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Dietary astaxanthin (Lucantin® Pink) mitigated oxidative stress induced by diazinon in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

The potential of commercial astaxanthin on growth, biochemical factors, and antioxidant-related gene expression following a challenge with diazinon were studied in rainbow trout (*Oncorhynchus mykiss*). Fish (~ 20.70 g) were fed diets containing commercial astaxanthin (ASX) at 0.00 (CTR and ASX0), 0.50 (ASX1), 2.00 (ASX2), and 5.00 (ASX3) g kg^{-1} for 60 days. Afterwards, the treated fish (ASX1, ASX2, ASX3) as well as the fish in ASX0 group were challenged with diazinon (0.11 mg L^{-1}) for 96 hr whereas fish in the CTR group was not challenged with diazinon. Results showed that growth pattern improved significantly with all enriched diets compared to the ASX0 group. Metabolic enzyme activities, including alanine aminotransferase and alkaline phosphatase decreased in ASX2 and ASX3 groups with respect to the ASX0 group. Serum antioxidant status also showed the same pattern with enhancement in the fish fed with the ASX2 and ASX3 supplemented diets. Feeding the fish with astaxanthin, particularly in the ASX3 group, up-regulated the expression of some antioxidant-relevant genes, including superoxide dismutase (*SOD*), catalase (*CAT*), glutathione peroxidase (*GPx*), glutathione S-transferase (*GST*), and nuclear factor erythroid-2 related factor 2 (*Nrf2*) in the kidney and liver. Besides, the histopathological damages in kidneys and liver induced by diazinon were less pronounced in the ASX2 and ASX3 groups compared to the ASX0 group. In conclusion, commercial astaxanthin, especially at 5.00 g kg^{-1}, enhanced the growth performance and ameliorated the oxidative stress induced by diazinon in rainbow trout.

Introduction

The aquaculture industry has seen a rapid growth in recent years, posing different stressors, e.g., pathogens and pollutants, on the fish during the rearing period.[1] Various contaminants, such as organic compounds and metals enter the aquatic environment and are absorbed by aquatic organisms through the water column and food. These substances stimulate reactive oxygen species (ROS) production by several mechanisms such as the impairment of transport by membrane-bound electron, accumulation of reduced intermediates, antioxidant enzymes inactivation, and the depletion of free radical scavengers.[2] Inside a normal cell, antioxidant components, namely low molecular weight free radical scavengers and some antioxidant enzymes can detoxify ROS and pro-oxidant products.[1] A high amount of pro-oxidant products can overcome antioxidant defenses due to the organism's inability to quench them, subsequently damaging cellular components.[3]

Diazinon is an organophosphorus insecticide frequently used in agriculture. Previous reports showed the continual input of this compound into the water (up to 1.00 mg L^{-1}), with persistence up to 6 months in the aquatic environments.[4] Therefore, this compound can cause several impairments to health condition in aquatic animals, including biochemical alterations and behavioral changes.[4,5]

The administration of some additives like probiotics, synbiotics, nucleotides, plant-derived products, and

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carotenoids to fish diet can improve the fish immune and antioxidant defense systems against various stressors.[6,7] Astaxanthin (ASX), a carotenoid pigment, has a significant potential in animal nutrition and health, with application in protecting the living organism against various stressors and infectious agents.[8-12] Various researchers have shown the boosting effects of astaxanthin on antioxidant and immune systems of many fish and crustaceans species.[13-16] However, its protective role against chemical pollutants such as diazinon has not been investigated in rainbow trout. Hence, this experiment was designed to assess the effects of dietary commercial astaxanthin against oxidative stress induced by the chemical pollutant diazinon.

Materials and Methods

Experimental fish and diet. Current study was performed in a local fish farm (Firouzkooh, Iran). Juvenile rainbow trout (~ 20.70 g) were located in 15 fiberglass tanks (150 L) with 15 fish per tank. The physicochemical parameters analyzed by a portable device (Aquacombo HM3070, Singapore, Singapore) were as: pH 7.60 ± 0.20, temperature 12.10 ± 0.40 ˚C, dissolved oxygen 8.60 ± 0.50 mg L^{-1}, and 0.60 L sec^{-1} flow rate. During the adaptation period, fish in all tanks received the control diet (Table 1) up to satiation for ten days.

