Summary
5. SUMMARY

The present work was undertaken to assess the variation in growth responses, tissue biochemical changes and carotenoids accumulation in the skin and muscle tissues of freshwater ornamental Gold fish (C.auratus) and Gourami (T.trichopterus). The candidate fishes were reared in laboratory condition in 200l glass tanks at the rate of five fishes per tank in triplicate and fed with control (CD) and experimental diets supplemented respectively with carrot waste (CWD), beetroot waste (BWD), hibiscus petels (HPD) and spirulina (SPD) at the rate of 5.0g /100g dry feed. This study was lasted for 40 days and observation were made at an intervals of 10,20,30 and 40 days. After the, said experimental duration, to start with growth responses were assessed, during first and second 20 days and then overall growth responses were also calculated.Simultaneously the skin and muscle samples were obtained from both in control and experimental diets fed fishes and the variation in biochemical constituents (Protein, Carbohydrate, and Lipid) and also the accumulation of carotenoids were measured. The carotenoids were studied by using two different solvents namely acetone and methanol. In both control and experimental diets fed fishes, total carotenoid was assessed by Spectrophotometric method and qualitative estimation was also made by Thin Layer Chromatographic method (TLC). Further spectral analysis was also made to assess the changes in carotenoids in the tested tissues. The salient findings of the present study are summarized below.
The results on growth responses of *C. auratus* and *T. trichopterus* fed with control and experimental diets during first and second 20 days were found to be influence by the variation in diets. This variation was much more obvious in *T. trichopterus* when compared with *C. auratus*. However, the overall growth responses registered a definite trend.

The overall growth responses of *C. auratus* and *T. trichopterus* clearly indicated the dietary dependent variation. In *C. auratus* food consumption was high (6.64 ± 0.040g) in control diet (CD) fed groups and it was low in experimental diets (CWD, BWD, HPD, SPD) fed fishes. An inverse trend was established for the production, when compared to consumption. Accordingly high production range of 1.89 ± 0.087g to 2.67 ± 0.025g was registered in experimental diet fed fishes when compared to the low value recorded in control diet fed group (1.35 ± 0.020g). Also the SGR and FCE values were high in experimental diet fed fishes. The better FCR (1.65 ± 0.012g) and higher SGR (3.21 ± 0.11‰) values were recorded in spirulina diet (SPD) fed *C. auratus*.

Likewise in *T. trichopterus* the trend in overall growth responses recorded was similar to that of noticed in *C. auratus*. But the overall growth efficiently of this fish species was low when compared to *C. auratus*. Here, the range of feed conversion efficiency and SGR noticed in experimental diets fed fishes were; 29.94 ± 0.65 to 51.11± 0.83 and from 1.46 ± 0.05 to 2.00 ± 0.06 respectively. Likewise the FCR range of 3.02 ±
0.14 to 1.82 ± 0.08 was noticed in those fishes fed experimental diets and these were better values when compared with control diet fed group.

- The results on biochemical constituents in the selected tissues of *C. auratus* and *T. trichopterus* fed with experimental diets showed dietary dependent variation at different experimental duration. Accordingly in carrot supplemented diet (CWD) fed *C. auratus*, skin protein content varied from 4.84 ± 0.17 to 6.49 ± 0.16 mg 100mg wet weight when compared with the value recorded in those fishes fed control diet (4.32 ± 0.38 mg 100mg wet tissue). In *T. trichopterus* fed with the same experimental diets the skin protein content varied from 3.79 ± 0.09 to 5.72 ± 0.24mg 100mg wet tissue. In the muscle tissue, the protein content varied from 7.86 ± 0.05 to 9.30 ± 0.01mg100mg wet tissue (*C. auratus*) and from 6.31 ± 0.21 to 8.04 ± 0.14mg 100mg wet tissue (*T. trichopterus*) and these values were higher when compared to control diet (CD) fed fishes. More or less a similar variations were also noticed in *C. auratus* and *T. trichopterus* fed beetroot waste (BWD), Hibiscus petals (HPD) and spirulina (SPD) supplemented diets fed fishes. The variation in skin and muscle biochemical constituents of *C. auratus* and *T. trichopterus* fed SPD diet was much more obvious when compared to other experimental diets (CWD, BWD, and HPD) fed fishes. Here, the skin and muscle protein content of experimental group varied from 5.45 ± 0.42 to 6.98 ± 0.38mg100mg wet tissue, from 8.16 ± 0.36 to 9.92 ± 0.52mg100mg wet tissue for *C. auratus* and also varied from 4.03 ± 0.75
to 6.12 ± 0.19mg/100mg wet tissue, from 6.48 ± 0.23 to 8.34 ± 0.14mg/100mg wet tissue for *T. trichopterus*.

