mentioned above. Pellets were made by Kenwood Multi-processors and dried at room temperature for four days and crushed to obtained fine particles. Daily feeding with 3% of body weight was achieved twice a day at 9:00 a.m. and 2:00 p.m. Fishes in every tank

were weighed together bimonthly. The feeding levels were then recalculated according to the new weights. The feeding trial continued for 12 weeks.

Growth and Feed Utilization Parameters: Fishes were weighed (g) together for all replicates every two weeks. Feed consumption of each replicate was then readjusted.

Weight gain (g/fish) = Mean of weight (g) at the end of the experimental period – Weight (g) at the beginning of the experimental period. Weight gain (g/fish) = W2–W1 where W_2: Fish weight (g) at the end of experimental period and W_1: Fish weight (g) at the beginning of the experimental period.

Daily weight gain (DWG) (g/day) = Weight gain/ Experimental period, = W2–W_1/ T where T: time between W2 and W1 (84 days).

Relative growth rate (RGR %) = Weight gain/ Initial weight x 100 = W2–W_1/ W_1 x 100 according to Brown (1957).

Specific growth rate (SGR) % = (Ln final body weight–Ln initial body weight)/ experimental period) x 100 = ((Ln W2–Ln W1)/ T) x 100 according to Lagler (1956).

Feed conversion ratio (FCR) = Total feed fed (g)/ Total wet weight gain (g) according to Uten (1978).

Feed efficiency ratio (FER) = Total weight gain (g)/ Total feed fed (g) according to Uten (1978).

Protein efficiency ratio (PER) = Total wet weight gain (g/fish)/ Amount of protein fed (g/fish) according to Uten (1978).

Proximate Composition: All fish samples were used for the chemical analysis of the muscle (percentage of moisture, crude protein, ether extract and ash) according to A.O.A.C. (2000) analytical methods.

At the end of the experimental period, three fishes were randomly taken from each experimental group. All fish samples were weighed and their length was measured individually. Blood samples from each fish of the different groups were collected by cutting the caudal vein. Whole blood samples were collected in small plastic vials containing heparin and stored under cooling condition at refrigerator temperature (Al-Koye, 2013).

Complete Blood Count: Erythrocyte count (RBCs: 10^{12} cells/l), mean corpuscular hemoglobin (MCH; pg), mean corpuscular hemoglobin concentration (MCHC, g/dl), mean corpuscular volume (MCV, fL), hemoglobin (Hb, g/l) and platelets (PLT, 10^9 cells/l). Leukocyte count (10^9 cells/l), and % granulocytes, lymphocytes and monocytes.

Biochemical Parameters: Alanine aminotransferase activity (ALT), aspartate aminotransferase activity (AST), total proteins, globulin (g/dl), albumin (g/dl) and albumin/ globulin (A/ G) ratio.

All blood tests were done by using a hematological analyzing device type ACCENT 200 Poland's origin. The levels of enzymes were assayed according to the instructions provided with the corresponding enzymatic kits.

Statistical Analysis: The trial was conducted by one way (ANOVA) with completely randomized design (CRD) and general linear models (GLM) procedure of XLSTAT 2016 Version.02.28451. Duncan's test was used for comparison among treatment means at $P<0.05$.

Results and Discussion

Significant differences were observed as astaxanthin added to fish diet as shown in Table 2 in each of weight gain, daily, relative and specific weight gain. Control was significantly higher in FCR.

The diet 7.5 g astaxanthin/kg had significantly ($P<0.05$) higher effect on each of HGB, RBC count and HCT as shown in Table 3, while the control without any astaxanthin was significantly ($P<0.05$) higher in each of MCV, PLT, PCT and MPV. No significant differences ($p>0.05$) were noted in both MCH and MCHC.

Algal astaxanthin enhanced the immunity indicators in mean of blood biochemical parameters such as blood sugar, creatine, ALT, AST, TP, ALB and globulin with increasing astaxanthin levels in fish diet as observed in Table 4.

The WBC differential count was significantly higher ($P<0.05$) in case of adding 7.5 g astaxanthin, while in monocytes count, the 5 g astaxanthin resulted in higher significance, and the granulocytes count was higher in each of 5 g and 7.5 g astaxanthin as shown in Table 5.

Results in Table 6 showed that astaxanthin has a positive effect on lipid profile in which it decreased case of cholesterol, LDL and VLDL, and increases the HDL, without significant differences ($P<0.05$) in triglyceride levels.

The relation between fish weight and length appeared in Table 7. The condition factor according to the total length was significantly higher in each of 5 and 7.5g astaxanthin. According standard length and forked length, the control, 5 and 7.5g astaxanthin were significantly higher.

The results in Table 8 indicated that adding of astaxanthin increased the chemical composition of carp meat in percentage of protein, lipids, ash and without significance in moisture.

