Levels of antioxidant enzyme activities in cultured rainbow trout (*Oncorhynchus mykiss*) fed with different diet compositions

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Abstract

The recent trend for rainbow trout diets to be higher in lipid content may increase the lipid concentration in fish and lipid peroxidation, which could lead to oxidative stress and affect fish health. In the present work, an evaluation of the possible effects of different fish diets on antioxidant enzyme levels was carried out on two aquaculture groups of rainbow trout *(Oncorhynchus mykiss)*. The fish from one group were fed a diet containing 5% more crude lipids, than the diet used for the other group. The objective was to determine the effects of different concentrations of dietary lipid on the antioxidant defense enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST) in the liver and muscle of rainbow trout. All enzymes showed an increasing trend in fish fed with a diet containing more lipids. GST was observed to be the most sensitive antioxidant enzyme, followed by SOD and GPx, and finally by CAT and GR. These results provided data indicating the prooxidative effects of higher dietary lipid levels and suggested that dietary lipid plays an important role in determining fish susceptibility to oxidative stress.

Introduction

In the current situation of constantly decreasing biodiversity and stagnating world fishery resources, with fish stocks in a state of deterioration, aquaculture represents a reliable source of increasing fish reserves and is one way of ensuring a sufficient amount of fish for food in the future.

The economic importance of rainbow trout (*Oncorhynchus mykiss*) in the global aquaculture industry, makes it one of the most leading species for inland water fish farming. Because of its rapid growth, and rich and valuable meat composition, rainbow trout is a preferred species for human consumption. Today in Serbia, more than half of consumed fish from total domestic resources are obtained from aquaculture. Rainbow trout represents an increasingly popular species of cultured fish in the Serbian aquaculture industry (Marković and Poleksić, 2013). It is reared in different production systems and specially designed facilities (Stoyanova et al., 2016). Although rainbow trout are mostly farmed in cages and classic outdoor raceways, due to the need to protect

farmed fish from disease transmission and the need for fresh fish, this species is increasingly farmed in recirculation aquaculture systems (RAS). This system rears fish at high densities, in indoor tanks with an optimised and controlled environment, with recirculating systems which filter and clean the water for recycling back through fish culture tanks.

When reared in high-density indoor systems, fish must be given a high-quality, nutritionally complete, and balanced diet (Craig and Helfrich, 2009). The use of feed lacking or containing an excess of components can lead to various physiological disorders in fish (Oliva-Teles, 2012). Rainbow trout is carnivorous and needs a diet rich in protein. Because protein is the most expensive component of fish feed, higher percentages of lipids are used in fish feeds. This can lead to the accumulation of unnecessary fat in the liver, thereby threatening the health of the fish and meat quality (Craig and Helfrich, 2009). Generally, fish tissues are rich with n-3 polyunsaturated fatty acids (PUFAs), a property that has potential benefits for human health (Ruxton, 2005), but the positive nutritional value of long-chain n-3 PUFAs derived from fish oils and meals can become a negative factor for fish (Hsieh and Kinsella, 1989). Fish are potentially at risk of peroxidative attack because of the presence of large quantities of PUFAs in both fish tissues and diets (Oliva-Teles, 2012). Polyunsaturated fatty acids are susceptible to oxidation because oxygen impacts double bonds, creating lipoperoxides, which produce reactive oxygen species (ROS), and finally induce oxidative stress (Jin et al., 2017). The liver is a target for metabolism in the fish body, and liver biomarkers are useful as indicators of the general health of fish and are considered to reflect physiological exposure to a variety of redox factors (Sadekarpawar and Parikh, 2013).

Harmful effects of oxidative stress on the lipids of cellular membranes are neutralised by effective antioxidant defense systems operating in fish (Martínez-Álvarez et al., 2005). The antioxidant enzyme system includes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and the enzyme of phase II biotransformation glutathione-S-transferase (GST). These enzymes, besides scavenging ROS, are good biomarkers of oxidative stress, and are useful diagnostic and prognostic tools for assessing the effects of a large number of individual and interactive processes in aquatic organisms (Ross et al., 2001).

To minimize production expenses in fish farming, potential threats to fish health need to be taken into account. The present study aimed to provide data about the possible effects of two commercial diets with different compositions on the levels of antioxidant enzymes (SOD, CAT, GPx, GR, and GST) in tissues of aquaculture rainbow trout.

