EFFECT OF DIETARY POMEGRANATE PEEL (*PUNICA GRANATUM*) ON GROWTH PERFORMANCE, BLOOD INDICES, AND IMMUNE RESPONSES OF NILE TILAPIA FINGERLINGS

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SUMMARY

The study examined the effects of dietary pomegranate peel (PP) on the growth performance, feed utilization, and immune response of Nile tilapia fish (*Oreochromis niloticus*). Four diets were formulated to include pomegranate peel at 0, 50, 70, and 90 g (PP) kg^{-1} diets and fed to the Nile tilapia fish (initial body weight, 9.8 ± 0.2 g/ fish) for 84 days. Compared to the control group, the average final weight, weight gain, specific growth rate, and feed utilization parameters of the fish group fed 50 g (PP) kg-1 diets were improved. Results indicated that fish fed a 90 g (PP) kg^{-1} diet exhibited significant improvements ($P<0.05$) in carcass composition, including dry matter and ether extract, there was a significant decrease in the body's crude protein content. Fish groups fed diets including PP displayed decreased ($P < 0.05$) in AST and ALT values. However, the treatment with 90 g (PP)/ kg diet increased total protein. The highest values of hematocrit, hemoglobin, red blood cells, and white blood cell counts were observed in fish fed a 70 g (PP) kg^{-1} diet. Likewise, the supplemented diet with (PP) recorded the highest levels of SOD, CAT, GSH, and GPx but the lowest MDA value. In summary, the study demonstrated that pomegranate peel at levels of 50 to 70 g (PP)/ kg diet may enhance growth performance and stimulate immunostimulatory responses of Nile tilapia.

Keywords: *O. niloticus, pomegranate peel (Punica granatum), growth performance, feed utilization, carcass composition, antioxidant enzymes, and immune responses.*

INTRODUCTION

Global aquaculture production has expanded year after year to suit the growing demand for aquatic animal proteins. Given the ongoing increase in demand for aquaculture as a secure and inexpensive source of seafood required for food security (FAO, 2018). Previous studies showed that using herbal extract as a supplement improves fish development, while also protecting them from diseases (Johnson *et al.*, 2007). It contains numerous bioactive substances, including quercetin, ellagic acid, punicalagin, pedunculagin, tannic acid, anthocyanins, rutin, catechin, and polyphenols.

Pomegranate components have antioxidant, neuroprotective, anti-inflammatory, anti-angiogenic, anti-cancer, anti-mutagenic, cytoprotective, cardiovascular protective, anti-ulcerogenic, hepatoprotective, antibacterial, and antifungal properties. They can also improve male fertility and protect against UV-induced skin damage.

The production of juices generates large quantities of waste and by-products; however, recycling the remaining by-products is crucial to prevent environmental pollution (Red Corn *et al*., 2018). Pomegranate is one of the most important fruits used in the manufacture of juices and medicines products; however, large amounts of by-products are left behind **(**Iqbal *et al*., 2008**).** The peel of pomegranate contains polyphenols and bioactive compounds that act as antioxidants and immunostimulants **(**Al-Zoreky, 2009).

The results of many studies showed that using pomegranate peel increased growth rate, feed efficiency, immune status, and the ability to resist oxidation in Nile tilapia *(Oreochromis niloticus*) (Badrey *et al*., 2019**;** El-Sayed *et al*., 2014; Toutou *et al*., 2019).

Pomegranate peel is a waste product of the pomegranate industry with higher antioxidant levels than the juice itself. It is an attractive candidate as a nutritional supplement for animal feed. Pomegranate extract is used to prevent and treat many major health problems. In particular, pomegranate peel has been extensively studied for its strong anti-microbial effect, high antioxidant activity **(**Ibrahim, 2010**),** cytotoxic effect, hypoglycemic effect (Rajput *et al*., 2011), hypolipidemic effect **(**Belkacem *et al.*, 2010), hepatoprotective effect, and anti-inflammatory activity **(**Jurenka 2008**).** Also, pomegranate peel has no side effects and no known drug interactions and may be the most potent way to prevent cancer by strengthening the immune system (**Lee** *et al.,* 2008); preventing heart disease; prevent liver fibrosis; promoting wound healing; and strengthening connective tissue, which may keep cancer cells from spreading **(**Murthy *et al.,* 2004**).** Hence, the current study aimed to examine the potential effects of pomegranate peel on the growth, biochemical parameters, antioxidant capacity, and immunity of the Nile tilapia fingerlings.

