http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (20): 1194-1198, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.1194.1198

Alterations in Fatty Acids of Polar Lipids in *Salmo trutta* on Long-term Exposure to a Glyphosate-Based Herbicide (Roundup®)

[1]Mehtap Bayir, [1]A. Necdet Sirkecioglu, [2]Abdulkadir Bayir and [2]Mevlut Aras
[1]Department of Agricultural Biotechnology, Faculty of Agriculture, Ataturk University, 25240, Erzurum, Turkey
[2]Department of Aquaculture, Faculty of Fisheries, Ataturk University, 25240, Erzurum, Turkey

Abstract: In present study, the effects of sublethal doses (10 and 20 mg L^{-1}) of Roundup® on fatty acid pattern in muscle and liver of brown trout were investigated. For this purpose, fish were held in experiment tanks for 1 month. While total MUFA wasn't influenced, the highest total SFA and total n-6 PUFA were determined in group 10 mg L^{-1} and the lowest values were determined in control group and group 20 mg L^{-1} in muscle, respectively. The highest and the lowest total n-3 PUFA was found in control group and group of 10 mg L^{-1} in muscle, respectively. Total n-3/n-6 PUFA ratio and EPA+DHA level of group 10 mg L^{-1} were lower than other groups in muscle. The amount of total n-3/n-6 PUFA, EPA+DHA and total n-3 PUFA of control group were found higher than treatment groups in liver. While the highest total SFA was determined in group 10 mg L^{-1}, there wasn't difference between control group and group 20 mg L^{-1} in liver. Both of doses herbicide had higher value than control for total MUFA in liver. While Roundup® didn't inhibit n-3 PUFA synthesis in the muscle, both concentrations, exhibited inhibitory effect on n-3 PUFA synthesis in the liver. This result probably consequence of its indirect effect on the some enzyme activities or gene expressions in fatty acid metabolism of brown trout.

Key words: Brown trout, herbicide, n-3 fatty acids, toxicity, DHA

INTRODUCTION

Glyphosate (N-(phosphonomethyl) glycine), the world top-used non-selective foliar-applied herbicide, is widely used for elemination of annual and perannual plants including grasses, sedges and weeds in agricultural, industrial and residential areas. It can also be used in aquatic environments for aquatic weed control (Tsui and Chu, 2008). Roundup® contains the isopropylamine salt of glyphosate as the active ingredient and a surfactant, polyoxyethylene amine (POEA). It has been known that surfactant POEA makes Roundup® highly toxic for aquatic organisms such as frog, bacteria, algae, protozoa and zooplankton (Tsui and Chu, 2003; Anonymous, 2013a, b).

Fish naturally contain high levels of n-3 polyunsaturated fatty acids (n-3 PUFA) such as eicosapentaenoic acid (EPA, 20: 5 n-3) and docosahexaenoic acid (DHA, 22: 6 n-3) that are recognized essential biochemical components of the human diet because of their beneficial effects of human health. Fatty acid composition of fish can be affected by many different factors such as the type and amount of feed available, water temperature, pH and salinity (Bayir *et al*., 2010). Although, there are a lot of study on the effect of glyphosate in metabolic, hematological and immunological parameters for fish (Glusczak *et al*., 2006; Kreutz *et al*., 2010, 2011) and ecotoxical risk assessment (Giesy *et al*., 2000), no study on relations between herbicides and fatty acid profiles of fish are available. It is clear that consumption of fish which are contaminated by pesticides or herbicides is highly dangerous for human health. But, it is also well known that many people consume these contaminated fish all over the world due to rapid pollution in water sources. Moreover, studies on fatty acid profiles, especially in liver, are necessary to get a deeper understanding of the deleterious effect of agrochemicals on fish. Animal lipids, including fish lipids, can be divided into two main groups, polar lipids composed principally of phospholipids and neutral lipids composed principally of triacylglycerols (Tocher, 2003). The general assumption is that polar lipids physiologically more important than neutral storage lipids which are mainly influenced by trophic levels (Bayir *et al*., 2011). For these reasons, the aims of the present study were to evaluate the effects of two sublethal doses (10 and 20 mg L^{-1}) of Roundup®, a glyphosate-based herbicide, on fatty acid pattern of polar lipids in muscle and liver of brown trout, *Salmo trutta*.

