*Indian J. Fish.*, 67(1): 72-79, 2020
DOI: 10.21077/ijf.2019.67.1.86990-10

# Effect of polyphenols supplemented canola meal based diet on growth performance, nutrient digestibility and antioxidant activity of common carp (*Cyprinus carpio* Linnaeus, 1758) fingerlings

SYED MAKHDOOM HUSSAIN[1], HINA GOHAR[1], AZHAR RASUL[1], MUHAMMAD MUDASSAR SHAHZAD[2], AFIA MUHAMMAD AKRAM[2], MUHAMMAD TARIQ[2], MAJID HUSSAIN[3], MUKHTIAR ALI[1] AND ANAM KHALID[4]

[1]*Fish Nutrition Lab, Department of Zoology, Government College University, Faisalabad, Pakistan*
[2]*Department of Zoology, Division of Science and Technology, University of Education Township, Lahore, Pakistan*
[3]*Department of Zoology, University of Okara, Okara, Pakistan*
[4]*College of Fisheries and Life Sciences, Shanghai Ocean University, China*
*e-mail: drmakhdoom90@gmail.com*

## ABSTRACT

A 70-days feeding trial was conducted to assess the impact of polyphenols supplemented canola meal based diets on growth performance, nutrient digestibility and antioxidant activity of common carp (*Cyprinus carpio* Linnaeus, 1758) fingerlings. Seven experimental diets *viz*., T0, T1, T2, T3, T4, T5, T6 and T7 with graded levels of polyphenols at 0, 100, 200, 300, 400, 500 and 600 mg $kg^{-1}$ respectively were formulated. Each treatment diet was fed to fishes in triplicate tanks and in each replicate, fifteen fingerlings were stocked. Effect of each treatment on the weight gain, feed conversion ratio (FCR), specific grwth rate (SGR), nutrient digestibility and antioxidant activity were assessed. Maximum growth performance, highest SGR (1.41) and best FCR (1.31) was observed in fish fed test diet T4 having 400 mg $kg^{-1}$ of polyphenols. Nutrient digestibility in terms of crude protein (72%), crude fat (76%) and gross energy (67%) also significantly increased ($p<0.05$) by supplementation of polyphenols at 400 mg $kg^{-1}$ level. With respect to antioxidant activity, increasing trend was observed with increasing levels of dietary polyphenols with the highest antioxidant activity recorded in fish fed T6 diet supplemented with 600 mg $kg^{-1}$ of polyphenols.

Keywords**:** Common carp, Lipid oxidation, Nutrients digestibility, Phenolic compounds, Weight gain

## Introduction

Feed, a major input in aquaculture production, is a fundamental challenge hindering the development and growth of aquaculture sector (Gabriel *et al.,* 2007). Aquaculture feed mainly relies upon the utilisation of fishmeal because of its high palatability and nutritious esteem (NRC, 2011). In fish feed, replacement of fish meal with ingredients which are less costly and are plant or animal derived, is essential as a result of growing cost and unpredictable availability of fish meal (Mahboob, 2014). Canola meal (CM), having highly digestible protein content (38%), is an appropriate alternative to fish meal. It has the best amino acid balance than all other currently accessible commercial sources of protein from plants (Enami, 2011). In comparison with soybean meal and fish meal, canola meal is reported to be more economical (Hussain *et al.,* 2015).

Antioxidative status of fish needs to be necessarily improved with the increase in water pollution and its stimulating consequences on oxidative stress. Accordingly, any push to support this framework might be related with useful impacts on wellbeing of fish (Gabor *et al.,* 2012). Fish is susceptible to peroxidation of n-3 polyunsaturated fatty acids (PUFA) which results in restriction of storage and processing possibilities (Sampels, 2013). So, as a result of oxidation in body of fish, there is great risk of loss in its quality (Medina *et al.,* 2009). Lipid oxidation contributes to harmful consequences, leading to off flavour and rancid taste as well as formation of various substances which have unfavourable impacts on human wellbeing (Sampels, 2013). Therefore, in order to increase the storage stability and nutritional value of farmed fish (Kazimierczak *et al.,* 2008), inclusion of antioxidants in fish feed is essential. Antioxidants scavenge free radicals and have health promoting effects (Biglari *et al.,* 2008). Polyphenols exhibit antibacterial, antioxidant and anti-inflammatory properties which protect the fish against disease and oxidative stress (Kamatou *et al.,* 2010; Samec *et al.,* 2010). Plant parts exhibit high antioxidant capacity especially because of phenolic contents *i.e.* anthocyanins and ascorbic acid, which play the role of radical scavengers