Table 2: Effect of three levels of algal astaxanthin on growth performance and feed utilization of *C. carpio*.

Treatments	Initial weight	Final weight	Weight gain	Daily weight gain	Relative weight gain	Specific weight gain	Survival	FCR	FER
T1 Control	309.000^{a}	368.067^{b}	59.067^{cd}	4.933^{c}	19.209^{cd}	0.159^{bc}	93.333^{a}	3.524^{a}	29.349^{c}
T2 (2.5 g astaxanthin)	309.667^{a}	373.934^{ab}	64.267^{c}	5.522^{b}	25.792^{c}	0.165^{bc}	100.000^{a}	2.886^{a}	31.054^{bc}
T3 (5 g astaxanthin)	308.333^{a}	380.866^{b}	72.533^{b}	6.128^{a}	32.639^{b}	0.201^{b}	93.333^{a}	2.635^{a}	37.451^{b}
T4 (7.5 g astaxanthin)	306.667^{a}	392.534^{a}	85.867^{a}	6.989^{a}	41.860^{a}	0.291^{a}	80.000^{b}	2.163^{a}	44.543^{a}

Different letters in one column mean significant differences at P<0.05.

Table 3: Effect of three levels of algal astaxanthin in some of blood picture of *C. carpio*.

Treatments	Hb (g/l)	RBC	HCT (%)	MCV	PLT	PCT	MCH	MCHC	MPV
T1 Control	116.500^{c}	1.123^{c}	47.075^{c}	145.075^{a}	42.000^{a}	0.045^{a}	61.500^{a}	251.000^{a}	10.475^{a}
T2 (2.5 g astaxanthin)	125.000b^{c}	1.313^{b}	49.625^{c}	135.675^{b}	35.000^{b}	0.037^{b}	61.150^{a}	245.500^{a}	8.375^{b}
T3 (5 g astaxanthin)	131.000^{b}	1.595^{ab}	53.925^{b}	130.350^{bc}	33.250^{b}	0.031^{b}	71.150^{a}	284.500^{a}	8.900^{b}
T4 (7.5 g astaxanthin)	139.750^{a}	1.878^{a}	61.450^{a}	129.800^{bc}	30.000^{bc}	0.029^{ab}	62.650^{a}	252.500^{a}	8.800^{b}

Different letters in one column mean significant differences at P<0.05.

Table 4: Effect of three levels of algal astaxanthin in some blood biochemical parameters of *C. carpio*.

Treatments	Blood sugar (mg/dl)	Creatinine (mg/dl)	ALT (IU/l)	AST (IU/l)	TP (g/dl)	Albumin (g/dl)	Globulin (g/dl)
T1 Control	2.91^{b}	0.583a^{b}	24.17^{bc}	429.77^{bc}	26.793^{b}	1.385^{bc}	20.365^{c}
T2 (2.5 g astaxanthin)	4.08^{ab}	0.523^{ab}	26.050^{bc}	448.925^{bc}	29.430^{b}	1.403^{bc}	25.018^{bc}
T3 (5 g astaxanthin)	3.53^{b}	0.700^{ab}	34.92^{b}	453.700^{b}	30.255^{ab}	1.688^{b}	28.568^{b}
T4 (7.5 g astaxanthin)	5.82^{a}	1.273^{a}	39.47^{a}	569.125^{a}	35.315^{a}	1.950^{a}	33.675^{a}

Different letters in one column mean significant differences at P<0.05.

Table 5: Effect of three levels of algal astaxanthin in differential WBC of *C. carpio*.

Treatments	WBC	Lymphocyte count	Monocyte count	Granulocyte count
T1 Control	218.800^{bc}	20.475^{b}	76.600^{ab}	119.225^{b}
T2 (2.5 g astaxanthin)	228.875^{bc}	24.450^{ab}	70.825^{b}	121.600^{b}
T3 (5 g astaxanthin)	281.300^{b}	28.150^{a}	78.725^{a}	126.325^{ab}
T4 (7.5 g astaxanthin)	325.175^{a}	29.825^{a}	74.600^{b}	129.750^{a}

Different letters in one column mean significant differences at P≤0.05.

Table 6: Effect of three levels of algal astaxanthin in lipid profiles of *C. carpio*.

Treatments	Cholesterol (Mmol/l)	Triglyceride (Mmol/l)	LDL (Mmol/l)	HDL (Mmol/l)	VLDL (Mmol/l)
T1 Control	3.478^{a}	1.850^{a}	0.980^{a}	1.025^{b}	0.310^{c}
T2 (2.5 g astaxanthin)	2.638^{b}	1.475^{a}	0.723^{ab}	2.350^{b}	0.295^{bc}
T3 (5 g astaxanthin)	2.640^{b}	1.825^{a}	0.683^{ab}	2.850^{ab}	0.265^{b}
T4 (7.5 g astaxanthin)	2.513^{b}	1.875^{a}	0.543^{b}	2.990^{a}	0.225^{a}

Different letters in one column mean significant differences at P<0.05.