MATERIALS AND METHODS

For 84 days, four experimental groups with triplicates were carried out at the Faculty of Agriculture, Ain Shams University to evaluate the impact of dietary PP on growth performance, feeding utilization, carcass composition, blood parameters, and immunity of Nile tilapia fingerlings (*Oreochromis niloticus*). This experiment was approved by the Research Ethics Committee decision authority at the Faculty of Agriculture- Ain Shams University.

The rearing system & experimental design:

One hundred and twenty mono-sex *O. niloticus* fingerlings (10 ± 0.05 g) were randomly divided into twelve quadrate fiberglass tanks (of 60 × 60 × 30 cm) in length, width, and depth respectively; hence, fish fingerlings were obtained from World Fish Center (WF Center) located at El Abbasa El Sharkia Governorate. Four experimental groups were allocated (in triplicate) with 10 fingerlings in each tank.

Water quality assessments:

Every fortnight, all water quality measurements were detected in the Limnology and Plankton Laboratories of the Central Laboratory for Agricultural Climate according to the standard methods of the American Public Health Association (APHA, 1985) and Boyd (1990). Water temperature and oxygen saturation were measured daily at 8.00 am by using oxygen meter (Lutron model Do – 5509). The pH values were measured in water samples using a combined electrode connected to a pH meter (Coming Co. pH meter model 345). Ammonia-nitrogen (NH^{+4}), (NH_3), and nitrate (No_3) were determined according to the methods described by Sauter and Stoub (1990). The water quality parameters were within the permissible range and presented in (Table 1).

Table (1): Averaged water quality measurements during the experimental period.

Parameters	Reading
Temperature average (C°)	26
Oxygen (mg / L)	6
pH	7.1
Ammonia (mg / L)	0.28
Nitrate (mg / L)	1

Experimental diets:

Pomegranate peel powder obtained from a hypermarket located in Cairo was chemically analyzed (Table 2) and was included in Nile tilapia diets. Fish feed was formulated according to the recommendation of the NRC (2012). Four isonitrogenous (~ 35% crude protein) and isoenergetic (~ 4315 Kcal GE/kg) were made to cover all nutrients required in experimental diets. The formulation and chemical composition of the experimental diets (% dry matter bases) were ascertained by the AOAC (2007) method and shown in Table 3.

Table (2): Chemical composition of Pomegranate powder extracted as dry matter basis.

Parameters	(%)
Dry matter (DM)	90
Crude protein (CP)	8.80
Crude fiber (CF)	13.50
Ether extract (EE)	0.2
Ash	7.90
Nitrogen-free extract NFE	59.60

Table (3): Formulation and chemical composition of experimental diets (% dry matter bases).

Ingredients	T0 (basal diet)	T 1 (5%)	T2 (7%)	T3 (9%)
Yellow corn	29.00	26.00	24.00	22.00
Pomegranate peel	0	5.00	7.00	9.00
Soybean meal	25.00	25.00	25.00	25.00
Sunflower meal	7.00	7.00	7.00	7.00
Poultry by-products meal	13.00	13.00	13.00	13.00
Corn gluten meal	7.00	7.00	7.00	7.00
Rice bran	17.00	15.00	15.00	15.00
Vit. & min. mix*	1.00	1.00	1.00	1.00
Fish oil	1.00	1.00	1.00	1.00
Total	**100**	**100**	**100**	**100**
Chemical composition % (FM bases)				
DM%	92.29	90.4	89.65	89.26
CP %	35.27	34.70	34.75	34.70
EE%	4.1	4	4.3	5.2
NFE%	49.2	47.5	46.4	44.6
CF %	3.72	4.20	4.20	5.00
GE Kcal/kg	4390.42	4408.25	4240.25	4245.50

Each 1 kg contains: Vitamin A, 4.0 m.i.u; Vitamin D_3, 0.8 m.i.u; Vitamin E, 4.0g; vitamin K, 0.8 m.i.u; Vitamine K, 0.8g; Vitamine B_1,0.4g; Vitamine B_2, 1.6g; Vitamine B_6, 0.6g; Vitamine B_{12}, 4.0g; Pantothenic acid, 4.0g; Nicotinic acid, 8.0g; Folic acid, 400.0mg; Biotine, 20.0g; Chlorine chloride, 200.0g; Copper, 4.0g; Iodine, 4.0g, Iron, 12.0g, Zink, 22.0g and Selenium, 0.04g

