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Substitution of Fish Oil with Selected Dietary Oils: Effects on Growth Performance, Nutrient Digestibility and Body Composition of *Catla catla* Fingerlings

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Key words
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ABSTRACT

A feeding trial of 70-days was conducted on *Catla catla* fingerlings (average weight 6.73±0.04). Total six experimental diets were formulated containing levels I (control), II, III, IV, V and VI. Diet I constituted fish oil (FO), while diets II, III, IV, V and VI were composed of palm oil, corn oil, sunflower oil, canola oil and mixture of all mentioned vegetable oils respectively. Triplicate tanks were used and every tank had 15 fingerlings. Results demonstrated that significant ($p<0.05$) improvement in growth performance i.e. weight gain % (WG %) (210.48), minimum feed conversion ratio (FCR) (1.11) and maximum specific growth rate (SGR) (1.61) of fingerlings were noted when fed with test diet-VI-based on plant mixture oil. When compared to a control diet (0%) and other oil based experimental diets, such results were significantly different. While minimum growth i.e. WG % (148.13), maximum FCR (1.43) and minimum SGR (1.29) was noted when fingerlings were fed with test diet-IV. In the current study, the best digestibility results of crude fat (CF) (81.25%), crude protein (CP) (69.39%), and gross energy (GE) (71.74) were seen in VI-level diet and these results were statistically ($p<0.05$) different from all other test diets. However, the test diet-IV had the least digestibility value of nutrients, including CF (73.55%), CP (49.17%) and GE (65.96%). It was concluded that *C. catla* fingerlings showed improvement in growth performance, nutrient digestibility and body composition when fed plant mixture oil based diet.

INTRODUCTION

Aquaculture relies highly on nutritionally balanced feed to fulfil the needs of aquatic species (Dawood, 2021). The aquaculture industry has notably expanded due to the increasing need for sustainable and profitable animal protein sources to feed the world's rapidly increasing population (FAO, 2020). According to predictions, world's population will reach 9 billion people in 2050. To meet the expanded population's need, about 50% additional food will be required (Diana *et al*., 2013). Increased demand for nutritional quality has resulted from rapid population increase in developing countries across the world (Abdulkadir *et al*., 2016). The fish nutritionists are primarily focused at forming diet that is of high quality as well as economical. Feed cost is predicted to account for 50 to 60% of total aquaculture expenses (Shahzad *et al*., 2018).

Fish oil (FO) has vital lipid sources because of its important fatty acid composition (Kok *et al*., 2020). Main source of lipids is FO and can be added in fish diets at higher levels. FO has large amount of polyunsaturated fatty acids (PUFA), particularly highly-unsaturated fatty acids (HUFA) so that's why it has been used as a primary source of lipids (Fukada *et al*., 2017). Furthermore, due to the high cost of these substances and the scarcity of resources, non-traditional substitutes of lipid sources are being used (Gasco *et al*., 2018). Plant oils are the greatest substitute for FO because of their high availability, rising production, and low cost (Turchini *et al*., 2009).

Lipid substances in aquaculture feeds should be healthy and accessible at reasonable levels (Alhazzaa *et al*., 2019). Vegetable oil (VOs) supply is becoming more consistent and costs are cheaper as compared to FO (Ayisi *et al*., 2019; Turchini *et al*., 2019). VOs are less susceptible to pollution and lipid peroxidation than FO (Larbi *et al*., 2018; Ayisi *et al*., 2019). For this approach, a variety of

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plant lipid resources, such as soybean, palm, sesame, linseed, olive, rapeseed, and sunflower oils, are commonly employed in the formulation of aquaculture feed (Cottrell *et al*., 2020). These oils increase fish growth performance and also helpful for the formulation of economical aquaculture feed.

Numerous researchers have found that substituting VOs with FO improves growth performance as in Nile tilapia (Apraku *et al*., 2017). At optimum temperature, the results showed that groups fed VOs based diets had significantly greater growth performance and feeding efficiency (p<0.05) than those provided FO-based diets (Dernekbasi *et al*., 2021). Fish that received 2% VOs based diet such as coconut oil based diet showed higher specific growth rate (SGR), feed intake, weight gain (WG) and final weight (FW) (Dawood, 2021). Growth performance and body composition were significantly improved when yellow drum fed with mixed oil based diet (Wabike *et al*., 2020).

