Asian Journal of Biological and Life Sciences | Short Communication

Study of acute and chronic treatment of tetracycline on total lipid contents in various tissues of freshwater mussels, *Lamellidens corrianus* (Lea) & *Parreysia cylindrica* (Annandale & Prashad)

H.P. NANDURKAR . , S.P. ZAMBARE*

Department of Zoology, Sant Gadge Baba Amravati University, Amravati. (M.S.) 444 602. INDIA.
* Department of Zoology, Dr. B.A. Marathwada University, Aurangabad (M.S.) 431 004. INDIA.

Submitted : 06.01.2012 Accepted :22.2.2012 Published : 10.5.2012

Abstract

Freshwater bivalves, *Lamellidens corrianus* were exposed to acute dose of tetracycline one of the broad spectrum antibiotics, 369.10PPM, and *Parreysia cylindrica* 166.54PPM up to 96 hrs. In *Lamellidens corrianus* the concentration used for chronic treatment of tetracycline was 73.82 PPM, while *P. cylindrica* 33.30 PPM up to 21 days. The total lipid content was estimated after 24 and 96 hours of acute treatment and 7, 14 and 21 days of chronic treatment in the mantle, gills, foot, ovary, testis, whole body and digestive gland of the bivalves. After acute and chronic exposure of tetracycline, *L. corrianus* and *P. cylindrica* showed increase in total lipid contents. There was overall increase in lipid contents in different tissues of both the species of bivalves. Ovary showed maximum response against the tetracycline treatment. Maximum increase in lipid contents was found in ovary after chronic treatment of tetracycline. The increase in lipid contents may be due to the lipogenesis occurring in the ovary for production of gametes

INTRODUCTION

Tetracyclines are a family of broad-spectrum antibiotics, which inhibit protein synthesis by preventing the binding of amino acyl transfer ribonucleic acid to the bacterial ribosome (70S).

These tetracyclines provide safe, inexpensive and effective treatment for many bacterial infections[1] The selection of the antibiotic is due to its use in artificial pearl culture during post-operative care to reduce the mortality rate.

Lipid is the most efficient organic reserves of most of the bivalves and other animals [2]along with major structural components of the body tissues. It is therefore essential to study the effect of variables on the lipid content. Starvation resulted in a significant decline of the lipid content and a complete depletion of the triglyceride reserve. Impact of pesticides on the lipid content has been studied in the gypsy moth, *Porthretia dispar* [3] and in *Mythimna (Psedaletia) separata [4]*. The pesticides are known to inhibit cholinesterase and hydrolases. Dimilin intoxication increased the lipid content in the larvae of *Porthretia dispar*.[5] A high lipid contents was observed of the gonad at the time of most active gametogenesis in Pismo clam, *Tivela stultorum[6]*. The major organic reserves, glycogen and lipid, declined in the hepatopancreas of *Scylla serrata* during the period of reproductive activity while inclined in ovary during the same period [7].

MATERIALS AND METHOD

The freshwater bivalves, *L. corrianus* & *P. cylindrica* were acclimatized to laboratory conditions for 4 days prior to experimentation. During experimentation the *L. corrianus* & *P. cylindrica* showing apparent good health and movements were used for investigation. The animals were divided into five groups, two for acute and two for chronic exposures of tetracycline and one group was maintained as control in each case.

a) Acute exposure to Tetracycline:

The healthy bivalves, *Lamellidens corrianus* were exposed to acute treatment ($LC_{50/2}$) of tetracycline 369.10PPM, while *Parreysia cylindrica* were exposed to tetracycline (166.54PPM) up to 96 hrs.

b) Chronic exposure to Tetracycline:

The acclimatized *L. corrianus* were exposed to ($LC_{50/10}$) concentration of tetracycline 73.82 PPM while *P. cylindrica* were exposed to chronic concentration of tetracycline 33.30 PPM up to 21 days. During exposure period, no special food was provided and the water with required concentration of antibiotic was changed daily in the experimental set and also from control. Control set was provided with dechlorinated water only without addition of tetracycline.

After 24 and 96 hours of acute and after 7, 14 and 21 days of chronic exposure, the mantle, gill, foot, testis, ovary, digestive gland and the whole body flesh was isolated, blotted to remove excess water and dried in oven at 80 ^{0}C till constant weight was obtained. All tissues were ground separately into fine powdered form and total lipid contents were estimated.

