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RESEARCH PAPER

Effects of dietary orange peel on growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings

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Abstract

This study investigated the effects of dietary orange peels (OP) wasting from sweet orange (*Citrus sinensis*) industry on growth performance, feed utilization, intestinal morphology and proximate body composition of Nile tilapia (*Oreochromis niloticus*) fingerlings of initial body weight 5.16±0.09 g fish^{-1} (mean±SE). Fish fed for 60 days on four isonitrogenous (28% crude protein), isocaloric (18.5 MJ kg^{-1}) diets supplemented with different levels of orange peel, 0 (CTR), 1 (OP1), 2 (OP2) or 4 (OP4) g kg^{-1} diet. The results showed that fish fed on OP-based diets had the best weight gain (WG), final weight (FW), specific growth rate (SGR) and feed conversion ratio (FCR) with an optimum level of OP at 2 g kg^{-1} diet (P≤0.05). CTR treatment produced the poorest growth performance and feed utilization parameters (P≤0.05). Height of villi of the anterior intestine progressively increased as the OP level increased in the diets. This suggested that dietary OP could improve the nutrient absorptive ability of the intestine in Nile tilapia. This finding suggests that dietary orange peel can act as a growth promoter for Nile tilapia fingerlings with an optimum level at 2 g kg^{-1} diet.

Introduction

At present, tilapias are the second biggest farmed finfish group in the world after carps (FAO, 2017). In particular, Nile tilapia (*Oreochromis niloticus*) culture has sharply extended globally during the last three decades in more than 100 countries within Asia, Africa and the Americas (Gu *et al*., 2017). Along these lines, the global tilapia production has jumped from less than 0.5 million metric tons (MMT) in the early 1990s to about 5.7 MMT in 2015 (FAO, 2017), with an average annual growth rate of 13.5% (FAO, 2017).

Due to this rapid development of tilapia farming, the production systems have been steadily shifted from extensive and semi-intensive systems to more intensive systems, which raise a demand for artificial feeds. It is expected by 2025, tilapia culture would have already been completely dependent on feeds producing 12.9 MMT of tilapia (Tacon & Metian, 2015). Thus, formulating economic tilapia diets using untraditional and cheap feed resources remains a major challenge facing tilapia farmers, as well as fish nutritionists. Recently, there is a global trend to prevent the inclusion of the growth-promoting synthetic hormones in fish diets. As well as, quality, safety and the absence of pollutants or antibiotics have been increasingly demanded by the farmed fish consumers. These rigorous regulations of food safety are inspiring fish nutritionists to search for alternate growth promoters from phyto-compounds. For instance, investing of the agro-industrial by-products as a supplementation in the animal's diet appear as a promising alternate which can mitigate the industrial impact on the environment, exploit the agricultural by-products and multiply the

profit (Elferink, Nonhebel, & Moll, 2008).

Sweet orange (*Citrus sinensis*) is a plant member of Citrus family and principally cultivated in subtropical regions (Angew, 2007; Piccinelli *et al.,* 2008). Sweet orange mainly used regularly for the juice and jam production which bring huge amount of by-products, such as peels, seeds and pulps that account for 50% of the raw fruit (Espiard, 2002; Anwar *et al.,* 2008). The world orange production is assessed at 89 MMT in 2014 (USDA, 2014). Around 34% of this production was used for orange juice production, resulting in about 44% of orange peel as a by-product (Li, Lo, & Ho, 2006).

The two primary differences between orange peel and juice components, in terms of composition, are that the peel contains higher concentration of ascorbic acid than the juice (Hakim & Harris, 2001). Moreover, the peel contains much more concentrations of active materials than the juice and pulp (Hakim & Harris, 2001). The active components of orange peel mostly are alkaloids, saponins, terpenes, resins, flavonoids, phenols, and tannins (Al-Saadi, Ahmad, & Sa´eed, 2009). In addition, limonene, phytate, and oxalate were found in all orange peel meals (Oluremi, Ngi, & Andrew, 2007). Orange peels were also found to contain some elements such as, Fe, Mn, Zn, Ni, Cu, Cr, Pb, Cd and P with concentrations of 125, 88, 13, 1.6, 1.3, 1.2, 0.25, and 0.11 µg/ml, respectively, and 0.2% of phosphorus (Al-Saadi, Ahmad, & Sa´eed, 2009). Oil constitutes around 13.12% of the orange peels (Smith *et al.,* 2001). It contains nearly 85-99% volatile and 1-15% non-volatile components (Smith *et al.,* 2001). The volatile components are a mixture of monoterpene (limonene) and sesquiterpene, hydrocarbons and their oxygenated derivatives (Smith *et al.,* 2001). In fact, plant-derived compounds such as flavonoids, alkaloids, pigments, phenolics, steroids, terpenoids and essential oils have been found to promote biological activities like growth, antistress, appetite, immunity, and antimicrobial properties in fish culture (Citarasu, 2010).

