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Effects of Feeds Enriched with Exogenous Enzymes on the Growth, Flesh Composition and Platability of an Indian Major Carp, *Cirrhinus cirrhosus* (Bloch, 1795)

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Abstract

In response to the increasing use of exogenous enzymes in carp polyculture systems in Bangladesh, a study was conducted to evaluate the effects of feeds enriched with exogenous enzymes on the growth, flesh composition, and palatability of an Indian major carp, *Cirrhinus cirrhosus*. This feeding trial was conducted over 90 days in 12 cages in a fish pond at University of Rajshahi, Bangladesh. A commercial feed additive (Aquazyme Plus) was used as the enzyme source at concentrations of 0.0, 2.0, 4.0, and 6.0 g/kg feed to prepare four test feeds, designated as the following treatments: T_1 (control), T_2, T_3, and T_4. Growth and feed utilization parameters, flesh composition, and organoleptic qualities of cooked flesh were evaluated using standard formulas, methods of the Association of Official Analytical Chemists (AOAC), and an organoleptic technique, respectively. Results revealed superior weight gain and SGR in fish from group T_4, followed by those from groups T_3, T_2, and T_1. Group T_4 also exhibited the best FCR and PER, as well as the highest protein and lipid content. Organoleptic evaluation showed that the flesh of fish from groups T_3 and T_4 achieved higher scores for sensory qualities, while that of the control fish achieved the lowest scores. The incorporation of exogenous enzymes into the feed improves the growth, flesh quality, and organoleptic qualities of *C. cirrhosus*. The incorporation of this enzyme at a dose of 6.0 g/kg of feed resulted in significantly better results for this species.

Keywords: Aquafeeds, Aquazyme, Feed utilization, Flesh quality, Growth performance.

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Introduction

Aquaculture is one of the fastest-growing food production sectors in the world. Aquaculture production is heavily dependent on feedstock, i.e., the nutrients supplied to the aquaculture system (Tacon and Metian 2015). The use of economically viable, ecologically sustainable, and nutritionally balanced feedstock is essential for successful and sustainable aquaculture. The rise of feed-based aquaculture practices in Bangladesh has generated an unprecedented demand for feed. To meet this growing demand, numerous feed industries have developed in the country. However, most manufacturers are unable to supply high-quality feedstock due to a lack of quality ingredients, the use of contaminated ingredients, and other factors (Khatun et al. 2017).

Because aquaculture feed production is heavily reliant on fishmeal as a protein source, it will most likely face significant sustainability challenges in the future. This has led to increased interest in locally sourced plant-based ingredients to create sustainable aquaculture feeds. Animal-based feeds are currently being replaced by plant-based feeds to reduce costs, as they are more affordable, readily available, and environmentally friendly (Dalsgaard et al. 2009). However, plant-based feeds present digestibility issues due to the high molecular weight of non-starch polysaccharides found in plant cell walls (Cunningham and Klein 2007). Non-starch polysaccharides increase digestive viscosity, thereby reducing the access of digestive enzymes to other nutrients (Francis et al. 2001). This can lead to decreased feed efficiency and slowed fish growth. The most commonly used plant-based components, such as trypsin-inhibiting proteins, glucosinolates, and phytates, are associated with antinutritional factors. These factors are a major concern in fish feed formulation, as many plant-based components have drawbacks in terms of palatability (Nikmaram et al. 2017). They have detrimental effects on fish health and feed efficiency, which can lead to slowed growth (Felix and Selvaraj 2004).

To minimize feed viscosity, various exogenous enzymes, such as phytase, proteases, lipases, and galactosidases, can be added. The addition of exogenous enzymes improves the digestibility of proteins and carbohydrates, thereby optimizing feed conversion ratios and promoting fish growth (Adeola and Cowieson 2011). Enzyme supplementation also helps to eliminate the effects of antinutritional factors and improve feed energy utilization (Soltan 2009). Adding enzymes to fish feed has been shown to increase their ability to digest minerals and other nutrients (Kiarie et al. 2013). Formulating a fish diet with the addition of enzymes offers a wide range of benefits, including digestibility and growth performance, as shown in *Clarias batrachus* (Giri et al. 2003), *Pangasius pangasius* (Debnath et al. 2005), *Oreochromis niloticus* (Maas et al. 2017) and *Labeo rohita* (Rahman and Sarker 2019).

