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# EFFECT OF CHOLYMBI ON GROWTH, PROXIMATE COMPOSITION, AND DIGESTIVE ENZYME ACTIVITY OF FINGERLINGS OF LONG WHISKERED CATFISH, *MYSTUS GULIO* (ACTINOPTERYGII: SILURIFORMES: BAGRIDAE)

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**Background.** Long whiskered catfish, *Mystus gulio* (Hamilton, 1822), enjoys a high consumer preference and market demand in many Asian countries including India. However, the growth of this fish is very slow under normal aquaculture conditions. The non-hormonal growth promoter—cholymbi—which contains minerals and the essential amino acids lysine and methionine, is widely used in livestock and poultry as a feed additive to improve growth and survival. Supplementing feed with cholymbi may increase the growth and survival of *Mystus gulio*.
**Materials and methods.** A feeding trial was conducted to determine the effect of dietary cholymbi supplementation on growth, survival, and feed conversion ratio of long whiskered catfish fingerlings. Four isoproteic diets (crude protein 37%) were formulated to incorporate cholymbi at 0%, 0.25%, 0.50%, and 0.75% diet. The diets were fed to triplicate groups of fish twice daily to supply 5% of the total body weight per day for 120 days. Water quality parameters were maintained within the range suitable for catfish growth.
**Results.** Weight gain (WG), protein efficiency ratio (PER) and feed conversion ratio (FCR) were all affected by diet ($P < 0.01$, 0.001, and 0.001, respectively). The group fed the 0.50% cholymbi diet had the highest WG and PER and the lowest FCR, although not significantly different from the group fed the 0.25% cholymbi diet. There was no significant difference ($P > 0.05$) in carcass proximate composition of catfish fed diets containing these different levels of cholymbi. Gut protease, amylase, and lipase activities were stimulated by dietary inclusion of cholymbi at all levels compared to control.
**Conclusion.** As cholymbi is a newly introduced dietary supplement for fish, more research is needed to optimize its supplementation to improve growth, PER, FCR, and survival of long whiskered catfish. Until then, a diet with 0.50% cholymbi can be recommended to improve the aquaculture production of this species.

**Keywords:** cholymbi, *Mystus gulio*, growth, body composition, digestive enzymes

## INTRODUCTION

Long whiskered catfish, *Mystus gulio* (Hamilton, 1822), is an omnivorous bagrid fish which is distributed along the shores Bangladesh, India, Sri Lanka, Indonesia, Vietnam, Myanmar, Nepal, Pakistan, Java, Thailand, and Malay Archipelago, especially in estuarine and tidal waters (Talwar and Jhingran 1991, Jhingran 1997, Senarathne and Pathiratne 2007, Froese and Pauly 2012). This species can be cultured in fresh-, brackish-, and sea water because of its euryhaline and hardy nature (Rajkumar et al. 2004, 2005). It is very delicious and, therefore, enjoys a high consumer preference and market

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demand (Haniffa 2009, Begum et al. 2010) and is of commercial value in many Asian countries including India. Culture of this species first started in brackish paddy fields in West Bengal and Orissa, India (Troell 2009). Since then, aquaculture technology for this species has improved, but its growth is still very slow under normal aquaculture conditions.

Fish growth can be increased by adding different growth promoters to the feed (Kumaraguru vasagam et al. 2004, Rajkumar et al. 2004, Maheskumar et al. 2006). These are non-nutritive materials which, at very low levels in the feed, increase the efficiency of feed utilization (Viola and Arieli 1987). Addition of growth promoters not only increases growth but also reduces the culture period and feed cost. There are many published reports on a variety of hormonal and non-hormonal growth promoters used as fish feed additives, based on their effectiveness in livestock (Kumar unpublished[*], Shadakshari unpublished[**]). Non-hormonal growth promoters include vitamins, minerals, and antibiotics, all of which also improve animal health and prevent infection (Keshavanath et al. 1991). Some previous studies included non-hormonal growth promoters in the diet of *M. gulio*. For example, Maheskumar et al. (2006) incorporated Superliv at three graded levels namely 0.25%, 0.50%, and 0.75% in the diet of *M. gulio*. They observed that the 0.75% Superliv-added diet gave better weight gain, protein efficiency ratio, and food conversion ratio. In another study, Kumaraguru vasagam et al. (2004) observed the effects of Nutripro-Aqua at 0.0%, 0.25%, 0.50%, and 0.75% on the diet of *M. gulio.* After a 120-day culture period, they observed that a 0.50% Nutripro-Aqua diet showed the best performance in terms of weight gain, specific growth rate, protein efficiency ratio, and food conversion ratio.