Corresponding Author: Mehtap Bayir, Department of Agricultural Biotechnology, Faculty of Agriculture, Ataturk University, 25240, Erzurum, Turkey Tel: +90.442.2311079 Fax: +90.442.2360958

MATERIALS AND METHODS

Experimental fish and housing: Brown trout (*Salmo trutta*) with a mean body weight of 110.52±20.12 g were obtained from research and extension center of the Fisheries Faculty at the Ataturk University, Erzurum, Turkey. Fish were randomly distributed to flow-through tanks (volume = 75 L; flow rate = 4 L h^{-1}) with a density of 10 fish/tank. Prior to the experiment, all fish were acclimatized to the environmental condition by feeding commercial trout feed at 12°C for two weeks. A diurnal light: dark cycle of 12:12 h was provided by fluorescent lighting. The feed was offered at approximately 1% body weight per day based on the initial biomass for each tank. Toxicant did not affect the feed intake. Each treatment was replicated (2 test tanks/treatment; n = 10 $tank^{-1}$). At the completion of the 30 days trial fish were anesthetized using 100 mg L^{-1}t MS-222 for 5 min and the muscle and liver tissues (c. 1 g) were dissected and frozen immediately in liquid nitrogen. Samples were transferred to the laboratory and stored at -84°C until analyses.

Experimental design: Following acclimation, 20 fish $groups^{-1}$ were exposed to a range of concentrations of Roundup® UltraMax including 450 g L^{-1} glyphosate for 30 days under flow-through conditions. Sublethal glyphosate concentrations were selected to be 10 (group b) and 20 (group c) mg L^{-1}. Control (group a) tanks received no chemicals. Concentrations of glyphosate were maintained through continuous addition of concentrated stock solution from special bottles which made of fluorinated high density polyethylene to reduce herbicide adsorption with flow rates of 2 mL min^{-1}.

Lipid fraction separation: Crude muscle and liver lipids were separated into the polar (phospholipids) lipid fractions using sep pak silica cartridges. The residual lipid (c. 0.15 g) was applied to a silica filter and the neutral lipids were eluted with 30 mL chloroform. After the elution of the neutral lipids, the polar fractions were eluted with 30 mL methanol. Solvent from both fractions was then evaporated under nitrogen and the amounts of polar lipids were determined gravimetrically (Czesny and Dabrowski, 1998).

Lipid and fatty acid analysis: Fatty acid methyl esters (FAMEs) were prepared from polar lipids according to the method of Metcalfe and Schmitz (1961). The crude lipid extract was saponified with sodium hydroxide in methanol and FAMEs were prepared by transmethylation with boron trifluoride in methanol. FAMEs were obtained using an HP (Hewlet Packard) Agilent 6890 N model Gas Chromatography (GC) equipped with a flame ionisation detector and fitted with a DB 23 capillary column (60 m, 0.25 mm i.d. and 0.25 μm). The ejector and detector temperature program was 190°C for 35 min followed by an increase of 30°C min^{-1} up to 220°C; this temperature was maintained for 5 min. The carrier gas was hydrogen (2 mL min^{-1}) and split ratio was 30: 1. The individual Fatty Acids (FAs) were identified by comparing their retention times to those of a standard mix of FAs and the FAs were quantified by comparing their peaks to those of the standards (David *et al*., 2003).

Statistical analysis: The statistical analyses were performed with SPSS version 10.0 for Windows (SPSS, 1996). Data were presented as Mean±Standard Deviation (SD) of the mean. Data were analyzed by one-way analysis of variance (ANOVA). The significant means were compared by Duncan's multiple range tests at $\alpha = 0.05$ level.