D-Limonene, one of the active components of orange peel, comprises more than 90% of orange peel oil, and has shown a chemo preventive activity against different types of chemically induced rodent cancers (Hakim & Harris, 2001). Besides, they work physiologically as chemoattractants, and they are the main responsible for the distinctive fragrance of many plants (Crowell, 1999). Flavonoids, another major constituent of the orange peel, have been found able to modulate cellular response to various stimuli by protecting antioxidant defenses (Virgili & Marino, 2008).

Although, there are no studies have been carried out to investigate the effect of dietary OP on fish growth performance, many studies have revealed the positive role of herbs that contain similar potent bioactive components (Ji *et al.,* 2007; Kumar, Sharma, & Sharma, 2007; Immanuel *et al.,* 2009; Citarasu, 2010; Hashemi & Davoodi, 2011).

However, to the best of the authors' knowledge, no published data on the effects of orange peel on fish has been conducted. This prompted us to formulate orange dried peel supplemented diets that were fed to Nile tilapia (*Oreochromis niloticus*) fingerlings. Therefore, the aim of the present study was to evaluate the sweet orange peel in terms of growth enhancement of Nile tilapia fingerlings.

Material and Methods

Test diets and feeding regimes

Four isonitrogenous (28% crude protein), isocaloric (18.5 MJ kg^{-1}) diets were formulated with different levels of orange peel: 0, 1, 2 or 4 g kg^{-1}. The sweet orange peels used in this study were obtained from a local market in alexandria city, Egypt. The fresh peels were dried in the oven at 50^{o}C for 36 hours. All ingredients were first ground to a small particle size (approximately 250 µm) in a Wileys mill (Labx Company, Midland, ON, Canada). All dry ingredients of the diets were thoroughly mixed prior to adding water to 40% moisture. The diets were processed by a mincer with die into 3-mm diameter, spaghetti-like strands, sun-dried and stored in air tight containers. The formulation and proximate analysis of the experimental diets are shown in Table 1.

The experimental fish were fed the control diet for one week to adapt to the experimental diets. Then, fish in each aquarium were weighed, and their initial weights were recorded. Fish in each aquarium were fed the experimental diets twice daily (six days a week) at a rate of 3% of their live body weight (BW).

Experimental fish

This study was carried out at the Fish Nutrition Laboratory, the National Institute of Oceanography and Fisheries, Alexandria, Egypt. Nile tilapia (*Oreochromis niloticus*) were obtained from a commercial farm near Alexandria. Fish were placed randomly in 12 glass aquaria of 70 Liter capacity per aquarium. Three replicates per treatment were used. Each aquarium was stocked with ten fish of Nile tilapia with an average initial body weight of 5.16±0.09 g $fish^{-1}$. Fish from each aquarium were weighed every 15 days. Each aquarium was cleaned daily in order to prevent accumulation of fecal materials and reduce the growth of algae, and the same amount of fresh water was used to refill the aquaria. Filtered water was partially changed to one half every day using fresh filtered water. Aeration was continuously provided using an air blower. Temperature was maintained at 25±1°C. Lighting in culture unit was set at 12:12 light: Dark cycle using fluorescent lamps. The fish were fed the test diets for 60 days. At the end of the feeding trial, all fish in each aquarium were bulk weighed.

Table 1. Composition and proximate analyses of the experimental diets fed to Nile tilapia (*O. niloticus*) supplemented with orange peel.