Experimental sampling and nutritional criteria:

The individual initial fish body weight (9.8 ± 0.2 g) was recorded at the beginning of the experiment. All fish in each tank were weighed every two weeks during the whole experimental period. According to the data of body weights, the following parameters were estimated: average weight gain (AWG), average daily gain (ADG), specific growth rate (SGR), and nutritional parameters were calculated according to Cho and Kaushik (1985) as the following equations:

Average weight gain (AWG)

AWG (g/fish) = an average final weight – an average initial weight

Average daily gain (ADG)

Daily gain was estimated according to the following formula:

$ADG = (wt_2 - wt_1)/ T$

Where:

Wt 1 = first fish weight in grams.

Wt 2 = following fish weight in grams.

T = period in days.

Specific growth rate (SGR %/day)

$SGR = (Ln\ wt_2 - Ln\ wt_1) \times 100/t.$

Where:

$Ln = (\log l0x)^{3.303}$

wt1= first fish weight in grams.
wt2 =final fish weight in grams
t= period in day

Feed conversion ratio (FCR)

The feed conversion ratio was calculated according to the following equation:

$$FCR = \frac{\text{Total feed consumption (g)}}{\text{Final body weight (g) - initial body weight (g)}}$$

Protein efficiency ratio (PER)

The protein efficiency ratio was calculated according to the following equation:

$$PER = \frac{\text{Final body weight (g)} - \text{initial body weight (g)}}{\text{protein intake (g)}}$$

Protein productive value (PPV%)

(PPV%) = 100 [protein gain in fish (g) / protein intake in feed (g)]

Relative intestine length (RIL), hepatosomatic index (HSI), and spleen somatic index (SSI) were calculated using the following equations:
RIL= Intestine length(cm)/ whole body weight (g)
HSI%= 100× (Liver weight (g)/ whole body weight (g))
SSI%= 100× (Spleen weight (g)/ whole body weight (g))

Hematological and biochemical blood indices:

Blood samples were immediately divided into two half parts, Half was transferred to a tube containing an anticoagulant (heparin) for studying the hematological assay, while the other half was transferred to non-heparinized tubes for biochemical and immunological studies. Sera samples were obtained by blood centrifugation (3000 g, 15 min) and stored at -20 °C until use (Adel *et al*., 2015). The serum samples were used for the analysis within 30 days to avoid the spoilage of samples. Three samples were selected at random from each treatment for the determination of the hematocrit and hemoglobin values according to (Dacie and Lewis, 1975). Blood samples were obtained from the caudal vein of individual fish. Calculation of the Red blood cells (RBC_S) and White blood cells (WBC_S) were obtained by hemocytometer according to the method described by (Stoskopf, 1993). Mean corpuscular volume (M.C.V.), Mean corpuscular hemoglobin (M.C.H.) and mean corpuscular hemoglobin concentration (M.C.H.C.) were detected by the formula suggested by (Dacie and Lewis, 1975).

M.C.V. = (PCV / RBCs) x 10 as μ/mm^3

M.C.H. = (HB content gm/100ml/ RBCs) x 10 as μ/mm^3

M.C.H.C. = (HB content gm/100ml / PCV) x100 as %

The liver function activity was represented as Aspartate Amino Transaminase (AST) and Alanine Amino Transaminase (ALT). AST and ALT were assayed according to (Reitman & Frankel, 1957). Total serum protein and albumin were determined according to Henry (1974). Also, the total serum globulin was calculated by subtracting the total serum albumin from the total serum protein.

Activities of hepatic antioxidant enzymes:

Livers of four fish from each replicate were weighed and collected to analyze antioxidant enzymes. The collected supernatant was used for superoxide dismutase (SOD), Nishikimi *et al.* (1975), Peskin and Winterbourn (2000), catalase (CAT), Beers and Sizer (1952), Aebi (1984), glutathione peroxidase (GPX), Moin (1986), malonaldehyde (MDA), Dogru *et al.* (2008).

Statistical analysis:

SAS, version 6.03 (SAS 2009) was the software used to analyze all the data. The effects of dietary pomegranate levels were examined using one-way ANOVA. When Duncan's multiple-range test was used

to examine variations between means (Duncan, 1955). The data are shown as means with pooled standard error of the mean (±SE), and all differences were considered significant at ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of pomegranate peel on growth performance and feeding utilization parameters:

Results in Table (4) showed significant improvement ($P<0.05$) in the average final weight (AFW), average weight gain (AWG), and average daily gain (ADG) in treatment T1, which contains 50 g (PP) kg^{-1} diet compared to the other treatments, followed without significant differences ($P>0.05$) by T2 (70 g PP kg^{-1} diet).

