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Goose fat, a promising nutrient for fish feeding, activates antioxidant enzymes in rainbow trout, *Oncorhynchus mykiss*

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ABSTRACT

The objective of this experiment was to test effects of different dietary lipids in rainbow trout feeding on the activity and expression of antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST). Four iso-nitrogenous and iso-lipidic casein–gelatin based experimental diets were formulated. The sources of dietary lipids were cod liver oil (CO, rich in polyunsaturated fatty acids), goose fat (GF, rich in saturated fatty acids and monounsaturated fatty acids), soybean oil (SO, rich in linoleic acid), and a blend of CO, GF and SO. Dietary treatments had no significant effect on growth performance and survival was not affected. SOD, GPx and GST enzymes had the maximum activity in GF diet. However qPCR data showed that SOD and GPx mRNA levels were minimum in GF group. Overall data showed that rainbow trout liver enzymes were activated upon GF diet probably activating the enzyme structure itself without stimulating gene expression.

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1. Introduction

Fish oil (FO) is a common lipid source in fish diets due to its high proportion of long-chain, n-3 highly unsaturated fatty acids (n-3 HUFA), which are nutritionally essential to teleost fish (NRC, 1993). Because of predictable insufficient FO availability for fish feeds, alternative sources must be assessed (FAO, 2002; Menoyo et al., 2005). Studies on fish fed vegetable oil rich diets containing high-oleic sunflower oil (Torstensen et al., 2000), rapeseed oil (Bell et al., 2001), palm oil (Bell et al., 2002), soybean oil (Grisdale-Helland et al., 2002; Bayir et al., 2011), and linseed oil (Bell et al., 2003; Arslan et al., 2012) revealed that these dietary lipid sources have no detrimental effects on growth. Nevertheless, use of different oil sources in fish nutrition still needs to be evaluated in terms of flesh quality and metabolic use (Menoyo et al., 2005). A significant loss of n-3 HUFA in the flesh of Atlantic salmon was observed when FO was replaced with >66% of vegetable oils (Bell et al., 2003). Contradictory reports on the effects of

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vegetable oils on lipogenic and lipolytic enzymes (Torstensen et al., 2000; Regost et al., 2003; Menoyo et al., 2003, 2005) suggest that different oils may have different effects on fish metabolism and that different species respond in different ways.

Under physiological conditions, aerobic tissues continuously generate reactive oxygen species (ROS) as a byproduct of oxidative metabolism (Fridovich, 1998). In some specific cell types, ROS generation may be beneficial against a pathogen. However, in most cases, high amounts of ROS are harmful to cells and cause DNA damage, enzyme inactivation and structural protein degradation as well as peroxidation of poluunsturated fatty acid (PUFA) leading to pathologies and alteration of development (Halliwell and Gutteridge, 2007). Since fish contain high concentrations of n-3 HUFA, they are vulnerable to lipid peroxidation and to tissue damage resulting from lipid peroxidation (Hsieh and Kinsella, 1989). Oxidative stress is due to the overwhelming of antioxidant defenses of cells by pro-oxidants. This imbalance can be aggravated by diet composition. Feeds for fish and especially feeds for fish larvae contain high levels of PUFA derived from marine fish oils and meals. These fatty acids are particularly prone to oxidative damage during diet preparation and storage. Products derived from lipid peroxidation may be absorbed and transported to tissues, where they may induce oxidative stress (Hata and Kaneda, 1980). To decrease ROS levels and avoid lipid peroxidation, all aerobic organisms possess two types of antioxidant defence systems. One is represented by enzymes and includes superoxide dismutase, catalase and glutathione peroxidase and glutathione S-transferase. SOD is a metalloenzyme that catalyses the dismutation of the superoxide anion (O_2^-) into oxygen and hydrogen peroxide (H_2O_2) (Fridovich, 1997). Subsequently, H_2O_2 is reduced to water by CAT in the peroxisomes. GPx catalyses the same reaction as CAT in the cytosol and also converts lipid hydroperoxides into lipid hydroxides, which are more stable products. The activity levels of these antioxidant enzymes are very important for the antioxidant defense system and detoxification mechanisms because liver is a very active organ where antioxidant scavenging and detoxification reactions occur. Inhibition or activity loss in these enzymes causes disruption of antioxidant defense might result in collective fish deaths.