Ingredients (%)	Diets			
	CTR	OP1	OP2	OP4
Fish meal (CP 70%)	100	100	100	100
Soybean meal (CP 44%)	400	400	400	400
Wheat bran	300	300	300	300
Starch	130	130	130	130
Fish oil	20.0	20.0	20.0	20.0
Corn oil	20.0	20.0	20.0	20.0
Vitamins and minerals premix[1]	20.0	20.0	20.0	20.0
Calcium diphosphate	10.0	10.0	10.0	10.0
Orange peel	0.0	1.0	2.0	4.0
Proximate analyses (%)				
Crude protein	27.66	28.06	27.96	28.11
Crude fat	7.30	6.98	7.19	7.22
Fiber	4.67	4.33	4.98	4.54
Ash	6.06	6.10	6.20	6.47
NFE[2]	54.31	54.53	53.67	53.66
Gross energy (MJ kg^{-1})	18.76	18.77	18.67	18.72

[1] Vitamin and minerals mixture contains (mg/kg or IU/kg of dry vitamins & minerals powder): Vit. A 2.200.000 IU., Vit. D3 1.100.000 I.U., Vit. E 1.500 I.U., Vit. K 800 mg, Vit. B1 1,100 mg, Vit. B2 200 mg, Vit. B6 2.000 mg, Vit. H 15 mg, Vit. B12 4 mg, Vit. C 3.000 mg, Iron 160 mg, Magnesium 334 mg, Copper 21.6 mg, Zink 21.6 mg, Selenium 25 mg, Cobalt 2.38 mg.

[2] NFE = 100 - (% protein + % fat + % fiber+% ash).

Water quality parameters including O_2, pH, and total ammonia (NH_4) were monitored regularly. The average values of these parameters throughout the study were: 6.29 mg l^{-1}, 7.5 and 0.26 ppm, respectively.

Analyses

Growth performance detection

At the end of the feeding trial of 60 days, fish in each aquarium were individually weighed. Growth performance was calculated as follows:

Calculation of fish performance

Fish performance was calculated as follows:

Survival (%)=100x(Final fish number/Initial fish number)

Growth rates

$$\text{Percent weight gain (\% WG)} = \frac{\text{Wf(g)} - \text{Wi (g)}}{\text{Wi (g)}} \text{x} 100$$

$$\text{Average daily gain (ADG)} = \frac{\text{Wf(g)} - \text{Wi (g)}}{t}$$

$$\text{Specific growth rate (\% SGR)} = \frac{\ln \text{Wf} - \ln \text{Wi}}{t} \text{x} 100$$

Where: Wi and Wf are initial and final weights (g), and *t* is time of experiment (days).

Feed utilization efficiency

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Dry feed intake (g)}}{\text{Fish live weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Fish weight gain (g)}}{\text{Protein intake (g)}}$$

$$\text{Protein productive value (PPV)} = \frac{\text{Protein gain (g wet weight)}}{\text{Protein fed on dry weight basis (g)}} x\ 100$$

Chemical analysis of the diets and fish carcasses

Proximate analyses of the formulated diets and fish carcasses were carried out according to a standard methodology (AOAC, 2009). At the end of the experiment, nine randomly sampled fish from each treatment were collected for the carcass analysis. Crude protein content was analyzed by the Kjeldahl method using an Auto Kjeldahl System, crude lipid content by the Soxhlet extraction method, ash content by a furnace muffler (600°C for 2 h) and moisture content by a dry oven (105°C for 24 h).

Histological examination

About six fish per treatment were sacrificed and their gut removed. The intestines were fixed in 10% formalin for 36 hours and then transferred to alcohol (70%). After conventional histological processing, the sections (3 µm thick) were stained with haematoxylin and eosin and observed under a light microscope. Assessment of villi development followed the description of Holden & Raitt (1975).

Calculations and Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA) at a 95% confidence limit, using SPSS software (v 20.0, SPSS Inc., Chicago, IL). Duncan's multiple range test was used to compare means when *F*-values from the ANOVA were significant (P≤0.05). Least significant difference was used to compare means at P≤0.05. In order to determine the optimal OP level on growth performance of Nile tilapia (*O. niloticus*), polynomial regression was applied to determine the best regression model between the dietary orange peel and the weight gain (g) (Figure 1).

Results

Growth performance

The effect of different levels of orange peel on growth and survival rates of Nile tilapia (*O. niloticus*) is summarized in Table 2. The results revealed that fish fed on dietary orange peel 2 g kg^{-1} diet (OP2) significantly (P≤0.05) produced the best final body weight (FW), body weight gain (WG), average daily gain (ADG) and specific growth rate (SGR), followed by fish fed on orange peel 1 g kg^{-1} diet (OP1). Increasing dietary OP to 4 g kg^{-1} resulted in a significant reduction (P≤0.05) in growth rates, but it was still higher than growth rates of the control group (CTR). In fact, CTR group produced the worst values of FBW, WG, ADG and SGR (P≤0.05). There was no significant difference (P≥0.05) among the experiment treatments for survival rates.