RESULTS

Fatty acid compositions of polar lipids in muscle and liver: Fatty acid patterns of polar lipids in the muscle and liver of brown trout, following a 30 days trial, are shown in Table 1 and 2, respectively. As seen in these tables, palmitic acid (16: 0) in Saturated Fatty Acids (SFA), oleic acid (18: 1 n-9) in Monounsaturated Fatty Acids (MUFA), DHA in the n-3 PUFA and linoleic acid (18: 2 n-6) and arachidonic acid (20: 4 n-6) in n-6 polyunsaturated fatty acids (n-6 PUFA) were the predominant fatty acids in both tissues for all treatment groups.

Table 1: Fatty acid composition (% of total fatty acids[A]) of muscle polar lipids in *Salmo trutta* exposed to sublethal concentrations of Roundup® for a period of 30 days[B]

Fatty acids	Control	10 (mg L^{-1})	20 (mg L^{-1})
14:0	1.21±0.17^{a}	1.42±0.04^{a}	1.42±0.03^{a}
16:0	14.60±1.05^{a}	15.55±0.10^{a}	14.66±0.21^{a}
18:0	7.51±0.53^{b}	8.22±0.05^{a}	8.54±0.06^{a}
Σ SFA	24.17±0.24^{c}	26.15±0.18^{a}	25.54±0.18^{b}
16:1 n-7	1.08±0.07^{b}	1.17±0.04^{ab}	1.22±0.08^{a}
18:1 n-9	8.29±0.58^{a}	8.19±0.06^{a}	8.26±0.11^{a}
18:1 n-7	1.81±0.19^{a}	1.60±0.06^{a}	1.67±0.02^{a}
20:1 n-9	1.65±0.10^{b}	1.86±0.09^{a}	1.90±0.02^{a}
Σ MUFA	14.26±0.79^{a}	13.98±0.15^{a}	14.14±0.19^{a}
18:3 n-3	0.33±0.02^{a}	0.22±0.02^{b}	0.20±0.01^{b}
20:5 n-3	5.00±0.30^{ab}	5.47±0.40^{a}	4.86±0.34^{b}
22:5 n-3	1.31±0.24^{a}	1.10±0.10^{ab}	0.88±0.02^{b}
22:6 n-3	44.39±0.01^{a}	42.31±1.71^{c}	44.20±0.76^{b}
Σ n-3 PUFA	51.32±0.74^{a}	49.60±0.13^{c}	50.53±0.92^{b}
18:2 n-6	1.52±0.29^{ab}	1.37±0.02^{b}	1.75±0.02^{a}
20:4 n-6	4.61±0.53^{a}	4.46±0.11^{a}	4.36±0.07^{a}
Σ n-6 PUFA	10.24±0.66^{ab}	10.83±0.10^{a}	9.74±0.21^{b}
Σ n-3/n-6 PUFA	5.02±0.30^{a}	4.58±0.05^{b}	5.19±0.12^{a}
EPA+DHA	49.39±0.50^{a}	47.78±0,65^{b}	49.06±0.88^{a}

[A]Values are expressed as percentages of total fatty acids, [B](a-b-c) means in a row with identical letters are not significantly different. Values were presented as mean SD (n = 4) ($p<0.05$)

Table 2: Fatty acid compositions (% of total fatty acids[A]) of liver polar lipids in *Salmo trutta* exposed to sublethal concentrations of Roundup® for a period of 30 days[B]

