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Utilization tow extracts of pomegranate (*Punica granatum*) peel on growth performance and serum biochemical parameters of the common carp (*Cyprinus carpio*) fingerlings

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ABSTRACT

The purpose of this research was to investigate how common carp (*Cyprinus carpio*) fingerlings' growth performance, survival rate, chemical composition, and serum biochemical were influenced by pomegranate (*Punica granatum*) peel powder and its extracts (aqueous and alcoholic). A commercial meal with ~35% crude protein was supplemented with 0.5 and 1% of raw pomegranate peel (RPP), aqueous (PPW), and alcohol (PPA), respectively (three replicates for each treatment). Fish were stocked in aquariums (60 x 40 x 50 cm) at a density of 15 fingerlings per group, and they were fed twice daily for 70 days. The results for specific growth rate (SGR) showed that, excluding RPP0.5 and PPW0.5, as well as for feed conversion (FCR) and protein efficiency (PER), all groups ($P<0.05$) outperformed the control group (C). Near-infrared spectroscopy (NIR) measurements revealed that protein improved in the RPP1, PPA0.5, and PPW1 groups ($P<0.05$), whereas fat was improved in the RPP0.5 and PPA1 groups ($P<0.05$). Ash did not change significantly ($P>0.05$). PPA showed a significant difference ($P<0.05$) for AST, however, RPP0.5 and PPW1 did not demonstrate a significant difference ($P>0.05$) for ALT or ALP compared to the control (C). Regarding kidney efficiency, it was found that RPP, PPA, and PPW were preferable to urea, uric acid, and creatinine. Summary of findings: pomegranate peel enhanced growth as well as chemical composition and biochemical serum parameters when added at a rate between 0.5 and 1%.

INTRODUCTION

Addressing the issues that prevent aquaculture's sustainability is critical given the ongoing rise in demand for it as a low-cost source of food chain security, and the safety of fish as food is a crucial aspect of safeguarding consumers **(Mchazime & Kapute, 2018)**. In addition to some vitamins (A, D, B6, B12, etc.) and minerals like iron, zinc, iodine, selenium, potassium, sodium, etc., fish is a good source of proteins and fats that contain fatty acids, the most well-known of which are omega-3, particularly icosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) **(Chowdhury *et al.*, 2017)**. In order to achieve maximum production per unit area, fish farmers must raise stock density. However, this practice stresses fish, increases the risk of infectious disease transmission, degrades fish health, and impairs their immune systems, especially in intensive farming systems **(Zaki *et al.*, 2020)**.

There are common mistakes in aquaculture which include an imbalanced diet, decreased growth, inadequate feed conversion, and stress. Fish diets frequently contain antibiotics. These substances do, however, have negative side effects. The World Health Organization (WHO) has so encouraged researchers to look for low-cost, safe, and efficient

natural alternatives such as herbs, vegetables, and other edible, environmentally friendly plants for use as growth stimulants or immune stimulants **(Toutou *et al.*, 2019)**. Many researchers have used some medicinal and herbal plants in feeding animals, such as the water hyacinth (*Eichhornia crassipes*) and bay laurel (*Laurus nobilis*) in feeding fish (**Sayed-Lafi *et al.*, 2018; Taher *et al.*, 2018)**, additionally the pomegranate peel in feeding quail **(Abbas *at al.*, 2017)** and others herbal plants.Pomegranate (*Punica granatum*) with its famous medicinal history, rich in bioactive molecules (Hydroxybenzoic acids, Anthocyanidins and Flavonoids) has shown many medicinal properties due to its high content of phenols **(Akhtar *et al.*, 2015)**. Pomegranate is growing in popularity and demand all over the world due to its multi-functionality and health-promoting effects in the human diet. The grains represent 45-52% of the weight of the entire fruit and the peel represents 49-55% depending on the variety **(Magangana *et al.*, 2020)**.

It was required to shed more light on these plants and their extracts in order to better understand how they affect fish performance. As a result, the goal of the present study was to investigate how common carp (*Cyptinus carpio*) diets including raw pomegranate peel (RPP) and pomegranate peel extract (PPE) influenced growth performance, body composition and some blood parameters.

