Subcritical water extraction as an efficient technique to isolate biologically-active fucoidans from Nizamuddinia zanardinii

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Complete List of Authors:	Elia, Antonia; Universita degli Studi di Perugia, Prearo, Marino; Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta Dörr, Ambrosius; Universita degli Studi di Perugia Pacini, Nicole; Universita degli Studi di Perugia Magara, Gabriele; Universita degli Studi di Perugia Brizio, Paola; Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta Gasco, Laura; Department of Agricultural, Forest and Food Sciences, University of Torino, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy Abete, Maria; Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta
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Effects of astaxanthin and canthaxanthin on oxidative stress biomarkers in rainbow trout

Antonia Concetta Elia[a], Marino Prearo[b], Ambrosius Josef Martin Dörr[a], Nicole Pacini[a], Gabriele Magara[a], Paola Brizio[b], Laura Gasco[c], Maria Cesarina Abete[b]*

a Department of Chemistry, Biology and Biotechnology, via Elce di sotto 8, University of Perugia, 06123 Perugia, Italy

b Veterinary Medical Research Institute for Piedmont, Liguria and Aosta Valley, Via Bologna, 148, 10154 Torino, Italy;

c Department of Agricultural, Forest and Food Sciences, University of Torino, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy.

Running title: Xanthophylls mediated-oxidative stress in *Oncorhynchus mykiss*

** Corresponding author: Dr. Antonia Concetta Elia*

Department of Chemistry,

Biology and Biotechnology,

University of Perugia, Italy

Email: antonia.elia@unipg.it

ABSTRACT

Farmed trout are commonly fed carotenoid-enriched diets during the finishing period to acquire the typical red-to-pink flesh color in salmonid muscle. The aim of this study was to examine the effects of the two xanthophylls, astaxanthin (Ax) or canthaxanthin (Cx), administered individually or in combination, on oxidative stress biomarkers in kidney and liver of rainbow trout. Specimens were fed Ax and/or Cx enriched diets with 75 or 25 mg of each compound/kg for eight weeks. Changes in concentration of oxidative stress biomarkers, such as glutathione, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione *S*-transferase, were recorded in Ax- and Cx-dosed trout. The two carotenoids, mainly Cx, impelled antioxidant defense in rainbow trout, while lipid peroxidation process was not recorded for all Ax or Cx-dosed trout. Furthermore, both combined xanthophylls did not exert remarkable combined effects in liver and kidney. Biomarker responses were generally altered in both tissues throughout both experimental endpoints, suggesting that different time-dependent pathways may be involved in strengthening the anti-oxidative defense in Ax and/or Cx-fed trout.

Keywords: astaxanthin, canthaxanthin, oxidative stress biomarkers, rainbow trout, tissues

Introduction

Salmonid farming strongly depends on market demand. Excellent organoleptic fish grade can be very attractive for consumers, and industries are struggling to conceive efficient strategies to enhance the appeal of aquaculture products. The aspect of fish fillet mostly influences customer's choice, and therefore farmed salmonids are often nourished during the finishing period with carotenoid-enriched diets. Likewise, pigmentation degree of meat is an important criterion of quality for farmers and consumers.

Oxocarotenoids (xanthophylls), such as Astaxanthin (Ax, 3,3'-dihydroxy-β,β-carotene-4,4'-dione) and canthaxanthin (Cx, 4,4-diketo-beta-carotene) have been widely used in order to develop the typical red-to-pink flesh color in salmonid muscle, due to fish ability to combine Ax and Cx with tissue actomyosinic complexes. Furthermore, a previous study reported that astaxanthin had a greater binding affinity for muscle fibers then canthaxanthin (Henmi et al., 1991). However, xanthophylls are considered as safe additives for animal and human consumption, and the maximum content in feed for Ax has been established by EU legislation (Commission implementing Regulation (EU) 2015/1415), while for Cx, also known as E161g, the maximum level as an additive in aquaculture feed was reduced from 100 to 25 mg/kg (Commission Directive 2003/7/EC).

Changes in oxidative stress biomarkers may be related with oxidative catabolism and nutritional factors (Martínez-Álvarez et al., 2005). In addition to its role as food additive, astaxanthin as well as other carotenoids are recognized as efficient physical and chemical scavengers of reactive oxygen species (ROS; hydrogen peroxide and hydroxyl radicals), quenchers of singlet oxygen ($^1O_2$) and inhibitors of lipid peroxidation (Chew, 1995; Sies and Stahl, 1995; Chien et al., 2003; Shimidzu et al., 1996; Stahl and Sies, 2003).

In fact, the Ax double bonds conjugated with relatively unstable electronic orbitals can reduce the concentration of prooxidant molecules (Conn et al., 1991). Astaxanthin can also enhance total antioxidant status (TAS) (Chien et al., 2003) and modulate lipid peroxidation and antioxidant

enzymes activity (Shih et al., 2008). Therefore, xanthophyll may be involved in improving the defense mechanisms against ROS, thus preventing oxidative stress impairment. However, although Ax and Cx are widely used in fish industry, the potential oxidative stress modulating role of the two xanthophylls on rainbow trout (*Oncorhynchus mykiss*) has been poorly investigated (Nakano et al., 1999).

