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IMPACT OF LYCOPENE SUPPLEMENTED CANOLA MEAL-BASED DIET ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY AND ANTIOXIDANT STATUS OF *CATLA CATLA* FINGERLINGS

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ABSTRACT

A 70 days feeding trial was conducted to determine the effects of lycopene supplemented canola meal-based diet on growth performance, nutrient digestibility and anti-oxidant status of *Catla catla* fingerlings. Seven experimental diets *viz*. T1, T2, T3, T4, T5, T6 and T7 were formulated with graded lycopene levels i.e., 0 (control), 10, 20, 30, 40, 50 and 60 mgkg^{-1}, respectively and fed to fingerlings using completely randomized design (CRD). During experimental period, fish were fed at 5% of their total biomass. After performing one-way Analysis of Variance, it was observed that maximum weight gain % (193%) and best FCR (1.95) was observed in fish group fed diet having 40 mgkg^{-1} of lycopene. Optimum gross energy (68%), ether extract (77%) and crude protein (75%) values were found in fish at the same level and these values showed significant ($p \leq 0.05$) difference than the control diet. Lowest percentage of oxidation (3.57%) was observed at test diet having 40 mgkg^{-1} with the highest antioxidant activity recorded in fish fed T-V diet. It was concluded that lycopene supplemented canola meal-based diet optimally improved growth parameters, nutrient utilization and antioxidant status of *C. catla* fingerling at 40 mgkg^{-1}.

Keywords: Lycopene, aquaculture, antioxidant status, canola meal, fish meal

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INTRODUCTION

Fish are well known as nutritionally valued portion of the human diet, usually due to long chain polyunsaturated fatty acids and proteins. These fatty acids are involved in many metabolic pathways and hence essential for human health. Moreover, fish meat is also rich in vitamins, minerals, calcium, selenium and phosphorus (Khalili and Sampels, 2018). Thaila is the common name of *Catla catla* (Hamilton, 1822) which is a surface feeder. In Pakistan, it is cultured with other major carps in a poly-culture system (Aslam *et al.,* 2016). As compared to other food sectors, aquaculture industry is growing rapidly. However, the limitations in the development of fish feed caused the increase in the cost of fish feed (Yıldırım *et al.,* 2014). Due to high nutritional value and palatability, aquaculture industry partially or solely depends on fish meal usage in aqua feeds (NRC, 2011).

In aquaculture sector, the cost of fish feed accounts for up to 50% of the total cost (Essa *et al.,* 2010). The fish meal prices are increasing constantly; probably due to scarce supply and increased demand (Joint FAO, 2010). Furthermore, constrained supplies of fish meal cannot fulfil the requirements of the growing fish-feed industry (Bostock *et al.,* 2010). Therefore, to search an alternate for fishmeal (FM) is very important (Kumar *et al.,* 2010). Plants are very important source of proteins and some other essential amino acids. For the development of eco-friendly and low-cost fish feeds, plants can play key role (Cheng and Hardy, 2004; Khan *et al.,* 2011; Hussain *et al.,* 2015). Canola meal (CM) is being frequently used as an alternative of FM in aquaculture feed formulations. Commercially and economically, CM is preferred than the FM as well as the soybean meal (Sajjadi and Carter, 2004). Due to the presence of cystine and methionine, the CM is highly digestible for the fish and serves as an alternative of fishmeal. It is a good source of energy and proteins and these proteins of plants are less expensive than the fish meal (Glencross *et al.,* 2007; Hussain *et al.,* 2015).

Lycopene is one of the important carotenoids which is present in synthetic pathway and provide the molecular basis for the production of other carotenoids. It acts as a powerful antioxidant for the reduction of oxidative stress and related pathological issues (Cardona *et al.,* 2006). Due to its activity as an antioxidant agent against single oxygen and other free radical species, it has created interest during the last decade. So, lycopene may have preventive role against the diseases (Yonar, 2013). Against the biological reactive oxygen species, lycopene act as an antioxidant and ameliorate the oxidative damage to the cell and different tissues during both in vivo and in vitro studies (Banerjee *et al.,* 2017). Hence, the main

objective of current research is to check the effects of lycopene supplemented canola meal-based test diets on growth, nutrient utilization and antioxidant status of *C. catla* fingerlings.