The mrigal carp (*Chirrhinus cirrhosus*) is a popular species and is farmed in Bangladesh using an aquaculture system based on artificial feeding due to its rapid growth. Faced with strong market demand and consumer preference for larger fish, fish farmers in the country use exogenous enzymes in their feed to promote carp growth. Unfortunately, some fish farmers use these enzymes indiscriminately, without understanding their effects or the appropriate dosage. Therefore, it is necessary to study the effects of feed enriched with exogenous enzymes on carp by analyzing their growth, flesh composition, and palatability. Several studies have been conducted on the effects of feed enrichment with exogenous enzymes on fish growth in various countries around the world (Ghomi et al. 2012, Maas et al. 2017, Rahman and Sarker 2019), but research on the effects of this enrichment on carp growth is scarce in Bangladesh. Therefore, this study was conducted to evaluate the effects of a diet enriched with exogenous enzymes on the growth, flesh composition, and palatability of *C. cirrhosus*, a candidate Indian major carp species reared in an artificial feeding aquaculture system in Bangladesh.

Materials and Methods

Experimental site and period

The study was conducted in twelve cages submerged in an earthen pond at the Department of Fisheries, University of Rajshahi, Rajshahi, Bangladesh, over a period of ninety days (March to May 2021). The twelve cages, with a metal frame and a unit volume of 2.72 m^3, were covered with knotless synthetic nylon netting (5 mm mesh) with an opening at the top for feeding and sampling. A suspended feeding tray was attached above.

Exogenous multi-enzyme used

A commercial exogenous multi-enzyme, Aquazyme Plus (EON Group, Bangladesh), was used in this study. Each 100g of Aquazyme Plus contains amylase (100,000 IU/kg), xylanase (1,000,000 IU/kg), protease (2,000,000 IU/kg), phytase (300 IU/kg), protein (0.75%), phosphorus (0.12%) and calcium (0.10%).

Experimental design

The study was conducted using four treatments (T_1, T_2, T_3, and T_4) with four experimental feeds. A commercial feed for ACI group carp (Godrej) served as the control (T_1). In addition to this control, three experimental feeds were prepared by adding Aquazyme to the selected commercial feed at doses of 2.0, 4.0, and 6.0 g/kg, respectively. These feeds were designated T_2, T_3, and T_4. The four treatments were administered in a completely randomized controlled trial (CCT) in rearing cages, with three replicates per treatment.

Feed preparation

To prepare the experimental feeds, the commercial feed was softened and made more malleable by adding a small amount of water. To form the paste, fixed doses of Aquazyme Plus were then added and thoroughly mixed. The paste was then extruded into pellets using a pellet mill. After drying, the feeds were vacuum-packed in labeled polyethylene bags and stored in a refrigerator until use. The AOAC method (2005) was used to evaluate the percentage composition of the feeds. Percentage analysis revealed no significant differences between the percentage compositions of the tested feeds (Table 1).

Table 1: Percentage composition of experimental feeds (on a dry basis).

Components (%)	Experimental feeds used in the treatments			
	T_1	T_2	T_3	T_4
Protein	21.21±0.82[a]	21.27±0.55[a]	21.38±0.77[a]	21.47±0.75[a]
Lipid	6.05±0.85[a]	6.08±0.82[a]	6.14±8.7[a]	6.21±0.90[a]
Carbohydrate	38.66±0.76[a]	38.91±0.71[a]	39.48±0.78[a]	39.09±0.78[a]
Ash	10.92±0.80[a]	11.01±0.85[a]	10.95±0.75[a]	11.08±0.71[a]
Moisture	14.90±0.70[a]	14.48±0.77[a]	14.02±0.72[a]	14.01±0.71[a]

Group T_1 consisted of fish fed a control diet (without the enzyme), while groups T_2, T_3, and T_4 consisted of fish fed a diet containing the enzyme at concentrations of 2.0, 4.0, and 6.0 g/kg, respectively. Values in the same row followed by different letters are statistically different ($p = 0.05$).

