Growth performance, feed utilization and gut histology of monosex Nile tilapia (*Oreochromis niloticus*) fed with varying levels of pomegranate (*Punica granatum*) peel residues

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Abstract. A large quantity of pomegranate peel (PP) residues is produced in juice manufacturing, representing a valuable waste of the food industry as they contain bioactive compounds. A study was conducted to investigate the appropriate levels of PP residues in the diet of monosex Nile tilapia (*Oreochromis niloticus*) to improve survival, growth performance, feed utilization, whole body composition and gut histology. Monosex *O. niloticus* (7.0 g) were tested with eight levels of PP: 0, 1, 2, 3, 5, 10, 15, and 20%. Fish were stocked in aquaria (100×40×50 cm), at a density of 15 fingerlings per group and fed on a pelleted diet (~25% crude protein, CP), 3 times daily for 90 days. Fish quality in terms of whole body composition showed the best level at 5% PP and the lowest at 20% PP level. The results of the present work revealed that body weight, weight gain and specific growth rate for the fish fed diets contained lower different levels of PP were non-significantly reduced ($p > 0.05$) compared to the control group. Based on the obtained results, the fish fed on 5% and 3% PP levels exhibited the best performance and the best feed utilization indices. This was evidenced by the best protein efficiency ratio and the highest energy utilization for those fish. This was supported also by the increase in the length of villi and the number of goblet cells of monosex *O. niloticus* fed on 3 or/and 5% level PP. It is recommended to add the PP to the diets of monosex *O. niloticus* up to 5% to enhance body composition of fish, feed utilization and fish integrity with increasing economic efficiency.
Key Words: Nile tilapia, pomegranate peel, gut histology, waste, food industry.

Introduction. The use unconventional less expensive agro-industrial by-products attracted the attention of many researchers in the field of feed industry in Egypt to produce high quality products to satisfy customer needs in a cost-effective manner (El-Sayed et al 2014). Pomegranate (*Punica granatum*) is a native Mediterranean plant that has been widely used by ancient Egyptians in the folk medicine (Tayel & El Tras 2010). According to Food and Agriculture Organization (FAO), pomegranate production is about 1.5 million tons all over the world (Eikani et al 2012). It consists of edible part, seeds, and peel. Pomegranate fruits are usually consumed after separation of seeds from the peels and the seed are use in juice manufacturing. The pomegranate peel (PP) constitutes 5 to 15% of its total weight (Orzua et al 2009). The disposal of such large quantity of PP waste is an environmental problem (Kanatt et al 2010), representing a valuable waste of the food industry as they contain bioactive compounds especially, antioxidant polyphenols, mainly hydrolysable tannins and anthocyanins (Çam & Hışıl 2010; Ibrahium 2010). PP has higher anti-oxidant levels than the juice itself, an attractive candidate as a nutritional supplement for animals feed. Li et al (2006) reported that PP provides higher yield of phenolics, flavonoids and proanthocyanidins than the pulp. Siddhuraju & Becker (2003) postulated that PP extract (PPE) had a strong biological activity and also enhanced liver and kidney functions in animal models. In addition, PPE is used for the prevention and treatment of many major health problems. In particular, PP extract has extensively been studied for its strong anti-microbial effect, high anti-oxidant activity (Ibrahium 2010), cytotoxic effect, hypoglycemic effect (Rajput et al 2011), hypolipidemic effect (Belkacem et al 2010), hepatoprotective effect and anti-inflammatory activity (Jurenka 2008). Also, PP has no side effects and no known drug interactions and may be the most potent way to prevent cancer; strengthen the immune system (Lee et al 2008), prevent heart disease, prevent liver fibrosis, promote wound healing and strengthen connective tissue which may keep cancer cells from spreading (Murthy et al 2004). Studies have proved that herbal extract as an additives enhanced the growth of fishes and also protected from the diseases (Johnson & Banerji 2007; Toutou et al 2018) but studies related to pomegranate application in fishes on the growth and disease scanty. It was necessary to throw some more light on these plants concerning their effects on fish growth performance. The digestive system of teleost fishes has been widely studied and described morphologically to determine the function of many specialized anatomical structures in relation to the different feeding adaptation (Cataldi et al 1987). Histological analysis of the digestive system is considered a good and immediate indicator of the nutritional status of fish (Hall & Bellwood 1995; Caballero et al 2003). Raskovic et al (2011) reviewed the histological methods in the assessment of different feed effects on liver and intestine of fish. The intestine and the liver are the most important organs in digestion and absorption of nutrients from ingested food, and therefore monitoring of these organs is considered necessary for assessing the effects of ingredients used as raw materials of animal/ plant origin (Roberts 1989). Hence, the objective of the present study was designed to examine the effect of PP as natural feed additives or replacement on growth performance feed utilization and gut histology of monosex Nile tilapia (*Oreochromis niloticus*).

