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IMPACT OF ORANGE (*CITRUS SINENSIS)* PEEL MEAL AS FEED ADDITIVE IN THE DIETS FOR MONO-SEX NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FRIES

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ABSTRACT: This experiment was conducted to evaluate the effect of orange peel meal as feed additive in the diets for mono-sex Nile tilapia fries on growth performance, body composition, carotenoids and the histology of liver and intestine. Two hundred and twenty-five fries at average weight of 0.52 ± 0.01 g/fry were randomly distributed in 15 hapa (1 m × 1 m × 0.5 m) placed in 5 concrete tanks measured (2 m × 2 m × 1 m) in three replicates for each treatment. Fish from each hapa were counted and weighed each two weeks to evaluate the growth and readjust the feeding rate. 10% of fish body weight were fed to every three groups of the experimental fish with one of the experimental diets and decreased gradually to 8 and 6% by the end of the feeding trial. The experimental diets were formulated to be similar in crude protein (33.6 ± 0.1%) and crude lipids (7.1 ± 0.1%). The formulated diets were performed (g/kg diet) as, 0 (CTRL), 10 (OP_{10}), 30 (OP_{30}), 50 (OP_{50}) and 70 (OP_{70}) orange peel meal. Fish were fed the experimental diets for 6 days per week, three times per day (9.00 a.m., 11 a. m. and 1 p.m.) for 98 days. There were no significant differences ($P>0.05$) among all treatments in final body weight, gain and average daily gain except fish fed OP_{10} diet were significantly differed and gained the highest values. In addition, no significant differences were observed in specific growth rate (SGR) among all treatments and the highest value was found in fish fed OP_{30} diet. However, there were significant differences ($P<0.05$) among all treatments in feed consumption and fish fed OP_{10} diet consumed more feed than other groups and was significantly higher. The best FCR was obtained in fish fed OP_{70} and OP_{30} diets, respectively. Slight increase in PER was observed in fish fed OP_{70} followed by fish fed OP_{30} diets. No significant differences ($P > 0.05$) were found in the survival rate among all treatments. Fish fed OP_{70} and OP_{50} diets gained the highest protein content and significantly differed among other treatments. Ether extract content of fish fed CTRL, OP_{50} and OP_{30} diets were the highest, and significantly differed with the other treatments. Also, the carotenoids content significantly differed ($P<0.05$) among all treatments and the highest value was found in fish fed OP_{70} diet. It was increased by increasing the level of orange peel meal in the diets. However, no abnormalities were observed in the examined liver and intestine related to the addition of orange peel meal at different levels in the experimental diets. It can be concluded that orange peel meal could be added to diets for mono-sex Nile tilapia at 10 g/kg diet without any adverse effects.

Key words: Orange peel, Nile tilapia mono-sex, performance, carotenoids, histology.

INTRODUCTION

Over the past few decades, the aquafeed business has concentrated on creating functional diets that not only seek to enhance fish development, but also seek to improve fish overall health. Fruits, herbs, and their by-products have all been addressed as promising feed additives. This is because they have a broad range of biological activity (Vicente *et al*., 2019) also; they have no detrimental effects on fish, human health, or the environment (Baba *et al*., 2016).

A significant portion of citrus peels are not processed in Egypt and other Mediterranean nations, hence some steps were made formulated for use as animal nutrition in these by-products (Farhat *et al*., 2011). Citrus by-products are regarded as simple, accessible, and inexpensive

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feed dietary supplements that can offer a productive, affordable, and environmentally friendly platform for the production of unique nutritional resources (Rafiq *et al.,* 2016).

One of the most popular fruits in the world is the sweet orange, *Citrus sinensis*. It constitutes 50 % of the raw fruit and itis used to produce juice and jam, leaving large volumes of peels, seeds, and pulps (Anwar *et al*., 2008). According to Genovese *et al*. (2014), oranges and their trash are significant sources of phytochemicals that can defend humans, animals, and possibly even fish by supplying a plentiful supply of vitamin C, folic acid, potassium, and pectin. Compared to its juice and pulp, orange peel has a higher concentration of ascorbic acid and more active ingredients (Guimaraes *et al*., 2009) and it is a significant source of phenolic chemicals and fiber.

Fish are not able to biosynthesize carotenoids de novo; consequently, the required carotenoids must be added to fish diets (Ha *et al*., 1993). Orange peel is rich in bioactive compounds especially carotenoids. It has been revealed that the inclusion of 2 % of orange peel powder in the diets for Nile tilapia improved the digestion and nutrient absorption (Salem and Abdel-Ghany, 2018). Moreover, it has been indicated that orange peel had a positive effect on feed utilization, enzyme activities, immunoglobulin (IgG) and enhanced the immune response of mono-sex Nile tilapia fed diets supplemented with different forms of orange peel powder and oil and also, improved plasma total protein and liver health (Attalla *et al*., 2021).

Thus, the objective of this study was to assess the effects of dietary orange peel meal as a feed additive on growth performance, feed utilization, body composition and histology of liver and intestine of mono-sex Nile tilapia (*Oreochromis niloticus*) fries.

MATERIALS AND METHODS

The present study was carried out at the fish laboratory of Poultry and Fish production Department in the Faculty of Agriculture, Menoufia University during the period of 30 th June to 4 th October 2021.

