Effect of Dietary Turmeric (Curcuma longa) Powder on Growth, Haemoglobin and Muscle Biochemical Parameters of the Climbing Perch, Anabas testudineus

Dr. Sunitha A. Philip
Associate Professor, Post Graduate and Research Department of Zoology, Mar Thoma College, Kuttapuzha, Tiruvalla, Kerala, India

***Abstract*— Plant-derived feed additives are being explored as low-cost ways to improve fish growth. This study tested turmeric (Curcuma longa) powder, included at 5, 10, 15 and 20% (w/w) in a basal pellet diet, in the climbing perch Anabas testudineus. Fingerlings (75 fish) were fed five diets, with three replicate tanks per diet and five fish per tank, for four months (May–August). Cumulative weight gain was recorded monthly; haemoglobin and muscle protein (Lowry) and carbohydrate (anthrone) were measured at the end. Tank means (n = 3 per diet) were compared by one-way ANOVA with Dunnett's test against the control and Tukey's test among diets. Weight gain did not differ in May, but from June to August every turmeric diet exceeded the control ($P \leq 0.026$). Gain was highest at 15% turmeric (20.64 ± 0.58 g versus 12.48 ± 0.47 g in the control, +65%) and significantly higher than at 20% (18.96 ± 0.60 g; $P = 0.015$). Haemoglobin rose with dose, from 6.12 ± 1.31 g/dL (control) to 10.83 ± 2.23 g/dL (20%); the overall test was borderline ($P = 0.051$) and only the 20% diet differed from the control ($P = 0.030$). Muscle protein ($P = 0.57$) and carbohydrate ($P = 0.95$) did not differ significantly. Dietary turmeric at 5–20% improved growth, with the best response at 15%, but the haematological effect is only suggestive and no biochemical effect was demonstrated. The diets were not analysed or balanced for nutrients, and replication was small.**

***Index Terms*— Anabas testudineus, Curcuma longa, turmeric, fish growth, haemoglobin, aquafeed**

I. INTRODUCTION

Aquaculture is one of the fastest-growing food-producing sectors, and its further expansion depends on feeds that are affordable and that improve growth and health without routine use of antibiotics (FAO, 2024; Reverter et al., 2014). Plant-derived feed additives have attracted interest because they contain bioactive compounds that may stimulate appetite and digestion, act as antioxidants and modulate immunity (Citarasu, 2010; Harikrishnan et al., 2011; Dawood et al., 2018). Dietary plant materials have been reported to improve growth, immune activity or disease resistance in cultured fishes, for example green tea in Nile tilapia (Abdel-Tawwab et al., 2010), crude plant extracts in tilapia (Immanuel et al., 2009; Kareem et al., 2016) and garlic peel in African catfish (Thanikachalam et al., 2010).

Turmeric, the rhizome of Curcuma longa L. (Zingiberaceae), is a common spice whose main bioactive constituents, the curcuminoids, make up only a small fraction of the dried rhizome (Priyadarsini, 2014). In fish, dietary curcumin has raised digestive enzyme activity and the expression of growth-related genes in tilapia (Midhun et al., 2016). In Nile tilapia, moderate curcumin levels improved weight gain, antioxidant and immune indices, whereas high levels fed for a long period raised markers of inflammation and apoptosis (Amer et al., 2022). Those studies used purified curcumin at low inclusion rates. Much less is known about whole turmeric powder at the higher inclusion levels that a farmer might use, and few studies have examined turmeric in locally important species.

The climbing perch, Anabas testudineus (Bloch, 1792), is an air-breathing freshwater fish of South and Southeast Asia that is cultured for its market value; its dietary protein requirement has been studied in fry (Hossain et al., 2012). The present study evaluated the effect of 5, 10, 15 and 20% (w/w) dietary turmeric powder on weight gain, haemoglobin and muscle protein and carbohydrate of A. testudineus fingerlings

over four months, and aimed to identify the inclusion level giving the best response. It is a small tank trial with three replicate tanks per diet, and the conclusions are drawn accordingly.