Collection and rearing of experimental fish

One hundred and forty-four juvenile *C. cirrhosus*, of relatively uniform size and weight, were collected from a local fish farmer. The juveniles were transported with an aeration system and acclimatized for two weeks. After this acclimatization period, twelve fish were randomly distributed into each cage, according to four treatments. Throughout the trial, the fish were fed daily with a ration equivalent to 5% of their body weight. This ration was divided into two portions: half was distributed in the morning (9:00 am) and the other half in the evening (5:00 pm). Fish samples were taken and weighed every two weeks to adjust the ration.

Monitoring water quality parameters

Throughout the experimental period, water temperature, pH, dissolved oxygen (DO), total alkalinity, and ammonia nitrogen (NH_3-N) were measured every two weeks. A Celsius thermometer was used to record the temperature. A digital pH meter was used to measure the water pH. The concentrations of DO, total alkalinity, and NH_3-N were assessed using a HACH kit (model: DR/2010). No significant differences were observed in the water quality parameters between the different treatments, and all values remained within an optimal range (Table 2).

Table 2: Average values of water quality parameters for four treatments.

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Temperature (°C)	29.37±0.63[a]	29.41±0.51[a]	29.43±0.39[a]	28.56±0.59[a]
pH	7.53±0.17[a]	7.55±0.31[a]	7.52±0.27[a]	7.40±0.25[a]
DO (mg/l)	6.05±0.42[a]	6.44±0.70[a]	6.14±0.46[a]	6.17±0.45[a]
Alkalinity (mg/l)	145.28±4.17[a]	150.31±4.09[a]	145.70±10.35[a]	145.53±6.64[a]
NH_3-N (mg/l)	0.036±0.006[a]	0.040±0.004[a]	0.041±0.008[a]	0.038±0.006[a]

Group T_1 received a control diet (without enzyme), while groups T_2, T_3, and T_4 received a diet containing 2.0, 4.0, and 6.0 g/kg of enzyme, respectively. Values in the same row followed by different letters are statistically different ($p = 0.05$).

Analysis of growth and feed utilization

The final weight of each fish in the four experimental treatments was measured individually at the end of the trial. Mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate (SR) were calculated to assess growth performance and feed utilization using standard formulas.

$$\mathrm{MWG} = \text{Mean Final Weight (MFW)} - \text{Mean Initial Weight (MIW)}$$

$$\mathrm{SGR} = \frac{\text{In (MFW)} - \text{In (MIW)}}{\text{Culture Duration (Days)}} \times 100$$

[In denotes the natural logarithm]

$$\text{FI (g fish}^{-1}\text{day}^{-1}) = ¼\text{DI} \times 100/\ [(w_1 + w_2)/2]/\text{T}$$

[DI= dry matter intake; w_1= start weight, w_2= final weight and T = feeding days]

$$\mathrm{FCR} = \frac{\text{Feed Fed}}{\text{Live Weight Gain}}$$

$$\mathrm{PER} = \frac{\text{Protein Fed}}{\text{Live Weight Gain}}$$

$$\mathrm{SR} = \frac{\text{No. of Fish Harvested}}{\text{No. of Fish Stocked}} \times 100$$

Proximate analysis of feed and fish flesh

The standard method (AOAC 2005) was used to determine the proximate composition (crude protein, crude fat, carbohydrates, moisture, and ash) of the experimental feed and the flesh of the experimental fish.

Palatability test

Initially, the collected fish were scaled, gutted, and filleted. After washing, 500 g of flesh from each batch was cooked using standard methods and spices. The fillets from each batch were marked and cooked together to avoid any cooking bias. After cooking, the fish were presented to a panel of experts for organoleptic and sensory evaluation. Following a blind tasting, the experts assigned a score to the sensory attributes (flavor, taste, and texture) of the flesh, using the structured evaluation system (Table 3).

Table 3: Organoleptic rating scale for the palatability test.

Organoleptic attributes			Score
Flavor	Taste	Texture	
Species - specific	Meaty flavor	Firm Elastic	10
Fresh fish	Sweet	Firm Springy	8
Slightly fishy or slightly sour	Slightly fishy	Less Firm	6
Sour and stale	Slightly sour/some off flavor	Softer	4
Strong Ammonia	Slightly rotten	Very Soft	2
Rotten smell	Spoiled	Slippery	0

Statistical analysis

Data were statistically analyzed using a one-way analysis of variance (ANOVA) followed by Duncan's test (DMRT) at a significance level of $p = 0.05$, using SPSS-20 (Statistical Package for Social Sciences) software (SPSS, USA).