The aim of the study was to investigate the effects of Ax (100 mg/kg) or Cx (25 mg/kg) diets administered alone or in combination for eight weeks on antioxidant stress biomarkers in liver and kidney of rainbow trout *Oncorhynchus mykiss*. The chosen doses would mimic the regulatory limits for dietary inclusion of astaxanthin and canthaxanthin in aquaculture industry (Commission Directive 2003/7/EC).

Materials and Methods

Fish exposure

Control and pigment diets were manufactured in the laboratory at the Experimental Station of the Department of Agricultural, Forest and Food Sciences of the University of Turin. Briefly, in-house pelleted feeding stuffs were prepared from the same basal composition (control diet) and supplemented with Ax and/or Cx. Ingredients of the experimental diets (on weight basis) were: fish meal (50%), corn gluten meal (10%), dehulled barley meal (18%), corn meal (9%), cod liver oil (7%), binder (merigel, 3%), mineral mixture (1.5%) and vitamin mixture (1.5%). Astaxanthin and canthaxanthin doses were chosen according to the previous study of Brizio et al. (2013). The three pigment-diets were: 1) Ax (75 mg/kg feed); 2) Cx (25 mg/kg feed); 3) Ax + Cx (75+25 mg/kg feed, respectively). Control diet was supplied to fish without carotenoids.

Females of rainbow trout (*O. mykiss* n=80) were divided into 8 tanks (10 specimens each tank) placed at same time (e.g 2 control groups, and 2 Ax, 2 Cx and 2 Ax+Cx groups) and then acclimatized for 15 days in flow-through open system with artesian well water (13 ± 1°C) with each tank (1500 L) having a water inflow of 8 L/min. Dissolved oxygen was measured daily and ranged

between 7.6 and 8.7 mg/L. The trial lasted for 8 weeks and specimens were fed by hand twice a day, 7d per week and the daily quantity of feed distributed was set at 1% of the tank biomass. No feed refusals were recorded during the experiment. Mortality was checked every day.

At week four and eight, the specimens of each experimental group were sampled and killed by over anesthesia (MS-222; PHARMAQ Ltd., UK). Each fish was weighed and total length was measured from the tip of the snout to the tip of the longer lobe of the caudal fin. Liver and kidney were collected immediately and stored in dry ice and next at -80°C, until used for analyses of antioxidant enzymes and total glutathione in liver and kidney.

The experiments were conducted in accordance with the European and national guidelines for the protection of animals used for experimental and other scientific purposes (European Commission, Directive 86/609/EC and Italian Directive 116/1992, respectively).

Biochemical analyses

The biochemical analyses were performed on the cytosolic fractions of liver and kidney as previously reported (Elia et al., 2017; Pacini et al., 2013).

Levels of lipid peroxidation products (MDA) were analyzed in each liver or kidney (1:10 and 1:5, respectively) in 20 mM 2-amino-2-hydroxymethylpropane-1,3-diol (TRIS/HCl), pH 7.4, and 0.5 M butylated hydroxytoluene (BHT), centrifuged at 3000×*g* for 20 min at 4 °C. The supernatant was derivatized in 1-methyl-2-phenylindole (10.32 mM in acetonitrile/methanol 3:1), HCl, and dilution buffer (TRIS/HCl, pH 7.4), sample or MDA standard (0-4 μM of 1,1,3,3-tetramethoxypropane). All samples were incubated at 45 °C for 60 min, then centrifuged at 15000×*g* for 10 min and read spectrophotometrically at 586 nm.

For antioxidant biomarkers, liver and kidney (0.2 g) were homogenized (1:10 w/v) in 100 mM TRIS buffer, pH 7.8, 100 μM phenylmethylsulphonyl fluoride (PMSF), 0.008 TIU mL^{-1} and bacitracin 0.1mg mL^{-1}, and then centrifuged at 100,000 g for 1 h at 4 °C.

Superoxide dismutase (SOD) activity was evaluated according to the original method of McCord and Fridovich (1969) and slightly modified (Pacini et al., 2012). The reduction of cytochrome C by the system xanthine/hypoxanthine was measured at 550 nm and a standard curve of SOD units was considered. One unit of SOD is defined as the amount of enzyme that inhibits by 50% the reduction of cytochrome C.

Catalase (CAT) activity was measured by following the decrease in absorbance at 240 nm due to H_2O_2 consumption. The assay was carried out in Na-phosphate buffer pH 7 and 12 mM H_2O_2.

