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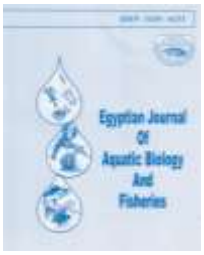

The role of dietary astaxanthin in European sea bass (*Dicentrarchus labrax*) growth, immunity, antioxidant competence and stress tolerance

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ABSTRACT

European sea bass (*Dicentrarchus labrax*) fingerlings (0.4 ± 0.05 g initial weight) were fed on 4 diets contain 0, 60, 80 and 100 mg axtaxanthin /kg diet for 60 days. Results showed marked enhancement in fish growth, feed utilization efficiency, survival rate and fish protein content when 100 mg astaxanthin was supplemented in fish diet. The activities of hepatic antioxidation enzymes; superoxide dismutase (SOD) and glutathione peroxidase (GPx) have reduced as the level of dietary astaxanthin increased. Results showed simultaneous gradual increase in intestinal mucosal phagocytic and lysozyme activities as astaxanthin inclusion level elevates in diets indicating effective role of astaxantin as an immunostimulant agent in sea bass diet. By the end of the trial, fish were exposed to a sudden drop in water salinity (37 to 0.3‰) and that continued for 24 hours period. Survival rate was significantly the highest in fish that consumed 100mg/kg diet and when compared with control group an increment of 36.9% was recorded suggesting an improvement in fish tolerance against osmotic stress. Results demonstrate that astaxanthin is a qualified feed additive for sea bass.

INTRODUCTION

Adequate nutrition is considered critically crucial for preserving healthy status of fish and for its resistance against diseases outbreak. Supplemental antioxidants reduce the effect of stressors and repair DNA, protein and lipid oxidative damage. In addition, supplemental antioxidants boost immunity and preserve the metabolic equilibrium towards anabolism. To achieve these specific aims, complementary supplemental antioxidants usage has become essential (Aklakur, 2016). Seabra and Pedrosa (2010) mentioned that astaxanthin is a cartenoid classified as a xanthophyll, it is extracted mostly from red yeast *Xanthophyllomyces dendrorhous* and green microalgae *Haematococcus pluvialis* and it is considered as an effective antioxidant agent. Also, they added that farmed fish often cannot synthesize astaxanthin *de novo*, so it is necessary to add it to their own diet.

Xie *et al.* (2017) claimed that astaxanthin supplemented in diet can enhance the golden pompano (*Trachinotus ovatus)* growth performance and hepatic antioxidant ability, not only in vivo, but also in vitro by terminating the reactive oxygen.

Earlier studies have reported that adding 30–50 mg axtaxanthin kg^{-1} to the diet for the rainbow trout (*Oncorhynchus mykiss*) could significantly diminish oxidative stress induced by oil, transaminase effectiveness and serum triglyceride (Nakano *et al*. 1995, 1999). Also, Rahman *et al*. (2016) suggested that adding 50 mg astaxanthin Kg^{-1} fish diet may be sufficient for enhancing juvenile rainbow trout antioxidation capability and that may affect positively human health; however, fish growth rates and muscular composition were not influenced by dietary astaxanthin concentrations. Salarzadeh and Rajabi (2015) concluded that at least 100 mg astaxanthin/kg is recommended in post larval white leg shrimp (*Litopenaeus vannamei*) diet to get positive effect on growth and survival. Smith *et al.* (2013) concluded that astaxanthin supplementation in fish diet improves flesh color and neutralizes reactive oxygen species (ROS) in order to protect the fish, so is considered as an effective antioxidant. Concerning the effect of astaxanthin supplementation on fish immunity, Li et *al.* (2014) did not assure if astaxanthin was able to improve it.

Using of intestinal mucus as an indicator of fish immunity was reviewed in many teleost fish by Salinas and parra (2015). Press and Evensen (1999) used intestinal mucosa for assessment of fish immunity where it is rich in immune cells; lymphocytes, granulocytes, plasma cells, eosinophilic and macrophages and accordingly it can stimulate immunological responses.

