



Efficiency of Black Soldier Fly Larvae Meal (BSFLM) as Partial or Total Replacement of Fishmeal (FM) on Growth Performance, Nutrient Utilization and Haematological Changes in Walking Catfish *Clarias magur* (Hamilton, 1822)

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ABSTRACT

Background: *Clarias magur* is an airbreathing walking cat fish native to South Asia with high market value. Successful culture of this species depends on proper feed supplementation and utilization. Therefore, an experiment was conducted to study the effect of black soldier fly larvae meal (BSFLM) incorporated diet on growth performances, nutrient utilization and haematological changes by replacing fish meal (FM) in *Clarias magur*.

Methods: The experimental fishes were reared in controlled condition for 120 days and fed with 6 different isonitrogenous sinking experimental diets having 35% crude protein. The main ingredient in experimental diet was BSFLM, which was incorporated as an alternative source to FM along with soyabean meal (SM), mustard oil cake (MOC) and wheat bran (WB) with varying percentage. Healthy *Clarias magur* fingerlings were (average individual weight of 2 g) stocked in experimental tanks at a stocking density of 4 fish per m² area.

Result: The highest final mean weight of *Clarias magur* was found to be 52.67 g (T2) followed by 42.59 g (T1) which was significantly different ($P < 0.05$) when compared within the treatments. Final biomass (755.13 ± 27.6 g) was found to be significantly high in T2 ($P < 0.05$) when compared to other treatments. The lowest value of feed conversion ratio (2.10 ± 0.03) was observed for treatment T2 followed by T1 (2.18 ± 0.02) and protein efficiency ratio was highest for treatment T2 (1.41 ± 0.02) followed by T3 (1.40 ± 0.04) with significant variation ($P < 0.05$) among treatments. Haematological indices also showed significant differences ($P < 0.05$) among treatments, T2 recorded the highest RBC (2.10 million/ m^3) followed by T3 (1.92 million/ m^3). On the contrary, WBC level was found highest in Treatment T5 (49.64 million/ m^3) followed by T4 (48.17 million/ m^3). Thus, from the above findings, it can be stated that 50% replacement of fish meal with BSFLM enhanced growth performance and health status of *Clarias magur*. Thus, BSFL may be considered as a viable source of animal protein for *Clarias magur* in the aquafeed business.

Key words: Black soldier fly larvae, *Clarias magur*, Fish meal, Growth, Haematology.

INTRODUCTION

Intensification and adoption of feed-based aquaculture practices is developing quickly in North-Eastern region of India where 95% of the population are fish eater (Singh *et al.*, 2017). FM is a major component in aquafeed due to its highly digestible protein as well as good palatability. However, an increasing demand and unstable production of FM has led to an increasing cost of aquaculture production. Therefore, there is a practical interest for partial or total replacement of FM with less expensive and protein-rich animal or plant ingredients has become a focus of research (Ashour *et al.*, 2021; Sharawi, 2024). Among the insect species used as protein source in aquaculture, Black Soldier Fly (*Hermetia illucens* L. 1758) is one of the most interesting source for its sustainability related to its capacity to convert organic waste material into biomass containing proteins (Caligiani *et al.*, 2018). In terms of protein quality, BSF larvae contain a favourable essential amino acid profile closer to fish meal than that of soybean meal (Anankware *et al.*, 2018) and has been suggested as a promising alternative for the fast-growing feed-based aquaculture sector.

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Several studies have shown that feeds with FM partially replaced by BSFLM as well as feeds without replacement in terms of growth of several fish species, e.g. *Oreochromis aureus* (Bondari and Sheppard, 1981) and *Scophthalmus maximus* (Kroeckel *et al.*, 2012). Additionally, Maranga *et al.* (2023) and Abd Hamid *et al.* (2022) reported that BSFLM can replace FM without impairing and affecting performance in growth and survival of *Clarias gariepinus*. It is also cited that BSFLM is a good ingredient for broiler in their early stage on both nutrient digestibility and growth (Alqazzaz *et al.*, 2019).

