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Influence of diets supplemented with pomegranate peel extract on performance in Oreochromus niloticus

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Abstract

A total of 300 Oreochromus niloticus with an average body weight of 15±1 gm were used. The fish were randomly divided into 5 equal triplicate groups (20 fish/replicate). A basal control diet was formulated to fulfil the nutrients requirement of fish according to[24)] and contained 30.80% CP and 2940 kcal/kg DE. In other 4 experimental diets supplemented by pomegranate peel extract (PPE) at rate of 0.1, 0.2, 0.3 and 0.5 %. The fish were fed isonitrogenous, isocaloric diets 3 times daily at rate of 3% of body weight for 10 weeks. Results revealed that the growth performance of fish fed the experimental diets was decreased but none significantly (P>0.05). Also, results indicated that the lowest levels of cholesterol, triglyceride, LDL-cholesterol, ALT, AST, creatinine and urea were found in fish groups fed on diets contained PPE, while these groups had a significant (P<0.05) increased HDL-cholesterol concentration. Some immunological parameters (IgM and lysozyme) were significantly improved due to supplementation of the PPE. These results indicate that addition of pomegranate peel extract (PPE) in Oreochromus niloticus diets without any adverse effects on growth parameters or health status. In addition, it improved immune status, lipid profile and functions of liver and kidney.

Key Words: Oreochromus niloticus, PPE, performance, biochemical, immunity.

Introduction

The use of antibiotics and other chemotherapeutics for controlling diseases has been criticized for their deleterious side effects on animals, fish and human. So, we intend to find an alternative, safe, cheap and acceptable natural promoters. In this respect, vegetable, herbs, spices and edible plants & its extracts were suggested a non-traditional feed additive, growth promoters or immunostimmulants.

Pomegranate peel, a waste product of the pomegranate industry with higher anti-oxidant levels than the juice itself, an attractive candidate as a nutritional supplement for animals feed. Pomegranate extract is used for prevention and treatment of many major health problems. In particular, pomegranate peel extract has extensively been studied for its strong anti-microbial effect; high anti-oxidant activity[12)], cytotoxic effect; hypoglycemic effect[28)], hypolipidemic effect[3)], hepatoprotective effect and anti-inflamatory activity[15)]. Also, pomegranate peel extract has no side effects and no known drug interactions and may be the most potent way to prevent cancer; strengthen the immune

system[17], prevent heart disease; prevent liver fibrosis; promote wound healing and strengthen connective tissue which may keep cancer cells from spreading[21].

Studies have proved that herbal extract as an additives enhanced the growth of fishes and also protected from the diseases[14] 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 and its extracts concerning their effects on fish performance. Hence, the objective of the present study was designed to examine the effect of pomegranate peel extract (PPE) as natural feed additives in diets of Oreochromus niloticus on growth performance, some blood parameters and immune status.

Materials and Methods

Preparation of pomegranate peels (PPE) extract: Local pomegranate fruits were washed by distilled water then peeled and their edible portions were carefully separated. The peels were air dried in a hot air oven at 40°C until the moisture content reach about 8% (dry basis). The peels were grounded then used for extraction[19]. The ethanol 70% solvent was added to the dried and ground peel in glass flask with glass cover in a thermostatic shaking water bath. The mixture filtered through a Whatman No. 1 filter paper for removal of peel particles. The alcohol was removed from the filtrate by using high-capacity evaporator (EYELA Rotary vacuum evaporator system). The ethanol free extract was dried by using lyophilizer. The dried extracts were kept in dark at 4°C until used.

Experimental fish: A total number of 300 apparently healthy live Oreochromus niloticus with an average body weight 15±1 gm obtained from Abassa Fish Hatchery at Sharkia province. Fish were kept in glass aquaria (80 X 60 X 30 cm) filled with 90 L., de-chlorinated fresh water, aerator and thermostatically controlled at 22°C ± 2°C. Fish were divided into 5 groups; each group was divided into subgroups. Each subgroup has three replicates (20 fish/replicate). The fish were adapted to experimental condition for two weeks before the start of the experiment.

Fish diets and feeding: Diets were prepared at Fish Research Center, Faculty of Veterinary medicine, Zagazig University. Diets in the form of dry pellets and were formulated to meet the nutrient requirements of Oreochromus niloticus[24] as shown in Table 1. A basal control diet was formulated to fulfil the nutrients requirement of fish contained 30.80% CP and 2940 kcal/kg DE. In other 4 experimental diets was supplemented by PPE at rate of 0.1, 0.2, 0.3 and 0.5 %. The fish were fed diets 4 times daily at rate of 3% of body weight for 10 weeks. Feedstuffs used in diets formulation were analyzed for moisture, crude protein, ether extract and crude fiber[1].