MATERIALS AND METHODS

The current study was conducted to determine the parameters of growth, antioxidant status and nutrient utilization of *C. catla* fingerlings fed canola meal-based diets supplemented with lycopene. The experimentation was conducted in the Fish Nutrition Laboratory, Department of Zoology, Government College University, Faisalabad starting from April 2019 to March 2020.

Fish and Experimental Conditions: *C. catla* fingerlings were purchased from Government Fish Seed Hatchery, Faisalabad. These were kept for two weeks to acclimatize them in specially designed V-shaped tanks that have the water storage capacity of approximately 70L. The special design of tanks allowed the collection of feces. Completely randomized design (CRD) was used in the present study. Fifteen fingerlings were assigned randomly to each tank. After purchasing fingerlings from hatchery, these were treated with sodium chloride (5g L^{-1}) solution to prevent from fungal diseases and ectoparasites (Rowland and Ingram, 1991). Basal diet was provided once a day till apparent satiation (Allan and Rowland, 1992).

Feed Ingredients and Formulation of Experimental Diets: The feed was finely grinded to pass it through the sieve of 0.5mm size. All ingredients were mixed in a mixer by gradually adding the fish oil. In order to make appropriate dough for pelleting, 10-15% water was also added in it (Lovell, 1989). Processed dough then passed through pelleting machine in order to make graded levels of lycopene supplemented canola meal-based diets. Table 1 shows the ingredient composition of all the test diets including control.

Table 1. Ingredient's composition (%) of test diets.

Ingredients	Test Diet-I (Control)	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI	Test Diet-VII
Lycopene($mgkg^{-1}$)	0	10	20	30	40	50	60
Canola meal	52	52	52	52	52	52	52
Fish meal	16	16	16	16	16	16	16
Wheat flour*	12	12	12	12	12	12	12
Rice Polish	9	9	9	9	9	9	9
Fish oil	7	7	7	7	7	7	7
Chromic oxide	1	1	1	1	1	1	1
Vitamin Premix	1	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1	1
Mineral premix	1	1	1	1	1	1	1

* Lycopene was added at the cost of wheat flour

Feeding Protocol and Sample Collection: Initial weight of fish was noted. Fish were fed at the rate of 5% of live wet body weight of the fish on their suggested diet two times a day, once in the morning and once in the evening. For each test diet, three replicates were assigned and fifteen fingerlings were stocked in each replicate. After two hours of feeding period, the valves of tanks were used to remove the uneaten diet from each tank. The tanks were washed thoroughly to eliminate the remaining diet particles and then again filled. Then feces were collected with the help of valves from the fecal collecting tube of each tank. Care was taken to avoid breaking of the thin fecal strings so, to prevent leaching of nutrients. Collected feces of each replicate treatment were dried in oven and stored for further chemical analysis.

Growth Study: At the start and end of experiment, fish in each tank were bulk weighed to evaluate the growth parameters. Initial weight of fish was recorded as 8.08g. Growth performance of fingerlings was assessed by using the following standard formulae (AOAC, 1995):

$$\text{Weight gain \%} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \times 100$$

$$\text{FCR} = \frac{\text{Total dry feed intake (g)}}{\text{Wet weight gain}}$$

After feeding, uneaten feed was collected and difference between two feeds was the feed intake by the fish.

Chemical Analysis of Feed and Feces: Experimental feed ingredients and feces samples were homogenized by using a mortar and pestle and analyzed by standard methods (AOAC, 1995). Crude protein (N × 6.25) contents by a micro kjeldahl apparatus, moisture content of feed and feces by drying in oven at 105°C for approximately 12 hours, crude fat by petroleum ether extraction method through Soxtec HT2 1045 system, ash content in electric furnace by igniting it at 650°C for 12 h (Eyela-TMF 3100), crude fiber was determined as dried

residue is lost after ignition as they are lipid-free after digestion with 1.25% NaOH and 1.25% H_2SO_4 and oxygen bomb calorimeter was used to determine the gross energy.