and inhibit oxidation. For the most part, the antioxidant activity of vegetables as well as fruits increases with the increase in concentration of total phenolics and flavonoids (Ghasemi *et al.,* 2009).

Common carp (*Cyprinus carpio* Linnaeus, 1758), is one of the important farmed species around the world contributing significantly to aquaculture production of many countries (Rechulicz *et al.,* 2014). It is a bottom feeder and relies on benthic organisms as well as decaying matter for its nutritional requirements (Khan *et al.,* 2016). The objective of the present study was to check the effect of canola meal based diets supplemented with polyphenols on growth performance, nutrients digestibility and antioxidant activity in *C. carpio* fingerlings.

## Materials and methods

The experimental trial was conducted in the Fish Nutrition Laboratory, Department of Zoology, Government College University, Faisalabad, Pakistan.

*Experimental set up*

For experimental work, *C. carpio* fingerlings were procured from the Government Fish Seed Hatchery, Faisalabad and were stocked in indoor water tanks having 70 l water holding capacity and made of steel, specially designed for faecal collection. Fingerlings were acclimatised to the experimental conditions for 15 days and were fed on basal diet @4% body weight twice daily (Allan and Rowland, 1992). During the experimental period, water quality parameters particularly, temperature, dissolved oxygen and pH were monitored regularly which ranged from 24.9-28.7°C, 5.8-7.3 mg $l^{-1}$ and 7.4-8.6, respectively. Aeration was provided round the clock in all the experimental tanks. Prior to initiation of the experiment, the fish fingerlings were given immersion treatment in 0.5% saline solution for 1 to 2 min to kill any ectoparasites (if present) (Rowland and Ingram, 1991).

*Experimental design*

Experimental diets were divided into one control and six test diets. Seven canola meal (CM) based diets *viz*., T0, T1, T2, T3, T4, T5, T6 and T7 were prepared supplemented with graded levels of 0, 100, 200, 300, 400, 500 and 600 mg $kg^{-1}$ respectively of polyphenols (Total phenols, Flavonols, Anthocyanins, and Phenylpropanoids collected from cabbage). Triplicate tanks were used for each treatment and each replicate had 15 fingerlings each. The experimental trial was conducted following a completely randomised design (CRD) for a period of 70 days. *C. carpio* fingerlings fed with test diets were compared with control as well as between test groups to assess growth performance, antioxidant activity and nutrient digestibility parameters.

*Feed ingredients and formulation of experimental diets*

Polyphenols were procured from the Natural Product and Synthetic Chemistry Lab, Department of Applied Chemistry and Biochemistry, Government College University, Faisalabad. Other feed ingredients were purchased from a commercial feed mill and were analysed for chemical composition following AOAC (1995) prior to formulation of the experimental diet (Table 1). Proximate composition of the feed ingredients are given in Table 2. The feed ingredients were finely ground and sieved through a 0.5 mm mesh size sieve. All ingredients were mixed in an electric mixer for 10 min and fish oil was gradually added. During mixing of ingredients 15% water was also added to prepare a dough (Lovell, 1989). Then, these mixed feed ingredients were extruded through a lab

