Research Article

Growth, Survival and Body Protein Content of Swordtail (*Xiphophorus helleri*) Fed Live and Formulated Feeds

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Abstract | Swordtail (*Xiphophorus helleri*), a commercially important live-bearing ornamental fish, is widely used in aquariums. It is omnivorous in nature and feeds on both live and formulated feeds. There is no study assessing the effect of formulated egg custard, live and commercial feeds on the growth performance and survival of swordtail. Therefore, the study was carried out to investigate the effect of a live feed (*Tubifex*), formulated egg custard, and two commercial feeds (one aquarium feed and one fish feed) on survival, growth, and body protein content of swordtail in triplicates. Swordtail juveniles (initial weight: 0.46±0.03 g and length: 3.28±0.30 cm) were randomly stocked in glass aquaria feeding *ad libitum* two times a day. After four weeks of feeding trial, survival of swordtail juveniles was high (≥ 90%) in all treatments and remained unaffected by the dietary treatments ($P > 0.05$). Differences in growth performance were realized in terms of final weight (g), final length (cm), weight gain (g, %), SGR (%), and metabolic growth rate (g $kg^{-0.8}$ d^{-1}) ($P < 0.05$), while swordtail juveniles fed *Tubifex* and custard showed similar growth performances ($P > 0.05$). There was a tendency towards a marginally significant difference in daily growth (g d^{-1}, $P = 0.077$) with the highest growth in juveniles fed *Tubifex* and custard. Body protein content was remained unaffected by the dietary treatments ($P > 0.05$) but a trend in increasing body protein content with increasing feed protein was observed. Formulated egg custard used in this study could be a potential feed for swordtail juveniles in terms of availability, cost, ecological and health hazard issues.

Keywords | Egg custard, *Tubifex*, Commercial feeds, Growth, Swordtail fish

Received | November 14, 2021; **Accepted** | December 11, 2021; **Published** | January 05, 2022
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Citation | Debnath S, Sarker DS, Kundu P, Parvez MS, Arafat ST, Mathew RT, Alkhamis YA, Rahman MM, Rahman SM (2022). Growth, survival and body protein content of swordtail (*Xiphophorus helleri*) fed live and formulated feeds. Adv. Anim. Vet. Sci. 10(2): 335-341.
DOI | http://dx.doi.org/10.17582/journal.aavs/2022/10.2.335.341
ISSN (Online) | 2307-8316

INTRODUCTION

Swordtail (*Xiphophorus helleri*) is a live-bearing ornamental fish species belonging to the family Poeciliidae. It is widely reared in the aquarium and is considered as one of the commercially important ornamental fish after goldfish due to its aesthetic values, food habit, and reproductive traits (Ghosh et al., 2008). The food habit of swordtail includes omnivorous in nature and feeds on both live and artificial feeds (James and Sampath, 2003). Feed and feed quality play a vital role as an exogenous factor in fish rearing because it affects both reproduction and growth in all stages. Different life stages of fish express different food habits therefore, successful aquaculture mostly depends on selecting the right choice of food for a particular stage. The rearing of an earlier stage of fish, for example, mostly

depends on live feeds (Bryant and Matty, 1980). These feeds are enriched in higher protein (60-65%), moderate lipid (8-9%), and contained comparatively lower crude fiber (4-5%) (Sharma, 2020).

Live feeds of aquaculture fish species mainly include *Artemia, Tubifex, Moina, Daphnia,* and *Chirononus* (Fermin and Recometa, 1988; Cruz and James, 1989; Evangelista et al., 2005). Several studies investigated the effect of different live feeds on the growth performance of swordtail juveniles such as *Artemia* (Mousavi-Sabet et al., 2013; Anjur, 2017), *Daphnia* (Kruger et al., 2001b), *Tubifex* (Anjur, 2017), and earthworm (Anjur, 2017). *Artemia* is a widely accepted live feed for fish larvae and juveniles but due to its availability and higher price fish breeders are looking forward to alternative options, especially in developing countries (Evangelista et al., 2005). *Tubifex* could be one of the potential alternative options as live feed commodity due to its availability and lower cost. From a nutritional point of view, *Tubifex* is highly potential as it contains high protein and essential fatty acids (Yanar et al., 2003; Görelşahin et al., 2018). It has already been established as an alternative to *Artemia* for different catfishes (Evangelista et al., 2005; Arslan et al., 2009). However, one of the major concerns of this live feed is health hazard issues including spreading protozoan diseases (Brinkhurst, 1996).