Glutathione peroxidase (GPx) activity towards hydrogen peroxidase (H_2O_2) as substrate, was determined and the oxidation of NADPH was followed at 340. The assay condition was: 100 mM Na-phosphate buffer with pH 7.5, 1mM EDTA, 0.12 mM NADPH, 2 mM GSH, 1 mM NaN_3, 1 U GR, 0.6 mM H_2O_2.

Glutathione reductase (GR) activity was assayed following the decrease in absorbance at 340 nm due to the oxidation of NADPH. The assay condition was: 100 mM Na-phosphate buffer with pH 7, 1 mM GSSG and 60 μM NADPH.

Glutathione S-transferase (GST) activity was measured in 100 mM Na-phosphate buffer with pH 6.5 with 1 mM CDNB that forming the conjugate with 1 mM GSH at 340 nm.

Protein concentration was determined according to the method reported in Lowry et al. (1951).

Total glutathione was measured in liver and kidney. Weighted tissues (0.5 g) were homogenized (1:5 w/v) in 5% sulphosalicilic acid with 4 mM ethylene diamine tetraacetic acid (EDTA), and centrifuged at 30,000 x g for 15 min. Total glutathione (GSH+2GSSG) content was measured in potassium phosphate (KP) buffer 100 mM pH 7, EDTA 1 mM, glutathione reductase (GR) 1U, NADPH 4 mg ml^{-1} and 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) 1.5 mg ml^{-1}, both dissolved in $NaCO_3$ 0.5%. The oxidized glutathione was used as the standard and the absorbance was read at 412 nm.

All biochemical analyses were performed in triplicate on each sample with a Varian spectrophotometer (Cary 50 Thermostat Cell Holder) at a constant temperature of 25°C.

Statistical analyses

Data are reported as mean and standard deviation (SD). Homogeneity of variance was checked by Bartlett test. To highlight the statistically significant differences among specimens fed carotenoid-supplied diets and controls, we used one-way ANOVA and the Tukey's Multiple Comparison Test. Statistical significance was checked at $p<0.05$.

Results

No statistically significant differences in growth and length were recorded between treated and control rainbow trout within the same experimental period (4 or 8 weeks). However, length of control and Cx groups and weight of Cx dosed trout were slightly higher at 8 weeks, when compared to those recorded at 4 weeks (Table 1).

Ax- and Cx- enriched feed administered to rainbow trout, either singly or in combination, did not produce changes of MDA levels in liver, whereas same metabolite was higher (75%) in kidney of Ax + Cx trout at 4 weeks, if compared to the last experimental time (Figure 1).

Elevated hepatic SOD activity was noted in fish dosed with Cx (65%) and Ax + Cx (95%), if compared with control group and the Ax and Ax+Cx one at 4 weeks. Moreover, SOD levels were also higher in Cx fish (2 fold) and Ax+Cx (3 fold), if compared with the same groups at 8 weeks (Figure 2). Therefore, SOD activity did not show a monotonic exposure response relationship, since at 8 weeks activity in all carotenoids treated fish was similar to control ones.

No significant changes of SOD levels were recorded in kidney for the whole sample (Figure 2).

Administration of carotenoids, Ax and/or Cx, to rainbow trout produced only slightly higher CAT activity in Ax groups at both experimental times. No significant changes of CAT activity were recorded in kidney (Figure 3) and for GPx in liver (Figure 4).

GPx activity was higher (about 95%) in kidney treated with Cx dose administered singly or combined with Ax (Ax+Cx), when compared with own control at 4 weeks and with same experimental groups at 8 weeks (about 2 fold) (Figure 4). Therefore, GPx activity similar to SOD in

liver did not exhibit a monotonic exposure response relationship, since at 8 weeks activity for all experimental groups was not markedly different from controls.

GR activity (Figure 5) was enhanced (2.8-3.9 fold) in liver of fish fed with Ax, Cx and both combined carotenoids at 8 weeks, whereas in kidney activity was slight higher in Ax- and Cx-treated specimens, when compared to own controls.

GST activity (Figure 6) increased about 2-3 times in liver and kidney of fish exposed to both carotenoids for 8 weeks. When the two compounds were co-administered, activity in liver was at the same as when each molecule was administered singly. On the contrary, mainly at 8 weeks GST activity in kidney of Ax+Cx fed trout was significantly higher than for Ax or Cx groups.

No significant changes of thiol concentrations (Figure 7) were recorded in liver among all carotenoids treated groups. Higher total glutathione levels were recorded in kidney of Ax + Cx group, if compared with control (30%) and Ax-trout (40%) at 4 weeks. All three carotenoid treated groups showed increased thiol levels at 8 weeks, when compared own control (1.8-2.2-fold) and the same experimental groups (1.5-2-fold) at 4 weeks, with the exception of the Ax+Cx one (Figure 7).