II. MATERIALS AND METHODS

A Fish and acclimation

Healthy fingerlings of A. testudineus were obtained from a fish seed farm at Tiruvalla (National Fish Seed Farm), kept for 10 days in a common aquarium under ambient temperature in well-ventilated rooms and fed the common feed to acclimatise. They were then distributed at five fish per tank after the initial length and weight were recorded. The experiment had five dietary treatments, each with three replicate tanks (15 tanks, 75 fish in total; Table 1). The feeding trial lasted four months (May–August).

B Diet preparation

The common (basal) feed was prepared from crushed maize (200 g), wheat bran (200 g), soybean meal (200 g), rice bran (200 g), groundnut oil cake (100 g), dried prawn (50 g) and refined wheat flour (maida, 50 g). The ingredients were powdered, sieved and mixed, made into a dough with hot water, pressed into small pellets, sun-dried for five days and stored in containers. Turmeric diets were prepared by mixing powdered turmeric with the powdered common feed on a weight basis to give 5, 10, 15 and 20% turmeric (for example, 150 mg turmeric with 850 mg feed for the 15% diet), weighed on an electronic balance, pelleted and dried in shade. The control diet was the common feed without turmeric. Turmeric was therefore added in place of an equal weight of the common feed, and the diets were not formulated to be equal in protein or energy.

C Growth

Weight gain of the fish in each tank was recorded monthly from May to August, and the mean per tank was calculated. Growth is reported as cumulative weight gain (g per fish).

D Haemoglobin

Blood was drawn from the heart with a syringe and needle into a watch glass rinsed with EDTA. Haemoglobin was determined by the acid haematin method with a Sahli haemoglobinometer: the graduated tube was filled with 0.1 N HCl to the 2% mark, 20 µL of blood was added with the haemoglobinometer pipette, the pipette was rinsed twice in the acid, and the mixture was stirred and left for 10 minutes. Distilled water was then added drop by drop until the colour matched the standard tube, and the haemoglobin concentration was read in g/dL.

E Muscle protein and carbohydrate

One gram of muscle tissue was ground with 10 ml of water in a mortar and pestle and centrifuged, and the supernatant was used for the assays. Protein was estimated by the Lowry method (Lowry et al., 1951) with bovine serum albumin as the standard: 1 ml of extract was mixed with 5 ml of alkaline copper reagent, left for 10 minutes, then incubated in the dark at room temperature for 30 minutes after addition of Folin's reagent, and absorbance was read at 660 nm. Carbohydrate was estimated by the anthrone method (Roe, 1955): 0.5 ml of extract (made up to 1 ml with distilled water) was mixed with 4 ml of anthrone reagent in an ice bath, heated in a boiling water bath for 8 minutes, cooled rapidly, and absorbance was read at 630 nm against a glucose standard series. Because anthrone reacts with hexose sugars in general, the result is expressed as glucose equivalents. Values are given as mg per g of muscle tissue.

F Statistical analysis

The tank was the experimental unit. Values are tank means, and results are presented as mean ± standard deviation (SD) of the three tank means per diet. Each variable (haemoglobin, protein, carbohydrate and weight gain in each month) was compared among the five diets by one-way ANOVA (4 and 10 degrees of freedom). Each turmeric diet was compared with the control by Dunnett's test (Dunnett, 1955), and the diets were compared with one another by Tukey's HSD test. Monthly weight gains come from the same tanks and are not independent, so each month was analysed separately and no diet × time interaction was tested. With three tanks per diet the assumptions of normality and equal variance cannot be checked reliably, and the results are interpreted cautiously. The analyses were run in Python (SciPy) from the means, SDs and tank numbers; the significance level was $P < 0.05$.

III. RESULTS

A Weight gain

Cumulative weight gain did not differ among diets in May ($F_{4,10}$ = 1.18, P = 0.375). From June onward it differed strongly among diets (June F = 55.7, July F = 71.5, August F = 128.9; all P < 0.001), and every turmeric diet exceeded the control (Dunnett, P = 0.001 to P < 0.001 in June; P = 0.026 to P < 0.001 in July; P = 0.001 to P < 0.001 in August; Table 2, Fig. 1).

