EVALUATION OF POMEGRANATE PEEL MEAL AS FEED SUPPLEMENT IN THE DIETS FOR MONO-SEX NILE TILAPIA, *OREOCHROMIS NILOTICUS* FRIES.

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SUMMARY

Diet supplemented with pomegranate peel (3, 6, and 9 g/kg diet) as identified as POM3, POM6 and POM9 were fed to Nile Tilapia fries for 84 days to evaluate the nutritional values, antioxidant activity, histology of liver and intestine and growth of mono-sex Nile tilapia (*Oreochromis niloticus*) fries. No significant changes ($P \geq 0.05$) in the final weight, weight gain, and specific growth rate (SGR) were seen in all treatments. Meanwhile, a decreasing trend was detected in feed intake by increasing the pomegranate peel inclusion. When compared to the other groups, POM9 diet had the worst (2.02) ($P \leq 0.05$) feed conversion ratio (FCR value). While POM3 diet had the best FCR (1.63) without significant differences ($P \geq 0.05$) from the other groups except POM9. The survival rates did not differ significantly ($P \geq 0.05$) in all treatments but increased pomegranate peel resulted in a modest rise. Pomegranate peel powder contains significant phenolic acid concentrations, resulted a high DPPH scavenging activity (81.72). Pyrogallol was the first abundant phenolic acid (927.45 mg/100gm powder). Increased pomegranate peel consumption also resulted in significant increases ($P \leq 0.05$) in superoxide dismutase (SOD), total antioxidant capacity (TAC), catalase (CAT), besides glutathione peroxidase (GSH). Additional pomegranate peel dosage led to a considerable decreasing trend ($P \geq 0.05$) in malondealdihye (MDA) and alkaline phosphatase (ALP). Compared with the control group, fish fed diet supplemented with pomegranate peel had shown normal appearance of liver and intestine Therefore, Pomegranate peel supplementation may enhance growth performance as well as the health of mono- sex Nile tilapia fries.

Keywords: *pomegranate peel, food waste, Nile tilapia, growth, antioxidant activity, histology.*

INTRODUCTION

Aquaculture is the world's fastest growing food-producing sectors and is recognized as an integral part of many poverty reduction programs worldwide. Globally, fish and seafood are among the most popular foods and are significance source of animal protein. Nile tilapia (*Oreochromis niloticus*) is the second most farmed freshwater species after carp (FAO, 2020; Thodesen *et al.*, 2012; Gjerde *et al.*, 2012). In Egypt, Nile tilapia is the most farmed freshwater fish, accounting for 17.4% of global tilapia production, the only African country in the top ten tilapia production (FAO, 2020; Miao *et al.*, 2020).

In aquaculture, assessment of fish oxidative status is important to improve fish welfare and maximize production as it is closely related to the health, growth and quality of the fish. Natural antioxidants have been gaining a lot of attention since they are safer and more effective than synthetic antioxidants. Since conventional antioxidants are considered safer, they are easily accepted by consumers in various countries

(Prasad *et al.,* 2009). Numerous studies on herbs, vegetables and other edible plants have been conducted to explore alternatives to inexpensive, safe and effective natural ingredients (Hasani, 2020; Toutou *et al.,* 2019). Among the ingredients, Pomegranate (*Punica granatum*) belongs to the Punicaceae family is one of the potential candidates. The juice, peel, and oil of pomegranate contain high levels of antioxidant with strong anti-inflammatory properties (Acar *et al.,* 2018, Malviya *et al.,* 2014). Therefore, they are widely used in medicine, cosmetic and a promising animal feed nutritional additive (Badawi and Gomaa, 2016).

Pomegranate juice is one of the most common products made from pomegranate fruit ((Iqbal *et al.,* 2008). Juices manufacturing will generate significant waste and by-products, and therefore it's important to recycle them all. Red Corn *et al.* (2018) reported that on average of 46% of pomegranate is used as a juice, and the remaining portion is considered waste. Pomegranate peel, which accounts for approximately 50% of the fruit is one of the major food industry wastes (Ali *et al.,* 2019). In spite of being an agro-waste, pomegranate peels contain higher total phenolic content and antioxidant activity compared to its pulp, flower, leaf and seed (Malviya et al, 2014). Thus, such peels have the potential to be a unique source of bioactive chemicals in food processing and animal feed industry (Singh *et al.,* 2018; Andrade *et al.,* 2019). Study on the use of herbal extracts as feed additives in Nile tilapia (Oreochromis niloticus) diet has shown that this ingredient can improve growth rate, feed efficiency, immune function, and resistance to oxidation ((Johnson and Banerji, 2007; El-Sayed *et al.,* 2014; Badrey *et al.,* 2019 and Toutou *et al.,* 2019). Pomegranate peel extract has also been studied extensively for its anti-bacterial and regulatory effects (Jurenka, 2008; Sun *et al.,* 2021 and Kaderides *et al.,* 2021). However, the usage of pomegranate peel as feed additives in fish feed is limited. Therefore, it is important to provide more light on the impact of this material on fish performance. As little more than a direct consequence, the goal of this study was to investigate the effect of pomegranate peel meal in the Nile tilapia, *Oreochromis niloticus* diets on the antioxidant activity, growth performance, body composition and histological examination.