Discussion

In our study, Ax and Cx fed continuously for eight weeks to rainbow trout, individually or concurrently, exerted no noticeable effects on length and weight. Our outcome agrees with previous findings on *O. mykiss* (Řehulka 2000; Choi et al., 2016; Rahman et al., 2016) as well as on Atlantic salmon, *Salmo salar* (Bell et al., 2000) or gilthead seabream, *Sparus aurata* (Gomes et al., 2002) treated with astaxanthin. However, both Ax and Cx diets stimulated different responses of oxidative stress biomarkers in liver and kidney of xanthophyll-fed trout.

Carotenoids play an essential role in protection of cell membranes and lipoproteins from oxidative damage, offsetting the effects of peroxyl radicals (Angeles and Chien, 2016; Dose et al., 2016; Esatbeyoglu and Rimbach, 2017). The improvement of total antioxidant ability of trout following single administration of 100 mg/kg astaxanthin or cantaxanthin or combined (50 mg/kg Ax + 50

mg/kg Cx) for 60 days was previously reported by Cui et al. (2009). Moreover, rats fed with canthaxanthin and β-carotene for 6 weeks showed higher GPx and catalase activity in erythrocytes, when compared to rats fed diets with a high cholesterol and lipid content (CC) (Shih et al., 2008). Conversely, Ax lowered SOD in juvenile tiger prawn *Penaeus monodon* or SOD and GPx activity in *Hyphessobrycon callistus* (Chien et al., 2003; Wang et al., 2006).

In the present study, biomarkers response may corroborate the antioxidant property of xanthophylls, mainly for Cx and Ax + Cx-dosed specimens. Indeed, although the unchanged hepatic and renal MDA concentration during the eight weeks represent a reassuring outcome, detoxifying biomarker levels in Ax and/or Cx fed rainbow trout suggest that different mechanisms may be involved in strengthening the antioxidant shield in both tissues and through the two experimental endpoints. In liver, the transient raise of SOD concentration was followed by the unchanged activity of both main H_2O_2 scavengers, such as catalase and GPx. This outcome is surprising, since the high SOD levels in xanthophylls-dosed rainbow trout suggest an increase of H_2O_2 hepatic load. Hydrogen peroxide is a strong oxidizer that can reduce cell viability due to membrane oxidative damage (Xie et al., 2017). Therefore, the unaltered lipid peroxidation process contributes to outline a more intriguing scenario. In particular, astaxanthin is recognized as effective molecule in preventing lipid peroxidation (Dose et al., 2016; Xie et al., 2017) and in lowering MDA concentration in *O. mykiss* (Brambilla et al., 2009). According to these results we measured a slight lowering of MDA levels in Ax and Ax + Cx-treated rainbow trout liver, although our values did not reach the statistical significant differences. Lack of lipid peroxidation process in both tissues of rainbow trout fed with xanthophyll diets along with high levels of the main antioxidant biomarkers suggest an alternative route for CAT and GPx in scavenging H_2O_2. Previous studies suggested that carotenoids, and in particular canthaxanthin, can act as radical scavenger by quenching free radicals in unsaturated fatty acids systems (Daubrawa et al., 2005; Mansour et al., 2006). A mechanistic study focused on the antioxidant role of carotenoids against the hydroperoxides indicated that astaxanthin and canthaxanthin are able to retard the hydroperoxide formation on azo-initiated lipid peroxidation.

Linkage of carbonyl groups at the 4 and 4' positions that is different from other carotenoids may play a key role in increasing their efficiency to trap peroxyl radicals. Indeed, Ax and Cx carbonyl oxygen has the property to attract electrons, hence reducing the unpaired electron density throughout the carbon skeleton of radical molecules. As a consequence, the carbon-centred radical of hydroperoxides reduces its affinity for oxygen (Mortensen et al., 1997). Although H_2O_2 may trigger lipid peroxidation and weaken the total antioxidant capacity, xanthophylls can act as a direct oxyradical scavenger, as previously reported in golden pompano (*Trachinotus ovatus*) exposed to astaxanthin (Xie et al., 2017). Unexpected response was also observed in kidney, where increasing GPx activity, mainly in Cx and Ax + Cx-fed rainbow trout, was not accompanied by a rising SOD level. Moreover, transcription of several antioxidant enzymes, such as SOD, CAT and GPx, can be regulated by the NF-E2-related nuclear factor 2 (Nrf2) in fish (Jiang et al., 2014). Furthermore, a previous study showed that xanthophylls may modulate the mRNA expression of Nrf2 and associated genes, and an optimal Nrf2-mediated oxidative defense can be achieved in HepG2 cell line following exposure at low (12 μM) astaxanthin concentration (Saw et al., 2013). Therefore, it is feasible that the enhancement of renal GPx activity was related to the ability of xanthophyll to modulate the Nrf-2 mRNA expression, reinforcing the antioxidant shield of *O. mykiss*.