MATERIALS AND METHODS

Preparation of pomegranate peels (PPE) extract:

Aqueous extract (PPW):

The aqueous extract was created by mixing 25 g of pomegranate peel powder with 250 ml of distilled water (1:10), shaking the mixture for 30 minutes at a speed of 150 cycles per minute, and letting it soak for 24 hours in the refrigerator, as described by **Handa (2008)**. A concentrated extract was then obtained at the bottom of the drying vessel after the mixture had been filtered through several layers of gauze to remove the insoluble plant matter and again using filter papers (Whatmann No. 2). The filter was then removed and dried in an electric oven at 40 C°. until it was no longer visible. The extract was then put in clean, opaque glass bottles and kept in the refrigerator at four C° until use. The process was then repeated by using the same steps and conditions until a sufficient amount of extract was obtained.

Alcoholic extract (PPA):

The method of **Gülçin *et al.*, (2003)** was used in the preparation, as 25 g of pomegranate peel powder was mixed with 250 ml of ethanol alcohol with a concentration of 96% and the mixture was stirred for 24 hours on a magnetic stirrer, and then filtered through gauze for two successive times. Then, using filter paper (Whatmann No.1), the filtrate was concentrated using the rotary evaporator and then dried in the electric oven at a temperature of 40 C° and placed in sealed opaque bottles and kept in the refrigerator until use, and the process was repeated by following the same steps and conditions until a sufficient amount was reached from the extract.

Determination of total phenolics content:

Folin Ciocalteu reagent was used for analysis of total phenolics content **(Chun *et al.*, 2003)**. Briefly, 0.5 ml of the extract was mixed with 0.5 ml of Folin-Ciocalteu reagent. The solution was kept at 25 C° for 5-8 min before adding 2 ml of sodium carbonate solution 7.5 % and adjusting the volume to eight ml with water. After tow h, the absorbance was

measured at 725 nm. Gallic acid was used as standard for the calibration curve. Total phenolic content was expressed as mg gallic acid equivalents per gram of sample (mg/g).

Determination of total flavonoids content:

The total flavonoid content was measured by a colorimetric assay **(Zhishen *et al.*, 1999)**. One hundred micro liters of extract was added to 4 ml of distilled water. Then, 0.3 ml 5% sodium nitrite was added. After five min, 0.3 ml of 10% aluminium chloride was added. In six min, 2 ml of 1 M sodium hydroxide was added to the mixture. Immediately, the mixture was diluted by the addition of 3.3 ml distilled water and mixed thoroughly. The absorbance was determined at 510 nm versus a blank. Rutin was used as standard for the calibration curve. Total flavonoids content of the extract was expressed as mg rutin equivalents per gram of sample (mg/g).

Experimental fish: -

The fingerlings of common carp used in the experiments were obtained from the fish culture station of the Collage of Agriculture. Fish that were highly stressed or oversized were excluded. The fish were placed in a bowl of water and individually weighed to the nearest 13.5±1 g on an electronic scale. The fish were divided into seven treatments, each treatment had three replicates (five fish for each). The fish were allowed to acclimate to the laboratory conditions for two weeks before the start of the experiment, and has been used 60 × 40 × 50 cm aquaria (experimental units) when fish were distributed.

Fish diets and feeding regime:

An Iranian commercial diet of known chemical composition was used in **Table (1)**, had been used seven experimental diets were supplemented by pomegranate peel powder (RPP) at (0.5 %,1%) and its PPA and PPW (0.5 % and 1%), as well as the control diet. The fish were fed diets two times daily (9 am and 4 pm) at a rate of 2 % of body weight for 10 weeks. Water quality parameters were measured during the trial period (pH = 8.78, EC = 2.71 ds\cm, DO= 9.43 ppm, Temp.= 24.34 C°, Sal. = 1.30 psu).

Table (1). The proximate chemical composition of the commercial diet used in the experiment

Nutrition	Amount
Moisture (Max)	10
Crude protein %	35
Ash (Max)	12
TVN (Max) mg/100g	50
Metablisable Energy (Kcal/kg)	3700
Crude Fiber (Max)%	5.5
Crude Fat %	6
TVN (Max) mg/100g	50
Lysine %	1.8
Methionine %	0.48
Threonine %	1.15

Composition: Wheat Flour, Barley, Corn Gluten, Vegetable Meal, Fish Meal, Yeast, Fish Oil, Vegetable Oil, Choline Chloride, Lysine, Methionine, Threonine, Vitamin Premix, Special Mineral Premix, Anti-Oxidant, Inositol.

