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Effect of dietary supplementation of Giloy (*Tinospora cordifolia*) on growth, survival and gut histology of striped catfish (*Pangasianodon hypophthalmus*)

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Abstract
Giloy (*Tinospora cordifolia*) is known to be having amazing medicinal properties and has been used in indigenous systems of medicine in treating various human diseases, as well as to boost overall health. Giloy includes bioactive compounds like Alkaloids, Terpenoids, Flavonoids, Lignans, Sitosterol, Hydroquinone and Palmitic acid, which exhibit the overall health improvement effect. In the present study, the effect of dietary supplementation of *T. cordifolia* on the growth, survival and gut histology of *Pangasianodon hypophthalmus* was evaluated. Three test diets, T_1, T_2, T_3 and a Control (T_0) with 30% crude protein were formulated. 1% of Giloy (T_1), 1.5% of Giloy (T_2), 2.0% of Giloy (T_3) were incorporated in the test diets and diet without any Giloy, served as a control (T_0). In the present study, Weight Gain (216.32±0.69%), Specific Growth Rate (SGR) (1.28±0.03%) and Protein Efficiency Ratio (PER) (1.99±0.06) were significantly higher in T_3 when compared to the other treatments and the control (p<0.05). Significantly lower Food Conversion Ratio (FCR) was observed in T_3 (1.33±0.02) followed by other treatments and the Control (p<0.05). In the present study, dietary supplementation of Giloy improved the overall health of Striped catfish when compared to the control. Significantly higher survival percentage of 100% was observed in T_3, T_2 and T_1 when compared to the control (97.78±2.22) (p<0.05), in the present study. The treatment T_3 exhibited longer and wider villi when compared to the other treatments and the control. Also, lumen space was reduced in the treatment group fed with Giloy while Control showed higher lumen space. It is evident from the results of the present study that Giloy has overall health promoting effect when fed as a dietary supplement to Striped catfish at the rate of 2% in the feed for the best growth promoting effects.

Keywords: *Pangasianodon hypophthalmus*, *Tinospora cordifolia*, gut h istology, SGR, FCR, per, survival rate

Introduction
India's extensive and diverse aquatic resources position it as a global contender for aquaculture expansion (Das *et al*., 2022) [9]. But among all major challenges in aquaculture, indiscriminate use of antibiotics has accelerated the emergence of antimicrobial resistant (AMR) pathogens leading to heavy economic loss to the fish farmers (Founou *et al*., 2021; Kolawole *et al*., 2023) [11, 18]. In response to the increasing threat of multidrug resistant (MDR) fish pathogens and the limitations of conventional chemotherapeutic agents, more emphasis is being given to herbal nutraceuticals as functional dietary additives in aquaculture (Bhat *et al*., 2022) [7]. Beyond their therapeutic potential, the use of herbal nutraceuticals in aquafeeds is widely regarded as an environmentally friendly and sustainable approach to disease management. Herbal nutraceuticals also do not pose ecological and public health risks which are associated with chemotherapeutics (Dawood *et al*., 2018; Jeyavani *et al*., 2022) [10, 17]. Giloy (*Tinospora cordifolia*) is widely used in ayurveda medicine for treating various human ailments and for boosting general health and immunity (Gupta *et al*., 2024) [13]. Giloy includes bioactive compounds like Alkaloids, Terpenoids, Flavonoids, Lignans, Sitosterol, Hydroquinone and Palmitic acid, which exhibit the overall health improvement effect (Kumar *et al*., 2021; Saga r *et al*., 2025) [19, 24]. Striped catfish (*Pangasianodon hypophthalmus*) is one of the fastest-growing fish species with a high potential for enhancing production and export (Alam, 2011). *P. hypophthalmus* is being farmed in an area of

about 40,000 ha in India and contributes to 0.7 million tons to total fishery (Singh, 2025) [24]. The effect of *T. cordifolia* on improving the overall health of *P. hypophthalmus* has not been studied much and hence, the present research work was carried out with the objective of studying the effect of dietary supplementation of Giloy (*T. cordifolia*) on the growth, survival and gut histology of *P. hypophthalmus*.

