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# Effect of Probiotic Yeast, *Sacchromyces cerevisiae* on Tissue Biochemical and Carotenoid Variation in Freshwater Ornamental Fish *Carassius auratus* (Linnaeus, 1758)

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author MM@M performed the experiments and collected the data. Author BS assisted in the experimental design, data analysis and preparation of manuscript. Author AP computed statistical analysis, reviewed and edited the manuscript for publication. All authors read and approved the final manuscript.*

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## ABSTRACT

The present work aims at assessing Bakers's yeast, *Saccharomyces cerevisiae* as an efficient and eco-friendly probiotic feed additives for ornamental fish, *Carassias auratus*. For this study an indoor culture experiment was carried out 300l plastic trough containing 200l water in triplicate. In each tank five fishes at fingerlings stage (1.58 ± 0.14g) were reared for a period of 30 days. An uniform water quality parameters such as temperature (28.0 ± 1.0 $^0$C), pH (7.60 ± 0.12), dissolved oxygen (4.80 ± 0.82mg/l) and ammonia (< 0.08 mg/l) were maintained. The results inferred that, the percentage increase in the biochemical constituents in both skin and muscle tissue of *C. auratus* received 2% yeast added diet (EDY2) registered higher values when compared to control and other experimental diets fed fishes. The total carotenoid content in the acetone extracted skin tissues of *C. auratus* fed with 2% yeast supplemented diet varied from 0.748± 0.042 in 0 day to 0.944 ± 0.014 µg/g wet tissue on 30$^{th}$ day of experiment. Likewise, in the muscle tissue of *C. auratus* extracted with acetone, the total carotenoid varied from 0.376±0.014 (0 day) to 0.541 ± 0.008 µg/g wet tissue (30 day). The carotenoid content of both the tested tissues on methanol extract was low, when compared with acetone extract. The overall results inferred that *S. cerevisiae* supplementation @ 2g/ 100g diet distingly altered the carcass composition of *C. auratus* and also the accumulation of cartenoids in skin and muscle tissues. *S. cerevisiae* contains bioactive lead molecules, which accelerated the vital physical and metabolic activities in host fish species.


*Keywords: Carassius auratus; Saccharomyces cerevisiae; carotenoids; biochemical constituents.*

## 1. INTRODUCTION

In ornamental fish culture, pigments are responsible for the wide spectrum of colours which is an inevitable prerequisite for fish quality as they fetch higher price in the commercial market. As fishes cannot synthesize their own colouring pigments *de novo*, the colouring molecules which are synthesized by plants, algae and microorganisms, need to be incorporated in their diet [1]. Traditionally ornamental fishes are fed with live feed, which were often nutritionally deficient and can act as the transmitter of parasitic, bacterial and viral diseases if not stored properly [2]. Furthermore, conventional use of commercial feed additives to enhance skin colour also cause intestinal disorders [3]. Over and above, aquaculture require high quality feeds with optimum protein content, which should not contain only necessary nutrients but also complementary additives to keep organisms healthy, colourful and display better growth [4]. To reduce feed costs and to improve colour and growth, feed additives including microorganisms have been tested. Some of the most utilized growth-promoting additives are hormones, antibiotics, ionophores and salts [5]. However, some feed additives such as antibiotics can cause intestinal disorders [3] or resistance in pathogenic bacteria [4]. Residues of antibiotics in aquaculture products may cause problems to human health [6]. Many of the probiotic bacteria alone or in combination have been used as alternative feed additives in aquaculture [7-9]. To date, probiotics are considered as a good alternative to the use of antibiotics. Probiotics, which are microorganisms or their products with health benefit to the host, have found use in aquaculture for improving the health of the host and increasing growth. It was reported that vitamin C and Lactobacillus rhamnosus significantly (P > 0.05) improved the growth performance of rainbow trout Oncorhynchus mykiss [10]. Oscar fish, Astronauts ocellatus fed with Artemia urmiana, earth worm and beef heart supplemented diets enhanced the number of total bacteria, lactic acid bacteria, mesophyll bacteria and entrobacteriacea [11]. Considering the potential role of carotenoids containing compounds in various physiological and biochemical activities of organisms, the present study was undertaken to investigate the yeast supplemented diet induced carotenoid changes in the freshwater ornamental gold fish *Carassius auratus*.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Experimental Feed

The control (CD) and experimental diets (EDY1, EDY2, and EDY3) were formulated using locally available ingredients (Fish meal, Prawn head waste, Groundnut oil cake, Rice bran, Tapioca powder and Wheat flour). Feed formulation was done by "Square method" by giving due consideration to protein content of the different

ingredients. Commercially available Baker's yeast (*Saccharomyces cerevisiae*) was diluted in water supplemented to 1, 2 and 4g/100g feed respectively, to experimental diets (EDY1, EDY2 and EDY3); whereas, control diet (CD) was prepared with all basal feed ingredients but devoid of given in yeast. Control and experimental diets were prepared by using the chosen feed ingredients as per the formulation Table A1. The feeds were prepared in pellet form by using hand pelletiser with 0.5mm diameter and manually broken into 2 to 6 mm. The pellets were dried at 40 to $45^0$C in an temperature controlled incubator and stored in an air tight containers at room temperature until further use.