Chromic Oxide Estimation: The chromic oxide content that was used as an inert marker was assessed by the use of acid digestion (Divakaran *et al.,* 2002) through UV-VIS 2001 spectrophotometer at 350 nm in feces and test diets with per chloric reagent after oxidation.

Calculation of Digestibility: By using the appropriate formula, apparent nutrient digestibility coefficient (ADC%) for experimental diets were evaluated by using the following formulae:

$$ADC\ (\%) = 100 - 100 \times \frac{\%\ \text{marker in diet} \times \%\ \text{nutrient in feces}}{\%\ \text{marker in feces} \times \%\ \text{nutrient in diet}}$$

Evaluation of Antioxidant Activity: Evaluation of antioxidant activity of experimental diets and fish samples was done by means of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay by Brand-Williams (1995) with some modifications. To evaluate the antioxidant activity, 2g of the samples were taken in 20mL methanol (80%) solution and homogenized. Then it was set aside at room temperature for 10 minutes. Then the homogenized solution was centrifuged at 5000 rotations per minute at 4ºC for 10 minutes. Before assay, the homogenate was then filtered across a 0.45 nm syringe filter (Whatman Inc., Clifton, NJ). In order to obtain a total volume of 1mL in a 1.5 mL cuvette, 100µL of filtered extract was added along with 900µl DPPH methanol solutions (100µM). The absorbance of the mixture at 517 nm was measured for 10 min with 1 min interval using a spectrophotometer. The antioxidant activity of extract against the DPPH radical was calculated as percentage inhibition.

$$\%\ \text{inhibition} = [(A0 - As)/A0] \times 100$$

In the equation, A0 and As are the sample absorbance after 0 and s min, respectively.

Statistical Analysis: Data of growth performance, digestibility coefficients and anti-oxidant status were subjected to one-way analysis of variance (Steel *et al*., 1996). The differences among means were compared by Tukey's Honesty Significant Difference Test and considered significant at $p \leq 0.05$ (Snedecor and Cochran, 1989). For statistical analysis, the Co-Stat computer software (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used.

RESULTS AND DISCUSSION

Growth Performance: The growth parameters of *C. catla* fingerlings fed canola meal containing diet supplemented with different lycopene levels (0, 10, 20, 30, 40, 50 and 60 $mgkg^{-1}$ diet) are shown in Table 2. The results of growth study showed that fingerlings fed test diet-V at 40 $mgkg^{-1}$ level of lycopene significantly ($p \leq 0.05$) augmented the weight gain (15.67±0.14 g) as compared to control diet (11.86±0.18 g) and other lycopene supplemented test diets. The maximum FCR value (2.92) was observed when fish fed on 60 $mgkg^{-1}$ lycopene. In terms of FCR, decreasing trend was observed with increasing levels of lycopene up to test diet-V. Decreased FCR depicted that most of the feed taken by *C. catla* was converted into flesh; therefore, lycopene supplementation enhanced the muscle production rate. Lowest value of FCR (1.95) was observed in fish fed diet having 40 mg lycopene per kg of diet. The 40 $mgkg^{-1}$ level diet showed maximum weight gain (%) (193%) of *C. catla* fingerlings followed by 30 $mgkg^{-1}$ level-based diet (181.94%) and these values were significantly different ($p \leq 0.05$) than the control and other remaining test diets.

These results are in consistent with the results of Lira *et al.* (2010), they showed that dietary supplementation of lycopene improved feed intake in the periods of 30 days of age and aggravated feed conversion. The positive impact of supplementation of lycopene on growth parameters is in agreement with former studies done in other animal species (Sahin *et al*., 2014a). Lycopene supplementation enhanced feed intake and consequently the weight gain in heat-stressed Japanese quail (Sahin *et al.,* 2014b). Englmaierová *et al.* (2011) showed that dietary supplementation of vitamin E and lycopene together improved growth, antioxidant activity and meat quality in chicken meat. However, contrary results were also observed by Gawad *et al.* (2019), who showed that synthetic carotenoids improved growth and biochemical parameters of yellow perch and concluded that dietary carotenoids such as lycopene and canthaxanthin exhibited no significant effect on growth parameters.

