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EFFECT OF DIETARY OMEGA-3 AND MICRONUTRIENTS ON GROWTH, MEAT QUALITY AND HEALTH STATUS OF POLYCULTURED CYPRINID SPECIES

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This study was aimed to assess the effect of omega-3 fatty acid and micronutrients on growth, meat quality and health status of polycultured Cyprinid fish species (*Labeo rohita*, *Cirrhinus mrigala* and *Catla catla*). The proposed fish fingerlings (8.0±1.5g to 10.0±0.9 g and 7±1.1 to 8±1.6 cm) were polycultured in twelve earthen ponds, for eight months (April - November 2017). Experimental formulated feed (35% CP) having three concentrations of omega-3 fatty acid (0.3, 0.6 and 0.9%), and two concentration of vitamin C (0.75g/kg and1.5 g/kg) and Vitamin E (300 and 600 mg/g). Fish were fed by 5% of body weight daily. The experimental feed includes 9% omega-3, 1.5 g/kg Vitamin C and vitamin E 600 mg/g showed highest weight and length gain, higher survival rate (98%), maximum specific growth rate (1.37%) and minimum FCR as 1.85. The values of condition factor (K) in all fish species were found to be more than 1 which indicated the good health status. Total plate count (TPC) in fish species statistically varied among various treatments. The moisture, protein and carbohydrates contents showed significant difference while ash and fat contents showed non-significant differences statistically. The results of current study suggested that dietary supplementation of omega-3 and micronutrients would increase fish growth and also improve the fish health status and body composition.
Keywords: Unsaturated fatty acid, proximate composition, vitamins, Indian major carps.

INTRODUCTION

Seafood's play substantial role in human nutrition with their rich source of n-3 long-chain poly-unsaturated fatty acids, e.g., arachidonic acid (ARA), eicosapentaenoic acids, (EPA) and docosahexaenoic acid (DHA), high quality protein, essential amino acids, micronutrients. All around the world about two billion peoples suffered from serious mental disorders, avoidable blindness and even death during childbirth due to micronutrients deficiencies. Fish fatty acids have dynamic role against heart diseases, stroke, hypertension, diabetes, depression and rheumatoid arthritis (Mohanty *et al*., 2016).

Indian major carps especially *Labeo rohita* considered world's 10th highest cultured fish in production by volume. *Catla catla*, *Labeo rohita* and *Cirhinus mrigala* in Pakistan are important poly cultured species because of their fast growth rate, palatability, size and superior nutritional status. Fish is a healthy diet that contains essential omega-3 fatty acids. These essential fatty acids must be involved in the diets like the other essential amino acids which are not produced in the human body (Ismail, 2005).

Fish needs various micronutrients in appropriate quantity to regulate different body functions. The supplementation of essential omega-3 polyunsaturated fatty acid (PUFA) in greater amount in the fish diet will result in a quality fish meat of high nutritive values. Omega-3 PUFA contents have significant effect on human health along with n-6 PUFA which may minimize the risk of many health hazards (Simopoulos, 2011).

Increase in dietary omega-3 in fish diet up to 3% improved larval growth, lipid deposition and absorption considerably. The increase in dietary vitamins C and E protects essential fatty acids from oxidation, enhance the quality and quantity of these fatty acids and promote larval growth (Kertaoui *et al*., 2017). Dietary omega-3 highly unsaturated fatty acids, especially Eicosapentaenoic acid and docosahexaenoic acid play an important biological role in supporting growth performance, decreasing fatty acids accumulation and establishing the cell membrane structure of aquatic animals and therefore the fish oil superior lipid source rich in omega-3 has been used in aquaculture diets (Todor, 2009).

According to the American Heart Association, n-3 fatty acid has been proved to help in preventing cardiovascular diseases by minimalizing the risks of thrombosis, arrhythmia, decreasing blood pressure and plasma triglyceride levels (American Heart Association, 2002). Tocotrienols and tocopherols are building blocks of vitamin E, both have a same structure but a different concentration; α-tocopherol is the most energetic and abundant isomer in different membranes of different vertebrates (Faramarzi, 2012).