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 weight gain (g/fish) (WG) = W_1 – In W_0**

- **Specific growth rate (%/day) (SGR) = (In W_1 – In W_0) / T) X 100**
- **Relative Growth Rate (RGR %) = $W_1 – W_0 / W_0$**
- **Feed conversion ratio (FCR) =Total feed fed (g/fish)/ WG**
- **Protein efficiency ratio (PER) = WG /amount of protein fed (g/fish)**
- **Survival rate (SR %) = (N_1 /N_2) × 100**

Where W_0 and W_1 are the initial and final weights, respectively. T is the number of days in the feeding period. N_1 is initial number of fish and N_2 is final number of fish.

Sample preparation and NIR measurement:

The chemical analysis of the body components was carried out according to the method of **Khodabu *et al*. (2007)**. Common carp (n = 21) samples were kept frozen until NIR spectroscopy analysis were performed. Samples were cut and thawed before NIR measurements were performed. NIR spectra were recorded on an Analytical Spectral Device (XDS) Monochromator typpe XM-1000 NIR analyzer (made in Sweeden) by using a fibre optic probe (Bifurcated Fibreoptic Reflectance Probe). Samples were scanned at 25 different sites with an increment of 2 nm between 350 and 2500 nm with a fast scanning time of 0.1 s. Spectra were collected randomly all over fish surface. The spectra were exported for computation as JCAMP files. Each spectrum was assigned the corresponding concentration value obtained by the reference method. All determinations were performed in triplicate.

Biochemical analysis:

Blood samples were drawn through the heart by a 2 ml glass syringe. The drawn blood was placed in test tubes in two groups, the first group free of anticoagulant for the purpose of obtaining serum by centrifuge at 3000 rpm for 15 minutes. And kept in refrigeration until biochemical analysis. AST and ALT in serum were measured by kit from the Chinese company mindray by mindray BS-230 spectrophotometer where taken 1 ml of reagent was withdrawn and 100 µl of serum was withdrawn and mixed with stirring and reading directly, three readings between readings one minute, summed and divided by their number It is multiplied at 1746 and has a wavelength of 550 and 500 nanometers, respectively. While, ALP M withdraw 800 µl of reagent (A) and withdraw 200 µl of reagent (B) and 20 µl of serum and mix it and wait for three minutes, measured at wavelength 630 and then three readings within three minutes were taken the average of the readings. Urea, uric acid and creatinine were measured by a kit from the same company above, at wavelengths of 580, 546 and 560 nm, respectively.

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 by using IBM SPSS (2013) program, Version 22. Differences between means were compared by using LSD's multiple range tests at ($p< 0.05$) level.

RESULTS

Table (2) showed the PPE's antioxidant effects (the total phenols and flavonoids). It was noted that the content of phenols and flavonoids in the alcoholic extract increased, followed by the aqueous extract.

Table (2): Total phenolic and flavonoid contents of Pomegranate peel extrac (n = 3)

Extracts	Total phenolic mg GAE/ 100 g*	Flavonoid mg RE/ g*
Alcoholic extract	175.95±1.41	42.89±1.30
Aqueous extract	151.09±1.50	36.04±1.53

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

The results through **Table (3)** of the growth performance (SGR and RGR) showed a significant deffrance ($p<0.05$) for treatment PPA0.5 over the control, PP 0.5 and PPW 0.5, and it did not differ significantly ($P>0.05$) with the rest of the treatments. It was noted that there was an improvement in the growth performance of fish fed a diet containing RPP and extracts. It was observed that the PPE were significantly ($P<0.05$) superior to the control (C) in addition to PPW 1 and PP1, and the same was the case for PER. The survival rate results were good for all groups.

Table (3): The effect of dietary supplementation with RPP and PPE on allover performance of common carp.

Performance parameters	Control Diet	Experimental diets					
		RPP in diets		PPE in diets			
		PP 0.5	PP1	PPA 0.5	PPA1	PPW 0.5	PPW 1
Initial weight (g)	66.68±1.11	65.84±1.59	65.61±1.42	66.66±1.67	64.45±1.12	65.72±1.63	66.82±1.01
Final weight (g)	77.65±1.34	77.99±1.93	81.64±1.77	84.41±1.32	79.80±0.665	78.40±2.46	82.32±1.28
WG (g)	10.78±0.38	16.13±0.49	16.03±0.91	17.55±2.40	15.35±1.34	12.68±3.04	15.50±2.28
SGR (% / day)	0.21 ± 0.01^{c}	0.24 ± 0.01^{bc}	0.31 ± 0.02^{ab}	0.33 ± 0.05^{a}	0.31 ± 0.03^{ab}	0.25 ± 0.06^{bc}	0.30 ± 0.04^{ab}
RGR (%)	16.13±0.49	12.15±0.54	24.43±1.45	26.42±4.22	23.86±2.44	19.38±4.89	23.25±3.73
FCR	6.52 ± 0.18^{c}	5.76 ± 0.20^{bc}	4.19 ± 0.47^{ab}	3.91 ± 0.41^{a}	4.33 ± 0.69^{ab}	5.56 ± 1.61^{bc}	4.45 ± 0.72^{ab}
PER	0.01 ± 0.44^{c}	0.02 ± 0.50^{bc}	0.03 ± 0.64^{ab}	0.10 ± 0.69^{a}	0.06 ± 0.63^{ab}	0.12 ± 0.52^{bc}	0.09 ± 0.61^{ab}
SR (%)	100	100	100	100	100	100	100

