A BRIEF STUDY ON THE QUALITATIVE EVALUATION OF THE COLOUR ENHANCING ABILITY OF*ALLIUM SATIVUM* FEED AND COMMERCIAL FEED IN *XIPHOPHORUS HELLERI* (HECKEL,1840).

Divya. M. S[1]*, Dr Sreeja. J[2]

[1]Ph.D scholar, PG& Research Department of Zoology, Sree Narayana College, Kollam-691001, Kerala, India

[2]Associate professor, Department of Zoology, Sree Narayana College for Women, Kollam-691001, Kollam-691001, Kerala, India

Abstract: Colour enhancing ability of feed supplemented with *Allium sativum*versus commercial feed analysed qualitatively using HPTLC pigment extraction method in ornamental fish *Xiphophorus helleri*. The result obtained was higher for TD_1(containing garlic) with 15260.9AU than the fishes fed with CF (commercial feed) generated only 4531.2AU. The spectrum analysis revealed maximum signal of 321AU @ 453nm for TD_1 while it was 113AU @ 226nm for CF thereby clarified the positive effect of products.

Keywords: *Allium sativum*, *Xiphophorus helleri*, *HPTLC*, *spectrum analysis*.

I. Introduction

The World Ornamental fish trade expanding globally with a turnover of 9 billion dollars exposes the demand of ornamental fishes. Ornamental fish rearing became unavoidable element of interior decoration due to their aesthetics with flourishing export value. Nature expelled diversified scenic beauty with a gradation of colours especially to biotic and non-biotic elements. A similar approach evident with fishes i.e.pigments responsible for the intensified coloration, a prerequisite feature that fixes the market prize[1,2]. Even though bright colour hue preferred by hobbyists a very few environmentalists and artists prefer dull colour. [3,4].

Under captivity in a confined space to develop skin colour in rearing fishes, it is mandatory to feed sufficient feed as they lack provision to consume aquatic vegetation to synthesise carotenoids [5-7]. Accurate feeding by administering pigment enriched feed improves the quality ultimately increases price of fishes. Evidences suggests that carotenoids have profound role in photosynthesis performed by plants, algae, photosynthetic and non-photosynthetic bacteria, offers protection to yeast, moulds against destruction by light and free radicals [8,9].

Acting as a source of pro-vitamin A with antioxidant activity regulate immunological functions promotes reproduction in fishes. Moreover, it has been found out that fishes with high amount of carotenoids possess the ability to attack bacterial and fungal diseases[10-12]. Synthetic pigment sources like β carotene, castaxanthine and zeaxanthine currently in usage need to be replaced with cost effective plant products [13,14]. In this perspective the present study carried out to examine the efficiency of *Allium sativum* supplemented diet in promoting skin colour as an eco-friendly practice in *Xiphophorus helleri.*

II. Material and methods

Fish Rearing

Experimental fishes namely yellow molly was purchased from Arya's aquarium and pet shop from Kollam district, Kerala acclimatized to laboratory conditions for two weeks prior to commencement of experiment to equalize their body weight by giving commercial feed. The fishes were weighed individually and randomly distributed at the rate of ten numbers per tank. The experiments were conducted for a period of 60 days after an acclimatisation period of fourteen days reared for sixty days (January 2018-March 2/2/2018) to detect pigments qualitatively by feeding with CF and TD_1 with proper governance of hydrological parameters. The individual length and weight of fishes were tabulated monthly. Fishes were fed with 3% of feed but divided and supplied twice in a day to avoid wastage and accumulation of food. The test diets were designated as CF (commercial feed) and TD_1(garlicfeed) whose proximate analysis carried out by standard methods [15-19] with result in Table-1.

Extraction and Qualitative estimation of Carotenoids.

The extraction process [20] carried out by selecting three out of ten fishes (randomly selected) so that after removing their head and gut body weight of 1g obtained, homogenised separately by adding 5ml of Acetone in a 10ml screwcap bottle, kept in the refrigerator overnight at freezing temperature. After 24 hours the homogenate centrifuged at 4000rpm for ten minutes. The supernatant collected and diluted with 5ml of hexane subjected to spectrum analysis for screening ranged from 200nm to 700nm using Spectrophotometer. The qualitative estimation of skin coloration was carried out by HPTLC using solvent systems. The mobile phase consisted of acetone and hexane (1:5). Aluminium-backed TLC plates pre-coated with 0.2 mm layer of silica gel 60 F_{254} (20cm × 10cm) was used as stationary phase. The stationary phase after proper saturation in the HPTLC chamber were mounted with 0.5µl samples and values derived from chromatogram details made for comparison present shown in Table 2, 3 & 4.

III. Results and Discussion

The parameters of feed under proximate analysis was crude protein, crude lipid, moisture, fibre, nitrogen free extracts, gross energy content, metabolizable energy and ME/DE in Table-1. Crude protein content was 19 mg% in CF but 28.7 mg% in TD1.Crude lipid value was 1.6 mg% in CF while 3 mg% in TD1. Moisture content of 11% and 11.5% noticed in CF and TD1.In CF the ash content was 13.33% but in TD1 14.7%. NFE obtained was % for CF and % for TD1. The values of gross energy, ME:DE Coefficient and Metabolizable energy were higher for TD_1(3.55%, 99.83% and 11.24%) than CF (3.14%, 99.40% and 10.29%). The hydrological parameters promoted better rearing of fishes provided with two feeds. During the experimental period water temperature varied from 22^0C-26^0C. The pH fluctuated between 7.5-8, dissolved oxygen between 5.3mg/l to 5.6mg/l with a total hardness ranged from 94-118mg/l of $CaCO_3$ while the level of ammonia, nitrite and nitrate values were feeble to detect.