Results

Growth and feed utilization

At the end of the feeding trial, the differences between the calculated values of growth and feed utilization parameters for the fish groups in the four treatments are presented in Table 4. Based on these data, the enzyme-enriched diets significantly improved growth and feed utilization parameters compared to the control diet. Fish fed the enzyme-enriched diet at a dose of 6.0 g/kg showed the best results. Survival rates did not show a significant difference.

Table 4: Growth and feed utilization in experimental fish under different treatments.

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Initial weight (g)	200.87±10.35^{a}	205.50±12.47^{a}	203.10±11.70^{a}	210.17±10.48^{a}
Final weight (g)	485.42±14.47^{c}	533.29±10.94^{b}	537.04±11.15^{b}	554.38±12.07^{a}
Weight gain (g)	284.55±4.21^{c}	327.79±3.03^{b}	333.94±3.93^{b}	344.21±3.01^{a}
PWG (%)	141.83±5.28^{b}	159.94±10.65^{ab}	161.70±15.27^{ab}	168.87±9.82^{a}
SGR (% bwd^{-1})	0.95±0.06^{b}	1.03±0.06^{ab}	1.07±0.04^{ab}	1.10±0.09^{a}
FI (g fish-1d^{-1})	2.28±0.06^{a}	2.13±0.09^{b}	2.03±0.09^{bc}	1.92±0.07^{c}
FCR	2.47±0.08^{a}	2.31±0.06^{b}	2.20±0.05^{bc}	2.08±0.07^{c}
PER	1.92±0.11^{c}	2.15±0.08^{b}	2.18±0.09^{b}	2.47±0.06^{a}
Survival rate (%)	91.67±8.33^{a}	94.44±9.62^{a}	89.11±4.81^{a}	91.67±8.33^{a}

Group T_1 consisted of fish fed a control diet (without the enzyme), while groups T_2, T_3, and T_4 consisted of fish fed a diet containing the enzyme at concentrations of 2.0, 4.0, and 6.0 g/kg, respectively. Values in the same row followed by different letters are statistically different (p = 0.05).

Proximate composition of fish flesh

Proximate analysis revealed that the crude protein, fat, carbohydrate, and ash content of the fish flesh were highest in fish fed the enzyme-enriched diet at 6.0 g/kg, differing significantly from that of the control group (Table 5). However, the water content of the flesh decreased with increasing doses of exogenous enzymes in the feed.

Table 5: Percentage Composition of Fish Flesh from the four treatments (on a wet basis).

Components (%)	Treatments			
	T_1	T_2	T_3	T_4
Protein	16.28±0.16^{c}	16.82±0.19^{b}	17.80±0.14^{a}	18.03±0.17^{a}
Lipid	2.68±0.06^{c}	2.76±0.03^{b}	2.89±0.03^{a}	2.92±0.08^{a}
Carbohydrate	1.42±0.03^{c}	1.78±0.06^{b}	1.79±0.04^{b}	1.87±0.05^{a}
Ash	1.66±0.07^{b}	1.64±0.02^{b}	1.20±0.05^{c}	1.75±0.07^{a}
Moisture	76.79±0.31^{a}	75.78±0.40^{b}	75.17±0.38^{b}	74.13±0.29^{c}

Group T_1 consisted of fish fed a control diet (without the enzyme), while groups T_2, T_3, and T_4 consisted of fish fed a diet containing the enzyme at concentrations of 2.0, 4.0, and 6.0 g/kg, respectively. Values in the same row followed by different letters are statistically different (p = 0.05).

Palatability of cooked fish

After consumption of cooked fish, the average scores obtained for organoleptic and sensory attributes (flavor, taste, and texture) are presented in Table 6. These scores indicate that enzyme-enriched feed improves the flavor, taste, and texture of cooked fish compared to the control fish. Fish fed with enzyme-enriched feed at 4.0 g/kg were ranked first, followed by fish fed with enzyme-enriched feed at 6.0 g/kg.

Table 6: Results of organoleptic tests on cooked fish flesh.