MATERIAL AND METHODS

Experimental design:

The current investigation was conducted at the Fish research facility of Agriculture's Poultry and Fish Production Department at Menoufia University, Egypt. Mono-sex Nile tilapia, *Oreochromis niloticus* fries were provided by a local fish farm from the governorate of Kafer El-Sheikh, Egypt. Upon arrival, the fish were acclimated to laboratory conditions for two weeks. A total of 600 fries at an average of 0.33 g ± 0.1 were randomly distributed into 12 glass aquaria (80 L) supplied with aerated and choline free fresh water. Aeration was supplied using an air blower. Three aquaria presenting a replicate of each treatment (50 fries /replicate). The initial feeding rate was at 10% of body weight at the start of the feeding trial, and it was adjusted to 8%, 6%, and 3% by the completion of the12-week feeding period. Every two weeks, fish were counted and weighed in bulk to monitor their growth and survival to adjust the feeding rate. Experimental fish were fed three times at (9 a.m., 11a.m and 1 p.m.). Feeding was terminated 24 hours before the last weighing. Temperature and dissolved oxygen in each tank were measured everyday by digital YSI (APHA, 1995). Ammonia and pH were measured twice a week. Water quality measurements were maintained under the normal range for rearing tilapia and the values were in an average of (± SD): 26±2.5 °C, water temperature; 6.7± 0.3 mg/l, dissolved oxygen; 0.22± 0.12 mg/l, total ammonia and 7.2±0.1; pH.

Diets and pomegranate peel chemical analyses:

Moisture content, crude fat, ash, and crude proteins of the diets and pomegranate peel were assessed according to AOAC (2012). Total carbohydrate was determined as glucose after hydrolysis by HCl. Reducing sugars were extracting by 70% ethanol and determined according to Dubois *et al.* (1956).

Pomegranate peel preparation and experimental diets manufacturing:

Pomegranate fruits (*Punica granatum*) were procured from The Agriculture Research Center (Giza, Egypt). Fruits were peeled and washed before the process. Then, the peels were dried up to 36 hours in an air draught oven at 40°C. The dried peels were grounded and sieved through 50 mm sieve before used in the diets manufacturing. Table (1) shows the chemical content of the formulated diets. All the other ingredients used in the diet preparation were purchased commercially. The dried pomegranate peel was added to three diets at 3, 6 and 9 g/kg diet and defined as Pom3, Pom6 and Pom9. To meet the fry's nutritional needs, a control diet containing 30% crude protein and 4000 kcal/kg was prepared The ingredients were weighted and mixed thoroughly, followed by the addition of vegetable oil and water. A

pelletizer was used to produce the pellets. The pellets were sun-dried and kept in plastic bags at 5 ºC until use. Each replicate was fed each treatment 6 days per week.

Table (1): Composition and proximate analysis of the experimental diets (%).

	Dietary groups			
Ingredients (%)	**CTRL**	**Pom3**	**Pom6**	**Pom9**
Fish meal (65%)	10	10	10	10
Soybean (44%)	44	44	44	44
Wheat bran	13	12.7	12.4	12.1
Wheat (14%)	14	14	14	14
Corn (7.5%)	13	13	13	13
Pomegranate meal	0	0.3	0.6	0.9
Vegetable oil	2	2	2	2
Dicalcium phosphate	1	1	1	1
Premix[1]	3	3	3	3
Chemical analysis				
Dry matter	89.81	89.71	89.51	89.52
Crude protein	30.63	30.56	30.6	30.55
Ether extract	5.33	5.43	5.55	5.35
Ash	6.34	6.44	6.55	6.64
Crude fiber	3.71	3.61	3.51	3.62
NFE[‡]	53.99	53.96	53.79	53.84
GE (kcal/100g DM) [§]	466.83	466.8	467.39	465.45
ME (kcal/100g DM) [¶]	369.43	369.82	370.38	368.71

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

[§] GE= Gross energy based on protein (5.65 kcal/g), Fat (9.45 kcal/g), and carbohydrate (4.12 kcal/g) according to (NRC, 2011).

[¶] ME (kcal/100g DM) = metabolically energy was calculated by using factors 3.49, 8.1 and 4.5 kcal/g for carbohydrates, fat and protein, respectively according to Pantha (1982).