1. Fish, facility and feeding trial

Mono-sex Nile tilapia, *Oreochromis niloticus* fries averaged (0.52 ± 0.01 g) were obtained from the local hatchery at Kafer El-Sheck, Egypt and acclimated for one week on the laboratory conditions. Two hundred and twenty-five fries were randomly distributed in 15 hapa (1 m × 1 m × 0.5 m) placed in 5 concrete ponds measured (2 m × 2 m × 1 m), in three replicates for each treatment. All procedures and handling of animals were conducted in compliance with the guidelines of the Institutional Laboratory Animal Care and Use Committee, Menoufia University, Egypt. Fish from each hapa were counted and weighed at regular intervals (two weeks) to evaluate the growth and readjust the feeding rate. Fish were fed 10% of body weight with one of the experimental diets and decreased gradually to 8 and 6% by the end of the feeding trial. Fish were fed the experimental diets for 6 days / week, three times / day (9.00 a.m., 11 a. m. and 1 p.m.) for 98 days.

2. Orange peel meal preparation

Sweet orange fruits, *Citrus sinensis* were purchased from the local market, washed and peeled. The peels were dried at 50 °C for 48 h then grounded and sieved through 50 mm sieve, packaged in plastic bags and stored in the fridge until use as feed additive in the diets. Chemical composition of dried orange peel meal is presented in Table (1).

3. Experimental diets

Five experimental diets were formulated by including orange peel meal at different levels. All diets were iso-nitrogenous with 33.6 ± 0.1% crude protein and 7.1 ± 0.1% crude lipids and were offered to the experimental fish in powdered form as follows: CTRL (0 g/kg diet orange peel meal), OP_{10} (10 g/kg diet orange peel meal), OP_{30} (30 g/kg diet orange peel meal), OP_{50} (50 g/kg diet orange peel meal) and OP_{70} (70 g/kg diet orange peel meal), respectively. The experimental diets were prepared by thoroughly mixing ingredients as presented in

Table (2). All ingredients were first ground to small particle size, then, water was added to the ingredients of each diet and the mixture was undergone the kitchen machine to have pellets. Then dried, preserved and kept in -20°C freezer until use. Following, the feed pellets were ground by the kitchen machine to get powder form. The proximate composition of the experimental diets was determined as stated by AOAC (2012).

Table (1). Chemical composition of dried orange peel.

Chemical parameter	**(%)**
Moisture	83.5
Protein	7.94
Crude fiber	40.75
Ash	3.01
Crude fat	0.95
NFE	47.35

According to Attalla *et al*. (2021).

Table (2). The experimental diets composition (g/kg) and proximate analysis (%).

Ingredients	**Dietary groups (g/kg)[4]**				
	CTRL	**OP_{10}**	**OP_{30}**	**OP_{50}**	**OP_{70}**
Fish meal (65 %)	100	100	100	100	100
Gluten (63.1 %)	100	100	100	100	100
Soybean meal (44 %)	400	400	400	400	400
Yellow corn (7.5 %)	150	150	150	150	150
Wheat bran (11.3 %)	180	170	150	130	110
Soybean oil	30	30	30	30	30
Mono-calcium-Phosphate	20	20	20	20	20
Premix[1]	20	20	20	20	20
Orange peel	0	10	30	50	70
Total	1000	1000	1000	1000	1000
Proximate analysis (%)					
Dry matter	90.27	90.29	90.32	90.35	90.39
Protein	33.56	33.52	33.45	33.39	33.32
Lipid	7.1	6.98	7.08	7.18	7.28
Ash	7.37	7.38	7.42	7.47	7.52
Fiber	3.22	3.55	4.21	4.88	5.54
NFE[2]	48.75	48.57	47.84	47.08	46.34
GE[3] (kcal/100 g diet)	462.43	460.31	457.78	455.18	452.61

[1]Premix: HI-MIX®AQUA (Fish) each one kilogram (1 kg) contains; vitamin A, 4,000,000 International Unit (IU); vitamin D_3, 8,00,000 IU; vitamin E, 40, 000 IU; vitamin K_3, 1,600 mg; vitamin B_1, 4,000 mg; vitamin B_2, 3,000 mg; vitamin B_6, 3,800 mg; vitamin B_{12}, 3 mg; Nicotinic acid 18000 mg; Pantothenic acid, 8000 mg; Folic acid, 800 mg; Biotin, 100 mg; Choline chloride 120,000 mg; Iron, 8000 mg; Copper, 800 mg; Manganese, 6000 mg; Zinc, 20,000 mg; Iodine, 400 mg; Selenium, 40 mg; Vitamin C (coated), 60,000 mg; Inositol, 10,000 mg; Cobalt, 150 mg; Lysine, 10,000 mg; Methionine, 10,000 mg; Antioxidant, 25,000 mg.

[2]Nitrogen Free Extract (NFE) = 100 – (%Protein + %Fat + %Fiber + %Ash).

[3]GE= Gross energy based on protein (5.65 kcal/g), Fat (9.45 kcal/g), and carbohydrate (4.22 kcal/g) according to (NRC, 2011).

[4]CTRL= control; OP_{10}, OP_{30}, OP_{50} and OP_{70} diets with different levels of orange peel meal 10, 30, 50 and 70 (g/kg).