In the experimental diets, different levels of commercial astaxanthin (Lucantin® Pink; BASF, Lampertheim, Germany) at 0.00, 0.50, 2.00, and 5.00 g kg^{-1} were used. After thorough mixing of the feed components, mentioned doses of astaxanthin were added to each experimental diet. In different groups, each dose of commercial astaxanthin was substituted by equivalent amount of cellulose (Table 1). Prepared feed was dried for one night at room temperature and then stored in the plastic bags at 8.00 - 10.00 ˚C.

The chemical compositions of fish diets were determined by the conventional methods.[17] In the current study, three tanks with 15 fish per tank were allocated to each group. Fish in the treatment groups were administrated with 0.50 (ASX1), 2.00 (ASX2), and 5.00 (ASX3) g kg^{-1} astaxanthin, whereas two groups with three tanks for each group received the control diet (CTR and ASX0) up to satiation for 60 days. The fish care and handling procedures in this study were approved by the Animal Experimentation Committee of University of Tabriz.

Growth performance. In the ASX0, ASX1, ASX2 and ASX3 groups, the initial and final biomass of each individual fish were measured. Parameters, including specific growth rate (SGR) and condition factor (CF), were determined according to the routine formulae.[18] During the trial, daily mortality was also recorded to estimate the total survival rate (%).

Bioassay test. The acute toxicity test was conducted in accordance with the previous study.[2] Diazinon with 60.00% purity, was purchased from the Partonar Company (Tehran, Iran). After the biometric measurements on day 60, all the fish in the ASX0, ASX1, ASX2, and ASX3 groups were challenged with diazinon in a dose of 0.11 mg L^{-1}, as 1:10 of diazinon 96-hr LC_{50} in rainbow trout (1.17 mg L^{-1}) based on the U.S. aquatic life ambient water quality criteria for diazinon (U.S. EPA 2005),[19] for a 96-hr period. The fish in the CTR group (the negative control) were not exposed to diazinon. The fish were not fed 24 hr before and during the exposure period. The exposure solution was renewed every 24 hr and a minimum concentration of 90.00% of the desired figure was ensured using photometric detection by high performance liquid chromatography (HPLC).[2] In this bioassay test, the water in tanks was daily controlled to be the same as the feeding trial.

Table 1. Formulation and proximate composition of different diets in the current study.

Ingredients (g kg^{-1})	CTR, ASX0	ASX1	ASX2	ASX3
Kilka fish meal[a]	250.00	250.00	250.00	250.00
Soybean meal[b]	230.00	230.00	230.00	230.00
Wheat flour	88.00	88.00	88.00	88.00
Corn flour	90.00	90.00	90.00	90.00
Soy lecithin	12.00	12.00	12.00	12.00
Cellulose[c]	20.00	19.50	18.00	15.00
Vitamin premix[d]	20.00	20.00	20.00	20.00
Mineral premix[d]	20.00	20.00	20.00	20.00
Lucantin® Pink	0.00	0.50	2.00	5.00
Proximate composition				
Dry matter (%)	87.52	87.59	87.71	88.00
Crude protein (%)	43.16	43.19	43.22	43.29
Crude lipid (%)	13.93	13.93	13.93	13.93
Ash (%)	8.32	8.32	8.32	8.30
Gross energy (Cal g^{-1})	4,465.93	4,469.27	4,475.29	4,489.32

[a] Total crude protein: 60.60%. Produced in Behparvar Company, Karaj, Iran; [b] Total crude protein: 44.20%; [c] Purchased from Merck Company (Darmstadt, Germany); and [d] Vitamin and mineral contents as described previously.[18]

CTR: Negative control group without challenge with diazinon; ASX0: Positive control group challenged with diazinon; ASX1: Low dose group challenged with diazinon; ASX2: Medium dose group challenged with diazinon and ASX3: High dose group challenged with diazinon.

