Egypt. J. Aquat. Biol. & Fish., Vol.8, No.4: 85 - 105 (2004) ISSN 1110 - 6131

EFFECT OF FATTY ACIDS ON THE RESPONSE OF THE LIVER MICROSOMAL BIOTRANSFORMING ENZYMES IN THE NILE TILAPIA *OREOCHROMIS NILOTICUS* EXPOSED TO QUINTOZENE

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Key Words: Cytochrome P-450, Cytochrome b_5, EROD, ECOD, UDPGT , GST, Polyunsaturated fatty acids (PUDFAS), microsomes, cytosol, *Oreochromis niloticus*

ABSTRACT

This study was conducted to investigate the impact of nutritional environmental factors on the efficacy of microsomal biotransforming enzymes to organic xenophobic in the freshwater fish *O. niloticu* exposed to the pesticide quintozene .Two groups of fish, one was maintained on a normal diet (A) and the other on a diet supplemented with fish liver oil (B) for 4 weeks. Then the fishes were treated with quintozene by interperitonial injection of 150 µl (30 mg/kg of body weight) and the responses of liver microsomal biotransforming enzymes were assessed after 48 hours.

Treatment with quintozene of fishes maintained on diet B, produced an increase in microsomal contents of cytochrome P-450 and cytochrome b_5 reaching 1.44 and 2.88 fold respectively compared with group A, whereas, activities of 7-ethoxyresorufin -O- deethylase (EROD) and 7- ethoxycoumarin- deethlase (ECOD) reached 2.8 to 5.11 fold respectively.

Similarly, the phase II enzymes, Uridine diphosphoglucuronyl – transferase (UDPGT) activity was less induced in fishes fed diet A than diet B; whereas an increase of 1.64 and 4.7 fold respectively were noticed. Cytosolic glutathione- S- transferase (GST) was not affected by either treatments.

In addition, a fish liver oil rich dietary supply of polyunsaturated fatty acids (PUFAs) may prevent these changes, even in a long- term subchronic exposure.

The results indicated that fatty acid composition of diet of *Oreochromis niloticus* can affect significantly the biotransforming enzymes in response to xenobiotic pollutants.

INTRODUCTION

Environmental parameters such as temperature and nutrition can play an important role in the ability of organisms to respond to toxic chemicals, particularly in the aquatic environment (Jimenez *et al.*, 1988; Jimenez and Burtis; 1989; Storr *et al.* ,1995; Boon *et al.*,1997 ; Kakela *et al.*, 2001)

It is well established that fish have the ability to metabolize foreign compounds in a similar manner to that of mammalian species (Adamson, 1967). In mammals, numerous dietary factors are known to influence hepatic systems including phase 1 oxidative enzyme such as cytochrome P-450, dependent monooxygenase system and phase II conjugating enzymes.

Although, the role of these enzymes is primarily detoxification and metabolic activation, cytochrome P-450 can convert xenobiotic compounds to highly reactive and potentially carcinogenic forms (Wolf, 1986 ; Yuan *et al.*, 2001).

The mixed function oxidase (MFO) system plays a determinant role in the initial stage of detoxification of xenobiotic lipophilic compounds. One of the basic features of this system is its substrate inducibility which stimulates the synthesis of new functional proteins (Payne *et al.*, 1987; De Matteis, 1988 ; Wong *et al.*, 2001).

Ankley and Blazer (1988) reported that diet can affect the toxicity of xenobiotics to fish through alterations in hepatic enzyme systems.Jimenez *et al.*, (1988) and Audw & Wurs (2001) have studied the effects of different commercial diets on the response of hepatic ERCD and hepatocytological changes of EROD activity in an immature demersal fish and blue gill sunfish exposed to Benzo (a) Pyrene. Dietary modification by starvation (Andersson *et al.*, 1985: Jimenez *et al.*, 1987 and Jimenez : Burtis. 1989). or different protein levels in diet that are known to affect the enzymatic system and monooxygenase activities in some fish species (Stott and Sinnhuber. 1978).

El Wald. (1999) reported that chlorinated fatty acids do not induce cytochrome P-450 and EROD in fish. This lack of organism recognition of chlorinated fatty acids as xenobiotic compound gives a new perspective on the risk assessment of these compounds.