## 2.2 Experimental Setup

After acclimatization, well acclimatized healthy fingerlings weighing 1.58 ± 0.14g were selected and starved for about 24 hours prior to the start of the experiment in order to evacuate their gut contents. The fish were then weighed individually in a monopan balance to 0.1mg accuracy with least disturbance.

Further, laboratory rearing experiments were performed in 300*l* plastic trough containing 200*l* water in triplicate in order to evaluate the CD and EDY1, EDY2 and EDY3 diets. In each tank, five fishes were reared and offered with experimental diets (CD, EDY1, EDY2 and EDY3) at *ad libitum* at the rate of two times daily. Everyday morning, the unfed remains were collected and 30 to 40% water exchange was made. The experiments for both control and experimental diets were performed for thirty days. In experimental tanks, water quality parameters were checked periodically following the standard methods described by APHA (1958) and an uniform water quality parameters such as temperature (28.0 ± 1.0 $^0$C), pH (7.60 ± 0.12) and ammonia (< 0.08mg/l) were maintained. To assess the tissue biochemical changes, fishes were withdrawn at 0, 10, 20 and 30 days of the experiment and the tissues such as skin and muscle were dissected out aseptically at low temperature and stored in labelled vials at -20$^o$C and were used for further analysis [12].

## 2.3 Extraction of Carotenoids

Total carotenoid was extracted in the tested tissues (skin and muscle) by using two different solvent system such as acetone and methanol following the method for Torrissen and Naevdal [13] and the amount of carotenoid present in the tissues was estimated spectrophotometrically at 444nm (TECHOM, HongKong).

## 2.4 Biochemical Analysis

Biochemical constituents such as protein [14], carbohydrate [15] and lipid [16] were estimated in the tested tissues (skin and muscle) and feeds by following the respective standard methods.

## 2.5 Statistical Analysis

Results obtained in the present study were subjected to statistical analysis such as SD and Two-way ANOVA and SNK test following the standard methods described in Zar [17].

# 3. RESULTS

## 3.1 Biochemical Composition of Experimental Diets

Fig. 1 shows the biochemical composition of control (CD) and experimental diets (EDY 1 to EDY3; 1%, 2% and 4% yeast). The protein content in the feeds did not show much variation and it ranged from 35.20 ± 0.42 to 36.80 ± 0.72 g/100g feed. The carbohydrate and lipid content of control and experimental diets were also with in an uniform range and it was from 9.20 ± 0.18 to 9.67 ± 0.18 g/100 g feed and from 7.20 ± 0.42 to 7.52 ± 0.168 g/100 g feed, ($p > 0.05$).

## 3.2 Tissue Biochemical Changes

### 3.2.1 Variation in protein content

Results on the changes in protein content in the skin and muscle tissues of *C. auratus* fed on control (CD) and experimental diets (EDY1 to EDY3) are shown in Table 1. In both the tissues tested, the protein content showed increase with the advancement of rearing period. In control diet fed fishes, the skin protein content varied from 4.32 ± 0.38 to 5.71 ± 0.37 mg/100 mg wet weight. However, this variation was high in the skin of experimental diet fed groups and attained optimum [4.32 ± 0.38 (0 day) to 6.10 ± 0.09 mg/ 100 mg wet weight (30 day)] in those fishes fed with EDY2 diet contained 2% yeast. In other experimental diets (EDY1 and EDY3) fed fishes, this variation was less when compared with EDY2 diet fed groups. More or less a similar variation was noticed for the muscle protein content. In this tissue also, the maximum variation of 7.05 + 0.49 to 8.96 + 0.07 mg/100 mg wet tissue was registered for *C,auratus* received EDY2 diet. Two -way analysis of

variance for the data on skin and muscle protein content of *C.auratus* (Table 3a) inferred that the variation due to experimental duration was statistically highly significant ($p < 0.01$ to $p < 0.0001$) than that to the variation due to diet ($p < 0.05$).