Palatability attributes	Treatments			
	T_1	T_2	T_3	T_4
Flavor	7.4±0.92^{b}	7.6±0.70^{ab}	8.2±0.42^{a}	7.9±0.70^{a}
Taste	7.6±0.70^{b}	7.6±0.37^{b}	8.2±0.37^{a}	8.1±0.32^{a}
Texture	7.2±0.99^{b}	7.7±0.67^{b}	7.9±0.47^{a}	7.8±0.84^{a}
Mean score	7.4±0.33^{b}	7.6±0.42^{b}	8.1±0.32^{a}	7.9±0.37^{a}

Group T_1 consisted of fish fed a control diet (without the enzyme), while groups T_2, T_3, and T_4 consisted of fish fed a diet containing the enzyme at concentrations of 2.0, 4.0, and 6.0 g/kg, respectively. Values in the same row followed by different letters are statistically different (p = 0.05).

Discussion

Growth performance and feed utilization

According to the results of this study, adding enzymes to the diet has a significant effect on the growth of *C. cirrhosus*. Our study showed that fish fed a high-enzyme diet (6.0 g/kg) exhibited the best growth and feed utilization performance, which is consistent with the results of Bala et al. (2020), who reported a significant improvement in the growth performance of *Pangasius hypothalamus* fed an enzyme-enriched diet. The positive effects of dietary enzyme supplementation on fish growth have been widely documented in several species, including Japanese sea bass (Huang et al. 2020), Nile tilapia (Tahounl et al. 2011, Maas et al. 2020), grass carp (Jin et al. 2020), and rohu (Rahman and Sarker 2019), which are comparable to the species examined in the present study. However, Yigit et al. (2018) and Shakoori et al. (2018) observed no improvement in growth performance or body composition in *Oncorhynchus mykiss* following the incorporation of enzymes into the diet, which could be due to interspecific variations or other factors. Contrasting results were also reported by Stone et al. (2003), who found no effect or even negative effects, which could be explained by the use of different enzymes and fish species. The results also demonstrated that the incorporation of exogenous enzymes into the fish diet improves weight gain and specific growth rate (SGR) in the experimental fish. These results are consistent with those of Bala et al. (2020), who reported that enzymatic treatment with *P. hypothalamus* significantly increased PWG and SGR. Jin et al. (2020) observed higher weight gain in grass carp following xylanase supplementation. According to Jiang et al. (2014), body weight in *Cyprinus carpio* increases with the amount of enzymes incorporated into the diet. SGR in rainbow trout improved following the addition of enzyme mixtures to the diet, and this method could prove cost-effective (Karimi et al. 2009). Similar results to those of our study were also reported by Jin et al. (2020) in grass carp and by Jiang et al. (2014) in *C. carpio* with dietary enzyme supplementation.

The present study showed a lower and more favorable FCR in fish fed an enzyme-enriched diet (6.0 g/kg), while the highest FCR was observed in the control group. Bala et al. (2020) also observed a better FCR with an enzyme-enriched diet than with a control diet, which is consistent with the results of our study. They found an improvement in FCR in *Salmo trutta* with the incorporation of dietary enzymes, a result comparable to ours. Ahmed et al. (2013) reported a better FCR in experiments using enzymes in the diet of *P. hypophthalmus*. Our results are also consistent with those of Ghomi et al. (2012), who reported a better FCR in *Huso huso* fry fed an enzyme-enriched diet. The fish survival rate did not vary significantly during the study, regardless of the treatment. These results are consistent with those of Rahman and Sarker (2019), who observed no significant difference in the survival rate of *L. rohita* fed diets enriched with exogenous enzymes. According to Ranjan et al. (2018), the survival rate of *L. rohita* remained unchanged despite the use of an enzyme-containing diet. Haghbayan and Mehrgan (2015) also found that the survival rate of *H. huso* fry did not vary significantly following the incorporation of exogenous enzymes into their diet, which is comparable to the results of the present study.