Pomegranate peel extracts analysis: The total phenols, flavonoids and radical scavenging activity were measured as in PPE[22].

Growth performance parameters: The fish were weighed at the start and the end of the experiment. Average body weight was calculated by dividing the total weight of fish by the number of fish in each group. Body gain and feed conversion ratio[31]. Body gain percent[13] and specific growth rate %[27] were determined.

Biochemical analysis: Blood was obtained from caudal blood vessels into plastic Eppendorf tubes for serum samples preparation. Blood was collected into Eppendorf tubes without anti-coagulant in syringe then centrifuged (3,000g for 15 min). The serum samples were collected and stored immediately in freezer (zero °C) until use for biochemical and immunological analysis[2]. Serum total protein[11]; albumin[5], while globulin was calculated by difference. Serum total cholesterol[23], triglyceride[35], blood urea nitrogen, creatinine and uric acids[26]. Serum aspartata-aminotransferase (AST) and alanine-aminotransferase (ALT) were determined as described by[29]. *Immunological parameters:*

Assay procedure for of IgM: Immunoglobulin M (IgM) was determined using ELISA Kit. Catalog No. CSB-E12045Fh (96 test). CUSABIO BIOTECH CO., Ltd.

Assay procedure for lysozyme determination: The lysozyme activity was measured using the turbidity assay. Chicken egg lysozyme (Sigma) was used as a standard and 0.2 mg ml^{-1} lyophilized Micrococcus lysodeikticus in 0.04 M sodium phosphate buffer (pH 5.75) was used as substrate. Fifty ml^{-1} of serum was added to 2 ml of the bacterial suspension and the reduction in the absorbance at 540 nm was determined after 0.5 and 4.5 min incubation at 22°C. One unit of lysozyme activity was defined as a reduction in absorbance of 0.001min-1[25].

Statistical analysis: The obtained data in this study were statistically analyzed for variance ANOVA, LSD (Least significant difference) according to[32]. Differences among treatment means were compared using Duncan's multiple range tests[6]. Data were presented as mean ± SE and significance was declared at ($P < 0.05$).

Table 1. Ingredients and calculated composition of the experimental diets.

Ingredient	Experimental diets				
	Control Diet	PPE extract in diets			
		0.1%	0.2%	0.3%	0.5%
Yellow corn	35.00	34.90	34.80	34.70	34.50
Pomegranate peel extract	0.00	0.10	0.20	0.30	0.50
Wheat flour	10.00	10.00	10.00	10.00	10.00
Soybean meal	18.00	18.00	18.00	18.00	18.00
Fish meal	16.00	16.00	16.00	16.00	16.00
Poultry by-product meal	14.00	14.00	14.00	14.00	14.00
Vegetable oil	5.50	5.50	5.50	5.50	5.50
Vitamins and Minerals mixture	1.50	1.50	1.50	1.50	1.50
DM, %	84.37	84.37	84.37	84.37	84.36
CP, %	30.79	30.79	30.79	30.79	30.76
EE, %	10.26	10.26	10.26	10.25	10.24
CF, %	2.42	2.43	2.43	2.43	2.45
Ash, %	7.12	7.12	7.12	7.13	7.14
NFE, %	38.99	38.99	38.99	38.99	38.99
DE, Kcal/ kg diet*	2944.41	2944.16	2943.90	2943.65	2941.86

* digestible energy calculation based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm, NFE 2.5 kcal/gm[30].

Results and Discussion

Anti-oxidant effect of PPE (the total phenols, flavonoids and radical scavenging activity) are shown in Table 2. This content of phenols and flavonoids is high if compared with many other plants, also radical scavenging activity is high. This result helped prompt manner and strong improvement in the fish serum biochemical parameters and immunological parameters.

These results are in agreement with whose reported that PPE had high anti-oxidant capacity, considering the scavenging or preventive capacity against superoxide anion, hydroxyl and peroxyl

radical which exerted diverse pharmacological functions as anti-oxidant activity[18, 22]. Also, found a significant and positive linear correlation were found between total anti-oxidant capacity and phenolics content, indicating that phenolics are the dominant anti-oxidant constituent of PPE[33].

Pomegranate peel extract have several biological active compounds as ellagic acid, phenols, flavonoids and tannins which have high anti-oxidant activity and anti-inflammatory activity on human gut[7].