### 3.2.2 Variation in carbohydrate content

Table 2 provides the data on changes in carbohydrate content in the skin and muscle tissue of *C. auratus* fed with control (CD) and experimental diets (EDY1 to EDY3). In the tested tissues, the carbohydrate content showed changes between diets and also during experimentation. For instance, in control diet fed *C. auratus* the skin carbohydrate showed variation between 2.42 ± 0.33 to 3.55 ± 0.49 mg/100 mg wet weight. In the experimental diets fed groups, the variation was maximum (2.42 ±0.33 to 3.69 ± 0.81 mg/100 mg wet weight) in *C. auratus* received EDY2 diet. In other experimental diets (EDY1 and EDY3) fed *C. auratus,* the skin carbohydrate showed a similar range of variation compared with EDY2 diet fed groups. Likewise, the muscle carbohydrate of *C. auratus* fed control diet varied from 4.75+0.61 to 5.70+0.02 mg/100mg wet weight. Furthermore, a similar range of variation was noticed in muscle carbohydrate content of *C.auratus* and it was more (4.75± 0.61to 6.30 ± 0.37 mg/100 mg wet weight) in those fishes fed with EDY2 diet. Two - way analysis of variance (Table 3a) indicated that, the influence of experimental duration on the skin and muscle carbohydrate content of *C. auratus* was statistically highly significant ($p < 0.01$ to $p < 0.0001$) than that to the variation due to experimental diet ($p < 0.05$).

**Table A1. Composition of feed ingredients (g/100 g dry weight) used for the preparation of control and experimental diets**

| Feed ingredients | Composition (g dry wt) Control / Experimental diets | | | |
|---|---|---|---|---|
| | CD | EDY1 | EDY2 | EDY3 |
| Fish meal | 30.00 | 30.00 | 30.00 | 30.00 |
| Prawn head waste | 30.00 | 30.00 | 30.00 | 30.00 |
| Ground nut oil cake | 30.00 | 30.00 | 30.00 | 30.00 |
| Rice bran | 2.00 | 2.00 | 2.00 | 2.00 |
| Tapioca powder | 3.00 | 2.50 | 2.00 | 1.00 |
| Wheat flour | 3.00 | 2.50 | 2.00 | 1.00 |
| Fish oil | 1.0 | 1.0 | 1.0 | 1.0 |
| Vitamin & Mineral mix | 1.0 | 1.0 | 1.0 | 1.0 |
| Baker's yeast | - | 1.0 | 2.0 | 4.0 |

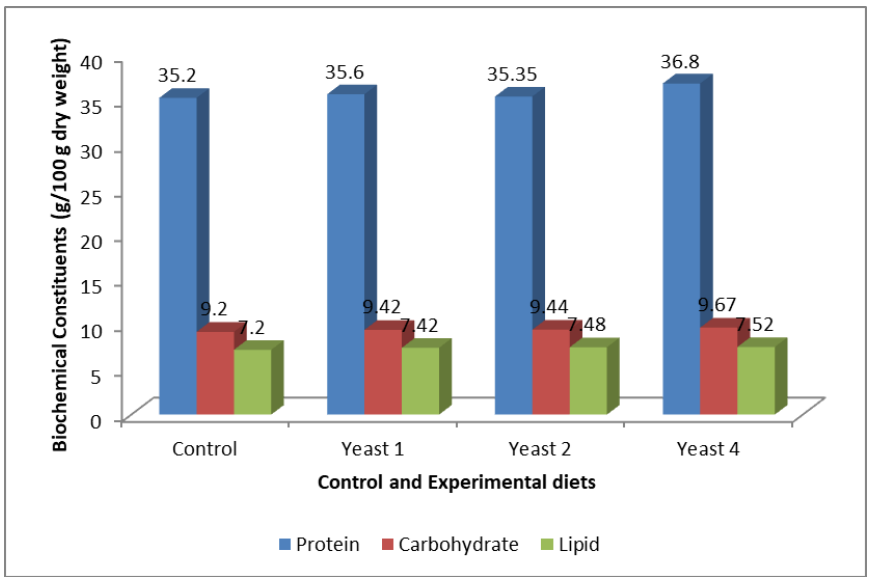


**Fig. 1. Proximate composition (% dry weight) of the control (CD) and experimental diets (EDY1, EDY2 and EDY3)**

**Table 1. Changes in Protein content in the skin and muscle tissue of gold fish (*C. auratus*) fed with control (CD) and experimental diet (EDY1 TO EDY3). Each value is the mean ± SD of three individual estimates**