[1]Premix Composition: Each 1 kg contains: Vit A 4.8 million IU; Vit D3, 0.8 million IU; Vit E 4 g; Vit K 0.8 g; Vit B1 0.4 g; riboflavin 1.6 g; Vit B6 0.6 g; Vit B12 4 mg; Vit C 150 mg; Nicotinic acid 8 g; Choline chloride 200 g; Folic acid 0.4 g; Biotin 20 mg; Pantothinic acid 4 g; Magnesium sulphate 22 g; Copper sulphate 4 g; iron sulphate 12 g; Zinc sulphate 22 g; Cobalt sulphate 100 mg; Selenium 0.4 g.

Growth performance and sample analysis:

At the start of the experiment, fifteen fish were sampled and frozen at -18°C for initial body proximate composition analysis (AOAC, 2012). At the end of the experiment, all fish from each tank were individually weighed and count to calculate the following:

- Weight gain (g) = (final w – initial w)
- Weight gain in percent (WG %) = [final weight – initial BW]x100/ days
- Specific growth rate (SGR%/fish/d) = [ln final BW – ln initial BW] x 100/days

The percentage of surviving fish at the end of the feeding period divided by the number of survived fish at the start of the feeding trial was used to calculate survival. Feed conversion ratio (FCR) was measured by dividing the amount of the dry feed eaten (g) by the weight gain (g) of fish and protein efficiency ratio (PER; WG/protein intake) were used to calculate feed utilization. Chemical compositional of the fish body and experimental diets were evaluated following the AOAC (2012). Three fish from each tank were randomly selected for the biochemical analysis. In addition, complete fish bodies were homogenized for a crude chemical analysis and then kept at -18 °C. Moisture content was determined using a dry oven (105°C for 24 hours), the Kjeldahl method was used to assess the protein content while, the Soxhlet extraction method was used for evaluating the lipids, and ash was measured using furnace muffler at 600°C for 2 hours.

Pomegranate peel antioxidant properties and phenolic compound:

Assay for scavenging DPPH radicals:

The free radical scavenging activity was used to calculate using a 2, 2-diphenyl-1picrylhydrazyl (DPPH) technique described by Brand-Williams *et al.* (1995). A 50 µl of extract (20.0 g/l) was add into a cuvette, and then 2 ml of 6×10^{-5}M DPPH methanolic solution was added. After 1 hour, the absorbance at 517 nm (UNICO 2802 C/PCS, USA) was measured. For 1 hour, on 5-minute intervals, the decrease in absorbance was measured. For comparison, alpha-tocopherol was employed. The DPPH radical inhibition % was determined as follow:

- % DPPH scavenging = [(AC (o) 517 - AA (t) 517) ÷ AC (o) 517] × 100
- Where: AC (o) 517 is the control absorbance at t = 0 min.
- The extracts absorbance at t = 1h is AA (t) 517

Total phenolic compounds determination:

The total phenolic content was estimated using a Folin–Ciocalteu reagent according to Taga *et al*. (1984) and tannic acid was used as the standard.

Sympathy of phenolic compounds:

Phenolic compounds have been identified according to Ricardo *et al.* (1993) by HPLC (Thermo Separation Products Inc.). The technique was made up of Consta METRIc 4100 sequences pump, spectra scheme FL 3000 fluorescence sensor (Ex: 250 nm – Em: 400 nm) and Column ODC-2 (3 µ M; 150 mm × 4.6 mm I.d., Alltech. USA). A flow rate (1 ml / min) of mobile phase comprises of Methanol: Ammonium acetate (12: 88, v/v at pH = 5.4) was applied.

Total flavonoids assay:

The aluminum chloride colorimetric method described by Chang *et al.* (2002) was used to evaluate the total flavonoids. As markers of fish tissue antioxidant activity, TAC, total antioxidant capacity; SOD, superoxide dismutase; GPx, glutathione peroxidases; ALP, alkaline phosphatase enzyme and CAT, catalase were examined. At the termination of feeding period, liver samples were taken from three fish from each aquarium (9 fish each group). Following collection, the livers sections were located directly in the freezer at -80 °C.

Histopathological analysis:

Two fish from each aquarium were randomly selected and sacrificed (n = 6 per treatment). The head and tail of each fish were removed, and the viscera and liver were dissected and stored for 48 hours in 10% neutral buffered formalin (Thermo Fisher, Kalamazoo, MI). The liver and intestinal samples were fixed in formalin for 48 hours. Then samples were washed, and dried in successive grades of ethyl alcohol. After then the samples were routinely treated to obtain 4 µm thick paraffin slices using an LEICA RM 2135 microtome. All tissues were sectioned longitudinally. For the microscopic analysis, hematoxylin and eosin stain (H&E) were used to stain the slices (Bancroft and Layton, 2013)

Analytical statistic:

A one-way ANOVA was applied using the SPSS 19 (SPSS Inc., IL, USA). Difference among treatments mean (means ± SD, standard deviation) were compared using a Duncan's novel multiple range test (Duncan, 1955). When ($P \leq 0.05$) differences between treatments were rated significant.