4. Sample collection and analysis

4.1. Chemical composition of whole-body fish

Samples of the experimental fish by the end of the feeding trials were taken to evaluate the moisture, protein, lipids, and ash contents by using the standard methods (AOAC, 2012). Six fish from each treatment were sampled for analysis. Blended samples were kept at −18 °C and used exclusively for chemical examination. The dry matter, crude protein and crude lipids were analyzed after dryness in drying oven (105°C for 5 h), by micro kjeldahl (N × 6.25), ether extraction (by soxhlet method) and ash content was determined by combusting dry samples in a muffle furnace at 550 °C for 6 h.

4.2. Carotenoids analysis

Fresh fillets were frozen at −18 °C and lyophilized for 48 h. The meat powder used to evaluate the carotenoid concentration was obtained by grinding the lyophilized samples in a mill (MA 630, Marconi, Brazil). Carotenoid content of fillets was extracted according to Teimouri *et al*. (2013). The solutions' absorptions were read in triplicate on UV-Vis spectrophotometer (Genesys 10-S, Thermo Fisher Scientific, USA) at wave length of 450 nm. Calibration curve was obtained using β-carotene (93%, Sigma-Aldrich, Brazil) as standard. Results were expressed as mg of β-carotene per 100 g of sample.

β -carotene analysis: One gram of ground fish meat was put in a test tube. Then, 10 ml of the mixed solvents of acetone and hexane in the ratio 4:6 (volume / volume) were added and mixed well using a spatula. Two other concentrations of extracted matter were made in the same way by adding 14 ml and 18 ml, respectively, of mixed solvent to 1 g of minced arils. The dilutions were selected to be just below the capability of the absorption range of the UV e visible spectrophotometer (Thermo Spectronic, GENESYS 10 UV e Vis; USA). The extracts were homogenized using a homogenizer (Polytron®, PT-MR 2100; Switzerland) at 15,000 rpm for 1 min. Then, the light absorption values (A) at 453, 505, 663 and 645 nm wave length were recorded for the determination of the β-carotene contents in each sample. The obtained values then were calculated further to be based on μg β-carotene g^{-1} sample.

$$\beta - \text{carotene (mg/100 ml)} = 0.216\ A_{663} - 1.22\ A_{645} - 0.304\ A_{505} + 0.452A_{453}$$

4.3. Growth performance parameters

The count and weight of the experimental fish were recorded every 2 weeks.

4.3.1. Weight gain (g/fish)

Weight gain was determined as following:

$$\text{Weight gain (g/fish)} = \text{Final body weight (g)} - \text{Initial body weight (g).}$$

4.3.2. Average daily gain. Was determined as

Average daily gain = weight gain (g) /days.

4.3.3. Specific growth rate

Specific growth rate (SGR %/ day) was calculated using the following equation:

$$\text{SGR (\%/ day)} = 100 \times (\text{Ln FBW} - \text{Ln IBW}) / \text{experimental period}$$

Where, FBW is the final fish body weight at the end of the experiment; IBW is the initial fish body weight at the start of the experiment; Ln is the natural log.

4.3.4. Survival rate

Survival rate (%) was estimated using the following equation:

Survival rate (%) = 100 × (No. of survived fish at the end of the experiment ÷ No. of survived fish at the beginning of the experiment).

4.4. Feed utilization parameters.

4.4.1. Feed conversion ratio (FCR)

Feed conversion ratio (FCR) was calculated according to the following equation:

FCR= Feed consumed (g) during the experimental period ÷ weight gain during the experimental period (g)

4.4.2. Protein efficiency ratio (PER).

Protein efficiency ratio (PER) was calculated according to the following equation:

PER = Weight gain (g) ÷ Protein intake (g)

4.5. Histological examination

For histological examination, two fish of each hapa were sacrificed (n=6 per treatment) by ice slurry and preserved in 10% neutral buffered formalin (Thermo Fisher, Kalamazoo, MI). The next day, fish were then washed with water the next day several times and preserved in 70% ethyl alcohol for further processing. The intestine and liver from each fish were separately dissected. Tissues were routinely dehydrated in ethanol, equilibrated in xylene and embedded in paraffin according to standard histological techniques. All tissues were sectioned longitudinally. Sections were cut at 4 µ m, mounted on glass slides and stained routinely with hematoxylin and eosin (H&E) followed by clearing through xylene and cover slipped over permount medium.

4.6. Statistical analysis

The differences between experimental groups were examined by one-way ANOVA test using SPSS (2003), version 19. Before the ANOVA analysis, the percentage of specific growth rates were arcsine converted. Differences were considered significant at $P<0.05$. The differences among means were determined by Duncan ´s multiple range test (Duncan, 1955).

Results

Growth performance and feed utilization

Growth performance parameters of mono-sex Nile tilapia (*Oreochromis niloticus*) fed diets supplemented with different levels of orange peel meal are presented in Table (3). There were no significant differences ($P > 0.05$) among all dietary treatments in final body weight except fish fed diet (OP_{10}) supplemented with 10 g of orange peel meal /kg diet are significantly differed and gained the highest value (Fig. 1). The same trend was observed in weight gain (Fig. 2) and average daily gain. No significant differences ($P > 0.05$) were observed in the specific growth rate (SGR) among all treatments and the highest value was found in fish fed $OP_{30,}$ which supplemented with 30 g of orange peel meal / kg diet.