Material and Method

Experimental design. One thousand monosex *O. niloticus* fingerlings were obtained from a private fish farm in Fayoum, Governorate, Egypt and were transported to aquarium Lab in the Department of Zoology, Faculty of Science, Al-Azhar University (Assiut branch) from August to November 2016. The average initial weight of the fish was 7.00±0.2 g and the average initial length was 5.3±0.6 cm. The fish were acclimatized for 7 days in glass aquaria and fed diet (25% crude protein) before the start of the experiments. Live fish were divided into 24 groups (15 fingerlings per group) and transferred to 24 aquaria (100×40×50 cm), providing eight experimental groups in triplicates, ensuring high water quality (conductivity 2000 µs cm^{-1}; pH ~7.6; oxygen 5.6±0.3 mg L^{-1}; temperature 25ºC; total NH_3-N 0.095±0.003 mg L^{-1}; photoperiod 12:12 light:dark). The aquaria were daily cleaned and excreta were siphoned. The siphoned water was replaced with freshwater before the first feeding in the morning. Water quality parameters were monitored on a weekly basis throughout the experimental period using standard APHA methodology (1995).

Experimental diets. All the ingredients of the experimental diets were purchased from local market at reasonable price. PP used for this study was locally prepared in the lab during the progress of the experiment. The diet used in the experiment was formulated to cover all nutrients required for tilapia as recommended by the NRC (1993). Pomegranate fruits were obtained from the local market at Assiut Governorate, Egypt. The fruits were washed by water then peeled and their edible portions were carefully separated. The peels were dried in an oven at 60°C for 48 hours until the moisture content reached about 8% (dry basis). Dry ingredients were finely ground by using a Lab-Mill, sieved, and then manually mixed to assure their homogeneity. A basal control diet was formulated to fulfill the nutrients requirement of fish contained 25% CP and 448.3 kcal/100g (Table 1). The basal diet was used as the control, and seven other experimental diets were formulated to represent eight dietary treatments. A three-month feeding trial was conducted in *O. niloticus* fingerlings to determine the amount of PP that could replace yellow corn and rice brine in formulated diets without reducing growth performance. PP powder was added to the components of experimental fish diet at rate of 1, 2, 3, 5, 10, 15, and 20%. The diet was molded into small balls and then passed through a commercial meat grinder to be a spaghetti-like. The diet was spread on a metal plate, and dried in an oven at 60°C for 36 hours. Dried diets were chopped into pellets and sieved through standard sieves to separate the pellets into an appropriate size suitable for the experimental fish size of 1 mm diameter. Diets were stored in labeled plastic bags and stored in the freezer at -20^{o}C until used. *O. niloticus* fingerlings were fed test diets of 5 to 7% of the total biomass of the fish per day. The amount of feed was divided into three equal portions and distributed by hand in one side of the aquaria three times daily at 9 a.m., 1 p.m. and 3 p.m., 6 days a week for 90 days. Fish in each aquarium were counted and weighed (collectively) biweekly throughout the feeding trials. The total amount of feed consumed by the fish in each aquarium during the study period was determined, and the feed consumed for each individual fish was calculated accordingly.

***Experimental analysis**.* Fish samples were netted from tank at the period of the feeding trial. They were pooled and homogenized for proximate composition. Moisture, total protein, lipid and ash contents were all determined by Standard Association of Official Analytical Chemist methodology (AOAC 2002). Feed sample was used for proximate analyses (Table 1).

***Evaluation of growth performance and feed utilization efficiency**.* Growth performance and feed utilization including weight gain (WG, g), specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR) and protein efficiency ratio (PER), were determined as follows: WG = (final weight, FW) – (initial weight, IW) (g/fish), SGR = 100 × [(FW) - (IW)] / experimental days, and FCR = feed fed (g) (dry weight) / WG (g). Protein efficiency ratio (PER) = (WG - FI), protein productive value (PPV) = 100 (PI/PF) and energy utilization (EU %) =100 (E.g. / EI). Where Iw and Fw = mean initial and final body weight (g), respectively, t is the duration of the experiment (98 days), FI is the total feed intake (g), PI is protein intake (g), PF is protein fed (g), EG is the energy gained (Kcal/100g) and EI is energy intake (Kcal/100 g).