At the end of the trial in August, cumulative gain was 12.48 ± 0.47 g in the control, 14.62 ± 0.39 g at 5% (+17%), 17.76 ± 0.45 g at 10% (+42%), 20.64 ± 0.58 g at 15% (+65%) and 18.96 ± 0.60 g at 20% (+52%). The 15% diet gave the highest gain in each month from June to August. It exceeded the 10% diet (August, P < 0.001) and the 20% diet (June P = 0.007, July P = 0.018, August P = 0.015), whereas the 10% and 20% diets did not differ significantly in August (P = 0.090).

B Haemoglobin

Haemoglobin increased with the turmeric level (Table 3, Fig. 2a): 6.12 ± 1.31 g/dL in the control, 6.51 ± 1.48 at 5%, 7.77 ± 1.83 at 10%, 9.11 ± 2.01 at 15% and 10.83 ± 2.23 g/dL at 20%. The overall ANOVA was borderline ($F_{4,10}$ = 3.46, P = 0.051). In Dunnett's test only the 20% diet differed from the control (P = 0.030; 5%, 10% and 15%: P ≥ 0.196). Because the overall test did not reach P < 0.05, this comparison should be regarded as exploratory.

C Muscle protein and carbohydrate

Mean muscle protein was 1.34 ± 0.38 mg/g in the control and 1.51 to 2.50 mg/g in the turmeric groups, the highest value being at 15% (2.50 ± 1.34), but the diets did not differ significantly ($F_{4,10}$ = 0.76, P = 0.574; Fig. 2b). Muscle carbohydrate was 1.89 ± 0.22 mg/g in the control and 2.02 to 2.54 mg/g in the turmeric groups, again without a significant difference ($F_{4,10}$ = 0.18, P = 0.945; Fig. 2c). The SDs among tanks in the turmeric groups were large (40–60% of the mean), so the study had little power to detect differences in these two variables (Table 3).

IV. DISCUSSION

Turmeric powder at 5–20% of the diet improved the weight gain of A. testudineus fingerlings, and the response was large and consistent: from June onward every turmeric diet exceeded the control, and gain was greatest at 15%. Growth followed an inverted-U pattern, with the 20% diet still above the control but below the 15% diet. Improved growth with plant additives is in line with reports for tilapia and catfish (Immanuel et al., 2009; Thanikachalam et al., 2010), and with the higher digestive enzyme activity and growth-related gene expression found in tilapia fed curcumin (Midhun et al., 2016). A decline in response at the highest level agrees with the observation that curcumin is beneficial at moderate doses but may become harmful at high doses in long-term feeding (Amer et al., 2022). These mechanisms were not examined here: feed intake, feed conversion, enzyme activity and tissue histology were not measured, so the study cannot show why growth improved.

Several features of the design limit what can be concluded about the cause. Turmeric was added in place of common feed, and the diets were not analysed or balanced for protein, energy or fibre, so a difference in nutrient content could contribute to the growth difference, not only a curcuminoid effect. The inclusion levels (50–200 g per kg of diet) are also much higher than the curcumin levels used in most feeding studies, and because curcuminoids are only a small part of turmeric (Priyadarsini, 2014), the curcuminoid dose cannot be compared directly with those studies. Finally, the 15% level was the best of the four levels tested; intermediate or lower levels, which could be more economical, were not tested.

Haemoglobin rose with the turmeric level, but the evidence is weak: the overall test was borderline (P = 0.051), the SDs were large, and only the highest dose differed from the control. The present result should be treated as a hypothesis for further testing, with more replicate tanks and additional blood indices such as red cell count and haematocrit. Muscle protein and carbohydrate did not differ significantly among diets. The higher mean values at 15% are therefore not evidence of an effect and should not be interpreted as an improvement in nutritional quality.