| Experimental duration (Days) | Protein content (mg/100 mg wet weight) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control diet (CD) | | Experimental diet @ 1% yeast (EDY1) | | Experimental diet @ 2% yeast (EDY2) | | Experimental diet @ 4% yeast (EDY3) | |
| | Skin | Muscle | Skin | Muscle | Skin | Muscle | Skin | Muscle |
| Initial | 4.32 ± 0.38 | 7.05 ± 0.49 | 4.32 ± 0.38 | 7.05 ± 0.49 | 4.32 ± 0.38 | 7.05 ± 0.49 | 4.32 ± 0.30 | 7.05 ± 0.49 |
| 10 | 4.65 ± 0.16 | 7.41 ± 0.28 | 4.84 ± 0.17 | 7.86 ± 0.05 | 4.94 ± 0.07 | 7.92 ± 0.06 | 5.06 ± 0.24 | 7.94 ± 0.24 |
| 20 | 5.42 ± 0.14 | 7.78 ± 0.09 | 5.55 ± 0.37 | 8.60 ± 0.31 | 5.57 ± 0.08 | 8.61 ± 0.27 | 5.46 ± 0.24 | 8.64 ± 0.34 |
| 30 | 5.71 ± 0.37 | 8.17 ± 0.19 | 6.02 ± 0.15 | 8.41 ± 0.06 | 6.10 ± 0.09 | 8.96 ± 0.07 | 5.92 ± 0.21 | 9.04 ± 0.41 |

**Table 2. Changes in Carbohydrate content in the skin and muscle tissue of gold fish (*C. auratus*) fed with control (CD) and experimental diet (EDY1 TO EDY3). Each value is the mean ± SD of three individual estimates**

| Experimental duration (Days) | Carbohydrate content (mg/100 mg wet weight) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control diet (CD) | | Experimental diet @ 1% yeast (EDY1) | | Experimental diet @ 2% yeast (EDY2) | | Experimental diet @ 4% yeast (EDY3) | |
| | Skin | Muscle | Skin | Muscle | Skin | Muscle | Skin | Muscle |
| Initial | 2.42 ± 0.33 | 4.75 ± 0.61 | 2.42 ± 0.33 | 4.75 ± 0.61 | 2.42 ± 0.33 | 4.75 ± 0.61 | 2.42 ± 0.33 | 4.75 ± 0.61 |
| 10 | 2.65 ± 0.08 | 4.97 ± 0.17 | 2.80 ± 0.02 | 5.25 ± 0.11 | 2.89 ± 0.07 | 5.40 ± 0.10 | 2.98 ± 0.09 | 5.36 ± 0.18 |
| 20 | 3.07 ± 0.10 | 5.32 ± 0.39 | 3.48 ± 0.34 | 6.14 ± 0.05 | 3.39 ± 0.18 | 6.19 ± 0.08 | 3.42 ± 0.11 | 5.92 ± 0.21 |
| 30 | 3.55 ± 0.49 | 5.70 ± 0.02 | 3.66 ± 0.09 | 6.28 ± 0.15 | 3.69 ± 0.81 | 6.30 ± 0.37 | 3.68 ± 0.16 | 6.42 ± 0.36 |

**Table 3. Changes in lipid content in the skin and muscle tissue of gold fish (*C. auratus*) fed with control (CD) and experimental diet (EDY1 TO EDY3). Each value is the mean ± SD of three individual estimates**

| Experimental duration (Days) | Lipid content (mg/100 mg wet weight) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control diet (CD) | | Experimental diet with 1% yeast (EDY1) | | Experimental diet with 2% yeast (EDY2) | | Experimental diet with 4% yeast (EDY3) | |
| | Skin | Muscle | Skin | Muscle | Skin | Muscle | Skin | Muscle |
| Initial | 1.95 ± 0.33 | 1.11 ± 0.07 | 1.95 ± 0.33 | 1.11 ± 0.07 | 1.95 ± 0.33 | 1.11 ± 0.07 | 1.95 ± 0.33 | 1.11 ± 0.07 |
| 10 | 2.03 ± 0.05 | 1.26 ± 0.04 | 2.16 ± 0.05 | 1.63 ± 0.05 | 2.41 ± 0.05 | 1.71 ± 0.04 | 2.54 ± 0.06 | 1.68 ± 0.05 |
| 20 | 2.42 ± 0.07 | 1.51 ± 0.07 | 2.87 ± 0.17 | 1.98 ± 0.01 | 2.95 ± 0.07 | 2.12 ± 0.45 | 2.94 ± 0.06 | 2.08 ± 0.06 |
| 30 | 2.81 ± 0.09 | 1.96 ± 0.06 | 3.32 ± 0.11 | 2.54 ± 0.07 | 3.22 ± 0.06 | 2.56 ± 0.06 | 3.12 ± 0.08 | 2.42 ± 0.07 |