Table 2. Analyses of pomegranate peel extract:

Parameters	Pomegranate peel extract (PPE)
Quantity of extract yield %	24.13
Total phenolics content	185 mg GAE*/gm extract powder
Total flavonoids content	32 mg RE*/gm extract powder
Radical scavenging activity (Inhibition percent I %) at concentration 10μg/ml	35.00

*(GAE)/g -mg gallic acid equivalents, * (RE)/g -mg rutin equivalents.

Growth performance of fish fed the experimental diets is shown in Table 3. The results revealed that the fish fed diets contained PPE weren't significantly ($P > 0.05$) different with all groups in total final BW, body gain, body gain % and total FCR compared with those fed the control diet. As addition of PPE, final BW, body gain, body gain % and total FCR were decreased but none significantly. The average daily feed intake and specific growth rate % weren't significantly ($P > 0.05$) different with all groups except for fish fed diet contained 0.5% PPE was had a significant ($P<0.05$) decrease if compared with the control diet.

Our growth performance results for fish groups fed diets contained PPE agreed with who reported that the rabbit bucks fed diets containing pomegranate peel 0.5, 1.0 and 1.5 % that cause decrease in final body weight, weight gain and feed intake compared to control[8]. Also, the rats fed on diets containing dry pomegranate peel can be used safely to manage body weight or even for weight reduction without any health hazards[20]. This effect on body weight gain or even cause weight reduction could be attributed to several factors as pomegranate peel contains considerable amounts of polyphenols together with the high fiber content which reduced food consumption and the restricted calorie intake[20]; contains polyphenols may suppress growth of the adipose tissue through their anti-angiogenic activity and by modulating adipocyte metabolism or reduce fat digestion and absorption, nutrient digestibility (CP and NFE) and nutritive value of TDN, DE and DCP[8].

Table 3. The effect of dietary supplementation with PPE on allover performance of Nile tilapia *(Oreochromus niloticus)*.

Ingredient	Experimental diets				
	Control Diet	PPE extract in diets			
		0.1%	0.2%	0.3%	0.5%
Initial body weight, g	14.86± 0.53	14.85± 0.54	14.88± 0.52	14.17± 0.41	15.72± 0.56
Final body weight, g	26.11± 1.38	25.47± 1.92	23.91± 1.40	22.77± 0.84	23.79± 0.48
Body weight gain, g	11.24± 0.86	10.62± 1.38	9.03± 0.87	8.59± 0.82	8.07± 0.52
Body weight gain, %	75.48± 3.13	71.07± 6.72	60.41± 3.86	60.80± 6.47	51.63± 4.90
Specific growth rate, %	0.76± 0.02^{a}	0.72± 0.05^{a}	0.63± 0.03^{ab}	0.63± 0.05^{ab}	0.56± 0.04^{b}
Feed consumption, g	25.07± 1.06^{a}	23.03± 1.58^{a}	19.51± 1.64^{ab}	17.51± 1.67^{ab}	16.45± 1.85^{b}
Feed conversion ratio	2.23± 0.01	2.19± 0.08	2.16± 0.04	2.05± 0.07	2.05± 0.14

^{ab}Mean in the same row with different superscripts are significantly different at ($P < 0.05$).

The results revealed that dietary supplements had a significantly (P <0.05) decreased the tested biochemical parameters of all groups compared to the control group as shown in Table 4. Results indicated that the lowest levels of total cholesterol, triglyceride, LDL-cholesterol, ALT, AST, creatinine and urea were found in fish groups fed on diets contained PPE, while these groups had a significant (P <0.05) increased HDL-cholesterol concentration.

Rat feeding PPE at level 800 mg/kg BW was able to reduce the levels of TC, TG and TL in hypercholesterolemic rat's serum[12]. Using of pomegranate peel had significant decrease in blood triglyceride, low density lipoprotein and very low density lipoprotein in rabbit[8]. Supplemental effects of purified bioflavonoids (genistein and hesperidin), as potential alternatives to plant/ herbs or synthetic anti-oxidants had significant decrease in cholesterol and triglyceride contents in serum and breast muscle[16].

A marked effect of PPE on rat liver functions that decreased ALT, AST and ALP by 1 to 1.5-fold compare to control group[12]. Also, the effect of chronic administration of PPE on liver fibrosis induced by bile duct legation in rats and found that serum ALT and AST were significantly decreased by PPE treatment[34]. These indicate that pomegranate peel preserved the structural integrity of the hepatocellular membrane and liver cell architecture which is confirmed by histopathological studies[9, 4].