Serum collection and biochemical parameters. After 96 hr of exposure to the toxin, five fish from each tank were euthanized with a clove oil solution (50.00 µL L^{-1}). Afterward, using a non-heparinized syringe, 2.00 mL of fish blood was collected, kept for 2 hr near ice, and centrifuged at 1,500 *g* for 15 min. The final serum samples were saved inside the Eppendorf tubes at – 80.00 ˚C for different assays. Liver enzyme activities, namely aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured using an Eppendorf, Epos 5060 biochemistry analyzer (Eppendorf, Hamburg, Germany) and commercial kits (Par Azmoon, Tehran, Iran). Total antioxidant power was determined according to Benzie and Strain.[20] The lipid peroxidation product, malondialdehyde (MDA), was determined according to Satoh.[21] The concentration of thiobarbituric acid-malondialdehyde products in serum samples were presented as nmol dL^{-1} of serum.

Real-time PCR. After the toxicity test, four specimens per tank were euthanized with a clove oil solution (50.00 µL L^{-1}). Then, the head kidney and liver tissues were stored inside the liquid nitrogen for molecular studies. For RNA extraction, 60.00 g of each tissue was manually homogenized and mixed with Gene All reagent (GeneAll Biotechnology, Seoul, South Korea). The RNA purity and quantity were determined by the spectrophotometer (Nanodrop 2000; Thermo Fisher Scientific, Waltham, USA). The first-strand cDNA was synthesized using a transcription kit (Thermo Fisher Scientific), according to previous study.[22] In the last step, PCR assay was performed to determine the expression of the different antioxidant genes, including superoxide dismutase (*SOD*), catalase (*CAT*), glutathione peroxidase (*GPx*), glutathione S-transferase (*GST*), and nuclear factor erythroid-2 related factor 2 (*Nrf2*) (Table 2). In this study, the housekeeping gene *β-actin* was used for normalizing the expression of the aforementioned genes. The PCR assay mixture for PCR consisted of 10.00 µL SYBR® Premix Ex Taq™ II (Takara, Kusatsu, Japan), 1.00 µL of cDNA template, 20.00 pmol of forward primer (Kiagene, Tehran, Iran) and 20.00 pmol of reverse primer (Kiagene). The real-time PCR program was as follow: an initial denaturing step of 95.00 ˚C for 4 min; 40 cycles of 95.00 ˚C for 20 sec and 60.00 ˚C for 30 sec. Relative mRNA level for the mentioned genes was expressed by $2^{-\Delta\Delta Ct}$ method. The real-time PCR reaction efficiency was determined using standard curve based on ten-fold dilution of three PCR replicates (Table 2).

Histopathological analysis. Samples of the distal intestine, pyloric caeca, head kidney (a pyramidal section from cortex to medulla), and liver from three fish per tank were dissected after anesthesia for histopathological tissue preparations. Then, they were fixed using 10.00% neutral buffered formalin for three days and prepared based on the previous study.[23] 5.00-µm histological sections were cut and then stained with Hematoxylin and Eosin. The prepared histological slides were examined with a light microscope (MBL2000; A. Krüss, Berlin, Germany) equipped with a digital camera. The length and thickness of folds in intestine and pyloric caeca, as well as the percentage of goblet cells among all epithelial cells in the fish distal intestine and pyloric caeca were measured. Twenty folds per fish and five sections in each sample were counted to assess the goblet cell percentage.[24]

Statistical analysis. Final results were presented as means ± standard error (SE). Shapiro–Wilk and Levene tests were performed to ensure the data normality and variance homogeneity. The one-way analysis of variance (ANOVA) and Tukey test in SPSS Software (version 21.00; IBM Corp., Armonk, USA) were used. The $p \leq 0.05$ was considered significant.