Feed utilization efficiency

The effects of different levels of dietary orange peel on feed utilization of Nile tilapia are shown in Table 3. Feed intake (FI) in OP2 fish group was slightly higher than other groups. However, FI in OP4 group was not significantly different (P≥0.05) from OP1 and OP2 groups. The lowest FI value was in CTR group. The best results of Feed conversion ratio (FCR) and protein efficiency ratio (PER) were recorded in OP2 fish group followed by OP1 group, and then OP4 group (P≤0.05). OP2 group produced the best protein productive value (PPV) followed by OP4 (P≤0.05). PPV in OP1 group was not significantly different from OP2 or OP4 groups (P≥0.05). The worst FCR, PER and PPV values were recorded in CTR fish group (P≤0.05).

Fish body composition

Chemical body composition of Nile tilapia fed on different concentrations of dietary OP is shown in Table 4. The highest values of crude protein (CP) were obtained in OP4 fish group (P≤0.05). The poorest CP

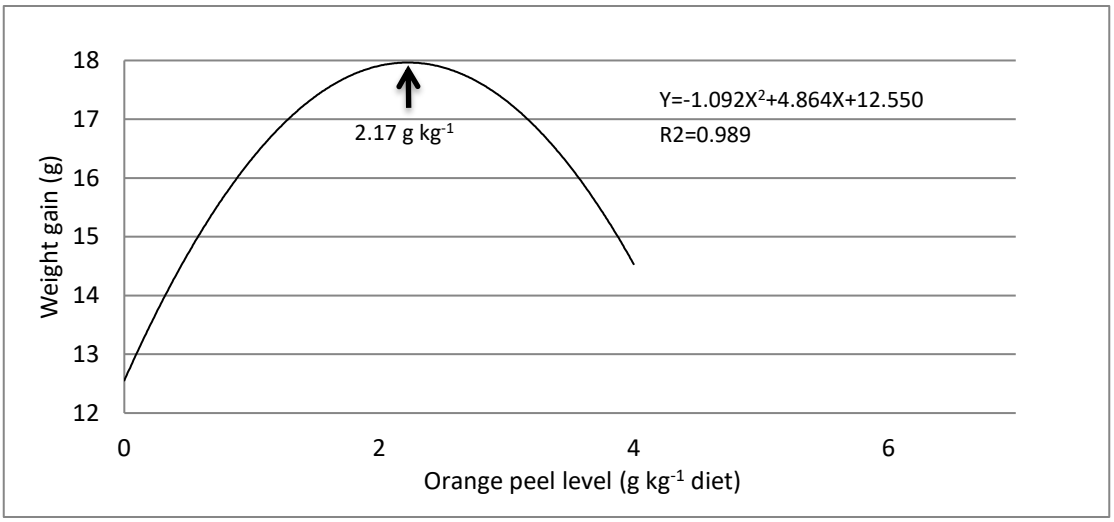

Figure 1. The relationship between fish weight gain and dietary orange peel levels determined by polynomial regression.

Table 2. Effects of different levels of dietary orange peel on growth performances of Nile tilapia (*O. niloticus*)

Treatment	Initial weight	Final weight	Weight Gain	ADG	SGR	Survival
OP1	5.12±0.08^{a}	21. 56±0.07^{b}	16.44±0.13^{b}	0.27±0.002^{b}	2.39±0.03^{b}	93.33±6.66^{a}
OP2	5.24±0.03^{a}	23.07±0.07^{a}	17.82±0.07^{a}	0.30±0.001^{a}	2.47±0.01^{a}	100.0±0.00^{a}
OP4	5.15±0.02^{a}	19.69±0.23^{c}	14.55±0.23^{c}	0.24±0.004^{c}	2.24±0.02^{c}	93.33±3.33^{a}
CTR	5.22±0.01^{a}	17.73±0.09^{d}	12.51±0.09^{d}	0.21±0.001^{d}	2.04±0.01^{d}	96.67±3.33^{a}

Means in the same column bearing different superscripts differ significantly at 0.05 levels. Values are means ± standard error (SE).