Clarias magur requires comparatively higher level of dietary animal protein for rapid growth (Mishra and Mukhopadhyay, 1996). Although several studies have been conducted on *Clarias magur* on efficiency of non-conventional animal protein like vermi meal and chicken viscera meal (Hussain *et al.*, 2021), a very few information is available regarding the use of sustainable animal protein sources in practical diet formulation for *Clarias magur* on growth performance and health status. Therefore, the present study was designed to determine the suitable proportion of dried BSFLM on growth performance and haematological parameters in *Clarias magur* as a substitute to FM.

MATERIALS AND METHODS

Study area and duration

The experiment was conducted at Fisheries Research Centre (Coordinates) lies at latitude 26.72°S and longitude 94.20°E and College of Fisheries (Coordinates) lies at latitude 26.22°S and 92.50°E, Assam Agricultural University, Jorhat, Assam, India from 31st July 2023 to 28th November 2023.

Animal and experimental design

Healthy and disease free *Clarias magur* (average wt. 2.13±0.04 g) stocked in experimental tanks at a stocking density of 4 fish per m². Fishes were acclimatized prior the experiment for two weeks in cement cisterns. The water quality parameters were in normal ranges i.e. pH (7.73-8.23), dissolved oxygen (5.3-7.1 mg/l), alkalinity (>70 mg/l), ammonia (0.23-0.42 mg/l) and nitrite (0.007-0.031 mg/l) during the entire experiment (APHA, 2023).

Experimental diets

The six-test supplementary iso-nitrogenous sinking feeds containing 35% crude protein level with a variation of composition level (Table 1). The main ingredient in feed is BSFLM, which is an alternate ingredient source to FM along with soyabean meal (SM), mustard oil cake (MOC) and wheat bran (WB) with different ingredient composition.

The mixed ingredients were subjected to pre-conditioning and pelletized in a mechanical pelletizer and were dried to a moisture level of 6-8% in an oven and the dried diets were stored in air tight polythene until used. Proximate analysis of all the dietary ingredients (Table 2) and experimental diets (Table 3) were conducted (AOAC, 2023).

Feeding and sampling of the experimental fish

Feeding frequency was twice in 24 hrs i.e. during forenoon and evening. The feeding rate varied between 1- 4% of

Table 1: Ingredient composition (% dry weight) of the experimental diets.

Ingredient	T1	T2	T3	T4	T5	C
BSFLM	35.0	17.5	0	0	17.5	0
FM	0	17.5	35.0	17.5	0	0
SM	30.0	30.0	30.0	37.0	37.0	50.0
MOC	08.0	10.0	15.0	35.0	35.0	45.0
WB	22.0	20.0	15.0	5.5	5.5	0
Vitamin	1.0	1.0	1.0	1.0	1.0	1.0
Oil	4.00	4.00	4.00	4.00	4.00	4.0

Table 2: Proximate composition (% dry weight) of experimental fish feed ingredients.

Ingredients	Moisture	Ash	Lipid	Protein	Fiber	NFE
BSFLM	71.15±1.03 ^c	15.39±0.70 ^c	5.43±0.66 ^b	69.96±1.02 ^d	02.13±0.43 ^a	07.10±2.25 ^a
FM	11.92±0.70 ^b	31.99±1.43 ^d	8.21±1.20 ^c	44.23±1.70 ^c	03.15±1.00 ^a	12.42±5.27 ^a
SM	08.75±0.77 ^a	06.65±0.46 ^a	2.18±0.18 ^a	44.40±1.92 ^c	15.51±0.83 ^b	31.27±1.92 ^b
MOC	12.13±0.82 ^b	10.11±1.09 ^b	8.29±0.72 ^c	31.41±1.91 ^b	12.95±1.09 ^b	37.24±4.40 ^b
WB	13.26±0.68 ^b	04.91±0.85 ^a	5.57±0.62 ^b	13.55±0.96 ^a	03.80±0.67 ^a	72.18±2.98 ^c

*Above values are expressed as mean±SE. Different superscripts indicate significant difference (P<0.05) in a column.

Table 3: Proximate composition (% dry weight) of different experimental diet.