Results

Growth performance. The initial weight and length of the fish in all groups were the same, whereas the final weight, final length and SGR were significantly higher in astaxanthin treated groups, especially ASX2 and ASX3, in comparison to the ASX0 group. However, CF and survival rate were not altered after the administration of astaxanthin compared to the control group (Table 3).

Table 2. The list of primers used in the current study for amplifying antioxidant-related genes.

Genes	Primer sequence	Accession No.	Product length (bp)	Efficiency value (%)
SOD	F: 5' TCCCTGACCTGACCTACGAC 3' R: 5' GGCCTCCTCCATTAAACCTC 3'	CA352127.1	201	96.45
CAT	F: 5' TGATGTCACACAGGTGCGTA 3' R: 5' GTGGGCTCAGTGTTGTTGAG 3'	TC99600	195	95.68
GPx	F: 5' CGAGCTCCATGAACGGTACG 3' R: 5' TGCTTCCCGTTCACATCCAC 3'	TC126469	183	94.54
GST	F: 5' CAGAGGACAGCTCCCTGCTT 3' R: 5' CTGAACCGGCTCTCCAGGTA 3'	NM_001160559.1	187	93.07
Nrf2	F: 5' TGAGCTGCAGCAATGTCTGA 3' R: 5' GTTGGGCAATGGGTAGAAGC 3'	CA360709.1	124	98.43
β-actin	F:5' ATGGAAGATGAAATCGCCGCAC 3' R:5'TGGCCCATCCCAACCATCAC 3'	AJ438158	191	93.43

SOD: superoxide dismutase, *CAT*: catalase, *GPx*: glutathione peroxidase, *GST*: glutathione S-transferase, and *Nrf2*: nuclear factor erythroid-2 related factor 2.

Table 3. Growth indices in rainbow trout administrated with dietary astaxanthin at the end of the trial. Data are presented as means ± SEM.

Parameters	ASX0	ASX1	ASX2	ASX3
Initial weight (g)	20.58 ± 0.18	20.75 ± 0.19	20.74 ± 0.15	20.71 ± 0.18
Initial length (cm)	12.64 ± 0.03	12.57 ± 0.04	12.67 ± 0.03	12.65 ± 0.03
Final weight (g)	65.26 ± 1.40^{a}	68.84 ± 1.65^{ab}	72.29 ± 1.43^{b}	82.48 ± 1.53^{c}
Final length (cm)	18.78 ± 0.34^{a}	19.64 ± 0.28^{b}	19.96 ± 0.31^{bc}	20.43 ± 0.37^{c}
Specific growth rate (%)	1.92 ± 0.01^{a}	1.999 ± 0.01^{b}	2.081 ± 0.01^{c}	2.30 ± 0.01^{d}
Condition factor (%)	1.02 ± 0.05	0.93 ± 0.05	0.93 ± 0.03	0.99 ± 0.04
Survival rate (%)	100	100	100	100

ASX0: positive control group challenged with diazinon; ASX1: low dose group challenged with diazinon; ASX2: medium dose group challenged with diazinon; and ASX3: high dose group challenged with diazinon. ^{abcd} Different letters in each row indicate significant differences ($p < 0.05$).

Serum biochemical parameters. Fish in ASX0 group had the highest liver enzyme activities, including AST, ALT and ALP after diazinon challenge. Liver enzyme activities decreased significantly in the treated groups. Astaxanthin's protective role was most pronounced in ASX3 group, in which the liver enzyme activity remained similar to that of the CTR group. Challenging the fish with diazinon increased the amount of lipid peroxidation products as well, which was attenuated by the supplementation of astaxanthin in treated groups. Supplementation of astaxanthin in the ASX3 group resulted in amounts similar to that of the CTR group. Serum antioxidant status also showed a significant improvement after ASX3 diet supplementation, where fairly the same antioxidant activity as CTR group was shown (Table 4).