To study the mechanistic basis of fish nutrition, it is essential to understand the biochemical mechanisms enabling efficient enzymatic catalysis under different nutritional conditions. There is not enough information on the biochemical consequences of goose fat feeding in the literature. Therefore, the objective of this research was to evaluate the influences of dietary lipid sources including goose fat, a new source in fish feeding, on the activity and expression levels of antioxidant enzymes in rainbow trout liver.

2. Materials and methods

2.1. Chemicals

Chemicals used for enzyme activity measurements were purchased from Sigma–Aldrich, Munich, Germany or Merck, Darmstadt, Germany. All other chemicals were of analytical grade and obtained from either Sigma–Aldrich or Merck.

Table 1 – Ingredient and proximate composition of the experimental diets (% dry matter).

Ingredients (%)	Lipid Sources			
	Cod liver oil	Soybean oil	Goose fat	Blend
Casein (vitamin free)	43.3	43.3	43.3	43.3
Gelatin	7.53	7.53	7.53	7.53
Dextrin (80% water soluble)	6.72	6.72	6.72	6.72
Wheat meal	16.1	16.1	16.1	16.1
Fish protein concentrate[a]	5.26	5.26	5.26	5.26
Cod liver oil	15.00	0	0	5
Soybean oil	0	15.00	0	5
Goose fat	0	0	15.00	5
Vitamin mixture[b]	4.08	4.08	4.08	4.08
Mineral mixture[c]	3.06	3.06	3.06	3.06
Carboxymethylcellulose sodium salt (CMC)	2.04	2.04	2.04	2.04
L-Arginine	0.50	0.50	0.50	0.50
L-Methionine	0.38	0.38	0.38	0.38
L-Lysine	0.60	0.60	0.60	0.60
Choline chloride (99%)	0.97	0.97	0.97	0.97

[a] Concentrate of fish-soluble protein (CPSP 90: crude protein, 82–84% wet weight (ww); crude lipid, 9–13%, ww); Sopropéche S.A., Boulogne-sur-mer, France.

[b] Roche Performance Premix (Hoffman-La Roche, Inc., Nutley, NJ, USA), composition in per gram of the vitamin mixture: vitamin A, 2645.50 IU; vitamin D3, 220.46 IU; vitamin E, 44.09 IU; vitamin B12, 13 mg; riboflavin, 13.23 mg; niacin, 61.73 mg; D-pantothenic acid, 22.05 mg; menadione, 1.32 mg; folic acid, 1.76 mg; pyridoxine, 4.42 mg; thiamin, 7.95 mg; and D-biotin, 0.31 mg.

[c] Bernhart Tomarelli salt mixture (ICN Pharmaceuticals, Costa Mesa, CA, USA), composition in g/100 g: calcium carbonate, 2.1; calcium phosphate dibasic, 73.5; citric acid, 0.227; cupric citrate, 0.046; ferric citrate (16–17% Fe), 0.558; magnesium oxide, 2.5; manganese citrate, 0.835; potassium iodide, 0.001; potassium phosphate dibasic, 8.1; potassium oxide, 6.8; sodium chloride, 3.06; sodium phosphate, 2.14; and zinc citrate, 0.133. Five milligrams of Se in the form of sodium selenite was added per kilogram of the salt mixture.

2.2. Experimental diets

Four iso-nitrogenous and iso-lipidic casein–gelatin based experimental diets with varying dietary sources were formulated. The sources of dietary lipid were cod liver oil (CO diet, rich in highly unsaturated fatty acids), goose fat (GF diet, rich in saturated fatty acids and monounsaturated fatty acids), soybean oil (SO diet, rich in linoleic acid), and a blend of cod liver oil, goose fat, soybean oil (CO + GF + SO diet).