Table 3. Effects of different levels of dietary orange peel on feed utilization of Nile tilapia (*O. niloticus*)

Treatment	FI	FCR	PER	PPV
OP1	23.86±0.11^{bc}	1.45±0.01^{c}	2.49±0.02^{b}	48.79±1.49^{ab}
OP2	24.25± 0.16^{a}	1.36±0.01^{d}	2.62±0.01^{a}	53.42±0.29^{a}
Op4	24.13±0.03^{ab}	1.66±0.02^{b}	2.16±0.03^{c}	47.63±0.38^{b}
CTR	23.62±0.05^{c}	1.88±0.02^{a}	1.88±0.02^{d}	38.95±1.20^{c}

Means in the same column bearing different superscripts differ significantly at 0.05 levels. Values are means ± SE.

Table 4. Whole body composition (dry weight basis) of Nile tilapia (*O. niloticus*) fed different levels of dietary orange peel

Treatment	Dry matter	Protein	Lipid	Ash
OP1	26.6333±0.86^{a}	56.0833±0.71^{bc}	20.85±0.24^{a}	15.4167±0.44^{a}
OP2	27.3833±0.34^{a}	57.5400±0.06^{ab}	22.07±0.49^{a}	15.2700±1.38^{a}
OP4	27.7233±0.21^{a}	58.8833±0.47^{a}	21.08±0.81^{a}	14.8000±0.44^{a}
CTR	26.4833±0.33^{a}	55.0433±0.85^{c}	22.03±0.81^{a}	16.3467±0.46^{a}

Means in the same column bearing different superscripts differ significantly at 0.05 levels. Values are means ± SE.

result was obtained in CTR group (P≤0.05). In the same time, CP value in OP2 group was not different (P≥0.05) from OP1 and OP4 groups. Similarly, CP value in OP1 was not different (P≥0.05) from OP2 and CTR group. Dry matter, lipid and ash values did not significantly differ (P≥0.05) among all treatments.

Histological analysis

At the end of the experiment, the microscopic structure of Nile tilapia intestine as affected by different levels of orange peel supplement is shown in Figure 2 and 3. In the present study, the intestinal villi of fish fed 0 g OP kg^{-1} (CTR group) showed the shortest as compared to OP supplemented diets. The intestinal villi height and density progressively increased as dietary OP levels increased and reach the maximum at 4 g OP kg^{-1} diet as shown in Figure 2 (A-D). However, at high dietary OP levels, 4 g kg^{-1} diet (Figure 2-C), it was noticed that intestinal villi were highly developed to an extent that might prevent the mobility of the food through it.

Discussion

This study aimed to evaluate the possible effects of orange peel, which are obtained as a by-product from orange juice industry, on growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings. In this context, the present study represents the first attempt to determine the growth performance of tilapia fed on dried sweet orange peel as a supplement in their diets. The results indicated that all fish groups fed on dietary orange peel had higher weight gain compared to CTR group. Among OP fish groups, OP2 group was significantly superior in terms of growth rates and feed utilization efficiency when compared to the other groups in the present study. This may be partly attributed to the improvement in morphological structure of the small intestine of Nile tilapia, where the histological analysis of intestine showed that the intestinal villi height increased as the dietary OP levels increased, and became well developed at OP levels 1 and 2 g kg diet^{-1}. At the same time, the intestinal villi of CTR fish group were poorly developed. In fact, intestinal villi provide a vast absorptive surface area, and the increasing in villi height and/ or density is important for the nutrients absorption (Dai, Shu, & Fang, 2007). This may explain that improvement in the growth performance of Nile tilapia in the present study. On the contrary, there was a depression in growth performance of fish fed a high level of dietary OP, 4 g kg^{-1} diet. In histological examination of intestine, it was noticed that at a high level of OP, 4 g kg diet^{-1}, intestinal villi were too highly developed in terms of height and density. That high degree of villi development led to block the intestine pathway which might hinder the movement of the food through it. Consequently, it prevented the nutrients absorption through the blood stream which might negatively affect the growth performance of Nile tilapia. This may explain why the growth of Nile tilapia has been depressed at the high level of OP 4 g kg diet^{-1} in this study. Thus, OP might play an important role in enhancing the digestion and absorption of nutrients to a certain level. The optimal response was found to occur at 2g OP kg^{-1} diet.