The current research aims at comparing the impacts of different levels of astaxanthin supplementation in diets on fish growth, feed efficiency, biochemical fish composition, activity of antioxidation enzymes, modulating immune responses of sea bass (*D. labrax*) and osmotic stress tolerance.

MATERIALS AND METHODS

Experimental protocol and diet preparation

Sea bass were gotten from El-Anfoushy marine fish hatchery (National institute of Oceanography and Fisheries, NIOF) and transported to fish nutrition Lab., Aquaculture Division (NIOF), Alexandria, Egypt. Fish were acclimatized to experimental conditions and they were fed on commercial diet for one week. At the beginning of the experiment, the fish (average initial body weight was 0.40 ± 0.05 g) were distributed in 12 tanks (200 L capacity / tank) with a stocking density of 50 fish per tank. The tanks were in an open system and continuously supplied with filtered sea water of 37 ppt salinity and water renewal was set 3 times total volume per day. Water temperature was 22.4 ± 1.2^{o} C. Oxygen was supplied by aeration with the minimum level observed during trial being 6.6 mg L^{-1}. Water quality parameters were recorded according to the standard methodology of APHA (1995) throughout the experimental period (pH: 7.5 ± 0.21, total ammonia: 0.02 ± 0.01 and nitrite 1.7 ± 0.06 mg/l). The natural photoperiod was 11 h light: 13 h dark throughout the feeding experiment.

Four diets were composed to fulfill fish dietary requirements and the test diets were slowly provided 3 times a day to visual satiation, at 08:00, 13:00 and 17:00 per day. All ingredients were milled well, screened and mixed together and then oil was added. The warm water was added slowly to form dough and pellets were then formulated in a proper size using a mincer. Pellets were dried in an oven adjusted at 60ºC for 24h and finally kept at -20°C until further use. Experimental diets were formulated (Table 1) as: astaxanthin-free added diet (control, CTR) and another 3 diets which contain ascending levels of astaxanthin (Carophyll ® Pink, DSM): 60

(AX1), 80 (AX2) and 100 (AX3) mg kg^{-1} diet. The four diets were tested on triplicate basis.

Table 1: Ingredients and proximate composition (% DM) of CTR diet as fed to European sea bass (*D. labrax*) for 60 days.

Ingredients	g/Kg
Fish meal[1] (70%)	450
Squid meal [2] (72%)	150
Soybean meal (44%)	165
Wheat flour	110
Fish oil	75
Vitamins and Minerals mix[3]	50
Proximate analyses (%DM)	
Crude protein (CP)	48.95
Total Lipids (L)	16.64
Ash	9.50
Fiber	2.45
Nitrogen Free Extract[4] (NFE)	22.46
Gross energy[5] (GE, KJ g^{-1})	21.99

[1, 2] lab made

[3] Vitamins and minerals premix (mg kg^{-1}): p-amino benzoic acid (9.48); D-biotin (0.38); inositol (379.20); niacin (37.92); Ca pantothenate (56.88); pyridoxine HCl (11.38); riboflavin (7.58);thiamine HCl (3.79); L-ascorbyl-2-phosphate Mg (APM) (296.00); folic acid (0.76); cyanocobalamine (0.08); menadione (3.80), vitamin A palmitate (17.85); a-tocopherol (18.96); calciferol (1.14). K2PO4 (2.011); Ca3 (PO4)2 (2.736); Mg SO47H2O (3.058); NaH2PO4 2H2O (0.795).

[4]Nitrogen-free extract (NFE) =100 - [%Ash+%lipid+%protein+%Fiber].

[5]GE (kJ g^{-1}) = (protein content×23.6) + (Lipid content×39.5) + (carbohydrate content×17.2)

Growth and feed utilization

Fish growth was evaluated depending on weight gain (WG, g), weight gain rate (WG, %), specific growth rate (SGR, % day^{-1}) parameters. Feed utilization was expressed as follows: feed conversion ratio (FCR), Relative feed intake (RFI, % BW day^{-1}), protein efficiency ratio (PER) and protein productive value (PPV). Survival rate was also recorded (S%).