***Histological examination**.* For histological examination, five fish from each tank were sacrificed by decapitation. Intestine and liver were immediately dissected out, fixed in 10% neutral buffered formalin for 24 hours, processed by conventional method, sectioned at 5 µm thickness using a rotary microtome, and then stained with haematoxylin & eosine (H&E) (Bancroft & Layton 2013). Periodic acid-Schiff (PAS) stain was also performed on the liver sections. Slides were examined under light microscopy (Motic microscop BA310 LED FL). Each slide was photographed with a DVC digital camera (HDCE-50 B). Twenty measurements for villi height (µm) were taken from each intestine slide using Image J (1.46) software. Baeverfjord & Krogdahl (1996) method was used to count goblet cells in each segment.

***Statistical analysis**.* Data were presented as mean±SD. The results were subjected to one-way analysis of variance (ANOVA) to test the effect of treatment inclusion on fish performance. Data were analyzed using SPSS program, Version 16. Differences between means were compared using Duncan's (1955) multiple range test at $p < 0.05$ level.

Table 1

The composition and chemical analyses (% on dry matter basis) of the experimental diets

Ingredient (g kg-1)	*Composition (%) of experimental diets*							
	Control	*1%*	*2%*	*1%*	*5%*	*10%*	*15%*	*20%*
Fish meal (65%)	70	70	70	70	70	70	70	70
Soybean meal	250	250	250	250	250	250	250	250
Corn gluten	80	80	80	80	80	80	80	80
Yellow corn	100	90	80	70	50	0	0	0
Pomegranate peel	0	10	20	30	50	100	150	200
Wheat bran	150	150	150	150	150	150	150	150
Rice brine	300	300	300	300	300	300	250	200
Fish oil	20	20	20	20	20	20	20	20
Premix[1]	30	30	30	230	30	30	30	30
Total	1000	1000	1000	1000	1000	1000	1000	1000
	Chemical composition (%)							
Dry matter (DM)	93.1	93	93.4	93.9	93.6	94.3	93.2	93.6
Crud protein (CP)	25	25.1	25.3	25.1	25.1	24.8	24.9	24.8
Ether extract	10.1	10.1	9.6	9.3	9.5	9.3	8.8	8.5
Crude fiber	5.7	5.6	5.8	5.7	5.9	5.9	6.1	6.2
Ash	7.7	7.7	7.8	6.9	7.2	8.6	7.9	7.8
Nitrogen free extract	51.5	51.5	51.5	53	52.3	52.2	52.3	52.7
M. E. (kcal/100g)[2]	374.0	374.4	371.3	373.2	372.4	365.5	365.8	364.3
D. E. (kcal/100g)[3]	396.1	396.6	393.1	394.7	394.0	386.4	386.7	384.9
G. E. (kcal/100g DM)[4]	448.3	448.9	445.2	447.4	446.4	437.9	438.7	436.9

[1]Premix composition: each 1 kg contains Vit A (400000 i.u.), Vit D3 (100000 i.u.), Vit E (230 mg) Vit K3 (165 mg) Vit B1 (300 mg), Vit B2 (80 mg), Vit B6 (200 mg), Vit B12 (1 mg), Vit C (650 mg), Niacin (1000 mg), Methionine (3000 mg), Choline chloride (10000 mg), Folic acid (100 mg), Biotin (2 mg), Pantothenic acid (220 mg), Magnesium sulphate (1000 mg), Copper sulphate (1000 mg), Iron sulphate (330 mg), Zinc sulphate (600 mg), Cobalt sulphate (100 mg), Calcium carbonate up to 1000 g; [2]Metabolizable energy (M E) was calculated as 4.5, 8.1 and 3.49 kcal/100g for protein, lipid and NFE, respectively according to Pantha (1982); [3]Digestible energy (DE) was calculated as 5, 9, and 3.5 kcal/100g for protein, lipid, and carbohydrates respectively; [4]Gross energy (GE) was calculated as 5.64, 9.44 and 4.11 kcal/100g for protein, lipid and NFE, respectively (NRC 1993).