Table 1. Ingredients and proximate composition (%) of test diets

| Ingredients | Control Diet (T0) | Test diet T1 | Test diet T2 | Test diet T3 | Test diet T4 | Test diet T5 | Test diet t6 |
|---|---|---|---|---|---|---|---|
| | Polyphenols (mg $kg^{-1}$) | | | | | | |
| | 0 | 100 | 200 | 300 | 400 | 500 | 600 |
| Canola meal | 55 | 55 | 55 | 55 | 55 | 55 | 55 |
| Fish meal | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| Wheat flour* | 11 | 10.9 | 10.8 | 10.7 | 10.6 | 10.5 | 10.4 |
| Soybean meal | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Fish oil | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Vitamin premix | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Mineral mixture | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Ascorbic acid | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Chromic oxide | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Proximate composition of test diets | | | | | | | |
| CP (%) | 32.47±0.18 | 32.46±0.14 | 32.48±0.11 | 32.47±0.15 | 32.45±0.15 | 32.46±0.12 | 32.47±0.16 |
| EE (%) | 7.41±0.03 | 7.46±0.14 | 7.48±0.11 | 7.47±0.15 | 7.45±0.15 | 7.95±0.04 | 7.76±0.20 |
| GE (kcal $g^{-1}$) | 3.18±0.06 | 3.31±0.06 | 3.46±0.12 | 3.40±0.10 | 3.47±0.15 | 3.54±0.05 | 3.40±0.17 |

Table 2. Proximate composition (%) of feed ingredients

| Ingredients | Dry matter (%) | Crude protein (%) | Crude fat (%) | Crude fibre (%) | Ash (%) | NFE (%) |
|---|---|---|---|---|---|---|
| Fish meal | 91.63 | 48.15 | 7.16 | 1.07 | 25.73 | 17.89 |
| Wheat flour | 92.45 | 10.10 | 2.35 | 2.65 | 2.08 | 82.82 |
| Canola meal | 94.14 | 37.02 | 1.27 | 1.42 | 9.21 | 51.08 |
| Soybean meal | 93.80 | 41.93 | 3.74 | 1.97 | 10.83 | 41.53 |

extruder (Model SYSLG30-IV Experimental Extruder), to form floating pellets (3 mm). All diets were equally treated in the given extruder to formulate seven CM-based test diets. All the prepared diets were air dried under a shady place and stored at 4°C until use.

*Feeding protocol and sample collection*

*C. carpio* fingerlings were fed with the respective diet at 4% of the body weight (in two split doses). Feeding session was of 2 h after which uneaten feed was collected to determine feed conversion ratio (FCR) and water was drained out. The tanks were cleaned and replaced with freshwater. Two hours after completion of the feeding session, faecal matter was collected from each tank using faecal collection tube taking care to prevent breakage to ensure minimum mineral discharge. Faecal matter collected were oven dried at 65°C and stored for further analysis.

*Growth assessment*

Fifteen fingerlings of initial average weight (8.07±0.041 g) were stocked in each replicate tank. Fingerlings from each tank were bulk weighed after every two weeks during the whole experimental period, to assess the growth performance of the fingerlings. FCR, specific growth rate (SGR) and weight gain percentage (WG) of fingerlings were estimated using the following formulae (NRC, 1993):

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

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

$$\text{SGR\%} = \frac{(\text{ln. final wt. of fish} - \text{ln. initial wt. of fish})}{\text{Experimental duration in days}} \times 100$$

*Digestibility studies*

For measurement of apparent digestibility co-efficient (ADC,%) of nutrients, chromic oxide was used as an inert marker at 1% inclusion level in test diets. Chromic oxide content in diets and faeces was estimated after oxidation with molybdate reagent using UV-VIS 2001 Spectrophotometer at 370 nm absorbance (Divakaran *et al*., 2002). ADC (%) of nutrients in the diets was calculated using the following formula (NRC, 1993):

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

*Antioxidant activity*

The effect of polyphenol supplemented diets on antioxidant activity of common carp was determined in terms of percent inhibition of oxidation following Hussain *et al*. (2011) with slight modification. Fishes sampled from each test group for estimation of antioxidant activity were dried and ground and 1 g each of the ground sample from each treatment group was taken in a test tube and hexane fraction was prepared, by adding 10 ml of n-hexane in each test tube. Then, this fraction was heated gently in water bath for 10 min. After that, 10 ml solution of 0.2M was prepared by adding phosphate buffer in each hexane fraction. The test tubes were gently shaken and then 200 µl from each test tube was transferred to a new test tube and 200 µl of 30% aqueous ammonium thiocyanate solution was added followed by addition of 200 µl of 35% ferrous chloride solution. Subsequently, 10 ml of 95% ethanol was added in each test tube and absorbance was measured in a spectrophotometer at 500 nm. The percentage inhibition of oxidation was evaluated using the formula:

