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### Research Article

### Zoology

#### ACUTE TOXICITY, BEHAVIOURAL RESPONSES AND ORGAN TOXICITY OF SOYBEAN FORMULATION DIET EXPOSURE TO *Catla catla* FINGERLINGS

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#### ABSTRACT

The objective of this study was to obtain data on the safety-in-use of Soybean as a dietary supplement by assessing its acute toxicity in fish. Soybean is the world's most important seed legume, which contributes to valuable ingredients in formulated feeds for poultry and fish. The fish are exposed to the test diet for a period of 24 hours. Mortalities are recorded, and where possible, the concentrations are estimated to kill 50% of the fish (LD<sub>50</sub>). Acute toxicity tests permit rapid assessment of the impacts of various toxicants on organisms. The aim of the present study is to assess the acute toxicity effect of the soybean formulation diet on fingerlings of *Catla catla* fish. The *Catla catla* fingerlings showed acute exposure to soybean supplementation diet caused significant non-sensitivity and the mortality rate was nil to all the concentration groups of fish. The various behavioural responses were observed in the present study of *Catla catla* exhibited a range of non-abnormal behaviours at all concentrations of the soybean (1 to 5%) supplementation diet and similar behaviours to control diet while soybean supplementation diet concentration was 1% to 5% espouse to fish, showing normal liver tissue with hepatocytes and a portal triad showing a prominent central vein. The obtained results showed that acute supplementation of Soybean is not toxic in fish, suggesting a safety use by fish. Present study concluded that in vivo acute toxicity study clearly showed the nontoxic nature of supplementation of different concentration of soybean diets up to the tested dose level from 1 to 5%. However, the fish mortality rate was nil and normal liver tissue histology should be considered for in vivo use of this supplementation diets. On the basis of acute toxicity studies, the 1, 2 and 3% diet has been taken as performed in the efficacy studies.

**Keywords:** Fish toxicity, LD<sub>50</sub>, Soybean diet, Fish behavioral responses, Acute toxicity.

#### INTRODUCTION

Soybean (*Glycine max* L. Merril) is the world's most important seed legume, which contributes to 25 % of the global edible oil, about two-thirds of the world's protein concentrate for livestock feeding. Soybean meal is a valuable ingredient in formulated feeds for poultry and **fish**. Soybeans, foods derived from soybeans (e.g., tofu, soymilk, soy infant formula, tempeh) and food and dietary supplement ingredients derived from soybeans (e.g., soybean oil, soybean proteins, isoflavones) have been under intensive

research for their health effects especially over the past 25 years (Hendrich, 2017). Use of herbal dietary supplements by the public is common and has been happening for centuries. The Food and Drug Administration has made efforts to prohibit the sale of unsafe herbal dietary supplements. Numerous reports have proliferated of adverse events due to these supplements, which may contain toxic botanical compounds that pose a public health risk. The investigation of bioactive plant compounds commonly used in herbal dietary supplements and their relative toxicities, with

special attention to those supplements whose exposure to the consumer is maximal, provides a unique contribution to the investigation of botanical supplements. The objective of this study was to obtain data on the safety-in-use of different concentrations of soybean (1, 2, 3, 4 and 5%) as a dietary supplement by assessing its acute toxicity in *Catla catla* fish.

**MATERIALS AND METHOD**

Collection and acclimation of experimental fishes Fingerlings of *Catla catla* (Average weight 5.00 ± 0.87gm) were procured from Fish farm, Thittai, Thanjavur District, Tamil Nadu, India (2020), using cast net and maintained in the laboratory in a glass aquarium tank and acclimated in aerated tap water with continuous aeration for two weeks prior to experimentation. During this period, fishes were fed with a known amount of fish food.

**Experimental designing of acute toxicity**

The experiment was conducted in 6 glass aquaria each having 50 L of water with 10 fish of *C. catla* fingerlings. The aquaria were maintained indoor under natural

photoperiod laboratory conditions (Kalita *et al.*, 2008). The temperature averaged 28±1.5°C, dissolved oxygen 7.4±0.6 mg/l, and total ammonia 0. 5±0.2 mg/l.

**Preparation of soybean floating pellet in acute toxicity**

The ingredient composition of the experimental diets feed preparations raw material were dried at room temperature, ground to powder. The ingredient composition powder were heated to about 50°C and then cooled to room temperature. These were then put to hand Murukku Maker (Alternative to pelletizer fitted) with a 2.0 mm diet (Kalita *et al.*, 2008). The experimental fish were fed with five different concentration of soybean (1 to 5%) diet once at a fixed feeding rate of 3% of the total body weight for a period of the experiment and monitor for 24 hours. After supplementation of the feeding, fish were monitored continuously for every 12 hours for 24 hrs. to detect any changes in behavioral responses and monitored for any mortality during the course of toxicity study.

Supplementation of diet (gm/ total B.W of fish) = Total B.W of fish/100 X % of food.