Diets were prepared with a laboratory pelleting machine after 35–40 g of distilled water was added into 100 g mixture of ingredients, and freeze-dried. Diets were then crushed to the approximate size of 0.7–2 mm and kept at −20 °C until used. The composition and fatty acid profiles of the experimental diets used in this study are shown in Tables 1 and 2, respectively.

Table 2 – Fatty acid composition of the oils and experimental diets (mg g^{-1} diet dry matter).

Fatty acid composition (g mg^{-1})	Dietary oils				Dietary treatments		
	Fish	Soybean	Geese	CLO	SBO	GF	BL
14:0	5.00	0.10	0.60	4.50	0.32	0.68	1.68
16:0	13.8	10.4	24.4	13.3	10.4	23.6	15.6
18:0	3.00	4.50	6.10	3.00	4.23	6.40	4.55
∑SFA	**26.8**	**15.6**	**31.6**	**25.8**	**15.7**	**31.3**	**23.9**
16:1 n-7	6.1	0.1	4.3	5.75	0.35	3.92	3.18
18:1 n-9	21.1	1.4	1.7	21.2	23.2	51.2	13.8
18:1 n-7	3.2	22.6	53.6	3.10	1.58	1.72	2.11
∑MUFA	**34.9**	**24.4**	**60.0**	**35.8**	**25.7**	**57.7**	**39.4**
18:3 n-3	2.7	7.8	0.8	2.65	7.78	0.88	3.78
20:5 n-3	10.0	0.40	–	9.82	0.70	0.33	3.34
22:5 n-3	2.70	–	–	2.37	0.13	0.07	0.72
22:6 n-3	10.6	–	–	10.8	0.50	0.45	3.66
∑n-3	**28.6**	**8.2**	**0.8**	**28.5**	**9.25**	**1.89**	**12.4**
18:2 n-6	5.9	51.6	7.8	6.56	48.9	8.71	23.2
20:2 n-6	0.50	–	–	0.60	0.06	0.09	0.22
20:4 n-6	0.50	–	–	1.00	–	0.02	0.31
∑n-6	**7.80**	**52.0**	**7.80**	**9.38**	**49.2**	**8.88**	**24.1**
∑PUFA	**37.7**	**60.4**	**8.7**	**39.0**	**58.6**	**11.0**	**36.9**
∑n-3/∑n-6	**3.65**	**0.16**	**0.10**	**3.04**	**0.19**	**0.21**	**0.52**

2.3. Fish husbandry and maintenance

Juvenile rainbow trout with an initial individual weight of ~1 g were distributed randomly in three replicate 50-L glass aquaria with a total of 25 fish per tank. Each tank received one of the four diets referenced above for 8 weeks. Well water was supplied at a rate of 1 L min^{-1}, and a semi-recirculation system with partial sedimentation and biofiltration was used to maintain stable conditions. Each aquarium was equipped with continuous aeration and the water temperature was 13.0 ± 0.2 °C. The fish were fed four times a day at (~3% body weight/day). At the end of the feeding trial, two fish from each aquarium were sampled and stored at −80 °C for enzyme analysis.

2.4. Enzyme assays

Samples were washed three times with 50 mM Tris–HCl + 0.1 M Na_2SO_4 (pH 8.0), and each was homogenized by liquid nitrogen, transferred to the same buffer, and centrifuged at 4 °C, 15,000 × *g* for 60 min. Supernatant was used in further studies.

The following enzyme activities were measured by using a spectrophotometer (Thermo Scientific, Multiskan Go, Japan).

2.4.1. SOD activity

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by inhibition of xantine/xantine oxidase generated O_2^- reduction of nitroblue tetrazolium (NBT) (Sun et al., 1988). NBT was reduced to blue formazon by O_2^- which has a strong absorbance at 560 nm. One unit was defined as the amount of protein producing 50% inhibition of reaction. The reaction mixture of 1 mL contained 1 M Tris–HCl, 5 mM EDTA buffer (pH 8.0), 150 μM NBT, 400 mM Na_2CO_3, 1 g L^{-1} bovine serum albumine, 3 mM L^{-1} xantine, 0.833 U mL^{-1} xantine oxidase, 0.8 mM $CuCl_2$ and tissue homogenate (30 μL).

