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Efficacy of Dietary Polyphenols Supplementation with Soybean Meal-Based Diet on Growth, Antioxidant Status and Carcass Composition of *Labeo rohita* Fingerlings

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MA conducted the study and wrote the manuscript. SMH acquired funds, administered and supervised the project. MA and MH curated and edited the data. MZHA, ZY and AAB edited and reviewed the manuscript.

Key words
Aquaculture, Polyphenols, Growth performance, Carcass composition, Antioxidant activity

ABSTRACT

A 70-days growth trial was carried out to ascertain how dietary supplementation of polyphenols with soybean meal-based diet influences the growth, antioxidant status and carcass composition of *Labeo rohita* fingerlings. Seven experimental diets were formulated with graded levels, 0, 50, 100, 150, 200, 250 and 300 mg/kg of polyphenols supplementation. Each test diet was allocated randomly to triplicate tanks with 15 fingerlings in every tank. These were fed @ 5% of live wet body weight. Results showed that *L. rohita* fingerlings fed on test diet T_4 with dietary supplementation of 150 mg/kg polyphenol showed significant (p<0.05) increase in growth performance having weight gain% (233.24%) and better feed conversion ratio (1.44). Similarly, significantly (p<0.05) improved body composition with crude protein (17.96%), crude fat (8.72%), ash (4.94%) and moisture (68.43%) were also recorded at same polyphenols supplementation level. However, in terms of antioxidant activity, increasing trend in inhibition of oxidation was recorded with increase in supplementation of polyphenols in test diets, having minimum oxidation (7.66) in fingerlings fed on T_7 with 300 mg/kg level of polyphenols supplementation.

INTRODUCTION

Feed in aquaculture is a major factor regarding its growth and development (Gabriel *et al*., 2007). More commonly used aqua-feeds contain fishmeal (FM) as a source of protein owing to its balanced amino acid content, high palatability and good digestibility (Nasr *et al*., 2021). But FM is primarily produced from fish harvested from wild resources that are constantly diminishing and it is supposed that in the near future, current FM yield will not be able to fulfill the expanding aquaculture production. This reinforces the need to search new FM alternatives to ensure long term aquaculture sustainability (Gasco *et al*., 2018). Due to low cost and world-wide availability, plant protein sources are considered as the best alternatives for FM (Saleh *et al*., 2021). Soybean meal (SBM) is considered as an appropriate protein source and good alternative for FM in aqua-feed because of its nutritional composition that is consistent with FM, comparatively better amino acid profile, easy availability and affordable price (Krishnan and Jez, 2018).

Plant extracts having many valuable functional properties, have also gained much attention due to their positive impacts on fish health (Parrino *et al*., 2019; Zargar *et al*., 2019). Particularly, polyphenols (secondary plant metabolites) are highly perceived for their antioxidant effects that inhibit harm due to oxidative stress, abolish inflammation, enhance metabolism of lipid and decrease chances of cancer and cardiac disorders (Hussain *et al*., 2016; Xu *et al*., 2019). Until now, only 8000 plant polyphenols have been explored and only about 10% of the possible plant polyphenols are produced by using plants (Tsao, 2010). These are found in nearly all residuals of agro industrial chains. By-products of seeds, fruits,

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nuts, cereals, and vegetables are rich in polyphenols (Castrica *et al*., 2019). Supplementation of polyphenols in fish feed is necessary for enhancing the efficacy of plant based products which in return improves fish growth rate and health status. Being immunity stimulants, polyphenols are regarded as natural antibiotics that increase resistance in fish body and are used as dietary additives (Zhu, 2020).

Fish is also subjected to repulsive flavor and malodorous smell during its handling and processing due to oxidation of n-3 polyunsaturated fatty acids (PUFA). So, oxidation of PUFA in fish body has a greater risk for loss of fish quality. Oxidation of lipids in meat of fish and other fish products not only give rancid taste but also produce different substances in fish meat which cause health issues in humans on its consumption (Secci and Parisi, 2016). Thus, for overcoming this problem of oxidation and low immunity, antioxidants may be included in fish feed. Antioxidants capture the reactive free radicals and are thus important for removing the potentially damaging oxidizing agents which results in better health of living organism. Polyphenolic compounds show redox properties due to which they act as strong antioxidants (Adedapo *et al*., 2008).