Table (3). Growth performance of Nile tilapia (*Oreochromis niloticus*) fries fed diets supplemented with different levels of orange peel meal for 14 weeks (means ± SE).

Parameters	Dietary groups (g/kg)*				
	CTRL	OP_{10}	OP_{30}	OP_{50}	OP_{70}
Initial body weight (IBW, g/ fish)	0.51 ± 0.02	0.49 ± 0.02	0.49 ± 0.02	0.58 ± 0.02	0.51± 0.02
Final body weight (FBW, g/ fish)	9.49^{b} ± 0.77	16.14^{a} ± 1.74	11.64^{b} ± 0.68	10.40^{b} ± 0.65	10.04^{b} ± 0.64
Weight gain (WG, g/ fish)	8.98^{b} ± 0.76	15.65^{a} ± 1.75	11.16^{b} ± 0.68	9.83^{b} ± 0.65	9.53^{b} ± 0.63
Average daily gain (ADG)	0.10^{b} ± 0.02	0.16^{a} ± 0.03	0.11^{b} ± 0.02	0.10^{b} ± 0.01	0.10^{b} ± 0.01
Specific growth rate (SGR)	2.98 ± 0.06	2.95 ± 0.01	3.24 ± 0.08	2.95 ± 0.01	3.04± 0.11
Total feed consumed (g/fish)	18.88^{bc}±0.39	30.85^{a} ±2.58	21.20^{b} ± 0.62	19.53^{bc} ±0.54	17.15^{c} ± 0.59
Feed conversion ratio (FCR)	2.10 ± 0.17	1.97 ± 0.09	1.90 ± 0.06	1.98 ± 0.12	1.80 ± 0.14
Protein efficiency ratio (PER %)	1.41 ± 0.14	1.51± 0.07	1.57 ± 0.06	1.50 ± 0.08	1.66 ± 0.15
Survival (%)	88.89 ± 5.87	88.89 ± 2.22	85.44± 3.44	88.89± 2.22	84.44 ± 8.01
EG[1] (Kcal)	22.34^{a} ± 2.14	16.19^{b} ± 0.89	13.24^{b} ± 0.85	12.22^{b} ±0.35	13.86^{b} ± 0.88
EU[2] (%)	11.47 ± 0.85	11.76 ± 0.54	10.57 ± 0.51	11.54 ± 0.30	11.44 ± 0.37

Means in the same row with different superscript letters are significantly different ($P \leq 0.05$).
*CTRL= control; OP_{10}, OP_{30}, OP_{50} and OP_{70} diets with different levels of orange peel meal 10, 30, 50 and 70 (g/kg).
[1]Energy gain (Kcal) = Et – E0
E0: energy content in whole fish at the start.
Et: energy content in whole fish at the end.
[2]Energy utilization (EU %) = 100 x (Et – E0)/ energy intake (kcal).

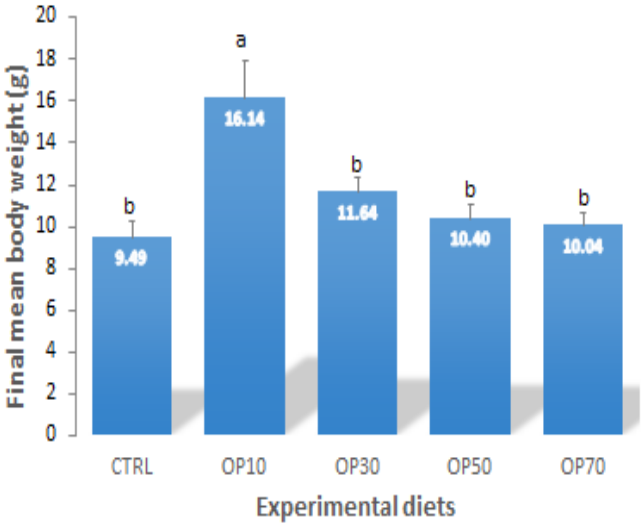

Figure (1): Final mean body weight (FBW) of Nile tilapia fries fed orange peel supplemented diets for 14 weeks. Different superscripts among columns indicate significant differences at *P* < 0.05.

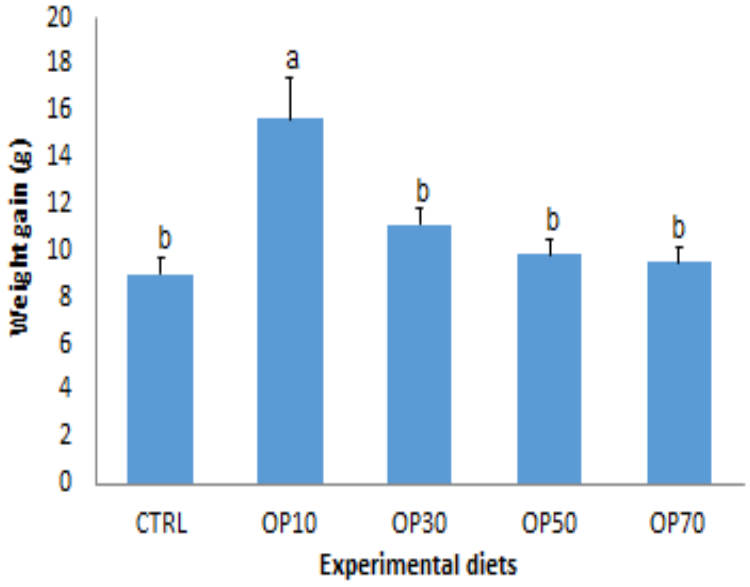

Figure (2): Weight gain (WG, g) of Nile tilapia fries fed orange peel supplemented diets at different levels for 14 weeks. Different superscripts among columns indicate significant differences at *P* < 0.05.