Table-1 showing the proximate chemical composition of 100 g.

Feed parameters	CF	DF
Crude protein	19.0	28.7
Crude lipid	1.6	3.0
Moisture	11.0	11.5
Ash	13.33	14.7
Fibre	2.2	3.1
NFE	52.97	39.0
Gross energy	3.14	3.55
ME:DE Coefficient	99.40	99.83
Metabolizable energy	10.29	11.24

The qualitative result by HPTLC produced a single peak in CF and TD1 but the data enlightened the pigment enhancing nature of plant products. The spectrum details screened at a wavelength ranging from 200nm to 700nm revealed only one signal for CF and TD1 fed fishes. The Rf value 0.81 and 0.83 obtained for CF and TD1. It demonstrated a minimum signal of 113AU @ 226nm in CF while a maximum signal of 321AU @ 453nm further clarified that carotenoids were higher in TD_1 shown in Table-2 highlighted the basic fact that carotenoids imparting better colour hue evident predominantly within 500nm than 226nm in CF.

Table-2 Spectrum analysis of *X. helleri* fed with CF and TD_1

Feed	Rf	Maximum signal
CF	0.81	113 AU@ 226nm
TD_1	0.83	321 AU@ 453nm

The qualitative study of carotenoids explored by HPTLC chromatogram come across with the findings(Table-3) that the start position (0.71 Rf), start height (1.4AU), Maximum Position (0.81 Rf), Maximum Height (77.0AU), Maximum%(100.0%), End position (0.90 Rf), End Height (1.6AU), Area (), Area% (100.0%) in CF found to be significantly lower comparatively lower than TD_1 with a start position (0.71 Rf), start height (1.7AU), Maximum Position (0.83 Rf), Maximum Height (255.1AU), Maximum%(100.0%), End position (0.90 Rf), End Height (0.7AU), Area (15260.9 AU) and Area% (100.0%)

Table-3 showing the HPTLC peak details obtained for CF

Peak	Start position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.71 Rf	1.4 AU	0.81 Rf	77.0 AU	100.00%	0.90 Rf	1.6 AU	4531.2 AU	100.00 %

Table-4 showing the HPTLC details obtained for TD_1

Peak	Start position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.71 Rf	1.7 AU	0.83 Rf	255.1 AU	100.00 %	0.90 Rf	0.7 AU	15260.9AU	100.00%

Naturally available plant materials as additives in feed required to be examined for developing cheaper feeds rather than promoting environment harnessing artificial synthetic sources. Macro and micro algae, seaweeds, animal based and plant based natural carotenoids employed in aqua feed as additives. Carotenoids derived from animal sources shows a declining trend due to the reduced supply of crustaceans with expensive cost of production further increased the feed cost. The common plant based carotenoid source obtained from the extracts of micro algae, marigold meal and red pepper.

Carotenoids, the key element of pigmentation provides various shades like yellow, red with its associated colours. It is usually obtained through aquatic food chain impossible for the ornamental fishes as dwelling in artificial environment, provided with zeaxanthin and lutein pigment sources (yellow corn, corn gluten meal and alfalfa apart from plant based and animal based carotenoids [21-22].

The study conducted envisaged the possibility of including *Allium sativum* (garlic) in the formulated feed of *X. helleri* (red sword tail) found effective in promoting skin colour compared to the commercial feed(CF). Being well-known that research works associated with qualitative study of pigments using HPTLC in fishes is little compared to other works. The present study clearly indicated that spectrum with peak details signified the potential of *A. sativum* in promoting skin colour in red sword tail than CF.

Our works are in conformity with Joseph et.al and Ahilan et.al [23,24] works who utilised four ornamental flowers and botanical additives in promoting growth and colour of sword tail (*Xiphophorus helleri)* and gold fish (*Carassius auratus)*. Reports of Harpaz and Padowicz also revealed carotenoid pigments from *Rosa rubiginosa*accelerated growth, survival and coloration of *Xiphophorus helleri* fish fry and dwarf cichlid *Microgeophagus ramirezi* [25]. Boonyaratpalin and Lovell [26] concluded that marigold petal meal augment colour in tiger barb supports our work.

The investigated work also approved the findings of Mc Graw and Ardia [27] clarified that the increased carotenoid content in fishes triggers a cascade of beneficial physiological process promote survival. The application of garlic feed evoked positive response in red sword tail remarked its effectiveness to deposit pigment is species specific stated by Divya et al [28,29] who stated that increased level of carotenoids resists infections hence promoting survival can be explored in future as an eco-friendly cost effective strategy.

IV. Conclusion

The application of *Allium sativum* incorporated feed yielded a positive approach in developing pigments in red sword tail. Being a natural plant product the production cost will be cheaper than the synthetic additives. As a precaution to avoid the deterioration generated by synthetic pigments it is mandatory to explore and flourish natural products.

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