Astaxanthin and canthaxanthin have been documented as weak inducers of the xenobiotic-metabolizing enzyme GST in liver or kidney of *O. mykiss* after short term treatment (3 weeks) (Page and Davies, 2002). Indeed, when dietary substances are absorbed at a higher rate than catabolism and excretion, astaxanthin and canthaxanthin can gradually accumulate in tissues. Brizio et al. (2013) reported muscle concentration of 2.2 and 3.70 mg/kg for Ax or 0.4 and 1.21 mg/kg for Cx, at 4 and 8 weeks, respectively. Moreover, both carotenoids are metabolized reductively, and geometrical E/Z-isomers of astaxanthin are utilized differently in rainbow trout. Although, all-E-astaxanthin accumulates in plasma and muscle, while 13Z-astaxanthin accumulates in liver (Ahmadi et al., 2006; Østerlie et al., 1999). In our study, no changes of GST activity were measured in xanthophyll-treated fish during the first 4 weeks of the trial, while enzymes level increased

noticeably mainly in liver after eight weeks. Although Ax and Cx concentrations were not measured in all tissues of rainbow trout, we may assume that the activation of this important defense line occurred along with higher accumulation levels of carotenoids or metabolites production.

Additionally, it is ascertained that antioxidant compounds may act as prooxidant factors when their concentration exceeds the optimal threshold (Dörr et al., 2008, 2013; Elia et al., 2011, 2014; Pacini et al., 2012). Accordingly, previous studies showed that high carotenoid concentrations may produce oxidative effects, such as alteration of the biological membrane properties and permeability to toxins, due to production of radical molecules (Stahl and Sies, 2003; El-Agamey et al., 2004). Furthermore, the highly reactive carotenoid-peroxyl molecules can impair the activity of antioxidant enzymes or cause lipid peroxidation (Lygren et al. 1999; El-Agamey et al., 2004). However, astaxanthin can activate the extracellular signal-regulated protein kinase (ERK) signaling pathway, following an up-regulation of expression of GST-α1, strengthen the biological defense against oxidative stress damages *in vitro* and *in vivo* (Fakhri et al., 2018). The induced GST activity in both rainbow trout tissues in this study could thus suggest a regulatory mechanism preventing Ax and/or Cx disturbance. Accordingly, unchanged activity of hepatic SOD and renal GPx during the last endpoint might have been balanced by higher GST levels offsetting the potential adverse effects of xanthophylls. Particularly, GST was differently supported in both analyzed tissues. While in liver the suitable GSH amount was ensured by the increased GR activity, in kidney an *ex novo* production of reduced thiol may be assumed, as suggested by the raised concentrations of total glutathione and the unchanged GR levels. Astaxanthin has also been described as enhancing factor of GR activity in giant freshwater prawn *Macrobrachium rosenbergii* following challenging conditions (Angeles et al., 2009). The role of GSH as enzymatic co-factor or radical scavenger may protect tissues from free radical damages (Saw et al., 2013). Furthermore, previous study showed that Ax in combination with fish oil enhances the immune response by improving the glutathione-based redox balance in rat plasma and neutrophils (Barros et al., 2012). This evidence could suggest

that in this study glutathione could act not only as antioxidant protector, but also reinforce promptly the immune system defense.

Conclusions

Astaxanthin and canthaxanthin stimulate the antioxidant defense in liver and kidney of rainbow trout involving different time-dependent routes. Furthermore, both combined xanthophylls diets did not exert additive effects in the investigated rainbow trout tissues throughout the trial. The current findings may deepen knowledge on the prooxidant effects of the considered carotenoids widely used in aquaculture, as safe additives, in order to sustain fish health and productivity.

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Table 1- Length and weight of treated rainbow trout fed astaxanthin (Ax) and/or canthaxanthin (Cx) diets

	Length	Weight
C T1	31.05 ± 0.83[a*]	306.5 ± 26.57[a]
Ax T1	31.7 ± 1.00[a]	329.5 ± 32.1[a]
Cx T1	31.25 ± 1.11[a*]	314.5 ± 22.66[a*]
Ax + Cx T1	31.5 ± 0.88[a]	315 ± 23.57[a]
C T2	32.65 ± 1.44[a*]	337.5 ± 48.95[a]
Ax T2	33.1 ± 1.24[a]	356 ± 45.39[a]
Cx T2	33.7 ± 1.25[a*]	367 ± 42.24[a*]
Ax + Cx T2	33.2 ± 0.82[a]	358.5 ± 32.83[a]

The results are reported as mean and standard deviation. Statistical significant differences ($p < 0.05$) are indicated by the following symbols: between the same group at both experimental times, asterisk (*), and between Ax, Cx and Ax + Cx samples at the same endpoint, different letters (a, b, c). T1= 4 weeks; T2 = 8 weeks.

Legend of Figures

Figure 1. Malondialdehyde concentration in rainbow trout fed Ax (astaxanthin), Cx (canthaxanthin), and Ax + Cx diets for 8 weeks. Liver (L) or kidney (K) of each group was analyzed individually (n = 10). The results are reported as mean and standard deviation. Statistical significant differences ($p < 0.05$) are indicated by the following symbols: between the same group at both experimental times, asterisk (*), and between Ax, Cx and Ax + Cx samples at the same endpoint, different letters (a, b, c). T1= 4 weeks; T2 = 8 weeks.

