Interdiscip Toxicol. 2018; **Vol. 11**(3): 200–203.
doi: 10.2478/intox-2018-0017

sciendo

interdisciplinary toxicology

ORIGINAL ARTICLE

Effects of mustard oil cake on liver proteins of *Channa punctatus* (Bloch)

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ITX110318A03 • Received: 22 July 2017 • Accepted: 15 May 2018

ABSTRACT
Mustard oil cake is a biofertilizer widely used in agriculture and fish cultivation almost in all South East Asian Countries including India. The study was carried out to observe the effects of this biofertilizer on the liver proteins of *Channa punctatus*. At sublethal concentration (0.42 g/L), fishes were exposed for a prolonged period of 35 days and amount of total liver protein (TLP) was measured. The investigation showed a low rate of liver protein synthesis in treated fish after 4 days of exposure. An increase in the amount of protein was observed between the 7th and 35th day. But such increment was below the amount of TLP of control fish, indicating physiological stress in the treated fish.

KEY WORDS: mustard oil cake; *Channa punctutas*; liver; protein

Introduction

Inorganic fertilizer is used to increase the growth and production of cultured plant and fish. But it has some adverse effect on the environment. Nowadays organic fertilizer as well as biofertilizer are used to reduce the adverse effects on the environment and induce growth performance, as reported by Abbas *et al.* (2001) on the major carp. Investigation showed a change in carbohydrate and nitrogen metabolism, depletion of protein glycogen and pyruvate stored in liver and muscle of fish during stress by pesticide induced hypoxia (Laul *et al.,* 1974). Mukhapadhyay and Dehadrai (1987) investigated the metabolic fate of non-protein nitrogenous substance for urea in *Heteropnustus fossilis.* The capacity of fish to survive on decayed or detritus matter and the capacity of tolerance of high concentration of ambient ammonia was revealed by Dehadrai (1980). Vasait and Patil (2005) studied the effect of monocrotophos on edible fish and observed a marked reduction in hemoglobin and total erythrocyte count. The study also revealed changes in liver and muscle protein concentration of *Channa punctatus* depending upon the period of exposure and concentration of xenobiotics applied (Sirohi & Saxena, 2006). Naveed *et al.* (2010) reported reduction in the level of total proteins and significant enhancement of free amino acid when *Channa punctatus* was exposed to triazophos. Malathi *et al.* (2012) studied the comparative hematological parameter on *Channa punctatus* in reference to physiological stress. Ahmed (2013) studied the effect of industrial waste discharge on the physiological parameter of *Tilapia niloticus*. Maitra and Nath (2014) studied the impact of urea on the hematological parameter of *Heteropnustus fossilis* and revealed the recovery pattern from the negative effect of toxic material. This toxic chemical after reaching sufficiently high concentration in body cell may cause alteration in physiological function of the aquatic organisms (Heath, 1987; Bartoskova *et al*., 2013; Torre *et al*., 2013; Fazio *et al*., 2014; Aliko *et al*., 2015; Faggio *et al*., 2016; Pagano *et al*., 2016; Pagano *et al*., 2017; Savorelli *et al*. 2017). Khan Niazi (1986) reported that mustard oil cake contained a high amount of the protein allylisothiocyanate, phytic acid, *etc.* Mondal *et al.* (2014) reported that the level of accumulation of mineral, nitrogen in soil was much more pronounced when mustard oil cake was applied along with other edible and non-edible oil cake.

So far there is no such study which can explore the effects of mustard oil cake on physiological aspects of liver protein of fish. Here an attempt has been made to observe the effect of mustard oil cake on *Channa punctatus* to liver protein levels during various days of exposure.

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Material and methods

Selection of specimen

Air breathing teleost, *Channa punctatus* (Bloch), commonly known as snake headed fish, were collected from the market of Dumdum, Kolkata, West Bengal. Adult fish of average weight (51.68±0.634), were selected for the experiment. Infected and diseased fish were avoided.