Fish need the same essential amino acids as most other animals and the requirements for individual amino acids are fairly consistent among species. Lysine and methionine are frequently the limiting amino acids (Tacon and Jackson 1985, El-Sayed 1990) and careful attention must therefore be paid to ensure sufficient dietary levels of these amino acids. Cholymbi (Lyka Labs Ltd., Bombay, India) is known to contain minerals and essential amino acids like lysine and methionine (Table 1) and is used widely in livestock and poultry as a feed additive to improve growth and survival. In this study, an attempt was made to increase the growth performance of *M. gulio* fingerlings using cholymbi as a feed additive. The effects of cholymbi on growth, proximate composition, and gut digestive enzyme activity were determined under laboratory conditions.

## MATERIALS AND METHODS

**Feed formulation and preparation.** Three test diets (T1, T2, and T3) were formulated to contain 0.25%, 0.50%, and 0.75% cholymbi, respectively, keeping the crude protein level at 37% and with the diet without cholymbi (T0) serving as the control (Table 2). Ingredients were sieved (200 μm) using a mechanical shaker and then mixed thoroughly to get a homogenate. Boiled water was then added to make a dough, which was steam cooked for 15 min in a kitchen cooker and then cooled. Vitamins and minerals were added to the cooled dough, which was then mixed for uniform distribution and pelletized (1-mm diameter) with a hand pelletizer. The pellets were oven dried and stored at 4°C in airtight containers.

**Growth trial.** *Mystus gulio* fingerlings were collected from the Vellar River estuary (11º29′N, 29º46′E) and nearby canals and transported to the laboratory, where they were acclimated to estuarine water (PSS 19 to 26) at 25 to 28°C, pH 7.0 to 8.5, and 6.5 to 8.2 mL · $L^{-1}$ dissolved oxygen (DO). The fish were acclimated in the same experimental tanks for two days. All fish were treated the same during acclimation period. The growth study was conducted for 120 days using 50 L plastic troughs, with 15 fingerlings stocked into each of 12 troughs (i.e., triplicates of each of the 4 treatments) and with 50% water exchange daily. Fish length (total length) and weight at stocking were 3.33 ± 0.06 cm (mean ± standard deviation) and 1.78 ± 0.15 g, respectively. Length and weight were measured every 14 days and feed quantity adjusted accordingly to provide 5% of total biomass daily, with equal amounts of feed given manually at 0730 h and 1930 h. Uneaten feed was collected and removed 6 h after each feeding. The photoperiod was approximately 12 h light and 12 h dark.

**Water quality analysis.** Temperature, salinity, pH, and DO were measured using a Zeal thermometer (Bliss Scientific Industry, Vivekananda Enterprises, Delhi, India), hand refractometer (AL-21a, Re-2361-51L, Atago Co. Ltd., Tokyo, Japan), pH pen (Scan 3, Japan) and DO meter (Incorporated YSI-55model/12FTSN: 95F34309), respectively.

**Calculation of growth performance.** Growth parameters, such as: specific growth rate (SGR), protein efficiency ratio (PER), and feed conversion ratio (FCR) were calculated using the following equations:

$$SGR = 100\ [\ln W_f - \ln W_i] \cdot T^{-1}$$

$$PER = WG \cdot PI^{-1}$$

$$FCR = FC \cdot WG^{-1}$$

**Table 1**

Composition of cholymbi

| Ingredient | Quantity (per 100 g) |
|---|---|
| Iron | 97.9 mg |
| Iodine | 15.6 mg |
| Cobalt | 4.5 mg |
| Magnesium | 211.4 mg |
| Copper | 31.2 mg |
| Zinc | 213.0 mg |
| Live yeast culture | 300.0 mg |
| L-Lysine HCl | 440.0 mg |
| DL-Methionine | 192.0 mg |
| Calcium | 30 g |
| Phosphorous | 8.25 g |

[*] Kumar M. 1994. Effect of two commercially available feed additives on growth and survival of cultivable carps. PhD thesis, University of Agricultural Sciences, Bangalore, India.