2.4.2. GPx activity

GPx (EC 1.11.1.9) activity was determined by following the rate of nicotinamide adenine dinucleotide phosphate (NADPH) oxidation at 340 nm by the coupled reaction with glutathione reductase (GR) (Beutler, 1975, 1984). The assay system contained 1 M Tris–HCl, 5 mM EDTA buffer (pH 8.0), 0.1 M GSH, 10 U mL^{-1} GR, 2 mM NADPH, 7 mM t-buthylhydroperoxide, distillated water and tissue homogenate (10 μL).

2.4.3. GST activity

GST (EC 1.5.1.18) activity was evaluated with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate in a final reaction mixture containing 0.11 M phosphate buffer (pH 6.5), 30 mM CDNB, 0.1 M GSH, distillated water and tissue homogenate (50 μL) (Habig et al., 1974). The activity rate was measured as change in optical density at 340 nm (ε = 9.6 mM^{-1} cm^{-1}).

2.5. Protein determination

Quantitative protein determination was spectrophotometrically measured at 595 nm according to Bradford's method (Bradford, 1976), with bovine serum albumin as a standard.

2.6. Lipid and fatty acid composition of experimental diets

Folch et al. (1957) method was used for lipid extraction. According to this method, experimental diet were homogenized in chloroform/methanol (2:1, v/v) containing 0.01% (w/v) of butylated hydroxytoluene [Sigma, ≥99.0% (GC)] as antioxidant 20 vol (w/v) for 1 min, then about 1 g sample was used for analysis. The organic solvent was evaporated under a stream of nitrogen and the amount of lipid was determined gravimetrically. Fatty acid methyl esters (FAME) were prepared from both fractions of lipids according to method of Metcalfe and

Table 3 – Sequence, amplification length, genbank accession number of primers and probes used for real-time PCR.

Gene	Sequence (listed 5′→3′)	Amplification length	GenBank accession no
SOD forward	TGCTTATGGAGACAACACCAA	156 bp	AF469663.1
SOD reverse	TGGATGTTGATCTTAGCCACA		
SOD probe	^{FAM}- CCACTGATGCTGTTCGGCACGT -^{TAMRA}		
GPx forward	GTGCCCTGCAACCAGTTT	134 bp	AF281338.1
GPx reverse	TTCCCATTCACATCCATCTTC		
GPx probe	^{FAM}- CCGTCCCGGAAATGGCTTTGA -^{TAMRA}		
GST forward	TGGCTGACGTTATTGTCTTCC	112 bp	NM_0011605591
GST reverse	CTGGGTCTGTCCTTCACCATA		
GST probe	^{FAM}- CGGCGCGTTACCCCAAACTG -^{TAMRA}		
β-Actin forward	TGGCCGTACCACCGGTAT	79 bp	AF254414
β-Actin reverse	GCAGAGCGTAGTCCTCGTAGATG		
β-Actin TaqMan probe	^{Cy5}- CTCCGGTGACGGCGTGACCC -^{BQ2}		

Schmitz (1961). Prior to transmethylation, a known amount of nonadecanoate acid (19:0), proportional to the amount of total, neutral or phospholipids detected (8 mg per 50 mg of lipids), was added to the samples as the internal standard for the quantification of fatty acids. The crude lipid extract was saponified with sodium hydroxide in methanol and FAMEs were prepared by transmethylation with boron trifluoride in methanol. FAME were obtained on a HP (Hewlet Packard) Agilent 6890 N model gas chromatography (GC), equipped with a flame ionization detector and fitted with a DB 23 capillary column (60 m, 0.25 mm i.d. and 0.25 μm) ejector and detector temperature program was 190 °C for 35 min than increasing at 30 °C per min up to 220 °C where it was maintained for 5 min. Carrier gas was hydrogen (2 ml min^{-1} and split ratio was 30:1). The individual FAs were identified by comparing their retention times to that of a standard mix of FAs (Supelco 37 component FAME mix) and quantification of the individual fatty acids (mg g total $lipids^{-1}$), were made against a C19:0 internal standard from Sigma (USA).

