

# Alterations in growth pattern relationships and condition factors of *Channa gachua* (Hamilton) exposed to a common biofertilizer

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## Abstract

Alteration in the length-weight and breath-weight relationship, as well as the condition factor of *Channa gachua*, was analysed after short-term (24, 48, 72 and 96 hours) and long-term (15, 30, 45, 60 and 75 days) exposure to the sublethal dose of 253.25 mg l<sup>-1</sup> of a common biofertilizer (mustard oil cake). The 96hr-LC50 dose of mustard oil cake of 5065.0 mg l<sup>-1</sup> showed that it was slightly toxic (500-5000 mg l<sup>-1</sup>) or virtually non-toxic (5000-15000 mg l<sup>-1</sup>) nature to this fish with a safe level estimate of 101.30 mg l<sup>-1</sup>. It was found that the weight, length and breadth of the fish showed a lower increase in treated fish when compared to control ones. Better results were obtained from the fish under long-term treatment compared to short-term experiments. The long-term experimental fish value of the exponent of the length-weight relationship of 1.398 suggests negative allometric growth. The condition factor 'K' for the experimental fish was above 1.0 (1.64-1.82) indicating the relative condition of the experimental fish. The decline in the growth of fish in biofertilizer-treated conditions is due to the presence of anti-nutritional factors of biofertilizer. The observation would help to formulate cost-effective fish food, assessment of optimal biofertilizer dose for the higher yield and computational biomass of the sample of this fish.

**Keywords:** *Biofertilizer, Channa gachua, Dose, Growth parameters, Sublethal concentration*

## Introduction

Fish and other animals become healthy only by the fitting extent of supplements in their food. The fish feed ought to be even and planned by their need (Aragona et al. 2017). It has been accounted that oil cakes are plentiful in protein and minerals but also supplements useful for the growth of plankton. Plankton is one of the components of fish food. Mustard oil cake (MOC) is one of the randomly used biofertilizers in pisciculture and agriculture in the preparation of seedbeds in India (Pandey

and Shukla 2005, Nath et al. 2018; Bannaee et al, 2023). Besides, environmental pollution may be reduced considerably by reducing increasing the use of mustard oil cake (Islam et al. 2007). With this speculation, the Government of India is attempting to promote the use of biofertilizers along with fertilizers and compost.

Length-weight relationship, width-weight relationship and condition factors are important tools in fish biology (Freon et al.

1997, Arredondo et al. 2016). These relationships estimate growth rate, length, breadth and age structures, the average weight at a given length group, the health status, and other components of the fish population dynamics (Kohler et al. 1995). It permits fisheries scientists to change growth-in-length equations to growth-in-weight in stock assessment model (Moutopoulos and Stergiou 2002), estimate biomass from length-frequency distributions (Dulcic and Kraljevic 1996), and compare life history and morphological aspect of the population inhabiting different regions (Stergiou and Moutopoulos 2001). Condition factors are used for comparing the condition, fatness, or well-being of fish (Tesch 1971). This relationship also enables the computation of condition indexes and allows for the comparison of species growth trajectories (Froese 2006).

*Channa gachua* (Dwarf Snakehead) is a hardy facultative air-breathing fish with a pretty tinge inhabiting paddy fields and ponds in India. The IUCN status of this fish shows that its number is diminishing (Chaudhry 2010). *Channa gachua* might be used as a biocontrol agent against mosquito larvae under laboratory conditions (Phukon and Biswas 2011). As of late, the mitochondrial genomic analysis of this fish has been conducted (Zhou et al. 2019) and Pandit and Kumari (2019) worked to determine 96 hr-LC50 doses and the toxicity status of mustard oil cake. But, the effect of mustard oil cake on the growth of *Channa gachua* is not measured.

Consequently, the objective of this work was to evaluate the effects of a sublethal dose of a biofertilizer on the growth pattern relationship and Condition factor of *Channa gachua*. The work will help in formulating cost-effective fish food, deciding the optimum dose of a

biofertilizer for the higher yield of this fish and better conservation strategy for the fishery resource.