[**] Shadakshari G.S. 1993. Effect of bioboost-forte, livol and Amchemin Aqon growth and body composition of common carp, *Cyprinus carpio* (Linn.). MSc thesis, University of Agricultural Sciences, Bangalore, India.

where: $W_f$ = mean final weight, $W_i$ = mean initial weight, $T$ = total experimental days, WG = weight gain [g], PI = protein intake [g], FC = feed consumed [g].

**Biochemical composition analysis.** Ash content was determined gravimetrically by burning in a Muffle furnace at 550°C for 6 h. The standard method of AOAC (Anonymous 1995) was followed for estimation of crude protein content. Carbohydrate content was determined by phenol sulphuric acid reagent at 490 nm using D-glucose as a standard (Dubois et al. 1956). Total lipid content was determined gravimetrically after extraction in chloroform/methanol (2 : 1 v/v) (Folch et al. 1957). Each sample was analysed in triplicate.

**Quantification of digestive enzymes.** Representative samples of 1 fish from each replicate were taken for the analysis of gut digestive enzyme (protease, amylase and lipase) activity at the end of the growth trial. The pancreas, foregut, midgut, and hindgut were dissected out, weighed, and homogenized with deionized water nine times. Homogenate was centrifuged for 30 min at 4472 × G and 4°C. The supernatant was separated from the top lipid layer, divided into subsamples for each different enzyme activity test and stored in 1 mL microfuge tubes at –20°C until analysis. Dilutions of the homogenate were made in respective buffers and tested in duplicate.

Protease activity was determined according to Kunitz (1947), using casein as a substrate. A mixture of 0.1 mL crude extract, 0.5 mL phosphate buffer (100 mM, pH 7.5), and 2 mL casein (1% in phosphate 50 mM, pH 7.5) was incubated at 28°C for 20 min. The reaction was stopped with the addition of 1 mL trichloroacetic acid (30%), the mixture clarified by centrifugation (1118 g for 15 min), and the absorbance measured at 440 nm. An additional negative control was generated by replacing crude extract with phosphate buffer. A standard curve of absorbance at 440 nm was established using tyrosine as a standard. One unit of protease activity was expressed as the number of μmol of tyrosine liberated per min per mg of protein at 28°C.

Amylase activity was determined according to Rick and Stegbauer (1984), with a 1% starch solution as a substrate in 50 mM phosphate buffer and pH 7.5. After adding 0.1 mL crude extract to 1 mL substrate, the mixture was incubated at 28ºC for 5 min. Amylase activity was determined by measuring the production of maltose resulting from starch hydrolysis. The maltose production was estimated by reading the colour intensity at 550 nm. A negative control was generated by replacing crude extract with phosphate buffer. A standard curve of absorbance at 550 nm was established using a standard maltose solution. One unit of amylase activity was calcu-

**Table 2**

Ingredients and proximate composition of experimental diets used for feeding *Mystus gulio* over a culture period of 120 days

| | Ingredient/component | Diet [g · kg$^{-1}$] | | | |
|---|---|---|---|---|---|
| | | T0 | T1 | T2 | T3 |
| Ingredient | Fish meal | 140 | 140 | 140 | 140 |
| | Soybean meal | 400 | 400 | 400 | 400 |
| | Wheat flour | 200 | 197.5 | 195 | 192.5 |
| | Rice bran | 110 | 110 | 110 | 110 |
| | Pea nut meal | 110 | 110 | 110 | 110 |
| | Fish oil | 15 | 15 | 15 | 15 |
| | Soylecithin | 5 | 5 | 5 | 5 |
| | DCPh | 10 | 10 | 10 | 10 |
| | V&M premix | 10 | 10 | 10 | 10 |
| | Cholymbi | 0 | 2.5 | 5.0 | 7.5 |
| Proximate composition | Dry matter | 88 ± 0.46 | 88.2 ± 0.22 | 88.1 ± 0.51 | 88.5 ± 0.64 |
| | Crude protein | 370 ± 0.73 | 369 ± 0.87 | 372 ± 0.59 | 372 ± 0.82 |
| | NFE | 238 ± 1.53 | 239 ± 1.19 | 237 ± 1.48 | 239 ± 1.76 |
| | Crude lipid | 57 ± 0.53 | 57 ± 0.66 | 57 ± 0.74 | 57 ± 0.58 |
| | Crude fibre | 113 ± 1.69 | 113 ± 1.26 | 113 ± 1.85 | 114 ± 1.60 |
| | Ash | 142 ± 0.87 | 142 ± 0.73 | 141 ± 0.67 | 141 ± 1.09 |