Rearing and culture

Live fresh water fish, *Channa punctatus*, was collected and cleaned with 0.2% $KMnO_4$ solution to avoid any fungal infection. Then the fishes were stored in a glass aquarium (90 cm × 50 cm × 31 cm) containing tap water and were acclimated under laboratory condition for 7 days. Water was changed every 48 hr. Dead fish (if any) were removed as soon as possible. Commercial fish food (containing crude protein 46%, fat 6%, fiber 5%) was provided during acclimatization as well as treatment period at the rate of only 2% of the fish body weight, once a day. The laboratory photo period was 12 hr. dark: 12 hr. light.

Biofertilizer

Bio-fertilizer, namely Mustard Oil cake (MOC), was used in the experiment. It is widely used in major cultivation in West Bengal, such as paddy, wheat, potato, *etc.* Dry MOC is mixed with the soil by ploughing to form seed bed. The measurement of applied MOC during cultivation is 50 kg/acre. Run off from the field in nearby water body may affect fishes and other animals at high concentration.

The chemical composition of MOC is: 43% protein, 2.05% oil, 1.22% Allylisothiocyanate (AITC) and 2.75% phytic acid. Phytic acid is usually regarded as an anti-nutritive factor (Khan Niazi, 1986).

Treatment

Acclimatized fishes were treated with MOC, a bio-fertilizer widely used in agriculture. LC_{50} was measured as 0.4625 g/L (96 hr.) during this experiment, according to the probit analysis method (Finney 1971). Acclimatized fishes were exposed (EP) to sub-lethal concentration (0.42 g/L) of MOC based on the result of 96 hr. LC_{50}. One aquarium (90 cm × 50 cm × 31 cm) was set for that particular dose and 30 fishes were kept in 60 L of tap water. The water temperature was kept at 28±1 °C during the whole experimental period. Another aquarium containing the same number of fish was maintained as control for the experiment. Water quality was maintained approximately as pH 7, alkalinity=293 mg/L, hardness=388 mg/L, nitrate=0.85 and DO=10.02 mg/L during the experiment. During the treatment period five fish were sacrificed at a time after 4 days, 7 days, 14 days, 21 days, 28 days and 35 days. The liver was isolated for protein estimation from five fish separately for each day of exposure. No fish were found to die either in the control or treated groups.

Protein concentration

Liver tissues were collected from the specimen and wet weight was measured. Then it was homogenized in a glass homogenizer (REMI Cat.No.RQ127A) using 10 mL of phosphate buffer solution (0.1 M, pH 7.4) as suggested by Saito *et al.* (1983) and centrifuged by cooling centrifuge (REMI serial No. EVCI 6169). Protein level was assessed according to the method of Lowry *et al.* (1951) using visual Spectrophotometer (SYSTRONICS 117). OD values were obtained and the protein concentration was calculated against bovine serum albumin as standard. Values have been expressed as mg/g.

Liver somatic index

Liver somatic Index (liver weight as % of body weight) was calculated with respect to the total body weight (Heidinger & Crawford, 1977).

Statistical analysis

ANOVA (single factor) was done by using Origin 6.0. Strength of association (ω^2) were also calculated with the recorded data (Das & Das, 1993).

Results

Channa punctatus exposed to water treated with 0.42 g/L of Mustard Oil Cake showed no mortality after prolonged period of exposure but the fishes were at physiological stress (Natarajan, 1984). In that condition the Total Liver Proteins (TLP) were estimated. In non-treated fish the level of TLP ranged between 85.8±0.59 mg/g and 128.4±0.464 mg/g and indicated a steady rate of increase with the advancement of days (Table 1). But there was a significant (p<0.05) decrease of protein content in the liver during various days of exposure. The rate of decrease in TLP was rapid on the 4th day, then an increase in the level of proteins was observed though the amount was lower than in the control fish as also revealed in the Liver Somatic Index (Table 2). Strength of Association (ω^2) is made to estimate the degree of relatedness between duration of exposure and liver protein concentration. The computed values show the proportion as 0.99(EP=35) >0.95(EP=07) >0.94(EP=28) >0.82(EP=21) >0.47(EP=4) >0.38(EP=14) of total variance of protein concentration as related to the duration of the exposure (Table 3).

