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Effects of Polyphenols Supplemented Canola Meal Based Diet on Proximate Composition, Minerals Absorption and Hematology of *Cyprinus carpio* Fingerlings

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SMH planned and supervised the research, provided research materials. HG conducted the trial and collected data. AR helped in statistical analysis. MH proffread the manuscript. HG prepared manuscript while MA and MMS helped her. MZHA and NA helped in feed analysis and compiling the results. AS helped in proximate analysis of fish.

Key words
Phenolic compounds, Blood parameters, Carcass composition, Minerals absorption, Common carp.

ABSTRACT

The research project was designed to check the effect of polyphenols supplemented canola meal-based diet on proximate composition, mineral absorption and hematology of *Cyprinus carpio* fingerlings. The diets were formulated in such a way that sufficient supply of all required nutrients were ensured for normal fish growth. Collection of feces from each tank was done twice a day. Impact of each treatment on the absorption of minerals, proximate analysis and hematology were determined using standard methods and formulae. Highest minerals absorption (Ca, Na, K, Fe, Cu, P, Mg and Al) was observed in the fish fed at 400mg/kg of polyphenols in canola meal based diets. Similarly, best hematological parameters (RBCs, WBCs, PLT, Hb, PCV, MCHC, MCH and MCV) as well as proximate composition (crude protein, crude fat, ash, moisture and carbohydrates) were noted in fish group fed on 400mg/kg of polyphenols in diet. Hence supplementation of polyphenols at 400mg/kg was found to be optimum for better hematology, minerals absorption and carcass composition of common carp.

INTRODUCTION

One of the rapidly developing animal food-producing sectors is aquaculture, which accounts for almost fifty percent of the total food fish and serves replacement for wild fish was well as plants (FAO, 2010). However, the basic limitation to aquaculture industry is disease which imposes severe losses on farming facilities throughout the world (Kim *et al*., 2013b). Muscle of fish is a complex system; it offers an appropriate environment for the lipids to be oxidized quickly. This eventually results in off-odor as well as new flavors and restrictsits shelf-life. Fish muscle is prone to oxidation because of occurrence of high amounts of poly unsaturated fatty acids (PUFAs), iron, and heme from myoglobin and hemoglobin (Maqsood *et al*., 2012). According to El-Banna and Atallah (2009) body weight, body weight gain, livability, immunity of fish can be improved and the level of mortality can be reduced by inclusion of feed additives to diet of fish, they also play role in improvement of productive and economic efficiency of fish farms. Polyphenols enriched extracts of plants have been detected to be the safe supplements, as reserving them from natural sources is easy and lipid oxidation can be hindered effectively under their influence (Maqsood *et al*., 2014). Ubiquitously present throughout most tissues of plants are polyphenols, which play significant role in plant physiology. From their respective sources, polyphenols can be extracted and after that can be included to diet because of their antioxidant effects as well as coloring properties (Maqsood *et al*., 2013).

The fundamental challenge which faces the development and growth of aquaculture is feed (Gabriel *et al*., 2007). With expanding aquaculture, fish oil and fish meal have become more costly and infrequent. As a result, aqua feed producers are facing pressure for replacing them with suitable options (Pickova and Morkore, 2007). In fish feed, as a result of increasing cost and unforeseeable availability of fish meal, it is essential to replace it with less costly ingredients which are either plant or animal derived (Higgs *et al*., 1995; Mahboob, 2014). An appropriate protein substitution for fish meal is canola meal as it has relatively high (380 g/kg) content of protein (Yigit and Olmez, 2009). Canola meal, in comparison with soybean meal and fish meal, is reported to be more economical

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(Sajjadi and Carter, 2004; Hussain *et al*., 2015). It is generally used in aquaculture diets for species such as perch, bass, catfish, turbot, tilapia, carp, sea bream and shrimp with positive effects (Enami, 2011).