Percentage Inhibition of oxidation (%) = 100 × (Absorbance of control sample- Absorbance of test sample/ Absorbance of control sample)

Oxidation (%) = 100 - 100 × (Absorbance of control sample- Absorbance of test sample/ Absorbance of control sample)

*Statistical analysis*

The data on growth performance and nutrient (crude protein, crude fat and apparent gross energy) digestibility was subjected to one-way analysis ANOVA (Steel *et al.,* 1996). The differences between means were compared and were considered significant at $p<0.05$ (Snedecor and Cochran, 1991) by Tukey's Honesty Significant Difference Test. For statistical analysis, the Co-Stat computer software (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used.

## Results

Maximum weight gain (16 g), WG% (261%) and SGR (1.43%) were recorded in fish fed diet T4 supplemented with 400 mg $kg^{-1}$ of polyphenol (Table 3) which was

significantly ($p<0.05$) higher compared to weight gain, WG% and SGR of fish fed control diet. However, in terms of FCR, decreasing trend was observed with increasing levels of polyphenols upto 400 mg $kg^{-1}$. Lowest value of FCR (1.31) was observed in fish fed diet T4 having 400 mg of polyphenols per kg of diet, whereas its value (1.77%) was the highest in control diet (T0).

The diets were isocaloric and isonitrogenous (Table 1). The excretion of nutrients from the body of fish was found to be the minimum in fish fed polyphenols supplemented diet. Best level of polyphenol supplementation was found to be 400 mg $kg^{-1}$, as excretion of nutrients at this level was the least. From the results of the current study, it was observed that at 400 mg $kg^{-1}$ level (diet T4), the digestibility value of crude fat (76%) was found to be the highest and significantly different ($p<0.05$) from, all other test diets (Table 4). Crude protein (72%) and gross energy (67%), were also found to be highsest for diet T4, which were found to be significantly ($p<0.05$) different from other diets, except with test diet T3 (supplemented with 300 mg $kg^{-1}$ of polyphenol) for crude protein and with test diet T5 (supplemented with 500 mg $kg^{-1}$ of polyphenol) for gross energy. Lowest digestibility values of nutrients, crude fat (59%), crude protein (56%) and gross energy (53%) were noted in fish fed control diet (0 mg $kg^{-1}$ polyphenols).

Table 5 shows the results of antioxidant activity of polyphenols supplemented canola meal based diet in *C. carpio.* Percentage oxidation in the body of fish was found to decrease with increasing levels of polyphenol supplementation in the diets which indicates increasing antioxidant activity with increasing levels of dietary polyphenols. Test diet T6 supplemented 600 mg $kg^{-1}$ of polyphenols was found to be the best compared to other test diets as the percentage of oxidation in the body of fish was found to be the lowest (4.25%) in fish fed diet T6.

## Discussion

Free radicals, that are reactive, are scavenged by antioxidants and protect living cells from oxidative damage (Biglari *et al.,* 2008). Polyphenols are antioxidants in nature and hance are expected to improve antioxidant a ctivity and in turn improves growth performance of fish as well. According to the results of the present study, growth performance of common carp in terms of FCR and weight gain was significantly improved at 400 mg $kg^{-1}$ level of polyphenols in canola meal based diet. Our findings are in accordance with the results reported by Zhai *et al*. (2014) who noted lowest FCR (1.31) and improved growth performance in Nile tilapia fed diets supplemented with the antioxidant, grape seed proanthocyanidins (GSP) at levels of 400 and 600 mg $kg^{-1}$.