Figure 2. Superoxide dismutase activity in rainbow trout fed Ax (astaxanthin), Cx (canthaxanthin), and Ax + Cx diets for 8 weeks. Liver (L) or kidney (K) of each group was analyzed individually (n = 10). The results are reported as mean and standard deviation. Statistical significant differences ($p < 0.05$) are indicated by the following symbols: between the same group of both experimental times, asterisk (*), and between Ax, Cx and Ax + Cx samples at the same endpoint, different letters (a, b, c). T1= 4 weeks; T2 = 8 weeks.

Figure 3. Catalase activity in rainbow trout fed Ax (astaxanthin), Cx (canthaxanthin), and Ax + Cx diets for 8 weeks. Liver (L) or kidney (K) of each group was analyzed individually (n = 10). The results are reported as mean and standard deviation. Statistical significant differences ($p < 0.05$) are indicated by the following symbols: between the same group of both experimental times, asterisk (*), and between Ax, Cx and Ax + Cx samples at the same endpoint, different letters (a, b, c). T1= 4 weeks; T2 = 8 weeks.

Figure 4. Glutathione peroxidase activity in rainbow trout fed Ax (astaxanthin), Cx (canthaxanthin), and Ax + Cx diets for 8 weeks. Liver (L) or kidney (K) of each group was analyzed individually (n = 10). The results are reported as mean and standard deviation. Statistical significant differences ($p < 0.05$) are indicated by the following symbols: between the same group of

both experimental times, asterisk (*), and between Ax, Cx and Ax + Cx samples at the same endpoint, different letters (a, b, c). T1= 4 weeks; T2 = 8 weeks.

Figure 5. Glutathione reductase activity in rainbow trout fed Ax (astaxanthin), Cx (canthaxanthin), and Ax + Cx diets for 8 weeks. Liver (L) or kidney (K) of each group was analyzed individually (n = 10). The results are reported as mean and standard deviation. Statistical significant differences ($p < 0.05$) are indicated by the following symbols: between the same group of both experimental times, asterisk (*), and between Ax, Cx and Ax + Cx samples at the same endpoint, different letters (a, b, c). T1= 4 weeks; T2 = 8 weeks.

Figure 6. Glutathione S-transferase activity in rainbow trout fed Ax (astaxanthin), Cx (canthaxanthin), and Ax + Cx diets for 8 weeks. Liver (L) or kidney (K) of each group was analyzed individually (n = 10). The results are reported as mean and standard deviation. Statistical significant differences ($p < 0.05$) are indicated by the following symbols: between the same group of both experimental times, asterisk (*), and between Ax, Cx and Ax + Cx samples at the same endpoint, different letters (a, b, c). T1= 4 weeks; T2 = 8 weeks.

Figure 7. Total glutathione concentration in rainbow trout fed Ax (astaxanthin), Cx (canthaxanthin), and Ax + Cx diets for 8 weeks. Liver (L) or kidney (K) of each group was analyzed individually (n = 10). The results are reported as mean and standard deviation. Statistical significant differences ($p < 0.05$) are indicated by the following symbols: between the same group of both experimental times, asterisk (*), and between Ax, Cx and Ax + Cx samples at the same endpoint, different letters (a, b, c). T1= 4 weeks; T2 = 8 weeks.