Results. The chemical composition of PP in the present experiment was analyzed. Averages of PP composition were recorded to be moisture (72.1±1.2), dry matter (27.9±1.2), crude protein (3.7±0.8), ether extract (2.2±0.3) and ash contents (4.2±0.4) at the initial phase of experiment. Survival, growth performance, and condition factor of monosex *O. niloticus* tested with different levels of PP are presented in Table 2. PP levels have a significant ($p \leq 0.05$) effect on the survival of the juvenile monosex *O. niloticus* after 90 days of feeding trial. Fish reared at 0% exhibited the lowest significant survival percent (83.3%). Significant elevation was observed in the survival rate of the fish fed on different levels of PP compared to the control non-significant differences ($p \leq 0.05$) were detected in the survival of fish fed on different level of PP (excluded 20%).

The growth performance parameters were investigated bi-weekly during the experiment. The average of initial weight of fish was 7.1±0.1g. Statistical insignificant ($p \leq 0.05$) differences in final weight, weight gain and SGR were found among the tested PP levels (excluded 15% and 20%) compared to the control (Table 2). The obtained data showed that growth performance was insignificantly affected by PP levels. Fish reared at 20% level showed the lowest final weight (28 g), weight gain (20.9) and SGR (2.3% day^{-1}).

At the end of the feeding assay, feed utilization efficiency indices, for all experimental treatments (0-20%) are given in Table 2. It is obvious that the lowest (best) FCR was recorded at 1% level (1.6) followed by 0% level (1.7), whilst the worst FCR (2.0) was obtained in 20% level. Also, the best PER (2.3) was recorded in 3 and 5% level dietary group, whereas the lowest value was at 10 and 15% level. Similarly, protein productive value (PPV) exhibited the highest values (43.3 and 42.8) for 5 and 3%, respectively, which were significantly ($p < 0.05$) higher than those for fish of all treatments. In the same trend, the highest energy utilization record was obtained for 5% fish; which was comparable with that of all treated groups.

Biochemical composition of fish. The effect of PP levels on whole body composition of fish in the present experiment are illustrated in Table 3. Moisture contents were the best when fish fed 5% PP levels also crude protein. The highest significant ($p \leq 0.05$) content of protein (18.1%) and the lowest significant ($p \leq 0.05$) content of crude lipids (5%) of fish body composition were detected at 20% levels. Ash content showed significant ($p \leq 0.05$) differences among the tested PP levels. However, the lowest retention of crude protein at 15% levels and moisture were observed at 20% levels.

At the end of experiment, the height and width of villi and the number of their goblet cells were measured in the anterior and posterior intestine for each of the control and treated groups. The results are shown in Table 4 and Figures 1 and 2. The present results showed remarkable variations among treatments. Significant increment in villi length, width and absorption area were recorded in fish fed 3 and 5% PP levels diets compared to the control group. Significant reduction in villi length and width in fish fed 15 and 20% levels PP. The highest number of goblet cells was recorded in 5% group (16.3) followed by 3% (15.6). In general, histological analysis showed satisfactory records in fish fed high levels compared to low levels of PP diets. In the anterior and posterior intestine of *O. niloticus* fed on 3 and 5% PP showing well-developed and branched villi (lamina propria) high distribution of goblet cells, with strong appearance of lymphocytes infiltration. Meanwhile of the anterior and posterior intestine of *O. niloticus* fed on 10% PP showing short branched villi (lamina propria), few distributions of goblet cells, with weak appearance of lymphocytes infiltration.

Table 2

Growth performance and feed utilization indices of fish of mono sex *O. niloticus* as affected with different levels of pomegranate peel (*Punica granatum*) during the experiment