Treatment	Moisture	Ash	Lipid	Protein	Fiber	NFE
C	4.68±0.07	13.70±0.12 ^d	5.47±0.03 ^{ab}	35.27±0.14 ^a	9.27±0.26 ^a	36.29±0.30 ^a
T1	4.51±0.03	7.45±0.03 ^b	5.84±0.06 ^c	35.04±0.14 ^b	13.74±0.05 ^b	37.94±0.16 ^b
T2	4.67±0.07	6.33±0.17 ^a	6.43±0.07 ^d	35.14±0.08 ^b	14.27±0.12 ^c	37.83±0.34 ^b
T3	4.58±0.11	7.93±0.09 ^c	5.73±0.12 ^{bc}	35.04±0.03 ^b	13.27±0.15 ^b	38.03±0.23 ^b
T4	4.63±0.09	7.40±0.15 ^b	5.30±0.12 ^a	35.33±0.27 ^{ab}	13.57±0.07 ^b	38.40±0.12 ^b
T5	4.47±0.09	7.37±0.18 ^b	5.30±0.12 ^a	35.47±0.15 ^{ab}	13.37±0.18 ^b	38.50±0.32 ^b

*Above values are expressed as mean±SE. Different superscripts indicate significant difference (P<0.05) in a column.

body weight of the total biomass according to the acceptance of the feed. The experimental tank was arranged in completely randomized design (CRD). The growth performance was assessed fortnightly.

Growth and nutrient utilization

Growth performance of the fish was monitored by the following parameters like

Specific growth rate (SGR)=

$$\frac{\ln(\text{Final weight}) - \ln(\text{Initial weight})}{\text{Culture period (days)}} \times 100$$

Net fish yield (NFY)=

$$\frac{\text{Total fish biomass at harvest} - \text{Total fish biomass at stock}}{\text{Area} \times \text{culture period}}$$

Daily weight gain (DWG)=

$$\frac{\text{Total final weight} - \text{Total initial weight}}{\text{Culture period}}$$

The feed utilization performance of the fish evaluated by the following parameters like

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Amount of dry feed intake}}{\text{Fresh weight gain in fish}}$$

Protein conversion efficiency (PCE) =

$$\frac{\text{Protein gain}}{\text{Protein consumed}} \times 100$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Fresh weight gain in fish}}{\text{Amount of protein fed}}$$

Proximate analyses of experimental fish were also carried out after the experiment (AOAC, 2023). Haematological changes of the fish were evaluated by the following parameters like

$$\text{Total erythrocytes count per mm}^3 = \text{No of cells counted in } 0.02 \text{ mm}^3 \times 50 \text{ (area counted)} \times 200 \text{ (dilution)}$$

$$\text{Total leucocytes count per mm}^3 = \text{No of cells counted in } 0.01 \text{ mm}^3 \times 10 \text{ (area counted)} \times 50 \text{ (dilution)}$$

Hemoglobin concentration in blood was determined by Sahlis haemometer (Microsil, India) and Packed cell

volume (PCV) was measured following modified method of Schaperclaus (1991).

Statistical analysis

The data from the experiment was subjected to Shapiro-Wilk test for assessment of normality. All the dependent variables were normal except SGR. Different transformation of non-parametric data was done which failed the normality assumption again, therefore Kruskal-Wallis's test, followed by Dunn's test for comparison of means. One way analysis of variance (ANOVA) was performed for all the parametric variables and Duncan multiple range test was used for post hoc comparison. The significant level was set at 5% level ($P < 0.05$). All the statistical test were performed in IBM SPSS Software 26.0.

RESULTS AND DISCUSSION

Growth, survival and nutrient utilization parameters

In general, experimental fish exhibited consistent increase in mean weights during the culture period (Table 4). The highest weight gain observed in T2 followed by T1 and the values were significantly different ($P < 0.05$). Increase in weights of *Clarias magur* become more evident with a weight of 50.47 ± 0.23 g (T2) followed by 40.46 ± 0.35 g (T1) and lowest in control (19.43 ± 0.20 g). Similarly, the Specific Growth Rate (% day⁻¹) of *Clarias magur* are presented in Table 4. The values were significantly different ($P < 0.05$) with highest SGR was found in T2 (0.42 ± 0.002) followed by T1 (0.34 ± 0.003) and lowest in control (0.16 ± 0.002).