RESULTS

Pomegranate peel chemical composition and antioxidant activity:

Pomegranate peels contained 6.22% moisture, 1.21% crude protein, 3.01 ash, 0.94 crude fat, 22.21 fiber and 66.50% total carbohydrate. The dried pomegranate peel's antioxidant scavenging activity was 81.72 % by way of DPPH (Table 2). The pomegranate peel powder contained 62.35 mg GAE/g which consider a significant amount compared to other different wastes sources. Identification of phenolic compound of pomegranate powder elucidates that it contains several phenolic acid such as Gallic, Benzoic, Ellagic, salicylic, chlorogenic catechin with a significant concentration. Pyrogallol was the first abundant phenolic acid (927.45 mg/100 g powder) followed by Benzoic acid (691.07 mg/100 g powder), Ellagic (640.35 mg/100 g powder) besides Catechin (504.82 mg/100 g powder) then chlorogenic and gallic (196.88 and 113.04 mg/100 g powder, respectively) while, cinnamic and protocatechuic acids showed the lowest content (4.81 and 17.92 mg/100 g powder, respectively).

Table (2): Phenolic Compounds and antioxidant activity of pomegranate peel.

Phenolic compounds	Pomegranate peels ethanol (mg/ 100 g)
Gallic	113.04
Chlorogenic	196.88
Pyrogallol	927.45
Protocatechuic	17.92
Catechin	504.82
Caffeic	133.63
Benzoic	691.071
Cinnamic	4.81
Salicylic	94.90
Ellagic	640.35
Total Phenolic	62.35 mg GAE/g
DPPH	81.72 %

Fish performance:

Summarized results after 84 days feeding the experimental meals to Nile tilapia, *Oreochromis niloticus* fries are performed in Table 3. Final weight, gain, gain percent, and specific growth rate (SGR %/d) across all treatments were insignificant ($P \geq 0.05$), with the Pom9, gained the lowest values. Final weight and gain were highest when fish given the CTRL diet, followed by fish given Pom3 which supplemented with 3 g/kg pomegranate peel. The survival rates of all treatments revealed insignificant variances ($P \geq 0.05$).

Table (3): Growth performance of mono-sex Nile tilapia, *Oreochromis niloticus* fries fed different levels of dietary pomegranate peel meal for 84 days.

Items	Levels of POM			
	CTRL	Pom3	Pom6	Pom9
Initial weight (g)	0.33±0.01	0.32±0.00	0.33±0.01	0.33±0.01
Final weight (g)	3.98±0.70^{a}	3.87±0.55^{a}	3.78±0.88^{a}	2.95±0.35^{a}
Gain (g)	3.65±0.69^{a}	3.55±0.55^{a}	3.45±0.87^{a}	2.62±0.34^{a}
Gain (%)	1089.69±175.3^{b}	1110.8±170.6^{b}	1029.8±229.5^{b}	785.4±84.2^{a}
Specific growth rate (SGR%/d)	2.94±0.18^{a}	2.96±0.16^{a}	2.87±0.25^{a}	2.59±0.11^{a}
Feed intake (g/fish)	6.01±1.31^{a}	5.78±0.81^{a}	5.62±0.59^{a}	5.24±0.58^{a}
Feed conversion ratio (FCR)	1.65±0.20^{b}	1.63±0.13^{b}	1.69±0.36^{b}	2.02±0.34^{a}
Protein efficiency ratio (PER)	2.04±0.27^{b}	2.05±0.16^{b}	2.04±0.44^{b}	1.68±0.26^{a}
Survival rate (%)	86.00±8.71	90.00±2.00	91.33±1.15	94.00±4.00

** Values are means ± SD. Means in the same row with different superscript letters are significantly different ($P \leq 0.05$).*

Best feed conversion ratio (FCR) (1.63) was detected when fish given Pom3 followed by fish fed CTRL without significant differences among all treatments and fish fed Pom9 was the worst value (2.02). Feed consumption decreased across all treatments, Fish given the Pom9 diet had the lowest value, but not differed substantially ($P \leq 0.05$). Entirely groups had also no significant changes ($P \geq 0.05$) in protein efficiency ratio (PER), and again fish fed Pom9, achieved the lowest value.

Fish body composition and antioxidant properties:

Table (4) displays how pomegranate peel supplementation in diets affects fish body composition. Dry matter, protein and ash contents of the fish body shows insignificant different among the treatments ($P \geq 0.05$). The lowest ash proportion was seen in fish fed the CTRL diet which is 14.71. Moreover, fish fed the Pom9 diet gained the lowest lipid content which differed significantly ($P \leq 0.05$) among all treatments.