Ankley and Blazer (1988) reported that, from a toxicological point of view, it was important to determine whether relatively minor differences in commercial diets affected xenobiotic metabolism in fish. High cyclopropenoid fatty acid levels in diet affected hepatic

monooxygenase activities in rainbow trout *Salmo gairdneri* (Elisle *et al.*, 1978; 1983 and Kakela *et al.*, 2001) while vitamin C deficiency was reported as being involved in lower hepatic monooxygenase activities in channel cat fish *Ictalurus punctatus*.(Ankley and Blazer, 1988).

Ankley *et al.* (1989) have shown that the type of lipid extracted from fishes added to the diet can affect basal biotransformation activities as a tool in biomonitoring.

Fishes are likely to face important diet change in relation to seasonal variations of available food. Therefore, there is relatively little information concerning effects of dietary lipid on response of biotransformation activities of pesticides in exposed fishes.

The present study aimed to investigate the response of liver monooxygenase (cytochrome P-450 and cytochrome b_5 contents and the activities of (EROD); (ECOD) and (UDPGT) and (GST) activity phase II enzymes to quintozene exposure in two groups of ***O. niloticus, one was*** fed a normal commercial diet and the other was maintained on another diet supplemented with fish liver oil.

MATERIALS AND METHODS

Quintozene pesticide Pentachloronitrobenzene (PCNB) (98%) an aromatic hydrocarbon derivative, used in this study was supplied as technical grade from Help Pesticides and Chemical Company, Free Zone, New Damietta, Egypt.

Fish oil, nicotinamide adenine dinucleotide phosphate oxidized (NADP); reduced NADPH; substrates were purchased from Sigma Chemicals Company. All other chemicals were of the highest available commercial grade.

Fishes

Nile tilapia *(Oreochromis niloticus)* weighing 50±5 g were obtained from El– Abassa fish farm and transported to a large water Aquarium (70 × 50× 40 cm). Fishes were acclimated to laboratory conditions for 4 weeks before experimentation. The fish were randomized in well aerated dechlorinated tap water in the aquarium with dissolved oxygen (6-8) mg/l; pH (7.4-7.7); temperature 24 ± 1°C; total hardness 80 mg/l as $CaCO_3$. Analysis was carried out according to APHA (1998).

Experiment 1:

In this experiment, fishes were divided into two groups ; 100 individuals each. The fish of the first group were fed on commercial diet (diet A) that contained total protein 45%; cereal 20%: total fat 12% and fish liver oil 0%, while the second group was fed on the same commercial diet but supplemented with 11% fish liver oil (diet B).

Twenty five fish of each group received a single intraperitonial injection of quintozene (150 µl, 30 mg/kg of the body weight) toluene.The remaining twenty five fish of each group received an injection of sterile toluene and served as control.

After treatment with quintozene fishes were placed in separate aquaria. Then they were sacrificed 48 hr from the beginning of treatment. This period was established from a preliminary experiment as given a marked response of the monooxygenase activities (Lemaire, 1990 ; Lemaire *et al.*, 1990 a.).

Microsomal isolation:

Fresh excised liver was rinsed in 150 mM KCl solution, following the procedures reported by Ariyoshi *et al.* (1970) and Arizono *et al.* (1982).

The minced livers were homogenized in 4 volume of 250 mM sucrose, 10 mM HEPES buffer (pH 7.4) with a Potter – Elvehjem glass and a Teflon homogenizer. The homogenates were centrifuged at 12,000 g for 15 min in Beckman. J_2 – Hs centrifuge. The supernatants were recentrifuged at 105.000 g for 60 min in a Beckman TL, 100 Ultracentrifuge.

Microsomal pellets were resuspended in 2.5 ml of 250 mM sucrose; 10 mM Hopes buffer (pH 7.4) containing 20% glycerol and the supernatant with a cytosol fraction. Microsomal suspension were frozen and stored in liquid nitrogen till analysis.