C. catla is a top feeder and being reared with other carp species in Pakistan (Aslam *et al*., 2016). Because of its nutritional quality, high commercial value, fast growth, good taste and acclimatization to laboratory conditions, this carp species is being cultured at large scale (Milstein *et al*., 2002). Because of its size, excellent flavor, rich in protein content, omega-3 fatty acids, and lower triglycerides, it is the most frequently farmed freshwater fish among Indian major carps (IMCs) (Vanitha *et al*., 2015). This study is aimed at asses the effect of replacement of FO with various oils on growth performance, nutrient digestibility and body composition and of *C. catla* fingerlings. Thus, it will be economically important for feed manufacturers.

MATERIALS AND METHODS

Acclimatization of fish and trial conditions

Fingerlings were bought from local fish hatchery, Satiyana road, Faisalabad, Pakistan. They were placed in V-shaped tanks with water holding capacity of 70L (specifically designed to collect feces). Fingerlings were bathed in a salt solution before beginning the feeding trial to eliminate parasites from their skin (Rowland and Ingram, 1991). Then fingerlings were acclimatized to be familiar with lab conditions for two weeks. The basal diet was fed during acclimatization period to the fingerlings for apparent satiation. All the experimental tanks were supplied with aeration by capillary system for 24 h daily.

Experimental layout

Different types of oils were used to make fish feed. FO was utilized to formulate the control diet whereas five test diets were formulated by using different vegetable oils e.g. corn oil, palm oil, canola oil, sunflower oil and one diet was prepared by mixing all of the selected oils. The feed was given to juveniles at 5% of their live wet weight every day. The trial lasted for total of 70 days.

Formulation of pellets

For the preparation of experimental diets**,** ingredients were bought from a commercial feed mill and tested chemically. For 10 min, all of the diet ingredients (Table I) were mixed using mixer. Different oils were added slowly to the diet while mixing the ingredients. Chromium oxide (1%) was utilized as an inert marker. Water was added at a rate of 10-15% to prepare suitable dough (Lovell, 1989). Then this dough was processed using pelleting apparatus to make feed pellets. All experimental diets were processed in the same manner in the pelleting machine to formulate six different oil based diets. The diet was completely dried in the shade and kept at 4°C until it was utilized.

Feeding protocol and sample collection

Different dietary oil based diets were given to fingerlings. Two hour after feeding practice, the tanks valves were opened and the uneaten feed was removed from each tank. For removal of feed residues, the tanks were washed thoroughly and filled with water. Fingerlings were then put back into tanks and fecal matter was collected from each tank through fecal collecting tube, once the valves were opened after a 2 h interval. Precautions were taken during collecting thin fecal fibers to reduce nutrient loss. Each treatment's feces were dehydrated, ground, and preserved for lab testing.

Chemical analysis of feed, feces and fish muscle

Samples of diet ingredients, feces and fingerling muscles were assembled by pestle and mortar, separately. The analysis was done using standard protocols (AOAC, 2005). Assessment of moisture was done through oven drying for 12 h at 105°C and calculation of crude protein (N×6.25) was done by using micro Kjeldahl equipment. The Soxtec HT2 1045 system extracted crude fat using the petroleum ether extraction technique. Ash was heated in an electric furnace for 12 h at 650°C to create a constant weight (Eyela-TMF 3100). Gross energy calculations were done using an oxygen bomb calorimeter.

Growth studies

For assessment of growth, fish from each tank was weighed at the end and start of trial. Growth performance of juveniles in term of WG %, FCR and SGR was estimated by using these standard formulae:

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

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

$$\text{SGR} = \frac{[\ln \text{(final weight)} - \ln \text{(initial weight)}]}{\text{Duration of trial in day}} \times 100$$

Digestibility determination

The following formula was used to figure out the apparent nutrient digestibility coefficients (ADC) for the experimental diets:

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

Chromic oxide estimation

Following oxidation with molybdate solution, the amount of chromic oxide in the diet was measured using a UV-VIS 2001 Spectrophotometer with an absorbance setting of 370 nm (Divakaran *et al*., 2002).

Statistical analysis

Fingerlings nutrient digestibility, growth performance and body composition were compared using one-way ANOVA (Steel and Torrie, 1996). Tukey's Honest Significant Difference Test was used to evaluate the differences between means, and p0.05 was assumed to be statistically significant (Snedecor and Cochran, 1991). Computer application Co-Stat was applied for statistical analysis.