Therefore, OP could act as a growth promoter in Nile tilapia diets. Following the same pattern, the effects of orange peels derived pectin (OPDP) on growth performance of *O. niloticus* were investigated. Nile tilapia fed diets supplemented with different levels of OPDP. The results revealed that OPDP improved SGR, WG, FW, and FCR. The authors revealed that OPDP can be used as functional feed additives for *O. niloticus* (Doan *et al.,* 2018). Similar results were obtained by Acar, Kesbiç, Yılmaz, Gültepe, & Türker (2015), who studied the effect of essential oil extracted (EO) from sweet orange peel (*Citrus sinensis*) on growth performance of Mozambique tilapia (*Oreochromis mossambicus*). The authors revealed that dietary orange EO can act as a growth promoter for Mozambique tilapia

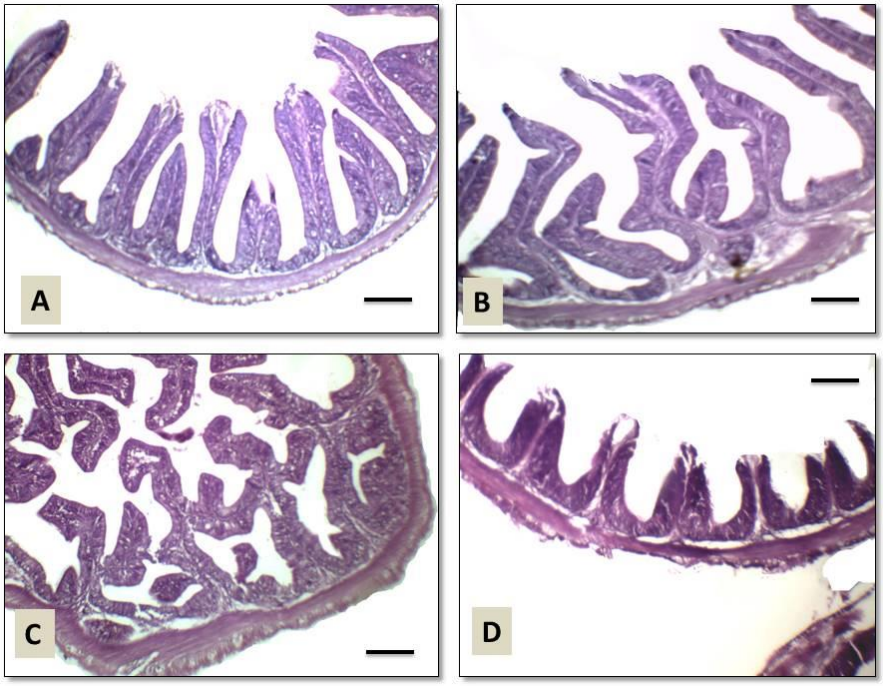

Figure 2. Light microscopy of hematoxylin and eosin staining sections of Nile tilapia (*Oreochromis niloticus*) feed experimental diets, A (OP1 diet), B (OP2 diet), C (OP4 diet) and D (CTR diet). Scale bar: 30 µm.

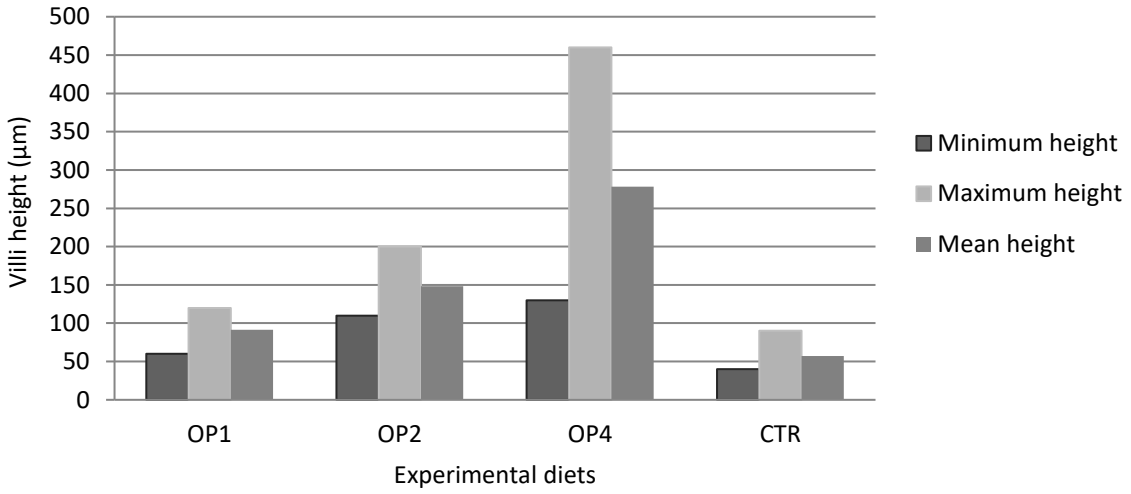

Figure 3. Maximum, minimum and mean heights of intestinal villi of Nile tilapia fed different levels of dietary orange peel.