Data are mean of triplicate samples (±standard deviation for proximate composition); Proximate composition was calculated on dry matter basis; V&M premix = vitamin and mineral premix: cholecalciferol 2000 IU, thiamine mononitrate 200 IU, riboflavin 20 mg, pyridoxine hydrochloride 6.0 mg, cyanocobalamin 30.0 mg, nicotinamide 200 mg, calcium pantothenate 32.6 mg, ascorbic acid 300 mg, α-tocophenyl acetate 50.0 mg, biotin USP 0.50 mg, calcium phosphate 258 mg, magnesium oxide 120.0 mg, dried ferrous sulphate 64.08 mg, manganese sulphate 4.06 mg, total phosphorus 51.60 mg, copper sulphate 6.78 mg, zinc sulphate 4.40 mg, sodium molybdate 0.50 mg, sodium borate 1.76 mg; DCPh = dicalcium phosphate; NFE = nitrogen free extract calculated by difference.

lated as the number of μmol of maltose liberated per min per mg of tissue protein at 28°C.

Bier's titrimetric method (Bier 1962) was used for calculating lipase activity by estimating the fatty acids liberated by the enzyme from triglyceride emulsion by titration with a standard alkali.

**Statistical analysis.** The effects of dietary cholymbi level on growth performance, proximate composition, and gut digestive enzyme activities were analysed through one-way ANOVA using SPSS Statistical Software System, Version 17.0 (SPSS, Chicago, IL, USA). Where effects were significant, differences between the means were analysed by Tukey's HSD test for multiple comparisons of means. $P < 0.05$ was considered to indicate a statistically significant result.

The presently reported research was carried out in accordance with the Indian regulation on animal experiments.

## RESULTS

The growth trials were conducted without interruption or disease problems. The water quality parameters across all treatments were: salinity PSS 22–25; temperature 25–28°C; DO 6.92–8.95 mL · $L^{-1}$; and pH 7.09–8.82. Although there was a significant effect of diet on survival (Table 3), survival rate was > 95% in all treatments and none of the cholymbi levels affected survival relative to the control.

There was a significant effect ($P < 0.05$) of diet on WG, PER, and FCR (Table 3). Weight gain did not differ among cholymbi diets, but was higher in fish fed 0.25% and 0.50% cholymbi than in controls. All levels of dietary cholymbi increased PER and decreased FCR relative to the control, with the 0.50% cholymbi diet giving a significantly higher PER and lower FCR than the 0.75% cholymbi diet. There was no significant difference ($P > 0.05$) in carcass proximate composition of catfish fed on diets containing different levels of cholymbi (Table 4). Gut protease, amylase and lipase activities were significantly stimulated by dietary inclusion of cholymbi at all levels compared to control, but with no consistent patterns (Table 5).

## DISCUSSION

In most cases, fish nutritionists give attention mainly to different macronutrients such as protein, lipid and carbohydrate during feed formulation. However, very limited attention is normally paid to the different micronutrients, which are very essential for the growth of fish. In the presently reported study, besides macronutrients a list of micronutrients (cholymbi) was added as supplement to increase the growth of *M. gulio*, which is not currently satisfactory in

**Table 3**

Effect of cholymbi on growth performance of *Mystus gulio* over a culture period of 120 days

