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

Susanta NATH ¹, Priyanka RAKSHIT ², Valerio MATOZZO ³

¹ Department of Zoology, Government G. D. College, Singur, Dist. Hooghly, West Bengal, India  
² Bidhannagar College, Department of Zoology, EB-2, Sector-1, Salt Lake, Kolkata, West Bengal, India  
³ Department of Biology, University of Padova, Via Ugo Bassi 58/B, Padova, Italy

**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 punctatus*; 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 *Heteropneustes 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 *Heteropneustes 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.
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% KMnO₄ 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 biofertilizer widely used in agriculture. LC₅₀ 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 (0.42g/L) of MOC based on the result of 96 hr. LC₅₀. 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 MOC was set for that particular dose and 30 fishes were kept as control for the experiment. Infected and diseased fish were avoided.

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 (ω²) were also calculated with the recorded data (Das & Das, 1993).

Results
*Channa punctatus* exposed to water treated with 0.42g/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 (<0.05) decrease in 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 (ω²) 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

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, pH7.4) 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.
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 Niaz, 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.

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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 | Day 4 | Day 7 | Day 14 | Day 21 | Day 28 | Day 35 |
|------------|-------|-------|--------|--------|--------|--------|
| F*         | 19.87 | 399.59 | 13.64  | 96.17  | 309.01 | 521.39 |
| ω²         | 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