However, there were significant differences (*P<0.05*) among all treatments in feed consumption and fish fed diet OP_{10} consumed more feed than other groups and was significantly higher. The best FCR was obtained in fish fed OP_{70} and OP_{30} diets, respectively (Fig. 3). However, as feeding rate was re-adjusted for fish weight (the same % of biomass) fish fed OP_{70} diet had lower feed consumption than other groups (Table 3). No significant differences (*P>0.05*) were observed in protein efficiency ratio (PER) among all treatments. Slight increase in PER was observed in fish fed OP_{70} followed by fish fed OP_{30} diets. No significant differences in survival rate of fish fed the experimental diets. In terms of energy gain and energy utilization, fish fed CTRL diet significantly differed among all treatments and gained the highest value of energy gain compared to other treatments. However, no significant differences (*P > 0.05*) were observed in energy utilization among all treatments and the highest value was found in fish fed OP_{10} diet (Table 3).

Whole-body composition

Body composition and carotenoids content of Nile tilapia (*O. niloticus*) fed the experimental diets are presented in Table (4). No significant differences (P > 0.05) were observed in terms of dry matter among all treatments. Fish fed OP_{50} and OP_{70} diets gained the highest protein content and significantly differed among other treatments. Ether extract content of fish fed CTRL, OP_{50} and OP_{30} diets were the highest, and significantly differed with the other treatments. However, ash content of fish fed CTRL diet was the lowest and significantly differed (*P<0.05*) among all treatments and the highest value was found in fish fed OP_{70} diet. The carotenoids content significantly differed (*P<0.05*) among all treatments and the highest value was found in OP_{70} diet. It was increased by the increasing level of orange peel meal in the diets (Table 4).

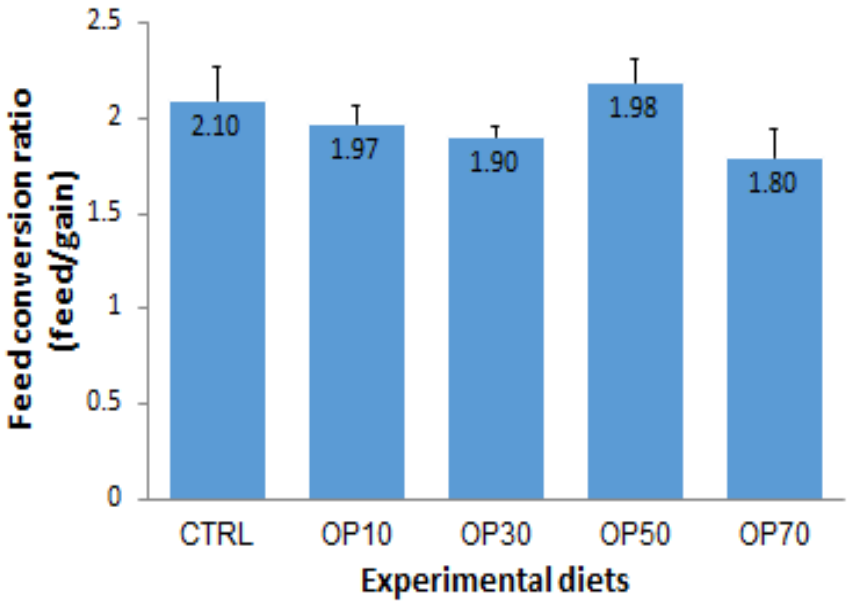

Figure (3): Feed conversion ratio of Nile tilapia fries fed orange peel supplemented diets at different levels for 14 weeks. Different superscripts among columns indicate significant differences at *P* < 0.05.

Table (4). Body composition (%) and carotenoids (µg/g tissue) of Nile tilapia (*O. niloticus*) fries fed diets supplemented with different levels of orange peel meal for 14 weeks (means ± SE).

*Treatments	Dry matter (%)	Protein (%)	Ether Extract (%)	Ash (%)	Carotenoids (µg/g tissue)
CTRL	25.28±0.28	58.29^{bc} ± 0.38	16.00^{a} ± 0.12	21.56^{b} ± 0.29	2.36^{c} ±0.01
OP_{10}	25.12±0.18	60.75^{ab} ± 0.30	13.76^{b} ±0.67	23.02^{ab} ±0.48	2.37^{c} ±0.01
OP_{30}	25.12±0.46	59.58^{b} ±0.69	15.92^{a} ± 0.17	23.05^{ab} ±0.42	2.37^{c} ±0.00
OP_{50}	24.99±0.06	61.30^{a} ± 0.30	15.93^{a} ± 0.06	23.68^{a} ±0.43	2.64^{b} ±0.06
OP_{70}	25.10±0.19	61.64^{a} ± 0.39	13.79^{b} ±0.29	24.38^{a} ± 0.68	2.86^{a} ±0.05

Means in the same column with different superscript letters are significantly different ($P \leq 0.05$).
*CTRL= control; OP_{10}, OP_{30}, OP_{50} and OP_{70} diets with different levels of orange peel meal 10, 30, 50 and 70 (g/kg).