Beside live feeds, different formulated feeds are also used in larval rearing. Generally formulated feeds are prepared for particular species with adequate nutrition to achieve maximum growth in a short period (Mohideen et al., 2014). One of the potential formulated feeds used in larval rearing could be egg custard although associated cost and digestibility are a big concern for breeders. It is reported that fish larvae fed egg custard gained better growth than commercially available larval feed (Malla and Banik, 2015). Commercial formulated feeds mainly contain a large portion of fish meals. The use of fish meal in aquarium feed means the conversion of potential consumable proteins sources into non-consumable luxury items (Tlusty, 2002). Therefore, in terms of sustainability alternative options should be explored.

To our knowledge, there is no study comparing the effect of formulated egg custard, live feed (*Tubifex*) and commercial feeds on the growth performance and survival of swordtail. Therefore, this study was aimed to investigate the effect of a live feed (*Tubifex*), formulated egg custard, and two commercial feeds (one aquarium feed and one fish feed) on survival, growth, and body protein content of swordtail juveniles. It was hypothesized that swordtail juveniles fed different dietary treatments would have similar growth performance.

MATERIALS AND METHODS

Diet preparation

The experiment was conducted using a completely randomized design for four weeks with four different dietary treatments; one live feed (*Tubifex*), one laboratory formulated egg custard, and two commercial feeds; one fish feed (Quality Feeds Limited, Bangladesh) and one aquarium feed (Sky Fish, China). Live *Tubifex* and aquarium feed were collected from local aquarium shops. The custard was prepared in the laboratory according to the procedures developed by Nik-Sin and Shapawi (2017) and stored in a refrigerator (4 °C) until further use. The ingredients of custard are presented in Table 1 and the moisture and protein content of four dietary treatments are given in Table 2. Twelve glass aquaria (50 × 30 × 30 cm^3) were used in this experiment, and four dietary treatments were randomly assigned among aquaria which resulted in three replications for each treatment.

Table 1: Ingredients (g/kg) used for preparing egg custard.

Ingredients	Unit (g)
Cornflower	50
Agar powder	20
Milk powder	500
Egg	325
Cod liver oil	5
Prawn meat	100

Table 2: Moisture (%) and protein (%, dry weight basis) of four different dietary treatments.

Dietary treatments	Protein (%)	Moisture (%)
Tubifex	55.64±3.3	82.28±0.04
Custard	43.08±2.54	70.23±0.36
Fish feed	34.86±0.96	10.94±0.88
Aquarium feed	18.80±0.73	11.26±0.35

Experimental procedures

The experiment was conducted in the Fish Physiology Laboratory of Fisheries and Marine Resource Technology Discipline, Khulna University, Bangladesh. Swordtail (*X. helleri*) juveniles were collected from local breeders and transported to the laboratory in oxygenated polythene bags and acclimatized in aquarium environment for one week. Fish juveniles (mean initial weight of 0.46 ± 0.03 g and length of 3.28 ± 0.30 cm) were randomly stocked in each aquarium (containing 15 liters of water) at a stocking density of 10 juveniles/aquarium. Before stocking, fish juveniles were starved for 24 hours to empty their stomach. To ensure continuous oxygen supply each tank was adorned with an air stone connected to an air pump.

Fish were fed twice a day at 9.00 am and 3.00 pm at their satiation level. Live *Tubifex* and egg custard were chopped to make adjustable size so that fish juveniles could easily consume. Before each feeding uneaten feed and faeces were removed through siphoning. Key water quality parameters such as pH, dissolved oxygen (DO), and temperature were measured daily at 8.30-9.00 am. About 40-50% water was changed every day in the morning before feeding with previously stocked groundwater.

Growth parameters

After four weeks of feeding trial, fish were harvested, and growth performance parameters were calculated using the following equations. Weight gain (g) = Final weight (g) – Initial weight (g); Daily growth (g d^{-1}) = Weight gain (g)/duration of the experiment (days); Specific growth rate (SGR, % d^{-1}) = {Ln (final weight) – Ln (initial weight)}/ duration of the experiment × 100; Survival (%) = (Final fish number/Initial fish number) × 100. Metabolic growth rate = Daily growth/$(\sqrt{(\text{Final weight} \times \text{Initial weight})/1000})^{0.8}$.