Table 7: Effect of three levels of algal astaxanthin in condition factor of *C. carpio*.

Treatments	Condition factor (total length)	Condition factor (standard length)	Condition factor (forked length)
T1 Control	1.41^{b}	2.87^{a}	2.06^{ab}
T2 (2.5 g astaxanthin)	1.45^{b}	2.42^{b}	1.91^{b}
T3 (5 g astaxanthin)	1.55^{a}	2.87^{a}	2.18^{a}
T4 (7.5 g Astaxanthin)	1.55^{a}	2.95^{a}	2.01^{ab}

Different letters in one column mean significant differences at P<0.05.

Table 8: Effect of three levels of algal astaxanthin in proximate analyses of *C. carpio*.

Treatments	Protein (%)	Lipids (%)	Ash (%)	Moisture (%)
T1 Control	19.312^{b}	5.351^{b}	1.243^{a}	71.112^{a}
T2 (2.5 g astaxanthin)	20.432^{ab}	6.011^{ab}	1.274^{a}	71.321^{a}
T3 (5 g astaxanthin)	21.648^{ab}	6.369^{ab}	1.401^{a}	71.521^{a}
T4 (7.5 g astaxanthin)	21.774^{a}	6.442^{ab}	1.494^{a}	71.612^{a}

Different letters in one column mean significant differences at P<0.05.

Sadraddin et al. (2019) showed a significant difference between treatments in terms of health indices, including the gonadosomatic index (GSI), growth performance including weight gain, relative and specific growth rate. Diets containing higher levels of astaxanthin powders improved the growth performance, biological status and GSI in *C. carpio* (Sadraddin et al., 2019) and these agree with the present results.

The biological and physiological status of fishes are a key factor underlying the attainment of the required performance. The results of regarding the biological

status of fishes which become an integral part of the routine observation of health, and have a potential position to interpret the results of feeding trials. The differences in the color intensity, caused by natural and artificial pigments, are due to the quality, concentration and the absorption period of these materials (Shapoori et al., 2012).

The effects of carotenoid supplementation in some fish species was achieved in gilthead seabream *Sparus aurata* by Scabini et al. (2011), yellow tail cichlid *Pseudotropheus acei* by Güroy et al. (2012) and goldfish *Carassius auratus* by Tizkar et al. (2013). The carotenoid enriched diets could lead to an increase in gonad weight of bighead catfish *Clarias macrocephalus* as demonstrated by Chainapong & Traichaiyaporn (2013).

Generally, increased lysozyme activity in the serum and mucus of fishes can be indicative of the immune system stimulation and improvement of the immune response. By enhancing the complement system and lysozyme, carotenoid pigments increase the total number of leukocytes and phagocytes, and thereby cause the stimulation of the immune system, increased immunity and resistance to pathogens. Wang et al. (2015) reported that dietary astaxanthin in Pacific white shrimp *Litopenaeus vannamei* significantly affects the hemolymph immunological index, including total hemocyte counts, phagocytic activity of hemocytes, serum anti-superoxide radical activity, serum phenoloxidase activity, serum antibacterial activity and serum bacteriolytic activity.

Astaxanthin is reported to improve growth performance of fishes with the reason that carotenoids may exert a positive influence on intermediary metabolism in aquatic animals. There are different views about the effect of carotenoid pigments on growth factors of different fish species. While some results indicated that these pigments cannot improve growth factors in rainbow trout *Oncorhynchus mykiss*, other researchers suggested the positive effect of these pigments on growth improvement (Sheikhzadeh et al., 2012). This difference may be attributed to fish species, development stages, and type of carotenoid as seen in the results of the present study.

Kalinowski et al. (2011) suggested that astaxanthin powder could enhance lipid utilization in whole fish and liver, providing more energy and consequently enhancing growth performance. The relative growth rate was higher in fishes feed astaxanthin powder throughout the experiment. In addition, they might lead to negative effects on the taste of food, physical quality of the pellets and nutrition balance of diets (Lim, 1989). However, the degree of this effect naturally depends on the feeding habit of the fish and the preparation of the diet.

Many earlier studies have reported that dietary astaxanthin has no significant influence on growth and flesh composition of fishes (Yi et al., 2014). Some factors such as environmental factors, especially due to the coldness of the fish, seasons, salinity, photoperiod, temperature, density, physiological parameters, species, reproductive cycle, puberty status, age, gender, nutritional conditions, sampling time and method, the accuracy and sensitivity of measurement methods can affect

growth factors and survival and make a difference in the interpretation of researchers (Tukmechi et al., 2011).

Conclusion

According to the results obtained, adding of astaxanthin with the used levels of the present study have different effects on fish performance, so we recommend to use more levels and more traits to show their immunity modulating effects.

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