More or less a similar range of variation was also noticed in the skin and muscle carbohydrate and lipid contents of *C. auratus* and *T. trichopterus* fed with control (CD) and experimental diets (CWD, BWD, HPD, and SPD). Also Two-way analysis of varience for the data on changes in skin and muscle biochemical constituents as a function of experimental duration and experimental diets revealed that, the variation on tissue biochemical constituents due to experimental duration was statistically highly significant (P<0.05 to<0.001), when compared to the independent influence shown by the experimental diets; wherein, the variation was significant at <0.05. Further, the influence of both the tested variables was high in skin and muscle protein and carbohydrate contents of experimental fish when compared to lipid content.

The variation in total carotenoids in the skin and muscle tissues of *C. auratus* and *T. trichopterus* yielded noteworthy information. It indicated that in the skin tissues of *C. auratus* extracted in acetone solvent system, the initial (0day) total carotenoid content was 1.40 ± 0.41µg/g tissue. In the experimental fishes fed with CWD supplemented diet for different durations, it ranged from 1.71 ± 0.038µg/g tissue (10day) to 2.55 ± 0.043µg/g tissue (40day) in *C. auratus* and from 0.88 ± 0.011µg/g tissue (10day) to 1.83 ± 0.056µg/g tissue (40day) in *T. trichopterus*. In the
muscle tissue of both the experimental fishes same variations were also noticed.

- Likewise in the same skin tissue extracted in methanol solvent system, the total carotenoid content of control diet fed fish was $0.45 \pm 0.20 \, \mu g/g$ tissue in $C. auratus$ and $0.25 \pm 0.093 \, \mu g/g$ tissue in $T. trichopterus$. But the values registered in experimental fishes were high and it ranged from $0.74 \pm 0.052$ to $1.15 \pm 0.097 \, \mu g/g$ tissue in $C. auratus$ and from $0.32 \pm 0.068$ to $0.61 \pm 0.021 \, \mu g/g$ tissue $T. trichopterus$ during 10th and 40th days of experiment. Similar trend was also noted in muscle tissues extracted in methanol solvent systems.

- The total carotenoid content in the skin tissue of $C. auratus$ and $T. trichopterus$ fed with BWD diet, but extracted with acetone varied from $1.40 \pm 0.41 \, \mu g/g$ tissue on 0 day to $2.13 \pm 0.018 \, \mu g/g$ tissue on 40th day in $C. auratus$ and from $0.74 \pm 0.20 \, \mu g/g$ tissue on 0 day to $1.96 \pm 0.051 \, \mu g/g$ in $T. trichopterus$ on 40th day of experiment. In the same tissue extracted in methanol solvent system, carotenoid content varied from $0.45 \pm 0.20 \, \mu g/g$ tissue on 0 day to $0.84 \pm 0.037 \, \mu g/g$ tissue on 40th day in $C. auratus$ and from $0.25 \pm 0.093 \, \mu g/g$ tissue 0day to $0.65 \pm 0.038 \, \mu g/g$ tissue in $T. trichopterus$ on 40th experiment.

- The variation in total carotenoid content in the skin tissue of $C. auratus$ and $T. trichopterus$ fed with HPD diet, but extracted with acetone, the total carotenoid content varied from $1.40 \pm 0.41 \, \mu g/g$ tissue in 0 day to $2.38 \pm 0.073 \, \mu g/g$ tissue on 40th day ($C. auratus$) and from $0.74 \pm 0.20 \, \mu g/g$ tissue
on 0 day to 1.95 ± 0.053 µg/g tissue on 40\textsuperscript{th} day (\textit{T.trichopterus}) experiment. In the skin tissue of \textit{C.auratus} and \textit{T.trichopterus} fed with same experimental diet, but extracted with methanol solvent, it respectively ranged from 0.45 ± 0.20 µg/g tissue (0day) to 0.85 ± 0.028 µg/g tissue (40\textsuperscript{th} day of experiment) and from 0.25 ± 0.093 µg/g tissue (0day) to 0.64 ± 0.038 µg/g tissue (40\textsuperscript{th} day of the experiment).