Fatty acids	Control	10 (mg L^{-1})	20 (mg L^{-1})
14:0	1.59±0.05^{c}	1.95±0.04^{a}	1.75±0.05^{b}
16:0	18.95±0.06^{a}	19.56±0.46^{a}	20.05±1.05^{a}
18:0	4.86±0.11^{b}	5.85±0.13^{a}	5.09±0.43^{b}
Σ SFA	26.40±0.11^{b}	29.07±1.14^{a}	27.46±0.37^{b}
16:1 n-7	1.27±0.08^{b}	1.38±0.07^{b}	1.54±0.05^{a}
18:1 n-9	5.27±0.13^{c}	6.71±0.33^{b}	7.36±0.36^{a}
18:1 n-7	1.52±0.08^{b}	1.96±0.03^{a}	1.86±0.13^{a}
20:1 n-9	0.57±0.07^{a}	0.48±0.04^{a}	0.54±0.08^{a}
Σ MUFA	10.79±0.20^{b}	12.77±0.27^{a}	13.43±0.59^{a}
18:3 n-3	0.66±0.04^{a}	0.62±0.03^{a}	0.59±0.07^{a}
20:5 n-3	7.64±0.28^{a}	6.05±0.18^{c}	6.82±0.20^{b}
22:5 n-3	1.77±0.03^{a}	1.61±0.08^{b}	1.68±0.11^{ab}
22:6 n-3	48.16±0.28^{a}	45.11±1.00^{b}	44.94±0.84^{b}
Σ n-3 PUFA	59.03±0.53^{a}	54.14±1.34^{b}	54.76±0.73^{b}
18:2 n-6	1.78±0.08^{b}	2.04±0.16^{a}	2.09±0.11^{a}
20:4 n-6	1.36±0.04^{a}	1.39±0.06^{a}	1.41±0.03^{a}
Σ n-6 PUFA	4.00±0.14^{a}	4.30±0.18^{a}	4.28±0.12^{a}
Σ n-3/n-6 PUFA	14.78±0.67^{a}	12.62±0.76^{b}	12.80±0.52^{b}
EPA+DHA	55.80±0.53^{a}	51.17±1.18^{b}	51.76±0.75^{b}

[A]values are expressed as percentages of total fatty acids, [B](a-b-c) means in a row with identical letters are not significantly different. Values were presented as mean SD (n = 4) ($p<0.05$)

As a general result of this study, it was found that glyphosate caused statistically significant differences in the fatty acid composition of phospholipids in the muscle and liver of brown trout. The highest total SFA values of muscle and liver were found in the group B, 26.15±0.18 and 29.07±1.14%, respectively. While glyphosate raised liver total MUFA amounts as 10.79±0.20% for group A, 12.77±0.27% for group B and 13.43±0.59% for group C ($p<0.05$); it had no effect those of muscle. Total n-3 PUFA amounts in the muscle (51.32±0.74%) and the liver (59.03±0.53%), EPA+DHA amount (55.80±0.53%) and n-3/n-6 PUFA ratio (14.78±0.67) in the liver of group A were higher than those of group B and C ($p<0.05$). However, group A and C which had the highest glyphosate dose, in the muscle had nearly same EPA+DHA levels and n-3/n-6 PUFA ratios (Table 1). As shown in the Table 1 and 2 while significant differences were found in the total n-6 PUFA and 20: 4 n-6 of muscle ($p<0:05$), there was no statistically important changes those of liver.

DISCUSSION

Herbicides kill plants by interfering with essential metabolic or bioenergetics pathways, usually through a specific interaction with a crucial target enzyme (Boger, 2003). Glyphosate inhibits an enzyme (5-Enolpyruvylshikimate-3-phosphate, EPSP) which is a vital part of the process by which plants make particular amino acids. Previous studies reported that since animals do not have this pathway, glyphosate has a very low toxicity to mammals, insects, fish and crustaceans (Anonymous, 2013a). However, surfactant POEA which is added to increase the efficacy of the herbicide makes Roundup® highly toxic for aquatic organisms (Tsui and Chu, 2003, 2008; Anonymous, 2013a). It is also known that K3 group of herbicides have inhibitory effects on elongase activity which catalyzes the elongation (Kato *et al*., 2005; Tanetani *et al*., 2011). Roundup® is a Group M herbicide and inhibits EPSP synthase preventing protein synthesis (Anonymous, 2013a).

In this study, as expected, Roundup® did not inhibit n-3 PUFA synthesis in the muscle (Table 1). But both concentrations, surprisingly, exhibited inhibitory effect on n-3 PUFA synthesis (total n-3 PUFA and EPA+DHA amounts and n-3/n-6 PUFA ratio) in the liver (Table 2). On the contrary, the amount of total SFA and MUFA in the liver and total SFA in the muscle (no effects was found in total muscle MUFA) increased with both concentration of Roundup®. Therefore, there was no harmony it's influence on fatty acid pattern of polar lipids in the liver and muscle of brown trout.