2.7. RNA isolation and cDNA synthesis

The liver tissue was isolated and then frozen. Total RNA was isolated from 500 mg of frozen tissues using TRIzol reagent. RNA was treated with DNase in order to avoid genomic contamination. RNA concentrations and quality were verified by means of spectrophotometer (Nanodrop) and RNA gel electrophoresis, respectively. Following isolation, cDNA synthesis was performed using the ThermoScript™ RT-PCR System for First-Strand cDNA Synthesis Kit (Invitrogen) according to the manufacturer's protocol. All cDNA was stored at − 20 °C until use.

2.8. TaqMan probe and primer design

Primers and TaqMan probes were designed in Primer3 software (v. 0.4.0) (http://frodo.wi.mit.edu/) using rainbow trout liver SOD (GenBank accession number AF469663.1), GPx (GenBank accession number AF281338.1) and GST (GenBank accession number NM_001160559.1) sequences and BLASTed to ensure correct mRNA sequences. β-Actin was used as a suitable reference gene since it was not affected by any of the treatments (GenBank accession number AF254414) (Johansen and Overturf, 2005). In order to perform Real Time PCR, TaqMan probe of the reference gene was conjugated with Cy5/Blackhole Quencher 2, the fluorophore and quencher molecules, whereas the probes for target genes were conjugated with FAM/TAMRA. The primer and probe sequences are provided in Table 3.

2.9. Real-time PCR

Quantification of gene expression by real-time PCR analysis was performed using a thermal cycler Stratagene MxPro3000. The real-time PCR was carried out in a reaction volume of 50 μl containing template DNA, 900 nM of both target and reference forward and reverse primers, 250 nM both target and reference TaqMan probes, and 25 μl FastStart TaqMan Probe Master (Applied Biosystems) which consists of AmpliTaq Gold DNA Polymerase, AmpErase uracil N-glycosylase (UNG), dNTP with dUTP, and optimized buffer component. Amplification and detection of the samples and the standards were performed using the following thermal cycling conditions: 50 °C for 2 min for activation of optical AmpErase UNG enzyme, 95 °C for 10 min as hot start to activate AmpliTaq Gold DNA polymerase followed by 45 cycles of denaturation at 95 °C for 15 s, and annealing and extension at 60 °C for 1 min. Real-Time PCR data were analyzed using the efficiency (*e*) (−ΔCt) method (Pfaffl, 2001; Ostaszewska et al., 2010), which is used to determine mRNA levels in gene expression against control group and reference gene *β*-actin. Analytical sensitivity was confirmed by running standard curves. Amplification efficiency (e) was calculated based on the slopes of the curves (slope) using the formula $e = 10^{(-1/slope)}$ (Pfaffl, 2001), and the slope value via Stratagene MxPro3000 software.

2.10. Statistical analysis

Results were expressed as mean ± SD ($n = 3$). Each experimental unit (aquarium) was considered a replicate. For enzyme activity analysis, two fish from each aquarium were used. Data were subjected to one way analysis of variance (ANOVA) to test the effect of dietary lipid sources on enzyme activity and expression level and subsequent comparison of means by Duncan's multiple range tests. Independent *t* test was used to compare activity and expression level of each enzyme for each experimental group with those for initial fish. Differences were considered statistically significant at $P < 0.05$.

Table 4 – Growth parameters and survival in rainbow trout fed the experimental diets for 8 weeks.