Table 3. Growth performance of *C. carpio* fingerlings fed polyphenols supplemented canola meal based diets

| Growth parameters | Test diet T0 (Control) | Test diet T1 | Test diet T2 | Test diet T3 | Test diet T4 | Test diet T5 | Test diet T6 |
|---|---|---|---|---|---|---|---|
| | Polyphenols (mg $kg^{-1}$) | | | | | | |
| | 0 | 100 | 200 | 300 | 400 | 500 | 600 |
| IW (g) | 6.17±0.20$^{a}$ | 6.16±0.13$^{a}$ | 6.15±0.11$^{a}$ | 6.14±0.15$^{a}$ | 6.15±0.09$^{a}$ | 6.17±0.09$^{a}$ | 6.16±0.06$^{a}$ |
| FW (g) | 16.57±0.71$^{c}$ | 17.09±0.70$^{c}$ | 17.82±0.77$^{bc}$ | 19.77±0.8 4$^{b}$ | 22.23±0.47$^{a}$ | 19.37±0.74$^{b}$ | 17.27±0.77$^{c}$ |
| WG (g) | 10.39±0.52$^{e}$ | 10.92±0.58$^{de}$ | 11.68±0.70$^{c}$ | 13.63±0.95$^{bc}$ | 16.08±0.39$^{a}$ | 13.20±0.69$^{b}$ | 11.12±0.72$^{b}$ |
| WG (%) | 168.30±3.77$^{d}$ | 177.16±6.11$^{d}$ | 189.92±9.50$^{cd}$ | 222.13±19.41$^{b}$ | 261.30±2.98$^{a}$ | 213.96±9.66$^{bc}$ | 180.50±10.04$^{d}$ |
| WG (g $day^{-1}$) | 0.148±0.007$^{d}$ | 0.156± 0.008$^{d}$ | 0.167±0.010$^{cd}$ | 0.195±0.014$^{b}$ | 0.230±0.006$^{a}$ | 0.189±0.010$^{bc}$ | 0.159± 0.01$^{d}$ |
| FI | 0.26±0.02$^{a}$ | 0.25±0.02$^{b}$ | 0.26±0.03$^{a}$ | 0.28±0.03$^{a}$ | 0.30±0.02$^{a}$ | 0.28±0.02$^{a}$ | 0.27±0.01$^{a}$ |
| FCR | 1.77±0.04$^{a}$ | 1.61±0.01$^{abc}$ | 1.55±0.10$^{abcd}$ | 1.42±0.03$^{cd}$ | 1.31±0.11$^{d}$ | 1.46±0.04$^{bcd}$ | 1.69±0.17$^{ab}$ |
| SGR | 1.10±0.02$^{d}$ | 1.13±0.02$^{d}$ | 1.18±0.04$^{cd}$ | 1.30±0.07$^{b}$ | 1.43±0.01$^{a}$ | 1.27±0.03$^{bc}$ | 1.15±0.04$^{d}$ |

Means within rows having different superscripts are significantly different ($p<0.05$)
Data are means of three replicates
IW= Initial weight, FW= Final weight, WG= Weight gain, FI= Feed intake, SGR= Specific growth rate, FCR= Feed conversion ratio

Table 4. Apparent nutrient digestibility of canola meal based diet with polyphenols supplementation in *C. carpio* fingerlings

| Experimental diets | Polyphenols (mg $kg^{-1}$) | Crude protein (%) | Crude fat (%) | Gross energy (kcal $g^{-1}$) |
|---|---|---|---|---|
| T0 | 0 | 56.40±0.79$^{e}$ | 58.50±0.94$^{d}$ | 52.93±0.98$^{e}$ |
| T1 | 100 | 59.63±0.93$^{d}$ | 55.63±0.77$^{e}$ | 53.49±0.75$^{e}$ |
| T2 | 200 | 64.87±0.80$^{c}$ | 63.26±0.94$^{c}$ | 59.76±0.97$^{c}$ |
| T3 | 300 | 70.59±0.82$^{a}$ | 72.41±0.74$^{b}$ | 63.40±0.95$^{b}$ |
| T4 | 400 | 72.36±0.90$^{a}$ | 76.38±0.73$^{a}$ | 66.59±0.71$^{a}$ |
| T5 | 500 | 67.72±0.85$^{b}$ | 71.27±0.92$^{b}$ | 66.36±0.75$^{a}$ |
| T6 | 600 | 59.72±0.97$^{d}$ | 63.18±0.53$^{c}$ | 56.65±0.86$^{d}$ |