The study has limitations. Only three tanks were used per diet, which restricts power and prevents checking statistical assumptions. The trial covered one season (May–August) and one batch of fish, and survival, feed intake, water quality, fish sex and proximate composition of the diets were not analysed here. The haemoglobin and biochemical measurements were made at a single time point and the protein and carbohydrate assays measured water-extractable

fractions of muscle, not total protein or carbohydrate. The study also did not assess safety, for example by liver histology, at the higher inclusion levels. Future work should use more replicate tanks, isonitrogenous and isoenergetic diets, a wider range of low-to-moderate inclusion levels, purified curcumin as a comparison, and measurements of feed conversion, digestive enzymes, immune and antioxidant indices and tissue histology.

V. CONCLUSION

Dietary turmeric powder at 5–20% (w/w) significantly improved the cumulative weight gain of Anabas testudineus fingerlings over four months, with the highest gain at 15% (+65% over the control in August) and a lower gain at 20%. Haemoglobin tended to rise with dose, but the evidence was borderline and only the 20% diet differed from the control. Muscle protein and carbohydrate were not significantly affected. A 15% inclusion was the best of the levels tested, but because the diets were not nutrient-matched and the trial was small, the findings should be confirmed in larger, nutrient-balanced trials before being applied in farming.

VI. DECLARATIONS

Ethical statement: The feeding trial was carried out as a postgraduate project in the Department of Zoology, Mar Thoma College, under the supervision of the author. Fish were reared in laboratory tanks and handled with care to minimise stress, and blood was collected from the heart at the end of the trial. The author confirms that the work followed the institutional requirements applicable to the study.

Conflict of interest: The author declares that there is no conflict of interest.

Funding: No external funding was received for this study.

Generative AI disclosure: Generative AI was used as an editorial aid during manuscript drafting, language refinement, statistical computation from the reported summary values and formatting. The author retains full responsibility for the study design, experimental procedures, data, statistical results, interpretation, references and final submitted version.

Data availability: The individual observations underlying the reported means and standard deviations are held by the author and can be made available by the corresponding author on reasonable request.

ACKNOWLEDGEMENTS

The author thanks Jibin Abraham Dolly, who carried out this M.Sc. project under the author's supervision in the Department of Zoology, Mar Thoma College, Kuttapuzha, Tiruvalla, Kerala. The author also thanks the department and its laboratory staff for facilities and support.

TABLES AND FIGURES

Treatment	Turmeric (% w/w)	Common feed (% w/w)	Replicate tanks	Fish per tank	Total fish
T1 (control)	0	100	3	5	15
T2	5	95	3	5	15
T3	10	90	3	5	15
T4	15	85	3	5	15
T5	20	80	3	5	15
Total			15		75

Table 1. Experimental design and dietary treatments for A. testudineus (four-month trial, May–August).

Turmeric in diet	May	June	July	August
Control	4.16 ± 0.27	6.32 ± 0.17	9.16 ± 0.64	12.48 ± 0.47
5%	4.04 ± 0.37	7.92 ± 0.58∗	10.64 ± 0.40∗	14.62 ± 0.39∗
10%	4.31 ± 0.23	8.98 ± 0.48∗	13.02 ± 0.46∗	17.76 ± 0.45∗
15%	4.54 ± 0.45	10.68 ± 0.16∗	15.88 ± 0.52∗	20.64 ± 0.58∗
20%	4.05 ± 0.30	9.26 ± 0.30∗	14.10 ± 0.68∗	18.96 ± 0.60∗
ANOVA $F_{4,10}$ (P)	1.18 (0.375)	55.7 (<0.001)	71.5 (<0.001)	128.9 (<0.001)

Table 2. Cumulative weight gain (g per fish) of A. testudineus fed diets containing 0–20% turmeric. Values are mean ± SD of three tank means.

Significantly different from the control (Dunnett's test, $P < 0.05$). In June, July and August the 15% diet also exceeded the 20% diet (Tukey, $P = 0.007$, 0.018 and 0.015).