Nutrient Digestibility: Table 3 and 4 illustrated the analyzed composition of nutrients in experimental diets and feces of *C. catla* fingerlings, respectively. It was apparent that maximum nutrients released in water when fish were fed on control diet and less nutrients were released at 40 $mgkg^{-1}$ level of test diet followed by 30 $mgkg^{-1}$ levels and so on. Calculations of apparent digestibility coefficient (ADC%) of nutrients is given in Table 5. Maximum values of crude protein (CP) (75%), crude fat (EE) (77%) and gross energy (GE) (68%) digestibility was observed in fish fed at 40 $mgkg^{-1}$ level of lycopene supplemented canola meal-based diet followed by 30.

The availability of nutrients in *C. catla* is basically associated with their utilization and digestibility. The digestibility values of crude fat, crude protein and gross energy of *C. catla* were optimally observed at test diet having 40 and 30 $mgkg^{-1}$ levels of lycopene supplementation. It can be concluded that the

range of these values is optimum for improving the digestibility of nutrients. Many reporters have appreciated the use of lycopene for improving the digestibility of nutrients. Fallahpour *et al.* (2014) reported that when fish was fed marshmallow extract; rich in lycopene, the results demonstrated increase in weight gain of fish. The diet supplemented with marshmallow (0.25%) improved fish growth by increasing the efficiency of nutrient absorption and utilization. Adamidou *et al.* (2009) suggested that incorporation of 150 and 300 gkg^{-1} faba bean in fish diets significantly improved the ADC of fats, proteins and gross energy as compared to the control diet. Thus, we may conclude that differences in nutrient digestibility values appear to be species specific and depend on the tolerance of the fish to tannin supplements. However, contrary results showed that there was an inverse dose-response relationship between the gradient of dietary tannin and ADC for protein (Omnes *et al.,* 2017). Frejnagel and Wroblewska, (2010) reported that supplementation of extracts of lycopene resulted in significant reduction of absorption of all measured nutrients from the intestine of monogastric animal.

Table 2. Growth performance of *C. catla* fingerlings fed lycopene supplemented canola meal-based diets.

Growth Parameters	Test Diet –I (Control diet)	Test Diet –II	Test Diet – III	Test Diet – IV	Test Diet –V	Test Diet –VI	Test Diet – VII
	Lycopene levels ($mgkg^{-1}$)						
	0	10	20	30	40	50	60
IW (g)	8.06±0.03^{a}	8.07±0.03^{a}	8.09±0.04^{a}	8.10±0.03^{a}	8.09±0.02 ^{a}	8.10±0.02 ^{a}	8.07±0.02^{a}
FW (g)	19.93±0.17^{e}	20.53±0.31^{d}	21.31±0.15 ^{c}	22.85±0.11^{b}	23.76±0.16^{a}	20.26±0.21^{de}	18.71±0.16^{f}
WG (g)	11.86±0.18 ^{e}	12.46±0.29^{d}	13.22±0.12^{c}	14.74±0.10^{b}	15.67±0.14^{a}	12.15±0.22^{de}	10.64±0.16^{f}
WG (%)	147.13±2.44^{e}	154.35±3.15^{d}	163.52±0.79^{c}	181.94±1.27^{b}	193.74±1.34^{a}	149.98±2.85^{de}	131.90±1.85^{f}
WG ($fish^{-1}day^{-1}$) g	0.17±0.00^{a}	0.18± 0.00 ^{c}	0.19±0.00 ^{c}	0.21±0.00 ^{a}	0.22±0.00 ^{c}	0.17±0.00 ^{a}	0.15± 0.00^{c}
FI	0.40±0.03 ^{a}	0.40±0.02 ^{a}	0.41±0.02 ^{a}	0.43±0.02 ^{a}	0.44±0.01 ^{a}	0.44±0.02 ^{a}	0.44±0.02^{a}
FCR	2.35±0.17^{bc}	2.26±0.11^{bcd}	2.17±0.12^{cd}	2.05±0.11 ^{cd}	1.95±0.07 ^{d}	2.56±0.16^{b}	2.92±0.10^{a}