Expressions of antioxidant-relevant genes. The evaluation of *SOD* gene expression in the kidney tissue revealed the lowest concentration in the CTR group compared with all other groups. Challenging the fish with diazinon in parallel with various amounts of astaxanthin supplementation could elevate the renal *SOD* gene expression. Similarly, challenging the fish with diazinon could increase the renal *GST* gene expression in comparison with the CTR group. Based on the results, providing astaxanthin, especially in the ASX3 group, produced a statistically significant improvement in the *Nrf2*, *GPx* and *GST* gene expressions in the kidney (Fig. 1A). Diazinon and astaxanthin co-treatment caused a significant up-regulation of the GST gene in the fish's liver compared to the CTR group. Meanwhile, treating fish with astaxanthin showed higher *Nrf2*, *GPX*, *CAT* and *SOD* gene expressions than the control groups (CTR and ASX0 groups), with the highest level being measured in the ASX3 group (Fig. 1B).

Table 4. Serum metabolic enzymes and antioxidant activity in rainbow trout administrated with dietary astaxanthin and challenged with diazinon (n = 15). Data are presented as means ± SEM.

Parameters	CTR	ASX0	ASX1	ASX2	ASX3
AST (IU L^{-1})	272.40 ± 23.51^{b}	616.20 ± 27.90^{a}	545.01 ± 23.95^{a}	521.20 ± 40.02^{a}	363.02 ± 40.72^{b}
ALT (IU L^{1})	9.47 ± 0.44^{d}	17.20 ± 0.66^{a}	16.01 ± 0.83^{ab}	13.80 ± 0.73^{bc}	12.20 ± 0.37^{c}
ALP (IU L^{-1})	182.28 ± 6.41^{b}	595.01 ± 60.35^{a}	279.85 ± 22.60^{b}	245.43 ± 19.11^{b}	169.85 ± 14.67^{b}
MDA (nmol dL^{-1})	7.93 ± 0.40^{c}	17.60 ± 1.89^{a}	15.62 ± 1.17^{ab}	12.86 ± 0.73^{b}	12.04 ± 0.62^{bc}
TAC (nmol dL^{-1})	67.29 ± 3.47^{b}	42.41 ± 1.09^{a}	52.93 ± 2.88^{a}	54.44 ± 2.20^{a}	70.95 ± 3.13^{b}

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase, MDA: malondialdehyde, and TAC: total antioxidant activity. CTR: Negative control group without challenge with diazinon; ASX0: Positive control group challenged with diazinon; ASX1: Low dose group challenged with diazinon; ASX2: Medium dose group challenged with diazinon and ASX3: High dose group challenged with diazinon. ^{abcd} Different letters in each row indicate significant differences ($p < 0.05$).