## Materials and Methods

The work was conducted at the Department of Zoology, Veer Kunwar Singh University, Arrah (Bihar), India during 2021-2022. Fresh and healthy specimens of either sex of *Channa gachua* (Hamilton 1822) (BW: 55-75 g, TL: 13-16 cm and width: 2.30-2.50 cm) were obtained from the local market of Arrah, Bihar. They were cleaned with 0.2% KMnO<sub>4</sub> solution to avoid any infection and acclimatized for a fortnight in Departmental Laboratory. Fish food having crude protein 45%, fat 5% and fiber 5%) was provided during acclimatization at the rate of 3% of the fish body weight daily. The water temperature of 26.0±1.5 °C was maintained with total alkalinity and hardness respectively of 326.0±14.5 and 195.0±8.2 mg l<sup>-1</sup>.

### Biofertilizer

Powder of biofertilizer namely mustard (*Brassica napus*) oil cake was selected for the experiment and was purchased from the local market of Arrah. It contains 43%-Protein, 2.05%-Oil, 0.086-1.22%-Allylisothiocyanate (AITC) and 2.75% Phytic acid (Swati and Das 2015). Its protein is rich in lysine and sulfur-containing amino acids which are limiting in cereal protein (Devi and Devi 2011). However, it also contains major anti-nutritional factors (ANFs) like aglucone, tannins, glucosinolates, erucic acid and phytates (Slominiski et al.1999).

### The 96hr-LC50 dose of biofertilizer

The 96hr-LC50 dose of biofertilizer was measured following the probit analysis method (Finney 1971). Acclimatized fishes were exposed daily to sub-lethal concentrations of

mustard oil cake along with Fish food at the rate of 3% also of the fish's body weight. The effect of a sub-lethal dose of mustard oil cake on the growth pattern of *Channa gachua* was observed after short-term (24, 48, 72 and 96 hours) and long-term (15, 30, 45, 60 and 75 days) durations.

#### Treatment

In the experiment, aquaria (90 × 50 × 31 cm<sup>3</sup>) were set for 30 fishes were kept in 60 L of tap water. Fishes were randomly selected from the stock and divided into two groups. One group was taken as a control or 'C' and another as a treatment or 'T'. Fishes in 'T' aquaria were exposed to the diet containing a sublethal dose of mustard oil cake but the fishes in aquaria 'C' were continued to feed fish food. Three observations were made from each group.

#### Measurement of Growth

Growth was monitored to body weight, length and breadth for 75 days. The body weight of the fish was measured using a pan balance. Length and breadth were measured with the help of measuring tape.

Length-weight and width-weight relationship: The relationships were determined by linear regression analysis and scatter diagrams of length, width and weight were plotted. The length-weight and width-weight relationship were worked out as per the cube law given by Le Cren (1951).

$$W = aL^b$$

Where, W = Weight of fish (g), L = observed total length (cm), L = observed total breadth (cm), a = intercept and b = slope.

Condition factor (CF): Fulton's condition factor (K) was calculated according to Htun-Han (1978) equation as per the formula given below:

$$K = \frac{\text{Weight of the fish} \times 100}{\text{Length of the fish}^3}$$

#### Statistical analysis

Statistical analysis was done using Graph Pad Prism 5.0 and applied to test for levels of difference between different treatments.

#### Results and Discussion

The 96hr-LC50 dose of mustard oil cake of 5065.0 mg l<sup>-1</sup> for *Channa gachua* (body weight: 55.75 g), following the method of Finney (1971) was determined by Pandit and Kumari (2019). Based on the 96hr-LC50 dose, mustard oil cake may be treated as a substance of slightly toxic (500-5000 mg l<sup>-1</sup>) or practically non-toxic (5000-15000 mg l<sup>-1</sup>) to this fish (Loomis and Hayes 1996). Safe level estimates of 101.30 mg l<sup>-1</sup> of mustard oil cake in *Channa gachua* were calculated by the method of CWQC (1972). A concentration of 253.25 mg l<sup>-1</sup> (5% of 96hr-LC50) of mustard oil cake was selected for this study.