Discussion

The result of the experiment revealed that protein concentration of the liver after 4 days of MOC treatment was significantly lower than the control. Decrease in the amount of liver proteins during the first 4 days of treatment showed that the fish were at physiological stress. According to Lett *et al.* (1976) the reduction in protein level may lead to increase in energy demand at the time of stress. As an important constituent of all the cells and tissue, proteins play an important role in physiological activity of living organisms (Adamu & Saikpere, 2011; Burgos-Aceves *et al.,* 2016; Lauriano *et al.* 2016). Moreover, proteins act as a source of energy during the

Table 1. Amount of liver protein mg/g in treated and non-treated *Channa punctatus*.

Type of specimen	Day 0	Day 4	Day 7	Day 14	Day 21	Day 28	Day 35
Control	85.8±0.59	65.6±2.16	110.72±0.28	109.04±0.66	108.64±0.24	127.34±0.57	128.4±0.464
Treated	–	54.5±0.94	94.01±0.73	103.15±1.36	94.51±1.35	107.93±0.92	85.4±0.38

Liver Protein as mg/g (mean±SE)

Table 2. Liver somatic index

Control	Day 4	Day 7	Day 14	Day 21	Day 28	Day 35
0.8126%	0.935%	1.21%	1.14%	1.10%	1.11%	0.88%

Table 3. Statistical relations between control and treated *Channa punctatus*

Statistics	Day4	Day7	Day14	Day21	Day28	Day 35
F*	19.87	399.59	13.64	96.17	309.01	521.39
ω^2	0.47	0.95	0.38	0.82	0.94	0.99
t-test†	4.45	19.98	3.69	9.8	17.57	72.21
Bonferroni modification	$p<0.002$	$p<0.002$	$p<0.002$	$p<0.002$	$p<0.002$	$p<0.002$

*Significant $p<0.05$; † Significant $p<0.001$

chronic period of stress (Umminger, 1977). But with advancement of days, there was rapid increase in protein concentration at first, then the rate of increase maintained a steady state which was higher than on 4th day as well as in control. This might indicate fish perseverance to cope with adverse stress situation. Moreover, TLP remained at low level in all treated fish compared to untreated. So more proteins were used to meet the increased energy demand, which led to increase the rate of protein synthesis. The used culture medium contaminated with MOC contains 43% protein (Khan Niazi, 1986) and it is also a rich source of nitrogen (Mondal *et al.,* 2014). Abbas *et al.* (2001) reported an average weight gain of fish when the pond was treated with urea. Whereas Tarar (1997) obtained higher net fish production from a pond which was urea treated as a source of non-protein nitrogen and a better nitrogen incorporation efficiency. MOC contains 43% protein. Based on the present experiment it was probable that increase in protein synthesis was accelerated by the consumption of protein and nitrogen from the culture medium by the fish. Increase in the synthesis of liver protein was probably due to metabolism of proteins synthesis enzyme activities in the fish and MOC stimulate the rate of synthesis during prolonged exposure. Adamu and Siakpere (2011) proposed that protein is the chief source of nitrogen metabolism. During the long-term exposure, protein concentration shows gradual increase. The rate of protein synthesis or its degradation regulates the quantity of protein. Moreover , impaired incorporation of amino acids in the polypeptide chain also affect the quantity of protein (Singh *et al*. 1996). On the other hand, inhibition of alkaline phosphatase activity reduces the protein level, as it plays an important role in protein synthesis along with the other secretory activities (Pilo *et al.,* 1972; Ibrahim *et al.,* 1974). The outcome of the study was that MOC alone was not sufficient for TLP synthesis, as artificial fish food which was provided at the rate of 2% of body weight, a very negligible amount as the body weight of experimental fish was concerned.

Acknowledgement

Authors are thankful to the Head, Post Graduate Department of Zoology, Bidhannagar College and Principal of the same college for providing necessary laboratory facilities. Susanta Nath has transferred from Bidhannagar College to Government G D College Singur recently.

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