The following formulae were used:

WG = final fish weight (W_f) - initial fish weight (W_I)

WG (%) = $[(W_f - W_I)/ W_I] \times 100$

SGR = $[(\ln W_f) - (\ln W_I)] /$ feeding trial days × 100

FCR = feed consumed /WG

RFI = 100 × Total feed consumed/ $[(W_I + W_f)/2]$ /feeding trial days.

PER = WG /protein consumed

Protein productive value (PPV) = protein gain/protein consumed × 100

Fish survival rate (S %) = (final count / initial count) × 100

Proximate analyses

At the end of the experiment, fish feeding was stopped for 24 h prior fish weighing and sampling to avoid the gut contents interference during the analyses. After fish anesthetization by clove oil (100 mg L^{-1}), they were counted and weighed per tank. Fifteen fish were randomly chosen from each tank then they were pooled and stored at -20°C for biochemical composition analyses. Contents of moisture, lipid, protein and ash were all analyzed according to AOAC (1995) methodology. Diet and fish were analyzed in triplicate (Tables 1, 2).

Collection of tissue samples

After fish were anesthetized and weighed, 15 fish from each tank were directly killed by decapitation and then dissected on ice. Liver and intestine were quickly excised, pooled each and stored at 4^{o}C for later analyses within half an hour.

Determination of hepatic total protein and antioxidative enzymatic activities

Pooled liver sample from each tank was homogenized (Wiggen Hauser, Berlin, Germany) in 5 to 10 ml ice-cold buffer solution (50 mM K_3PO_4, pH = 7.5, 1 mM EDTA) for each gram of liver. After homogenization, samples were centrifuged (100,000 xg, 4 °C) for 15 minutes and consequently, the aliquots of supernatants obtained after centrifugation were used for the protein determination (Bradford, 1976), superoxide dismutase (Marklund and Marklund, 1974) and glutathione peroxidase (Paglia and Valentine, 1967).

Total protein

Hepatic total protein was measured using a protein assay kit (No. B6916, Sigma) and bovine serum albumin was implemented as a standard (BSA, 66 kDa, Sigma). One analysis used 200-µl sample following the manufacturer instructions and in this analysis protein concentration was expressed by µg/ml.

***Superoxide dismutase* (SOD)**

` 20 µl of liver homogenate (test) or buffer (reference) and 10µl pyrogallol were added to 1 ml buffer solution. The absorbance of test or reference was read at 420 nm after 30 and 90 seconds. One unit of SOD activity is defined as the amount required for inhibiting pyrogallol autoxidation by 50% per min. The activity of SOD was expressed as unit mg^{-1} protein.

Glutathione peroxidase (GPx)

Antioxidant enzyme glutathione peroxidase activity was determined using glutathione reductase and NADPH. The method is based on the oxidation of NADPH at 25^{o}C, which is indicated by the decrease in absorbance at 340 nm. One unit of enzyme activity is defined as µmol NADPH oxidized min^{-1}mg protein^{-1}.

Determination of some immunological indices in fish intestinal mucosa

The intestine of the fifteen fish which were killed and directly slit lengthways were collected and then washed with phosphate-buffered saline (0.1 M, pH =7.4). The mucus was carefully scraped using a rubber spatula and then was put in formerly weighed micro-centrifuge tubes. Sodium phosphate buffer solution (2.7 mM Na_2HPO_4/1.3 mM NaH_2PO_4; 0.004 M, pH = 7.2) was pipetted to tubes carefully. The suspension was then centrifuged (10 000 xg, 4^{o} C) for 20 min and the supernatant was then stored at -80^{o} C until further analysis.