Labeo rohita is globally considered a dominant freshwater tropical Indian major carp which accounts for almost 3.70 % of global aquaculture yield in 2018 (FAO, 2018). It is column feeder fish, feeding both on plant and animal matter for getting nutrients. It is an economically important species with total yield of about two million tons, enchanting an amount of US$ 3.42 billion in 2018 (FAO, 2020). Considering the commercial value of *L. rohita* and promising use of polyphenols in critically improving the fish health and immunity, this study was designated to investigate the effects of polyphenols supplementation with SBM-based diets on growth, antioxidant activity and carcass composition of *L. rohita* fingerlings.

MATERIALS AND METHODS

Culture conditions and experimental design

Fingerlings were procured from Government Fish Seed Hatchery, Faisalabad and were acclimatized with laboratory environment for 14 days. To make fingerlings free from ecto-parasites, bathed with saline water (5% NaCl) prior to experiment (Rowland and Ingram, 1991). During acclimatization period, fingerlings were fed basal diet twice daily to apparent satiety. Water quality indicators i.e., water oxygen level, pH and temperature were measured on daily basis and were adjusted within following ranges as temperature (24.8-28, 6 ºC), pH (7.4-8.5), and water oxygen level (5.8-7.4 mg/L). To all experimental tanks, aeration was provided round the clock by using capillary system. 15 fingerlings were placed in each tank having three replicates for each test diet. These were fed according to 5% live body mass. Total duration of trial was of 70-day.

Test diet formulation

Seven diets including one control and six test diets with supplementation of polyphenols in SBM-based diet on following graded levels (0, 50, 100, 150, 200, 250, and 300 mg/kg) were formulated. For supplementation, polyphenols were procured from tomato extract at Department of Applied Chemistry and Biochemistry, Government College University, Faisalabad. While rest of feed items (Table I) were bought from private feed mill and analyzed chemically according to AOAC (2005). For pellets formation, all feed ingredients were ground, mixed thoroughly by using mixer, and then fish oil was added slowly. 10 to 15% of water was also added to obtain dough which was then passed via laboratory extruder to obtain floating pellets. All the experimental diets were then oven dried at 105ºC and kept at 4ºC until use.

Table I. Ingredients composition (%) of test diets (TD).

Ingredients	TD-I (Control)	TD-II	TD-III	TD-IV	TD-V	TD-VI	TD-VII
Polyphenols (mg/kg)	0	50	100	150	200	250	300
Soybean meal	50	50	50	50	50	50	50
Fish meal	17	17	17	17	17	17	17
Wheat flour*	12	12	12	12	12	12	12
Sunflower meal	10	10	10	10	10	10	10
Fish oil	7	7	7	7	7	7	7
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

*Polyphenols was supplemented at the cost of wheat flour.

Feeding procedure and sample collection

Fish were fed on test diets daily two times (morning and evening) at 5% of their live body mass. After 2 h. of feeding session, for measuring feed conversion ratio (FCR), unconsumed feed was collected after water drainage by disclosing the tanks valves. Tanks were cleaned properly and filled with water. After 120 min, fecal material was collected from tanks via fecal collecting tube. Proper care was done during collection of feces to prevent nutrient leaching. Then, fecal material was dried in oven at 105 ºC and after grinding stored for chemical analysis.

Growth measurement

L. rohita fingerlings with average weight (7.09 g) were stocked in each tank at the start of experiment. During experimental period, fingerlings were bulk weighted after every two weeks for assessment of growth. Weight gain % (WG%), and FCR were measured by following formulae:

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

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

Evaluation of antioxidant activity

By utilizing 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay stated by Brand-Williams (1995), antioxidant status of samples was checked. For this purpose, 2 g of samples were homogenized in 20 ml of 80% aqueous methyl alcohol and were placed 20-25°C for 15 min. The samples were centrifuged (5000 rpm) for 10 min at 4°C and passed via 0.45 nm syringe filter (Whatman Inc., Clifton, NJ) before analysis. Then in 1.5 ml cuvette, 100 µl of filtered extract and 900 µl DPPH methanol solution (100 µM) was added to get final volume of 1 ml. With interval of one minutke, mixture absorbance was noted at 517 nm for 10 min by using spectrophotometer. As per inhibition the extract's antioxidant capacity against the DPPH radicals was calculated.