Cyprinus carpio is the most commonly cultured species of fish in the world. In ponds of Asia, Near and Far East, this fish is preferred for cultivation in ponds, alone or in combination with other fishes, due to its omnivorous habit, excellent growth rate, hardy nature, breeding in confined waters and easy adaptability to feeds (Khan *et al*., 2016). The present research was conducted to determine the effect of polyphenols in canola meal based diet on hematology, minerals absorption and carcass composition of *C. carpio* fingerlings.

MATERIALS AND METHODS

The experiment was carried out in Fish Nutrition Laboratory, Department of Zoology, Government College University, Faisalabad.

Fish and experimental conditions

Fingerlings of common carp were taken from the local fish seed hatchery and were acclimatized for two weeks. They were stocked, in specially designed water tanks that were V-shaped (water capacity 70 L), for two weeks and were fed on basal diet (Allan and Rowland, 1992). Parameters related to water quality were monitored using electrical conductivity meter (HANNA: HI. 8633), dissolve oxygen meter (Jenway 970) and pH meter (Jenway 3510). The water quality parameters ranges were noted; temperature 24.9–28.7°C, pH 7.4–8.6, electrical conductivity 1.30–1.52 dS/m and dissolved oxygen 5.8–7.3 mg/L. By means of capillary system, aeration was constantly provided to all the experimental tanks. Prior to initiation of trial, fingerlings were treated with saline solution (0.5 % NaCl) for killing all the pathogens if present (Rowland and Ingram, 1991).

Experimental design

Triplicate tanks, having 15 fingerlings each, were used for each treatment. Fingerlings were given feed at the rate of 5% live wet weight. Experimental trial continued for 70 days. Polyphenols supplemented canola meal-based diets were compared with control as well as with each other to determine parameters of carcass composition, minerals absorption and hematology using completely randomized design (CRD).

Feed ingredients and formulation of experimental diets

From a commercial feed mill, the ingredients of feed were taken. Before formulating experimental diet, the ingredients were analyzed (Table I) for chemical composition following AOAC (1995). They were finely ground and mixed for 10 min in an electric mixer followed by inclusion of fish oil. Water (10–15%) was added during mixing of ingredients (Lovell, 1989). Then through Lab Extruder (SYSLG30-IV Experimental Extruder), these ingredients were extruded to form floating pellets (3 mm). In the extruder, all canola meal based diets were treated equally to formulate seven CM-based test diets.

Polyphenols were obtained from Natural Product and Synthetic Chemistry Lab, Department of Applied Chemistry and Biochemistry Government College University, Faisalabad. Seven test diets were formed from the experimental diet and then were supplemented with graded levels (0, 100, 200, 300, 400, 500 and 600 mg/kg) of polyphenols (Robinson *et al*., 2002). Each diet was then dried and stored at the temperature of 4°C until use.

Table I.- Composition (%) of diet ingredients.

Ingredients	Test diet-I (control diet)	Test diet-II	Test diet-III	Test diet-IV	Test diet-V	Test diet-VI	Test diet-VII
Polyphenols (mg/kg)	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	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mixture	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ascorbic acid	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0

*Polyphenols were added at the expense of wheat flour.

Chemical analysis of feed, feces and carcass composition

After the research period, the feed ingredients, samples of experimental diets and feces were homogenized by using a motor and pestle. Four fish were taken from each tank and were sacrificed followed by drying at room temperature. These samples were then homogenized separately using a pestle and mortar, to be analyzed by using standard methods (AOAC, 1995). Oven-drying method was used for determining moisture in whole fish body at 105°C for 12 h. Crude protein was analyzed by (Nx6.25) Micro Kjeldahl`s (InKjel M behr Labor Technik GmbH D-40599 Dusseldorf) method and crude fat by petroleum ether using Soxhlet system (Soxhlet Extraction Heating Mantels, 250 ml 53868601). Contents of crude fiber were determined as loss on ignition of dried lipid-free residues after digestion with 1.25% sodium hydroxide and 1.25% H_2SO_4, whereas ash by ignition in electric furnace (Naberthern B170) at 650°C for 12 h to constant weight. Total carbohydrates (N-free extract) were calculated by difference, *i.e.* total carbohydrates (%) = 100- (CP% + EE% + CF% + Ash% + Moisture %).