Material and methods
Ethical statement
All experimental procedures complied with the European Council Directive 86/609/EEC for the protection of animals used for experimental and other scientific purposes, the Serbian Animal Welfare Law (''Official Gazette of the Republic of Serbia'', No 41/09) and Regulations on the Welfare of Animals Intended for Experimental Purposes (''Official Gazette of the Republic of Serbia'', No 39/10).

Rearing conditions

Rainbow trout from the "Little Danube" Centre for Fisheries and Applied Hydrobiology (the University of Belgrade - Faculty of Agriculture, Serbia) were divided into two aquaculture groups, - AG1 and AG2. Each group was placed in a separate pool and maintained in small indoor recirculating aquaculture systems (RAS) with all conditions needed for effective trout production. Fish were stocked at a density of 200 individuals per m^{-3} (n ≈ 80 fish per tank). Water temperature (T), pH, dissolved oxygen (DO), and conductivity (EC) were checked daily with the aid of a MULTI 340i set (WTW, Germany). Physical and chemical parameters were obtained by immersing the corresponding electrodes 0.05 m below the water surface in both pools (Table 1).

Table 1. Physical and chemical parameters (PCP) (means ± SD) of water measured at the "Little Danube" Centre for Fisheries and Applied Hydrobiology, University of Belgrade - Faculty of Agriculture, Serbia (CEFAH)

PCP	
T (°C)	14.5 ± 1.0
DO(mg/L)	8.07 ± 0.59
pH	8.49 ± 0.04
TA (mg CaCO3/L)	4.87 ± 0.40
TH (°dH)	5.03 ± 0.26
HCO3- (mg/L)	296.9 ± 24.6
Cl- (mg/L)	1.63 ± 0.12
Ca2+ (mg/L)	21.50 ± 2.26
Mg2+ (mg/L)	11.88 ± 1.89
SO42- (mg/L)	28.77 ± 0.86
PO43- (mg/L)	0.27 ± 0.01
OP (mg/L)	0.21 ± 0.01
NH3 (mg/L)	0.01 ± 0.00
NO3- (mg/L)	1.16 ± 0.22

All fish used in the study were of homogeneous age and size. The fish from AG1 were fed with commercial diet 1, while fish from AG2 were fed with diet 2. Formulas and approximate composition of diets 1 and 2 are presented in Table 2. Fish were fed daily by hand at a feeding rate of 1.2-1.5% of total fish body mass in both fish groups following regulation of the temperature and oxygen level.

Table 2. Formulation and approximate composition of the diets 1 and 2 used for trout of the AG1 and AG2 groups, respectively, at the "Little Danube" Centre for Fisheries and Applied Hydrobiology, University of Belgrade - Faculty of Agriculture, Serbia (CEFAH)

Ingredient, g/100 g of diet Approximate composition	Diet 1	Diet 2
Dry matter (%)	91.5	93.00
Crude protein (% of DM †)	43.00	45.00
Crude lipids (% of DM)	22.00	27.00
Crude fiber – cellulose (% of DM)	3.00	1.00
Ash (% of DM)	6.50	7.30
Phosphorus (% of DM)	1.00	0.97
Digestible energy (MJ kg-1)	18.00	21.00

[‡]Additional ingredients: fish meal, fish oil, wheat and by-products of wheat processing, wheat gluten, peeled roasted soy flour, soybean oil, rapeseed oil, butylated hydroxytoluene (BHT).
[†]DM - dry matter. [§]Diet 1 and Diet 2. – Produced by Skretting, Italy.

Fish sampling

Fifteen healthy rainbow trout were randomly sampled using seine nets from each studied group (AG1 and AG2) and then transferred to a laboratory of Belgrade University's Faculty of Agriculture. Fish were sacrificed with an overdose of 2-phenoxyethanol. Individual fish body weights (BW), total lengths (TL), and liver weights (LW) were taken and used to determine the hepatosomatic index (HSI) and condition factor (CF) using the following formulae:

Hepatosomatic index (HSI) = ((liver weight (g) / fish weight (g)) × 100

Condition factor (CF) = weight (g) / length $(cm)^3$ x 100.