2. Materials and Methods

1000 L capacity cement tanks, in an outdoor system were used in the present study at the field lab of the Research and Instructional Fish Farm of the Aquaculture Department, College of Fisheries, Mangaluru to investigate the effect of dietary incorporation of Giloy (*Tinospora cordifolia*) on growth, survival and gut histology of Striped catfish (*Pangasianodon hypophthalmus*) for a period of 90 days. Fishmeal, groundnut oil cake, rice bran and tapioca flour were procured from local market in Mangaluru. Vitamin and mineral premix was obtained from a veterinary pharmacy. The laboratory grade Giloy powder was obtained from a super market at Mangaluru. All the components were powdered and sieved in a mesh size of 0.18mm. The sieved ingredients were stored in air tight, HDPE (High Density Poly Ethylene) bags and placed at room temperature till further use. The proximate composition of the feed ingredients was analysed before feed preparation by following standard protocol (AOAC, 2017) [3]. The moisture content of the samples was estimated by subjecting to heating at 105 °C for 30 minutes, followed by cooling and weighing until a stable weight was achieved. The crude protein was measured using the Kjeltron system (Tulin devices). The fat content was measured using the Soxtech system (Pelican) and the fibre content was evaluated using the Fibretech system (Pelican). The difference approach (Hasting, 1976) [15] was used to determine carbohydrate content as nitrogen free extract (NFE). The ash. content was determined by charring the sample and heating it in a muffle furnace at 550±10 °C for 6 hours. The estimated quantities of ingredients were weighed, blended and hand kneaded to the required consistency with enough water to make a smooth dough. The dough was then cooked for 25 to 30 minutes. The cooked formulated feed was cooled to room temperature in an enamel tray. The calculated quantity of vitamin and mineral pre-mix was added to the cooked and cooled formulated fish feed. This formed the basal diet. A portion of this was kept aside as control diet. Calculated quantity of Giloy powder was added to the three aliquots of basal diet, which formed the test diets T_1 (1% Giloy), T_2 (1.5% Giloy) and T_3 (2% Giloy) (Tables 1 and 2). A hand pelletizer with a diameter of 3 mm was used to extrude the feed. Pellets were dried in a hot air oven at 65 °C until the moisture content was lower than 10%. Feed was packed separately in HDPE bags, labelled and stored in dry place until further use.

Table 1: Composition of different ingredients in the experimental diet of different treatments

Ingredients (%)	Control	T_1	T_2	T_3
Fish meal	34.13	34.13	34.13	34.13
Groundnut oil cake	34.13	34.13	34.13	34.13
Rice bran	15.37	14.37	13.87	13.37
Tapioca flour	15.37	15.37	15.37	15.37
Vitamin-mineral mixture	1.00	1.00	1.00	1.00
Total	0	1.00	1.50	2.00

Table 2: Proximate composition of the formulated feed

Parameters	Control (T_0)	Treatments		
		T_1	T_2	T_3
Protein	30.25±0.06	30.55±0.06	30.92±0.01	30.89±0.05
Fat	6.82±0.24	7.02±0.09	6.95±0.13	6.79±0.14
Fibre	8.19±0.06	8.38±0.23	8.72±0.18	8.97±0.06
Moisture	9.32±0.03	9.25±0.07	9.42±0.08	9.16±0.07
Ash	7.14±0.03	7.25±0.06	7.39±0.08	7.58±0.04
NFE	38.28±0.05	37.55±0.11	36.60±0.01	36.61±0.12

Values presented as Mean ± SE

The fingerlings of *P. hypophthalmus* were procured from a fish farm located at Bobli, Andhra Pradesh. The fish were acclimatised to the experimental conditions and fed initially with floating pellet feed which formed the basal diet at the Research and Instructional Fish Farm, College of Fisheries, Mangaluru, Karnataka, India. Uniform sized Striped catfish fingerlings with an average length and weight of 7.34±0.09cm and 35.35±1.02g respectively were stocked @ 15 numbers/tank. The duration of experiment was 90 days. Water exchange was done fortnightly to get rid of the left-over feed and faecal matter. Fishes were fed at 5% of their body weight till the end of the experiment. The feed was broadcast over the surface of water twice daily in the morning and evening. Water samples were collected on each sampling days and were analyzed for pH, temperature, dissolved oxygen, ammonia, nitrite, nitrate, total alkalinity and total hardness by following the standard procedures (APHA, 1995) [4]. Water quality parameters were maintained within the optimal range throughout the experimental period. The fishes were sampled at an interval of 15 days to record the growth. The stocked fishes were measured for increase in length and weight. After the completion of the experiment, fish samples were collected for further analysis of different parameters.