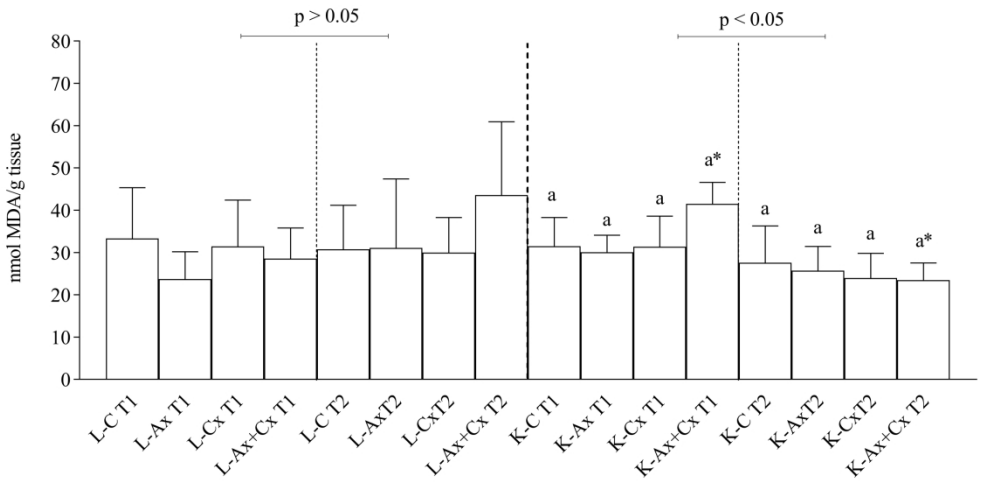

Figure 1

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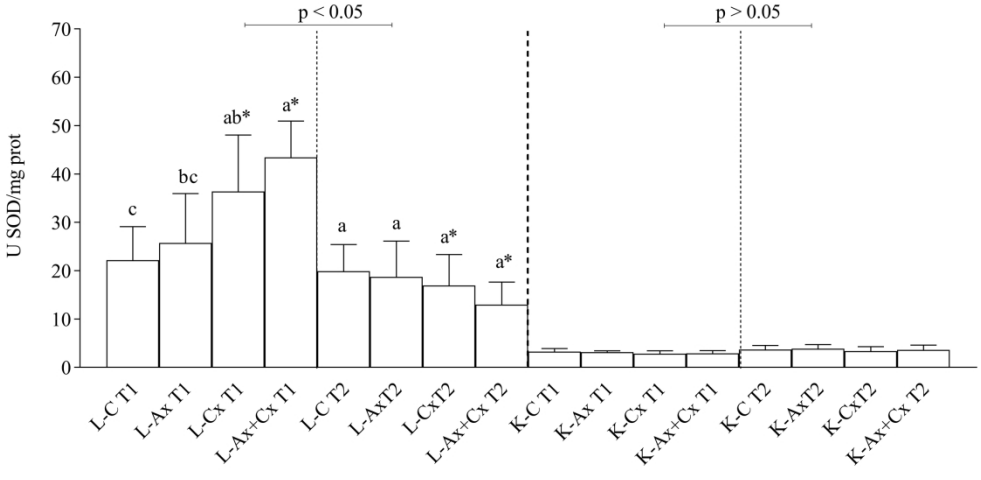

Figure 2

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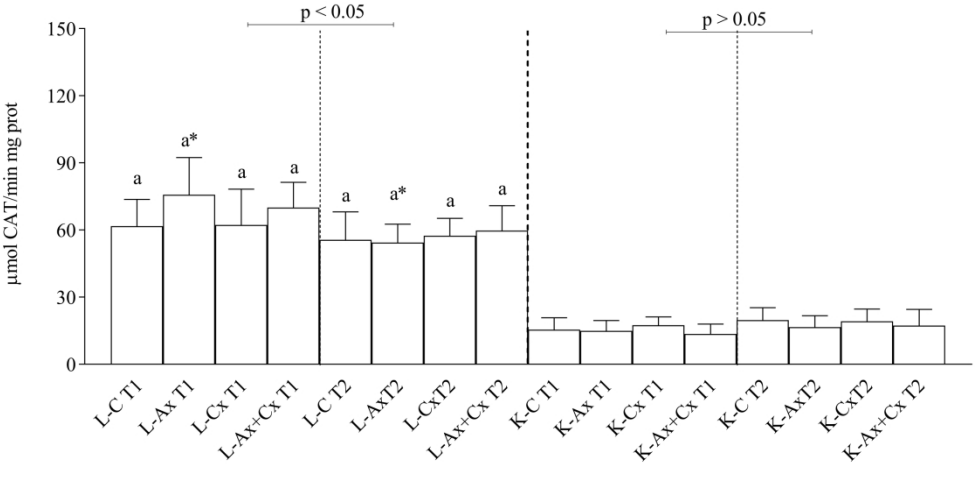

Figure 3

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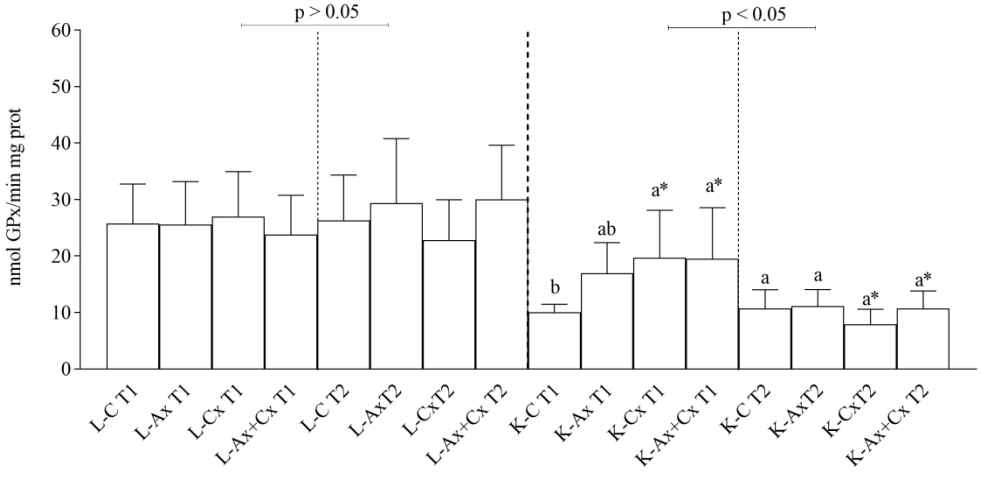