Table (4): Effect of pomegranate peel on Nile tilapia's growth performance and feeding utilization parameters.

Growth Items	T0 (basal diet)	T1 (5%)	T2 (7%)	T3 (9%)
AIW	9.86±2.22	10.04±2.22	9.56±2.22	9.84±2.22
AFW	57.42^{b}±9.89	69.00^{a}± 2.5	61.97^{ab}± 2.5	58.10^{b}±2.5
AWG	47.56^{b}±2.56	58.96^{a}±2.56	52.41^{ab}±2.56	48.09^{b}±2.56
ADG	0.55^{b}±0.03	0.69^{a}±0.03	0.61^{ab}±0.03	0.55^{b}±0.03
SGR	2.09±0.06	2.29±0.06	2.22±0.06	2.11±0.06
FCR	1.52±0.02	1.51±0.02	1.49±0.02	1.53±0.02
PER	2.06±0.12	2.30±0.12	2.26±0.12	2.09±0.12
PPV	37.07±2.09	41.04±2.09	34.77±2.09	34.31±2. 09

Values are the mean ± S.E, means in the same raw, with different superscripts are significantly different ($P<0.05$). AIW: average initial weight; AFW: average final weight; AWG: average weight gain; ADG: average daily gain; SGR: specific growth rate. FCR: Feed conversion ratio, PER: Protein efficiency ratio, PPV: Protein productive value.

However, there were no significant differences ($P>0.05$) in specific growth rate (SGR), although treatment T1 was also the best in this regard. The results in Table 4 also, showed that adding PP in all treatments has no significant effect ($p>0.05$) on feed conversion ratio (FCR), protein efficiency ratio (PER), and protein productive value (PPV). The best result values were observed when only 50 g PP was added in treatment T1. These findings agree with the results obtained by Hussein *et al.* (2023) who found that at a certain level, increasing the amount of pomegranate peel in diets can result in a decrease in Nile tilapia growth performance and feed intake. Toutou *et al*. (2019) found that lower levels of PP (1% to 5%) improve feed conversion ratio (FCR) and protein efficiency ratio (PER). In addition, Yousefi *et al*. (2023) observed deterioration in the growth of common carp (*Cyprinus carpio*) after increasing the percentage of pomegranate peel in the diet beyond 5%. They attributed this to the decrease in thyroid hormones T3 and T4 levels. The authors thought that the decrease in growth and feed utilization parameters with increasing percentage of pomegranate peel may be attributed to the higher fiber content in the diet, as well as the presence of certain phenolic compounds that affect the palatability of the pomegranate peel.

Effect of pomegranate peel on the chemical composition of fish carcass:

Results in Table 5 indicated that T3 differed significantly ($P<0.05$) from the other treatments in both DM and EE.

Table (5): Chemical analysis of Nile tilapia fish (%DM) fed different experimental diets.

Items	T0 (basal diet)	T1 (5%)	T2 (7%)	T3 (9%)
DM	28.61^{b}±0.01	27.43^{c}±0.01	25.84^{d}±0.01	29.68^{a}±0.01
CP	60.80^{a}±0.18	61.33^{a}±0.18	61.30^{a}±0.18	59.50^{b}±0.18
EE	12.60^{c}±0.11	13.30^{b}±0.11	10.96^{d}±0.11	14.50^{a}±0.11
Ash	13.36^{a}±0.09	11.13^{c}±0.09	12.23^{b}±0.09	11.93^{b}±0.09

a, b means of the same raw with different superscripts are significantly different ($p<0.05$).

This increase may be attributed to the high percentage of triglycerides, which will be discussed later. These results explained that the changes in blood cholesterol and triglyceride concentrations are sensitive

indicators of liver dysfunction because lipid homeostasis is one of the principal functions of the liver (Syed *et al.*, 2011). The results also showed a significant decrease (P<0.05) in CP for T3, which resulted in the lowest growth rates for fish. Additionally, T1 showed a significant increase in ash compared to the other treatments. Our findings are contrary to the results obtained from Hussein *et al.* (2023) who found no significant variations in dry matter (P>0.05) and ash across any of the treatments. However, there is a slight increase in the ash content of fish bodies with the increased levels of pomegranate peel in the diets.