A total of 360 healthy specimens from fish samples were examined for the length-weight relationship, width-weight relationship and conditions factors analysis. The weight ranged between 55.80 1.05 to 75.47 1.88 g with a maximum average weight of 67.58 6.39 g, the range of was length 14.41 0.86 to 17.63 1.75 cm with a maximum average length of 16.58 0.89 cm while the range width was between 2.46 0.15 to 2.85 0.28 cm with the maximum average of 2.72 0.13 cm (Tables 1 and 2).

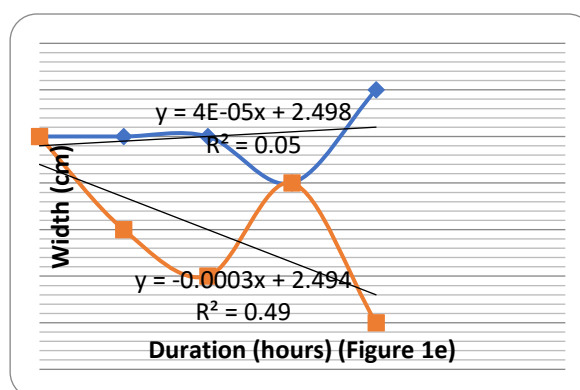
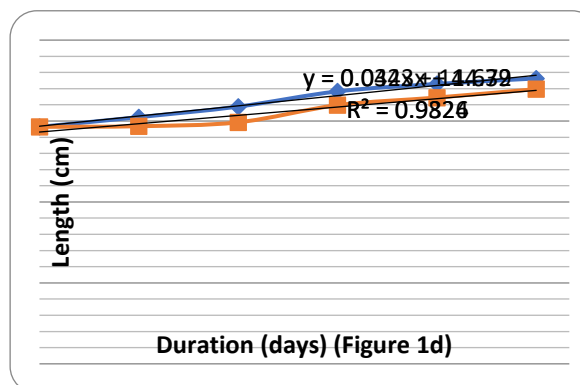
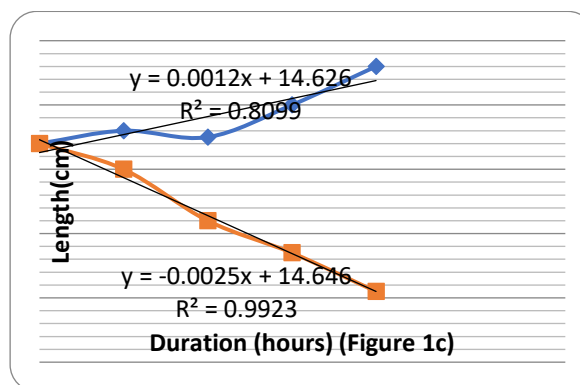
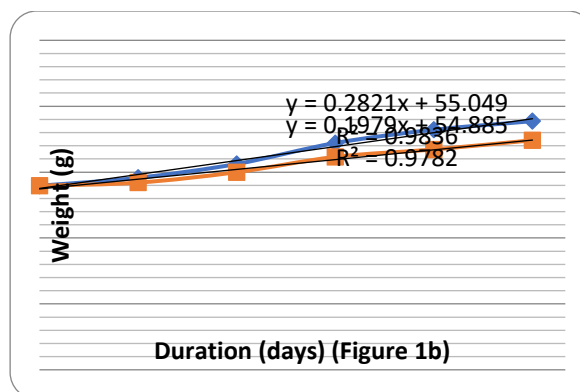
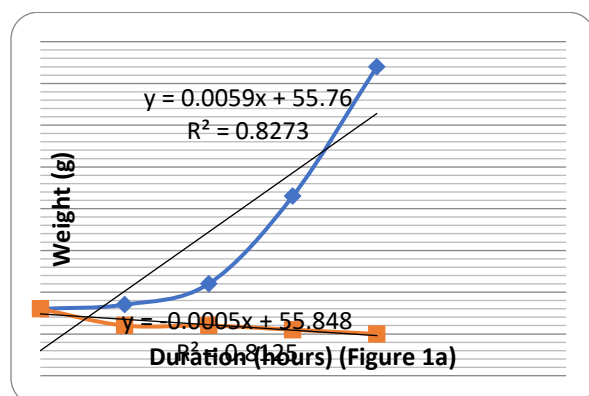
The weight of *Channa gachua* under controlled conditions was  $y=0.005x+55.76$  and  $y=0.282x+55.04$  (Fig. 1a). While observed weight from short-term treatment was  $y=-0.000x+55.84$  and long-term treatment was  $y=0.197x+54.88$  (Fig. 1b). The values of determination coefficient (R<sup>2</sup>) ranged from 0.812 to 0.983 indicate that 81% to 98% of variant can be explained by the model. While correlation coefficients higher