Dietary micronutrient ingredients are also important for fish health and welfare. Higher levels of vitamin C in diet of *Labeo rohita* enhanced protection significantly against the infection of *Aeromonas hydrophila*. Various serological and hematological parameters are influenced by supplementation of vitamin C (Tewary and Patra, 2008). Vitamins A, D, E, K and C are essential in the fish diet. Both vitamins C and E can act as antioxidants in a synergistic manner. Vitamin C acts as a terminal element in the protection against tissue damage, but when both vitamins are present, the major function of vitamin C is restoration of vitamin E (Torres *et al*., 2002).

Keeping in view the above considerations regarding nutritional importance and enhancement, this study was planned to enhance the nutritional value of farmed Major Carps and to determine the optimal inclusion levels of n-3 fatty acid and micro nutrients (Vitamins C and E) in fish feed.

MATERIALS AND METHODS

***Performance of experimental trial*:** An experiment of 8 months' duration (April - November 2017) was executed in 12 earthen ponds having the dimension of 22 X 10 X 1.5 meters (length- width-deep) each at Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. The three fish species including *L. rohita, C. catla* and *C. mrigala* were fed two time in a day at 5% of body weight with feed having 35% crude protein along with three concentrations of n-3 fatty acids and two concentrations of vitamins C and E (Table 1).

***Fish stocking*:** For growth studies, a total of 150 Cyprinid fish species fingerlings (50 fingerlings of each species) were reared in twelve earthen ponds. At the time of stocking, initial body weight (g) and total length (cm) of each species were recorded.

***Feed supplementation*:** The feed was prepared from locally available ingredients like soya bean meal, sunflower meal, wheat flour, fishmeal, molasses vitamins, minerals, omega-3 and vitamin (C and E). The feed ingredients were mixed in proper quantities contained 35% crude protein. Required components were weighed accurately and grounded and mixed well. All the above ingredients were mixed in an enamel tray and blended homogenously. The powdered feed has been used instead of extruded or pelleted feed for current study. Fishes were fed with formulated feed supplemented with three concentrations of omega-3 (0.3, 0.6 and 0.9%), two concentrations of vitamin C (0.75mg/kg, 1.5 mg/kg) and Vitamin E (300 and 600 mg/100g) @ of 5% body weight two times daily. The quantity of formulated feed was adjusted fortnightly.

***Growth monitoring*:** A sample of at least 10 fishes of each species was taken randomly and released back in to respective ponds after recording their body weight (g) and body length (cm). The specific growth rate, feed conversion ratio and condition factor were calculated to determine the growth of major carps.

***Specific growth rate (SGR)*:** The SGR (%) was calculated by the formula given by Dhawan and Kaur (2002) as:

$$\text{SGR} = \frac{\text{In (Final BW} - \text{In (Initial BW)}}{\text{Time duration (days)}} \times 100$$

Where, BW = Wet body weight

***Feed conversion ratio (FCR)*:**

$$\text{FCR} = \frac{\text{Feed given (g)}}{\text{Weight gain of fish}}$$

***Condition factor (K)*:** Condition factor (K) was determined for different lengths and weights data with (LeCren, 1951) equation.

$$\text{K} = (\text{W} * 100/\text{L}^3)$$

Where, W = fish weight (g), L = fish standard length (cm), W =fish weight (g)

***Harvesting of fish*:** At the end of experiment, 10 individuals of each species were harvested from each pond. The collected samples were stored at -20°C and following

Table 1. Formulation and composition% of experimental Feed (g/100 g of dry weight).

Ingredients/ FA	T_1 0.3	T_2 0.3	T_3 0.3	T_4 0.3	T_5 0.6	T_6 0.6	T_7 0.6	T_8 0.6	T_9 0.9	T_{10} 0.9	T_{11} 0.9	T_{12} 0.9
Fish meal	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Soy bean meal	33.00	33.00	33.00	33.00	33.00	33.00	33.00	33.00	33.00	33.00	33.00	33.00
Sunflower meal	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Wheat bran	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Rice polish	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Maize grain	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Molasses	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Minerals Mixture	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin Premix	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin C gm/kg	0.75	1.50	0.75	1.50	0.75	1.50	0.75	1.50	0.75	1.50	0.75	1.50
Vitamin E mg/100gm	300	600	600	300	300	600	300	300	300	600	600	300

analysis were done within 72 hours prescribed time limit as per AOAC (2016).