Table (5) shows the antioxidant activity in mono-sex Nile tilapia, *Oreochromis nioticus* fries after 84 days of feeding diets enriched with Pomegranate peel. As a result, SOD, superoxide dismutase; TAC, total activity capacity; Cat catalase and GSH, glutathione peroxidase levels increased significantly ($P \leq 0.05$) with the increase level of pomegranate peel. On the other hand, Malondealdihye (MDA) and, the levels of

ALP, alkaline phosphatase in the studied groups reduced significantly ($P \leq 0.05$) when the level of pomegranate peel is increase, with the lowest values seen in fish fed Pom9 diet.

Table (4): Effects of pomegranate peel meal supplemented diets on body composition of mono-sex Nile tilapia, *Oreochromis niloticus*.

Parameters (%)	Initial	CTRL	Pom3	Pom6	Pom9
Dry matter	26.92	26.81±0.35	27.09±0.42	27.96±0.42	27.00±1.81
Protein	67.54	64.74±1.64	64.21±0.98	64.35±0.01	64.70±0.38
Lipid	18.77	20.55±0.61^{b}	20.49±0.01^{b}	20.36±0.28^{b}	19.20±0.18^{a}
Ash	13.69	14.71±1.16	15.30±1.77	15.29±0.81	16.10±0.81

** Values are means ± SD. Means in the same row with different superscript letters are significantly different ($P \leq 0.05$).*

Table (5): Effects of dietary pomegranate peel on antioxidant status in the liver of mono-sex Nile tilapia, *Oreochromis niloticus* fries fed experimental diets for 84 days.

Items	Treatments			
	CTRL	Pom3	Pom6	Pom9
Superoxide Dismutase (SOD)	15.66 ±0.58^{a}	20.33±2.08^{b}	29.67±3.05^{c}	31.00±2.65^{c}
Total antioxidant capacity (TAC)	18.67±2.08^{a}	29.33±1.15^{b}	43.33±2.08^{c}	48.33±2.15^{d}
Catalase (Cat)	13.33±0.58^{a}	16.67±0.58^{b}	19.00±1.00^{c}	21.33±0.58^{d}
Glutathione peroxidase (GSH)	57.67±3.05^{a}	64.33±3.05^{a}	73.67±5.51^{b}	77.33±3.51^{b}
Malondialdehyde (MDA)	4.00±0.30^{c}	3.59±0.34^{ab}	3.53±0.17^{ab}	3.33±0.12^{a}
Alkaline Phosphatase (ALP)	59.33±3.21^{d}	49.33±1.53^{c}	43.33±2.08^{b}	38.33±1.15^{a}

** Values are means ± SD. Means in the same row with different superscript letters are significantly different ($P \leq 0.05$).*

Histopathological examination:

Nile tilapia, *O. niloticus* fed CTRL diet without any addition of pomegranate peel showing normal histological appearance of liver and intestine. Comparing with control group, tilapia fed diet supplemented with pomegranate peel at the levels of 3, 6 and 9 g/kg diet showing commonly normal appearance of liver and intestine. The liver only showed lipid accumulation in the cytoplasm of hepatocytes, which increased as the amount of pomegranate peel rose (Figure 1).

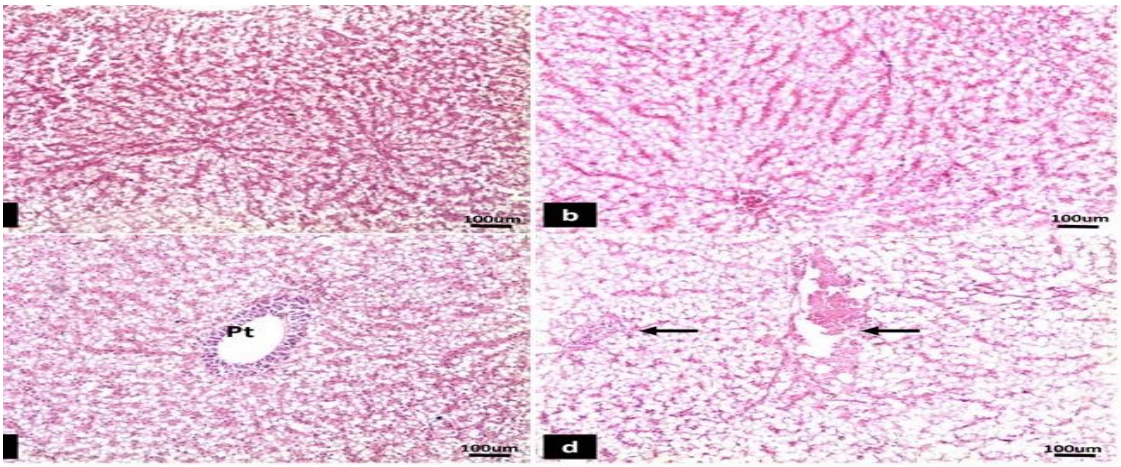

Figure (1): Liver of Nile tilapia, *Oreochromis niloticus* fed diets supplemented with different levels as 0, 3, 6 and 9 g/kg diet of pomegranate peel for 84 days defined as a, b, c, and d, respectively.