Histological analysis

Mono-sex Nile tilapia fed CTRL (diet without orange peel) and other groups fed diets supplemented with different levels of orange peel (10, 30, 50 and 70 g/kg) revealed histological architecture of normal liver (hepatocytes, hepatic sinusoids, and pancreatic tissue) in all observed groups as shown in (Fig. 4 a, b, c, d, and e); respectively. Fish fed OP $_{70}$ diet (Fig. 4 e) had increased lipid droplets compared to other groups. It was shown that lipid droplets were increased by increasing the OP in the diets.

Also, CTRL group and other groups fed diet supplemented with different concentrations of orange peel meal (10, 30, 50 and 70 g/kg) revealed normal intestinal villi, and increased goblet cell in orange peel supplemented groups compared to CTRL group as shown in (Fig. 5 a, b, c, d, and e), respectively. The height villi were observed to be short in fish fed CTRL diet (Fig. 5 a) and increased as dietary OP levels increased, and the highest was shown in fish fed OP_{70} diet (Fig. 5 e). No histological differences in liver and/or intestine related to the inclusion of orange peel meal were found among all dietary treatments.

Discussion

Growth performance and feed utilization

The present study represents the role of orange (*Citrus sinensis*) peel meal as growth promoter for Nile tilapia fries. The obtained results showed that all fish fed OP supplemented diets had higher final weight, ADG and weight gain compared to the control. These results actually, in fact, agree with Salem and Abdel-Ghany (2018) who did research on the effects of dietary OP at various doses (0, 1, 2 and 4 g/kg diet) on growth performance and feed using Nile tilapia (*Oreochromis niloticus*) fingerlings.

Fish fed OP-based diets had the best growth performance and feed utilization efficiency, according to our obtained results. It has been provided by Acar *et al*. (2015) that sweet orange peel essential oil (OPEO) has the ability to boost growth. The growth of Mozambique tilapia (*Oreochromis mossambicus*) fed OPEO supplemented diets were substantially higher than that of fish fed the control diet. Similarly, flavonoids may enhance nutrient absorption, according to Azima *et al*. (2004). Additionally, the consumption of green flavonoid-rich tea (*Camellia sinensis*) leaves improved the Nile tilapia growth, FCR and protein content (Abdel-Tawwab *et al*., 2010).

Decreasing trend was found in growth performance by increasing the OP level in the diets (Table 3; Fig. 1, 2). This may be due to the level of OP in the diets. It was explained by Holst and Williamson (2008) who indicated that a bio-available dosage may be the reason for the different extents of effect in different individuals and the supreme benefits may be obtained at an optimal amount while, both extreme and/or lacking levels may cause harmful effects. Consequently, further studies are needed to determine the acceptable threshold of dietary OP.