***Total plate count analysis*:** For total plate count (TPC) was determined with a standard test method as described in AOAC (2016)

***Proximate analysis*:** By following standard procedures of AOAC (2016), different proximate constituents viz. moisture, crude fat, crude proteins and ash were calculated separately for each fish species. Total carbohydrates in fish meat were estimated by difference of the entire proximate parameters i.e.

Total carbohydrate (%) = 100 – (moisture % + crude protein % + crude fat % + ash %)

***Statistical analysis*:** The differences between the mean values of parameters was examined and calculated by using analysis of variance (ANOVA) after normalization and homogeneity were checked and Tukey's test was applied to unequal variances among the mean values of samples at 5% level of significance.

RESULTS

The study was carried out to investigate omega-3 fatty acid and micronutrients (vitamins C and E) effect on growth and health status of Indian major carps.

***Weight gain (WG)*:** The maximum weight gains (WG) in *Labeo rohita* was seen in T_{10} and minimum weight gain in T_{12}. The maximum weight gain of *Cirrhinus mrigala* was observed in T_{10} and minim in T_{11} while, the maximum weight gains in *Catla catla* in T_{10} and minimum in T_{12} was recorded (Table 2).

***Length gain (LG)*:** Maximum and minimum increase in length in *C. mrigala* was recorded in T_8 and T_1, respectively. Increase in length of *C. catla* in all treatments showed similar trends except T_9 which showed minimum increase in length (Table 2).

***Specific growth rate (SGR) and feed conversion ratio (FCR)*:** All the treatments showed a little variation in specific growth rate and feed conversion ratio for major carps viz; *L. rohita*, *C. mrigala* and *C. catla*. T_{10} showed a maximum survival rate of 98%, while minimum survival rate 92% was recorded in T_8. Specific growth rate (SGR) was minimum in T_7 and 8 (1.24) and maximum rate was observed in T_{10} (1.37) (Fig. 1). Maximum feed conversion ratio (FCR) 2.92 in T_2 and its minimum value (1.86) was recorded in T_{10} which showed maximum growth (Fig. 2).

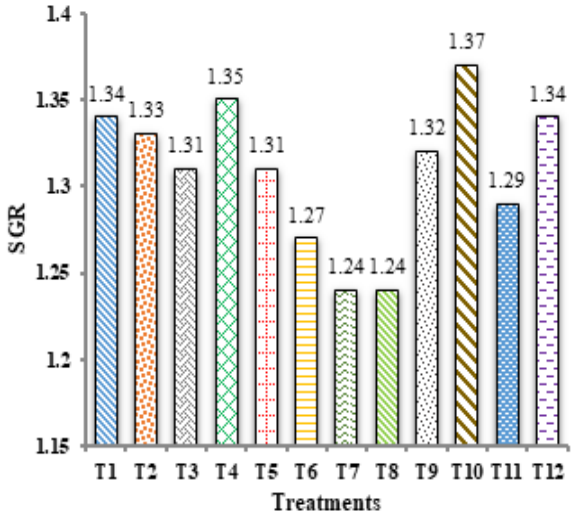

Figure 1. Specific growth rate (SGR) for major carps under various treatments.

Table 2. Comparison of means for *L. rohita, C. mrigala* and *C. catla* weight gain (g) fortnightly under various treatments.

Treatments	Species			Mean
	L. rohita	*C. mrigala*	*C. catla*	
T_1	206.50±4.47b-f	215.00±5.78a-d	188.60±3.81c-f	203.37±3.35BC
T_2	207.70±6.74b-f	209.30±8.43b-f	181.80±3.12f	199.60±4.30BC
T_3	215.50±3.95abc	211.40±6.18a-f	193.40±4.21c-f	206.77±3.25AB
T_4	203.10±6.77b-f	212.40±5.96a-e	184.00±3.02ef	199.83±3.77BC
T_5	200.30±6.99b-f	217.10±6.73abc	197.10±4.86b-f	204.83±3.85BC
T_6	203.00±6.55b-f	201.80±3.83b-f	196.60±3.22b-f	200.47±2.70BC
T_7	196.50±5.32b-f	200.70±4.90b-f	193.50±3.44c-f	196.90±2.63BC
T_8	196.10±5.18b-f	201.20±8.71b-f	191.40±3.45c-f	196.23±3.52BC
T_9	201.00±6.50b-f	196.30±6.23b-f	184.70±4.28def	194.00±3.45BC
T_{10}	224.30±3.39ab	241.50±8.51a	193.70±3.79c-f	219.83±4.86A
T_{11}	200.20±6.22b-f	188.20±6.47c-f	183.60±3.78ef	190.67±3.39C
T_{12}	186.80±6.62c-f	200.70±7.87b-f	189.60±3.89c-f	192.37±3.71BC
Mean	203.42±1.82A	207.97±2.21A	189.83±1.14B	

Average with similar letters in a column and row are non-significant (P>0.05) statistically. Capital letters are used for overall mean and small letters denote comparison between means interactions.