The experimental fish's intestines had more goblet cells in the mucosa, which rose steadily as the amount of pomegranate peel increased, as seen in (Figure 2).

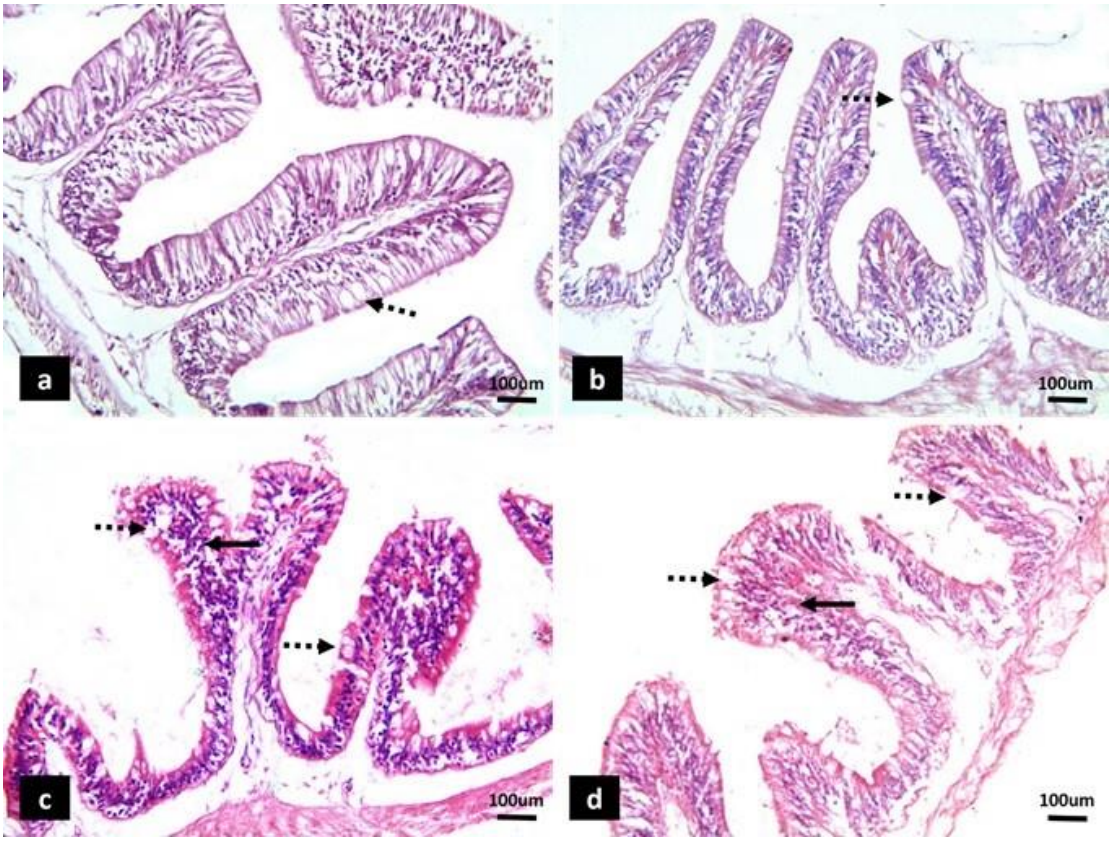

Figure (2): Intestine of Nile tilapia, *Oreochromis niloticus* fed diets supplemented with different levels as 0, 3, 6 and 9 g/kg diet of pomegranate peel for 84 days defined as a, b, c, and d, respectively.

DISCUSSION

Pomegranate peel chemical composition, phenolic constituents, and antioxidant properties:

Carbohydrates (66.50%) is a major abundant of chemical components in pomegranate peel meanwhile fat (0.94%), and protein (1.21%) were all lower than previously observed Rowayshed *et al.,* (2013) had. Whereas fiber in the pomegranate peels (22.12 %) showed a higher value. Generally, our findings are consistent with those published in the literature by various authors (Galaz *et al.,* 2017; Hasnaoui *et al.,* 2014; Topkaya and Isik, 2019).