Biological and hematological parameters of Nile tilapia:

Condition factor (K), % HSI, % SSI, and % RIL values are indicators of the fish's physiological status (Table, 6). The results revealed that the addition of pomegranate peel in *O. niloticus* diets has improved the HIS, SSI and RIL values. These may be due to the antimicrobial properties, promoting a healthier gut flora, which improves nutrient absorption and leads to a longer relative intestine length. Low levels of PP in diets may not provide an adequate concentration of beneficial compounds such as polyphenols, tannins and antioxidants. No significant effects in condition factor (K), HSI %, SSI %, and RIL were observed between the different fish groups (Table 6). These results agreed with the results of Abozahra *et al*. (2022); Kondera *et al.* (2017).

Table (6): Biological parameters of Nile tilapia juveniles fed diets supplemented with different levels of pomegranate peel (PP) for 84 days.

Biological parameters	T0	T1 (5%)	T2 (7%)	T3 (9%)
Condition factor (K)	0.016±0.001	0.017±0.002	0.017±0.001	0.017±0.002
Hepatosomatic index (HSI %)	1.78±0.09	2.72±0.58	2.00±0.27	2.38±0.41
Spleen somatic index (SSI %)	0.30 ±0.07	0.64±0.41	0.10±0.03	0.08±0.02
Relative intestine length (RIL; cm)	1.83±0.05	1.58±0.13	2.08± 0.27	2.10±0.17

Values are the mean ± S.E, means in the same raw, with different superscripts are significantly different (P<0.05).

Results in Table (7) show that there was a significant increase (P ≤ 0.05) in Hemoglobin, RBCs, HCT, MCV, and WBCs in the fish group fed T2 (7% PP) more than all other treatments. The hematological parameters displayed decreased RBCs, hemoglobin, and hematocrit levels following high feeding levels of pomegranate meal (9%). In parallel, Nile tilapia fed high concentrations of pomegranate meal displayed lower RBCs, hemoglobin, and hematocrit levels (Badrey *et al.,* 2019; Avazeh *et al.,* 2021). The highest significant (P ≤ 0.05) WBC counts were obtained in the fish group fed T2 (7% PP), followed by T1 (5% PP) in the current study suggesting a possible immunomodulatory effect of pomegranate peel.

Table (7): Hematological indices of Nile tilapia juveniles fed diets enriched with different levels of pomegranate peel meal for 84 days

Items	T0	T1 (5%)	T2 (7%)	T3 (9%)
HGB (g dl $^{-1}$)	11.50±0.50^{a}	9.10±0.50^{b}	12.40±0.50^{a}	8.30±0.50^{b}
RBCs (×10^{6}µl)	2.26±0.57^{b}	2.07±0.57^{b}	2.85±0.57^{a}	1.91±0.57^{c}
HCT (%)	30.20±0.58^{b}	30.80±0.58^{b}	40.60±0.58^{a}	25.70±0.55^{c}
MCV (fL)	133.6±0.58^{c}	148.0±0.58^{a}	142.0±0.58^{b}	134.0±0.58^{c}
MCHC (g dl $^{-1}$)	38.08±0.58^{a}	29.55±0.58^{c}	30.54±0.58^{bc}	32.30±0.58^{b}
Platelets count (×10^{3} µl)	32.00±0.58^{d}	80.00±0.58^{a}	49.00±0.58^{a}	66.00±0.58^{b}
WBC (×10^{3} µl)	136.9±0.58^{d}	178.6±0.58^{b}	191.0±0.58^{a}	148.6±0.58^{c}

Values are the mean ± S.E, means in the same raw, with different superscripts are significantly different (P<0.05). RBCs: Red blood cells, HB: hemoglobin concentration, HCT%: hematocrit, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, WBCs: white blood cells.

Biochemical parameters in Nile tilapia:

In the present study, Nile tilapia fingerlings exhibited significant increases in blood total protein levels after 84 days of feeding on inclusion levels of pomegranate peel, confirming good growth performance during the feeding experiment. In terms of, total protein, albumin, and globulin, T3 (9% PP) recorded significantly higher levels between all treatments followed by the control group (T0).