$$\text{Percent inhibition} = \frac{(\text{Absorbance of control sample} - \text{Absorbance of test sample})}{\text{Absorbance of control sample}} \times 100$$

Carcass composition analysis

For carcass composition analysis, 5 fingerlings were chosen at random from each tank and were air dried. Samples were homogenized by using motor and pestle prior to proximate analysis by using standard protocols (AOAC, 2005). Samples were dried in oven (105°C) for 12 h to measure moisture amount. For measurement of crude protein (CP) (N × 6.25), Kjeldahl apparatus was used while crude fat (CF) was measured by using Soxtec HT2 1045 system. Ash was analyzed by its ignition for 12 h. at 650 °C in electric furnace (Eyela-TMF 3100). For estimation of total gross energy, oxygen bomb calorimeter was utilized.

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the growth and proximate composition of data (Steel *et al*., 1996). For comparison of differences in means, Tukey's Honesty Significant Difference Test was used and found significant at $p<0.05$ (Snedecor and Cochran, 1989). All was done by utilizing the Co-Stat computer software (Version 6.303, Monterey, PMB 320, 93940 USA, CA).

RESULTS

According to results of growth performance (Table II), an increasing trend in WG and WG% was recorded in fingerlings fed on polyphenols supplemented diet up to T_4 (polyphenols supplementation of 150 mg/kg), with maximum and significant ($p<0.05$) WG% (233.24%) and WG (16.56g) with FCR (1.44), while from T_5 to T_7 a decreased trend in growth rate and WG was recorded. The least WG (11.43g) and WG% (161.29%) with FCR (1.94) was recorded at control diet. Antioxidant status in fingerlings fed SBM-based diet with polyphenols supplementation at various levels (0, 50, 100, 150, 200, 250 and 300 mg/kg of diet) is determined (Table III). In terms of oxidation, decreasing trend was noted with rising level of polyphenols that indicate increase in antioxidant activity with rise of dietary polyphenols level. Experimental diet T_7 with 300 mg/kg polyphenols was found to be best in terms

Table II. Growth performance of *L. rohita* fingerlings fed on soybean meal based diets supplemented with polyphenols.

Growth parameters	TD-I (Control)	TD-II	TD-III	TD-IV	TD-V	TD-VI	TD-VII
IW (g)	7.09 ± 0.02^a	7.09 ± 0.02^a	7.08 ± 0.02^a	7.10 ± 0.01^a	7.10 ± 0.02^a	7.09 ± 0.02^a	7.08 ± 0.02^a
FW (g)	18.52 ± 0.08^g	20.84 ± 0.07^d	21.53 ± 0.13^c	23.66 ± 0.10^a	22.77 ± 0.12^b	20.01 ± 0.12^e	19.24 ± 0.05^f
WG (g)	11.43 ± 0.06^g	13.75 ± 0.05^d	14.45 ± 0.11^c	16.56 ± 0.09^a	15.67 ± 0.10^b	12.92 ± 0.10^e	12.16 ± 0.04^f
WG (%)	161.29 ± 0.59^g	193.80 ± 0.38^d	204.14 ± 0.93^c	233.24 ± 0.94^a	220.60 ± 0.95^b	182.23 ± 0.90^e	171.62 ± 0.19^f
WG ($Fish^{-1}$ day^{-1})g	0.16 ± 0.01^g	0.20 ± 0.00^d	0.21 ± 0.00^c	0.24 ± 0.02^a	0.22 ± 0.01^b	0.18 ± 0.00^e	0.17 ± 0.00^f
FI	0.36 ± 0.01^c	0.39 ± 0.01^b	0.40 ± 0.01^b	0.43 ± 0.03^a	0.42 ± 0.02^a	0.39 ± 0.01^b	0.36 ± 0.01^c
FCR	1.94 ± 0.07^a	1.97 ± 0.04^a	1.56 ± 0.06^b	1.44 ± 0.02^c	1.55 ± 0.02^b	1.62 ± 0.07^b	1.83 ± 0.20^a