Minerals absorption

Homogenization of the samples of experimental diets and feces was done by using standard methods (AOAC, 1995). Moisture was determined by oven-drying at 105°C for 12 h. Feed as well as samples of feces were digested in boiling nitric acid and perchloric acid mixture (ratio of 2:1). After appropriate dilution, mineral contents such as calcium (Ca), iron (Fe), magnesium (Mg), copper (Cu) and aluminum (Al) were determined using Atomic Absorption Spectrophotometer (Hitachi Polarized Atomic Absorption Spectrometer, Z-8200). Calibrated standards for mineral estimation were prepared from commercially available standards (AppliChem® Gmbh Ottoweg4, DE-64291 Darmstadt, Germany). Potassium (K) and Sodium (Na) were estimated through flame photometer (Jenway PFP-7, UK). Using ammonium molybdate as reagent, calorimetric determination of phosphorus (P) was done (UV/VIS spectrophotometer) at absorbance of 720nm through standard methods (AOAC, 1995). Contents of chromic oxide in the feed and feces were determined after oxidizing with molybdate reagent using a UV-VIS 2001 Spectrophotometer at 370nm absorbance (Divakaran *et al*., 2002).

Calculation of apparent absorption coefficient

Apparent absorption coefficients of minerals in test diets were calculated using standard formula (NRC, 1993).

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

Hematological study

Clove oil (Sigma) was firstly dissolved in ethanol as it has poor solubility in water (Peake, 1998; Coyle *et al*., 2004) and then its concentrations of 60 mg/L were used to anesthetize fish fingerlings from each tank for 5 min. Blood was taken from each sample fish through caudal vein using heparinized syringe and after that blood samples were carried to the Molcare Lab, Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan. Hematocrit was determined using micro-hematocrit technique using capillary tubes (Brown, 1980). Red blood cells (RBC) and white blood cells (WBC) were counted using a haemo-cytometer having approved Neubauer counting chamber (Blaxhall and Daisley, 1973). Estimation of concentration Hb (hemoglobin) was done using method explained by Wedemeyer and Yastuke (1977). The formulas stated below were used to calculate MCHC (mean corpuscular hemoglobin concentration); MCH (mean corpuscular hemoglobin) and MCV (mean cell volume):

$$MCHC = Hb / PCV \times 100$$
$$MCV = PCV / RBC \times 10$$
$$MCH = Hb / RBC \times 10$$

Statistical analysis

Data of proximate composition, mineral absorption and hematological indices of test diets was subjected to one-way analysis of variance (Steel *et al*., 1996). By Tukey's Honesty Significant Difference Test, the differences among means were compared and were considered significant at $p<0.05$ (Snedecor and Cochran, 1991). The Co-Stat computer software (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analysis.

RESULTS

Proximate composition

Significant differences ($p<0.05$) were observed among the fish carcass in terms of crude fat, crude protein, ash and moisture (Table III). According to the results, fish fed at 400 mg/kg level based diet had maximum contents of crude protein (62%) and crude fat (15%) as compared to fish fed on control diet (protein 54% and fat 9%). Highest amount of crude ash (9%) was found in test diet II (*i.e.,* 100 mg/kg) whereas moisture (8%) was found to be highest in carcass of fish fed on control diet. However, lowest amount of crude ash (6%) was found in the fish fed at 400 mg/kg level based diet and lowest moisture (5%) was noted in group of fish which fed on test diet IV. On the basis of these results, it was found that fingerlings fed at 400 mg/kg level based diet showed highest amount of nutrients (protein, fat and gross energy) in carcass as compared to fish fed on control and other test diets.