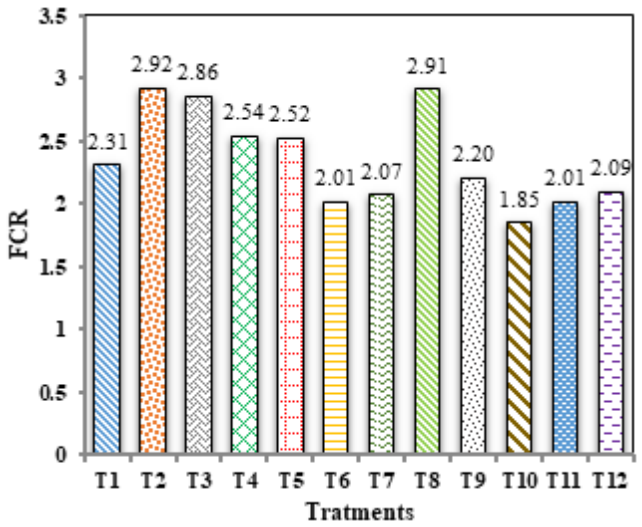

Figure 2. Feed conversion ratio (FCR) for major carps under various treatments.

***Condition factor (K)*:** The condition factor (K) characterizes health status or security of fish. The three fish species of major carps having value of K greater than 1 are supposed to be better in health conditions. In the current study, the value of 'K' in all the three fish species of major carps under different treatments were found higher than 1 which indicated the good health status of fish (Fig. 3 a, b, c).

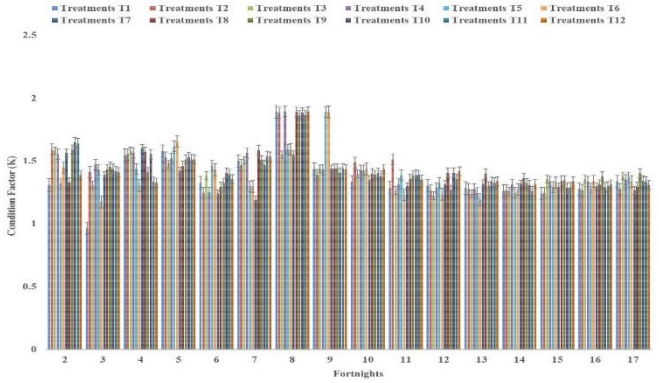

Figure 3a. Condition factor (K) for different treatments regarding *L. rohita* at different fortnights.

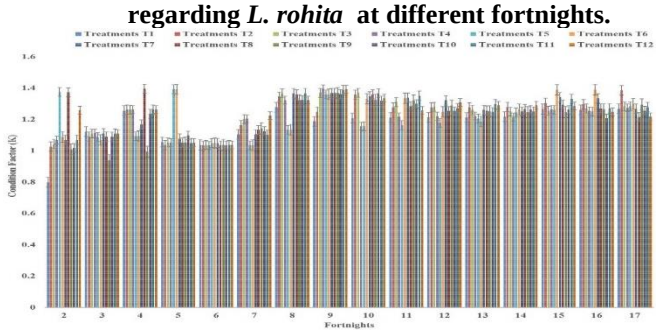

Figure 3b. Condition factor for different treatments regarding *C. mrigala* at different fortnights.