**Table 1: Ingredients composition of diets (Ramakrishnan *et al.*, 2008)**

Ingredients (gm)	Control Diet	Soybean				
		1%	2%	3 %	4%	5%
Fishmeal	35.0	35.0	35.0	35.0	35.0	35.0
Soybean meal	17.0	17.0	17.0	17.0	17.0	17.0
Rice bran	11.0	11.0	11.0	11.0	11.0	11.0
Groundnut oil cake	10.0	10.0	10.0	10.0	10.0	10.0
Tapioca flour	10.0	10.0	10.0	10.0	10.0	10.0
Mineral premix	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin premix	0.5	0.5	0.5	0.5	0.5	0.5
Wheat flour	15	14	13	12	11	10
Soybean	-	1	2	3	4	5

**Table 2: Acute toxicity feeding to *Catla catla* fingerlings**

Acute toxicity	Control Diet	Soybean				
		1%	2%	3 %	4%	5%
Number of total exposed fish (5± 0.87gm)	10 (50gm)	10 (50gm)	10 (50gm)	10 (50gm)	10 (50gm)	10 (50gm)
3% of diet (gm/50gm B.W of total fish)	1.50	1.50	1.50	1.50	1.50	1.50

**Analysis of organ toxicity**

At the end of the experimental period of the fish was sacrificed and the liver was dissected out, washed with ice-cold physiological saline and prepared for histopathological analysis carried out by Ochei and Kolhatkar, (2000).

**RESULTS AND DISCUSSION**

**Toxicity and behavioral responses of soybean supplementation diet to *Catla catla* fingerlings**

The fish are exposed to the test diet for a period of 24 hours. Mortalities are recorded, and where possible the concentrations estimated to kill 50% of the fish (LD<sub>50</sub>), are determined. Acute toxicity tests permit rapid assessment of the impacts of

various toxicants on organisms. The criterion of lethal toxicity is mortality, the final response of an organism. However, in natural waters, fish are mostly affected by long-term influence of low concentrations of substances or their mixtures. The studies effects on physiology, growth, biology and behavior of various organisms, to predict probable toxic effects and their consequences on adaptive

abilities of various animals including fish (Pane *et al.*, 2004). In the present study, fish species of *Catla catla* fingerlings showed acute exposure to supplementation of different concentrations (1 to 5%) soybean diet caused significant non-sensitivity to all the concentration groups of fish. The mortality rate was nil in all soybean supplementation diet concentrations, as represented in table 3.

**Table 3: Acute toxicity study of different concentration (1, 2, 3, 4 and 5%) soybean supplementation to *Catla catla* fingerlings**

Concentration of diet (%)	Number of total exposed fish	12 hours		24 hours	
		Number of fish mortality	% of mortality	Number of fish mortality	% of mortality
1.0	10	Nil	Nil	Nil	Nil
2.0	10	Nil	Nil	Nil	Nil
3.0	10	Nil	Nil	Nil	Nil
4.0	10	Nil	Nil	Nil	Nil
5.0	10	Nil	Nil	Nil	Nil
Control	10	Nil	Nil	Nil	Nil

The test fish were supplemented to various concentrations (1 to 5%) of the soybean diet, the behavioral responses and the mortality rate of the *Catla catla* fingerlings were observed and recorded at intervals of 12 and 24 hours. The *Catla catla* fingerlings fish in the control tank were monitored and they served as a reference to the behavioral responses observed in those exposed to the different concentrations (1 to 5%) of soybean supplementation diet the test tanks. The responses to be observed if any occurred were general activity, hyperactivity, hypoactivity, loss of equilibrium and death of fish. Mortality Observations on mortality rate of *Catla catla* were made at 12 and 24hours. *Catla catla* in

the control set were normal in behavior with gentle body movement. They remained active and vigorous throughout the experiments. All the fishes were morphologically and physically well balanced. Their swimming movement, gulping for air, body colour, eyes and fins were normal. No mucus secretion and mortality was observed. Behavioral and biological responses of *Catla catla* to soybean supplementation diet of are displayed on table 4. The various responses were observed in present study of *Catla catla* exhibited a range of non-abnormal behaviors at all concentrations of the soybean supplementation diet.

**Table 4: Behavioral responses exposure time 12 and 24 hrs of soybean supplementation diet to *Catla catla* fingerlings**

Behaviours	Control	Concentration of diet (%)									
		1.0		2.0		3.0		4.0		5.0	
		12h	24h	12h	24h	12h	24h	12h	24h	12h	24h
Fish active	+	+	+	+	+	+	+	+	+	+	+
Loss of reflex	-	-	-	-	-	-	-	-	-	-	-
Air gulping	-	-	-	-	-	-	-	-	-	-	-
Erratic swimming	-	-	-	-	-	-	-	-	-	-	-
Mucus secretion	-	-	-	-	-	-	-	-	-	-	-
Loss of scale	-	-	-	-	-	-	-	-	-	-	-
Haemorrhage	-	-	-	-	-	-	-	-	-	-	-