**Table 3a. Summary of Two-way Analysis of variance for the data on biochemical constituents in the skin and muscle tissue of *C. auratus* as a function of test diets and experimental duration**

| Tissue | Biochemical constitents | Source of Variance | MS (Df) | F-ratio | P-value |
|---|---|---|---|---|---|
| | Protein | Variance due to experimental duration | 2.011 (9/3) | 197.81 | < 0.0001 |
| | | Variance due to experimental diet | 0.033 (9/3) | 3.26 | < 0.05 |
| Skin | Carbohydrate | Variance due to experimental duration | 1.177 (9/3) | 148.06 | < 0.0001 |
| | | Variance due to experimental diet | 0.033 (9/3) | 4.26 | < 0.05 |
| | Lipid | Variance due to experimental duration | 1.082 (9/3) | 49.67 | < 0.001 |
| | | Variance due to experimental diet | 0.100 (9/3) | 4.63 | < 0.05 |
| | Protein | Variance due to experimental duration | 2.038 (9/3) | 41.22 | < 0.01 |
| | | Variance due to experimental diet | 0.268 (9/3) | 5.43 | < 0.05 |
| Muscle | Carbohydrate | Variance due to experimental duration | 1.648 (9/3) | 47.34 | < 0.01 |
| | | Variance due to experimental diet | 0.196 (9/3) | 5.64 | < 0.05 |
| | Lipid | Variance due to experimental duration | 1.141 (9/3) | 61.06 | < 0.01 |
| | | Variance due to experimental diet | 0.145 (9/3) | 7.77 | < 0.05 |

*Note : Value in paranthesis represents Error Df/ Factor Df and P < 0.05 is statistically significant.*

### 3.2.3 Variation in lipid content

The data on variation in lipid content of skin and muscle tissues of *C. auratus* fed control (CD) and experimental diets (EDY1 to EDY3) are given in Table 3. The results indicated that the lipid content of skin and muscle tissues of *C. auratus* varied much due to variation in experimental diets and duration. For example, the skin lipid content of CD diet fed *C. auratus* fluctuated between 1.95 ± 0.33 and 2.81 ± 0.09 mg/100 mg wet weight. The skin lipid content of *C. auratus* fed on experimental diets did not show much variation and the over all values ranged between 1.95 ± 0.33 and 3.22 ± 0.06 mg/100 mg wet weight. Similarly, the muscle lipid content of CD diet fed *C. auratus* varied from 1.11 ± 0.07 to 1.96 ± 0.06 mg/100 mg wet weight. In experimental diets fed groups the muscle lipid content showed enhancement and it was more (1.11 ± 0.07 to 2.56 ± 0.06 mg/100 mg wet weight) for those fishes received EDY2 diet. The next higher range of 1.11 ± 0.07 to 2.54 ± 0.07 mg/100 mg wet weight was recorded for the muscle lipid content of *C. auratus* received EDY1 diet ant it was low for those fishes fed on EDY3 diet. Two-way analysis of variance (Table 3a) indicated that in both the tissues tested the influence of experimental duration on the lipid content was statistically highly significant ($p < 0.01$ to $p < 0.0001$) when compared to the independence influence of experimental diet ($p < 0.05$).

## 3.3 Total Carotenoid Content (Quantative Analysis)

In order to assess the influence of control and experimental diets on accumulation of total carotenoids, in skin and muscle tissues of experimental fishes, it was extracted by using acetone and methanol solvent systems. Here, the total carotenoid content in the acetone extract of skin and muscle tissues of *C. auratus* fed with experimental diets was high when compared to methanol extracted tissues. Further among the tested diets, the total carotenoid content was more in experimental diets fed fishes, and it was less in control diet fed groups (Table 4).

### 3.3.1 Total carotenoid content in the skin tissue

The total carotenoid content in the acetone extracted skin tissue of *C. auratus* fed with EDY1 diet (1% yeast) showed variation between 0.748 ± 0.042 (0 day) and 0.826 ± 0.018 µg/g wet tissue ($30^{th}$ day). In fishes fed with EDY2 diet (2% yeast), it varied from 0.748 ± 0.042 (0 day) to 0.944 ± 0.014 µg/g wet tissue ($30^{th}$ day). Likewise, in fishes fed EDY3 diet (4% yeast), the total carotenoid content fluctuated from 0.748 ± 0.042 to 0.782 ± 0.007 µg/g wet tissue during 0 and $30^{th}$ day of experiment (Table 4).

Furthermore, the total carotenoid content in the methanol extracted skin tissue of *C. auratus* fed with control and experimental diets are also varied much (Table 4). In fishes received EDY1 diet (1% diet yeast) the total carotenoid content varied from 0.146 ± 0.002 in 0 day to 0.168 ± 0.014 µg/g wet tissue on $30^{th}$ day. In EDY2 diet (2% yeast) fed fishes, it varied from 0.146 ± 0.003 in 0 day to 0.182 ± 0.021 µg/g wet tissue on $30^{th}$ day. A similar range of variation was also recorded in fishes fed with EDY3 diet fed group.