In the present study, there were no significant differences ($P > 0.05$) in the survival (%) among the treatments. Overall survival of the fish varied from 88% (T5) to 96% (T1) in different treatments (Table 4).

The apparent FCR showed significant variation ($P < 0.05$) between different treatments (Table 4). However, the lowest value of FCR (2.10 ± 0.03) was noticed for T2 followed by T3 (2.16 ± 0.06) and the FCR (2.62 ± 0.06) was the highest for control (C). PCE also showed significant variation ($P < 0.05$) among treatments. The highest PCE (91.71 ± 0.92) was obtained in treatment T2 followed by that for T3 (90.31 ± 2.59) and the lowest for control (74.94 ± 1.72). Similarly, the apparent PER was the highest for Treatment T2 (1.41 ± 0.02) followed by T3 (1.40 ± 0.04), while the lowest value of apparent PER (1.20 ± 0.02) was obtained for control ($P < 0.05$).

Table 4: Growth and nutrient utilization $\pm$ SE (n=3) of *Clarias magur* in different treatments.

Treatment	Weight gain (g)	Survivability %	SGR (rate/day)	FCR	PCE	PER
C	19.43 ± 0.20^a	90 ± 2.08	0.16 ± 0.002^b	2.62 ± 0.06^b	74.94 ± 1.72^a	1.20 ± 0.02^a
T1	40.46 ± 0.35^e	96 ± 2.08	0.34 ± 0.003^{ab}	2.18 ± 0.02^a	88.86 ± 1.02^b	1.38 ± 0.01^b
T2	50.47 ± 0.23^f	94 ± 3.61	0.42 ± 0.002^a	2.10 ± 0.03^a	91.71 ± 0.92^b	1.41 ± 0.02^b
T3	35.40 ± 0.29^d	92 ± 2.08	0.30 ± 0.002^{ab}	2.16 ± 0.06^a	90.31 ± 2.59^b	1.40 ± 0.04^b
T4	31.50 ± 0.21^c	90 ± 5.51	0.26 ± 0.003^{ab}	2.24 ± 0.06^a	85.67 ± 2.49^b	1.35 ± 0.03^b
T5	26.40 ± 0.21^b	88 ± 3.61	0.22 ± 0.002^{ab}	2.22 ± 0.05^a	87.75 ± 1.39^b	1.38 ± 0.02^b

*Above values are expressed as mean $\pm$ SE. Different superscripts indicate significant difference ($P < 0.05$) in a column.

Yield parameters

There were significant differences ($P < 0.05$) in the final biomass, daily weight gain and NFY (Table 5). Notably the highest net gain in biomass (755.13 ± 27.60 g) was observed in T2 as compared to other treatments and lowest was found in control (275.10 ± 5.59 g).

Proximate composition of fish

The crude protein, crude lipid and NFE content showed significant variations ($P < 0.05$) among different treatments (Table 6) whereas crude ash and moisture content did not show significant variation ($P > 0.05$). The highest and lowest crude ash content was found in control (8.73 ± 0.19) and T2 (8.03 ± 0.24) respectively. The highest protein content was found in T2 ($64.83 \pm 0.32\%$) followed by T1 ($64.54 \pm 0.28\%$) and lowest in control ($62.57 \pm 0.47\%$). Similarly, lipid content was highest in T4 ($21.10 \pm 0.38\%$) followed by T3 ($20.95 \pm 0.27\%$) and lowest in control ($19.00 \pm 0.25\%$).

Haematological parameters

Haematological (RBC, WBC, Hb and PCV) parameters showed significant differences ($P < 0.05$) among the

treatments (Table 7). Fish in the T2, recorded the highest RBC (2.10 ± 0.01 million/ m^3) followed by T3 (1.92 ± 0.02 million/ m^3) whereas, the lowest RBC count was found in control (1.35 ± 0.02 million/ m^3). Similarly, haemoglobin content was highest in T2 (8.20 ± 0.05 g/dl) followed by T1 (7.89 ± 0.02 g/dl) and PCV was also highest in T2 ($24.63 \pm 0.05\%$) followed by T1 ($23.36 \pm 0.01\%$). On the contrary, WBC level was found highest in treatment T5 (49.64 ± 0.71 million/ m^3) followed by T4 (48.17 ± 0.16 million/ m^3) and lowest (33.36 ± 0.01 million/ m^3) in control ($P < 0.05$).