than 80% inform the strong relationship between weight and duration. The 't' values inferred significant ( $P < 0.05$ ) changes in short-term and long-term treatment (Table 3).

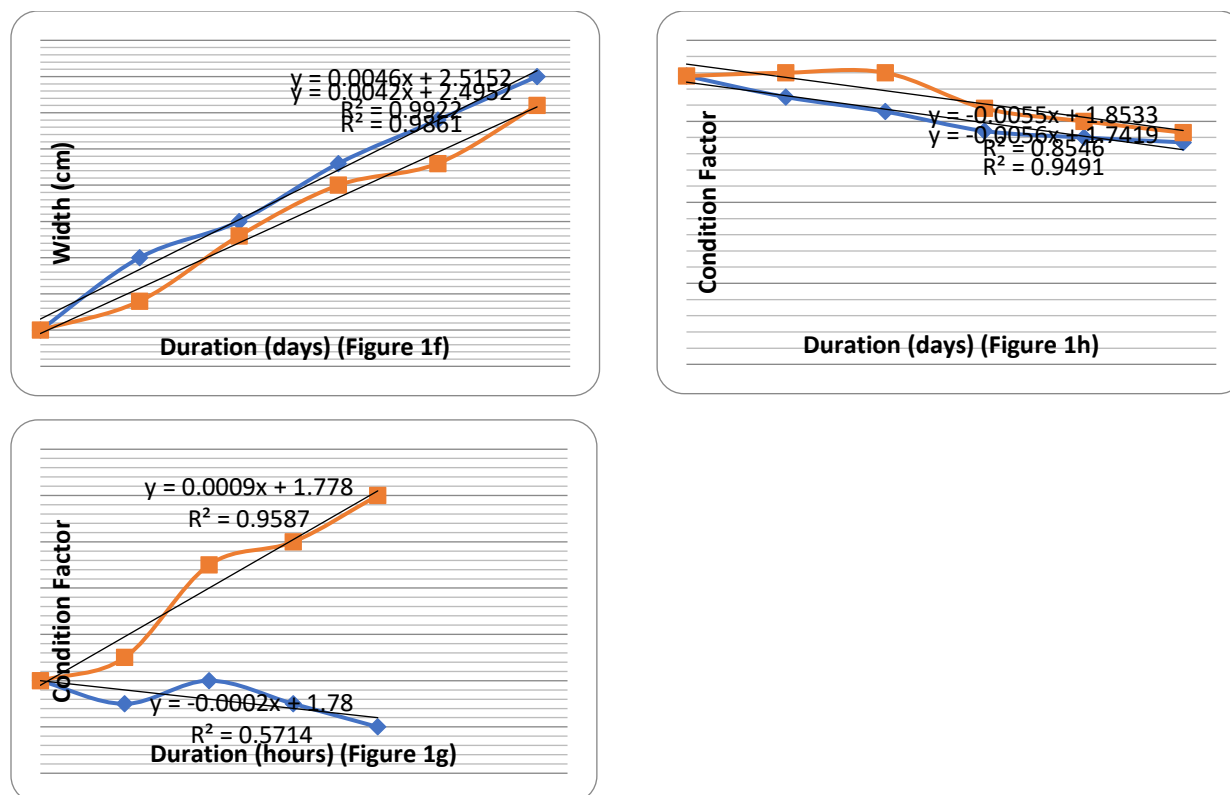
Under controlled conditions, the length of fish was  $y = 0.001x + 14.62$  and  $y = 0.042x + 14.67$  (Fig. 1c). While during short-term treatment, the observed length was  $y = -0.002x + 14.64$  and in long-term treatment was  $y = 0.034x + 14.32$  (Fig. 1d). The  $R^2$  values ranged from 0.808 to 0.992, suggesting that the model would describe 81% to 99% of the version. The close relationship between length and duration is stated by correlation coefficients above 80%. Significant ( $P < 0.05$ ) improvements were inferred from the 't' values in short and long-term treatment (Table 3).