Regarding the levels of uric acid, urea and creatinine, it could be noticed that lower levels were found in hypercholesterolemic rat group fed on basal diet containing 800 mg/kg PPE than other groups[12].

Table 4. The effect of dietary supplementation with PPE on some serum biochemical parameters of Nile tilapia *(Oreochromus niloticus)*.

Ingredient	Experimental diets				
	Control diet	PPE extract in diets			
		0.1%	0.2%	0.3%	0.5%
Effect of PPE on Lipid profile					
Total cholesterol, mg/ dl	192.51±10.02^{a}	161.85±6.92^{b}	142.44±4.45^{bc}	132.01±4.37^{c}	124.18±4.23^{c}
Triglyceride, g/dl	172.71±5.03^{a}	146.41±3.66^{b}	126.12±1.74^{c}	113.41±3.04^{d}	91.08±4.32^{e}
HDL, mg/dl	51.30±2.02^{a}	59.81±2.60^{b}	60.62±2.98^{b}	62.99±2.64^{b}	66.04±2.95^{b}
LDL, mg/ dl	157.37±4.71^{a}	93.79±2.99^{b}	84.10±6.38^{bc}	79.05±3.56^{c}	71.38±3.55^{c}
Effect of PPE on liver enzyme activities					
AST, IU/ dl	26.51±1.24^{a}	23.13±0.98^{b}	20.68±1.09^{b}	16.94±0.80^{c}	15.39±0.86^{c}
ALT, IU/ dl	16.89±0.93^{a}	14.23±0.80^{ab}	12.69±1.14^{bc}	10.58±1.18^{c}	9.85±0.92^{c}
Effect of PPE on kidney function					
Creatinine, mg/ dl	0.79±0.04^{a}	0.73±0.06^{ab}	0.65±0.03^{abc}	0.56±0.04^{bc}	0.49±0.04^{c}
Urea, mg/ dl	22.01±1.52^{a}	18.77±1.32^{ab}	16.92±0.38^{bc}	14.88±0.95^{cd}	12.68±1.05^{d}
Uric acid, mg/ dl	4.87±1.09^{a}	4.21±0.78^{a}	3.95±0.43^{a}	3.27±0.47^{a}	2.66±0.32^{a}

^{abcde}**Mean in the same row with different superscripts are significantly different at (P < 0.05).**

Table 5 shows the effect of experimental diets on some immunological parameters in different fish groups. There were significant differences (P <0.05) between fish groups. The fish groups fed on diet contained 0.5, 0.3, 0.2% PPE had a significant higher values of final IgM and lysozyme values as compared with other dietary treatments or the control.

The non-specific immune system of fish is considered to be the first line of defense against invading pathogens. IgM and lysozyme are some important indices of non-specific immunity in

fishes. The results indicated that the highest survival rate was in fish groups fed on diet contained 0.5, 0.3, 0.2% PPE as compared with other dietary treatments or the control. These results are in agreement with who reported that the cumulative mortality was high, 80%, in fish fed non-pomegranate enriched diet against bacterial infection[10]. The pomegranate enriched diet enhanced the innate immune response, which may possibly be an important factor in reducing the percentage cumulative mortality thereby protecting the fish from bacterial infection.

In conclusion, this study declared that the addition of pomegranate peel extract (PPE) in Oreochromus niloticus diets without any adverse effects on growth parameters or health status. In addition, it improved immune status, lipid profile and functions of liver and kidney. PPE has been suggested as immunostimulatant due to their biological effects.

Table 5. The effect of dietary supplementation w with PPE on on immune status of Oreochromus niloticus.

Ingredient	Experimental diets				
	Control diet	PPE extract in diets			
		0.1%	0.2%	0.3%	0.5%
Initial IgM value	15.47±0.47	14.95±0.60	15.08±0.85	15. 48±0.92	15.42±0.83
Final IgM value	17.85±1.71^{b}	25.61±3.79 ^{ab}	28.66±3.33^{a}	30.51±3.09^{a}	33.05±2.91^{a}
Initial lyzozyme value	14.44±0.38	15.58±0.59	16.04±0.68	15.57±0.77	16.14±1.28
Final lyzozyme value	18.40±1.22^{b}	26.17±4.16^{ab}	29.55±5.17^{ab}	32.47±1.80^{a}	35.92±3.27^{a}
Survival rate, %	85.55±2.00	89.45±1.46	91.11±2.00	91. 11±1.93	92.77±2.42

^{abcd} Mean in the same row with different superscripts are significantly different at (P < 0.05).

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