Xenobiotics in both plant and animal organisms are detoxificated by a glutathione-dependent detoxification system by conjugation (Miteva *et al*., 2003). This system includes some enzymes such as Glutathion Peroxidase (Gpx), Glutathion Reductase (GR) and Glutathion-S-Transferases (GST) whose main role, for instance, is biosynthesis of prostoglandin and leukotrienes which are subclases of eicosanoids (Van der Oost *et al*., 2003; Bayir, 2005). Eiocosanoids produced by oxidation of essential fatty acids such as EPA and DHA. In fish, also, xenobiotics usually caused an elevation of antioxidant enzymes including GPx, GR and GST (Wang *et al*., 2006; Wu *et al*., 2007; Li *et al*., 2011). In our study, probably, higher GST activity in the liver for detoxification of glyphosate resulted with higher prostoglandin and leukotriene production and higher oxidation of EPA and DHA. This might be the reason of lower EPA+DHA levels in the liver of brown trout. Moreover, Cavalcante *et al*. (2008), Guilherme *et al*. (2012) and Vera-Candioti *et al*. (2013) determined genotoxicity of Roundup® in *Prochilodus lineatus*, *Anguilla anguilla* and *Cnesterodon decemmaculatus*, respectively. So, glyphosare might also affect some gene expressions in fatty acid metabolism of brown trout.

CONCLUSION

Although, it has been suggested that glyphosate inhibits EPSP synthase in aminoasit metabolism and it does not affect elongase synthase in fatty acid metabolism, it reduced EPA and DHA synthesis in the

liver of brown trout. This result might be consequence of its indirect effect on the some enzyme dependent dexocification systems or gene expressions in fatty acid metabolism of brown trout. Finally, more detailed studies, especially in the molecular aspect, are needed to determine the effects of potential non-harmful chemicals on the aquatic animals.

REFERENCES

Anonymous, 2013a. Classification of herbicides according to site of action. Herbicide Resistance Action Committee. http://www.hracglobal.com/Publications/ClassificationofHerbicideSiteofAction.aspx

Anonymous, 2013b. Mode of action group M herbicides. Department of Agriculture and Food.

Bayir, A., 2005. The investigation of seasonal changes in antioxidant enzyme activities, serum lipids, lipoproteins and hematological parameters of siraz fish (*Capoeta capoeta umbla*) living in Hýnýs Stream (Murat Basin). Ph.D. Thesis, Ataturk University, Graduate School of Natural and Applied Sciences, Erzurum, Turkey.

Bayir, A., A.N. Sirkecioglu, N.M. Aras, E. Aksakal, H.I. Haliloglu and M. Bayir, 2010. Fatty acids of neutral and phospholipids of three endangered trout: *Salmo trutta caspius* Kessler, *Salmo trutta labrax* Pallas and *Salmo trutta macrostigma* Dumeril. Food Chem., 119: 1050-1056.

Bayir, A., A.N. Sirkecioglu, E. Aksakal, M. Bayir, H.I. Haliloglu, M. Gunes and N.M. Aras, 2011. Changes in the fatty acids of neutral and polar lipids of *Silurus glanis* and *Barbus capito* capito during an annual cycle. Ital. J. Food Sci., 23: 173-179.

Boger, P., 2003. Mode of action for chloroacetamides and functionally related compounds. J. Pestic. Sci., 28: 324-329.

Cavalcante, D.G.S.M., C.B.R. Martinez and S.H. Sofia, 2008. Genotoxic effects of Roundup® on the fish *Prochilodus lineatus*. Mutat. Res., 655: 41-46.

Czesny, S. and K. Dabrowski, 1998. The effect of egg fatty acid concentrations on embryo viability in wild and domesticated walleye (*Stizostedion viterum*). Aquat. Living Res., 11: 371-378.

David, F., P. Sandra and P.L. Wylie, 2003. Improving the analysis of fatty acid methyl esters using retention time locked methods and retention time databases. Application Note, http://www.chem.agilent.com/Library/applications/5988-5871EN.pdf

Giesy, J.P., S. Dobson and K.R. Solomon, 2000. Ecotoxicological risk assessment for Roundup herbicide. Rev. Environ. Contam. Toxicol., 167: 35-120.