Sample preparation and measurement of antioxidant enzymes

After death, liver, abdominal muscle, and dorsal muscle samples from the fish were excised aseptically, frozen immediately in liquid nitrogen,

and stored at −80°C until further analysis. Before the measurement of antioxidant enzymes, tissue samples were thawed, washed with sterile physiological saline, and then dried with filter paper. Approximately 0.5 g of each tissue sample (n=15) was minced and then homogenised in a homogenisation medium (0.25M sucrose, 0.5mM EDTA, and 10 mM Tris–HCl; pH 7.4) with an IKA-Werk Ultra-Turrax homogeniser (Janke and Kunkel, Staufen, Germany) at 4°C (Rossi et al., 1983). The resulting homogenates were sonicated for 15 s at 10 kHz on ice to release enzymes (Takada et al., 1982). Sonicates were centrifuged at 100 000×g for 90 min (4°C). After centrifugation, the supernatant was separated and used for the determination of total protein concentration and activity assays. Total protein concentration in all samples was quantified by the Bradford method (Bradford, 1976) adapted from Bio-Rad's Bradford micro-assay, using bovine serum albumin as the standard.

Superoxide dismutase activity was analysed following the method of Misra and Fridovich (1972) based on the autoxidation of adrenaline to adrenochrome. The change in absorbance was monitored at 480 nm. The activity of CAT was quantified by the decomposition of H_2O_2 at 240 nm according to the method of Claiborne (1985). Glutathione peroxidase activity was determined by following the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) as a substrate with t-butyl hydroperoxide (Tamura et al., 1982). The activity of GR was detected from the oxidation of NADPH during the reduction of GSSG (Glatzle et al., 1974). The activity of GST towards 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate was evaluated according to the technique described by Habig et al. (1974). All measurements were performed in duplicate to ensure representativeness.

Data analysis and statistical methods
Data were analysed using the SAS statistical package and the Microsoft Excel data analysis tool package. Each column on the graph represents the mean of 15 fish ± the standard error of the means (SE). The data were first tested for normality (Kolmogorov–Smirnov normality test) and homogeneity of variance (Levene's test). One-way analysis of variance (ANOVA) was used to compare means in the two different aquaculture groups. Following analysis of variance, significant between-group differences were detected by Tukey's posthoc test. Differences were considered significant at $P < 0.05$. The data presented in the figures for each biomarker inside each group were pooled and correlated with each other. Pearson's correlation coefficients were calculated for pairs of biomarkers using the Microsoft Excel data analysis tool. Statistical analyses were performed with STATISTICA 8.0.

Results
The higher crude protein and lipid content in diet 2 resulted in an increase in BW and LW in AG2 fish (average weight 135.8 ± 22.1), by ~9 and 11%, respectively, with concerning AG1 fish (average weight 125.3 ± 23.9). The condition factor exhibited no difference between the two groups, while HSI was 7% higher for individuals sampled from AG2 compared to samples from AG1.