Assays:

Cytochrome P-450 and cytochrome b_5 contents. NADPH cytochrome C reductase and Uridine diphosphogluronyl transferase (UDPGT) activity were determined in the microsomal fraction. Glutathione –S – transferase (GST) activity in cytosol. Cytochrome P-450 cytochrome b_5 were assayed after reduction by sodium dithionite according to Stegeman *et al.* (1987): NADPH - Cytochrome C reductase as described by Masters *et al.* (1967): 7 – ethoxyresorufin – O – deethylase (EROD) according to Klotz *et al.* (1984). 7 – ethoxy coumarin –O – dealkylase (ECOD) as described by Ullrich and Weber (1972): GST activity as described by Hebig *et*

al., (1974) with 1 – chloro – 2, 4- dinitrobenzene (CDNB) as substrate and UDPGT as described by Frei (1970). Microsomal and cytosolic protein concentrations were determined according to Lowry *et al.* (1951). Enzyme activities were measured by using UV/ Visible Spectrophotometer Perkin – Elmer model Lambda$_3$ (Cytochrome P-450: Cythochrome b_5: NADPH – cytochrome C reductase: GST and UDGT), on Jenway 6200 Spectrofluorimeter (EROD and ECOD) . All enzymatic assays were demonstrated to be linear for time and protein concentrations used in experiments.

Diet composition:

Analysis of the diet using the method reported by lemaire *et al.* (1991 b), the two diets differed mainly with respect to fish liver oil (0% level in diet A and 11% in diet B). Fish oil contained essential fatty acids, fatty acid composition was analyzed according to Flock *et al.* (1957), Diet B contained 26% docosahexanoic acid and 54% linolinic acid, whereas no trace of these polyunsaturated fatty acids (PUFA),could be detected in diet A, as (Table 1).

Statistical analysis:

Statistical analysis of results was performed by student t-test (Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

Treatment with quintozene did not increase cytochrome P-450 level, cytochrome b_5 and NADPH-cytochrome c reductase activity in fishes fed diet A (Fig.1) . In contrast, with diet B, cytochrome P-450, cytochrome b_5 and NADPH-Cytochrome C reductase were increased respectively by 1 and 1.44 , 1.48 and 2.8 and 1 and 1 fold in diet B respectively, as a consequence of this difference (Table 2)

Quintozene treated fish fed the commercial diet B had higher level of cytochrome P-450 than cytochrome b_5 and NADPH-cytochrome C reductase fed diet A (Figs. 1 a, b, c)

The contents of cytochrome P-450, cytochrome b_5 and NADPH cytochrome C were slightly increased in diet A but the activity increased in diet B. In mammals Cytochrome P-450 has multiple forms which differ with respect to substrate specificity and degree of infusibility by drugs and other chemical (Ryan *et al.*, 1975 ; Haugen *et al.*, 1975).

Numerous studies have shown that dietary lipid markedly affects xenobiotic metabolism in mammals (Wade *et al.*, 1985). In fish, the information is more limited with respect to conjugating ability of enzymes in fish treated with pollutants, but the effect of nutrition and lipid composition on xenobiotic metabolism have been demonstrated (Ankley and Blazer 1988; Jimenez *et al.*, 1988 ; Ankley *et al.*, 1989).

Both categories of Congeners are probably metabolized by different families of cytochrome P-450 (1A and 2B), the levels of which apparently differed with the species of cat fish and the induction of cytochrome P-450 enzymes offers the most likely explanation for this phenomenon, but starvation could have a similar effect on occasion, (Boon *et al.*, 1997) . It was shown that the main constituents of fish oil are the polyunsaturated fatty acid (PUFA) linolenic and docosahexaenoic acid (Lemaire *et al.*, 1991b). Sarasquete and Segner (2000) have shown that cytochrome P-450 monooxygenase has an important function in the biotransformation of many xenobiotics including polynuclear aromatic hydrocarbons (PAH), and planer organochlorine compounds. The metabolism of PAH can lead to detoxification or production of reactive intermediates. In fish, docosahexaenoic acid is an essential fatty acid either absorbed during digestion or synthesized from linolenic acid. Fatty acids undergo bioconversion by elongation and desaturation by desaturase. Ariyohi *et al.* (1970), sated that the capacity to elongate and desaturate linolelic acid varies with the species, but desaturation activity is much greater in freshwater fish (Dwen *et al.*, 1975) than in marine fish (Cowey *et al.*, 1976. Fuji *et al.*, 1976 ; Tinoco, 1982).