Glusczak, L., D. dos Santos Miron, M. Crestani, M.B. da Fonseca, F.A. Pedron, M.F. Duarte and V.L. Vieira, 2006. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens*). Ecotoxicol. Environ. Saf., 65: 237-241.

Guilherme, S., M.A. Santos, C. Barroso, I. Gaivao and M. Pacheco, 2012. Differential genotoxicity of Roundup® formulation and its constituents in blood cells of fish (*Anguilla anguilla*): Considerations on chemical interactions and DNA damaging mechanisms. Ecotoxicology, 21: 1381-1390.

Kato, S., A. Tanaka, H. Watanabe, Y. Sato, Y. Ikeda, P. Boger and K. Wakabayashi, 2005. Inhibitory activity of indanofan and its enantiomers on biosynthesis of very-long-chain fatty acids. J. Pestic. Sci., 30: 7-10.

Kreutz, L.C., L.J. Gil Barcellos, A. Marteninghe, E.D. dos Santos and R. Zanatta, 2010. Exposure to sublethal concentration of glyphosate or atrazine-based herbicides alters the phagocytic function and increases the susceptibility of silver catfish fingerlings (*Rhamdia quelen*) to *Aeromonas hydrophila* challenge. Fish Shellfish Immunol., 29: 694-697.

Kreutz, L.C., L.J. Gil Barcellos, S.F. Valle, T.O. Silva and D. Anziliero *et al*., 2011. Altered hematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate. Fish Shellfish Immunol., 30: 51-57.

Li, Z.H., V. Zlabek, J. Velisek, R. Grabic and J. Machova *et al*., 2011. Acute toxicity of carbamazepine to juvenile rainbow trout (*Oncorhynchus mykiss*): Effects on antioxidant responses, hematological parameters and hepatic EROD. Ecotoxicol. Environ. Saf., 74: 319-327.

Metcalfe, L.D. and A.A. Schmitz, 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. Anal. Chem., 33: 363-364.

Miteva, L., S. Ivanov, V. Alexieva and E. Karanov, 2003. Effect of herbicide glyphosate on glutathione levels, glutatathione-s-transferase and glutathione reductase activities in two plant species. Compt. Rend. Acad. Bulg. Sci., 56: 79-84.

SPSS, 1996. SPSS for Windows Release 10.01 SPSS Inc, USA.

Tanetani, Y., T. Fujioka, K. Kaku and T. Shimizu, 2011. Studies on the inhibition of plant very-long chain fatty acid elongase by a novel hericide, pyroxasulfane. J. Pestic. Sci., 36: 221-228.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci., 11: 107-184.

Tsui, M.T. and L.M. Chu, 2003. Aquatic toxicity of glyphosate-based formulations: Comparison between different organisms and the effects of environmental factors. Chemosphere, 52: 1189-1197.

Tsui, M.T.K. and L.M. Chu, 2008. Environmental fate and non-target impact of glyphosate-based herbicide (Roundup®) in a subtropical wetland. Chemosphere, 71: 439-446.

Van der Oost, R., J. Beyer and N.P.E. Vermeulen, 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environ. Toxicol. Pharmacol., 13: 57-149.

Vera-Candioti, J., S. Soloneski and M.L. Larramendy, 2013. Evaluation of the genotoxic and cytotoxic effects of glyphosate-based herbicides in the ten spotted live-bearer fish *Cnesterodon decemmaculatus* (Jenyns, 1842). Ecotoxicol. Environ. Saf., 89: 166-173.

Wang, C., Y. Zhao, R. Zheng, X. Ding, W. Wei, Z. Zuo and Y. Chen, 2006. Effects of tributyltin, benzo[a]pyrene and their mixture on antioxidant defense systems in *Sebastiscus marmoratus*. Ecotoxicol. Environ. Saf., 65: 381-387.

Wu, Y.Q., C.G. Wang, Y. Wang, Y. Zhao, Y.X. Chen and Z.H. Zuo, 2007. Antioxidant responses to benzo[a]pyrene, tributyltin and their mixture in the spleen of *Sebasticus marmoratus*. J. Environ. Sci., 19: 1129-1135.