2.1 Total Weight Gain

Total weight gain was expressed as weight gain percentage of initial weight.

Weight Gain (g) = Mean final weight (g) - Mean initial weight (g)

$$\text{Weight Gain (\%)} = \frac{\text{Final Weight (g)-Initial Weight(g)}}{\text{Initial Weight(g)}} \text{ X } 100$$

2.2 Specific Growth Rate (SGR %)

SGR is a coefficient that measures the percentage increase in fish weight per day. Specific growth rate. was calculated by using the following. formula:

$$\text{SGR(\%)} = \frac{[\text{Ln(Final weight)} - \text{Ln(Initial weight)}]}{\text{No. of days of Rearing}} \times 100$$

2.3 Protein Efficiency Ratio (PER)

PER can be. defined as the weight gain of test group/protein consumed by the test group. Protein efficiency. ratio was delineated with the following formula:

$$\text{PER} = \frac{\text{Gain in body. weight}}{\text{Protein intake}}$$

2.4 Feed Conversion Ratio (FCR)

FCR represents kilograms of feed needed to produce one kilogram of fish in culture.

Food conversion ratio was calculated by using the following formula:

$$FCR = \frac{\text{Dry weight of the feed given}}{\text{Gain in wet weight of fish}}$$

This indicated the given amount of feed required to produce a unit weight of a fish.

2.5 Survival

After the end of experiment, the survived fishes were. collected and calculated.

$$\text{Survival (\%)} \quad \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

At the end of the experiment after 90 days, the fish were sacrificed and the intestine was collected from the three treatment groups and the control group and stored in 4% Neutral Buffered Formalin (NBF). Collected intestinal tissue samples were processed by routine paraffin embedding techniques. The tissue sections of 5µ thickness of the midgut were obtained with the help of a Microtome and stained with haematoxylin and eosin (Bullock, 1989) [8]. The histological changes manifested in the midgut of fish samples were observed under the light microscope attached with an ultrascope 9.1-v.

3. Results and Discussion

The study was carried to investigate the effect of Giloy (*T. cordifolia*) on growth, survival and gut histology of Striped catfish (*P. hypophthalmus*) reared in cement tanks with treatment diets T_1, T_2 and T_3 with a control (T_0) for a period of 90 days at the Research and Instructional Fish Farm, Department of Aquaculture, College of Fisheries, Mangaluru, Karnataka, India.

3.1 Growth Performance and Survival of Striped Catfish

In the present study, weight gain percentage was significantly higher in T_3 (216.32±0.69 %) when compared to T_2 (197.58±0.59%), T_1 (187.51±0.43%) and the control (156.4±0.31%) (p<0.05) (Table 3). The findings of the present study are in agreement with those of Turaihi *et al.* (2025) [27] who reported very high weight gain percentage of 303.35% with 0.6% Giloy stem powder dietary supplementation in Common Carp (*Cyprinus carpio*) when compared to the control (167.23%). In the present investigation, significantly higher Specific Growth Rate (SGR) was observed in T_3 (1.28±0.03%) when compared to the other treatments and the control (1.05±0.01%) (p<0.05) (Table 3). The findings of the present study are in accordance with Basuini *et al.* (2022) [6] who reported significantly higher SGR (2.81 ± 0.06%) when fed with diet supplemented with Giloy powder at 0.6% when compared to the control (2.30 ± 0.04%) in Nile Tilapia. In the present investigation, significantly higher Protein Efficiency Ratio (PER) was observed in T_3 (1.99±0.06) followed by T_2 (1.95±0.05), T_1 (1.89±0.04) and the Control (1.64±0.03) (p<0.05) (Table 3). Similar results were recorded by Latha *et al.* (2020) [20] who observed significantly higher PER of 2.49 ± 0.12 when fed with a diet supplemented with leaf extract of Giloy at 0.04% in Striped catfish when compared to the control (1.80±0.07). In the present study, significantly better Food Conversion Ratio (FCR) was observed in T_3 (1.33±0.02) followed by T_2 (1.37±0.01), T_1 (1.41±0.03) and the Control (1.62±0.05) (p<0.05) (Table 3). In the present study, there was no significant difference (p<0.05) observed within the three treatment groups for the FCR values (Table 3). Similar results were reported by Turaihi *et al.* (2025) [27] who observed that there was no significant difference between the FCR values with 4% Giloy stem powder (1.39±0.01) and 6% Giloy root powder (1.42±0.03). However, a very high FCR (2.41±0.20) was reported by Upreti and Chauhan (2018) [28] in Catla when fed with a diet supplemented with 1% leaf powder of Giloy. In the present study, the growth parameters of weight gain, SGR, FCR and PER were greatly improved by the dietary supplementation of Giloy. This clearly indicates that the dietary supplementation of Giloy greatly improved the overall health of Striped catfish when compared to the control. Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) are commonly used to measure the efficiency and growth promoting effect of herbal nutraceuticals in fish feed (Afifah *et al*., 2021; Meena *et al*., 2022; Hassan *et al*., 2025) [1, 23, 14].