Lack of polyunsaturated fatty acid (PUFA) elicits numerous hepatic pathological damages (Mosconi, 1987). This lack of the organism for recognition of chlorinated fatty acid as xenobiotic compound gives a new perspective on the risk assessment of these compounds (El Wald, 1999) . Stegman *et al.* (1997) have shown that the total cytochrome P-450 contents of 7 species of fishes varies between 0.1 and 0.5 n mol/mg protein and cytochrome b_5 content, between 0.025 and 0.25 n mol/mg protein. In contrast to these 7 species, fishes had microsomal P-450 contents near 1.7n mol/mg, among the highest value as reported in untreated fishes. Fishes fed diet A showed lower activity of phase I and phase II enzymes than those fed the diet B supplemented with fish liver oil. No differences were observed in cytochrome P-450, cytochrome b_5 and NADPH – cytochrome C reductase compared with the control level in fish fed with either one of the two different diets. However, higher induction

response was observed in diet B than in diet A. Kakela *et al.* (2001) reported that, in the minks fed fish – based diets and exposed to Aroclor 1242, the different changes in the microsomal fatty acids minks fed a diet rich in fat and low in polyunsaturated fatty acids (PUFA), the PCB increased the percentage of oleic acid (18- In-9, characteristic of the storage TGS) at the expense of $n\text{-}_3$ PUFAS. In contrast to some changes in cytochrome, P-450 content (Nave and Engalhaedt, 1982; Stegman, 1987), cytochrome b_5 has rarely been observed in these studies in fish. Although the role of cytochrome P-450 and cytochrome b_5 in pesticides has not yet been defined in fish, it is possible that as in mammals it may increase the overall efficiency of electron transport to cytochrome P-450 (De- Marco and Mccoy, 1985) . Treatment with quintozene increased microsomal UDPGT activity by 1.64 and 4.68 fold in fishes fed with diet A and B respectively, (Fig. 2 and Table 2). In contrast, cytosolic glutathione – S – transferase activity was not affected in fishes fed diet A, whereas, it was significant by 57.33% in fishes fed diet B.

UDPGT activity was similarly more induced in quintozene treated fish fed with polyunsaturated fatty acid (PUFA) contained in diet B. This result is in agreement with those data reported by (Ankley *et al.*, 1986 and Ankley and Blazer 1988).

Gadabui and Goksoys (1996) observed in *Tilapia* fish and Mud fish exposed to the aromatic chlorinated hydrocarbon, elevated levels of EROD, UDPGT and GST activities.In contrast, cytosolic GST activity in *O.niloticus* showed 57.33% inhibition after quintozene treatment in diet (B). Conversely, in channel cat fish, GST activity toward CDNB shows higher level with a diet containing numerous $n\text{-}_3$ series fatty acids (Garcia *et al.*, 2000 ; kakela *et al.*, 2001). Koss *et al.* (1991) have observed also the activities in phase II enzymes are known to be weak in fish and greatly varied according to the used substrate. However, CDNB appeared to be good substrate for GST activity with least specific difference (Gregus *et al.*, 1983). The results indicate that diet can significantly affect the enzymatic metabolism and thereby the toxicity potential of organic pollutants in *O.niloticus* and other fish fed a diet containing essential fatty acids could be more sensitive to xenobiotic enzyme induction than those lacking polyunsaturated fatty acid in diet. Liver microsomal EROD activity followed the same qualitative pattern with respect to quintozene treatment and diet. However, the responses were more marked for fish fed diet (B), 2.8 compared with 1.9 fold for diet A

respectively, (Fig. 3 and Table 2). Concerning ECOD activity, a slight difference was observed with diet A between the control and quintozene treated fish. whereas , higher level appeared in quintozene treated fish with diet B compared to control (4.42 and 5.11 fold, respectively. The induction effect of quintozene on liver microsomal EROD activity were quite similar in groups with diet (A). but the induction for diet B was higher in polyunsaturated fatty acid. Similarly, ECOD activity was slightly induced only in diet (B), but greater induction was observed for diet (A) (Lacking polyunsaturated fatty acids) .The difference between the responses of ECOD and EROD activites is probably because the EROD are more specific for polycyclic aromatic hydrocarbon induction (Lech *et al.*, 1982; Fisk *et al.*, 1997 ; Straus *et al.*, 2000).