The peelings contained 12.61–62.09 % dietary fiber, 0.40–9.79 % oil, 0.70–9.08 % protein, 2.70–5.49 % ash, and 1387.00–86700.00 mg GAE/100 g total phenolic compounds, according to the author. As a result, the peelings could provide a good source of fiber, ash, besides carbs. Polyphenols, a type of natural radical scavenging molecule, are important in preventing the effect of free radicals in the body. A real amount of phenolic compounds (punicalin, punicalagin, ellagic acid, and gallic acid) induced the biological activity of pomegranate peel. Among the phenolic acids (Gallic, ellagic, vanillic, caffeic, ferulic, cinnamic, and p-coumaric acids) were found in pomegranate peel (Singh *et al.*, 2018 and El-Hadary and Ramadan, 2019). Pomegranate varieties might be differed in the contents of phenols (Singh *et al.*, 2018 and Bar-Ya'akov *et al.,* 2019). Tunisian pomegranate peel, contained 7.3 mg/g of Ellagic (Li *et al.,* 2016), but Spanish varieties contained 16.5 mg/g (Rosas-Burgos *et al.,* 2017).

On the contrary, El-Hadary and Ramadan (2019) found that the primary phenolic acids in Egyptian pomegranate varieties (Wonderful variety) were 12.56 mg/g ellagic, 2.5 mg/g gallic, 2.5 mg/g cinnamic, 1.56 mg/g chlorogenic, besides 0.91 mg/g coumarin. Meanwhile, Li *et al.* (2016) discovered higher quantities 2.59 mg/g and 2.83 mg/g of gallic acid and ellagic acid in several extracts of Chinese peelings.

Influence of dietary pomegranate peel on growth performance of mono-sex Nile tilapia fries:

The use of antibiotics and other chemotherapeutics for treating diseases has been criticized because of their negative effects on both animals and humans. Natural promoters such as vegetables, herbs, spices, and edibles are considered safe, inexpensive, and effective alternatives. Plants and their extracts are commonly used as an unconventional treatment option for feed additives, growth enhancers, and immune stimulants (Badri *et al.*, 2021). Our finding showed that the at certain level, increasing the amount of pomegranate peel in the diets can resulted in a decrease in fish growth and feed intake and this is concordance with the finding from Fayed *et al.* (2012) who found that The final weight and growth of rabbit bucks fed diets supplemented with pomegranate peel at levels of 5, 10 and 15 g/kg decreased compared to the control group. Similarly, Nile tilapia fed high quantities of pomegranate meal showed slower growth and lower feed consumption (Badrey *et al.*, 2019). Several factors could be responsible for the decrease in the growth such as pomegranate peel contain antioxidants, polyphenols, as well as high fiber content that can cause a decrease in food consumption and calorie intake as well as impairing digestive enzymes (Madrigal-Carballo *et al.*, 2009). Pomegranate peel addition in the diets enhanced the feed conversion ratio (FCR). The rainbow trout, *Oncorhynchus mykiss*, showed similar outcomes (Avaveh *et al.*, 2020).

When pomegranate peel was added to the diet of mono-sex Nile tilapia, *Oreochromis niloticus* fries, in comparison to control group, the increasing trend was observed in their survival rate without substantial variances ($P \geq 0.05$).

Fish body composition and antioxidant activity as a result of pomegranate peel:

In regards to the fish body composition (Table 3), there were no significant variations in dry matter and ash across any of the treatments. However, a slight increase in the ash content of fish body with the increased levels of pomegranate peel in the diets was found.

Superoxide dismutase (SOD); glutathione peroxidase, and others (GSHPx), as well as catalase (CAT), are among the antioxidants found in aquatic species and in the enzymatic system, they are the first line of defence against oxidation (Ighodaro and Akinloye, 2017). The findings demonstrated that adding pomegranate peel to the diet had a positive effect on the antioxidant activity of the fish liver. In general, the activities of total antioxidant capacity (TAC); glutathione peroxidase (GSH); superoxide dismutase (SOD) and catalase (CAT) in fish given dietary pomegranate peel increased significantly (Table 4). On the contrary, Malondealdihye (MDA), is the metabolic consequence of lipid peroxides and ALP, Alkaline Phosphatase is a cell damage indicator enzyme.

Result from this study showed that feed supplemented with pomegranate peel meal can decrease tissues damage and improve oxidative stress resistance in mono-sex Nile tilapia, *Oreochromis niloticus* fries, and this could be due to pomegranate peels contain antioxidants materials. Our findings are in line with the previous research which examined the effect of dietary orange peel in sea bream diets at dosage of 0, 1, 3, and/or 5 g/kg diet (Salem *et al.*, 2019). Furthermore, different doses of the orange peelings in the diets for Nile tilapia under stress at 0, 2, 4, 6, and/or 8 g/kg were examined by Vicente *et al.*, (2019) who revealed an improvement in Superoxide Dismutase (SOD); Total antioxidant capacity (TAC); Catalase (CAT), and Glutathione peroxidase (GSH), caused by heat and dissolved oxygen. Giri *et al.* (2016) discovered that dietary banana peel flour increased *Labeo rohita*'s antioxidant activity. This showed that some fruits peels contain active substances which may inhibit antioxidant activity and reduce responsiveness of the oxygen intermediate system.