Table 3: Growth performance and Survival rate of Striped catfish under different treatments and control

Parameters	Control (T_0)	Treatments		
		T_1	T_2	T_3
Weight gain (%)	156.4±0.31^{d}	187.51±0.43^{c}	197.58±0.59^{b}	216.32±0.69^{a}
SGR (%)	1.05±0.01^{c}	1.17±0.03^{bc}	1.21±0.02^{b}	1.28±0.03^{a}
PER	1.64±0.03^{b}	1.89±0.04^{a}	1.95±0.05^{a}	1.99±0.06^{a}
FCR	1.62±0.05^{a}	1.41±0.03^{b}	1.37±0.01^{b}	1.33±0.02^{b}
Survival rate (%)	97.78±2.22^{b}	100±0^{a}	100±0^{a}	100±0^{a}

Values expressed as Mean ± Standard Error (M ± SE). Values with different superscripts in the same column are significantly different (p<0.05)

In the present study, a significantly higher Survival Rate was recorded in all the Giloy supplemented treatments, T_1, T_2, T_3 (100±0.0%) when compared to the Control, T_0 (97.78±2.22) (p<0.05). However, there was no significant difference observed within the treatment groups, T_1, T_2 and T_3 (Table 3 and Fig.1). It is clearly evident from the results of the present study that Giloy is having a overall health promoting effect when fed as a dietary supplement to Striped catfish. Similar results were obtained by Basuini *et al.* (2022) [6] who reported significantly higher Survival Rate (100±0.0%) when fed with diet supplemented with Giloy powder at 0.8% level when compared to the control

(95.00±2.89) in Nile Tilapia. The findings of the present study are in agreement with those of Upreti and Chauhan (2024) [26] who reported similar findings of significantly higher Survival Rate (100±0.00%) in Catla