Flesh composition

Composition analysis revealed that the incorporation of exogenous enzymes into the diet significantly influenced the flesh composition of *C. chirochus*. Compared to control fish, those fed a diet containing 6.0 g/kg of exogenous enzymes had higher levels of crude protein, fat, carbohydrates, and ash, as well as lower water content. Ranjan et al. (2018) observed that the addition of dietary enzymes increased the protein content of *L. rohita*, which supports the findings of the present study. Ghomi et al. (2012) also reported similar results, showing higher crude protein levels in *H. huso* when the fish received 750 mg/kg of dietary enzymes. Hussain et al. (2011) observed higher protein content in *L. rohita* fry fed a diet enriched with 1,000 FTU/kg of enzymes, which is broadly consistent with the results of the present study.

The lipid content of the carcass of fish treated with dietary enzymes was increased and higher in those fed a diet containing 6.0 g/kg of enzymes. This result is consistent with the observations of Ghomi et al. (2012), who reported higher carcass lipid content in fish fed an enzyme-enriched diet than in control fish. Lin et al. (2007) found no significant difference in lipid content in fish fed an enzyme-containing diet, which, contrary to the current findings, could be explained by interspecific variability and the enzymatic composition of the fish. Carcass carbohydrate content also increased in fish fed an enzyme-enriched diet, and this value was higher when the diet contained 6 g/kg of enzymes. Maas et al. (2020) found that enzyme supplementation in the diet increased carcass carbohydrate content in *O. niloticus*, which is consistent with the present study. In a notable study, Rahman and Sarker (2019) reported a substantial improvement in carcass carbohydrate content in *L. rohita* fed enzyme-supplemented diets.

According to Rahman and Sarker (2019), adding enzymes to the diet had positive effects on the ash content of *L. rohita*, which is consistent with the results of the present study. Goda et al. (2012) reported a substantial increase in ash content in Nile tilapia fed a diet containing exogenous enzymes, a result comparable to those of the present study and those of Adeoye et al. (2016). Our results showed the highest water content in the control fish and the lowest in those fed a diet containing 6.0 g/kg of enzymes. According to Wheeler and Morrissey (2003), water content is inversely proportional to lipid content. Maas et al. (2020) observed an inverse relationship between carcass protein and moisture in Nile tilapia supplemented with exogenous enzymes. The results of the present study are in agreement with those of Siti-Noritaac et al. (2015), who observed the highest lipid content and the lowest moisture content due to the addition of a higher level of an enzyme to the diet of *O. niloticus* × *O. aureus*.

Palatability of cooked flesh

Based on the organoleptic scores assigned by the expert panel, the cooked flesh of fish fed with a diet enriched with exogenous enzymes had improved flavor, taste, and texture compared to the control fish. According to the average organoleptic score for the three sensory attributes (flavor, taste, and texture), the enzyme-enriched diets significantly improved the overall palatability of *C. chirochus*, but the flesh of fish fed with 4.0 g/kg of enriched diet obtained the highest score and was the most palatable. This could be due to changes in the percentage composition of the flesh of enzyme-treated fish. However, no scientific study has yet established a link between palatability and the percentage composition of fish flesh. Rahman and Sarker (2019) conducted a study showing that the palatability of cooked *L. rohita* flesh was altered by enzyme-enriched feed, which corroborates the results of the present study. However, studies on the impact of enzyme-enriched feed on fish palatability remain scarce; further research is therefore needed to draw definitive conclusions.

Conclusion

This study concludes that adding exogenous enzymes to the diet improves the growth, carcass quality, and palatability of mrigal carp (*C. cirrhosus*) compared to a commercial control diet. This study suggests that a dose of 6.0 g/kg of exogenous enzymes is optimal for *C. cirrhosus*. Given the encouraging results of this study and previous research, further studies are needed to optimize the dose of exogenous enzymes to improve the growth, nutritional composition, and palatability of mrigal carp.

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Conflict of interest: The authors declare that they have no conflict of interest, financial or otherwise, nor any personal relationship that could have influenced the work described in this manuscript.

Author's contribution: MAUZ, MSI, and MRH conducted the experiment in the field and performed the laboratory work, data collection, and data processing. MAUZ and MSI wrote the first draft of the manuscript. MSI and IKS corrected the manuscript. MMR designed the experiment, collected funding source and supervised the whole investigation. All authors have read and approved the final manuscript.

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Data availability: All data generated in the study are reported in the manuscript, and unprocessed data will be available to the corresponding author upon request.

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