Items	*Experimental diets*							
	0%	*1%*	*2%*	*3%*	*5%*	*10%*	*15%*	*20%*
IW (g $fish^{-1}$)	7.1±1.0	7.1±1.0	7.1±1.0	7.1±1.0	7.1±1.0	7.2±0.5	7.1±1.0	7.1±1.0
FW (g $fish^{-1}$)	$33.6±1.7^{a}$	$32.8±3.6^{a}$	$31.0±2.7^{ab}$	$30.7±1.7^{ab}$	$30.7±0.8^{ab}$	$30.5±1.7^{b}$	$30.3±1.3^{b}$	$28.0±1.3^{c}$
TWG (g $fish^{-1}$)	$26.5±2.7^{a}$	$25.7±4.7^{a}$	$23.9±3.6^{ab}$	$23.6±2.2^{ab}$	$23.6±3.7^{ab}$	$23.3±1.8^{ab}$	$23.2±3.8^{ab}$	$20.9±0.8^{b}$
SGR (% d^{-1})	$2.7±0.3^{a}$	$2.6±0.2^{a}$	$2.5±0.1^{a}$	$2.5±0.2^{a}$	$2.5±1.0^{a}$	$2.5±0.3^{a}$	$2.4±0.2^{b}$	$2.3±0.2^{c}$
FCR	$1.7±0.0^{b}$	$1.6±0.1^{bc}$	$1.9±0.2^{a}$	$1.9±0.2^{a}$	$1.9±0.1^{a}$	$1.9±0.0^{a}$	$1.9±0.1^{a}$	$2.0±0.1^{a}$
PER	$2.0±0.1^{bc}$	$2.1±0.3^{ab}$	$2.2±0.2^{ab}$	$2.3±0.2^{a}$	$2.3±0.2^{a}$	$1.7±0.3^{c}$	$1.7±0.4^{c}$	$2.0±0.4^{bc}$
PPV (%)	$40.2±1.6^{ab}$	$40.8±1.5^{ab}$	$40.9±0.9^{ab}$	$42.8±0.6^{a}$	$43.3±0.8^{a}$	$38.4±1.4^{c}$	$37.8±1.2^{c}$	$40.3±1.6^{ab}$
EU (%)	$9.8±0.3^{c}$	$10.3±0.2^{ab}$	$10.0±0.2^{bc}$	$10.9±0.4^{a}$	$11.1±0.4^{a}$	$9.7±0.1^{c}$	$9.8±0.2^{bc}$	$9.8±0.3^{c}$
SR (%)	$83.3±2.3^{c}$	$96.4±1.0^{a}$	$96.6±1.0^{a}$	$93.3±1.0^{ab}$	$93.3±1.0^{ab}$	$93.3±1.0^{ab}$	$92.8±1.0^{b}$	$90.0±1.0^{bc}$
K	$1.2±0.1^{b}$	$1.2±0.2^{b}$	$1.2±1.2^{b}$	$1.3±1.1^{ab}$	$1.2±1.5^{b}$	$1.2±1.6^{b}$	$1.4±1.4^{a}$	$1.4±1.5^{a}$

Means in the same row with different superscript letters are significantly different at ($p < 0.05$).

Table 3

Biochemical composition of fish feeding trial (wait weight basis)

Items	*Experimental diets*								
	Initial	*0%*	*1%*	*2%*	*3%*	*5%*	*10%*	*15%*	*20%*
Moisture	74.7±0.4	$71.3±1.0^{ab}$	$71.1±0.4^{ab}$	$70.0±1.2^{bc}$	$70.0±0.8^{bc}$	$68.1±1.5^{c}$	$72.4±1.0^{a}$	$72.9±1.3^{a}$	$73.1±0.8^{a}$
Crude protein	16.9±1.3	$17.5±0.4^{ab}$	$18.0±0.3^{a}$	$17.9±0.1^{ab}$	$18.0±0.4^{a}$	$18.1±0.6^{a}$	$16.7±0.3^{ab}$	$16.5±0.6^{b}$	$17.4±0.6^{ab}$
Crude lipid	3.6±1.1	$6.3±0.2^{d}$	$7.1±0.1^{cd}$	$7.9±0.1^{b}$	$8.0±0.2^{b}$	$9.4±0.3^{a}$	$7.1±0.2^{cd}$	$6.6±0.2^{cd}$	$5.0±0.3^{f}$
Ash	4.6±0.1	$3.9±0.1^{ab}$	$3.9±0.1^{ab}$	$3.6±0.2^{bc}$	$3.9±0.0^{ab}$	$3.9±0.1^{ab}$	$3.5±0.1^{c}$	$3.7±0.1^{bc}$	$4.2±0.2^{a}$

Means in the same row with different superscript letters are significantly different at ($p < 0.05$).

Table 4

Measurements of anterior posterior-gut morphology of mono sex Nile tilapia *Oreochromis niloticus* fish at end of feeding trial