Ankley and Blazer (1988) and Straus *et al.* (2000) suggested that their results on differential induction of EROD and ECOD in channel cat fish with Arocolor 1254 could have been related to the presence of polyunsaturated fatty acids (PUFA) in the most inductive diet, which is in agreement with our results .Recently, Ankley *et al.* (1989) and Kakela *et al.* (2001) have shown that the relative abundance in $n\text{-}_3$ series fatty acids (essentially 18:3, 20:5 and 22:6) in diet containing medhaden oil, increased basal EROD and ECOD activities. On the other hand. Ankley and Blazer, (1988) and Kakela *et al.*, (2001) suggested that variation in hepatic activities could be due to vitamin C or vitamin E and PUFAS. Fat deficiency may prevent these changes even in a long term subchronic exposure. The present results indicate an effect of PUFA presence on induction responses. Similar results were found in rats fed with diet rich in n-series fatty acids,where hepatic monooxygenase and GST activities were higher than those obtained with other diets devoid in this series (Century. 1973; Wills 1980 : Doi AM *et al.*, 2000). Several hypotheses are proposed to account for variation of hepatic biotransformation activities in relation to diet. Wills (1980) and Wade *et al.* (1986) reported that the higher inductions of hepatic biotransformation activity with C_{22} : 6 fatty acid diet content could be explained by improvement of catalytic possibilities. i.e with membrane topology and/or fluidity alternations or better catalytic sities as shown in mammals by Ankley *et al.* (1989) who postulated that fatty acids influence enhanced monooxygenase activity in fishes in a similar way like mammals.

The previous studies showed the relatively weak induction of phase II conjugation enzyme in fishes (Kirby *et al.*, 1990. Koss *et al.*,

1991 ; Flammarian *et al.*, 1998) . Phase II metabolism is a potent detoxyifing system of the carcinogens produced by phase I enzymes.

In *O.niloticus*, the combination of a low phase II to a high phase I induction, indicates the possibility of a diet related increase of neoplasm potential. Moreover the effect of PUFA on intoxication response in fishes, and as in mammals, monooxyenase and phase II activities were affected by the type of lipids fishes received in diet. Also, a standardization of fishes diet is necessary to reduce variability in organic xenobiotic toxicity. Moreover, such results revealed the necessity of more knowledge about fish biology and nutrition in order to use biotransformation activities in biomonitoring studies. The fatty acid rate in the diet should be taken in account to correlate pollutants level and biotransformation activities in freshwater fish in freshwater environments.

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Table (1): Level of different components in commercial diets A and B percent of total diet

Component	**Diet A**	**Diet B**
Total protein	**45 %**	**45 %**
Cereal	**20 %**	**20 %**
Total fat	**12 %**	**12 %**
Fish liver oil (% of total fat)	**0 %**	**11 %**

Table (2): Induction factors (relative to control) of liver microsomal biotransformation enzymes in *O. niloticus* fed with diet A or B.

Liver microsomal enzymes	**Diet A**	**Diet B**
Cytochrome P_{-450}	1.0	1.44
Cytochrome b_5	1.48	2.88
NADPH cytochrome– c-reductase	0.9	1.04
UDPGT	1.64	4.68
GST	0.75	0.57
EROD	1.90	2.80
ECOD	4.42	5.11