Protein and moisture content analysis

At the end of the experiment, three fish from each tank were randomly sampled for determining body protein content. Protein content of fish and feed samples were analyzed according to the procedures depicted in Association of Official Analytical Chemists (AOAC, 2005). Crude protein content (N × 6.25) was measured by determining nitrogen (N) content using the Kjeldahl digestion and distillation process. Moisture content of feed was determined using an oven at 105 °C for 24 hours until achieving constant weight.

Statistical analysis

Statistical analysis was done using SPSS 23 software (NY, USA). Normality was checked using the Shapiro–Wilk test. Data were subjected to one-way ANOVA followed by Tukey multiple comparison test as post-hoc test. Significant was considered at $P < 0.05$. All data in the text are presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Water quality parameters

Water quality parameters for four weeks feeding trial are given in Table 3. There was no significant difference in water quality parameters among four different dietary treatments ($P > 0.1$). Water temperature varied from 26.96 to 27.81 °C. Water pH and DO were ranged from 8.35 to 8.59 and 6.69 to 7.81 mg l^{-1}, respectively in different dietary treatments.

Survival

Survival of swordtail juveniles was high (≥ 90%) in all treatments with the highest survival rate (97%) in juveniles fed *Tubifex* (Figure 1). The dietary treatments did not affect the survival rate of swordtail ($p > 0.05$) after a four-week feeding trial.

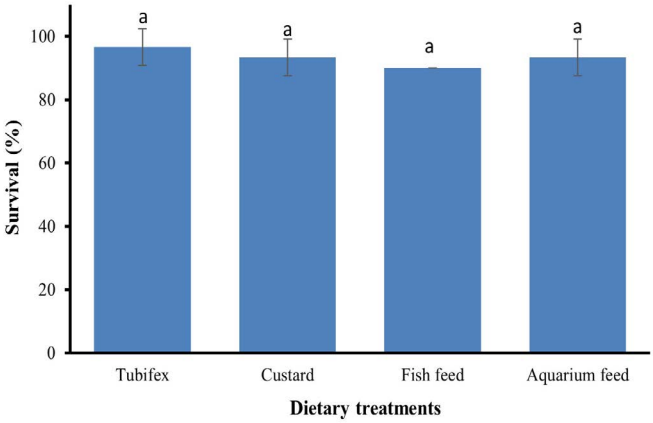

Figure 1: Survival of *X. helleri* fed different experimental diets over four weeks. The same superscripts in the bars indicate no significant differences ($P > 0.05$).

Growth performance

The growth performances of swordtail juveniles under the four dietary treatments are given in Table 4 and Figures 2, 3. There was no significant difference in initial body weight and length ($P > 0.05$), but after four weeks of the feeding trial, differences in growth performances were realized in terms of final weight (g), final total length (cm), weight gain (g, %), SGR (%) and metabolic growth rate (g $kg^{-0.8}$ d^{-1}). Weight gain (g, %) was the lowest in juveniles fed aquarium and fish feed, while juveniles fed *Tubifex* had the highest weight gain but not significantly higher than those fed custard ($P > 0.05$). Juveniles fed *Tubifex* had the highest SGR (3.05%) followed by custard, aquarium feed, and fish feed. A similar pattern was also noticed in metabolic growth rate that juveniles fed *Tubifex* had the highest metabolic growth rate. There was a tendency towards significant difference in daily growth (g d^{-1}, $P = 0.077$) with the highest growth in juveniles fed *Tubifex* and custard. It was observed that the weight and length of swordtail juveniles under different dietary treatments were almost similar until the second week while significant differences were observed after the third week onward (Figures 2, 3).

Body protein

Body protein content (%) of swordtail after four weeks feeding trial is given in Figure 4. Different dietary treatments did not affect the body protein content of swordtail juveniles ($P > 0.05$). The highest body protein content was found in juveniles fed *Tubifex*, followed by those fed custard, fish feed, and aquarium feed. A tendency in increasing body protein content (%) was observed in swordtail juveniles fed feed with high protein (%) (Figure 2, Table 4).