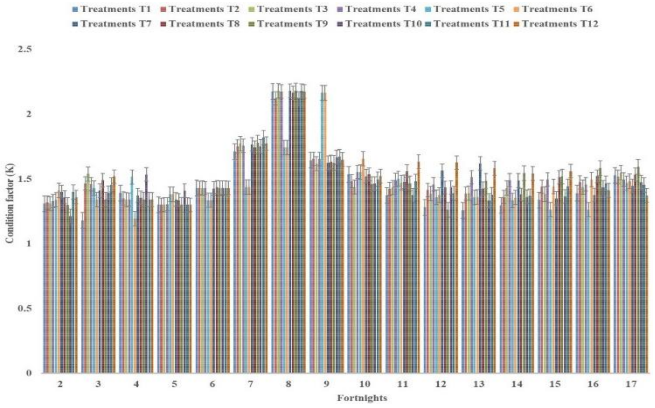

Figure 3c. Condition factor for different treatments regarding *C. catla* at different fortnights.

***Total plate count (TPC)*:** Total plate count (TPC) widely varied under various treatments. The maximum mean count was recorded in T_9 (9157±578 cfu/g) and minimum count was determined in T_8 (6998±706 cfu/g). TPC was counted 9688±214 cfu/g, 10104±150 cfu/g and 3692±297 cfu/g in *L. rohita*, *C. mrigala* and *C. catla*, respectively (Fig. 4). Analysis of variance on TPC showed highly-significant difference among species, treatments and in three fish species ($P<0.05$) (Table 3).

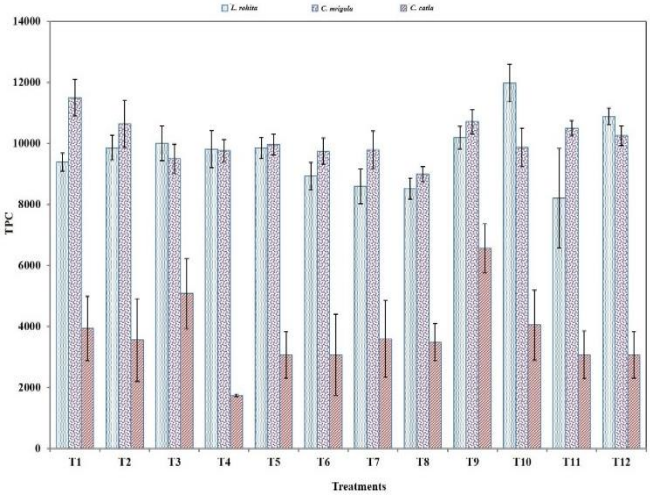

Figure 4. Comparison of total plate count (cfu/g) in *L. rohita, C. mrigala* and *C. catla*.

***Proximate analysis*:** The moisture content percentage in three fish species showed a least difference at $P<0.05$ among various treatments. The maximum mean value (73.98±0.14%) of moisture content and minimum values of moisture content (74.67±0.07%) were observed in T_9 and T_2, respectively. Analysis of variance on moisture content showed significant difference among species and treatments in three fish species ($P<0.05$). The interaction between species and treatment for moisture content was non-

Table 3. Comparison of means for *L. rohita, C. mrigala* and *C. catla* increase in length (cm) fortnightly under various treatments.

Treatments	Species			Mean
	L. rohita	*C. mrigala*	*C. catla*	
T_1	18.10±0.64a-g	14.20±0.55i	20.10±0.31a	17.47±0.54BC
T_2	17.40±0.78a-h	15.40±0.67ghi	19.40±0.45a-d	17.40±0.47C
T_3	17.00±0.47b-i	16.40±0.43d-i	19.90±0.38ab	17.77±0.37ABC
T_4	19.50±0.37abc	18.00±0.65a-h	19.90±0.31ab	19.13±0.30A
T_5	19.30±0.63a-d	17.60±0.78a-h	20.40±0.54a	19.10±0.42A
T_6	17.80±0.59a-h	15.00±0.56hi	19.30±0.68a-d	17.37±0.48C
T_7	17.50±0.45a-h	16.00±0.71ghi	19.20±0.55a-e	17.57±0.41BC
T_8	16.50±0.52c-i	16.20±0.68e-i	19.70±0.42ab	17.47±0.43BC
T_9	19.10±0.59a-f	16.10±0.75f-i	17.60±0.56a-h	17.60±0.42BC
T_{10}	18.10±0.43a-g	19.20±0.44a-e	20.10±0.57a	19.13±0.31A
T_{11}	19.50±0.54abc	16.50±0.43c-i	19.80±0.53ab	18.60±0.39ABC
T_{12}	19.40±0.45a-d	17.00±0.52	20.30±0.52a	18.90±0.38AB
Mean	18.27±0.18B	16.47±0.21C	19.64±0.15A	

Average with similar letters in a column and row are non-significant (P>0.05) statistically. Capital letters are used for overall mean and small letters denote comparison between means interactions.