Items		*Experimental diets*							
		0%	*1%*	*2%*	*3%*	*5%*	*10%*	*15%*	*20%*
Villi length	Anterior	$110±5.9^{ab}$	$98.3±6.0^{b}$	$110±5.8^{ab}$	$125±2.9^{a}$	$120±5.8^{a}$	$71.6±6.0^{c}$	$66.6±8.8^{c}$	$71.6±6.0^{c}$
(μm)	Posterior	$28.3±1.7^{bc}$	$35±2.8^{cd}$	$46.6±3.1^{bc}$	$75±3.4^{a}$	$50±5.9^{bc}$	$56.6±3.3^{b}$	$33.3±8.9^{d}$	$36.6±2.1^{cd}$
Villus width	Anterior	$31.6±4.4^{ab}$	$31.6±1.7^{ab}$	$26.6±3.3^{bc}$	$35.0±2.9^{a}$	$31.6±4.4^{ab}$	$33.3±3.3^{ab}$	$24.0±3.0^{bc}$	$18.3±1.7^{c}$
(μm)	Posterior	$25.0±2.9^{bc}$	$28.3±1.7^{b}$	$16.6±3.3^{cb}$	$23.3±3.1^{bc}$	$36.6±3.5^{a}$	$23.3±1.8^{bc}$	$21.6±3.0^{bc}$	$11.6±1.5^{d}$
Absorbiton	Anterior	$3.1±0.1^{bc}$	$3.1±0.2^{bc}$	$3.0±0.5^{bc}$	$4.4±0.2^{a}$	$3.8±0.6^{ab}$	$2.3±0.2^{cd}$	$1.6±0.1^{df}$	$1.3±0.2^{f}$
area (mm^2)	Posterior	$1.1±0.2^{bc}$	$1.0±0.1^{bc}$	$0.8±0.0^{bc}$	$1.7±0.3^{a}$	$1.8±0.3^{a}$	$1.3±0.0^{ab}$	$0.7±0.2^{bc}$	$0.4±0.2^{c}$
Goblet cells	Anterior	$11.6±0.3^{b}$	$14.6±0.5^{ab}$	$15.3±1.7^{a}$	$15.6±0.9^{a}$	$16.3±0.9^{a}$	$14±1.1^{ab}$	$14.3±0.3^{ab}$	$13.6±0.8^{ab}$
(numbers)	Posterior	$10.6±1.7^{c}$	$13.3±0.7^{abc}$	$13.6±0.9^{ab}$	$14±0.7^{ab}$	$15±0.8^{a}$	$11.3±0.7^{bc}$	$12.3±0.9^{abc}$	$11±0.6^{bc}$

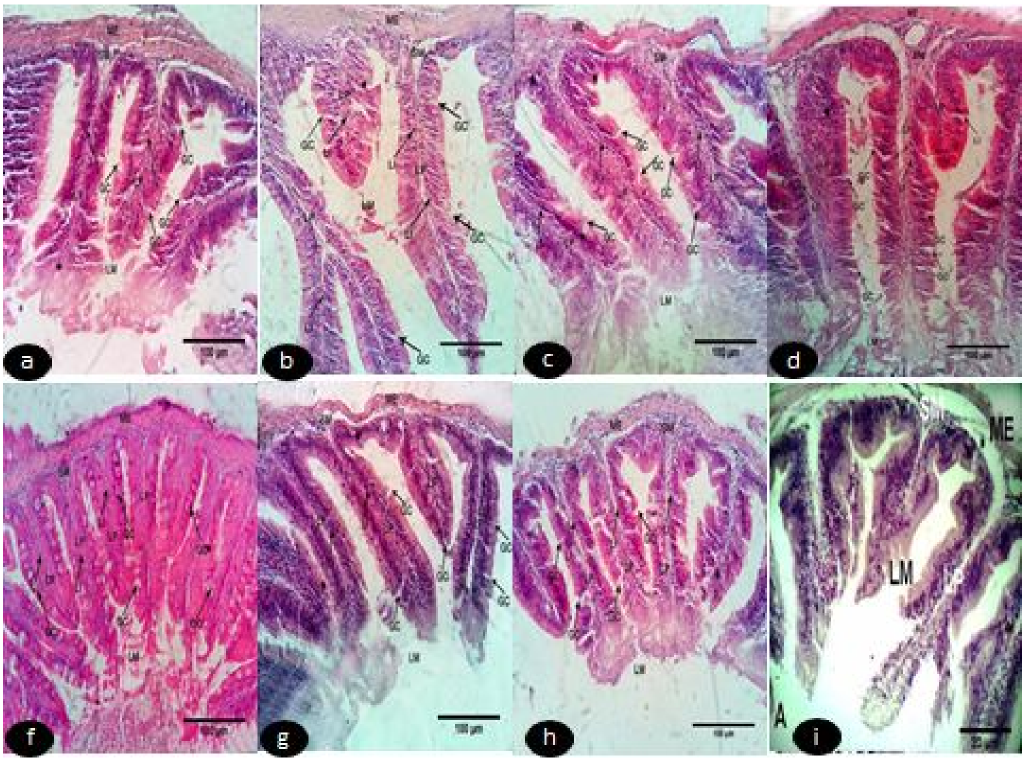

Figure 1. Photomicrographs of the anterior intestine of *Oreochromis niloticus* fed different levels of pomegranate peel (0, 1, 2, 3, 5, 10, 15 and 20%; a, b, c, d, e, f, g, h and i, respectively) showing well-developed and branched villi (lamina propria, LP), muscular layer (ME), mucosal layer (LM) and a high distribution of goblet cells (GC). H&E; bar = 40 µm.