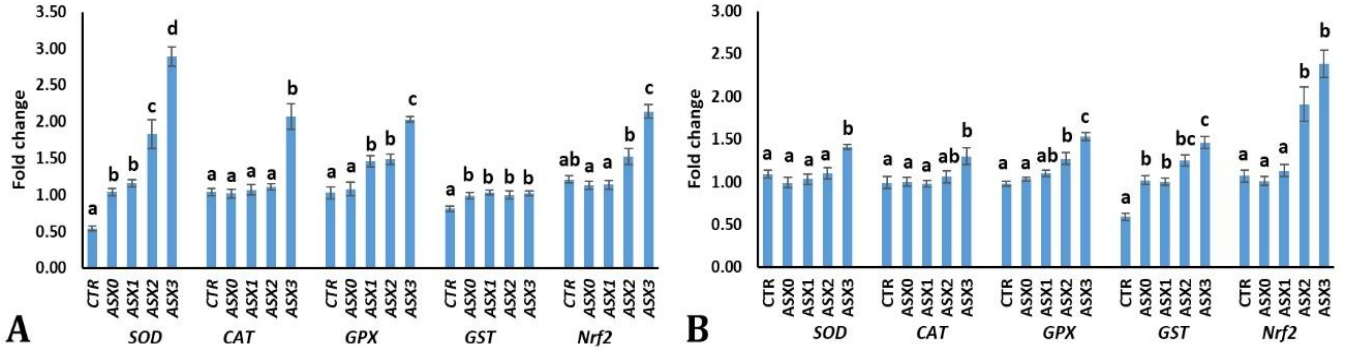

Fig. 1. The expression pattern of antioxidant genes relative to *β-actin* in **A)** kidney and **B)** liver of rainbow trout administrated with dietary astaxanthin and challenged with diazinon (n = 12). CTR: negative control group without challenge with diazinon; ASX0: positive control group challenged with diazinon; ASX1: low dose group challenged with diazinon; ASX2: medium dose group challenged with diazinon; and ASX3: high dose group challenged with diazinon. ^{abcd} Different letters indicate significant differences ($p < 0.05$).

Histopathological analysis. Challenging the fish with diazinon could cause a decrease in the length and goblet cell density of the intestinal and pyloric caeca folds in the ASX0 and ASX1 groups. In comparison with ASX0 and ASX1 groups, fish in the ASX2 and ASX3 groups presented higher fold length and goblet cell density, similar to values in the CTR group. However, the thicknesses of the intestinal and pyloric caeca folds were the same in all groups (Table 5). Renal tissue appeared normal in the CTR group; however, in the ASX0 group, a slight hemorrhage in the interstitial connective tissue and scattered necrosis with cell nuclei pyknosis in the urinary tubular epithelium were observed. In the ASX1 group, the vacuole formation in the tubular epithelium and corrupted tubules were noted, which shows the persistent toxic effects of diazinon in this treatment group. In the ASX2 group, mild pathological lesions with scattered necrosis and pyknosis were observed in urinary tubules. In the ASX3 group, the renal tissue was normal, and no pathological alternations were noted (Fig. 2).

No pathological lesions were observed in the hepatic tissues of the CTR group. Conversely, active hyperemia, intensive fatty change, and slightly scattered pyknosis of the hepatocytes were observed in the liver tissues of fish in the ASX0 group. In the ASX1 group, fatty change and hyperemia were lower than the ASX0 group. In the higher doses of astaxanthin administration, especially in the ASX3 group, a mild fatty change was visible, which shows the protective effects of astaxanthin in the hepatic tissue (Fig. 2).

Table 5. Morphological features of the distal intestine and pyloric caeca in rainbow trout administrated with dietary astaxanthin and challenged with diazinon (n = 15). Data are presented as means ± SEM.

Parameters	CTR	ASX0	ASX1	ASX2	ASX3
Distal intestine					
Fold length (µm)	711.25 ± 18.25^{a}	618.75 ± 8.26^{b}	613.75 ± 11.06^{b}	688.75 ± 15.59^{a}	690.00 ± 20.41^{a}
Fold thickness (µm)	126.25 ± 7.46	120.09 ± 10.80	125.00 ± 6.45	123.75 ± 6.25	126.25 ± 8.02
Goblet cell (%)	34.75 ± 1.54^{a}	16.75 ± 1.97^{c}	22.75 ± 0.94^{bc}	20.25 ± 1.25^{bc}	25.00 ± 2.38^{b}
Pyloric caeca					
Fold length (µm)	441.25 ± 10.87^{a}	261.25 ± 25.85^{b}	295.00 ± 14.43^{b}	372.50 ± 19.31^{a}	440.01 ± 5.42^{a}
Fold thickness (µm)	102.50 ± 8.53	100.10 ± 9.12	81.25 ± 4.26	88.75 ± 6.88	106.25 ± 4.26
Goblet cell (%)	21.50 ± 0.64^{a}	9.25 ± 2.65^{b}	10.85 ± 1.77^{b}	19.75 ± 2.05^{a}	21.75 ± 1.70^{a}

CTR: negative control group without challenge with diazinon; ASX0: Positive control group challenged with diazinon; ASX1: Low dose group challenged with diazinon; ASX2: Medium dose group challenged with diazinon and ASX3: High dose group challenged with diazinon. ^{abc} Different letters in each row indicate significant differences ($p < 0.05$).