Table 4. Analysis of variance for proximate body composition and total plate count of *L. rohita, C. mrigala* and *C. catla* among various treatments.

SOV	DOF	Mean squares					
		Protein	Ash	Moisture	Fat	Carbohydrates	TPC
Species (S)	2	7.0780**	0.12029 ^{NS}	0.7915*	0.05029 ^{NS}	7.4501**	77.634**
Treat. (T)	11	0.1083 ^{NS}	1.06045**	0.5778*	0.28250**	0.8623**	13.399**
T x S	22	0.1590 ^{NS}	0.15491 ^{NS}	0.1308 ^{NS}	0.03425 ^{NS}	0.3855 ^{NS}	1.681**
Error	324	0.2761	0.09715	0.2461	0.08406	0.2495	1.384

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

significant (P>0.05). The protein content percentage in three fish species showed a least difference among various treatments. The maximum mean value (18.51±0.14%) of protein content and minimum values of moisture content (18.20±0.07%) were observed in T_6 and T_4, respectively. Analysis of variance on protein content showed significant difference among species and non-significances in treatments in three fish species (P<0.05). The interaction between species and treatment for protein content was also non- significant (P>0.05). The fat content percentage in three fish species showed a least difference among various treatments. The maximum mean value (2.90±0.09%) of fat content and minimum values of fat content (2.45±0.03%) were observed in T_9 and T_2, respectively. Analysis of variance on fat content showed non-significant difference among species and highly significances difference in treatments in three fish species (P<0.05). The interaction between species and treatment for fat content was also non-significant (P>0.05).

Ash content of major carps widely varied under different treatments. The highest ash content (2.45±0.06%) were recorded in T_8, while minimum value of ash content (1.79±0.07%) was observed in T_1. Statistical analysis on ash content showed non-significant difference among species and highly significant difference in treatments in three fish species (P<0.05). The interaction between species and treatment for ash content was also non- significant (P>0.05). Carbohydrates content of major carps widely varied under different treatments. The highest carbohydrate content ((2.98±0.12%) were recorded in treatment no T_4, while minimum value of carbohydrate content (2.09±0.06%) were observed in T_8. Analysis of variance on carbohydrate content showed highly-significant difference among species and treatments in three fish species (P<0.05). The interaction between species and treatment for carbohydrates content was also non- significant at P>0.05 (Table 4).

DISCUSSION

After the completion of research, numerous parameters of chemical composition, survival and growth in terms of, WG, LG, SGR, FCR and K were found different significantly under different treatments. During this study, it was observed that micronutrients had no significant effect on proximate body composition, but a direct effect was observed on the growth performance and health condition of major carps. The importance of vitamin E and C along with artemia on growth, survival and stress resistance in

fingerlings of Angelfish (Norouzitallab *et al.,* 2009). The supplementation of higher levels of vitamin C helped to recognized effects on growth and health of *L. rohita*. Similar effects were reported by Tewary and Patra (2008) for *L. rohita*. By increasing vitamin E supplementation, the serum content minimized while gonad somatic index increased in Rohu (Tan *et al*., 2007). The supplementation of vitamin C at rate of 500 mg positively affected the growth and immunity of Nile tilapia (Ibrahem *et al.*, 2010). By increasing the levels of dietary vitamin E from 100 mg/kg to 200 mg/kg and vitamin C 500 mg to 1000 mg/kg innocently affected the growth of Japanese flounder (Gao et al., 2014)

Fresh fish body weight is a basic indicator for growth performance. During this study, final body weight and net fish production of major carps after a period of 240 days, showed a significant increase under all treatments having various doses of n-3 fatty acids, vitamins and time duration on growth and biomass of major carps. During the present study the values of overall mean weight gain was shown by *C. mrigala* followed by *L. rohita* and *C. catla*. The maximum length gain was shown by *C. catla* followed by *L. rohita* and *C. mrigala*, respectively under various treatments. The T_{10} showed maximum fish production. On the other hand, T_{11} showed minimum fish production. Kertaoui *et al.* (2017) suggested that dietary essential fatty acids up to 3%, improved fish larval lipid, absorption, deposition, survival and growth significantly. Along with 3% fatty acids and increase in micronutrients improved total lipid, n-3 fatty acid content and body weight of fish juvenile.