Two-way analysis of variance (Table 4a) for the data on skin total carotenoid indicated that both variation due to experimental diets and also variation due to experimental duration were statistically significant for both acetone (F-ratio : 6.52 and 3.64 ; $P < 0.05$) and methanol solvent (F-ratio : 10.90 and 3.36; $P < 0.05$).

### 3.3.2 Total carotenoid in the muscle tissue

The total carotenoid content in the acetone extracted muscle tissue of *C. auratus* fed EDY1 diet (1% yeast) showed variation from 0.376 ± 0.014 in 0 day to 0.482 ± 0.014 µg/g wet tissue on $30^{th}$ day. In those fishes fed EDY2 diet (2% yeast), it varied from 0.376 ± 0.014 in 0 day to 0.541 ± 0.008 µg/g wet tissue on $30^{th}$ day. Likewise, the total carotenoid varied from 0.376 ± 0.014 in 0 day to 0.424 ± 0.014 µg/g wet tissue on $30^{th}$ day in the muscle tissue of fishes fed EDY3 diet (4% diet) (Table 5).

The total carotenoid content in the methanol extracted muscle tissue of *C. auratus* fed with control and experimental diets are also showed dietary dependent variation (Table 5). In those fishes fed EDY1 diet (1% yeast), it varied from 0.086 ± 0.003 in 0 day to 0.098 ± 0.007 µg/g wet tissue on $30^{th}$ day. Likewise, in the muscle tissue of EDY2 diet (2% yeast) fed in fishes, it varied from 0.086 ± 0.003 in 0 day to 0.142 ± 0.010 µg/g wet tissue on $30^{th}$ day. Also in fishes received EDY3 diet (4% yeast) it varied from 0.086 ± 0.003 to 0.090 ± 0.004 µg/g wet tissue respectively during $10^{th}$ and $30^{th}$ days of experiment.

**Table 4. Total carotenoid content in the skin tissues of *Carassius auratus* fed with CD, EDY1, EDY2 and EDY3 diets supplemented with yeast, *Saccharomyces cerevisiae* for different days and extracted in acetone and methanol solvent system. Each value is the mean ± SD of the three individual estimates**

| Solvent system | Experimental duration (Days) | Total carotenoid (µg/g wet tissues) | | | |
|---|---|---|---|---|---|
| | | CD | EDY 1 | EDY 2 | EDY 3 |
| Acetone | Initial | 0.748 ± 0.042 | 0.748 ± 0.042 | 0.748 ± 0.042 | 0.748 ± 0.042 |
| | 10 | 0.685 ± 0.031 | 0.720 ± 0.016 | 0.724 ± 0.007 | 0.716 ± 0.014 |
| | 20 | 0.721 ± 0.008 | 0.794 ± 0.011 | 0.826 ± 0.011 | 0.752 ± 0.008 |
| | 30 | 0.756 ± 0.007 | 0.826 ± 0.018 | 0.944 ± 0.0174 | 0.782 ± 0.007 |
| Methanol | Initial | 0.146 ± 0.002 | 0.146 ± 0.003 | 0.146 ± 0.004 | 0.146 ± 0.007 |
| | 10 | 0.138 ± 0.002 | 0.140 ± 0.012 | 0.144 ± 0.014 | 0.140 ± 0.011 |
| | 20 | 0.144 ± 0.014 | 0.154 ± 0.011 | 0.161 ± 0.017 | 0.156 ± 0.017 |
| | 30 | 0.148 ± 0.012 | 0.168 ± 0.014 | 0.182 ± 0.021 | 0.161 ± 0.015 |

**Table 4a. Summary of Two-way Analysis of variance for the data on total carotenoid content in the skin tissue of *C. auratus* as a function of variation due to feed and also variation due to experimental duration**

| Solvent system | Source of Variance | EDf / FDf | F-ratio | P-value |
|---|---|---|---|---|
| Acetone | Variance due to experimental diet | 15/3 | 6.525223 | < 0.05 |
| | Variance due to experimental duration | 15/3 | 3.465689 | < 0.05 |
| Methanol | Variance due to experimental diet | 15/3 | 10.90274 | < 0.05 |
| | Variance due to experimental duration | 15/3 | 3.365753 | < 0.05 |