Table II.- Chemical composition (%) of feed ingredients.

Ingredients	Dry matter (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	Carbohydrates (%)
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

Table III.- Proximate composition (%) of *C. carpio* fed on polyphenols supplemented canola meal based diet.

Carcass parameters	Polyphenols levels (mg/kg)	Protein	Fat	Ash	Moisture	Carbohydrates
Test diet –I	0	53.62±0.65^{d}	8.86±0.59^{e}	9.29±0.14^{a}	7.49±0.37^{a}	19.45±0.84^{a}
Test diet –II	100	55.27±0.86^{cd}	10.94±0.66^{d}	9.40±0.40^{a}	6.60±0.83^{a}	16.42±0.88^{b}
Test diet –III	200	58.88±0.64^{b}	13.23±0.91^{ab}	8.75±0.13^{ab}	6.43±0.39^{ab}	11.43±0.83^{d}
Test diet –IV	300	59 .60±0.95^{b}	14.04±0.48^{ab}	7.58±0.46^{c}	5.17±0.18^{c}	12.59±0.88^{c}
Test diet –V	400	62.17±0.64^{a}	14.78±0.19^{bc}	6.43±0.35^{d}	5.30±0.18^{c}	10.07±0.32^{c}
Test diet –VI	500	58.41±0.24^{b}	13.15±0.65^{bc}	7.82±0.75^{bc}	5.45±0.17^{bc}	13.98±1.01^{d}
Test diet –VII	600	55.92±0.88^{c}	11.69±0.34^{ab}	8.83±0.16^{ab}	6.92±0.24^{a}	15.29±1.28^{c}

All values of Means within rows are different significantly (*p<0.05).* Data values are mean (Mean ± Standard deviation) of three replicate.

Table IV.- Percentage of minerals in test diets of *C. carpio* fingerlings fed on canola meal based diet supplemented with polyphenols.

Minerals / Polyphenols levels (mg/kg)	Test diet –I (control diet) 0	Test diet –II 100	Test diet –III 200	Test diet –IV 300	Test diet –V 400	Test diet –VI 500	Test diet –VII 600
Ca (%) in diets	0.93±0.05^{ab}	0.96±0.08^{a}	0.79±0.07^{abc}	0.79±0.09^{abc}	0.69±0.03^{c}	0.96±0.07^{a}	0.74±0.10^{bc}
Na	0.14±0.02 ^{a}	0.15±0.01^{a}	0.14±0.02 ^{a}	0.14±0.03^{a}	0.13±0.02^{a}	0.13±0.02^{a}	0.15±0.02^{a}
K	0.017±0.003^{a}	0.016±0.001^{a}	0.018±0.001^{a}	0.017±0.001^{a}	0.0170±0.002^{a}	0.017±0.0009^{a}	0.018±0.001^{a}
Fe	0.0543±0.005^{a}	0.054±0.004^{a}	0.054±0.005^{a}	0.053±0.007^{a}	0.0533±0.004^{a}	0.053±0.004^{a}	0.055±0.006^{a}
Cu	0.006±0.0004^{a}	0.006±0.0006^{a}	0.006±0.0004^{a}	0.006±0.0004^{a}	0.006±0.0004^{a}	0.006±0.0004^{a}	0.006±0.0005^{a}
P	2.11±0.06^{a}	2.11±0.05^{a}	2.12±0.10^{a}	2.12±0.04^{a}	2.11±0.09^{a}	2.11±0.06^{a}	2.10±0.04^{a}
Mg	0.009±0.0004^{a}	0.009±0.0003^{a}	0.009±0.0003^{a}	0.009±0.0006^{a}	0.009±0.0006^{a}	0.009±0.0004^{a}	0.009±0.0005^{a}
Al	0.0005± 0.00006^{a}	0.0005± 0.00007^{a}	0.0005± 0.00004^{a}	0.0005± 0.00006^{a}	0.0005± 0.00007^{a}	0.0005± 0.00005^{a}	0.0005± 0.00006^{a}

Data are means of three replicates. Ca, calcium; Na, sodium; K, potassium; Fe, iron; Cu, copper; P, phosphorus; Mg, magnesium; Al, aluminium.