Total lipid content was estimated by using Vanillin reagent and cholesterol was used as a standard [8]

RESULTS AND DISCUSSION

Tables 1 to 2 indicate changes in total lipid level of different tissues of *L. corrianus* & *P. cylindrica* on acute and chronic exposure to tetracycline. After acute and chronic exposure of tetracycline in *L. corrianus* and *P. cylindrica* lipid contents showed great increase. There was overall increase in lipid contents in different tissues of both the species of bivalves. Ovary has given maximum response against the antibiotic treatment. Maximum increase in lipid contents was found in ovary after chronic treatment of tetracycline. The increase in lipid contents

Table 1: Impact of tetracycline on lipid content (mg %) of *Lamellidens corrianus* after acute and chronic exposure

Tissues	24 h		96 h		7 d		14 d		21 d	
	Control	Tetra	Control	Tetra	Control	Tetra	Control	Tetra	Control	Tetra
M	5.086 ±0.2980	5.694 ±0.2866 +11.972*	5.120 ±0.2908	5.953 ±0.5817 +16.272*	4.053 ±0.7695	4.390 ±0.5037 +8.333NS	3.609 ±0.2908	5.811 ± 0.7887 +14.285**	4.011 ±0.5037	5.013 ±0.2908 +24.999*
G	5.422 +0.0791	6.075 +0.1375 +12.0536	5.361 +0.2103	6.740 +0.1379 +25.727**	5.849 +0.079	6.748 +0.1375 +15.384**	6.222 +0.796	7.556 +0.2103 +21.444*	5.749 +0.1375	7.028 +0.0796 +22.263*
F	6.204 ±0.1379	6.746 ±0.796 +8.750NS	6.229 ±0.2103	7.168 ±0.1375 +15.076**	7.568 ±0.2103	8.364 ±0.1379 +10.526N	8.156 ±0.505	8.859 ±0.1375 +8.631*	8.085 ±0.079	9.118 ±0.4013 +12.789**
O	13.129 ±0.7688	17.306 ±1.332 +31.818*	12.497 ±1.332	18.328 ±2.774 +46.666**	10.028 ±0.8179	11.899 ±2.774 +18.666N	9.878 ±0.1375	12.654 ±0.0796 +28.107**	8.784 ±0.8179	11.858 ±2.097 +35.000**
T	10.559 +0.5817	11.937 +0.5021 +13.055*	10.851 +0.7719	11.271 +0.5021 +3.875***	9.491 +0.7695	10.684 +0.5021 +12.571*	8.022 +0.5037	9.265 +0.7673 +15.499*	8.151 +1.267	9.667 +0.7743 +18.604*
WB	7.459 ±1.048	8.857 ±0.7719 +18.750*	7.129 ±0.5817	8.778 ±0.7695 +23.142**	8.917 ±0.2936	9.923 ±0.5037 +11.285**	6.814 ±0.2936	8.114 ±0.5035 +19.090*	6.159 ±0.8179	7.945 ±0.7719 +29.00*
DG	9.056 ±1.007	10.723 ±2.195 +18.412N	8.447 ±0.8753	10.981 ±1.007 +29.999**	10.288 ±0.2986	11.806 ±0.7887 +14.761**	9.356 ±0.7695	11.655 ±0.5817 +24.580**	8.511 ±0.2908	11.269 ±0.7887 +32.411**

M = Mantle; G = Gill; F = Foot; O = Ovary; T = Testis; WB = Whole body; DG = Digestive gland.
Values are expressed as mg/100mg dry weight of tissue. ± indicates standard deviation of three independent replications.
+ or - indicates % variation over control. Significance: * P < 0.05; ** P < 0.01; *** P 0.001; NS = Non-significant.

Table 2: Impact of tetracycline on lipid content (mg %) of *Parreysia cylindrica* after acute and chronic exposure

Tissues	24 h		96 h		7 d		14 d		21 d	
	Control	Tetra	Control	Tetra	Control	Tetra	Control	Tetra	Control	Tetra
M	4.060 ±0.2103	4.486 ±0.1379 +10.500**	4.039 ±0.1379	4.768 ±0.505 .+18.058*	3.539 ±0.1375	3.892 ±0.079 +9.999**	3.418 ±0.879	3.877 ± 0.137 +13.456 **	4.235 ±0.210	4.969 ±0.138 +17.333*
G	5.422 +0.0791	6.075 +0.1375 +12.0536*	5.361 +0.2103	6.740 +0.1379 +25.727***	5.849 +0.079	6.748 +0.1375 +15.384***	6.222 +0.796	7.556 +0.2103 +21.444*	5.749 +0.1375	7.028 +0.0796 +22.263*
F	6.204 ±0.1379	6.746 ±0.796 +8.750NS	6.229 ±0.2103	7.168 ±0.1375 +15.076***	7.568 ±0.2103	8.364 ±0.1379 +10.526NS	8.156 ±0.505	8.859 ±0.1375 +8.631*	8.085 ±0.079	9.118 ±0.4013 +12.789**
O	13.129 ±0.7688	17.306 ±1.332 +31.818**	12.497 ±1.332	18.328 ±2.774 +46.666**	10.028 ±0.8179	11.899 ±2.774 +18.666NS	9.878 ±0.1375	12.654 ±0.0796 +28.107***	8.784 ±0.8179	11.858 ±2.097 +35.000**
T	10.559 ±0.5817	11.937 +0.5021 +13.055***	10.851 +0.7719	11.271 ±0.5021 +3.875***	9.491 ±0.7695	10.684 ±0.5021 +12.571*	8.022 ±0.5037	9.265 ±0.7673 +15.499*	8.151 ±1.267	9.667 ±0.7743 +18.604*
WB	7.459 ±1.048	8.857 +0.7719 +18.750*	7.129 ±0.5817	8.778 +0.7695 +23.142**	8.917 ±0.2936	9.923 +0.5037 +11.285**	6.814 +0.2936	8.114 +0.5035 +19.090*	6.159 +0.8179	7.945 +0.7719 +29.00*
DG	8.602 ±1.332	9.662 ±0.7695 +12.333NS	8.381 ±0.5021	9.642 ±0.5037 +15.053***	8.819 ±0.8179	10.110 ±1.267 +14.647NS	7.574 ±0.5035	9.337 ±2.097 +23.285**	5.218 +0.562	6.865 ±0.8179 +31.568*