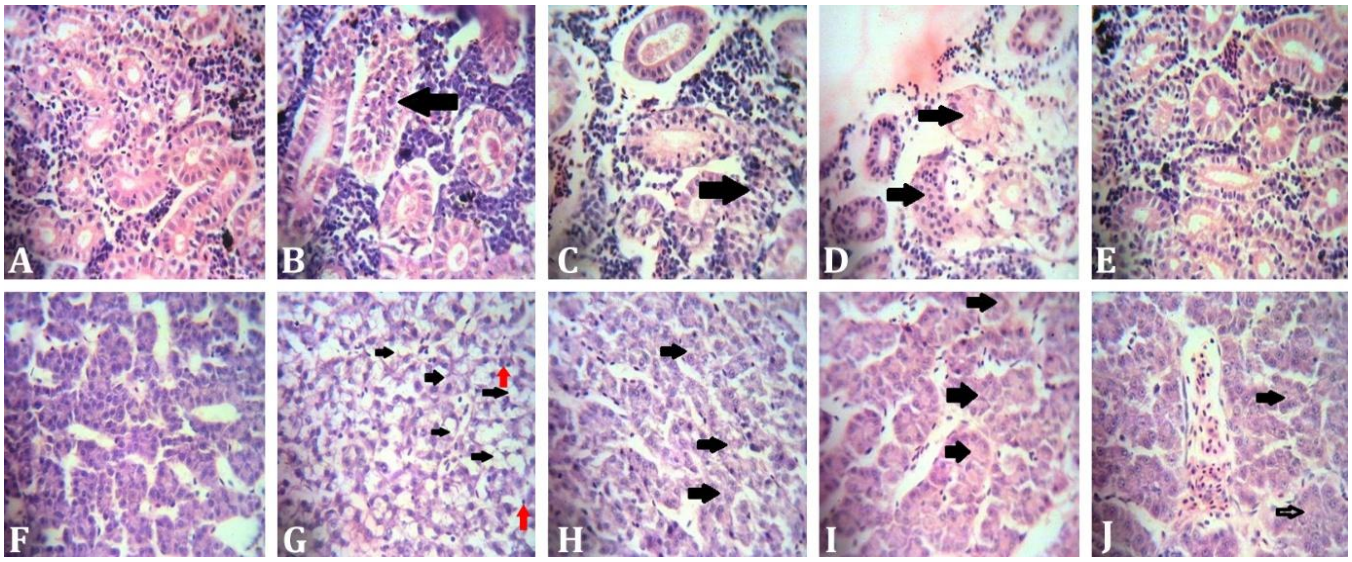

Fig. 2. Pathological lesions in the kidney (A-E) and liver (F-J) tissues of rainbow trout administrated with dietary astaxanthin and challenged with diazinon (n = 9). **A)** CTR group: normal architecture is present; **B)** ASX0 group: necrosis with scattered pyknosis of the nuclei in tubular epithelium and corruption of tubules (black arrow) are shown; **C)** ASX1 group: necrosis with nuclear pyknosis in tubules with low severity are noted (black arrow); **D)** ASX2 group: some corruption in tubules is obvious (black arrows); **E)** ASX3 group: a fairly normal appearance is shown; **F)** CTR group: normal architecture is present; **G)** ASX0 group: vast fatty change (black arrows), as well as a few pyknotic nuclei (red arrows) are obvious; **H)** ASX1 group, fatty change is still present (black arrows); **I)** ASX2 group: fatty change decreased remarkably (black arrows) and **J)** ASX3 group, a slight vacuolation is still present (black arrows), (Hematoxylin and Eosin staining; 200×).