> Likewise in those fishes fed with SPD diet, the total carotenoid content in the skin extracted in acetone varied from 1.40 ± 0.41 µg/g tissue to 2.89 ± 0.081 µg/g tissue in \textit{C.auratus} and from 0.74 ± 0.20 to 2.43 ± 0.058 µg/g tissue in \textit{T.trichopterus} during 10\textsuperscript{th} to 40\textsuperscript{th} days of the experiment. In the skin of same diets fed fishes, but extracted with methanol, the total carotenoid content varied from 0.45 ± 0.20 to 1.03 ± 0.04 µg/g tissue in \textit{C.auratus} and from 0.25 ± 0.093 to 0.74 ± 0.057 µg/g tissue in \textit{T.trichopterus} respectively during 10\textsuperscript{th} to 40\textsuperscript{th} days of the experiment. The muscle tissue of \textit{C.auratus} and \textit{T.trichopterus} fed with same diet, extracted in acetone and methanol solvent system, more or less same variation were registered.

> In the present study attempts have also been made to assess the qualitative changes in carotenoids through TLC and spectral analysis. Here also analysis was made in the skin and muscle tissues of \textit{C.auratus} and \textit{T.trichopterus} fed control and experimental diets in two different solvent systems (Acetone and Methanol). In general, the numbers of carotenoids were more in experimental diet fed fishes, when compared to those fishes...
received control diet. Also more number of carotenoids were registered in the tested tissues during 30\textsuperscript{th} and 40\textsuperscript{th} days of experimental duration. Further among the nutrients tested, SPD diet fed fishes registered more number of carotenoids, when compared with those fishes fed control and other experimental diets. This trend was true in both the tested tissues.

- In specific, the TLC analysis of skin and muscle tissues of \textit{C.auratus} fed with CWD diet, but extracted in acetone solvent system, seven and nine different carotenoids were noticed. Here, Asteraxanthin, Canthaxanthin, Lutein, Isozeaxanthin and \(\alpha\)-carotene were dominated. In addition to these carotenoids, the other carotenoids noticed were; \(\beta\)-carotene, Asteraxanthin monoester, Neoxanthin and \(\beta\)-cryptoxanthin. Also one unidentified carotenoid was recorded in muscle tissue. Compared with acetone solvent system, the number of carotenoids registered in the skin and muscle tissues of \textit{C.auratus} extracted in methanol solvent system was less. In this solvent system, Asteraxanthin was alone showed its dominant occurrence. The other carotenoids noticed were; \(\alpha\)-carotene, lycopene, isozeaxanthin, \(\beta\)-carotene and leutin. Also both in skin and muscle tissues each one unidentified carotenoid was noticed.

- More or less a similar variation was noticed in the skin and muscle tissues of \textit{T.trichopterus} fed with CWD diet. Also a similar variation was noticed in the distribution of carotenoids in the skin and muscle tissue of \textit{C.auratus} and \textit{T.trichopterus} fed with BWD, HPD,and SPD diets, extracted in acetone and methanol solvent systems. Compared with other
diets fed groups, the number of carotenoids registered in skin and muscle tissue of *C. auratus* and *T. trichopterus* fed with SPD diet was more and it is may be attributed to the availability of specific nutrients in this diet. In SPD diet fed *C. auratus*, in acetone solvent system, the skin tissue recorded fourteen different carotenoids including four unidentified carotenoids. In the same solvent system the muscle tissue showed thirteen different carotenoids along with two unidentified ones. The dominant carotenoids registered in skin tissue were; Asteraxanthin, Free Asteraxanthin, α-carotene, and Echinenone; whereas, in the muscle tissue Canthaxanthin, Asteraxanthin, α-carotene, β-cryptoxanthin, and β-carotene were dominantly occurred. Likewise in the skin tissue of *T. trichopterus* fed SPD diet, extracted in acetone solvent system eight different carotenoids were noticed and here the dominant carotenoids recorded were; β-cryptoxanthin, Canthaxanthin, Asteraxanthin, Neoxanthin and Lutin. But in muscle tissue of the same fish, nine different carotenoids were noticed along with an unidentified one. Here, the dominant carotenoids noticed were; Asteraxanthin, Lutein, Canthaxanthin, Isozeaxanthin and Neoxanthin. The carotenoids present in the skin and muscle tissues of *C. auratus* and *T. trichopterus* fed with the same SPD diet, but extracted in methanol solvent system was less.