The important effect of polyunsaturated fatty acids on survival, post hatchling size and improving fertility in zebrafish up to 30% (Nowosad *et al*., 2017). The maximum growth was observed in fish fed higher amount and good quality commercial feed (Watters *et al.,* 2013). By inclusion of 1% omega-3 showed greater immunological responses and greater than 1% caused immunosuppression in *L. rohita* juveniles along with reduction in chemicals and antibiotics. Increase in dietary omega-3 in fish diet up to 3% improved larval growth, lipid deposition and absorption considerably (Baharthi and Kunda,2011) and increase in dietary vitamins C and E protects essential fatty acids from oxidation, enhance the quality and quantity of these fatty acids and promote larval growth (Kertaoui *et al*., 2017).

The survival rate ranged from 92-98%. The maximum survival rate was recorded in treatment no 10 having 0.9% n-3 fatty acid, 1.5 g/kg vitamin C and 600 mg/100g vitamin E and minimum in T_8. Similar values of FCR, SGR survival were recorded for tilapia (Tawwaby *et al.,* 2008). The minimum value of FCR was observed in treatment no 10. These results showed that major carps feed with 0.9% omega-3 fatty acids achieved best feed conversion ratio. Baharthi and Kunda (2011) reported similar findings by the inclusion of 1 % n-3 fatty acids in feed of fingerlings of *L. rohita*.

The supplementation of vitamin C greater than the normal is required for growth and survival. The fish diet with 500 mg vitamin C /kg enhanced FCR and SGR in *labeo rohita* (Misra *et al*., 2007). Better growth, immune response and disease resistance in the *Epinephelus malabaricus* juveniles feeding with vitamin C and growth parameters were found to be healthier with feeding of intermediate levels of vitamin C and suggested that diets with 200 mg/kg vitamin C would be best for *Labeo rohita*. culture (Lin and Shiau, 2005). While the optimum supplemented levels of vitamin E for GIFT were about 573.8–582.3 mg/kg and 655–752.5 mg/kg (Wu *et al.*, 2017).

The value of 'K' in all fish species of major carps under different treatments were found to be more than 1 which directed the good health status of fish. The condition factor was also found to be more than 1 showed good health condition of fish (Singh and Serajuddin, 2017).

Total plate counts widely varied under various treatments. The maximum mean count was recorded in 9^{th} treatment and minimum count was determined in 8^{th} treatment. The highest total plate count was recorded in both catfish and tilapia collected from lakes and cages paralleled to fish species from tanks, ponds and hatcheries (Kato *et al.*, 2016).

The analysis of proximate body composition of three freshwater fish species of major carps is a significant aspect for estimation of nutritional status for clients and nutritionists. The culture environment has direct effect on meat quality. The ranges of proximate body composition of major carps under different treatments in our study were within ranges investigated by other researchers (Job *et al.*, 2015).

The fish weight increases with increase in temperature. The results of present study revealed that maximum growth of major carps was observed in treatment no 10 in which maximum and minimum temperature were recorded 35 and 28°C, respectively. While the minimum growth in T_{11} at minimum range of temperature 27.7°C and upper limit of temperature 34.0°C were observed. The levels of temperature 28-32°C was good for tropical major carps as suggested by Bhatnagar *et al*. (2004). The water quality parameters of fish pond directly and indirectly plays a conclusive role in survival, growth performance and total fish production. Due to these conditions aqua culturist always kept these aspects under concern. The Best fish production is dependent on the physical, chemical and biological potentials of water. Comparable inferences were reported by Singh *et al*. (2008) in *C. mrigala*.

***Conclusion*:** Based on the results of the current study, it can be concluded that supplementation of omega-3 fatty acid level of 0.9 g/100g, vitamin C (1.5g/kg) and vitamin E (600mg/100g) levels of dry weight of feed showed better growth, survival, production, healthier body composition and improved feed conversion ratio in major carps than other

treatments. Micronutrient (Vitamin C and E) did not showed any substantial effect on proximate fish body composition.

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