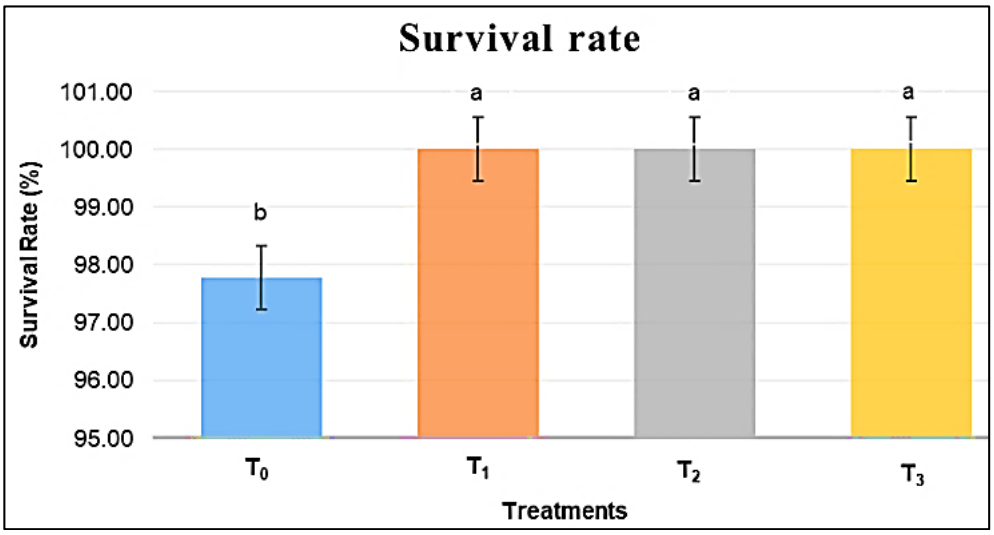

Fig 1: Survival rate of Striped catfish under different treatments and control

fed with diet supplemented with 1% Leaf powder of Giloy when compared to the control (95.3 ± 0.8%). On the contrary, Latha *et al*., (2020) [20] reported significant difference between the treatment and the control group as well as within treatments with respect to survival and observed significantly higher Survival in 0.04% dietary supplementation of Giloy leaf extract (94.67±2.08%) when compared to the control (75.34±2.06%) in Striped catfish.

3.1 Gut Histology of Striped Catfish

In the present study, the midgut tissues of Striped catfish fed with both control and experimental diets (Fig. 2) showed distinct structural features. Transverse sections of the midgut revealed well preserved musculosa and mucosa layers along with a noticeably thick serosa. The intestinal villi appeared stout and robust accompanied by a higher number of Goblet cells in fish fed the experimental diets (Fig. 2). The treatments T_3 and T_2 revealed notably longer villi when compared to T_1 clearly indicating that higher dose of Giloy increased the nutrient absorptive area. While, mid-gut section of the fish fed with the control diet revealed non-intact musculosa with short and stout architecture of villi and increased lumen space. Whereas, the mid-gut of fish fed with Giloy (*T. cordifolia*) diet showed increased length of villi with intact mucosa, musculosa and serosa, prominent lamnia propria and decreased lumen space. It is evident from the results of the present study that the antioxidant compounds of Giloy, neutralize reactive oxygen species in the gut, protecting epithelial cells and maintaining mucosal health. The histology of fish gut is crucial for understanding their digestive and metabolic functions. The gut is divided into specialized regions including the foregut, midgut and hindgut each with distinct histological features tailored for nutrient absorption and digestion but majority absorption is found in the mid gut section (Marco *et al.*, 2023; Guillén and Yúfera, 2025) [22, 12]. The findings of the present study are in agreement with those of Thayes *et al*. (2024) [26] who observed that dietary Ashwagandha (*Withania somnifera*) showed higher growth of intestinal villi length and width when administered at the rate of 6% in fingerlings of Catla. Intestinal histology of Grey Mullet (*Liza ramada*) fed with Ajwain (*Trachyspermum ammi*) revealed intact structures of the intestinal wall and villi with an outer serosal layer (Basuini *et al*., 2025) [5]. Dietary inclusion of Maidenhair Tree (*Ginkgo biloba*) leaf at the rate of 0.9% observed to increase villi length of proximal, middle, and distal intestinal parts in Nile Tilapia (Latif *et al*., 2021) [21]. In the present study, increased number of goblet cells were found in the midgut sections of fish of T_2 (1.5%) and T_3(2%) compared to the control. The findings of the present study are in agreement with those of Yaqoob *et al*., (2024) [29] who reported that dietary supplementation of Ginger (*Z. officinale*) at the level of 0.5% showed unbroken epithelial barrier with more goblet cells in Nile Tilapia compared to the control. Similar results were reported by Heidarieh *et al*. (2013) [16] who observed that dietary inclusion of *Aloe vera* at 0.1% exhibited high density of the goblet cells per villus in the gut histology of Rainbow Trout (*Oncorhynchus mykiss*). Similarly, dietary supplementation of Turmeric (*Curcuma Longa*) at inclusion level of 0.2%, resulted in high number of goblet cells in the gut epithelium compared to the control in Nile Tilpia (Yusuf *et al*., 2017) [20]. The increase in number of goblet cells indicate enhanced mucus secretion leading to trapping of fish pathogens and fish parasites thereby offering protection from diseases. Hence, the increased goblet cell numbers clearly indicate enhanced disease resistance (Zahran *et. al.*, 2020) [31].