In the present study, *Clarias magur* juveniles fed with 50% BSFLM (T2) exhibited highest growth performance and feed/nutrient utilization. Similar results were found by Fawole *et al.* (2020), where feeding African catfish, *Clarias gariepinus* with 50% partially defatted BSFLM increased the growth performance. The results were in accordance with a study on Atlantic salmon (*Salmo salar*) showed a good growth response when fed with BSFLM (Belghit *et al.*, 2018) and also in loach (*Misgurnus anguillicaudatus*) where superior growth performance achieved while BSFLM incorporation level was 50% (Nguyen and Tran, 2025). Similarly, 50% replacement of FM with BSFLM in the diet of tilapia (*Oreochromis niloticus*) also showed positive effects on growth and feed utilization parameters (Muin *et al.*, 2017).

The knowledge of the body composition of fish and factors affecting, a tool to know about fish health, determination of efficiency of transfer of nutrients from the food to the fish made it possible to predictably modify carcass composition (Al-Ogaily *et al.*, 1996). In the present study crude protein, crude lipid and NFE showed significant variations ($P < 0.05$) among different treatments and superior proximate composition (higher crude protein and lower crude lipid) was observed in T2. The findings of the present study were in contrast to previous studies in Jian carp (Zhou *et al.*, 2018) and Atlantic salmon (Belghit *et al.*,

Table 5: The relevant yield parameters $\pm$ SE (n=3) for different treatments.

Treatment	Net gain biomass (g)	Daily weight gain (g d ⁻¹)	Net fish yield (g m ⁻² d ⁻¹)
C	275.10 $\pm$ 5.59 ^a	2.29 $\pm$ 0.05 ^a	0.57 $\pm$ 0.01 ^a
T1	618.95 $\pm$ 12.78 ^e	5.16 $\pm$ 0.11 ^e	1.29 $\pm$ 0.03 ^e
T2	755.13 $\pm$ 27.60 ^f	6.29 $\pm$ 0.23 ^f	1.57 $\pm$ 0.06 ^f
T3	516.23 $\pm$ 7.98 ^d	4.30 $\pm$ 0.07 ^d	1.08 $\pm$ 0.02 ^d
T4	448.00 $\pm$ 6.13 ^c	3.73 $\pm$ 0.05 ^c	0.93 $\pm$ 0.01 ^c
T5	374.73 $\pm$ 9.82 ^b	3.12 $\pm$ 0.08 ^b	0.78 $\pm$ 0.02 ^b

*Above values are expressed as mean $\pm$ SE. Different superscripts indicate significant difference ($P < 0.05$) in a column.

Table 6: Proximate composition (% dry weight) of fish (*Clarias magur*) in different treatments.

Treatment	Moisture	Ash	Lipid	Protein	NFE
C	75.34 $\pm$ 0.28 ^b	8.73 $\pm$ 0.19	19.00 $\pm$ 0.25 ^a	62.57 $\pm$ 0.47 ^a	9.70 $\pm$ 0.50 ^b
T1	74.53 $\pm$ 0.24 ^a	8.37 $\pm$ 0.24	20.50 $\pm$ 0.29 ^b	64.54 $\pm$ 0.28 ^b	6.59 $\pm$ 0.38 ^a
T2	74.73 $\pm$ 0.20 ^{ab}	8.03 $\pm$ 0.24	20.43 $\pm$ 0.35 ^b	64.83 $\pm$ 0.32 ^b	6.70 $\pm$ 0.40 ^a
T3	75.00 $\pm$ 0.25 ^{ab}	8.37 $\pm$ 0.24	20.95 $\pm$ 0.27 ^b	64.50 $\pm$ 0.29 ^b	6.19 $\pm$ 0.41 ^a
T4	75.17 $\pm$ 0.17 ^{ab}	8.10 $\pm$ 0.23	21.10 $\pm$ 0.38 ^b	63.67 $\pm$ 0.44 ^{ab}	7.13 $\pm$ 0.58 ^a
T5	75.07 $\pm$ 0.23 ^{ab}	8.10 $\pm$ 0.26	20.90 $\pm$ 0.42 ^b	63.60 $\pm$ 0.38 ^{ab}	7.40 $\pm$ 0.40 ^a

*Above values are expressed as mean $\pm$ SE. Different superscripts indicate significant difference ($P < 0.05$) in a column.