*Note : P < 0.05 is statistically significant*

**Table 5. Total carotenoid content in the muscle tissues of *Carassius auratus* fed with CD, EDY1, EDY2 and EDY3 diets supplemented with 1,2&4% yeast, *Saccharomyces cerevisiae* for different days and extracted in acetone and methanol solvent system. Each value is the mean ± SD of the three individual estimates**

| Solvent system | Experimental duration (Days) | Total carotenoid (µg/g wet tissues) | | | |
|---|---|---|---|---|---|
| | | Control Diet (CD) | Experimental diets | | |
| | | | EDY 1 | EDY 2 | EDY3 |
| Acetone | Initial | 0.376 ± 0.014 | 0.376 ± 0.014 | 0.376 ± 0.014 | 0.376 ± 0.014 |
| | 10 | 0.348 ± 0.032 | 0.352 ± 0.021 | 0.358 ± 0.032 | 0.340 6± 0.026 |
| | 20 | 0.884 ± 0.014 | 0.416 ± 0.024 | 0.482 ± 0.016 | 0.382 ± 0.015 |
| | 30 | 0.389 ± 0.022 | 0.482 ± 0.014 | 0.541 ± 0.008 | 0.424 ± 0.014 |
| Methanol | Initial | 0.086 ± 0.003 | 0.086 ± 0.003 | 0.086 ± 0.003 | 0.086 ± 0.003 |
| | 10 | 0.074 ± 0.002 | 0.082 ± 0.004 | 0.084 ± 0.002 | 0.076 ± 0.002 |
| | 20 | 0.082 ± 0.003 | 0.092 ± 0.002 | 0.098 ± 0.010 | 0.088 ± 0.007 |
| | 30 | 0.092 ± 0.008 | 0.098 ± 0.007 | 0.142 ± 0.010 | 0.090 ± 0.004 |

**Table 5a. Summary of Two-way Analysis of variance for the data on total carotenoid content in the muscle tissue of *C. auratus* as a function of variation due to feed and also variation due to experimental duration**

| Solvent system | Source of Variance | EDf / FDf | F-ratio | P-value |
|---|---|---|---|---|
| Acetone | Variance due to experimental diet | 15/3 | 0.203705 | > 0.05 |
| | Variance due to experimental duration | 15/3 | 0.599251 | > 0.05 |
| Methanol | Variance due to experimental diet | 15/3 | 0.044879 | > 0.05 |
| | Variance due to experimental duration | 15/3 | 0.135674 | > 0.05 |

*Note : P < 0.05 is statistically significant*

Summary of two-way analysis of variance for the data on total carotenoid content in the muscle tissue indicated that irrespective of the solvents tested, both the variation due to diets and also variation due to experimental duration was not statistically significant ($p>0.05$) (Table 5a).

## 4. DISCUSSION

Carotenoids are the important natural pigments principally produced by photosynthetic organisms and accumulated by many animals through their diet [18]. One of the greatest challenges in the ornamental fish industry is to replicate the accurate natural colour of the fish in the captive environment. Numerous operations that have been propagated, tailered for the successful marketing of ornamental fishes without fadded colour. Also various products have been introduced to alleviate this problem, but none has performed so effectively and consistently as carotenoid pigment. Varieties of carotenoid pigments are used as a dietary supplement for colour enhancement. The most promising carotenoids proved to be successful in enhancing colour is astaxanthin that showed marked improvement in colour on most species of brightly coloured ornamental fishes like Tetras, Cichlids, Gouramis, Gold fish, Koi, Danios and many other species [19]. In the present study, *C. auratus* fed on control (CD) and experimental diets (EDY1 to EDY3) supplemented with yeast @ 1,2 and 4% as carotenoid feed additives yield noteworthy information. The biochemical constituents such as protein, carbohydrate and lipid of skin and muscle tissues of *C. auratus* fed with experimental diets showed an obvious enhancement, when compared to those fishes received control diet (CD). The enhanced synthesis of macromolecules in the tested tissue of *C.auratus*, is mainly due to the dietary supplementation of carotenoid source (yeast). Compared to all the tested experimental diets, fishes received EDY2 diet (2% yeast) registered better values. Jelel Opeyemi Agboola et al. [20] reported that compared to fish meal, the different yeast species have favourable amino acid profiles, except for methionine, lysine, arginine and phenylalanine which are the frequently limiting essential aminoacids in Atlantic salmon and rainbow trout. Also the crude protein content of yeast (40-45%) is comparable with soya bean meal. Hence, the higher parameters attained in fishes fed with diet containing 2% yeast may be attributed to their protein and amino acids contribution to the host fish species apart from accelerating the digestion processes. Earlier studies also indicated that, changes in protein and lipid contents in the fish tissues could be linked with changes in their synthesis, deposition rate in muscle and or different growth rate [21-24].