(*Oreochromis mossambicus*), where the weight gain of fish fed orange EO supplemented diet was significantly higher than those fed on other diets.

Orange peels have been found to contain alkaloids, saponins, terpenes, resins, flavonoids, phenols, and tannins (Al-Saadi, Ahmad, & Sa´eed, 2009). It has been reported that herbs that contain such potent bioactive components may influence digestive processes in a positive way by enhancing enzyme activity, improving digestibility of nutrients and feed absorption, consequently resulting in an improvement infish growth (Immanuel *et al.,* 2009; Citarasu, 2010; Kaleeswaran, Ilavenil, & Ravikumar, 2010; Hashemi & Davoodi, 2011). Therefore, all these effects of phytochemicals have been found to promote growth in fish (Immanuel *et al.,* 2009).

For instance, Sweet orange peel was found to contain considerable amount of saponin (Oluremi, Ngi, & Andrew, 2007). When, Nile tilapia (*Oreochromis niloticus*) fingerlings fed diets supplemented with the dietary ginseng herb containing saponin as an active chemical component, it greatly enhanced the growth and diet utilization efficiency (Goda, 2008). In similar, the dietary administration of green tea (*Camellia sinensis*) leaves that contain flavonoid for 12 weeks enhanced the growth, FCR and protein content of Nile

tilapia (Abdel-Tawwab, Ahmad, Seden, & Sakr, 2010). This supports the results of the present study, as the body protein content slightly increased with the increasing of OP levels. In rabbits, Azima, Muchtadi, Zakaria, & Priosoeryanto (2004) suggested that flavonoids, saponins and tannins could improve nutrient absorption.

Following the same pattern, the oriental medicinal herb liquorice (*Glycyrrhiza glabra*) comprising flavonoids and pentacyclic triterpene saponins as major constituents, was reported to have a growth-promoting effect in Indian major carp (*Cirrhinus mrigala*) fingerlings (Kumar, Sharma, & Sharma*,* 2007).

Furthermore, Ji *et al.* (2007) investigated the growth promoting effect of a medicinal herbs mixture, containing many antioxidant compounds such as carotenoids, flavonoides, and ascorbic acid as main bioactive phytochemicals, in Japanese flounder (*Paralichthys olivaceus*) diets for 8 weeks. Fish fed herbal mixture-based diets showed higher weight gain and feed efficiency than fish in the control group (Ji *et al*., 2007).

Although there are no studies investigating the effect of dietary OP on fish, it was studied on broiler. A study was conducted for the evaluation of various levels of orange and banana peels on growth performance of broiler chicks. Both peels increased the growth performance of broilers (Siyal *et al.,* 2016). In similar, El-Boushy & Van der Poel (2013) revealed that orange fruits by-products enhanced the FCR of animals in poultry sector when compared with traditional feed.

In the present study, it has been observed that growth rate was higher in OP1 and OP2 fish groups than in OP4 group, which indicated that effect of OP was dose dependent. This is explained by Holst & Williamson (2008) who revealed that a bio-available dose may cause different magnitudes of effects in different individuals and the maximum benefit may be obtained at an optimal amount, while both deficient and excessive levels may cause deleterious effects. For example, catfish was not able to tolerate the high content of bioactive compounds (tannins and flavonoids) and inhibit the nutrient absorption due to the limited ability of the fish digestive enzymes (Setiawati, Jusadi, Rolin, & Vinasyiam, 2016). Therefore, tolerable threshold of dietary OP needs further studies.

For conclusion, dried orange peels can efficiently be used as a growth promoter in Nile tilapia fingerlings diets but to a certain limit. The optimal response was found to occur at 2g OP kg^{-1} diet. However further research are demanded with incremental dose response to explore its effects on growth and blood biochemistry indices in different fishes models.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: “All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.”

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