The width of *Channa gachua* was  $y = 4E-05x + 2.498$  and  $y = 0.004x + 2.515$  under controlled conditions (Fig. 1e). Although, the width observed for short-term treatment was  $y = -0.000x + 2.494$  and long-term treatment was  $y = 0.004x + 2.495$  (Fig. 1f). The values of  $R^2$  ranged from 0.051 to 0.992 indicating that 05% to 99% of version can be explained by the model.

**Figure 1. Alteration in growth parameters under controlled and biofertilizer-treated *Channa gachua* (a) and (b) weight of fish, (c) and (d) length of fish, (e) and (f) width of fish, (g) and (h) condition factor of fish.**



Alterations in growth pattern relationships and condition factors of *Channa gachua* (Hamilton) exposed to a common biofertilizer



**Table 1: Growth of *Channa gachua* (n = 30) exposed to short exposure of 253.25mg l-1 of Biofertilizer**

S. No.	Duration of treatment (hours)	Weight (g)			Length (cm)			Width (cm)		
		Control	Treated	Difference	Control	Treated	Difference	Control	Treated	Difference
1	0	55.86 $\pm$ 1.26			14.64 $\pm$ 0.99			2.50 $\pm$ 0.21		
2	24	55.87 $\pm$ 1.15 (+0.02)	55.82 $\pm$ 1.03 (-0.07)	-0.05	14.66 $\pm$ 0.95 (+0.13)	14.60 $\pm$ 0.75 (-0.27)	-0.06	2.50 $\pm$ 0.17 (0.00)	2.48 $\pm$ 0.15 (-0.80)	-0.02
3	48	55.92 $\pm$ 1.07 (+0.11)	55.82 $\pm$ 1.06 (-0.07)	-0.10	14.65 $\pm$ 0.91 (+0.06)	14.52 $\pm$ 0.84 (-0.82)	-0.13	2.50 $\pm$ 0.19 (0.00)	2.47 $\pm$ 0.17 (-1.2)	-0.03
4	72	56.13 $\pm$ 0.95 (+0.48)	55.81 $\pm$ 1.02 (-0.09)	-0.32	14.70 $\pm$ 0.99 (+0.41)	14.47 $\pm$ 0.93 (-1.16)	-0.23	2.49 $\pm$ 0.15 (-0.40)	2.49 $\pm$ 0.13 (-0.40)	-0.00
5	96	56.44 $\pm$ 1.01 (+1.02)	55.80 $\pm$ 1.05 (-0.11)	-0.64	14.76 $\pm$ 0.92 (+0.68)	14.41 $\pm$ 0.86 (-1.57)	-0.35	2.51 $\pm$ 0.18 (+0.40)	2.46 $\pm$ 0.15 (-1.60)	-0.05
	Average	56.04 $\pm$ 0.25	55.82 $\pm$ 0.023		14.68 $\pm$ 0.049	14.53 $\pm$ 0.094		2.50 $\pm$ 0.007	2.48 $\pm$ 0.017	
		(r = -0.717, P>0.05) (t=1.784, P>0.05)			(r = -0.868, P<0.05) (t=3.24, P<0.05)			(r = -0.671, P>0.05) (t=2.717, P>0.05)		

(Figures in parenthesis represent percent change in comparison to control)

**Table 2: Growth of *Channa gachua* (n = 30) exposed to long term exposure of 253.25mg l<sup>-1</sup> of Biofertilizer**