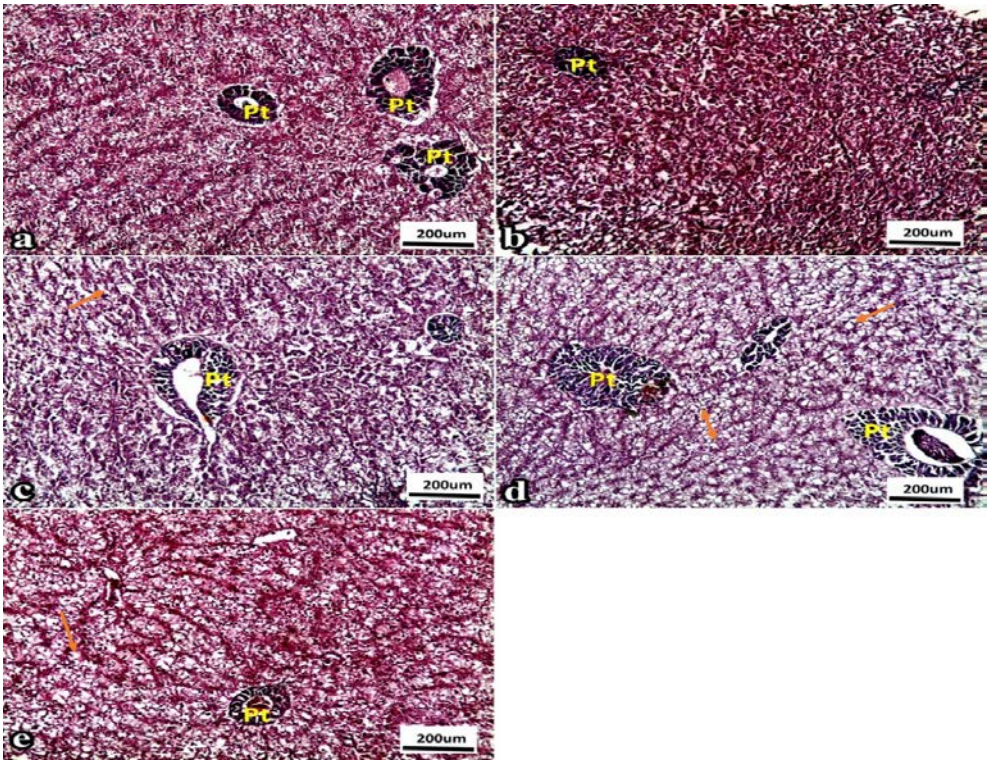

Figure (4): Liver of Nile Tilapia fries fed the experimental diets with different levels of orange peel meal (0, 10, 30, 50 and 70 g/kg diet) for 14 weeks; (a) CTRL fish fed diet without orange peel, (b) fish fed OP_{10}, (c) fish fed OP_{30}, (d) fish fed OP_{50} and (e) fish fed OP_{70}. Red arrows point to lipid droplets.

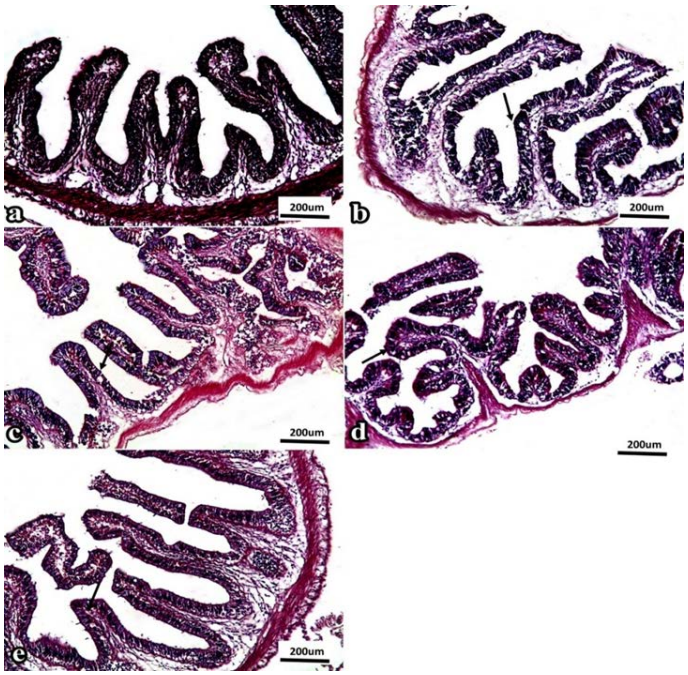

Figure (5): Intestine of Nile Tilapia fries fed the experimental diets with different levels of orange peel meal (0, 10, 30, 50 and 70 g/kg diet) for 14 weeks; (a) CTRL fish fed diet without orange peel, (b) fish fed OP_{10}, (c) fish fed OP_{30}, (d) fish fed OP_{50} and (e) fish fed OP_{70}.

The obtained results indicated that the inclusion of OP in the diets improved feed utilization. Feed conversion ratio was improved by the inclusion of OP in the diets and the best value (1.80) was observed in fish fed OP_{70} compared to the control (Table 3; Fig. 3). These findings are in line with Attalla *et al*. (2021). Furthermore, it was recorded that, orange peel powder added at 2% in the diets for tilapia improved the digestion and nutrient absorption (Salem and Abdel-Ghany, 2018). The same results were found by Gültepe (2020) who recorded the improvement of growth performance of rainbow trout fed diets supplemented with orange peel extracted oil.

Body composition and carotenoids

Significant differences were observed in protein, lipid and ash content among all treatments. The highest protein and ash contents were found in fish fed OP_{70} (Table 4). Fish fed CTRL diet gained the highest lipid and the lowest ash values. This may be due to the mineral content in orange peel powder which expected to increase the mineralization in fish muscles (Nwanna *et al*., 2011). These findings are agreed with Attalla *et al*. (2021).

Total carotenoids content was significantly increased as the level of OP increased in the diets. These results are in the line with Salem *et al*., (2019) when fed Gilthead Sea bream larvae diets supplemented with 0, 1, 3 and 5 g / kg OP for 60 days. Carotenoids, a crucial component of orange peel, really have the ability to scavenge peroxyl radicals, protecting cellular membranes and lipoproteins from reactive oxygen species (ROS) (Rodrigo *et al*., 2003, 2004; Stahl and Sies, 2003).

Histology

Regarding to the histological examination of liver and intestine tissues of Nile tilapia fries after feeding OP supplemented diets for 98 days (Fig. 4 and 5, respectively) showed no alterations in fish liver structure and/or intestine were discovered during the histological investigation, demonstrating the biosafety of dietary OP on fish. The lipid droplets in fish liver were increased as OP increase in the diets and the highest was observed in fish fed OP_{70} diet (Fig. 4 e). The same results were reported by Salem *et al*., (2019) when fed Gilthead Sea bream OP supplemented diets at 0, 1, 3 and 5 g/kg diet for 60 days. However, the height of the intestinal villi were shown to increase as dietary OP increased and the highest was shown in fish fed OP_{70} diet (Fig. 5 e), it was detected that the intestinal villi were extremely developed to size that possibly preventing the movement of feed from passing through it. This may explain the less feed consumed by this group (Table 4). These results are in step with Salem and Abdel-Ghany (2018). But these results are at odds with Chung *et al*., (2021) when Nile tilapia (*Oreochromis niloticus*) juveniles fed diets with essential oil from ginger (EOZO) supplementation and found liver dysfunction in fish fed EOZO above 1 ml/ kg diet and the increase of EOZO added in the diets increased hepatocyte numbers, hepatocyte area and perimeter, also reduced intestinal villus width and height.