Generally speaking, antioxidant activities in the AG2 fish were higher compared to the AG1 fish. As expected, the highest antioxidant enzyme activities in both populations were found in the liver compared to both muscle tissues analysed. The most pronounced significant difference ($P < 0.001$) between the two groups was found
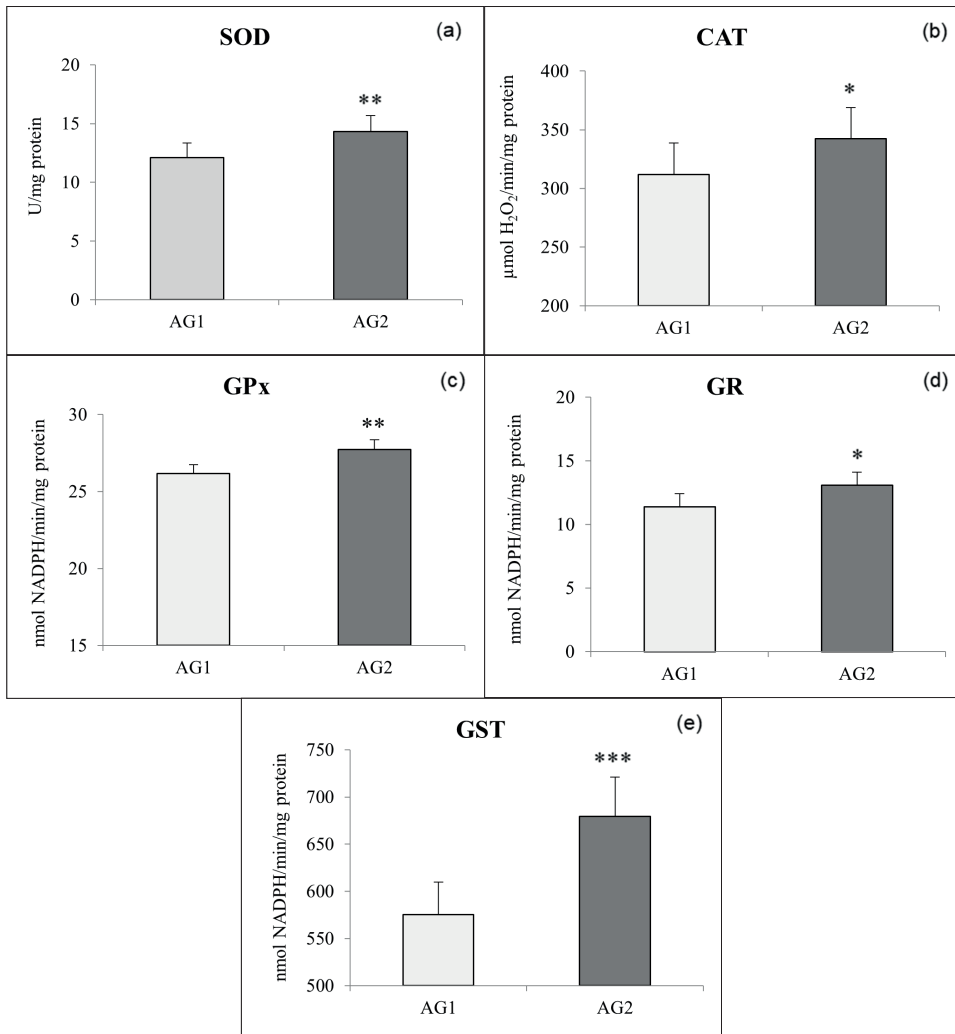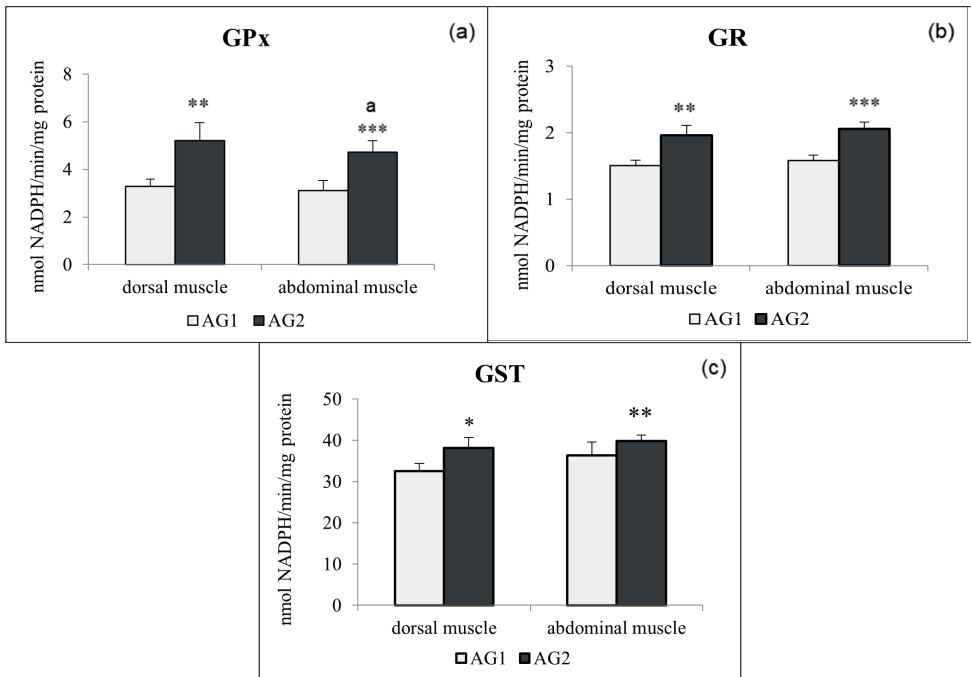
Figure 1. Activities of antioxidant biomarkers in the liver of rainbow trout fed with diet 1 - AG1 (n=15) and diet 2 - AG2 (n=15) from the "Little Danube" Centre for Fisheries and Applied Hydrobiology, University of Belgrade-Faculty of Agriculture, Serbia (CEFAH). (a) Superoxide dismutase (SOD) activity; (b) Catalase (CAT) activity; (c) Glutathione peroxidase (GPx) activity; (d) Glutathione reductase (GR) activity; (e) Glutathione-S-transferase (GST) activity (mean ± standard deviation). Asterisks (*) indicate significant differences among samples from AG2 compared to AG1 (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