Turmeric in diet	Haemoglobin (g/dL)	Muscle protein (mg/g)	Muscle carbohydrate (mg/g)
Control	6.12 ± 1.31	1.34 ± 0.38	1.89 ± 0.22
5%	6.51 ± 1.48	1.51 ± 0.39	2.29 ± 1.37
10%	7.77 ± 1.83	1.96 ± 1.12	2.33 ± 1.03
15%	9.11 ± 2.01	2.50 ± 1.34	2.54 ± 1.33
20%	10.83 ± 2.23∗	2.13 ± 1.00	2.02 ± 1.02
ANOVA $F_{4,10}$ (P)	3.46 (0.051)	0.76 (0.574)	0.18 (0.945)

Table 3. Haemoglobin and muscle protein and carbohydrate of A. testudineus after four months on diets containing 0–20% turmeric. Values are mean ± SD of three tank means.

Different from the control (Dunnett's test, $P = 0.030$); the overall ANOVA for haemoglobin was borderline, so this comparison is exploratory. Carbohydrate is expressed as glucose equivalents.

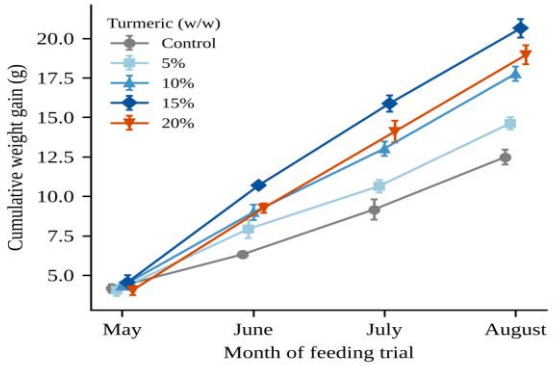

Fig. 1. Cumulative weight gain (g per fish) of A. testudineus fed diets with 0–20% turmeric from May to August. Points are means and bars are SD of three tank means.

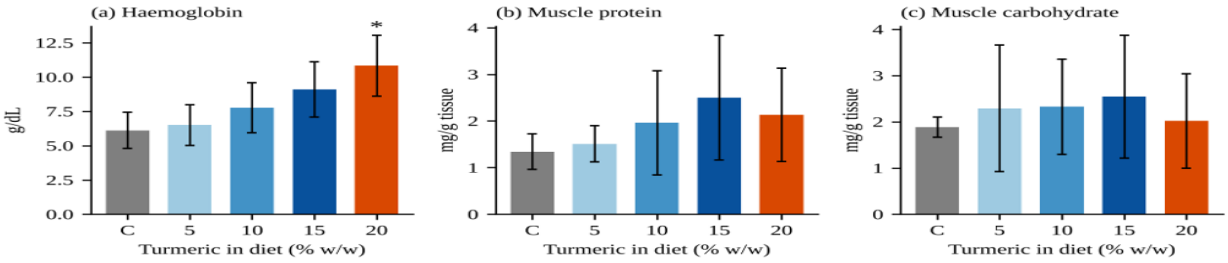

Fig. 2. (a) Haemoglobin, (b) muscle protein and (c) muscle carbohydrate of A. testudineus after four months on diets with 0–20% turmeric (C = control). Bars are means and error bars are SD of three tank means. ∗ Different from the control (Dunnett's test, $P = 0.030$).

REFERENCES

[1] M. Abdel-Tawwab, M. H. Ahmad, M. E. A. Seden, and S. F. M. Sakr, "Use of green tea, *Camellia sinensis* L., in practical diet for growth and protection of Nile tilapia, *Oreochromis niloticus* (L.), against *Aeromonas hydrophila* infection," *Journal of the World Aquaculture Society*, vol. 41, no. S2, pp. 203–213, 2010.