Means within rows having different superscripts are significantly different at (p<0.05). Data are means of three replicates. IW, initial weight, FW, final weight; FI, feed intake; FCR, feed conversion ratio; WG, weight gain.
For details of test diets (TDs), see Table I.

of antioxidant activity having lowest oxidation value (7.66) as compared to other test diets. In terms of *L. rohita* carcass composition (Table IV), increasing trend was recorded in fingerlings fed from T_1 to T_4, and fish fed on T_4 (150 mg/kg polyphenols supplementation) has significantly (p<0.05) higher CP (17.96%), CF (8.72%), ash (4.94%) and moisture (68.43%), while fish fed T_5 to T_7 showed the decreasing trend in proximate body composition. Among all test diets, fingerlings fed on control diet (T_1) showed the minimum proximate body composition with CP (15.03%), CF (5.71%), ash (4.04%) and moisture (67.38%).

Table III. Antioxidant activity of polyphenols supplemented soybean meal-based diet in *L. rohita.*

Experimental diets	Polyphenols levels (mg/kg)	Oxidation (%)
TD-I (Control)	0	100.00±0.00
TD-II	50	96.88±0.36
TD-III	100	78.34±0.84
TD-IV	150	46.28±0.62
TD-V	200	33.15±0.34
TD-VI	250	18.23±0.38
TD-VII	300	7.66±0.44

DISCUSSION

Polyphenols may be recognized as credible alternatives to antibiotics and other chemicals in fish farming as they may defend fishes against oxidative stress and many other diseases (Dawood *et al*., 2018; Van *et al*., 2019). A fish fed on polyphenols supplemented feed is like having a body armed with antioxidants and is more likely to combat with diseases, exhibit better carcass composition and increase in growth performance.

According to results of present study, *L. rohita* fingerlings exhibited significant improvement in carcass, growth performance and WG with lowest FCR value at 150 mg/kg polyphenols supplementation level in SBM-based diet. These results are in line with study of Shin *et al*. (2010) who noted that olive flounder showed better growth performance and lower FCR, when fed on diet supplemented with quercetin (polyphenols) at 150 mg/kg. Better growth performance was also reported in *Pangasianodon gigas* when fed on diet in which 5% of FM replaced with spirulina (rich in polyphenols) (Tongsiri *et al*., 2010). Xu *et al*. (2019) recorded significant improvement in growth, antioxidative activity and meat quality of *Ctenopharyngodon idella* fed on diet rich in polyphenols with quercetin supplementation. Significant decrease in FCR and higher growth performance in Nile tilapia was reported when fed on diet supplemented with grape seed proanthocyanidins (Zhai *et al*., 2014). Babaheydari *et al*. (2014) observed that with increase in supplementation of *Stachys lavandulifolia* up to 40 mg/kg, there was an increase in growth performance of tilapia. Better growth performance was reported in *Cyprinus carpio* when fed on diet supplemented with marshmallow extracts (Fallahpour *et al*., 2014). *Megalobrama amblycephala* showed improved immunity, growth performance and lipid metabolism when fed on high fat diet supplemented with polyphenols (Jia *et al*., 2019). Munglue (2016) reported higher growth performance and maximum WG in tilapia when fed on 1% *Nelumbo nucifera* peduncle extract. *Oreochromis niloticus* showed better growth performance at 0.5, 1 and 1.5% supplementation of roselle calyx, another rich source of polyphenols (El-Mesasllamy *et al*., 2016).

Fallahpour *et al*. (2014) reported that inclusion of marshmallow extract (0.25%) in feed of fish attributed to better absorption of nutrients, resulting in higher WG.

Table IV. Proximate composition (%) of *L. rohita* carcass fed on soybean meal based diet supplemented with polyphenols.