for liver GST activity (Figure 1e). A significant increase in the SOD ($P < 0.01$) and GST ($P < 0.001$) (Figure 1a, e) levels of liver tissue (both by 15%) was found in AG2 compared to the AG1 group. The activities of liver CAT, GPx, and GR in liver tissue displayed significant increases of 9, 5, and 13%, respectively, in the AG2 group concerning AG1 (Figure 1b,c,d). In the AG1 fish, liver SOD activity was positively correlated with GPx activity and GPx was positively correlated

Table 3. Overall Pearson correlations among antioxidant biomarkers (SOD, CAT, GPx, GR, and GST) measured in the liver of the AG1 fish group (n=15) and AG2 fish group (n=15) from the "Little Danube" Centre for Fisheries and Applied Hydrobiology University of Belgrade-Faculty of Agriculture, Serbia (CEFAH). Statistically significant values are presented in bold ($P < 0.05$)

Liver	AG1					AG2				
	SOD	CAT	GPx	GR	GST	SOD	CAT	GPx	GR	GST
SOD	-	0.22	**0.63**	0.47	-0.16	-	-0.14	-0.12	0.29	**0.58**
CAT			0.40	0.40	0.29			0.38	0.07	0.42
GPx				**0.54**	0.31				-0.08	**0.82**
GR					0.47					**0.74**

Figure 2. Activities of antioxidant biomarkers in the dorsal muscle, and abdominal muscle of rainbow trout from AG1 and AG2 fish groups (the number of individuals for each group was n=15) from the "Little Danube" Centre for Fisheries and Applied Hydrobiology, University of Belgrade-Faculty of Agriculture, Serbia (CEFAH) fed diets with different approximate compositions. (a) Glutathione peroxidase (GPx) activity; (b) Glutathione reductase (GR) activity and (c) Glutathione-S-transferase (GST) activity (mean ± standard deviation). Asterisks (*) indicate significant differences among samples from AG2 compared to AG1 (*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$); the letter (a) denotes a significant difference (*$P < 0.05$) between dorsal muscle and the respective abdominal muscle of individuals from AG2.

with GR, while in fish from AG2, on the other hand, GST activity was positively correlated with SOD, GPx, and GR (Table 3). The levels of GR and GST in the two types of muscle tissues did not differ significantly in individuals of the same group (Figure 2c), in contrast to the case of GPx activity in individuals from AG2 (Figure 2a), where abdominal muscle activity

Table 4. Overall Pearson correlations among antioxidant biomarkers (SOD, CAT, GPx, GR, and GST) measured in (A) dorsal muscle (n=15) and (B) abdominal muscle (n=15) of both analysed fish groups, AG1 and AG2, from the "Little Danube" Centre for Fisheries and Applied Hydrobiology, University of Belgrade-Faculty of Agriculture, Serbia (CEFAH). Statistically significant values are presented in bold ($P < 0.05$)

(A)

Dorsal muscle	AG1					AG2				
	SOD	CAT	GPx	GR	GST	SOD	CAT	GPx	GR	GST
SOD		-0.06	0.25	0.34	0.26		0.37	0.13	0.64	0.2
CAT			0.32	0.19	0.23			-0.3	0.05	0.34
GPx				0.42	**0.69**				**0.82**	0.46
GR					**0.73**					0.64

(B)

Abdominal muscle	AG1					AG2				
	SOD	CAT	GPx	GR	GST	SOD	CAT	GPx	GR	GST
SOD		0.31	0.47	0.35	0.45		0.11	0.37	0.55	-0.15
CAT			0.04	0.06	0.18			-0.51	-0.15	0.4
GPx				**0.81**	0.38				**0.77**	0.2
GR					0.7					**0.83**

was significantly higher compared to dorsal muscle activity of the same individuals.