RESULTS

Growth performance

Findings of growth of fingerlings given various dietary oil based diets are provided in Table II. Initial weight of fingerlings was nearly identical across all treatments, however weight of fingerlings assessed after the trial was considerably different from others. The maximum values of weight gain (14.20±0.03g), weight gain% (210.48±0.99%) of *C. catla* fingerlings were noted in fish fed at VI-level of different plant mixture oil based diet followed by fish (12.48±0.04g; 185.16±0.75%) fed at V-level of palm oil based diet. When compared to the control diet (11.33 0.05 g; 168.35 1.08%) and other test diets, these results were shown to be statistically (p 0.05) different. The minimum weight gain (9.99±0.08 g), weight gain % (148.13±1.99%) were recorded at IV level of canola oil based diet. FCR of fingerlings was also significantly increased. The lowest value of FCR was noted (1.11±0.02) at test diet VI while highest value of FCR was observed (1.43±0.02) at test diet IV. In case of SGR (%) results showed that the VI-level of plant mixture oil based diet had the maximum SGR value (1.61±0.004%) of fish. Minimum value of SGR (1.29±0.01%) was indicated in fish fed at the IV-level of canola oil based-diet. Overall, it was assessed that fingerlings showed improvement in growth with respect to WG, WG (%) FCR and SGR (%) at VI level of plant mixture oil based diet.

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

Ingredients	Test diet-I (Control)	Test diet-II	Test diet-III	Test diet-IV	Test diet-V	Test diet-VI
Canola meal	32	32	32	32	32	32
Fish meal	20	20	20	20	20	20
Corn gluten meal (60%)	15	15	15	15	15	15
Wheat flour	16	16	16	16	16	16
Rice polish	7	7	7	7	7	7
Fish oil	6					
Canola oil		6				
Sunflower oil			6			
Corn oil b				6		
Palm oil					6	
Oil mixture						6
Vitamin premix*	1	1	1	1	1	1
Mineral premix**	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1

Table II. Growth performance of *C. catla* fingerlings fed different oil based diets.

Growth parameters	Test diet-I (Control diet)+ fish oil	Test diet-II+ sunflower oil	Test diet-III +corn oil	Test diet-IV+ canola oil	Test diet-V+ palm oil	Test diet-VI+ plant mixture oil
IW (g)	6.73±.02^{a}	6.73±.01^{a}	6.74±.03^{a}	6.75±.04^{a}	6.74±.01^{a}	6.75±.03^{a}
FW (g)	18.06±0.04^{c}	17.95±0.04^{d}	16.89±0.03^{e}	16.74±0.04^{f}	19.22±0.03^{b}	20.95±0.04^{a}
WG (g)	11.33±0.05^{c}	11.22±0.03^{c}	10.14±0.04^{d}	9.99±0.08^{e}	12.48±0.04^{b}	14.20±0.03^{a}
WG (%)	168.35±1.08^{c}	166.67±0.35^{c}	150.42±0.94^{d}	148.13±1.99^{d}	185.16±0.75^{b}	210.48±0.99^{a}
WG (fish/day)g	1.41±0.002^{c}	1.40±0.001^{c}	1.31±0.005^{d}	1.29±0.011^{d}	1.49±0.003^{b}	1.61±0.004^{a}
FCR	1.31±0.02^{a}	1.35±0.09^{ab}	1.42±0.02^{bc}	1.43±0.02^{c}	1.22±0.01^{a}	1.11±0.02^{a}
SGR	1.41±0.002^{c}	1.40±0.001^{c}	1.31±0.005^{d}	1.29±0.011^{d}	1.49±0.003^{b}	1.61±0.004^{a}

At p<0.05, means with various superscripts are statistically different. The data is based on three replicates. IW, initial weight; FW, final weight; WG, weight gain; FCR, feed conversion ratio.

Table III. Analyzed compositions of CP, CF and GE in the feed and feces, apparent nutrient digestibility of *C. catla* fingerlings fed different oil based diets.