Determination of phagocytic activity

Mucosal phagocytic activity was measured following Puangkaew *et al.* (2004) procedure with slight modifications. Aliquots of 0.5 ml containing 10^7 cells ml^{-1} in L-15 medium supplemented with PS-G (polysaccharide from *G. lucidum*) were seed onto 20 mm diameter glass coverslips in 6-well plates (Nunc, Roskilde, Denmark). The phagocyte monolayer was incubated with 10 µl of 10^9 colony-forming units, CFU ml^{-1}, at the desired multiplicity of infection (MOI), for 1 h at 22^{o} C. The cells were stained with Diff Quick solution (Panreac, Spain) after washing with sodium phosphate buffer. One hundred leucocytes with phagocytic capability per slide were counted and the phagocytic capacity was determined as the percentage of cells with phagocytic ability. All samples were analyzed in triplicate.

Lysozyme activity assay

Turbidimetric method (Ellis, 1990) with slight modifications was used for the determination of lysozyme activity based on the ability of lysozyme to lyse the

bacterium *Micrococcus lysodeikticus*. A suspension of *M. lysodeikticus* (0.2 mg/ml 0.05 M sodium phosphate buffer, pH = 6.2) was mixed with varying sample amounts (10-200 µl) to give 2 ml as a final volume in 96-well microtray. The microtray was incubated at 25^{o} C and the absorbance was measured at 530 nm after 0.5 and 4.5 min. Lysozyme activity unit is defined as the amount of sample which causes absorbance depletion by 0.001 min^{-1}.

Osmotic stress test

At the end of the feeding trial, 15 fish from each tank were transferred to 100 L aerated glass aquarium filled with fresh water to be exposed to a sudden change in water salinity (37 to 0.3‰) for 24 hours and then survival rates were recorded.

Statistical analysis

The results are expressed as mean ± standard error. Data were analyzed by one-way analysis of variance (ANOVA) to calculate the statistical significance of data, using statistical package for social sciences (SPSS) software (version 20.0). Post hoc analysis was used and then Tukey test was chosen to compare the means. If $P<0.05$, then the difference was considered significant.

RESULTS

Fish growth and feed efficiency

Growth and feed efficiency indices are illustrated in Table (2). Survival rate was improved when astaxanthin was supplemented to fish diets at all supplementation levels. WG, WG % and SGR values increased significantly ($P<0.05$) in fish that consumed AX3 diet relative to the other groups ($P<0.05$). Results showed marked enhancement in feed utilization indices (FCR, PER and PPV) in fish that consumed AX3 diet. In general, the growth and the utilization of diets were improved in fish fed diets that comprised astaxanthin as a feed additive (AX1, AX2 and AX3) when compared with fish fed CTR diet.

Table 2: Growth and feed utilization indices (mean ±SE) of sea bass (*D. labrax*) fed astaxanthin (AX) supplemented diets for 60 days.

Parameter	Diets			
	CTR	AX1	AX2	AX3
Initial body Weight (g)	0.38±0.04	0.40±0.01	0.39±0.02	0.38±0.03
Final body Weight (g)	2.43±0.24b	2.77±0.15b	3.33±0.20ab	3.80±0.17a
Weight gain (g)	2.06±0.13b	2.36±0.13b	2.94 ±0.21ab	3.42 ±0.18a
Specific growth rate (% d^{-1})	3.10±0.08b	3.21 ±0.03b	3.57 ±0.13ab	3.82 ±0.09a
Weight gain rate (%)	545.57±31.34b	585.19±13.66b	756.61±64.78ab	892.13±52.55a
Feed conversion ratio (FCR)	1.69 ±0.05a	1.37 ±0.06b	1.35 ±0.02b	1.29 ±0.06b
Protein efficiency ratio (PER)	1.23±0.04b	1.53±0.06a	1.54±0.02a	1.62±0.08a
Protein productive value (PPV, %)	16.18±0.76b	18.83±1.49b	21.01±0.43ab	25.74±1.17a
Relative feed intake (% BW day^{-1})	4.12±0.66a	3.40±0.12b	3.56±0.47b	3.52±0.13b
Survival rate (%)	94.0±1.1b	95.3±2.1ab	96.8±1.4ab	98.7±1.2a

Different letters represent significant difference ($P < 0.05$) within each row.