Means within rows having different superscripts are significantly different at $p \leq 0.05$
Data are means of three replicates
IW= Initial Weight, FW= Final Weight, WG= Weight gain, FI= Feed Intake, FCR= Feed Conversion Ratio
$mgkg^{-1}$. While least ADC% of CP (53%), EE (58%) and gross energy (51%) were present in fish fed on control diet.

Table 3. Analyzed composition (%) of apparent CP, EE and GE in diet of *C. catla* fingerlings based on canola meal supplemented with lycopene.

Experimental diets	Lycopene Levels ($mgkg^{-1}$)	CP	EE	GE (kcal g^{-1})
Test Diet –I (Control diet)	0	30.41±0.07	8.16±0.11	4.24±0.12
Test Diet –II	10	30.41±0.03	8.14±0.09	4.26±0.03
Test Diet –III	20	30.41±0.07	8.26±0.09	4.25±0.02
Test Diet –IV	30	30.42±0.06	8.23±0.09	4.26±0.02
Test Diet –V	40	30.41±0.04	8.17±0.05	4.25±0.02
Test Diet –VI	50	30.41±0.06	8.16±0.06	4.25±0.02
Test Diet –VII	60	30.42±0.04	8.20±0.11	4.25±0.02

Data values are mean (Mean ± Standard deviation) of three replicates
CP= Crude protein, EE= Ether Extract (crude fat), GE= Gross energy

Table 4. Analyzed composition (%) of nutrients in feces of *C. catla* fingerlings fed on canola meal-based diets supplemented with lycopene.

Experimental diets	Lycopene Levels ($mgkg^{-1}$)	CP	EE	GE (kcal g^{-1})
Test diet-I (control)	0	15.59±0.46 ^{b}	3.80±0.09 ^{a}	3.80±0.09 ^{a}
Test diet-II	10	11.83±0.39^{a}	3.34±0.19^{ab}	3.34±0.19^{ab}
Test diet-III	20	10.90±0.64 ^{b}	3.10±0.21 ^{b}	3.10±0.21 ^{b}
Test diet-IV	30	9.27±0.83 ^{b}	2.65±0.14 ^{a}	2.65±0.14 ^{a}
Test diet-V	40	8.29±1.01^{ab}	2.05±0.08^{ab}	2.05±0.08^{ab}
Test diet-VI	50	13.74±0.57^{bc}	2.37±0.18 ^{a}	2.37±0.18 ^{b}
Test diet-VII	60	14.05±1.03 ^{b}	2.85±.06^{ab}	2.85±.06^{ab}

Data values are mean (Mean ± Standard deviation) of three replicates
All values of means within columns are different significantly ($p \leq 0.05$)

Table 5. Apparent digestibility coefficient (ADC%) of nutrients in *C. catla* fingerlings fed on canola meal-based diets supplemented with lycopene.

Experimental diets	Lycopene Levels (mgkg^{-1})	CP	EE	GE
Test diet-I (control)	0	53.72±0.93 ^{a}	58.01±1.57 ^{b}	51.87±1.70 ^{ab}
Test diet-II	10	63.43±2.06 ^{a}	61.43±3.14 ^{a}	53.05±1.13 ^{b}
Test diet-III	20	66.05±2.00 ^{ab}	64.42±2.74 ^{ab}	55.21±3.84 ^{a}
Test diet-IV	30	71.89±2.95 ^{a}	70.31±1.39 ^{a}	64.42±2.35 ^{ab}
Test diet-V	40	75.34±3.19 ^{bc}	77.30±0.90 ^{ab}	68.74±3.40 ^{b}
Test diet-VI	50	58.91±2.86 ^{a}	73.58±.53 ^{a}	62.85±3.00 ^{a}
Test diet-VII	60	57.11±2.53 ^{b}	67.12±0.85 ^{b}	57.84±4.00 ^{ab}