Minerals absorption

The analyzed values of minerals in diet and feces have shown that there is equal composition of all the minerals in control diet as well as polyphenols supplemented diets (Table IV). It was noted that minerals such as Ca (71%), Fe (69%) and Al (64%) were found highly absorbed when the fingerlings were fed at test diet VI as compared to other test diets and control diet. Whereas, maximum absorption of K (74%) and P (76%) were found at 400 mg/kg level based diet and Cu (69%) as well as Mg (67%) were at 300 mg/kg level based diet. On the other hand all the minerals were found lowest when fingerlings were fed on control diet as compared to test diets except Cu that was lowest at test diet II as shown in Table VI.

Hematology

Values of WBCs ($8\times10^{3}mm^{-3}$) and RBCs ($3\times10^{6}mm^{-3}$) were noted to be highest in test diet V (400 mg/kg of polyphenols) whereas highest Hb (9 g/100ml) was noted at test diet IV (300 mg/kg of polyphenols). These value were significantly different (P>0.05) from fish group which consumed control diet, whereas values of WBCs and Hb were statistically similar with fish fed on test diet IV (Table VII). However, values of RBCs, WBCs and Hb were found to be lowest in the fish fed on control diet. On the other hand, PCV (25%) in test diet V and PLT (67%) in test diet VI were noted to be highest, and lowest values were noted in fish fed control diet. Highest values of MCV (188 fl) as well as MCHC (35%) were observed in fish fed

on test diet V and MCH (57 pg) was reported to be highest in fish fed on test diet VI. These noted values significantly varied from the values noted in fish fed on control diet. The hematology of fish showed that 400 mg/kg level is optimum for monitoring stress response, healthy fish growth as well as measuring immune response of fish through WBCs count.

Table V.- Analyzed mineral compositions in feces of *C. carpio* fingerlings fed on canola meal based diet supplemented with polyphenols.

Minerals / Polyphenols levels (mg/kg)	Test diet –I (control diet) 0	Test diet –II 100	Test diet –III 200	Test diet –IV 300	Test diet –V 400	Test diet –VI 500	Test diet –VII 600
Ca	0.51±0.02^{a}	0.49±0.03^{ab}	0.42±0.04^{bc}	0.38±0.04^{c}	0.27±0.02^{d}	0.29±0.02^{d}	0.39±0.02^{c}
Na	0.07±0.01^{ab}	0.07±0.01^{a}	0.05±0.01^{bc}	0.05±0.01^{bc}	0.04±0.05^{c}	0.04±0.004^{c}	0.07±0.01^{ab}
K	0.008±0.001^{a}	0.007±0.0005^{ab}	0.007±0.0006^{ab}	0.006±0.0005^{bc}	0.004±0.0005^{c}	0.007±0.0006^{ab}	0.008±0.0005^{a}
Fe	0.030±0.003^{a}	0.027±0.002^{ab}	0.025±0.002^{abc}	0.021±0.002^{bcd}	0.020±0.001^{cd}	0.018±0.002^{d}	0.023±0.002^{bcd}
Cu	0.003±0.0002^{a}	0.003±0.0003^{a}	0.002±0.0002^{ab}	0.002±0.0001^{c}	0.002±0.0002^{bc}	0.002±0.0002^{ab}	0.003±0.0004^{a}
P	1.08±0.05^{a}	0.94±0.04^{b}	0.71±0.02^{c}	0.67±0.01^{c}	0.53±0.04^{d}	0.64±0.04^{c}	0.86±0.02^{b}
Mg	0.005±0.0003^{ab}	0.004±0.0002^{abc}	0.003±0.0002^{de}	0.003±0.0003^{e}	0.003±0.0003^{cde}	0.004±0.0003^{bcd}	0.005±0.0007^{a}
Al	0.0003± 0.00003^{a}	0.0002± 0.00003^{ab}	0.0002± 0.00002^{ab}	0.0002± 0.00003^{ab}	0.0002± 0.00003^{b}	0.0002± 0.00002^{b}	0.00025± 0.00003^{ab}