S. No.	Treatment (days)	Weight (g)			Length (cm)			Width (cm)		
		Control	Treated	Difference	Control	Treated	Difference	Control	Treated	Difference
1	0	55.86 $\pm$ 1.26			14.64 $\pm$ 0.99			2.50 $\pm$ 0.21		
2	15	58.28 $\pm$ 1.11 (+4.33)	56.82 $\pm$ 1.62 (1.72)	1.46	15.24 $\pm$ 0.96 (+4.10)	14.68 $\pm$ 0.77 (0.27)	0.56	2.60 $\pm$ 0.14 (+4.00)	2.54 $\pm$ 0.42 (1.60)	0.06
3	30	62.48 $\pm$ 1.21 (+11.85)	59.98 $\pm$ 1.27 (7.37)	2.50	15.89 $\pm$ 0.64 (+8.64)	14.92 $\pm$ 1.24 (1.91)	0.97	2.65 $\pm$ 0.24 (+6.00)	2.63 $\pm$ 0.62 (5.20)	0.02
4	45	68.83 $\pm$ 1.49 (+23.22)	64.62 $\pm$ 1.24 (15.68)	4.21	16.83 $\pm$ 1.49 (+14.95)	15.98 $\pm$ 0.76 (9.15)	0.85	2.73 $\pm$ 0.23 (+9.20)	2.70 $\pm$ 0.24 (8.00)	0.03
5	60	72.85 $\pm$ 1.81 (+26.00)	66.89 $\pm$ 1.86 (19.75)	5.96	17.29 $\pm$ 0.94 (+18.10)	16.45 $\pm$ 0.89 (12.36)	0.84	2.79 $\pm$ 0.36 (+11.60)	2.73 $\pm$ 0.31 (9.20)	0.06
6	75	75.47 $\pm$ 1.88 (+35.10)	69.67 $\pm$ 1.26 (24.72)	5.80	17.63 $\pm$ 1.75 (+20.42)	16.97 $\pm$ 0.92 (15.92)	0.66	2.85 $\pm$ 0.28 (+14.00)	2.81 $\pm$ 0.29 (12.40)	0.04
	Average	67.58 $\pm$ 6.39	63.60 $\pm$ 4.64		16.58 $\pm$ 0.89	15.80 $\pm$ 0.88		2.72 $\pm$ 0.13	2.68 $\pm$ 0.09	
		(r = 0.998, P<0.001) (t=31.18, P<0.001)			(r = 0.964, P<0.001) (t=7.41, P<0.001)			(r = 0.986, P<0.001) (t=11.74, P<0.001)		

(Figures in parenthesis represent percent change in comparison to control).

**Table 3: Statistical relationship between growth parameters and duration of Biofertilizer exposure on *Channa gachua***

Short term Exposure of Biofertilizer					Long term Exposure of Biofertilizer				
Variable	Experimental Condition	Regression Equation	Correlation Coefficient	t value (df=3)	Variable	Experimental Condition	Regression Equation	Correlation Coefficient	t value (df=4)
Weight	Control	y=0.005x+5.76	0.909 (P<0.05)	3.787 (P<0.05)	Weight	Control	y=0.282x+55.04	0.991 (P<0.001)	14.15 (P<0.001)
	Treated	y=-0.000x+55.84	0.901 (P<0.05)	3.590 (P<0.05)		Treated	y=0.197x+54.88	0.989 (P<0.001)	14.13 (P<0.001)
Length	Control	y=0.001x+14.62	0.899 (P<0.05)	3.458 (P<0.05)	Length	Control	y=0.042x+14.67	0.991 (P<0.001)	14.16 (P<0.001)
	Treated	y=-0.002x+14.64	0.996 (P<0.001)	24.90 (P<0.001)		Treated	y=0.034x+14.32	0.965 (P<0.01)	6.89 (P<0.01)
Width	Control	y=4E-05x+2.498	0.225 (P>0.05)	0.400 (P>0.05)	Width	Control	y=0.004x+2.515	0.996 (P<0.001)	22.63 (P<0.001)
	Treated	y=-0.000x+2.4	0.700 (P>0.05)	1.707 (P>0.05)		Treated	y=0.004x+2.495	0.993 (P<0.001)	16.55 (P<0.001)