Parameters	Test diet-I (Control diet)+ fish oil	Test diet-II+ sunflower oil	Test diet-III +corn oil	Test diet-IV+ canola oil	Test diet-V+ palm oil	Test diet-VI+ plant mixture oil
Analysis of feed						
CP (%)	30.79±0.04^{a}	30.82±0.10^{a}	30.73±0.07^{a}	30.74±0.06^{a}	30.62±0.11^{a}	30.58±0.03^{a}
CF (%)	7.31±0.015^{a}	7.27±0.01^{a}	7.28±0.03^{a}	7.31±0.02^{a}	7.29±0.03^{a}	7.30±0.03^{a}
GE(kcalg^{-1})	3.44±0.01^{a}	3.43±0.02^{a}	3.42±0.01^{a}	3.41±0.01^{a}	3.45±0.02^{a}	3.46±0.01^{a}
Analysis of feces						
CP (%)	12.24±0.45d	13.07±0.06^{c}	14.23±0.07^{b}	15.22±0.07^{a}	11.28±0.05^{e}	10.18±0.04^{f}
CF (%)	1.79±0.035^{c}	1.86±0.036^{b}	1.89±0.03^{b}	1.98±0.005^{a}	1.70±0.01^{d}	1.58±0.02^{e}
GE(kcalg^{-1})	1.10±0.03^{a}	1.11±0.02^{a}	1.13±0.02^{a}	1.13±0.01^{a}	1.08±0.03^{a}	1.06±0.01^{a}
Apparent nutrient digestibility						
CP (%)	60.98±1.19^{bc}	54.38±5.52^{cd}	53.69±1.26^{d}	49.17±0.14^{d}	64.78±0.20^{ab}	69.39±0.08^{a}
CF (%)	77.21±0.23^{c}	75.80±1.00^{d}	75.50±1.00^{d}	73.55±0.14^{e}	78.92±0.24^{b}	81.25±0.31^{a}
GE(kcalg^{-1})	68.52±1.35^{c}	67.63±0.40^{d}	66.96±0.28^{e}	65.96±0.51^{f}	70.00±0.66^{b}	71.74±0.22^{a}

At p<0.05, means with various superscripts are statistically different. The data is based on three replicates. CP, crude protein; CF, crude fat; GE, gross energy.

Nutrient digestibility calculation

Table III show the examined nutrient content of the diet including crude protein (CP), crude fat (CF), and gross energy (GE) and excretions of fingerlings fed on various oil based diets. In comparison to the digestibility values of the control diet and all other diets, it was found that the diet based on plant combination oil had the greatest (69.39%) and significantly different (p0.05) crude protein digestibility. The results demonstrated a dramatic increase in nutrient digestibility up to VI-level when it achieved its highest, with a mixture of various plant oils in the diet, whereas subsequent increased in plant mixture oil level resulted in lower nutritional digestibility. The digestibility findings at VI level were found to be the greatest and statistically (p0.05) different from all other test diets for CF (81.25%), CP (69.39%), and GE (71.74) (Table III). While, minimum digestibility results of nutrients such as CF (73.55%), CP (49.17%) and GE (65.96%) were obtained at diet IV. These results showed that a plant combination oil based diet gave fingerlings the best nutrient digestion and caused the least quantity of nutrients to be released into the environment.

Body composition

Results of body composition of fingerlings in term of CP, CF, ash and moisture are represented in the Table IV. The best value of CP content (20.25±0.07 %) and lowest value of fat (5.34±0.01 %) collected in the body of fingerlings were obtained when fingerlings fed with plant mixture oil based diet while minimum value of CP content

Table IV. Body composition of *C. catla* fingerlings fed with different oil based diets.

Body composition parameters	Test diet-I (Control diet)+ fish oil	Test diet-II+ sunflower oil	Test diet-III +corn oil	Test diet-IV+ canola oil	Test diet-V+ palm oil	Test diet-VI+ plant mixture oil
Protein (%)	18.68±0.17^{c}	18.05±0.15^{d}	17.54±0.08^{e}	16.77±0.21^{f}	19.33±0.10^{b}	20.25±0.07^{a}
Fat (%)	5.81±0.08^{d}	5.97±0.15^{c}	6.03±0.13^{b}	6.15±0.15^{a}	5.63±0.03^{e}	5.34±0.01^{f}
Ash (%)	3.16±0.02^{bcd}	3.24±0.05^{bc}	3.29±0.02^{b}	3.47±0.02^{a}	3.09±0.01^{c}	3.02±0.01^{cd}
Moisture (%)	72.35±0.19^{d}	72.74±0.12^{c}	73.14±0.12^{b}	73.61±0.18^{a}	71.95±0.11^{e}	71.39±0.08^{f}

At p<0.05, means with various superscripts are statistically different. The data is based on three replicates.

(16.77±0.21 %) and maximum value of CF (6.15±0.15 %) collected in the fingerlings body were observed when fingerlings fed with canola oil based diet. These values showed that there was significant difference (p<0.05) among them. Highest values of ash content (3.47±0.02 %) and moisture (73.61±0.18 %) in the fingerlings body were recorded when fingerlings fed with canola oil based diet while lowest values of ash and moisture contents (3.02±0.01% and 71.39±0.08%, respectively) in the body of fingerlings were observed when fingerlings fed with plant mixture oil based diet.