Means in the same **raw** with different overscript letters are significantly ($P<0.05$) different.

The results in **Table (4)** and by the NIR showed that RPP and PPE improved the biochemical content of the fish body, with the crude protein content significantly increased ($P<0.05$) and the crude lipid content also improved ($P<0.05$). No significant differences ($P>0.05$) were observed in the ash content.

Table (4): Proximate carcass composition (% wet weight) of common carp fingerlings at the end of experiment (Mean + SD) (n=3).

Items	Initial	Control Diet	Experimental diets					
			RPP in diets		PPE in diets			
			PP 0.5	PP1	PPA 0.5	PPA1	PPW 0.5	PPW 1
Moisture	73.66	77.41 ± 1.83^{a}	75.05 ± 1.23^{a}	75.18 ± 1.98^{a}	74.29 ± 0.87^{a}	74.57 ± 1.75^{a}	75.45 ± 1.89^{a}	74.57 ± 2.32^{a}
Crude protein	13.80	13.45 ± 0.62^{c}	14.4 ± 0.51^{bc}	16.0 ± 1.30^{ab}	16.66 ± 1.04^{a}	15.3 ± 1.06^{abc}	15.6 ± 1.23^{abc}	16.4 ± 0.96^{ab}
Crude lipid	7.11	4.23 ± 0.29^{b}	5.81 ± 0.83^{a}	5.43 ± 0.62^{ab}	5.06 ± 0.38^{ab}	6.46 ± 0.40^{a}	5.19 ± 1.19^{ab}	5.08 ± 0.58^{ab}
Ash	4.43	3.60 ± 0.49^{a}	4.02 ± 1.07^{a}	2.74 ± 0.34^{a}	3.65 ± 1.06^{a}	3.11 ± 0.45^{a}	3.16 ± 0.84^{a}	3.24 ± 0.88^{a}

Means in the same **raw** with different overscript letters are significantly ($P<0.05$) different.

Table (5) displays the liver enzymes, with the highest value occurring in the control (C). Ast and ALT were decreased in the blood serum by the effect of pomegranate peels and its alcoholic and aqueous extracts. ALP, however, didn't demonstrate any significant difference (P>0.05) between any of the groups. Therefor,the results show a significant improvement in the liver enzymes AST, ALT, and ALP when compared to the control treatment (C).

Table (5): The effect of dietary supplementation with RPP and PPE on some serum biochemical parameters of Common carp.

Items	Control Diet	RPP in diets		PPE in diets			
		PP 0.5	PP1	PPA 0.5	PPA1	PPW 0.5	PPW 1
AST (U/L)	173.4±1.94^{b}	168.5±5.17^{ab}	169.2±1.68^{ab}	165.9±4.65^{a}	165.0±2.76^{a}	169.5±1.61^{ab}	1.71.1±3.35^{ab}
ALT (U/L)	74.0±4.05^{c}	36.9±2.76^{a}	58.9±2.54^{b}	59.9±3.80^{b}	63.0±2.05^{b}	62.5±3.00^{b}	38.9±1.57^{a}
ALP (U/L)	135.5±1.96^{a}	134.9±7.32^{a}	134.1±3.81^{a}	130.7±5.60^{a}	130.1±1.49^{a}	134.5±2.73^{a}	134.4±3.64^{a}

Means in the same **raw** with different overscript letters are significantly (P<0.05) different.

Fig. (1) showed that the total efficiency of urea improved as all groups decreased (P>0.05) compared to the control (C). The results showed that uric acid decreased in the raw pomegranate peel and extracts treatments (P>0.05) compared to the control (C), and the same was true for creatine, as all treatments (P>0.05) outperformed the control (C).