The results in the present study reflect enhanced immunity and the ability to resist the infection. Pomegranate peel has several active compounds that act as immunostimulants to strengthen the immune system, these results agreed with Løvoll *et al.* (2006) and Dügenci *et al.* (2003).

In terms of cholesterol, triglyceride, HDL, LDL, and VLDL, there were significantly higher in T3 (9% PP) than in the control group. Results indicated that total cholesterol, triglyceride, and LDL were elevated with the increase of pomegranate peel levels in diets. Similar results showed in Nile tilapia (*O. niloticus*) demonstrated significantly lower cholesterol levels in all feeding diets of Nile tilapia feeding trial (after 90-day) on 1, 2, 3, 5, 10, 15, and 20% pomegranate peel extracts, than the control group; except the higher levels of PP extract (15 and 20%), that recorded the highest cholesterol levels Badrey *et al.,* (2019). These results are in contrast with previous studies by Basiri (2015); Hygreeva *et al.* (2014), who showed that PP extract can lower cholesterol levels in humans and other animals. Pomegranate peel exerts inhibitory effects on pancreatic lipase activity, inhibiting fat absorption from the intestinal tract (Kumar *et al.,* 2018).

Data in Table (8) illustrated that the lowest values of ALT and AST were recorded by T2 (7% pp). The decrease in ALT and AST values is regarded as an indicator of the protective effect of pomegranate on cells, tissues, and organs (Babalola *et al*. 2009; Ibrahim, 2010). Similar results were recorded by Badawi and Gomaa (2016) and El-Sayed *et al.* (2014) suggested that dietary supplementation with pomegranate peel has no adverse effects on different fish organs or fish health status.

Table (8): Biochemical parameters in Nile tilapia (*O. niloticus*) fed diets containing different levels of pomegranate for 84 days

Items	T0	T1 (5%)	T2 (7%)	T3 (9%)
Total protein (g/dL)	1.30±0.006^{b}	1.18±0.006^{c}	1.13±0.006^{b}	1.41±0.006^{a}
Globulin (g/dL)	0.52±0.006^{c}	0.46±0.006^{d}	0.54±0.006^{b}	0.59±0.006^{a}
Albumin (g/dL)	0.77±0.006^{b}	0.72±0.006^{c}	0.77±0.006^{b}	0.82±0.006^{a}
Cholesterol (mg/dL)	139.0±0.58^{b}	133.0±0.58^{c}	139.0±0.58^{b}	178.0±0.58^{a}
Triglyceride (mg/dL)	156.0±0.58^{c}	126.0±0.58^{d}	172.0±0.58^{b}	204.0±0.58^{a}
HDL (mg/dL)	48.00±0.58^{b}	39.00±0.58^{d}	46.00±0.58^{c}	58.00±0.58^{a}
LDL (mg/dL)	59.80±0.58^{c}	68.80±0.58^{b}	58.60±0.58^{c}	79.20±0.58^{a}
VLDL (mg/dL)	31.20±0.58^{c}	25.20±0.58^{d}	34.40±0.58^{b}	40.80±0.58^{a}
ALT (U/L)	2092.0±0.58^{a}	806.0±0.58 ^{c}	251.0±0.58 ^{d}	1223.0±0.58 ^{b}
AST (U/L)	1472.0±0.58^{a}	932.0±0.58^{b}	733.0±0.58^{d}	864.0±0.58^{c}

Means followed by different small letters in the same row are significantly different (P< 0.05, one-way ANOVA). High-density lipoprotein (HDL), Low-density lipoprotein (LDL), Very low-density lipoprotein (VLDL), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST).

Table (9): The activity of oxidative enzymes in Nile tilapia (*O. niloticus*) fed on diets containing different levels of pomegranate for 84 days

Items	T0	T1 (5%)	T2 (7%)	T3 (9%)
GPX	15679.4±0.29^{a}	7800.80±0.29^{c}	7003.22±0.29^{d}	13325.6±0.29^{b}
CAT	20.06±0.006^{d}	24.79±0.006^{b}	23.33±0.006^{c}	33.04±0.006^{a}
GSH	138.12±0.006^{a}	50.66±0.006^{d}	55.46±0.006^{c}	116.52±0.006^{b}
MDA	341.92±0.006^{b}	298.19±0.006^{c}	237.62±0.006^{d}	502.35±0.006^{a}
SOD	729.00±0.006^{c}	1179.0±0.006^{a}	1318.00±0.006^{a}	1019.0±0.006 ^{b}

Means followed by different small letters in the same row are significantly different (P< 0.05, one-way ANOVA).