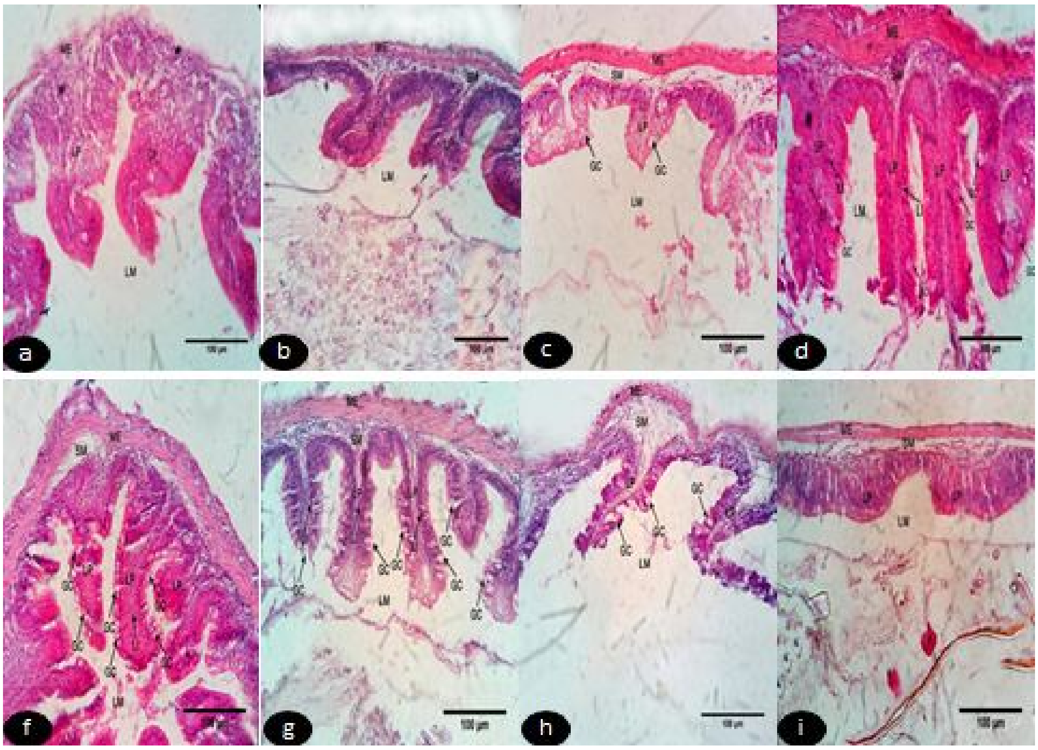

Figure 2. Photomicrographs of the posterior intestine of *Oreochromis niloticus* fed different levels of pomegranate peel (0, 1, 2, 3, 5, 10, 15 and 20%; a, b, c, d, e, f, g, h and i, respectively) showing well-developed and branched villi (lamina propria, LP), muscular layer (ME), mucosal layer (LM) and a high distribution of goblet cells (GC). H&E; bar = 40 µm.

Discussion. A large quantity of PP residues is produced in juice manufacturing; its disposal is an environmental problem locally in Assuit governorate. The results of the present work revealed that FBW, WG, and SGR for the fish fed diets contained different levels of PP were non-significantly reduced ($p > 0.05$) compared to the control group. These results agreed with those reported by Badawi & Gomaa (2016) using PP extract. The same results were also recorded for rabbit bucks fed diets containing different PP levels (0.5, 1.0 and 1.5 %), causing decrease in final body weight and weight gain compared to the control (Fayed et al 2012). The observed weight reduction in the present work could be attributed to presence of considerable amounts of polyphenols together with the high fiber content in the PP which reduced food consumption and the restricted calorie intake (Mahmoud et al 2011).