Histopathological investigation:

The liver composition of the fish was examined after 84 days of feeding on dietary pomegranate peel and showed normally. Comparable findings were reported (Salem *et al.*, 2019) when the larvae of Gilthead Sea bream, *Sparus aurata* were fed diets including orange peel for 60 days, the tested fish's intestine had a normal structure. Increasing the inclusion level of the peelings resulted in an increase in the number of goblet cells. These results demonstrating the biosafety of dietary pomegranate peel on fish.

CONCLUSION

The results show that supplementary pomegranate peel meal boosted Superoxide Dismutase (SOD); Total antioxidant capacity (TAC); Catalase (CAT); Glutathione peroxidase (GSH), besides lowered Malondealdihye (MDA) and Alkaline Phosphatase (ALP) in mono-sex Nile tilapia, *Oreochromis niloticus* fries. This improves the health status of fish because of the stimulating substances in the pomegranate

peelings by way of natural antioxidants to maintain fish health and enhancing the growth. Further studies are necessary to conduct transcriptional investigations on the medicinal activities of pomegranate peel.

DECLARATIONS

Human and Animal Ethics

The experiment was conducted in accordance to the recommendations of the Guide for the Care and Use of Laboratory Animals approved by the Committee on the Ethics of Animal Experiments, University of Menoufia, Egypt. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Competing Interests

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors have no relevant financial or non-financial interests to disclose

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Authors' contributions

Ebtehal EL-Sayed Hussein contributed in the conceptualization, data curation and wrote the original draft. El-Beltagy, Alaa designed the methodology, monitored and supervised the experiment. Naeem, M.A. conducted the formal analysis. Eman A. Hussein performed the statistical analysis and edited the final draft of the paper. All authors reviewed the manuscript.

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تقييم مسحوق قشر الرمان كإضافة غذائية في علائق زريعة أسماك البلطي النيلي وحيد الجنس

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تم إضافة مسحوق قشر الرمان بمعدل 3 , 6 و9 جم لكل كجم عليقة وتغذيتها لزريعة البلطي النيلي لمدة 84 يوم لتقييم القيمة الغذائية ونشاط مضادات الأكسدة وهستولوجي الكبد والأمعاء ونمو زريعة البلطي النيلي وحيد الجنس. لم تظهر أي اختلافات معنوية في معدل النمو النهائي والزيادة في الوزن ومعدل النمو النوعي بين كل المعاملات. وكذلك لوحظ الاتجاه التناقصي في معدل تناول الغذاء بزيادة إضافة مسحوق قشر الرمان. معدل التحول الغذائي للعليقة التي تحتوي على 9 جم لكل كجم عليقة من مسحوق قشر الرمان (POM9) كان الأسوأ (2.02) مقارنة بالمجموعات الأخري، بينما العليقة التي تحتوي علي 3 جم لكل كجم عليقة من مسحوق قشر الرمان كانت الأفضل في معدل التحول الغذائي (1.63) بدون اختلافات معنوية مع المجموعات الأخري فيما عدا مجموعة (POM9). لم تظهر اختلافات معنوية في معدلات الحيوية بين كل المعاملات، لكن زيادة مسحوق قشر الرمان نتج عنه زيادة متوسطة.

يحتوي مسحوق قشر الرمان على تركيزات معنوية من حمض الفينوليك والذي نتج عنه نشاط عالي لل DPPH scavenging (81.72) وكان Pyrogallol متوفر بمعدل (927.45 mg/100gm powder). زيادة مسحوق قشر الرمان أدي إلى زيادات معنوية في نشاط مضادات الأكسدة مثل السوبر أوكسيد ديسموتيز (SOD) والسعة الكلية لمضادات الأكسدة (TAC)والكتاليز (CAT)بجانب الجلوتاثيون بيروكسيد (GSH) وأدي إضافة مسحوق قشر الرمان إلى انخفاض ملحوظ في الMDA والالكالين فوسفاتيز (ALP). الأسماك التي تغذت على علائق مضاف لها مسحوق قشر الرمان أظهرت مظهر طبيعي لأنسجة الكبد والأمعاء عند مقارنتها بالكنترول. ومن ذلك فإن إضافة مسحوق قشر الرمان ربما يعزز من أداء النمو وكذلك صحة زريعة البلطي النيلي وحيد الجنس.