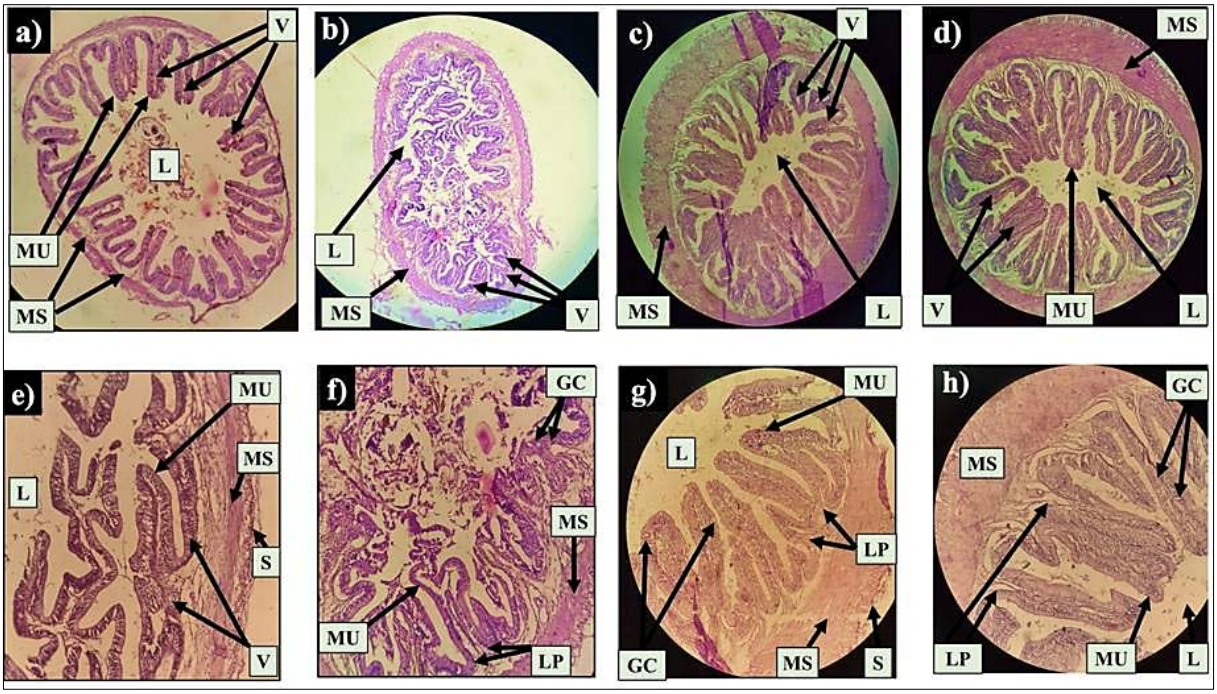

Fig 2: Histology of midgut of Striped Catfish: a) Control 10x; b) T_1 10x; c) T_2 10x; d) T_3 10x; e) Control 40x; f) T_1 40x; g) T_2 40x; h) T_3 40x, L- Lumen, V-Villi, MU-Mucosa, MS- Musculosa, S-Serosa, GC-Goblet Cell, LP-Lamina Propria

4. Conclusion

In the present study, weight gain was significantly higher in T_3 (216.32±0.69%) when compared to the other treatments and the control (p<0.05). In the present investigation, significantly higher SGR was observed in T_3 (1.28±0.03%) followed by the other treatments and the control (p<0.05). Significantly better FCR was observed in T_3 (1.33±0.02) followed by other treatments and the control (p<0.05). In the present study, significantly higher PER was observed in T_3 (1.99±0.06) followed by other treatments and the control (p<0.05). Growth parameters of Weight Gain, SGR, FCR and PER were enhanced by the dietary supplementation of Giloy which improved the overall health of Striped catfish when compared to the control. In the present investigation, significantly higher Survival percentage of 100% was observed in all the three Giloy incorporated treatment diets when compared to the control (p<0.05). The treatment T_3 exhibited longer and wider villi when compared to T_2, T_1 and control. The villi were longer and wider in all the treatments when compared to that of the control clearly indicating increased absorptive area and better nutrition. There was increase in the number of goblet cells in the treatment groups, indicating better disease resistance. It is clearly evident from the results of the present study that Giloy is having overall health promoting effect when fed as a dietary supplement to Striped catfish and it is recommended to use Giloy as a dietary supplement in Striped catfish at a rate of 2% in the feed for the best growth promoting effects.