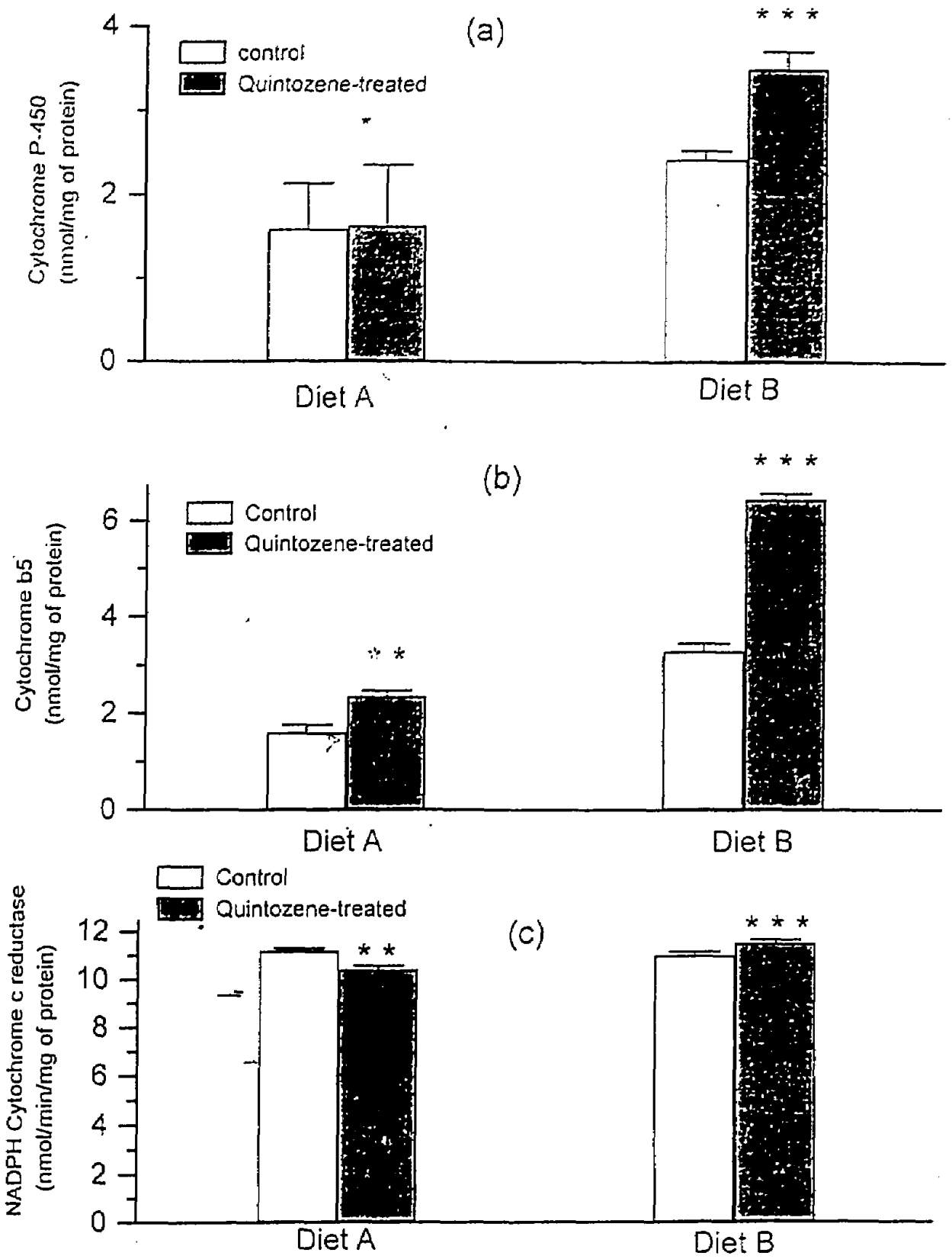

Fig. (1): Effect of diets A and B on the induction of liver microsomal (a) Cytochrome P-450, (b) Cytochrome b5 content and (c) NADPH cytochrome C reductase after quintozene treatment (30 mg/kg body weight) in *O. niloticus* at 48 hrs. Mean + S.D. (n = 5).