M = Mantle; G = Gill; F = Foot; O = Ovary; T = Testis; WB = Whole body; DG = Digestive gland.
Values are expressed as mg/100mg dry weight of tissue. ± indicates standard deviation of three independent replications.
+ or - indicates % variation over control. Significance: * P < 0.05; ** P < 0.01; *** P 0.001; NS = Non-significant.

may be due to the lipogenesis occurring in the ovary for production of gametes. In mantle the lipid contents increased with least rate (Tables 1 & 2).

In *P. cylindrica* the lipid content was maximum in ovary and then in digestive gland after chronic exposure of tetracycline. This tetracycline exposure caused an overall continuous and consistent increase in the lipid content of ovary, digestive gland, whole body, gill, mantle and foot of *P. cylindrica*. The different rate of increase was a function of their dose and period of exposures

The different factors like age, sex, food supply, seasonal variations etc. influence the lipid content of the organisms. It was observed that the lipid contents increased when the animals came across the stressed conditions. There was increase in lipid content after dimilin intoxication in larvae of *Porthretia dispar [5]*. One of the reasons for lipid increase as inhibition of lipase activity after organophosphate treatment [9]. The transformation of glycogen into lipid through triose phosphate pathway as one of the causes for lipid elevation [10]. The same reason for lipid enhancement in *L. marginalis* after flodit and metacid treatment [11]. Elevated level of lipid on pesticide and heavy metal stress was observed in *Mythimna (P) separata [4]* in *Oziotelphusa senex senex*,[12] in *Bellamya bengalensis[13]* and in *Corbicula striatella [14]*. The increase in lipid contents may be due to the production of corticosteroids to resist the toxic condition made by different chemicals [9]. The prominent reason to raise the lipid level may be the biotransformation of the other organic constituents like carbohydrates and proteins into lipid and the cessation of lipolytic enzyme activity. The similar results were recorded in *Notopterus notopterus* [15].

The anaerobic or hypoxic conditions also lead toward the lipid synthesis in molluscs [16]. The effect of starvation (nutritive stress) in *Tapes philippinarum* indicated that the clams lost 26 % of their initial total lipid contents [17]. Bioabsorption of cadmium and mercury revealed the decrease of lipids in the tissue of *Perna viridis* [18]. During oogenesis, the oocytes acquire their lipid reserves from three main sources: (1) the adductor muscle, whereby muscle glycogen is converted to lipid [19][20][21] (2) lipid transfer from reserves in the digestive gland to the female gonad [21] [22] [23] and (3) directly from food when adults are put under nutritional stress [24] [25].

The results obtained in present study are in agreement of most of the above observations and showed proportionate increase in the lipid contents with the period of exposure to tetracycline.

REFERENCES:

1. Wilson, W.R., (1977) Antimicrobial agents: tetracyclines, chloramphenicol, erythromycin and clindamycin. Mayo Clin. Proc. 52: 635-640.

2. Anderson, P. D. and Webber, L. J. (1977) The toxicity to aquatic populations of mixtures containing certain heavy metals. Proc. Int. Conf. Heavy Metals Environ., Toranto, Ontario, Canada, 2: 933-955.

3. Baumler W. and H.S. Salama (1976) Some biochemical changes induced by dimilin in the gypsy moth Porthretia dispar L.Z.Ang. Ent. 81: 304-310.

4. Patil P.N. (1986) Impact of different pesticides on some physiological and neuroendocrinological aspects of Mytgimna (Pseudaletia) separata. Doctoral theis Marathwada Univ., Aurangabad, India.