Diets	IBW[a]	FBW[b]	WG[c]	SGR[d]	FCR[e]	SR[f]
CO	1.18 ± 0.04	6.51 ± 0.13	453.93 ± 8.14	3.11 ± 0.03	1.04 ± 0.01	96.00 ± 4.00
GF	1.18 ± 0.02	6.36 ± 0.24	439.42 ± 20.16	3.06 ± 0.07	1.02 ± 0.04	94.66 ± 6.14
SO	1.18 ± 0.01	6.49 ± 0.24	450.23 ± 37.57	3.09 ± 0.12	1.16 ± 0.20	93.33 ± 6.11
BL	1.18 ± 0.03	6.51 ± 0.21	463.67 ± 23.51	3.14 ± 0.07	1.05 ± 0.04	94.66 ± 6.13

Data are presented as means ± SD ($n = 3$).
[a] IBW, Initial body weight (g/fish).
[b] FBW, Final body weight (g/fish).
[c] WG, Weight gain (% g/100 g) = 100 × (final body weight – initial body weight)/initial body weight.
[d] SGR, Specific growth rate (%/day) = 100 × [ln (final body weight) – ln (initial body weight)]/feeding days.
[e] FCR, Food conversion ratio = dry feed intake (g)/weight gain (g).
[f] SR, Survival rate = 100 × (final fish number/initial fish number).

3. Results

Dietary treatments had no significant effect on growth performance of juvenile rainbow trout ($P < 0.05$) (Table 4). Fish fed Blend (CO + GF + SO) diet had the highest growth, while SO fed fish exhibited the poorest growth performance. Although some concern exists that feeding diets with oil may affect fish health (Thompson et al., 1996; Bell et al., 2001), survival was not affected by the dietary treatments in the current study (Table 4). The composition and fatty acid profiles of the experimental diets used in this study are shown in Tables 1 and 2, respectively. Dietary treatments had significant effect on the activity and gene expression of antioxidant enzymes. Changes in the enzyme activity in rainbow trout fed diets with different lipid sources are presented in Figs. 1–3. In almost all cases, the activity of all enzymes significantly increased ($P < 0.05$) in comparison to their activities in initial fish. However, expression levels of the enzymes did not exhibit compatible results and represented in Figs. 1–3. The activity of liver SOD and GPx enzyme were at the highest level in GF diet whereas the minimum expression levels were also observed in this group. Similarly, GST activity was the highest upon GF diet but the minimum mRNA level was not observed in this group.

4. Discussion

Compound diets are formulated to meet the specific nutritional requirements of fish. Lipids play an important role as a source of dietary energy for fish. They are well utilized by fish and necessary for normal growth and development. They also provide the essential fatty acids for normal functions of organisms. In rainbow trout, diets with different lipid sources have been rarely tested. This study attempted to establish a relationship between differing lipid nutrition and antioxidant enzymes SOD, GPx and GST in rainbow trout. Although few studies focused on lipid nutrition in rainbow trout, there is no information about enzymatic consequences. In this experiment, we have demonstrated that different dietary lipid supplementation altered both the activity and expression levels of the antioxidant enzymes.

Several methods have been established as markers of oxidative stress in animal cells, caused either by excessive production of ROS or reduced antioxidant defense (Armstrong, 2002; Ekinci and Beydemir, 2009; Balaydin et al., 2012; Cavdar et al., 2012a). SOD catalyzes the dismutation of $^{\bullet}O_2^-$ into O_2 and H_2O_2 (Fridovich, 1986). In the current study, we analyzed both enzyme activity and mRNA expression profiles of liver SOD enzymes in rainbow trout. Liver SOD enzyme had the maximum activity in GF diet and minimum in CO and SO diets. However, the result of gene expression analysis does not confirm this data. It is known that antioxidant enzymes are very active in liver in many organisms (Aksakal et al., 2010; Cankaya et al., 2007; Ekinci and Beydemir, 2010; Gulcin et al., 2008; Cakmak et al., 2011). From combination of activity and expression data, it could be said that CO diet induces the expression of SOD gene in muscle, but it may also cause an