Table 3: Water quality parameters (mean ± SD) during four weeks feeding trial. The same superscripts in a row indicate no significant differences ($P > 0.05$).

Parameters	Dietary treatments				*P* -value
	Tubifex	Custard	Fish feed	Aquarium feed	
Temperature (°C)	26.96±0.79[a]	27.35±0.38[a]	27.81±0.56[a]	26.74±0.83[a]	0.287
pH	8.59±0.18[a]	8.47±0.08[a]	8.36±0.19[a]	8.35±0.05[a]	0.190
DO (mgl^{-1})	7.81±0.54[a]	7.52±0.42[a]	6.69±0.58[a]	7.34±0.58[a]	0.146

Table 4: Growth performances of *Xiphophorus helleri* fed experimental diets over 4 weeks. Different superscripts in the same row indicate significant differences ($P > 0.05$).

	Dietary treatments				*P*-value
	Tubifex	Custard	Fish feed	Aquarium feed	
Initial weight (g)	0.45±0.03[a]	0.47±0.05[a]	0.47±0.02[a]	0.46±0.05[a]	0.884
Initial total length (cm)	3.23±0.49[a]	3.30±0.29[a]	3.36±0.10[a]	3.32±0.08[a]	0.962
Final weight (g)	1.06±0.02[ab]	0.93±0.09[bc]	0.84±0.04[bc]	0.83±0.13[c]	0.039
Final total length (cm)	4.24±0.05[a]	4.19±0.20[ab]	3.96±0.05[bc]	3.76±0.05[c]	0.002
Weight gain (g)	0.61±0.03[a]	0.46±0.06[ab]	0.37±0.03[b]	0.37±0.10[b]	0.006
Weight gain (%)	135.13±14.24[a]	99.66±13.27[ab]	78.91±6.52[b]	80.94±19.34[b]	0.004
Length gain (cm)	1.01±0.45[a]	0.89±0.10[a]	0.60±0.07[a]	0.43±0.13[a]	0.066
Growth ($g\ d^{-1}$)	0.02±0.001[a]	0.02±0.002[a]	0.01±0.001[a]	0.01±0.004[a]	0.077
SGR (%)	3.05±0.21[a]	2.46±0.23[ab]	2.08±0.13[b]	2.10±0.37[b]	0.005
Metabolic growth rate ($g\ kg^{-0.8}\ d^{-1}$)	7.33±0.50[a]	5.81±0.57[ab]	4.82±0.33[b]	4.88±0.97[b]	0.004

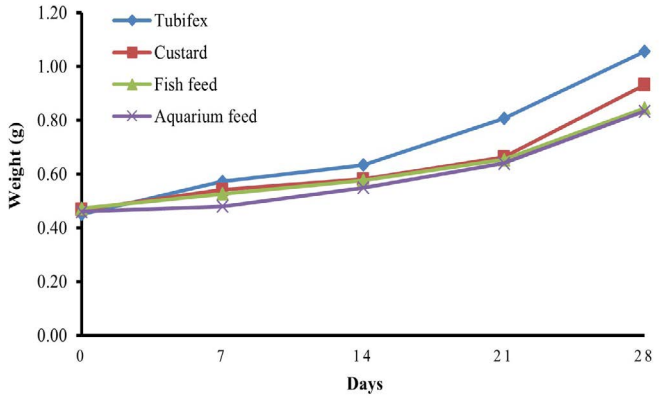

Figure 2: Weekly weight increment of *X. helleri* over four weeks period under different dietary treatments.

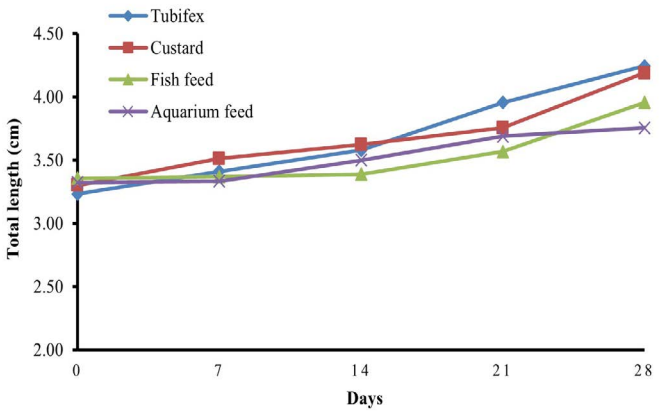

Figure 3: Weekly total length increment of *X. helleri* over four weeks period under different dietary treatments.