Means within rows having different superscripts are significantly different ($p<0.05$)
Data are means of three replicates

Table 5. Antioxidant activity of polyphenols supplemented canola meal based diet

| Concentration of polyphenol (mg kg$^{-1}$) | Absorbance | Oxidation (%) |
|---|---|---|
| 0 (T0) | 0.0282±0.00010 | 100.00±0.00 |
| 100 (T1) | 0.0273±0.00010 | 96.81±0.35 |
| 200 (T2) | 0.0266±0.00021 | 94.44±0.73 |
| 300 (T3) | 0.0243±0.00015 | 86.29±0.84 |
| 400 (T4) | 0.0065±0.00021 | 23.17±0.82 |
| 500 (T5) | 0.0046±0.00015 | 16.19±0.56 |
| 600 (T6) | 0.0012±0.00010 | 4.25±0.35 |

Shin *et al*. (2010a, b) when fed olive flounder *Paralichthys olivaceus* on diet containing 250 or 500 mg kg$^{-1}$ quercetin (dietary polyphenolic compound) for 30 and 60 days, observed weight gain to be significantly higher than that of fish fed on control diet. Shin *et al*. (2010a) also observed that administration of quercetin in diet improved FCR in olive flounder. Significant increase in the SGR rate as well as weight gain of *O. niloticus* was observed when 2400 mg kg$^{-1}$ of structural analogue of quercitin, Dihydromyricetin was included in feed (Cai *et al.,* 2010). Spirulina, being rich in polyphenols, has been used by many researchers and positive effects of spirulina are reported on the growth of fish species, including small scale black fish (Nakazoe *et al.,* 1986); red seabream (Mustafa *et al.,* 1994); rohu (Nandeesha *et al.,* 2001); Siberian sturgeon (Palmegiano *et al.,* 2005) and Nile tilapia (Lu *et al.,* 2004; Abdel-Tawwab *et al*., 2010). Tongsiri *et al*. (2010) replaced 5% of fish meal with spirulina and reported better growth performance in *Pangasianodon gigas*. Pham *et al*. (2006) reported that with the increase in supplementation levels of *Hizikia fusiformis* (another potent source of polyphenols); in the diet, the growth of olive flounder improved, which showed that its supplementation has positive effect on growth of fish. Fallahpour (2015) observed significant improvement in weight gain in commmon carp fed on diet supplemented with marshmallow (*Althaea officinalis*) extract (0.25%). Jian and Wu (2004) reported higher weight gain in carp when diet was supplemented with a mixture of Chinese angelica root and astragalus root as sources of polyphenols. Munglue (2014) found growth performance to be significantly better in Nile tilapia (*Oreochromis niloticus*) fed with 1% *Nelumbo nucifera* (Lotus) peduncle extract (NNPE), rich in polyphenols.