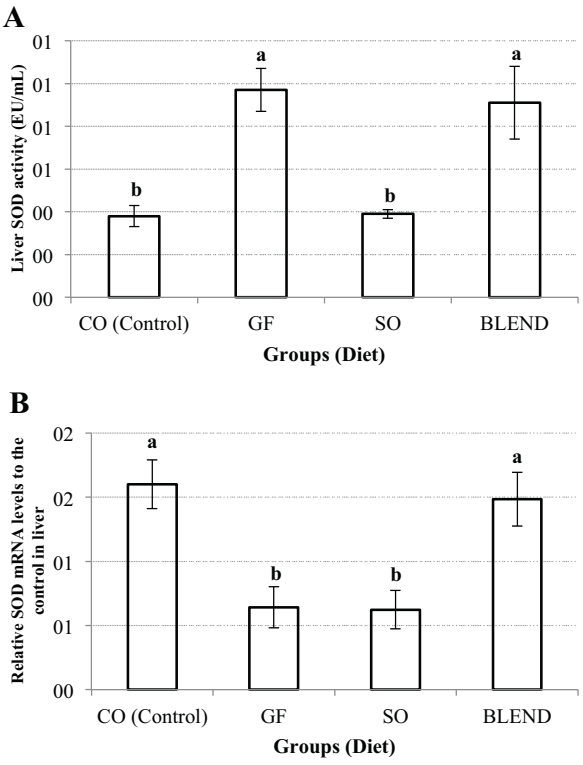


Fig. 1 – (A) *Oncorhynchus mykiss* liver SOD activity upon different diets. a, b, c, d = Statistical significance between diets ($p < 0.05$). (B) Relative mRNA levels of SOD in Oncorhynchus mykiss liver upon different diets ($p < 0.05$). β-Actin was used as the referenge gene. a, b, c, d = Statistical significance between diets.

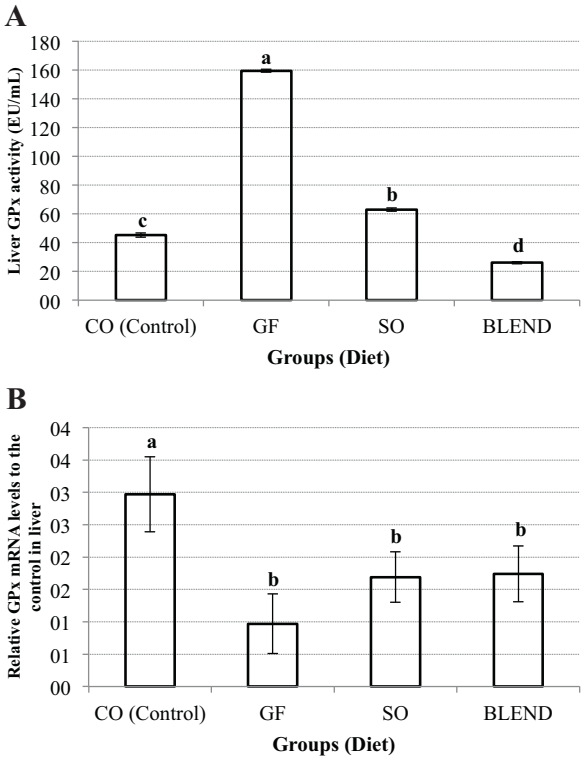


Fig. 2 – (A) *Oncorhynchus mykiss* liver GPx activity upon different diets. a, b, c, d = Statistical significance between diets ($p < 0.05$). (B) Relative mRNA levels of GPx in *Oncorhynchus mykiss* liver upon different diets ($p < 0.05$). β-Actin was used as the referenge gene. a, b, c, d = Statistical significance between diets.