Biochemical body composition

No marked variations (Table 3) in moisture and whole body lipid contents were recorded amongst all dietary fish groups. However, fish that consumed AX3 diet showed a significant ($P<0.05$) increase in protein content (16.33%) as compared with all tested groups.

Table 3: Biochemical composition (mean ±SE) of sea bass (*D. labrax*) fed astaxanthin (AX) supplemented diets for 60 days.

	CTR	AX1	AX2	AX3
Moisture	73.48±1.93	74.27±1.89	74.06±2.33	73.28±2.43
Protein	13.93±0.82b	14.24±0.42b	14.18±0.37b	16.33±0.24a
Lipid	6.54±0.18	5.98±0.16	6.56±0.20	6.13±0.13
Ash	6.07±0.10	5.67±0.26	5.18±0.17	5.22±0.97

Different letters represent significant difference ($P < 0.05$) within each row of data.

Hepatic antioxidation enzymes activity and protein concentration

Fish fed CTR diet registered the highest SOD and GPx enzymes activity and the activity for both decreased as the astaxanthin inclusion level increased in diets ($P<0.05$). SOD and GPx activities in fish fed CTR and AX1 diets were insignificantly different ($P> 0.05$). No marked differences in protein contents were observed among all fish groups (Table 4).

Immune response

Table (4) shows a direct relationship between both lysozyme and phagocytic activities and the supplementation level of astaxanthin in fish diets. Fish fed on basal diet showed the least activities ($P < 0.05$) of lysozyme and phagocytic activities as compared with other experimental diets. Data show that there are no significant variations ($P> 0.05$) between fish fed AX2 and AX3 diets in both measured immunity parameters.

Table 4. Antioxidation enzymes and some immunological parameters (mean ±SE) of sea bass (*D. labrax*) liver and intestine fed astaxanthin (AX) supplemented diets for 60 days

Organ	Parameter	CTR	AX1	AX2	AX3
Liver	SOD	65.2±3.80a	57±3.24a	42.4±3.11ab	32.8±3.77b
	GPx	59.20±3.85a	56.60±6.19a	43.80±4.04ab	31.0±3.48b
	Protein	209.6±14.08	202.2±11.83	213.0±12.10	219.4±18.02
Intestine	PA%	36.40±3.09b	48.20±4.24ab	49.4±4.60ab	58.2±3.92a
	Lysozyme	281.8±13.11b	293.8±15.43b	366.0±14.93a	396.4±14.81a

SOD and GPx activities are expressed as U mg^{-1} protein.
Different letters represent significant difference ($P < 0.05$) within each row.

Osmotic stress test

The survival rate (%) increased (Fig.1) as astaxanthin concentration in diets was increased and the best survival rate (70.2%) was noticed in fish fed AX3 diet which is 36.9 % more than in CTR group (33.3%).

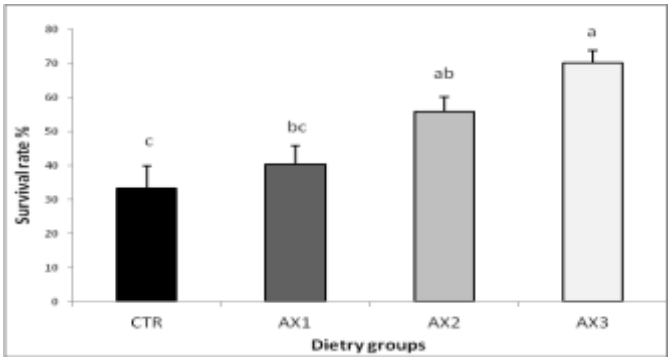

Fig 1: Survival rates of sea bass (*D. labrax*) fed astaxanthin (AX) supplemented diets after exposure to osmotic stress.