DISCUSSION

Current study found that a mixture of VOs can be used to substitute FO in the feed of fingerlings without compromising their growth, nutrient digestibility and body composition. Lipids in aqua-feeds must be safe and readily available in moderate proportions (Alhazzaa *et al*., 2019).

It is shown that replacement of FO with various sources of oils significantly influenced the growth performance of fingerlings. While opposed to a FO-based diet, a plant mixture oil based diet improved the growth performance of fish. Our results are similar with (Dernekbasi *et al*., 2021) who suggested that plant mixture oil based diets enhanced the growth performance of fish without adverse effect on environment. Shahrooz *et al*. (2018) also stated that fish, when fed on plant mixture oil based diet resulted highest feed intake and maximum growth rate as compare to the fish that fed on FO based diet. Similar to our results, FW, WG, SGR and PER were indicated significant improvement in yellow drum when fish fed mixed oil based diet (Wabike *et al*., 2020). The growth results of the current study did not match those of Mu *et al*. (2020), who discovered a significant decline in growth and feed intake in large yellow croaker on a diet in which plant oils completely replaced FO. In contrast to the findings of this study, Peng *et al*. (2016) found that juvenile Nile tilapia fed a VOs-based diet, such as soybean oil, had lower WG, PER, FCR, and SGR than fish fed a FO-based diet.

It was also indicated that highest nutrient digestibility in terms of CP, CF and GE at plant mixture oil followed by palm oil based diets. Duan *et al*. (2014) observed highest protein in fish that fed on partial or total replacement of FO with palm oil and plant mixture oil based diets, respectively. The findings of this study revealed that a minimum number of nutrients such as CP, CF, and apparent GE were eliminated through feces at levels containing plant mixture oil followed by palm oil. According to Larbi *et al*. (2018) fingerlings fed a palm oil based diet had no deleterious impacts on nutrient discharge, while a palm oil based diet had negative effects on innate immunological parameters and antioxidant activity. Findings of current research work revealed that GE digestibility was maximum at the level of plant mixture oil and that it differed considerably from results obtained in other test diets. When comparing the apparent digestibility coefficient values of control diet and different levels of plant oil based diets, the findings revealed that the plant mixture oil level offered the highest apparent digestibility coefficient value.

Body composition of fingerlings was improved when fingerlings were fed with plant mixture oil based diet. Our results also matched with the Milián-Sorribes *et al*. (2021) who found that vegetable mixture oil improved the protein content in *Seriola dumerili*. Results of Wabike *et al*. (2020) also similar with the current study, who found that CF, moisture and ash contents significantly higher in yellow drum when fish fed with mixed oil based diet. Also similar to our outcomes, Ayisi *et al*. (2019) stated that whole body protein of Nile tilapia improved when fish fed with VOs based diets. According to the findings of this study, better growth has been directly corresponded to the high content of protein for these diets used by these authors which showed that fish growth is positively affected by protein accumulation in skeletal muscles. In opposite to our findings, Erondu *et al*. (2021) concluded that the protein content in Nile tilapia was lowest when fish given with VOs based diet. Some results of our study were same as to Milián-Sorribes *et al*. (2021) who described that fat content was lowered when *S. dumerili* fed with vegetable mixture oil based diet. Similar to our findings

Ayisi *et al*. (2017) also found in his study that whole body fat composition of fish was lowered. Dissimilar to current research findings, Erondu *et al*. (2021) concluded that fish fed with VOs based diets increased the whole body fat content of fish. The results found in this work also mismatched with the findings of (Sankian *et al*., 2019; El-Asely *et al*., 2020) who suggested that there was more improvement in the body lipids of several fish species including Nile tilapia when fishes fed with VOs based diet than FO based diet. The contradiction between these authors and current study's results might be associated with changes in the preparation of diet, fish species, size of fish, lipid contents in the diet, and different environment as well as experimental conditions.

CONCLUSION

The ideal amount of a distinct plant combination oil based diet for increasing growth metrics, body composition and nutrient digestibility in *C. catla* fingerlings was identified in this study. It has also been recommended that substituting plant mixture oil for FO in the production of cost-effective and environmentally friendly aquaculture feed for *C. catla* fingerlings has been highly beneficial.

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IRB approval

The experiment was carried out in line with the institutional review board guidlines of Government College University, Faisalabad.

Ethical statement

Ethical approval is not required for this study.

Statement of conflict of interest

The authors have declared no conflict of interest.

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