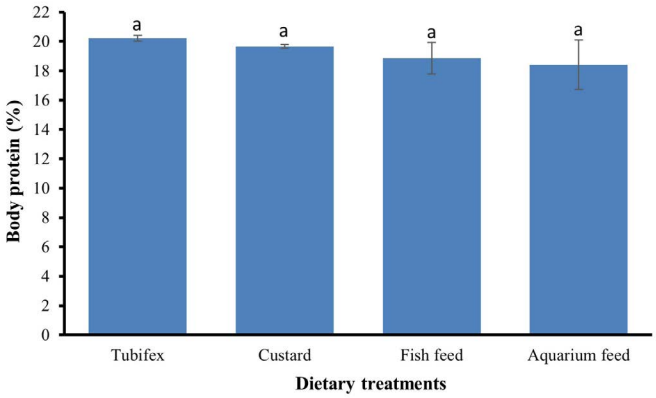

Figure 4: Body protein content (%) of *X. helleri* after four weeks feeding trial. The same superscripts in the bars indicate no significant differences ($P > 0.05$).

Generally, food fish farmers focus on mass production of fish while ornamental fish traders consider the number and minimum acceptable market size of fish (Olivier and Kaiser, 1997). Therefore, both the survival rate and size of swordtails are major concerns for ornamental fish traders and they aim to produce the highest number of fish with the least size variation in the shortest possible time. In the present study, there was no significant difference in survival rate in swordtail juveniles fed different dietary treatments ($P > 0.05$). This finding indicates that all dietary treatments were acceptable to swordtail juveniles. Juveniles fed *Tubifex* had the highest survival rate (97%) followed by those fed custard (93%), aquarium feed (93%), and fish

feed (90%) (Figure 1). Different studies have reported a higher survival rate in ornamental fish species fed *Tubifex*. A high survival rate, for example, in goldfish juveniles fed *Tubifex* has been reported by Mellisa et al. (2018) and Mohanta and Subramanian (2002). Likewise, in another study the highest survival rate in goldfish juveniles fed *Tubifex* followed by custard, aquarium feed, and fish feed have been found (under review). The higher survival rates with *Tubifex* have also been reported in Asian catfish, *Pangasius bocourti* (Hung et al., 2002); Siamese fighting fish, *Betta splendens* (Mandal et al., 2010); South American catfish, *Pseudoplatystoma fasciatum* (Arslan et al., 2009) and guppy, *Poecilia reticulata* (Görelşahin et al., 2018).

In the present study, growth performances in terms of weight gain (g, %), SGR (%) and metabolic growth rate (g $kg^{-0.8}$ d^{-1}) of swordtail juveniles fed *Tubifex* and custard were comparable ($P > 0.05$) while *Tubifex* showed significantly better performance than those fed fish and aquarium feeds ($P < 0.05$). This discrepancy in growth performances can be attributed to differences in the protein content of feeds. Both *Tubifex* and custard are proteinaceous feeds containing a protein content of 55.64 and 43.08%, respectively, while fish and aquarium feed contained comparatively lower protein content (34.86 and 18.80%, respectively) (Table 2). The dietary protein requirement of a particular fish species can be varied depending on several factors such as protein to energy ratio, source of protein, age and size of target fish species, and water temperature (Kruger et al., 2001a). Generally, swordtail juveniles require higher protein content in the feed. Kruger et al. (2001a) reported that swordtail of 6-8 weeks age required at least 45% protein to gain better SGR. Ling et al. (2006) found that swordtail fed 30% protein showed improved growth and better reproductive performance than those fed 20% protein.