* P>0.05 = non significance, ** P< 0.05 = significance

*** P< 0.001 = highly significance

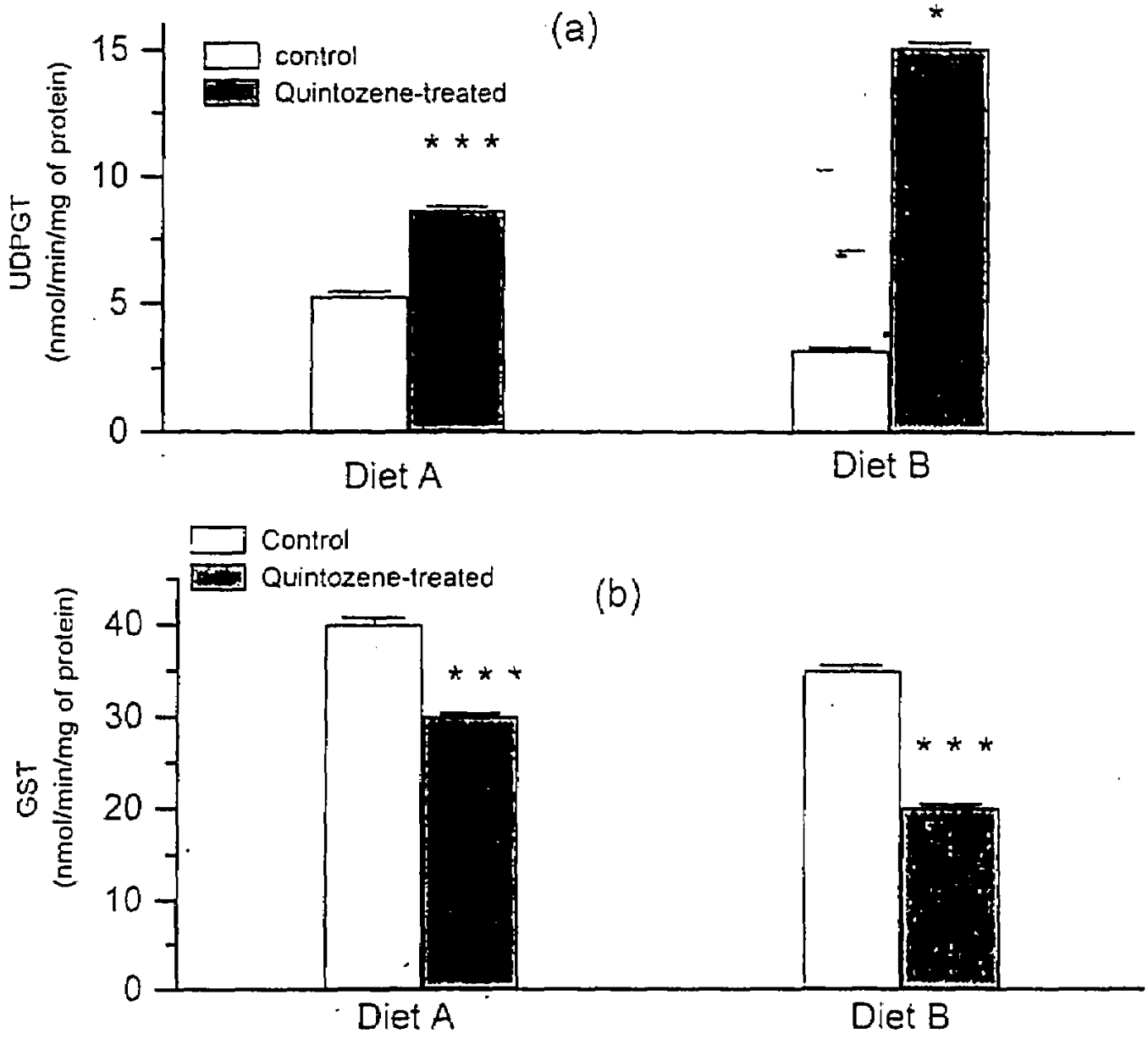

Fig. (2): Effect of diets A and B on the inductionof liver (a)microsomal UDPGT and (b) Cytosolic GST activities after quintozene treatment (30 mg/kg body weight) in O. *niloticus* at 48 hrs. Mean + S.D. (n = 5).