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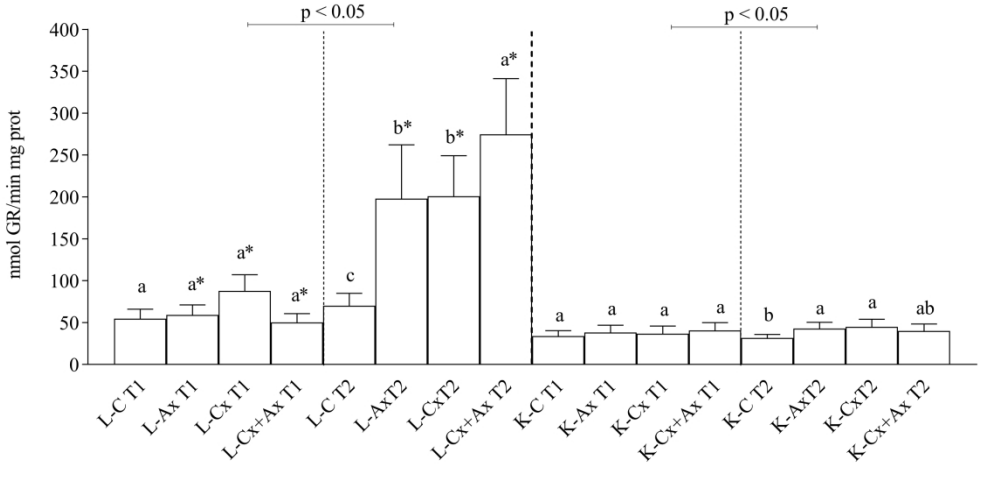

Figure 5

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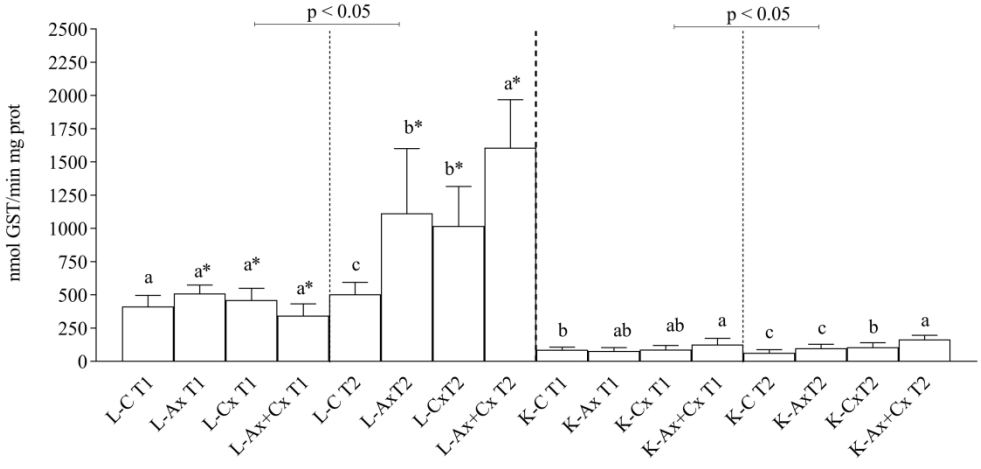

Figure 6

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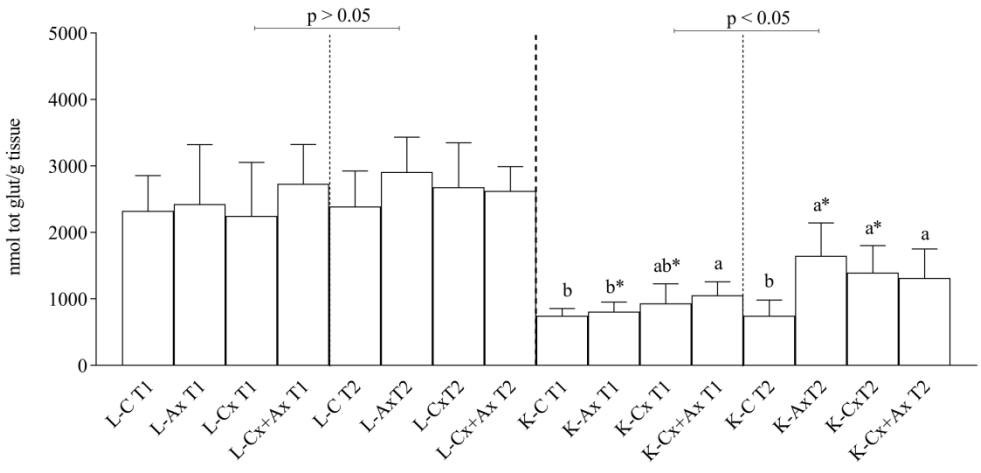

Figure 7

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