5. Salama H.S., Z.A. Matagally and V. Skatulla (1976) On the mode of action of dimilin as a moulting inhibitor in some lepidopterous. Ects. Z. Ang.Ent. 20:396-407.

6. Giese, A.C. and Hart, M.A. (1967) Seasonal changes in component indices and chemical composition in Katharina tunicata. J. Exp. Marine boil. Ecol.1: 34

7. Nagabhushnam, R. and farooqui, U.M. (1982) Mobilization of protein, glycogen and lipid during ovarian maturation in marine crab, Scylla serrata Forskal. Indian J. of Marine Sciences (11): 184-186.

8. Barnes, H. and Blackstock, J. (1973) Estimation of lipids in marine animals and tissues: detailed investigation of the sulpho-phosphovanillin method for total lipids. J. Expt. Mar. Biol. Ecol.. 12(1): 103-118.

9. Coley, R. M. and Jensen, R. G. (1973) Effect of parathion on lipolysis in isolated rat adipose tissue cells. Lipids, 8: 43-44.

10. Gabbott, P. A. (1976) Energy metabolism. In: Marine mussels, their ecology and physiology. (Ed, Bayne, B. L), I. B. P. Cambridge Univ. Press, PP. 293-337.

11. Swami, K. S., Rao, K.S.J., Reddy, K. S., Moorthy, R.S., Chetty, C.S. and Indira, K. (1983) The possible metaboilic diversions adopted by the fresh water mussel to counter the toxic metabolic effects of selected pesticides. Ind. J. Comp. Anim. Physiol., 1: 95-106.

12. Bhagyalakshmi, A. (1981) Physiological studies on the fresh water field crab, Oziotelphusa senex senex (Fab) in relation to pesticide impact. Ph.D. Thesis, Sri Venkateswara University Tirupati, India.

13. Chaudhari, T. R., (1988) Pesticidal impact on some physiological aspects of Bellamya (Viviparus) bengalensis. Ph.D. Thesis, Marathwada University, Aurangabad (M.S.), India.

14. Zambare, S. P. (1991) Reproductive physiology of the fresh water bivalve, Corbicula striatella. Ph. D. Thesis, Marathwada University, Aurangabad, India.

15. Verma, S. R. and Tonk, I. P. (1983) Effect of sub-lethal concentration of mercury on the composition of liver, muscle and ovary of Notopterus notopterus. Water, Air, Soil Pollut., 20: 287-299.

16. Hochachka, P. W., Fields, J. and Mustafa, T. (1973) Animal life without oxygen. Basic biochemical mechanisms. Amer. Zool., 13: 513-555.

17. Caers M., Coutteau P., Sorgeloos P., (2000) Incorporation of different fatty acids, supplied as emulsions or liposomes, in the polar and neutral lipids of Crossostrea gigas spat. Aquaculture, Amsterdam 186: 157-171.

18. Bhavani G, Dawood Shareif S (Sch Environ Sci, PG Res Dept Zoo, The New College, Chennai 600014). Bioabsorption of cadmium and mercury by mussel Perna viridis. Eco Env Conserv, 9(3)(2003), 285-289 [29 Ref].

19. Gabbott PA (1975) Storage cycles in marine bivalve molluscs. A hypothesis concerning the relationship between glycogen metabolism and gametogenesis. In: Proceedings of the 9th European Marine Biology Symposium. Aberdeen University Press, Aberdeen, pp 191211 (Gabbott1975;

20. Bayne BL, Bubel A, Gabbott PA, Livingstone DR, Lowe DM, Moore MN (1982) Glycogen utilization and gametogenesis

in Mytilus edulis L. Mar Biol Lett 3:89105

21. Barber BJ, Blake NJ (1985a) Intra-organ biochemical transformations associated with oogenesis in the bay scallop Argopecten irradians concentricus (Say as indicated by 14C incorporation. Biol Bull (Woods Hole) 168:3949

22. Barber BJ, Blake NJ (1985b) Substrate catabolism related to reproduction in the bay scallop Argopecten irradians concentricus, as determined by O/N and RQ physiological indexes. Mar Biol 87:1318

23. Vassallo MT (1973) Lipid storage and transfer in the scallop Chlamys hericia Gould. Comp Biochem Physiol 44:11691175

24. Barber BJ, Blake NJ (1981) Energy storage and utilization inrelation to gametogenesis in Argopecten irradians concentricus.J Exp Mar Biol Ecol 52:121134

25. Gallager SM, Mann R (1986) Growth and survival of larvae of Mercenaria mercenaria (L.) and Crassostrea virginica (Gmelin) relative to broodstock conditioning and lipid content of eggs. Aquaculture 56:105121

26. Soudant P, Marty Y, Moal J, Samain JF (1996b) Fatty acids and egg quality in great scallop. Aquacult Int 4:191200