*P> 0 05 = non significance. ** P< 0.05 = significance

*** P< 0.001 =highly significance

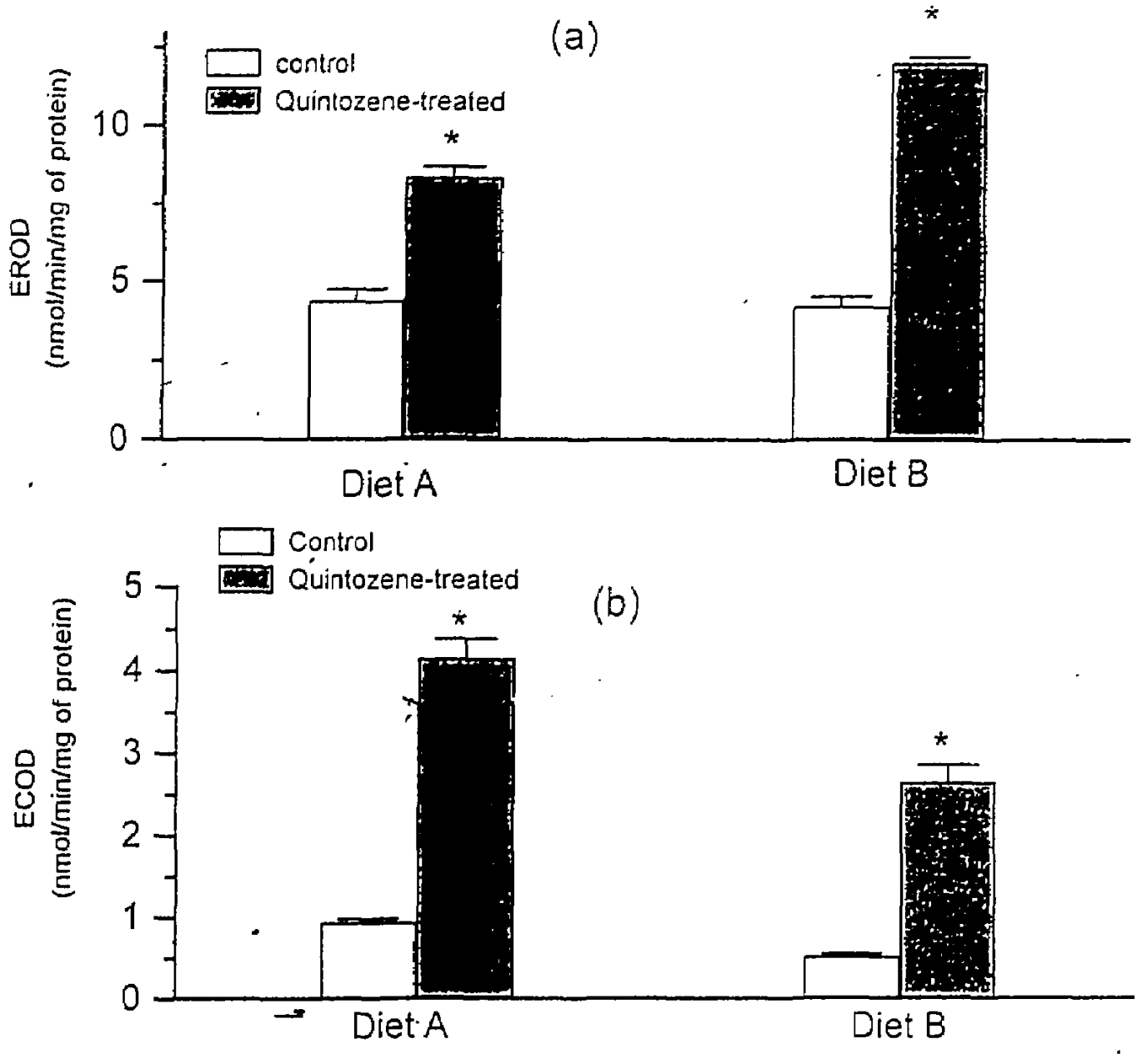

Fig. (3): Effect of diets A and B on the induction of liver microsomal (a) EROD and (b) ECOD activities after quintozene treatment (30 mg/kg body weight) in *O. niloticus* at 48 hrs. Mean + S.D. (n = 5).

* $P > 0.05$ = non significance,