Regarding muscle tissue, Pearson correlations showed positive correlations between GSH-dependent enzymes in the dorsal muscle of the AG1 group, while a positive correlation was noted between SOD and GR activities in the dorsal muscle of the AG2 group (Table 4A). Activities of antioxidant enzymes in the abdominal muscle of both fish groups followed almost the same pattern as that shown for dorsal muscle in Table 4B.

Discussion

Values of body weight, LW, and HIS were higher in the group (AG2) fed with the diet having higher fat content (diet 2) than in the AG1 fish, as was expected (Table 3). In line with the study of Librán-Pérez et al. (2015) on rainbow trout, our results showed that a diet 2 compared to a diet 1 improved fish body weight. Since a high level of HSI is associated with high energy reserves and metabolic activity, these results indicate that fish fed a diet

with a higher content of crude lipids had a good nutritional status, but there are several drawbacks to increasing the lipid concentration in a fish feed (Sinclair, 2000). Both lipid intake and protein intake cause an acute increase in ROS generation, which indicates that nutrient intake is probably a major modulator of ROS generation (Mohanty et al., 2002). This finding raises the issue of the relative effect of different diets on ROS generation, ROS load, and potential oxidative damage.

Numerous studies have already shown an increase in the activity of antioxidant enzymes when fish were fed oxidised diets (Trenzado et al., 2006; Fontagné et al., 2008) and other papers have discussed the possibility of a PUFA-elicited antioxidant enzyme response in animals (Garrido et al., 1989; Nowak, 2013). We assume that the higher % of lipids in diet 2 given to the AG2 group caused an excessive release of superoxide anion radicals, which can cause a fairly intense activation of SOD and CAT. It has been previously shown that induced increases in CAT and SOD activity result both in a reduction of

ROS and in oxidative damage (Wilhelm-Filho et al., 1993). Alternatively, the relatively high activity of CAT in the liver (15-20 times higher than SOD activity) in the AG2 population might suggest the existence of permanent protection against the cytotoxic action of H_2O_2. The low GPx activities recorded in the present study in the liver of both fish populations were linked with extremely high activities of CAT in this organ (Figure 2b, c). This reflects competition between CAT and GPx for the same reactive oxygen species, i.e., H_2O_2.

Concerning glutathione-related enzymes, it seems that diet 2 evokes an increase of these enzymes in both the liver and the muscles of *O. mykiss*. As far as GPx activity is concerned, it was significantly weaker than the activity of CAT, but an increase in GPx activity was nevertheless present in the AG2 compared to the AG1 fish, which would greatly improve the ability of the fish to detoxify H_2O_2 or organic peroxides and could promote (via Fenton reactions) lipid peroxidation and oxidative stress (Halliwell and Gutteridge, 2006). The activity of GR and GST in both fish tissues also displayed relatively similar increasing trends as shown by GPx activity in the AG2 compared to the AG1 group. Glutathione peroxidase activity is considered complementary to CAT activity, being especially suited for H_2O_2 detoxification at low substrate concentrations. In addition, GPx reduces a variety of peroxides to their corresponding alcohols with its major detoxification function being to terminate radical chain propagation by quick reduction to yield further radicals (Kono and Fridovich, 1982). Glutathione peroxidase is considered the most effective enzyme against lipid peroxidation. In the present study, an increase in

the level of GPx activity was recorded in all analysed fish tissues. The existence of a small difference between dorsal muscle GPx activity and abdominal muscle GPx activity in fish fed with different diets indicates a weaker ability of a dorsal muscle to neutralise the impact of peroxides. The results suggest that the higher content of lipids in diet 2 evoked higher GPx activity in the liver of trout from AG2, which follows previous findings in trout and sturgeon (Trenzado et al., 2006).