DISCUSSION

Growth and fish composition

The present results showed that astaxanthin supplementation, at all inclusion levels, positively influenced the fish performance where it improved the final body weight, feed utilization indices and survival rates of tested fish groups as compared with CTR group. Former researches have suggested that astaxanthin could not enhance the growth of many fish species as Atlantic salmon (*Salmo salar*), gilthead sea bream (*Sparus aurata*), red porgy (*Pagrus pagrus*), Atlantic cod (*Gadus morhua*), Arctic char (*Salvelinus alpinus*) and as well as rainbow trout (*Oncorhynchus mykiss*) (Storebakken and Goswami 1996; Gomes *et al*. 2002; Tejera *et al*., 2007; Sawanboonchun *et al*., 2008; Mansour *et al*., 2006; Sheikhzadeh *et al*., 2012 respectively). In contrast, Chen *et al*. (2012) and Li *et al*. (2014) recorded an enhancement in weight gain for large yellow croaker (*Pseudosciaena crocea*) when astaxanthin was supplemented to fish diet. Also, Weigeng *et al*. (2011) mentioned that adding astaxanthin to the diet of black tiger shrimp (*Penaeus monodon*) increased weight gain and specific growth weight compared with CTR group.

In the present study, the significant growth and feed utilization improvement in fish fed AX3 diet coincided with a significant increment in carcass protein content. This improvement may be explained according to Segner *et al*. (1989) and Amar *et al*. (2001) who suggested that carotenoids may have a positive effect on the intermediary metabolism of fish leading to the enhancement of nutrient utilization and the improvement in growth. The other possible explanation is that astaxanthin adjusts the capabilities of intestinal flora to break down the indigestible components as to extract more nutrients and to motivate the activity of digestion enzymes (James *et al*., 2006). Kalinowski *et al*. (2011) noticed significant lower lipid percentage in red porgy (*Pagrus pagrus*) that was fed diets containing astaxanthin when compared with fish fed on the control diet and concluded that astaxanthin improved lipid utilization and consequently supplied excess energy and enhanced growth performance. In contrast, Xie *et al*. (2017) concluded in their study that there were no significant differences in the whole-body composition of golden pompano (*Trachinotus carolinus*) fed 0 and 200 mg astaxanthin/kg diet. The effects of axtaxanthin on fish growth and their whole body biochemical composition are still dialectical and more studies are required to reveal the mechanisms which lead to such positive influence.

Production of oxygen reactive enzymes and immunity assessment

The ultimate equilibrium shift towards oxidants rather than antioxidants is called oxidative stress and this cellular structures disruption in reducing and oxidizing (redox) potential leads to cell damage and consequently slow growth, immune repression and accordingly pathological symptoms. Therefore, regulation of redox status is pivotal for cell' viability and the functions of organs (Aklakur, 2016). According to Smith *et al*. (2013) various biovital activities of astaxanthin are attributed to its antioxidant properties, as it possesses effective oxygen quenching activity, thus sharing it in the protection of organisms against ROS (Shimidzu *et al*., 1996).

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) are effective antioxidant enzymes that scavenge superoxide anions (O^{2-}) and protect cells against oxidative stress. According to Campa-Córdova *et al*. (2002 a, 2002 b), SOD is used to indicate fish immune response. In the present experiment, a gradual depletion in SOD and GPx activities were perceived as the concentration of dietary astaxanthin