Experimental diets	Polyphenols levels (mg/kg)	Protein %	Fat %	Ash %	Moisture %
TD-I (Control)	0	15.03±0.03[d]	5.71±0.37[d]	4.04±0.03[c]	67.38±0.12[cd]
TD-II	50	15.15±0.14[d]	6.39±0.04[bc]	4.15±0.03[b]	67.94±0.23[bc]
TD-III	100	15.49±0.09[c]	6.42±0.16[bc]	4.20±0.03[b]	67.08±0.11[d]
TD-IV	150	17.96±0.01[a]	8.72±0.04[a]	4.94±0.03[a]	68.43±0.59[b]
TD-V	200	15.99±0.08[b]	6.58±0.12[b]	4.19±0.09[b]	69.33±0.10[a]
TD-VI	250	15.05±0.05[d]	6.02±0.12[cd]	4.17±0.06[b]	67.73±0.22[bcd]
TD-VII	300	15.06±0.02[d]	5.92±0.04[cd]	4.13±0.04[b]	67.67±0.49[bcd]

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

Enhanced nutrient digestibility leads to improved carcass composition of fish in terms of fat, protein, ash and moisture. Jiang *et al*. (2016) reported that supplementation of curcumin (polyphenols) in feed of *Carassius auratus* is beneficial in enhancing the absorptive ability and digestion of nutrients leading to higher growth rates. According to Nandeesha *et al*. (2001), *L. rohita* fingerlings showed significant improvement in lipid content when fed on spirulina supplemented diet. Similarly, protein content was significantly different from control group when fish fed on diet supplemented with green tea extracts and propolis extracts (Wafaa *et al*., 2013).

Antioxidants capture the free radicals, thus have health promoting benefits by preventing the peroxidative damage to the body (Biglari *et al*., 2008). The results of this study proved that antioxidant activity of *L. rohita* increased with increased polyphenols level in diet. Maximum inhibition of oxidation was reported with 300 mg/kg of polyphenols level among all test diets while the fish fed on controlled diet showed the maximum oxidation. Amer (2016) reported that spirulina supplementation worked as antioxidant, hence enhances the immunity in body of Nile tilapia. Darsini *et al*. (2013) reported that *Limonia acidissima* supplementation significantly (p<0.01) enhances the number of antioxidant enzymes in common carp leading to decrease lipid oxidation. Hwang *et al*. (2013) studied that supplementation of green tea extracts in diet of *Sebastes schlegeli* improved lipid utilization along with increased lysozymal activity for better immunity. Curcumin supplementation boosted the immunity and antioxidative status of *C. carpio* and *C. idella* (Giri *et al*., 2019; Ming *et al*., 2020). Wang *et al*. (2020) observed that quercetin supplementation fortified the antioxidative status along with improvement in immunity of *Danio rerio*.

However, contradictory results have also been noticed by other researchers. Spirulina supplementation showed no significant effects when fed to olive flounder (Kim *et al*., 2013). Hwang *et al*. (2013) mentioned that fish fed on diet having 5% supplementation of green tea extracts showed significantly lowered specific growth rate, WG, and feed efficiency in comparison with fish fed on control diet. Frejnagel and Wroblewska (2010) observed that polyphenols supplementation resulted in decreased nutrients absorption in gut of monogastric animals resulting in poor growth performance. These contradictions in results can be due to differences in fish species, nutrient requirements, feeding conditions and polyphenols supplementation levels (Zhai and Liu, 2013).

CONCLUSION

Results of current study showed that polyphenols are good antioxidants and its inclusion in SBM-based diet at 150 mg/kg level has significant positive impacts on growth and proximate body composition. By increase in polyphenols supplementation level in diet, effective inhibition of oxidation occurred indicating improved antioxidant activity. Hence, SBM-based diet with polyphenols supplementation ensured cheap and effective feed additive referred for use in aqua-feed that will help in producing healthy fish, overcoming the issue of shortage and high price of FM leading to aquaculture industry development.

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IBR approval

The experiment was carried out in-line with the IRB guidelines of Government College University, Faisalabad.

Ethical statement

All the procedures and methods used in this study followed the ethical guidelines provided by Government College University, Faisalabad.

Statement of conflict of interest

The authors have declared no conflict of interest.

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