Means within same column having different superscripts are significantly different at $p < 0.05$. Data are means of three replicate. Ca, calcium; Na, sodium; K, potassium; Fe, iron; Cu, copper; P, phosphorus; Mg, magnesium; Al, aluminium.

Table VI.- Minerals absorption of *C. carpio* fingerlings fed on canola meal based diet supplemented with polyphenols.

Minerals / Polyphenols levels (mg/kg)	Test diet –I (control diet) 0	Test diet –II 100	Test diet –III 200	Test diet –IV 300	Test diet –V 400	Test diet –VI 500	Test diet –VII 600
Ca	48.54±0.92^{f}	52.40±0.58^{de}	50.77±0.25^{e}	54.71±0.65^{c}	63.44±0.89^{b}	71.34±0.49^{a}	52.97±0.79^{cd}
Na	48.59±0.35^{a}	51.73±0.75^{a}	50.99±0.88^{a}	52.47±0.87^{a}	59.57±0.83^{a}	53.76±0.72^{a}	67.74±0.73^{a}
K	55.62±0.90^{e}	57.78±0.91^{de}	61.60±0.88^{c}	66.68±0.91^{b}	73.64±0.79^{a}	61.49±0.92^{c}	58.55±0.96^{d}
Fe	47.49±0.80^{f}	53.43±0.89^{e}	56.82±0.94^{d}	61.39±0.89^{c}	64.30±0.97^{b}	68.53±0.89^{a}	62.18±0.89^{bc}
Cu	53.67±0.97^{d}	50.40±0.94^{e}	57.17±0.90^{c}	69.38±0.59^{a}	65.66±0.90^{b}	58.43±0.63^{c}	52.49±0.68^{de}
P	52.51±0.43^{f}	58.39±0.19^{e}	68.46±1.06^{c}	70.26±0.53^{bc}	76.42±0.16^{a}	71.43±0.81^{b}	63.43±0.86^{d}
Mg	51.75±1.30^{d}	53.79±1.45^{d}	64.31±0.97^{ab}	67.32±0.94^{a}	62.77±0.90^{b}	57.52±0.95^{c}	50.86±0.97^{d}
Al	47.70±0.85^{d}	52.17±0.92^{c}	58.55±0.97^{b}	60.57±0.75^{b}	63.27±0.78^{a}	64.41±0.75^{a}	58.28±0.81^{b}

Means within same column having different superscripts are significantly different at $p < 0.05$. Data are means of three replicates. Ca, calcium; Na, sodium; K, potassium; Fe, iron; Cu, copper; P, phosphorus; Mg, magnesium; Al, aluminium.

Table VII.- Hematological parameters of *C. Carpio* fingerlings fed different levels of canola meal based diet supplemented with polyphenols.