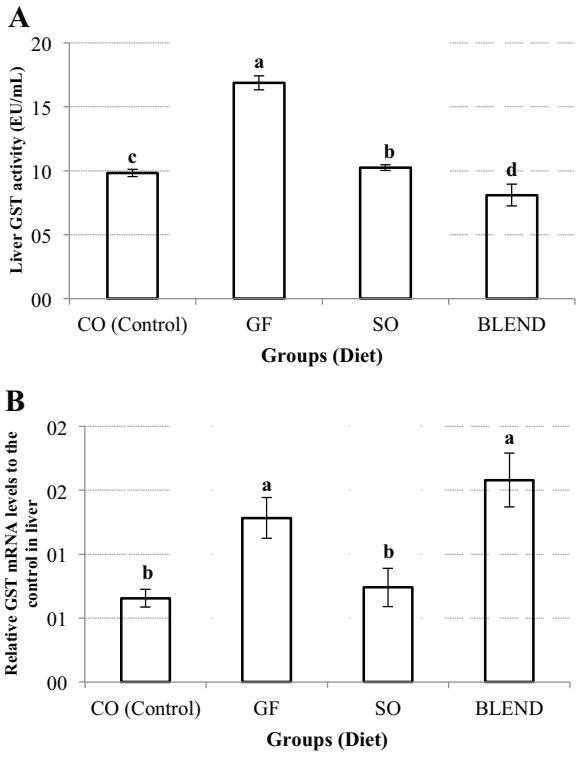


Fig. 3 – (A) *Oncorhynchus mykiss* liver GST activity upon different diets. a, b, c, d = Statistical significance between diets ($p < 0.05$). (B) Relative mRNA levels of GST in *Oncorhynchus mykiss* liver upon different diets ($p < 0.05$). β-Actin was used as the referenge gene. a, b, c, d = Statistical significance between diets.

inhibition on the active protein structure. Interestingly, GF diet showed the opposite action. The activity of liver SOD enzyme was at the highest level in GF diet whereas the minimum expression level was also observed in this group. Although it is speculative to explain this situation, it is possible that GF suppress the expression of this gene, but it may also activate the enzyme structure itself probably by interacting with the protein sulfhydryl groups.

As for the GPx enzyme, maximum activity was shown by GF group and minimum by blend. Only GF showed considerably high activity and others were quite low. However, expression data does not support this finding; the highest mRNA level was observed upon CO and minimum upon GF although there are not significant differences among GF, CO and Blend. The activity and expression data of SOD and GPx enzymes showed quite similar behavior especially upon GF treatment.

Glutathione S-transferases, are members of a multigene family of isoenzymes ubiquitously expressed in most living organisms (Cavdar et al., 2012b). It was subsequently shown that these enzymes catalyze the conjugation of GSH to a variety of electrophilic compounds, thus establishing the now widely accepted role of GSTs as cell housekeepers involved in the detoxification of endogenous as well as exogenous substances (Ekinci and Senturk, 2013). In our study, similar to other two enzymes, maximum GST activity was observed in GF. The mRNA levels were also compatible with the activity profiles except for the Blend group.

Although there were inconsistencies between activity and expression data, overall results indicate goose fat significantly activates the antioxidant enzymes in rainbow trout liver. Even though there are many studies regarding the effects different parameters on fish enzymes (Ekinci et al., 2011; Erdogan et al., 2011; Demirdag et al., 2012; Ceyhun et al., 2011) few literatures on the effect of different diets on some metabolic parameters in fish (Yildiz et al., 2006) and we have not encountered any data on the effect of the diets used in this study on activity and expression of antioxidant enzymes. Especially, the present study is the first one to measure the expression of genes coding for antioxidant enzymes and activity profiles upon GF diet. There are not enough investigations on GF regarding fish metabolism. The results of gene expression analyses in part confirm data of antioxidant enzyme activities. It is known that CO is widely used as control in fish feeding studies and results of our study show that GF treatment causes significant induction of the corresponding enzyme activity levels compared to CO diet.

5. Conclusions

In conclusion, GF diet caused significant increase in antioxidant enzymes SOD, GPx and GST in liver tissue which indicates

that goose fat may represent a promising class of fish nutrient and results discussed in this study may assist to agriculturists to design novel fish diets with enhanced efficiency and other tailored properties.

Conflict of interest statement

Nothing declared.

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