Polyphenols may suppress growth of the adipose tissue through their antiangiogenic activity and by modulating adipocyte metabolism (Mulvihill & Huff 2010; Baile et al 2011). Some researchers have suggested that mild calorie restriction might provide multiple health benefits such as extending the lifespan of diverse organisms (Gonzalez et al 2004), regulation of immune function (Sun et al 2004). This was also confirmed by Mahmoud et al (2011) who reported that, the rats fed on diets containing dry PP can be used safely to manage body weight or even for weight reduction without any health hazards. Badawi et al (2014) study the effect of partial replacement of yellow corn by PP at rate of 5 or 10% with and without addition of Allzyme at rate of 0.02% on growth performance and health status of *O. niloticus* for 10 weeks. Badawi & Gomaa (2016) reported that the fish fed diets contained different levels of PP extract weren't significantly different with all groups in total final BW, body gain, body gain (%) and total FCR compared to those fed the control diet. The non-significant difference in the growth and nutrient utilization parameter at varying levels support the study of previous result that PP was accepted and utilized by fish. The average daily feed intake and specific growth rate (%) weren't significantly different in all groups except for fish fed diet contained 0.5% PP extract which exhibited a significant decrease if compared to the control diet. According the present results, the best PER, the highest PPV and the highest energy utilization were recorded in 3 or/and 5% level dietary group. This means that the fish fed on 5% and 3% PP levels achieved the best feed utilization indices.

Most of the research conducted on PP and its extracts did not address the chemical composition of the fish body and the flesh quality which is very important because it is a priority of nutrition objectives. The results of the whole body composition in monosex *O. niloticus* exhibited its best level at 5% PP and the lowest level at 20% PP compared to the control. This remarkable improvement in the chemical composition of the fish body may be due to the improvement seen in gastrointestinal histology of the fish fed on the 3% and 5% levels of PP.

The histological structure of digestive system of fish has been well documented (Smith 1981; Domeneghini et al 1998). The intestinal histology of *O. niloticus* as one of aquaculture species is important to understand histological changes promoted by nutritional source (Gargiulo et al 1997). Although fish histological researches gives several information about the gastro-intestinal tract (Calzada et al 1998), the literature needs more information regarding morphological adaptations to diet nutrient variations, which could be driven to diet formulation. Histological analysis of the digestive system is considered as a good indicator of the nutritional status of fish (Hall & Bellwood 1995; Caballero et al 2003) and the variations in structure are useful for the nutritional development researches (Rotta 2003). Histopathological changes in the intestine may vary depending on the species and feed used in the experiments (Dayal et al 2013).

Intestine is crucial to guarantee the nutritional efficiency of the diet as well as animal health. For this reason, this work studied different parameters related to anterior and posterior-intestine morphology of fish fed experimental diets. In the present study, the intestine of fish fed on PP at 3% and 5% level exhibited remarkable increase in the height and width of villi. Length of villi is a useful histological parameter that could be followed in experiments not just related to the use of PP, but also in evaluating different types of commercial feed. An increase in the length of villi is associated with an increase in the surface area for absorption of nutrients (Aanyu et al 2014). The longer villi found in fish fed on PP indicate higher efficiency in the absorptive process (Caballero et al 2003; Da Silva et al 2012). This was evidenced by the best PER and the highest energy utilization recorded in 3 or/and 5% level dietary group. This fact shows that the PP diet promoted an increase in the length of villi of those fish.

The number of goblet cells could vary with the food habit or starvation (De Silva & Anderson 1995). The higher number of goblet cells was recorded in the intestine of fish fed on 3% and 5% diet compared to those fed on control diets and other groups. Goblet cells are associated to the immune system, and act through the mucus as a lubricant. The increase in the number of goblet cells may be an indication of increased irritation (Da Silva et al 2012) as these cells produce mucus lining the brush border. This mucus serves as a lubricant providing protection against chemical and mechanical damage. The increase in goblet cell number may also be an immune response against the anti-nutrients (Marchetti et al 2006). This was evidenced by higher in the blood white blood cell count for fish fed on diets supplemented with pomegranate peel compared to the control after three months of feeding trial (unpublished data).

Conclusions. Based on the obtained results, the fish fed on 5% and 3% PP levels exhibited the best performance and the best feed utilization indices. This was evidenced by the best PER and the highest energy utilization for those fish. This was supported also by the increase in the length of villi and the number of goblet cells of monosex *O. niloticus* fed on 3 or/and 5% level PP. It is recommended to add the PP to the diets of monosex *O. niloticus* up to 5% to enhance body composition of fish, feed utilization and fish integrity with increasing economic efficiency.

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