Alterations in growth pattern relationships and condition factors of *Channa gachua* (Hamilton) exposed to a common biofertilizer

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Length - weight	Control	$y=49.54x-16.69$	0.987 ( $P<0.001$ )	10.81 ( $P<0.001$ )	Length - weight	Control	$y=6.686x-43.04$	0.995 ( $P<0.001$ )	31.78 ( $P<0.001$ )
	Treated	$y=.0211x+52.74$	0.868 ( $P>0.05$ )	3.036 ( $P>0.05$ )		Treated	$y=5.560x-24.47$	0.988 ( $P<0.001$ )	14.09 ( $P<0.001$ )
Width-weight	Control	$y=15.5x+17.29$	0.444 ( $P>0.05$ )	0.858 ( $P>0.05$ )	Width-weight	Control	$y=61.11x-98.95$	0.986 ( $P<0.001$ )	14.09 ( $P<0.001$ )
	Treated	$y=-9.5x-79.60$	0.608 ( $P>0.05$ )	2.04 ( $P>0.05$ )		Treated	$y=47.13x-62.68$	0.989 ( $P<0.001$ )	14.13 ( $P<0.001$ )
Condition Factor	Control	$y=-0.000x+1.78$	0.571 ( $P>0.05$ )	1.215 ( $P>0.05$ )	Condition Factor	Control	$y=-0.005x+1.741$	0.974 ( $P<0.001$ )	8.854 ( $P<0.001$ )
	Treated	$y=0.000x+1.778$	0.979 ( $P<0.01$ )	8.16 ( $P<0.01$ )		Treated	$y=-0.005x+1.853$	0.924 ( $P<0.01$ )	4.86 ( $P<0.01$ )

Correlation coefficients above 05% clarify the relationship between width and duration. In long-term treatment, highly significant ( $P<0.001$ ) changes were inferred from the 't' values (Table 3).

Under controlled conditions, the weight, length and width gradually increased in short-term and long-term treatment fish but the increase was less in biofertilizer-exposed fish. While working with *Channa punctatus*, Nath et al. (2018) observed a similar trend of growth in mustard oil cake intoxicated specimens. A dose-dependent growth after 56 days of feeding of mustard oil cake added diets compared to controlled conditions in *Labeo rohita* was observed by Latif et al. (2008). Reduction in growth exposed to sublethal concentration of biofertilizer was probably due to suppression of food intake posed by the biofertilizer along with its erucic acid anti-nutritional factors (Slominiski et al. 1999). Erucic acid was found to be harmful to rats in high doses but had no harmful effect on human beings (Manchanda and Passi 2016). It has been also reported that most of the

physiological aspects of *Channa gachua* and *Channa punctatus* are similar.

The length-weight relationship of *Channa gachua* under controlled conditions was  $y=49.54x-16.69$  and  $y=6.686x-43.04$ . While the observed length-weight relationship from short-term treatment was  $y=.0211x+52.74$  and long-term treatment was  $y=5.560x-24.47$ . The values of  $R^2$  ranged from 0.753 to 0.990 indicating that 75% to 99% of variants can be explained by the model. While correlation coefficients higher than 80% inform the strong relationship between length and weight. The 't' values inferred significant ( $P<0.05$ ) changes except under short-term treatment (Table 3).

Under controlled conditions, the width-weight relationship of *Channa gachua* was  $y=15.5x+17.29$  and  $y=61.11x-98.95$ . While during short-term treatment, the observed breadth-weight relationship was  $y=-9.5x-79.60$  and in long-term treatment was  $y=47.13x-62.68$ . The  $R^2$  values ranged from 0.197 to 0.978, suggesting that the model would describe 20% to 98% of the version. The close relationship between width and

weight is told by correlation coefficients above 80%. Significant ( $P < 0.05$ ) improvements were inferred from the 't' values in long-term treatment (Table 3).