The activity of GR closely followed that of GPx, as was to be expected (Stoliar and Lushchak, 2012). The higher GR activity observed in trout fed diet 2 indicates its active role in pathways of ROS recycling. The induction of GPx and GR activities could be interpreted as a greater demand for reductant power with higher lipid content. Additionally, GSH-associated enzymes such as GR, GPx, and GST cumulatively protect fish against ROS (Srikanth et al., 2013).

Glutathione-S-transferase is highly inducible in animals and humans, and its expression is affected by dietary factors that include lipids (Girón et al., 1999). The present study demonstrated an increase of GPx and GST levels positively correlated with GR activity in all tissues of *O. mykiss* (Tables 3 and 4). Because the presence of PUFA in the diet is known to increase the levels of peroxides, increased GST activity in fish fed diet 2 compared to fish fed diet 1 could be an indication of a detoxification process aimed at protecting tissues from oxidative damage.

In the liver of fish of the two studied groups, a positive relationship was found between SOD and GPx in the AG1 group and of SOD with

GPx and GST in the AG2 group (Table 3), while some findings showed a positive relationship between SOD and CAT in the liver of other fish species (Wilhelm-Filho et al., 1993). This could mean that even minor increases of lipid content in the fish diet could lead to significant physiological changes in *O. mykiss*.

The findings discussed in the present study suggest that a diet containing more lipids and digestible energy is effective in enhancing enzymatic antioxidant capacity in *O. mykiss*. Rainbow trout seem to activate antioxidant defense mechanisms in response to increasing amounts of dietary lipids, doing so in a manner efficient enough to prevent serious injury to the tissues. The observed changes probably represent a normal physiological adaptation to different compositions of the two diets used. These data enable us to assert that these changes can have an important role in monitoring the health of fish in conditions of aquaculture. Gaining an understanding of such links should guide the development of stress-based strategies to balance the lipid content of fish diets.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgment

References

Bradford MM (1976). Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry* **72**, 248-254.

Claiborne A (1985). Catalase activity. *In* "**CRC Handbook of Methods for Oxygen Radical Research**" (R. A. Greenwald, Ed.), pp. 283-284. Florida: CRC Press, Boca Raton.

Craig S and Helfrich L (2009). Understanding Fish Nutrition, Feeds, and Feeding. Cooperative Extension Service, Publication 420–256 http://pubs.ext.vt.edu/420–256.

Fontagné S, Lataillade E, Breque J and Kaushik S (2008). Lipidperoxidative stress and antioxidant defence status during ontogeny of rainbow trout (*Oncorhynchus mykiss*). *British Journal of Nutrition* **100**, 102-111.

Garrido A, Garrido F, Guerra R and Valenzuela A (1989). Ingestion of high doses of fish oil increases the susceptibility of cellular membranes to the induction of oxidative stress. *Lipids* **24**, 833-835.

Girón MD, Salto R, González Y, Girón JA, Nieto N, Periago JL, Suárez MD and Hortelano P (1999). Modulation of hepatic and intestinal glutathione S-transferases and other antioxidant enzymes by dietary lipids in streptozotocin diabetic rats. *Chemosphere* **38**, 3003-3013.

Glatzle D, Vuilleumier JP, Weber F and Decker K (1974). Glutathione reductase test with whole blood, a convenient procedure for the assessment of the riboflavin status in humans. *Experientia* **30**, 665-667.

Habig WH, Pabst MJ and Jakoby WB (1974). Glutathione S-transferase. *Journal of Biological Chemistry* **249**, 7130-7139.

Halliwell B and Gutteridge JMC (2006). "**Free Radicals in Biology and Medicine**". Clarendon Press, Oxford. ISBN 9780198717478.

Hsieh R and Kinsella J (1989). Oxidation of polyunsaturated fatty acids: mechanisms, products, and inhibition with emphasis on fish. *Advances in Food and Nutrition Research* **33**, 233-341.

Jin M, Monroig O, Lu Y, Yuan Y, Li Y, Ding L, Tocher DR and Zhou Q (2017). Dietary DHA/EPA ratio affected tissue fatty acid

profiles, antioxidant capacity, hematological characteristics, and expression of lipid-related genes but not growth in juvenile black sea bream (*Acanthopagrus schlegelii*)**.** *PLoS One* **12**, e0176216.

Kono Y and Fridovich I (1982). Superoxide radical inhibits catalase. *Journal of Biological Chemistry* **257**, 5751-5754.