Experimental diets	Polyphenols levels (mg/kg)	WBCs (10^3mm^{-3})	RBCs (10^6mm^{-3})	PLT	Hb (g/100ml)	PCV (%)	MCHC (%)	MCH (pg)	MCV (fl)
Test diet-I (control)	0	6.63±0.27^{b}	1.84±0.40^{b}	54.42±0.29^{e}	6.37±0.11^{d}	21.20±0.50^{c}	24.68±0.69^{e}	37.64±0.09^{e}	90.26±0.10^{g}
Test diet-II	100	6.90±0.50^{b}	2.14±0.28^{ab}	60.69±0.33^{d}	6.57±0.10^{cd}	22.38±0.10^{bc}	27.69±0.31^{d}	38.61±0.35^{e}	111.30±0.90^{f}
Test diet-III	200	7.35±0.20^{ab}	2.31±0.14^{ab}	63.47±0.44^{c}	7.35±0.18^{bc}	23.72±0.65^{ab}	31.38±0.18^{c}	41.70±0.44^{d}	181.05±0.48^{d}
Test diet-IV	300	7.76±0.12^{a}	2.76±0.32^{ab}	65.96±0.13^{b}	8.47±0.08^{a}	24.51±0.13^{a}	33.81±0.22^{b}	49.97±0.13^{c}	187.11±0.16^{b}
Test diet-V	400	7.85±0.08^{a}	2.98±0.29^{a}	65.65±1.00^{b}	8.45±0.15^{a}	24.91±0.46^{a}	35.24±0.46^{a}	52.10±1.78^{b}	188.36±0.39^{a}
Test diet-VI	500	7.32±0.38^{ab}	2.26±0.64^{ab}	67.34±0.11^{a}	7.78±0.32^{ab}	23.91±0.78^{a}	33.82±0.71^{b}	56.84±0.30^{a}	183.01±0.27^{c}
Test diet-VII	600	6.87±0.29^{b}	1.85±0.16^{b}	66.66±0.16^{ab}	7.89±0.32^{bcd}	23.66±0.28^{ab}	33.12±0.21^{b}	50.02±0.21^{c}	173.69±0.24^{e}

RBC, red blood cell; WBC, white blood cell; PLT, platelet; Hb, hemoglobin. Means within rows having different superscripts are significantly different at $p< 0.05$. Data are means of three replicates.

DISCUSSION

Supplementation of polyphenols significantly influenced whole-body composition of fish in present study. The studies conducted by other researchers are in agreement with our results, who observed significant changes in whole-body crude protein (Nandeesha *et al*., 2001; Abdel-Tawwab and Ahmad, 2009; Promya and Chitmanat, 2011), crude lipid (Nandeesha *et al*., 1998, 2001; Abdel-Tawwab and Ahmad, 2009), ash (Nandeesha *et al*., 2001; Tongsiri *et al*., 2010) and moisture contents (Tongsiri *et al*., 2010; Promya and Chitmanat, 2011) of fish fed on feed with supplementation of spirulina (source of polyphenol). Significant increase in whole-body lipid was reported for *Labeo rohita* by spirulina intake (Nandeesha *et al*., 2001). Similarly, the results of study conducted by Wafaa *et al*. (2014) showed that crude protein was significantly ($p<0.05$) increased in groups of fish fed on green tea (GT), black seed and propolis extract (polyphenols sources), when compared to control group. Likewise, results of Abdel-Tawwab *et al*. (2010) are also in agreement with our findings, who confirmed that addition of GT extract in diet up to 0.5g and 1 g/kg diet increased content of protein and 0.5 g/kg diet significantly increased total lipids whereas, 1g/kg diet significantly decreased total lipids content. Supplementation of propolis, at the level of 50 g/kg, increased whole-body content of lipid and protein in juvenile upto peak values.

Cho *et al*. (2007) reported increase in total protein (TP) in flounder fed green tea polyphenols in diet. Using roselle calyx in the experimental diets, Mesallamy *et al*. (2016) observed a trend that with its increasing levels, crude protein of fish bodies increased whereas total lipid decreased significantly ($p<0.05$). However, contradictory results were also observed, Kim *et al*. (2013a) found that spirulina had no significant effect on whole-body composition of parrot fish, same results were reported in studies with silver sea bream, siberian sturgeon and red tilapia hybrid (El-Sayed, 1994; Palmegiano *et al*., 2005; Ungsethaphand *et al*., 2010). Kim *et al*. (2013a) used spirulina as replacement for fishmeal for parrot fish and did not find any significant differences in carcass or flesh protein content of common carp and mekong giant catfish (Nandeesha *et al*., 1998; Tongsiri *et al*., 2010) at any level of spirulina administration. Similarly, Zhai *et al*. (2013) reported that supplementation of polyphenols i.e. quercetin, lowered the crude lipid level in body of Nile Tilapia. No significant differences were noted in the whole body compositions of trout juveniles fed propolis for 10 weeks (Deng *et al*., 2011). Abdelwahab *et al*. (2012) observed that crude protein and crude ash contents of Asian sea-bass were not significantly affected by supplementation of 5 and 10g/kg black cumin seed to the diet. Likewise, results of study conducted by Amer (2016) depicted no significant difference in protein and ash content among treatments, but there was significant decrease in fat content between control group and other groups.