| Parameter | Significance | Treatment (% cholymbi) | | | |
|---|---|---|---|---|---|
| | | T0 (0%) | T1 (0.25%) | T2 (0.50%) | T3 (0.75%) |
| Initial length [cm] | NS | 3.33 ± 0.06 | 3.33 ± 0.12 | 3.33 ± 0.15 | 3.30 ± 0.10 |
| Initial weight [g] | NS | 1.78 ± 0.15 | 1.66 ± 0.27 | 1.62 ± 0.25 | 1.55 ± 0.10 |
| Final length [cm] | NS | 12.33 ± 0.08 | 12.45 ± 0.13 | 12.47 ± 0.05 | 12.27 ± 0.15 |
| Final weight [g] | $P < 0.01$ | $21.95 \pm 0.66^{a}$ | $24.01 \pm 0.51^{b}$ | $24.18 \pm 0.40^{b}$ | $22.82 \pm 0.64^{ab}$ |
| WG [g] | $P < 0.01$ | $20.17 \pm 0.81^{a}$ | $22.35 \pm 0.58^{b}$ | $22.57 \pm 0.25^{b}$ | $21.27 \pm 0.66^{ab}$ |
| Feed consumed [g] | $P < 0.05$ | $74.98 \pm 0.99^{b}$ | $71.28 \pm 2.35^{ab}$ | $69.42 \pm 1.13^{a}$ | $72.05 \pm 0.96^{ab}$ |
| SGR | NS | 2.02 ± 0.10 | 2.17 ± 0.15 | 2.20 ± 0.03 | 2.18 ± 0.07 |
| PER | $P < 0.001$ | $0.71 \pm 0.03^{a}$ | $0.83 \pm 0.01^{bc}$ | $0.88 \pm 0.03^{c}$ | $0.80 \pm 0.03^{b}$ |
| FCR | $P < 0.001$ | $3.78 \pm 0.11^{c}$ | $3.27 \pm 0.05^{ab}$ | $3.07 \pm 0.09^{a}$ | $3.39 \pm 0.11^{b}$ |

Values are mean ± standard deviation (of triplicate samples); NS = non-significant; Mean values in the same row with no superscript in common differ significantly ($P < 0.05$); WG = weight gain, SGR = Specific growth rate (% body weight per day), PER = protein efficiency ratio, FCR = feed conversion ratio.

**Table 4**

Effect of cholymbi on whole-body proximate composition (dry matter basis) of *Mystus gulio* over a culture period of 120 days

| Parameter [%] | Significance | Treatment (% cholymbi) | | | |
|---|---|---|---|---|---|
| | | T0 (0%) | T1 (0.25%) | T2 (0.50%) | T3 (0.75%) |
| Dry matter | NS | 23.77 ± 0.38 | 23.43 ± 0.55 | 23.73 ± 0.32 | 23.53 ± 0.55 |
| Crude protein | NS | 62.74 ± 0.79 | 62.88 ± 0.36 | 64.05 ± 1.09 | 62.34 ± 0.68 |
| Total carbohydrate | NS | 5.61 ± 0.36 | 5.60 ± 0.14 | 5.83 ± 0.07 | 5.41 ± 0.16 |
| Crude lipid | NS | 8.68 ± 0.30 | 8.58 ± 0.07 | 8.92 ± 0.09 | 8.47 ± 0.23 |
| Ash | NS | 13.75 ± 0.20 | 13.52 ± 0.12 | 13.88 ± 0.31 | 13.69 ± 0.15 |

Values are mean ± standard deviation (of triplicate samples); NS = non-significant.

**Table 5**

Gut digestive enzyme activities of *Mystus gulio* fed experimental diets among treatments differing in % content of cholymbi (in brackets) over a 120-day culture period