- Spectral analysis of carotenoid in the tested tissues of *C. auratus* and *T. trichopterus* fed with experimental diets and extracted in mixed solvent system (Hexane, Ethanol, and Petroleum ether; 2:1:1) also showed notable variation. In the skin tissue of fishes fed with CWD
diet, maximum number of carotenoids such as Phytoene (286nm), Phytofluene (331nm), δ-carotene (377nm), Auroxanthin (381nm) and Capxanthin (450nm) were showed their occurrence. In the muscle tissues of same diet fed *C. auratus* and extracted in acetone solvent, five peaks were recorded; which were related to Phytoene (286nm), α-zeacarotene (398nm), Asteraxanthin (480nm), Rubixanthin (409nm) and Lycopene (503nm).

- By the same way in *T. trichopterous* fed with CWD diet, five peaks were observed at the spectral range of 286nm to 480nm and the related carotenoids identified were; Phytoene, δ-carotene, α-cryptoxanthin, Asteraxanthin, and γ-carotene. In the muscle tissues of the same diet fed fishes, the carotenoids such as Phytofluene (331nm), Crocetin (413nm), Canthaxanthin (482nm), β-cryptoxanthin (485nm) and Asteraxanthin (480nm) showed their occurrence.

- The results on the spectral analysis of carotenoids in the skin tissues of BWD diet fed *C. auratus* and extracted in mixed solvent system indicated the presence of Phytoene (286nm), Phytofluene (331nm), δ-carotene (377), β-cryptoxanthin (485nm), γ-carotene (446nm) and α-carotene (444nm). In the muscle tissue of the same diet fed fishes, five carotenoids were identified. Similarly in the skin tissue of *T. trichopterous* fed with BWD supplemented diet and extracted with mixed solvent system, five peaks were identified at the spectral range of 286nm to 510nm and the relevant carotenoid identified were; Pytoene, β-carotene, δ-carotene,
Asteraxanthin and Capsorubin. By the same way in the muscle tissue of the BWD diet fed *T. trichopterus*, five carotenoids were showed their occurrence, in the spectral range of 331nm to 480nm.

- Likewise in the skin tissue of *C. auratus* fed with HPD diet and extracted in mixed solvent system, maximum number of carotenoids such as Phytoene, Phytofluene, β-cryptoxanthin, Antheraxanthin, and Asteraxanthin were registered. In muscle tissue of the same diet fed fishes, the number of carotenoid noticed were less. In the skin tissue *T. trichopterus* fed with HPD diet, five peaks were identified. Also in the muscle tissue of the same diet fed *T. trichopterus* the carotenoids like Crocetin (413nm), α-zeacarotene (398nm), Neoxanthin (448nm), Mutatoxanthin (426nm), unidentified (468nm) were showed their existence.

- In the skin tissue of *C. auratus* fed with SPD diet but extracted in mixed solvent system, more number of carotenoids were noticed when compared to CWD, BWD, and HPD diets fed fishes. Here, the carotenoids like Phytoene (286nm), Phytofluene (331nm), α-zeacarotene (398nm), Violaxanthin (419nm), Asteraxanthin (480nm), Capxanthin (450nm), and Rubixanthin (509nm) were occurred along with one unidentified carotenoid. In the muscle tissues of same diet *C. auratus* and extracted in the same mixed solvent system, seven peaks were observed at the spectral ranged 286nm to 503nm and the related carotenoids recorded were;
Pytoene, β-carotene-5,8,5’-8’-diepoxide, Neurosporene, Zeaxanthin, Antheraxanthin, Lycopene, and Asteraxanthin.

- Also in the skin tissue of *T. trichopterus* fed with SPD diet, seven peaks were identified and they were; Phytoene (286nm), β-carotene (452nm), β-5,8-epoxide (407nm), Antheraxanthin (422nm), α-carotene (444nm), Asteraxanthin (480nm) and Lycopene (503nm). In the muscle tissue of the same experimental diet fed fishes, but extracted in mixed solvent system, seven peaks were identified in the spectral range of 286nm to 503nm.

- This study inferred that addition of carotenoid contained feed ingredients in the diet of *C. auratus* and *T. Trichopterus* exerted their influence not only on the growth responses of the candidate fish species, but also accelerated the synthesis of tissue biochemical constituents. These physiological and biochemical variations in turn affected the total carotenoid content in the skin and muscle tissues of *C. auratus* and *T. trichopterus* during the course of experimentation. This trend is also evidenced in the TLC and Spectral analysis of carotenoids in the tested tissues of *C. auratus* and *T. trichopterus*. Overall, this present work explored the use of carotenoid contained feed ingredients such as Spirulina, carrot waste, beetroot waste and hibiscus petals in the diet of ornamental fishes, *C. auratus* and *T. trichopterus* for enhancing growth responses and pigmentation.