Conclusion

According to the findings, orange (*Citrus sinensis*) peel meal as feed additive mostly in diets for Nile tilapia fries has a positive impact on fish growth, feed utilization, body composition, as well as the histology of liver and intestine. As a result, orange peel meal could be added in the diet at a rate of 10 g/kg diet, with no detrimental consequences on fish performance. Further studies are necessary to investigate the effects of orange peel meal on the immune parameters of fish and the microbial communities in fish gut to improve the quality of fish health.

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تأثير مسحوق قشر البرتقال كإضافة غذائية في علائق زريعة أسماك البلطي النيلي وحيد الجنس

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الملخص العربي

أجريت هذه الدراسة لتقييم تأثير مسحوق قشر البرتقال كإضافة غذائية في علائق زريعة أسماك البلطي النيلي وحيد الجنس علي النمو وتركيب الجسم والكاروتينات وهستولوجي الكبد والأمعاء. تم توزيع عدد ٢٢٥ زريعة أسماك البلطي النيلي وحيد الجنس بمتوسط وزن (٠,٥٢±٠,٠١ جم) عشوائياً في ١٥ هابة (١ ×١ × ٠,٥ متر^{٣}) موضوعة في ٥ أحواض أسمنتية (١ × ٢ ×٢ متر^{٣}) في ثلاث مكررات لكل معاملة. تم عدّ ووزن الأسماك كل أسبوعين لتقييم النمو وتعديل معدل التغذية. تم تغذية الأسماك بمعدل ١٠٪ من وزن الجسم بواحدة من العلائق التجريبية لكل معاملة ثم تخفيضها إلي ٨ ثم ٦ % في نهاية فترة التغذية.

تم تكوين العلائق التجريبية لتكون متشابهة في نسبة البروتين ٣٣,٦ % والدهن ٧,١٪ وتعريفها كالآتي: كنترول (صفر)، ١٠ (OP_{10}) ، ٣٠ (OP_{30}) ، ٥٠ (OP_{50}) و٧٠ (OP_{70}) مسحوق قشر البرتقال (جم| كجم عليقة) و تم تغذية الأسماك ٦ أيام في الأسبوع ثلاث مرات يومياً (٩ ص , ١١ ص و ١ ظ) لمدة ٩٨ يوم. لم تكن هناك فروق معنوية ($P > 0.05$) بين جميع المعاملات في وزن الجسم النهائي، ومعدل الزيادة في الوزن ومعدل الوزن اليومي بإستثناء الأسماك المغذاة علي ١٠ جم مسحوق قشر البرتقال (OP_{10}) اختلفت معنوياً وسجلت أعلى قيمة. أيضاً لم يلاحظ وجود فروق معنوية في معدل النمو النوعي (SGR) بين جميع المعاملات وسجلت الأسماك المغذاة علي ٣٠ جم قشر البرتقال OP_{30} أعلي قيمة ومع ذلك، كانت هناك فروق معنوية ($P < 0.05$) بين جميع المعاملات في استهلاك العلف، واستهلكت الأسماك المغذاة علي عليقة OP_{10} المضاف لها قشر البرتقال ١٠ جم أكثر كمية علف من المجموعات الأخرى. كذلك سجلت المعاملات OP_{70} ثم OP_{30} أفضل معدل تحويل غذائي علي التوالي. أيضاً سجلت الأسماك المغذاة علي عليقة مضاف إليها ٧٠ جم مسحوق قشر البرتقال إرتفاع طفيف في معدل الاستفادة في البروتين تلاها الأسماك المغذاة علي ٣٠ جم مسحوق قشر البرتقال. اكتسبت الأسماك المغذاة علي OP_{70} ثم OP_{50} أعلي محتوي من البروتين واختلفت معنوياً عن المجموعات الأخري وسجل محتوي جسم الأسماك من المستخلص الإيثيري في الأسماك المغذاة علي علائق CTRL, OP_{50} و OP_{30} الأعلي علي التوالي واختلف معنوياً عن المعاملات الأخري. أيضاً ظهرت فروق معنوية في محتوي الجسم من الكاروتينات بين كل المعاملات وكانت أعلي قيمة في الأسماك المغذاة علي عليقة مضاف إليها ٧٠ جم مسحوق قشر البرتقال ولوحظ زيادة محتوي جسم الأسماك من الكاروتينات بزيادة مستوي مسحوق قشر البرتقال في العلائق التجريبية. لم تظهر فروق معنوية في حيوية الأسماك التي تغذت على العلائق التجريبية. كذلك لم تظهر أي تشوهات عن الكنترول في الأنسجة المختبرة (الكبد والأمعاء) مرتبطة بإضافة مسحوق قشر البرتقال بمستويات مختلفة في العلائق التجريبية. ونستنتج من ذلك أنه يمكن إضافة مسحوق قشر البرتقال في علائق زريعة أسماك البلطي وحيد الجنس بمعدل ١٠ جم لكل كجم عليقة بدون أي تأثيرات سلبية.