Abdel-Latif and Khalil (2014) reported non-significant differences among treatments for lipid content, moisture and ash, but the protein content in muscle of fish fed 1000 g/kg spirulina diet was the highest. Hwang *et al*. (2013) found that contents of crude protein and moisture in whole body, dorsal muscle as well as in liver were not significantly different between the control and test diet groups ($p<0.05$). Significantly lowered lipid and ash contents in whole body of the group fed on 500 g/kg level based diet than those of control diet group ($p<0.05$) were observed. Fallahpour *et al*. (2015), in their study found that polyphenols-rich marshmallow extract brought slight variations in body composition of fish as compared to controls. There were no significant changes in moisture levels in all groups. Compared to controls, lipid levels for the dried body were the lowest after feeding the fish marshmallow extract 50g/kg. The whole body ash reduced after administering marshmallow extract at 250 g/kg as compare to control groups. Katya *et al*. (2014) found that replacement of fish meal by fermented by-product of mushroom in the diets ($p<0.05$) did not significantly affect whole-body proximate composition of fish.

Our results have shown that 400 mg/kg is the optimum level of polyphenols which can increase the retention of minerals in body of *C. carpio* fingerlings fed polyphenols supplemented canola based diets. Since less work has been conducted on this parameter, therefore few relevant data was found. Contrary to our finding, Frejnagel and Wroblewska (2010) reported that they used various polyphenolic extracts in feed of a monogastric animal and conducted a comparative study. They reported that polyphenolic extracts reduced the concentrations and apparent absorption of some minerals as Zn and Cu. However, the concentrations of Ca, Fe, P and Mg in the femur ash were not affected. Differences encountered might be due to different specie used, source of polyphenols, conditions and concentration.

Hematological indices are indeed an important tool for judging the health status of fish (Abdel-Tawwab *et al*., 2010). Use of polyphenols aided in significantly higher Ht, Hb and RBC levels as compared to diet which lack polyphenols, *i.e.* control diet. Our findings are in tandem with the work of Mesallamy *et al*. (2016), who analyzed hematological parameters (Hb, Ht, and RBCs) for Nile tilapia fed on *Hibiscus sabdariffa* calyx, a potent source of polyphenols. They reported that there was a numerical increase in Hb, Ht and RBC, as the level of *H. sabdariffa*

calyx increased in concentration. Similar results were found from the work of Olusola (2011), who found that the anti-oxidative potency of Roselle calyx extract, resulted in a gradual increase in Hb, Ht and RBC as the concentration increased. Another contradiction is witnessed in results of Hwang *et al*. (2013) who reported that Hb and Ht levels of group fed on control diet were found to be significantly higher than group fed on 5 % Green tea extract diet ($p<0.05$) in *Sebastes schlegelii*. Contradictions among our results and results of other researchers might be due to different source of polyphenols used, variation in species, their nutrient requirements, environmental conditions, varying levels and other such factors (Zhai *et al*., 2013).

CONCLUSION

Polyphenols, used directly or indirectly (*i.e.* through rich source), has significant effect on body composition of common carp as a result of increased mineral absorption, improved body composition and hematology of Common carp fingerlings. Supplementation of the polyphenols in canola meal based diet at the rate of 400 mg/kg showed maximum improvement in fingerlings of common carp.

Statement of conflict of interest

The authors have declared no conflict of interests.

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