[2] S. A. Amer, D. A. El-Araby, H. Tartor, M. Farahat, N. I. A. Goda, M. F. M. Farag, E. M. Fahmy, A. M. Hassan, M. F. Abo El-Maati, and A. Osman, "Long-term feeding with curcumin affects the growth, antioxidant capacity, immune status, tissue histoarchitecture, immune expression of proinflammatory cytokines, and apoptosis indicators in Nile tilapia, *Oreochromis niloticus*," *Antioxidants*, vol. 11, no. 5, Art. no. 937, 2022, doi: 10.3390/antiox11050937.

[3] T. Citarasu, "Herbal biomedicines: A new opportunity for aquaculture industry," *Aquaculture International*, vol. 18, pp. 403–414, 2010.

[4] M. A. O. Dawood, S. Koshio, and M. Á. Esteban, "Beneficial roles of feed additives as immunostimulants in aquaculture: A review," *Reviews in Aquaculture*, vol. 10, no. 4, pp. 950–974, 2018.

[5] C. W. Dunnett, "A multiple comparison procedure for comparing several treatments with a control," *Journal of the American Statistical Association*, vol. 50, pp. 1096–1121, 1955.

[6] Food and Agriculture Organization of the United Nations, *The State of World Fisheries and Aquaculture 2024: Blue Transformation in Action*. Rome, Italy: FAO, 2024, doi: 10.4060/cd0683en.

[7] R. Harikrishnan, C. Balasundaram, and M.-S. Heo, "Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish," *Aquaculture*, vol. 317, pp. 1–15, 2011.

[8] M. A. Hossain, Z. Sultana, A. S. M. Kibria, and K. M. Azimuddin, "Optimum dietary protein requirement of a Thai strain of climbing perch, *Anabas testudineus* (Bloch, 1792) fry," *Turkish Journal of Fisheries and Aquatic Sciences*, vol. 12, pp. 217–224, 2012, doi: 10.4194/1303-2712-v12_1_04.

[9] G. Immanuel, R. P. Uma, P. Iyapparaj, T. Citarasu, S. M. Punitha Peter, M. Michael Babu, and A. Palavesam, "Dietary medicinal plant extracts improve growth, immune activity and survival of tilapia *Oreochromis mossambicus*," *Journal of Fish Biology*, vol. 74, pp. 1462–1475, 2009.

[10] Z. H. Kareem, Y. M. Abdelhadi, A. Christianus, M. Karim, and N. Romano, "Effects of some dietary crude plant extracts on the growth and gonadal maturity of Nile tilapia (*Oreochromis niloticus*) and their resistance to *Streptococcus agalactiae* infection," *Fish Physiology and Biochemistry*, vol. 42, pp. 757–769, 2016.

[11] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," *Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265–275, 1951, doi: 10.1016/S0021-9258(19)52451-6.

[12] S. J. Midhun, D. Arun, L. Edatt, *et al*., "Modulation of digestive enzymes, GH, IGF-1 and IGF-2 genes in the teleost, tilapia (*Oreochromis mossambicus*) by dietary curcumin," *Aquaculture International*, vol. 24, pp. 1277–1286, 2016, doi: 10.1007/s10499-016-9984-1.

[13] K. I. Priyadarsini, "The chemistry of curcumin: From extraction to therapeutic agent," *Molecules*, vol. 19, no. 12, pp. 20091–20112, 2014, doi: 10.3390/molecules191220091.

[14] M. Reverter, N. Bontemps, D. Lecchini, B. Banaigs, and P. Sasal, "Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives," *Aquaculture*, vol. 433, pp. 50–61, 2014.

[15] J. H. Roe, "The determination of sugar in blood and spinal fluid with anthrone reagent," *Journal of Biological Chemistry*, vol. 212, no. 1, pp. 335–343, 1955, doi: 10.1016/S0021-9258(18)71120-4.

[16] K. Thanikachalam, M. Kasi, and X. Rathinam, "Effect of garlic peel on growth, hematological parameters and disease resistance against *Aeromonas hydrophila* in African catfish *Clarias gariepinus* (Bloch) fingerlings," *Asian Pacific Journal of Tropical Medicine*, vol. 3, no. 8, pp. 614–618, 2010.