Apart from variation in tissue biochemical constituents of *C. auratus*, the experimental diets also exerted their influence on accumulation of carotenoid in the tested tissues. The results revealed that the total carotenoid content of skin and muscle tissues of *C. auratus* showed dietary dependent variation. It was also noted that, the total carotenoid in the tested tissues of *C. auratus* was more in those fishes fed EDY2 (2% yeast) and it was less in fishes received EDY1 (1% yeast), EDY3 (4% yeast) and control diets fed groups. Sutthi and Thaimuangphol [18] reported that Nile tilapia fed with yeast supplemented diet 0.5% showed improved growth performance, body composition and blood chemistry under salinity treatments (0 and 5 ppt). Further, they also inferred that crude protein content of fish fed with yeast supplemented diet reared at 0 and 10ppt salinity was higher than those fed with basal feed devoid of yeast ($P >0.05$). Mahdy et al. [25] reported that yeast supplementation and yeast containing feed ingredients led to the higher protection against disease and to the better productivity of fishes resulting in the greater growth of the aquaculture industry. They further concluded that yeast has been appeared as a novel and vital component of aquatic animals feed in modern aquaculture and yeast as probiotic product often improve immunity of fishes as well as attempt to enhance the water quality of aquaculture resulting in enhanced production. In the present study, the total carotenoid content was measured in skin and muscle tissues of *C. auratus* by using two different solvent systems.

Moreover, Irianto and Austin [26] reported that Brewer's yeast, have been used in aquaculture as a probiotic due to their fast growth, low cost, high stability and fact that they are not common constituents of feed. In the present study, supplementation of commercial yeast *S.cerevisiae* @ 2.0g/ 100g feed improved the synthesis of macromolecules and carotenoid accumulation in *C.auratus*. Tovar et al., [27] reported that incorporation of yeast in diet of improved the digestive enzyme activity of sea bass and it leadto great improvement of the survival and growth rates. They further inferred that yeast adherence to the gut when supplied

with diet lead to enhance amylase secretion and stimulation of brush border membrane enzyme. Wache et al. [28] and Abdel-Tawwab et al. [24,29] have opinioned that the addition of live yeast improved diet and protein digestibility which may explain the better growth and feed effiency with yeast supplements. Extracted in acetone solvent system Ibrahim et al. [30] reported that fishes can be pigmented by including processing wastes and plant sources. Also Hata and Hata [31] and Stevan [32] reported that the carotenoid pigmentation of fishes resulted from the pigment present in the diet. It implied that the colour enhancing diet should contain additional natural pigments to enhance the pigments in ornamental fishes. Further in natural environment, fishes met their carotenoids requirements by ingesting aquatic plants having carotenoids or through their food chains.

These results suggest that yeast supplementation plays a role in enhancing food intake with subsequent acceleration of fish vital physiological and biochemical performances [33]. Cnaani et al. [34], Rehulka et al. [35] and Abdel-Tawwab et al. [29] have observed that the better feed intake in yeast supplemented diets (from 1.01 upto 3.0g/kg diet) may have been due to increase fish appetite resulting in a higher feed intake and therefore improved growth performance, physiological and biochemical analysis often provide vital information for health status about cultured fish. Furthermore, this study inferred that *C. auratus* fed diets containing 2.0g yeast per 100g feed established higher protein, carbohydrate, lipid synthesis in skin and muscle tissue; which in turn affect the bioaccumulation of total carotenoids. These results indicated on enhancement in the synthesis of macromolecules and bioaccumulation of carotenoids in skin and muscle tissue of *C.auratus* when fed EDY2 diet (2% yeast) an is in consistence with the findings of Li and Gatllin [36] and Abdel-Tawwab et al. [24,29]. Further, in these fishes reviewed *S.cerevisiae* at 4% the biomolecule synthesis and accumulation of carotenoid was lowered it may be due to the availability of exclusive protein and amino acids, in the host system which may led to energy expenditure on related deamination process. On the other hand, acceleration of digestive processes takes place at a higher level at the optimum concentration of *S. cerevisiae* (2%) and over and above the optimum level may attain saturation point. This may be also a reason for non-enhancement of tested parameters in the experiment fish. The overall results of this present study inferred that Brewer's yeast supplementation significantly affect the carcass biochemical composition of *C. auratus* and also the accumulation of carotenoids in tissues [37-39].

## 5. CONCLUSION

*Saccharomycese cerevisiae* as probiotics accelerated the biosynthesis of macromolecules in both skin and muscle tissue of *Carrasius auratus.* Besides enhancing biochemical constituents, *S. cerevisiae* added diet fed fishes at the rate of 2.0g/100g displayed higher carotenoid level in the tested tissues in both the solvent system used. Overall results inferred that *S.cerevisiae* could be effectively used as probiotics in ornamental fish culture for accelerating the synthesis of biomolecules and also for bio-accumulation of carotenoids.

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## AVAILABILITY OF DATA AND MATERIALS

This is original work carried by the authors. All the analysed data are available. Upon request it will be made available.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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