A wide range of biological activities, are shown by polyphenols (Kamatou *et al.,* 2010; Samec *et al.,* 2010), including health-promoting effects (Biglari *et al.,* 2008). Recent studies have also shown that polyphenolic compounds possess protective effects on immune system (Aquilano *et al.,* 2008; Franova *et al.,* 2010). The results of our study clearly showed that increased dietary polyphenol level was positively correlated with antioxidant capacity of the diets. The highest antioxidant activity was observed in 600 mg kg$^{-1}$ of polyphenols supplemented diet group, whereas least was noted in fish fed control diet. Our findings are in accordance with the results of study by Shin *et al*. (2010) who reported that inclusion of quercetin, a polyphenol, in diet of olive flounder gave positive results. They found lower cholesterol levels and increased levels of superoxide dimutase and catalase (both are antioxidant enzymes) which is a good sign in inhibiting oxidation. Zheng *et al*. (2009) treated channel catfish with antioxidant oregano and reported increased activity of plasma lysozyme. Tang *et al*. (2001) added tea catechins in muscle patties of fish and observed that the prooxidative effect of NaCl was significantly ($p<0.01$) inhibited at level of 300 mg kg$^{-1}$ of minced muscle and controlled lipid oxidation in all cooked muscle patties. Amer (2016) reported that by feeding dietary *S. platensis* (rich polyphenols source) to Nile tilapia (monosex), catalase activity was found to be increased along with glutathione reductase and formation of malondialdehyde reduced, which is secondary product formed during peroxidation of lipids. Ragap *et al*. (2012) reported that lysozyme activity was maximum in Nile tilapia fed on diet supplemented with spirulina (10 mg kg$^{-1}$ fish) in comparison to fish fed diet supplemented withspirulina at 1 mg kg$^{-1}$ fish and fish fed control diet. Similarly, Ibrahem *et al*. (2013) also reported that spirulina supplementation in diet upto 10%, improved level of lysozyme in Nile tilapia.

El-Mesallamy *et al*. (2016) observed that inclusion of dried flowers of *Hibiscus sabdariffa* (source of polyphenols) in the diet of Nile tilapia enhanced immune/ health status; and improved disease resistance as well as growth performance. Darsini *et al*. (2013) supplemented *Limonia acidissima* fruit, in diet of common carp and reported that superoxide dismutase (SOD) activity in liver, muscle homogenates and serum, significantly increased in fish groups fed with the experimental diets as compared to control diet. Similarly, Papuc *et al*. (2012) incorporated polyphenols from sea buckthorn fruits in the minced carp muscles, at levels of 250 mg kg$^{-1}$ and reported that it aided in inhibiting oxidation of lipids and proteins in all types of carp muscle. The role of polyphenols in enhancing antioxidant activity is clearly evident from all these studies. Higher digestibility values of crude fat (64%), crude protein (76%) and gross energy (66%) were also reported for test diet supplemented with 400 mg kg$^{-1}$ polyphenols. Many reports have appreciated the use of polyphenols for improving the digestibility of nutrients. Fallahpour *et al*. (2015) attributed the improvement in growth of fish fed diet supplemented with marshmallow extract to the influence of marshmallow on improving the nutrient digestibility, increasing the efficiency of nutrient absorption and feed utilisation. Adamidou *et al*.

(2009) found that incorporation of 150 and 300 g $kg^{-1}$ faba bean in extruded diets for juveniles of European seabass significantly improved the ADC for protein, fat and energy as compared to the control diet.

However, contradictory results have also been reported. Omnes *et al.* (2017) observed inverse dose response relationship between dietary tannin level and ADC for dry matter (DM) as well as energy in European seabass, while positive correlation was observed between tannin level and ADC for protein. Tannins from a *Fabaceae* plant extract at levels equal to or greater than 6.3 g $kg^{-1}$ also significantly affected DM and protein digestibility in Nile tilapia (Pinto *et al.,* 2000). Frejnagel and Wroblewska (2010) reported that supplementation of extracts of polyphenols resulted in significant reduction of absorption of all measured nutrients from the intestine of monogastric animals. Differences between the results of the present study with the above findings could be attributed to the difference in species, nutrients need, their feeding condition and levels of dietary polyphenols (Zhai *et al.,* 2013).

Our study clearly showed that polyphenols are potent source of antioxidants and they have significant positive impact on growth and digestibility of nutrients in fish at a dietary level of 400 mg $kg^{-1}$. Increased levels of supplementation of polyphenols in diet aided in reduced oxidation indicating improved antioxidant activity. Hence, canola meal along with supplementation of polyphenols ensures productively effective and affordable fish diet which is more likely to produce healthy fish and overcome the issue of ever increasing cost of fish meal that is expected to escalate in future.

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Date of Receipt : 14.02.2019
Date of Acceptance : 13.01.2020