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References

1. Afifah D, Arief M, Al-Arif MA. The effect of garlic (*Allium sativum*) and turmeric (*Curcuma longa*) extract addition in commercial feed on feeding rate, feed efficiency and feed conversion ratio of gouramy fish (*Osphronemus gouramy*). IOP Conf Ser Earth Environ Sci. 2021;679(1):012073.
2. Alam F. Measuring technical, allocative and cost efficiency of pangas (*Pangasius hypophthalmus* Sauvage, 1878) fish farmers of Bangladesh. Aquac Res. 2011;42(10):1487-1500.
3. AOAC. Official methods of analysis. Washington (DC): AOAC International; 2017.
4. APHA. Standard methods for the examination of water and wastewater. Eaton AD, Clesceri LS, Greenberg AE, editors. Washington (DC): APHA; 1995.
5. Basuini EMF, Hussein AT, El-Hais AM, Elhetawy AI, Soliman AA, Gabr SA, *et al*. Impacts of dietary Ajwain (*Trachyspermum ammi* L.) on growth, antioxidative capacity, immune responses, and intestinal histology of grey mullet (*Liza ramada*). Aquaculture. 2025;595:741706.
6. Basuini EMF, Teiba II, Shahin SA, Mourad MM, Zaki MA, Labib EM, *et al*. Dietary Guduchi (*Tinospora cordifolia*) enhanced the growth performance, antioxidative capacity, immune response and ameliorated stress-related markers induced by hypoxia stress in Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol. 2022;120:337-44.
7. Bhat R, Tandel R, Pandey PK. Alternatives to antibiotics for combating the antimicrobial resistance in aquaculture. Indian J Anim Health. 2022;61:1-18.
8. Bullock AM. Laboratory methods. In: Roberts RJ, editor. Fish pathology. London: Bailliere Tindall; 1989. p. 374-406.
9. Das SK, Mandal A, Khairnar SO. Aquaculture resources and practices in a changing environment. In: Sustainable agriculture systems and technologies. 2022. p. 169-99.
10. Dawood MA, Koshio S, Esteban MÁ. Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Rev Aquac. 2018;10(4):950-74.