Table 7: The haematological parameters $\pm$ SE (n=3) for different treatment.

Treatment	RBC (Million/ m^3)	WBC (Million/ m^3)	Hemoglobin (g/dl)	PCV (%)
C	1.35 $\pm$ 0.02 ^a	33.36 $\pm$ 0.58 ^a	6.74 $\pm$ 0.01 ^a	17.97 $\pm$ 0.01 ^a
T1	1.86 $\pm$ 0.01 ^b	41.53 $\pm$ 0.51 ^b	7.89 $\pm$ 0.02 ^d	23.36 $\pm$ 0.01 ^d
T2	2.10 $\pm$ 0.01 ^d	43.48 $\pm$ 0.35 ^c	8.20 $\pm$ 0.05 ^e	24.63 $\pm$ 0.05 ^e
T3	1.92 $\pm$ 0.02 ^c	45.73 $\pm$ 0.26 ^d	7.32 $\pm$ 0.01 ^c	19.36 $\pm$ 0.01 ^c
T4	1.91 $\pm$ 0.01 ^{bc}	48.17 $\pm$ 0.16 ^e	7.23 $\pm$ 0.01 ^c	18.93 $\pm$ 0.02 ^b
T5	1.86 $\pm$ 0.01 ^b	49.64 $\pm$ 0.71 ^e	6.89 $\pm$ 0.01 ^b	18.05 $\pm$ 0.01 ^a

*Above values are expressed as mean $\pm$ SE. Different superscripts indicate significant difference ($P < 0.05$) in a column.

2019) where BSFLM incorporated diet showed no significant impact on flesh quality. This phenomenon may be due to the fact that *Clarias magur* is a carnivore fish and thus, is probably more capable of digesting insect larvae which contains chitin than the other fish species mentioned above.

Haematological indices are essential for evaluating formulated feed efficacy, physiological stress and fish health and growth performance (Dawood *et al.*, 2020). The Haemoglobin, RBC and PCV is a function of oxygen supply, absorption and transportation within a living cell and depletion in the count may cause anaemia and lead to death in fish. In the present study, highest RBC, Hb and PCV values were noticed in T2 group of fish as compared to the other treatments ($P < 0.05$) and the values were within the normal ranges recommended for *Clarias gariepinus* (Adedeji, 2009), a congeneric species of *Clarias magur*. This finding is supported by a study where dietary supplement of BSFLM (even at 100%) did not have deleterious effect on health status of *Clarias gariepinus* (Ayodeji *et al.*, 2020).

WBC count increases due to metabolic stress (Misra *et al.*, 2006). The WBC count obtained in this study have not been associated with any detrimental health effect in Magur. This explained the efficacy of BSFLM as a protein source in maintaining good and healthy condition in Magur. In this study, comparatively lower WBC values were noticed in T2 group of fish as compared to the other treatments which may be due to lower metabolic stress in T2 group of fishes. However, future research is required to study the inclusion level of BSFLM on metabolic stress in *Clarias magur*.

CONCLUSION

Our study demonstrated that *Clarias magur* can be fed pelleted feed containing BSFLM up to 50% combined with 50% FM which has the higher growth performance, nutrient utilization and overall health status of Magur as compared to other inclusion level. Thus, it suggests that BSFL could be considered a promising protein source for *Clarias magur* in aquafeed industry contributing to low-cost and healthy fish feed production. However, further research efforts are necessary to find out the Chitin composition of BSFL and its effect on metabolic and physiological activity in *Clarias magur*.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for Animal care and handling techniques were approved by the Institutional Ethics Committee of College of Fisheries, Assam Agricultural University, Raha, Nagaon, India. The animals used in this study were not endangered or protected species. The study was conducted with compliance to the provisions of the guidelines of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests, Government of India, for care and use of animals for scientific experiments.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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