(+ = Present- = Absent)

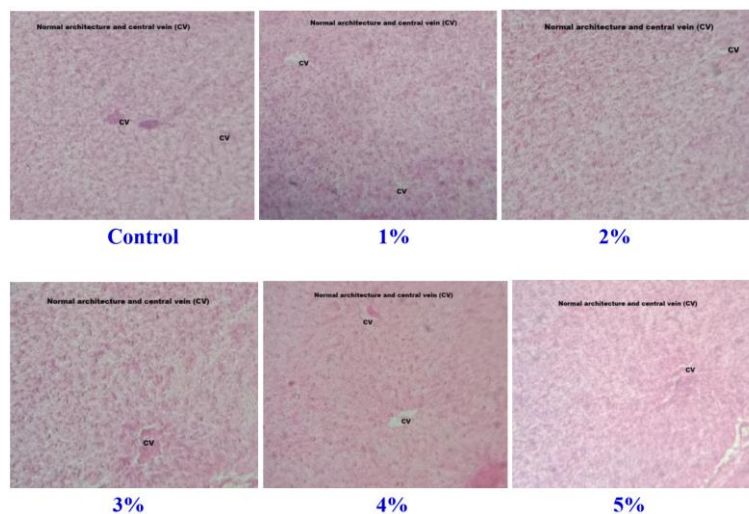
Soybean meal (SBM), having high protein content and favorable amino acid profile that closely meets the requirements of fish, is

consistently available and reported to be palatable to most species of fish (Lim and Akiyama 1992). Studies have shown

considerable success in partial or total replacement of FM with SBM in diets for many fish species without toxicity (Reinitz, 1980; Mohsen and Lovell, 1990; Vivyakarn *et al.*, 1992; Olli *et al.*, 1995; Boonyaratpalin *et al.*, 1998; Thompson *et al.*, 2006; Biswas *et al.*, 2007; Hernandez *et al.*, 2007; Abdel-Tawwab *et al.*, 2010). Many studies shown that approximately 20 to 40% FM protein can be replaced in diets for carnivorous fish species (Chou *et al.*, 2004; Lim *et al.*, 2004; Hernandez *et al.*, 2007; Pham *et al.*, 2007; Lim and Lee, 2008).

**Organ toxicity of soybean supplementation diet to *Catla catla* fingerlings**

At the end of the experiment, the liver of fish, as well as the moribund fish during the experiment, were analyzed by histopathological evaluation (Behmer *et al.*, 1976). The histological sections were analyzed in terms of the prevalence of pathologies/toxicological between treatments and the degree of histopathological severity of each organ according to the methodology of Cengiz and Unlu (2006). In the present study, soybean supplementation diet concentration was 1% to 5%. Fish shows normal liver tissue with hepatocytes and a portal triad showing a prominent central vein, as represented in plate 1.



**Plate 1: Organ toxicity of soybean supplementation diet to *Catla catla* fingerlings using histopathological observation**

Regarding the liver, this is one of the organs most affected when toxic substances or elements are present in the water and food. It is associated with the detoxification and biotransformation process due to its function and blood supply (Van Der Oost *et al.* 2003). Muhammad Awais Nazir *et al* (2021) study was undertaken to investigate the impact of replacement of fishmeal (FM) with soybean meal (SBM) in diets of rainbow trout (*Oncorhynchus mykiss*). The inclusion of SBM in diets 1-4 was at the following respective levels of 0% (control), 29%, 35% and 51%. Growth performance of fish fed diet 4 was significantly lower than that of fish fed the other diets (P<0.05). The dietary inclusion of SBM up to level of 35% did not affect the blood biochemical parameters of rainbow trout, and was associated with acceptable growth performance. Results showed that inclusion of dietary 35% SBM had no toxic effect on fish liver. The fish of *Catla catla* fingerlings presented in hepatocytes at concentrations of 1, 2, 3, 4 and 5% in soybean

supplementation diet, concentrations much normal architecture and central vein of liver tissue present in this study. The presence of hepatocytes and central vein observed using soybean supplementation diet in this study may have owed to non-excessive liver activity. However, histopathology of *Catla catla* fingerlings liver exposed to soybean supplementation diet in acute toxicity test, a control fish normal morphology of liver (24 h) and different concentration (1, 2, 3, 4 and 5%) soybean supplementation to *Catla catla* fingerlings liver tissue similar to control diet.

**CONCLUSION**

In the present work concluded that *in vivo* acute toxicity study clearly showed the nontoxic nature of supplementation of different concentration of soybean diets up to the tested dose level from 1 to 5%. The fish mortality rate was nil and normal liver tissue histology should be considered for *in vivo* use of this supplementation diets. The obtained results showed that acute supplementation of

Soybean is not toxic in fish, suggesting a safety use by fish. On the basis of acute toxicity studies, the 1, 2 and 3% diet has been taken as performed in the fish efficacy studies.

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