The length-weight and width-weight relationships help in assessing the variations from the expected weight for the known length and breadth groups. These relationships provide information about the growth, health, habitat conditions, calculation of condition factors etc. (Schneider et al. 2000, Froese 2006). Length-weight relationships may be useful as a character to differentiate "small taxonomic units". In practice, fish with thin elongated bodies have 'b' values  $>3$  while fish having thicker bodies  $<3$ . A value of b smaller, equal and larger than 3 indicates negative allometry, isometry and positive allometry respectively. When  $b < 3$ , either the large specimens have changed body shape to become more elongated (Wootton 1992).

The slope values of 0.061 to 1.652 for the length-weight relationship and 0.378 to 0.658 for the width-weight relationship indicate negative allometric growth in all the experimental and controlled conditions. In a growth pattern study, Datta et al. (2013) calculated 'b' varied from 2.7675 to 4.3922 in *Channa punctatus* under 7.84 to 26.92% mustard oil cake added to the diet and inferred negative to positive allometric growth. Negative allometric growth patterns have also been reported in *Channa maurilius* (Rathod et al. 2011) and *Channa striatus* (Khan et al. 2011). Variation in slope may be attributed to sample size variation, life stages, type of feed and environmental factors (Kleanthidis et al. 1999). The poor growth responses of *Channa gachua* fed a diet containing mustard oilcake were presumably due to the presence of glucosinolate in the mustard seed, which after hydrolysis by thioglucosidase release

isothiocyanates. Moreover, thioglucosidase may also act on glucosinolate to produce intestinal irritants (Gohi 1981).

The condition factor (Kn) measures the deviation of a fish from the average weight to assess the suitability of the environment for its growth (Mensah 2015). Kn reflects biological circumstances and fluctuations by interaction among feeding conditions, parasitic infections and physiological factors. This also indicates the changes in food reserves and therefore an indicator of the general fish condition. Overall fatness for fish species is assumed when Kn values are equal or close to 1. Kn value  $<1$  indicates a better condition of fish (Le Cren 1951) and a value  $>1$  indicates a stressed condition of fish. Information on condition factors can be vital to culture system management because they provide the producer with information on the specific condition under which living organisms are developing (Araneda et al. 2008).

The condition factor of *Channa gachua* was  $y = -0.000x + 1.78$  and  $y = -0.005x + 1.741$  under controlled conditions (Fig. 1g). Although, the condition factor observed for short-term treatment was  $y = 0.000x + 1.778$  and long-term treatment was  $y = -0.005x + 1.853$  (Fig. 1h). The values of  $R^2$  ranged from 0.326 to 0.958 indicating that 33% to 96% of the version can be explained by the model. Correlation coefficients above 5% clarify the relationship of the condition factor. Significant ( $p < 0.05$ ) changes were inferred from the 't' values except for the controlled condition of short-term experiments (Table 3).

In this experiment, Kn was found in the range of 1.54-1.78 during controlled conditions while 1.64-1.82 during the biofertilizer added to the diet. Datta et al. (2013) also calculated condition factors varied from 1.094 to 1.334 in

*Channa punctatus* under 10.85 to 9.38% mustard oil cake added to the diet. Conversely, while working on *Channa gachua*, Sarma et al. (2016) calculated the Kn value of 0.52-0.94 at Garjan Beel of Assam. It seems that the more values of condition factor in biofertilizer treated condition indicate a selective better condition of fish than the controlled conditions.

### Conclusions:

The key factors that influence the growth of fish are the quantity of food available, the number of fish utilizing the same food source and other water quality factors besides the size, age and sexual maturity of the fish. In this study, growth pattern, condition factor and coefficient of determination value were recorded in *Channa gachua* under controlled and biofertilizer-treated conditions. The less growth pattern exhibited by the fish during biofertilizer-treated conditions was within the tolerance range for the species and nutrient composition of the cake. The findings of the present study support that the species may be cultured on a large scale as food fish to meet the nutritional demand.

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