| E | Tissue | S | Enzyme activity [U] | | | |
|---|---|---|---|---|---|---|
| | | | T0 (0%) | T1 (0.25%) | T2 (0.50%) | T3 (0.75%) |
| Protease | Pancreas | $P < 0.001$ | $33.38 \pm 0.58^{b}$ | $26.68 \pm 1.41^{a}$ | $38.73 \pm 0.96^{c}$ | $38.87 \pm 0.80^{c}$ |
| | Foregut | $P < 0.001$ | $25.67 \pm 0.48^{a}$ | $37.49 \pm 0.99^{c}$ | $45.12 \pm 0.49^{d}$ | $32.97 \pm 0.91^{b}$ |
| | Midgut | $P < 0.001$ | $24.84 \pm 1.03^{b}$ | $43.38 \pm 0.49^{d}$ | $22.86 \pm 0.66^{a}$ | $35.46 \pm 0.67^{c}$ |
| | Hindgut | $P < 0.001$ | $23.50 \pm 0.57^{a}$ | $36.00 \pm 0.17^{c}$ | $25.99 \pm 0.17^{b}$ | $38.50 \pm 0.63^{d}$ |
| Amylase | Pancreas | $P < 0.001$ | $14.22 \pm 0.92^{ab}$ | $17.84 \pm 0.28^{c}$ | $14.05 \pm 0.18^{a}$ | $15.51 \pm 0.24^{b}$ |
| | Foregut | $P < 0.001$ | $14.58 \pm 0.42^{c}$ | $11.95 \pm 0.43^{b}$ | $9.90 \pm 0.59^{a}$ | $14.49 \pm 0.32^{c}$ |
| | Midgut | $P < 0.001$ | $9.63 \pm 0.20^{a}$ | $17.14 \pm 0.21^{d}$ | $13.70 \pm 0.43^{b}$ | $14.73 \pm 0.45^{c}$ |
| | Hindgut | $P < 0.001$ | $11.40 \pm 0.37^{b}$ | $12.15 \pm 0.75^{b}$ | $7.63 \pm 0.34^{a}$ | $13.85 \pm 0.11^{c}$ |
| Lipase | Pancreas | $P < 0.001$ | $1.98 \pm 0.06^{b}$ | $0.59 \pm 0.03^{a}$ | $3.85 \pm 0.25^{d}$ | $3.13 \pm 0.09^{c}$ |
| | Foregut | NS | $3.29 \pm 0.18$ | $2.55 \pm 0.44$ | $3.19 \pm 0.05$ | $2.71 \pm 0.49$ |
| | Midgut | $P < 0.05$ | $1.77 \pm 0.34^{a}$ | $2.66 \pm 0.36^{b}$ | $2.34 \pm 0.24^{ab}$ | $2.52 \pm 0.32^{ab}$ |
| | Hindgut | $P < 0.001$ | $1.76 \pm 0.09^{a}$ | $2.42 \pm 0.16^{b}$ | $3.64 \pm 0.43^{c}$ | $3.24 \pm 0.10^{c}$ |

Values are mean ± standard deviation (of triplicate samples); NS = non-significant; E = enzyme; S = significance; Mean values in the same row with no superscript in common differ significantly ($P < 0.05$).

aquaculture ponds. However, we observed a better growth with diet containing 0.25% and 0.50% cholymbi than the feed with no cholymbi and 0.75% cholymbi. There are no previous studies comparing the effects of cholymbi on the growth performance of *M. gulio*. However, Moheskumar (data are not published yet) investigated the effects of three different level of cholymbi (0.25%, 0.50%, and 0.75%) on the growth performance of goldspot mullet, *Liza parsia* (Hamilton, 1822). In that study, diet with 0.75% cholymbi was better than the diet with 0.25% and 0.50% cholymbi. This suggests that the dose of cholymbi in promoting growth might be depended on species.

Similar results were reported by Swain et al. (1996) and Nandeesha et al. (2000) using non-hormonal growth promoters in mrigal, *Cirrhinus cirrhosus* (Bloch, 1795), and common carp, *Cyprinus carpio* L., respectively. Swain et al. (1996) observed better growth and nutrient utilization in mrigal fed with the non-hormonal growth promoter Bioboost forte, with lower FCR and a PER of 1.26 at the dietary level of 150 mg · $kg^{-1}$. Nandeesha et al. (2000) observed an increased SGR, PER, and net protein retention, and decreased FCR, in common carp by adding 1.5% NaCl in the diet. In the presently reported study, diets with 0.50% and 0.25% cholymbi were statistically the same on growth performance parameters. However, 0.50% cholymbi diet comparatively performed better than with 0.25% cholymbi diet in case of weight gain, specific growth rate, protein efficiency ratio, and feed conversion ratio. When comparing price, the price of cholymbi is very inexpensive in India, therefore it is widely used as chicken and cow feed additives. However, additional price of 0.25% cholymbi is negligible. So, the cost of additional 0.25% cholymbi will not make up the improved weight gain, protein efficiency ratio, and feed conversion ratio. Based on the above discussion, 0.50% cholymbi diet is comparatively better than all other diets. More research is needed to further optimize the supplementation of cholymbi on growth performance of this fish. Until then, a diet with 0.50% cholymbi can be recommended to the aquaculturists to improve the production of long whiskered catfish.

In this study, fish were fed two times per day. This is the common practice in India for the majority of the aquaculture species. However, more frequent feeding or feeding with satiation may improve the growth performance of *M. gulio*. Therefore, more research is needed to optimize the feeding frequency for better growth performance of *M. gulio*.

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