11. Founou LL, Founou RC, Essack SY. Antimicrobial resistance in the farm-to-plate continuum: more than a food safety issue. Future Sci OA. 2021;7(5):FSO692.
12. Guillén NC, Yúfera M. Understanding rhythms in the digestive functionality of fish gut. J Exp Biol. 2025;228(14):jeb249942.
13. Gupta A, Gupta P, Bajpai G. *Tinospora cordifolia* (Giloy): An insight on the multifarious pharmacological paradigms of a most promising medicinal ayurvedic herb. Heliyon. 2024;10(4):e03529.
14. Hassan HU, Ali A, Al Sulivany BS, Bilal M, Kanwal R, Raza MA, *et al*. Investigation of the effects of phytogenic dietary additives on growth performance, nutrient utilization, economic efficiency and health of *Pangasius hypophthalmus*: implications for sustainable aquaculture development. Sci Rep. 2025;15(1):22661.
15. Hastings WH. Fish nutrition and fish feed manufacture. FAO Technical Conference on Aquaculture; 1976; Kyoto, Japan. FIR:Aq/Conf/76/R.23.
16. Heidarieh M, Mirvaghefi AR, Sepahi A, Sheikhzadeh N, Alishahbazfar A, Akbari M. Effects of dietary *Aloe vera* on growth performance, skin and gastrointestine morphology in rainbow trout (*Oncorhynchus mykiss*). Turk J Fish Aquat Sci. 2013;13(2):361-70.
17. Jeyavani J, Sibiya A, Sivakamavalli J, Divya M, Preetham E, Vaseeharan B, *et al*. Phytotherapy and combined nanoformulations as a promising disease management in aquaculture: a review. Aquac Int. 2022;30(2):1071-86.
18. Kolawole TO, Mustapha AY, Mbata AO, Tomoh BO, Forkuo AY, Kelvin-Agwu MC. Innovative strategies for reducing antimicrobial resistance: a review of global policy and practice. 2023.
19. Kumar P, Kaur S, Goswami M, Singh S, Sharma A, Mehta N. Antioxidant and antimicrobial efficacy of giloy (*Tinospora cordifolia*) stem powder in spent hen meat patties under aerobic packaging at refrigeration temperature (4±1°C). J Food Process Preserv. 2021;45(10):e15772.
20. Latha C, Krishnakumar V, Munuswamy N. Growth, survival and haemato-biochemical profiles of the freshwater catfish, *Pangasius sutchi* (Fowler, 1937) fingerlings fed on *Tinospora cordifolia* leaf extract supplemented diet. Egypt J Aquat Biol Fish. 2020;24(1):249-62.
21. Latif AHM, Hendam BM, Nofal MI, El-Son MA. *Ginkgo biloba* leaf extract improves growth, intestinal histomorphometry, immunity, antioxidant status and modulates transcription of cytokine genes in hapa-reared *Oreochromis niloticus*. Fish Shellfish Immunol. 2021;117:339-49.
22. Marco DG, Cappello T, Maisano M. Histomorphological changes in fish gut in response to prebiotics and probiotics treatment to improve their health status: a review. Animals. 2023;13(18):2860.
23. Meena DK, Sahoo AK, Jayant M, Sahu NP, Srivastava PP, Swain HS, *et al*. Bioconversion of *Terminalia arjuna* bark powder into a herbal feed for *Labeo rohita*: can it be a sustainability paradigm for green fish production? Anim Feed Sci Technol. 2022;284:115132.
24. Sagar PK, Candra H, Sagar A, Murugeswaran R. Anti-cancerous, anti-tumour, anti-carcinogenic agents-herbal medicinal plants and their immense pharmacological, therapeutics medicinal potent values. Asian J Pharm Res Dev. 2025;13(1):238-58.
25. Singh AK. Assessing the sustainability and food security implications of introduced *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822) into Indian aquaculture: a OneHealth perspective. Rev Aquac. 2025;17(1):e12990.
26. Thayes C, Chauhan S, Ali B, Rawal YK. Ashwagandha (*Withania somnifera*) potential effects on growth, intestinal histomorphology, and antioxidant enzyme activity in fingerlings of *Catla catla* (Hamilton, 1822). Environ Conserv J. 2024;25(3):71-9.
27. Turaihi AZMR, Al-Niaeem KS, Ahmed AR. Assessment of growth performance, blood biochemical parameters, and intestinal microbiota in common carp (*Cyprinus carpio*) following dietary *Tinospora cordifolia* root and stem powder supplementation. Vet Integr Sci. 2025;23(3):1-11.
28. Upreti P, Chauhan RS. Effect of leaf powder of giloy (*Tinospora cordifolia*) in fish feed on survival and growth of post larvae of *Catla catla*. J Appl Nat Sci. 2018;10(1):165-9.
29. Yaqoob MA, Khalid MS, Javed R, Raza M, Ashraf Z, Bashir Z, *et al*. Ameliorative effect of ginger on hematology and histopathology of Nile tilapia (*Oreochromis niloticus*). J Wildl Ecol. 2024;8:235-44.
30. Yusuf MS, Hassan MA, Tag HM, Sarivistava K, Reddy PG, Hassan AM. Influence of turmeric (*Curcuma longa*) on performance, histomorphology and microbiota of intestine in juvenile tilapia (*Oreochromis niloticus*). Int J Agric Sci Vet Med. 2017;5(1):7-16.
31. Zahran E, Sebaei MGE, Awadin W, Elbahnaswy S, Risha E, Elseady Y. *Withania somnifera* dietary supplementation improves lipid profile, intestinal histomorphology in healthy Nile tilapia (*Oreochromis niloticus*), and modulates cytokines response to *Streptococcus* infection. Fish Shellfish Immunol. 2020;106:133-41.