increased. Additionally, a significant quenching of endogenous enzymes was recorded when fish were fed AX2 and AX3 diets indicating that the sea bass antioxidation system was improved by astaxanthin diet supplementation. The present results are parallel to Li *et al*. (2014) who found that activities of both SOD and GPx decreased as the level of astaxanthin increased in diets and the highest activities were in yellow croaker (*Larimichthys polyactis*) which fed CTR diet. Also, Yang (2010) results showed that, SOD activity in pacific white shrimp (*Litopenaeus vannamei*) muscles was depleted significantly if compared to the control group when the organism consumed diet supplemented with yeast. He concluded that this reduction of antioxidant enzymatic activity may be attributed to depletion of free radical efficiency and oxidative stress depending on the principle that when the oxidative stress is depleted then less antioxidant enzymes are created (Rahmat *et al*., 2006). Moreover, Tocher *et al*. (2002) interpreted SOD activity decrease in *Sparus aurata* fed Vitamin E supplemented diets as a probable indication of a lowering requirement to superoxide radical detoxification.

As astaxanthin is considered as an antioxidant, this suggests that it should participate in immunomodulation. In the current study, astaxanthin supplementation to fish diets was found to increase both lysozyme and phagocytic activities, so it may be considered as a method of cell's protection against excess free radicals and accordingly enhance the production of specific immune responses. Dietary carotenoids can enhance immunity, survival rate and play a role in the prevention against pathogens in many fish species; *Oncorhynchus mykiss* (Amar *et al*., 2004), *Cyprinus carpio* (Anbazahan *et al*., 2014; Sowmya and Sachindra 2015) and *Larimichthys crocea* (Li *et al*., 2014). Furthermore, Amar *et al*. (2004) found that lysozyme activity and phagocytic indices are increased in rainbow trout that consumed astaxanthin in their diets. Also, carotenoids' immunostimulant effects were recorded in *Marsupenaeus japonicus* (Chien and Shiau, 2005), *Penaeus monodon* (Supamattaya *et al*., 2005), *Macrobrachium rosenbergii* (Angeles *et al.*, 2009) and *Litopenaeus vannamei* (Chuchird *et al.*, 2015). This enhancement in organism immunity may be attributed to carotenoids inducement of phagocytic cell activity alike almost of the immunostimulants. In contrast, Thompson *et al.* (1995) mentioned that astaxanthin possesses a limited role as immunostimulator in rainbow trout (*Oncorhynchus mykiss*) diet. Contradiction between these results might be due to that the physiological response to any feed additive is species-specific and might also be due to differences in experimental conditions.

In the current study, the fish survival rate was improved by adding astaxanthin to the fish diet and this improvement can be explained by the enhancement in antioxidantion and immunity parameters when fish were fed astaxanthin as a feed additive. The results of Palma *et al.* (2017) demonstrate a significant benefit of astaxanthin in terms of improved growth and survival of long snout seahorse (*Hippocampus guttulatus*). Chien and Jeng (1992) mentioned an affirmative interconnection between tissue pigment intensity and survival rate of kuruma prawn (*Marsupenaeus japonicas*) when astaxanthin was used as a feed additive. Furthermore, the survival rate of the tiger shrimp post larvae was increased due to the supplementation of astaxanthin in their diet (Thongrod *et al*., 1995).

Osmotic stress test

Sea bass, in the present study, tolerated osmotic stress efficiently when fish were fed astaxanthin supplemented diets and maximum survival rate was registered in fish that consumed AX3 diet. Growing resistance to osmotic pressure, as astaxanthin was added to fish diet, may be due to its antioxidant action (Shimidzu *et*

al. 1996). The improvement in tiger shrimp post larvae resistance against salinity stress was accompanied with an elevation in astaxanthin concentration both in diet and body (Darachai *et al*., 1998; Chien *et al.*, 2003).

CONCLUSION

Astaxanthin can be used as a growth promoter in sea bass diets at 100 mg kg^{-1} diet and it can also enhance the fish survival rate and its antioxidant capacity. In addition, fish showed an enhancement in some immune parameters when fed AX supplemented diets. The current results suggest that supplementing AX as a feed additive could deplete osmotic stress. Nevertheless, astaxanthin is a promising dietary supplement for sea bass not only for growth and survival objectives but also for the health benefits and to reduce the impact of stressors without any side effects on fish.

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