All values of means within columns are different significantly ($p\leq0.05$)
Data values are mean (Mean ± Standard deviation) of three replicates

Anti-oxidant Status: Percentage of oxidation depicted the degree of oxidation in fish, by comparison of oxidation % calculated from fish fed on control diet with oxidation % estimated from fish fed on lycopene supplemented test diets (table 6). Higher the value, the more oxidation occurred with test diet 2, it was observed that with increasing levels of lycopene in diet, oxidation % decreased. However, lowest percentage of oxidation (3.57%) was observed at test diet-V. Hence, role of lycopene in enhancing antioxidant activity is clear from these findings as test diet having 40 mgkg^{-1} is found to be best of all other test diets.

Table 6. Antioxidant activity of *C. catla* fingerlings fed on lycopene supplemented canola meal-based diets.

Experimental Diets	Lycopene Levels (mgkg^{-1})	Absorbance	Inhibition (%)	Oxidation (%)
Test diet-I (control)	0	0.028	0.00	100.00±5.01
Test diet-II	10	0.028	0.00	100.00±6.01
Test diet-III	20	0.027	3.75	96.43±4.0
Test diet-IV	30	0.026	7.14	92.86±5.56
Test diet-V	40	0.001	96.43	3.57±0.28
Test diet-VI	50	0.004	85.71	14.29±0.76
Test diet-VII	60	0.006	78.57	21.43±1.3

Same results were observed in lycopene experiments conducted on monogastric animals (Zdunczyk *et al.,* 2000; Gonzalez-Mujica *et al.,* 2003). Gawad *et al.* (2019) also showed that lycopene has a defensive role because of its antioxidant activity and regulate the immune-related gene (*il-1b*) to improve immunity in yellow perch. Mezbani *et al.* (2019) suggested that the supplementation of the 100mg per kg dietary lycopene could increase the oxidative capacity of broiler. Exogenous lycopene as compared to dietary provision of lycopene was significantly effective ($p\leq0.05$) in hindering oxidation of lipid in *C. catla* fingerling's muscles. In the meantime, as both the endogenous and exogenous lycopene increased, values of lipid oxidation indicators reduced linearly. This reduction in lipid oxidation is the most effective factor in decelerating the lipid deterioration as compared to the other groups. Ibrahim and Harabawy, (2014) showed that exposure of CF minimizes levels of GSH in African catfish tissues. Reduction in GSH levels in response to CF exposure might reflect its ability to oppose oxidative damage resulting from ROS (reactive oxygen species). There was a substantial increase in MDA value, (a lipid peroxidation marker) in CF-exposed fish as compared to that in control. Increase in SOD activity upon CF exposure might be attributed to increase in production of superoxide anion radical (Zeid and Khalil, 2014). In all organisms, antioxidant enzyme converts reactive oxygen species (ROS) into harmless metabolites (Clasen *et al.,* 2018). Pesticide exposure in fish promotes removal of ROS from cells, clarifying the increased levels of SOD in fish tissues (Jin *et al.,* 2010). ROS usually reacts with lipids, nucleic acids and proteins and caused cellular injury. Bainy *et al*. (1996) indicated that superoxide radicals promote the production of TAC enzyme, which reduces the TAC levels in African catfish tissues. These results are confirmed by Ibrahim and Banaee, (2014) who reported that after Nile tilapia tissue was exposed to an insecticide, there was a significant drop in SOD level and an obvious increase in TAC levels. Similar results were also obtained by Yonar, (2012).

Conclusion: On the basis of results, it was concluded that lycopene supplemented canola meal-based diet improves growth performance, nutrient digestibility and antioxidant activity of *C. catla* fingerling at 40 mgkg^{-1}. Furthermore, lycopene inhibited the oxidation in muscles of fish by

scavenging free radicals in the body of fish that oxidizes lipids in its body.

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