Librán-Pérez M, Geurden I, Dias K, Corraze G, Panserat S and Soengas JL (2015). Feeding rainbow trout with a lipid-enriched diet: effects on fatty acid sensing, regulation of food intake and cellular signaling pathways. *Journal of Experimental Biology* **218**, 2610-2619.

Marković Z and Poleksić V (2013). Aquaculture in Serbia. *Aquaculture Europe* **38**, 32-37.

Martínez-Álvarez RM, Morales AE and Sanz A (2005). Antioxidant defenses in fish: biotic and abiotic factors. *Reviews in Fish Biology and Fisheries* **15**, 75-88.

Misra HP and Fridovich I (1972). The role of superoxide anion in the autooxidation of epinephrine and a sample assay for superoxide dismutase. *Journal of Biological Chemistry* **247**, 3170-3175.

Mohanty P, Ghanim H, Hamouda W, Aljada A, Garg R and Dandona P (2002). Both lipid and protein intakes stimulate increased generation of reactive oxygen species by polymorphonuclear leukocytes and mononuclear cells. *American Journal of Clinical Nutrition* **75**, 767-772.

Nowak JZ (2013). Oxidative stress, polyunsaturated fatty acids-derived oxidation products and bisretinoids as potential inducers of CNS diseases: focus on age-related macular degeneration. *Pharmacological Reports* **65**, 288-304.

Oliva-Teles A (2012). Nutrition and health of aquaculture fish. *Journal of Fish Diseases* **35**, 83-108.

Ross SW, Dalton DA, Kramer S and Christensen BL (2001). Physiological (antioxidant) responses of estuarine fishes to variability in dissolved oxygen. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **130**, 289-303.

Rossi MA, Cecchini G and Dianzani MU (1983). Glutathione peroxidase, glutathione reductase, and glutathione transferase in two different hepatomas and normal liver. *Medical Science-Biochemistry* **11**, 805-806.

Ruxton CHS, Calder PC, Reed SC and Simpson MJA (2005). The impact of long-chain n–3 polyunsaturated fatty acids on human health. *Nutrition Research Reviews* **18**, 113-129.

Sadekarpawar S and Parikh P (2013). Gonadosomatic and hepatosomatic indices of freshwater fish *Oreochromis mossambicus* in response to a plant nutrient. *World Journal of Zoology* **8**, 110-118.

Sinclair R (2000). Good, bad or essential fats: what is the story with Omega-3? *Nutrition and Food Science* **30**, 178-182.

Srikanth K, Pereira E, Duarte AC and Ahmad I (2013). Glutathione and its dependent enzymes' modulatory responses to toxic metals and metalloids in fish - a review. *Environmental Science and Pollution Research* **20**, 2133-2149.

Stoliar OB and Lushchak VI (2012). Environmental pollution and oxidative stress in fish. *In* "**Oxidative Stress - Environmental Induction and Dietary Antioxidants**" (V. Lushchak, Ed.), pp. 131-166. INTECH Open Access Publisher, Rijeka.

Stoyanova S, Staykov Y, Gelqzkov G, Sirakov I and Nikolov G (2016). Fish production and some traits of meat quality in rainbow trout (*Oncorhynchus mykiss*) farmed in different production systems. *Agricultural Science and Technology* **8**, 346-350.

Takada Y, Noguchi T, Okabe T and Kajiyama M (1982). Superoxide dismutase in various tissues from rabbits bearing the Vx-2 carcinoma in the maxillary sinus. *Cancer Research* **42**, 4233-4235.

Tamura M, Oschino N and Chance B (1982). Some characteristics of hydrogen and alkyl-hydroperoxides metabolizing systems in cardiac tissue. *Journal of Biochemistry* **92**, 1019-1031.

Trenzado C, Hidalgo MC, García-Gallego M, Morales AE, Trenzado CE, Furné M, Domezain A, Domezain J and Sanz A (2006). Antioxidant enzymes and lipid peroxidation in sturgeon *Acipenser naccarii* and rainbow trout *Oncorhynchus mykiss*, A comparative study. *Aquaculture* **254**, 758-767.

Wilhelm-Filho DW, Giulivi C and Boveris A (1993). Antioxidant defenses in marine fish I. Teleosts. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **106**, 409-413.