The activity of antioxidant enzymes is presented in Table (9). GPX and GSH activity was significantly higher in the control group followed by T3 (9% PP) than in the other groups. GPX and GSH are two important antioxidant enzymes that help to protect cells from oxidative damage. The higher activity of these enzymes in the control group and T4 suggests that pomegranate may help to enhance the antioxidant capacity of Nile tilapia. SOD was significantly lower in T2 (7%PP) followed by the control group than in the other treatments. These results suggested that using PP may help to reduce oxidative stress in Nile tilapia. The current oxidative stress biomarker values measured in this study showed an increase in the activity of SOD and GPX, which enhances the activity of NADPH oxidase for the scavenging of superoxide anions (Sheikh Asadi et al., 2018). CAT and MDA decreased with the decrease of pomegranate

peel levels. MDA is a non-enzyme used as a baton of oxidative stress (Khosravi-Katuli et al., 2018), so the pomegranate diets have enhanced the antioxidant enzyme capacity and health status of Nile tilapia.

CONCLUSION

Pomegranate peel exhibits a strong immunostimulant function, as seen in their antiviral and antibacterial effects. Dietary inclusion of 50–70 g pomegranate peel extracts kg-1 diet can enhance the immune system response and disease resistance and control disease outbreaks, which have consequent positive impacts on growth performance, nutrient utilization efficiency, antioxidant enzymes, and immune response of the Nile tilapia. These findings demonstrate that pomegranate peel extract could be considered a potential feed ingredient to boost the growth performance and the immune response system of the Nile tilapia.

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تأثير قشر الرمان الغذائي (*Punica granatum*) على أداء النمو ومؤشرات الدم والاستجابات المناعية لإصبعيات البلطي النيلي

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تم في هذه الدراسة فحص آثار استخدام قشر الرمان الغذائي (PP) على أداء النمو والاستفادة من الغذاء والاستجابة المناعية لإصباعيات البلطي النيلي (*Oreochromis niloticus*). تم تركيب أربعة علائق يدخل في تركيبها قشر الرمان المجفف بأربعة مستويات (0 و 50 و 70 و 90 جم /كجم عليقة) تم تغذيتها لإصباعيات البلطي النيلي (بمتوسط وزن بداية 9.8 ± 0.2 جم / سمكة) استمرت التجربة لمدة 84 يوما. وتم تغذية الأسماك بمعدل 5% من وزنها يوميا. بالمقارنة مع المجموعة المقارنة (T0) حدث تحسن في متوسط الوزن النهائي ، وزيادة الوزن ، ومعدل النمو النوعي ، ومعلمات استخدام الأعلاف لمجموعة الأسماك التي تغذت على 50 جم (PP) كجم$^{-1}$. أشارت النتائج إلى أن الأسماك التي تتغذى على 90 جم (PP) كجم $^{-1}$ أظهرت تحسنا كبيرا ($P<0.05$) في مكونات الجسم ، بما في ذلك المادة الجافة ومستخلص الأثير ، وكان هناك انخفاضا معنويا في محتوى البروتين الخام في الجسم. أظهرت مجموعات الأسماك التي تغذت على العلائق الغذائية والتي يدخل قشر الرمان في تركيبها انخفاضا معنويا ($P < 0.05$) في قيم AST و ALT. ومع ذلك ، فإن المعاملة بنظام غذائي يحتوي على 90 جم / كجم زاد من البروتين الكلي. كما تلاحظ أعلى قيم في عدد خلايا الهيماتوكريت والهيموجلوبين وخلايا الدم الحمراء وخلايا الدم البيضاء في الأسماك التي تغذت على نظام غذائي 70 جم قشر رمان لكل كجم عليقة ، وبالمثل ، سجل النظام الغذائي المحتوي على قشر رمان أعلى مستويات SOD و CAT و GSH و GPx ولكن أقل قيمة MDA. وباختصار، أظهرت الدراسة أن قشر الرمان بمستويات 50 إلى 70 جم (PP) / كجم من العليقة المقدمة قد يعزز أداء النمو ويحفز الاستجابات المناعية للبلطي النيلي.

الكلمات المفتاحية: *O. niloticus، قشر الرمان (Punica granatum)، أداء النمو، استخدام الأعلاف، تكوين الذبيحة، الإنزيمات المضادة للأكسدة، والاستجابات المناعية.*