Discussion

In the present study, the astaxanthin-supplemented diet, especially in the ASX3 group, significantly increased the growth performance in rainbow trout. Similarly, administration of pure astaxanthin at 40.00 and 80.00 mg kg^{-1} had improved the growth performance in large yellow croaker (*Pseudosciaena crocea*).[25] However, these findings are not consistent with previous studies that astaxanthin had no beneficial effects on fish growth. For example, administration of astaxanthin at 50.00 – 100 mg kg^{-1} did not affect rainbow trout growth performance.[26] A reduction in the growth was also observed in Atlantic salmon, *Salmo salar*, after diet supplementation with 5.30 mg kg^{-1} astaxanthin.[27] Differences in the astaxanthin source can also be responsible for the variations in the fish's biological response. When added to large yellow croakers' diet at the same concentration, astaxanthin derived from *Haematococcus pluvialis* (a natural source of astaxanthin) proved more potent at improving growth than synthetic astaxanthin.[25] In addition to variations in astaxanthin dosage and source, other factors, including diet composition, fish size, growth rate, and feeding length may also influence dietary carotenoid use and muscle deposition.[13,26] In the present study, the mechanism behind the improved growth parameters can partly be attributed to higher villus height, since raising villus length in the fish intestine can improve gut efficiency through improving digestion, absorption, and ultimately growth performance.[28] Meanwhile, the increased goblet cell density in the intestine and pyloric caeca of the treated fish can protect the mucosal layer from noxious substances.[29]

Analyzing the serum metabolic enzyme activity of ALP and AST can reveal the hepatotoxic changes.[30] In this study, a lower metabolic enzyme activity was recorded in the fish supplemented with astaxanthin. In accordance with this finding, feeding astaxanthin could ameliorate the hepatic pathological lesions in the treated fish. Similar to our study, feeding astaxanthin to rainbow trout could influence the liver function by decreasing the AST activity.[31]

Diazinon can induce oxidative damages by diminishing the metabolic pathways, including redox enzymes and mitochondrial electron permeability in different tissues.[4] In recent years, the application of natural antioxidants for decreasing oxidative damage has attracted many researchers.[3,32-35] The antioxidant potential of astaxanthin is reported as 10 times higher than that of other carotenoids, i.e., zeaxanthin, lutein, canthaxanthin, and α-carotene.[36] In the same way, the high antioxidant capability and health benefits of astaxanthin led researchers to the conclusion that this carotenoid can serve as an efficient food additive and nutritional supplement in aquaculture.[13] In this study, astaxanthin was able to reduce the MDA content, the main lipid peroxidation product, in rainbow trout serum after being challenged with diazinon. In parallel, the improvement in total antioxidant activity occurred in fish serum after being treated with astaxanthin. The present results also showed the ameliorating impact of astaxanthin through increasing the expression of different antioxidant enzyme genes. Similarly, dietary astaxanthin could increase mRNA expression of antioxidant enzyme genes (*CAT*, *SOD*, and *GPx*) in the hepatopancreas of Pacific white shrimp *Litopenaeus vannamei*.[37] In parallel, significant up-regulation in the expression of *SOD* and *CAT* genes in tiger pufferfish administrated with astaxanthin was shown.[38] The *Nrf2* is a major nuclear transcription factor that binds the anti-oxidant response element and regulates antioxidant gene transcription in fish species.[39] The *Nrf2* signaling pathway plays a vital role in elevating the transcriptional levels of various protective genes, namely antioxidant protease genes, phase II detoxification enzyme genes, and molecular chaperone genes.[39] In the current study, diets in the ASX2 and ASX3 groups up-regulated the *Nrf2* gene expression in the liver and kidney. Like supplemental astaxanthin, lycopene as another carotenoid showed improved *Nrf2* expression in rainbow trout's muscle tissue.[40]

In conclusion, challenging the fish with diazinon could cause severe damage in kidneys and liver with depression in tissue antioxidant status. Based on the results of this study, commercial astaxanthin, particularly at 5.00 g kg^{-1} could improve growth performance and mitigate diazinon-induced oxidative stress in the liver and kidney tissues. Therefore, astaxanthin can be considered as a potent feed supplement against various forms of pesticide-induced toxicities.

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Conflict of Interest

The authors had no conflict of interests to declare.

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