Several studies reported improved growth performance in fish fed *Tubifex*. Different ornamental fish species, for instance, goldfish (Mohanta and Subramanian, 2002; Mellisa et al., 2018), zebrafish (Bouguenec, 1992), guppy (Görelşahin et al., 2018), *Siamese fighting fish* (Mandal et al., 2010), and Sailfin molly (Mohideen et al., 2014) fed *Tubifex* showed better growth performance. Furthermore, improved growth performance providing *Tubifex* has also been reported in food fish such as Asian catfish (Evangelista et al., 2005) and South American catfish (Arslan et al., 2009). Since *Tubifex* is proved as a potential source of spreading protozoan diseases in fish juveniles (Brinkhurst, 1996) the safe application of *Tubifex* should be ensured or incorporation of other safe live feed could be included in any aquaculture operation. Different studies reported that supplementation of other live feeds improved the growth performance of swordtail. Kruger et al. (2001b) reported that supplementation of *Daphnia* sp. as a live feed to swordtail enhanced growth and reproductive performance. Mousavi-Sabet et al. (2013) stated that swordtail juveniles fed commercial pellet feed with *Artemia* supplementation showed better growth performances than those fed only commercial pellet. Boaru et al. (2016) reported enhanced growth in swordtail juveniles fed earthworm meal supplemented commercial feed. However, providing live feeds to homestead aquarium animal is sometimes costly and requires sophisticated facilities.

The findings of this study revealed that swordtail juveniles fed custard and *Tubifex* showed almost similar growth performance ($P > 0.05$) although there was a large variation in protein content in custard and *Tubifex*. The ingredients of custard are locally available, and its preparation process is comparatively easy, therefore, homemade custard could be an alternative to *Tubifex* in terms of availability, cost, and health hazard issues. It was observed that juveniles fed egg custard exhibited numerically higher growth performances than those fed commercial pelleted feeds. The lower growth performance of swordtail juveniles fed two commercial feeds compare to those fed *Tubifex* and custard can be attributed to acceptance and energy expenditure for processing after ingestion. It is suggested that formulated pellet feed can be used for finfish juveniles when the feed intake with time and acceptance level is high enough to support growth (Pillay, 1993). Although fish feed contained a protein content of 34.86% the growth performance was not satisfactory compared to those fed aquarium feed with lower protein (18.80%). This can be explained by the fact that fish feed might contain indigestible protein for swordtail juveniles resulted in lower protein utilization.

The results of the present study showed that dietary treatments did not affect the body protein content of swordtail juveniles ($P > 0.05$). But a trend in increasing body protein with increasing feed protein content was observed (Table 2, Figure 4). It was found that although not significant, swordtail juveniles fed *Tubifex* (with the highest dietary protein) had the highest body protein content. Likewise, Ling et al. (2006) reported that swordtail fed feed with 30% protein had higher muscle protein content than those fed feed containing 20% protein. However, the trend in increasing body protein content of juveniles fed feed with high protein content was not reflected in the growth performance. Although fish feed contained 85% more protein than the protein of aquarium feed a significant difference was not observed in growth ($P > 0.05$). This finding indicates that not only the protein content of feed but also the source of protein, protein composition, and digestibility play a significant role in modulating growth performance. In the present study, swordtail juveniles were fed *ad libitum* while feed intake and nitrogen balance were not measured therefore,

this finding requires further elucidation.

In conclusion, swordtail juveniles fed *Tubifex* and custard had similar growth performances, survival, and body protein content. In terms of availability, cost, ecological and health hazard issues homemade custard could be a potential feed for swordtail juveniles. However, determining feed intake, digestibility, and assimilation through measuring balances (nitrogen and energy) of the tested feeds should be investigated.

ACKNOWLEDGEMENTS

The authors would like to thank the staff of Fish Nutrition Laboratory for helping to analyze protein and moisture content of fish and feed samples.

NOVELTY STATEMENT

This study investigates growth performance, survival and body protein content of swordtail under different dietary treatments following experimental data.

AUTHOR'S CONTRIBUTION

Sudip Debnath: Data analysis, writing original draft, visualization. Dipta Sundar Sarker: Conduct experiment, lab analysis, data collection. Pankaj Kundu: Conduct experiment, lab analysis, data collection. Md. Shahin Parvez: data curation, writing-review and editing. Shaikh Tareq Arafat: data curation, writing-review and editing. Roshmon Thomas Mathew: Writing and editing data, visualization. Yousef A Alkhamis: Supervision, project administration, visualization. Md Moshiur Rahman: Formal analysis, data curation, writing-review and editing. Sheikh Mustafizur Rahman: Conceptualization, methodology, investigation, data curation, writing-original draft, visualization.

Conflict of interest

The authors have declared no conflict of interest.

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