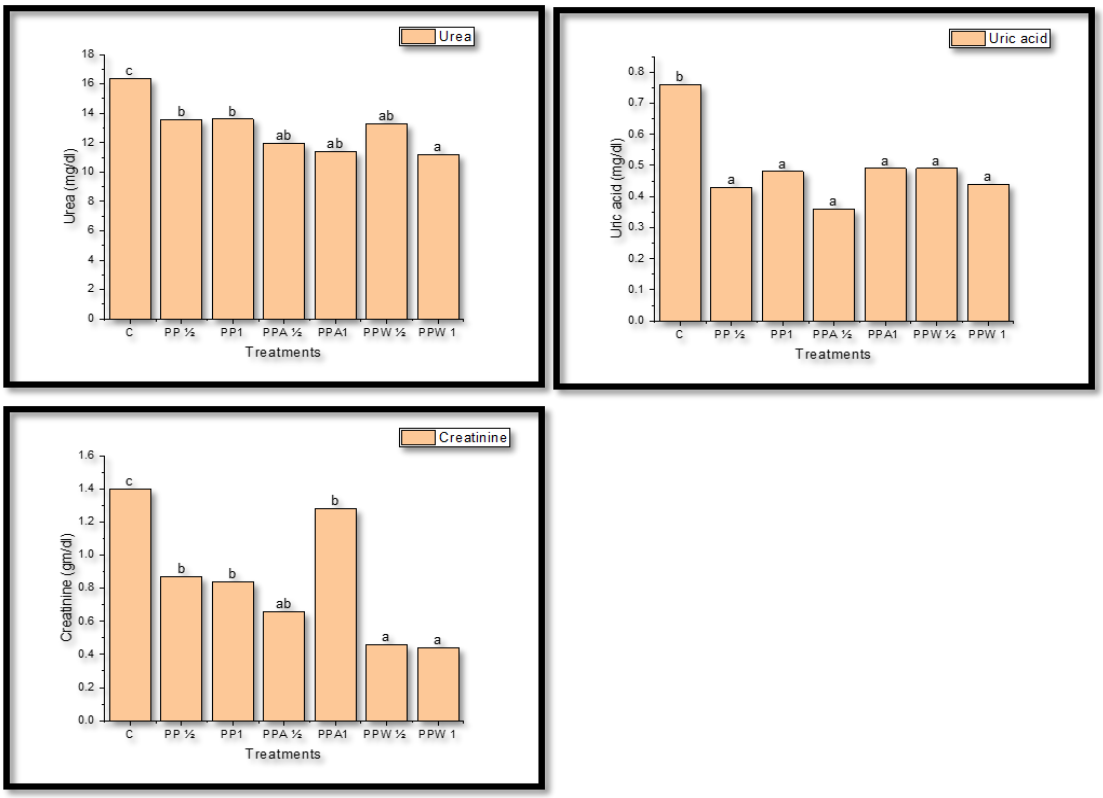

Fig (1). Changes in the Urea, Uric acid and Creatinine in treatments fed supplementation with RPP and PPE. Data are represented as mean ± S.D. (n = 3, in replicate)

DISCUSSION

The antioxidant results were consistent with those from studies by **Badawi & Gomaa (2016)** and **Nuamsetti *et al.*, (2012)** who found that the alcohol extract contained phenols (185 mg GAE/100 g) and flavonoids (32 mg rutin/100 g), as well as a total of 166.83 mg GAE/100 g, 152.6 mg GAE/100 g, and 85.48 mg GAE/100 g of phenols, respectively. Hence, If one compares this plant's antioxidant content to that of many other plants, it is high. PPE includes various active compounds that have antioxidant and anti-inflammatory effects on the human gut, including ellagic acid, phenols, flavonoids and tannins **(Colombo *et al.*, 2013)**. This result assisted in increasing performance and a significant improvement in fish growth (SGR and RGR). It is observed that the polarity which influences the extraction rate accounts for the PPE is superior to the PPW. It has been shown that the solvent is more extractive for the antioxidant chemicals the more semi-polar is and the less extractive it is the more non-polar the other half of the solvent is **(Wang *et al.*, 2011)**. The growth rate was improved by numerous therapeutic herbs, perhaps as a result of the higher feed intake caused by the increased palatability of the herbs **(Sarhadi *et al.*, 2020)**. According to these findings, plant-based feed additives are likely to alter the intestine's microbiota and digestive system, which is why these supplements are enhancing fish growth **(MacLennan *et al.*, 2002)**. It is noticed from the results that the values of PER increased in the treatments of pomegranate peels and its extracts. This is due to the improvement of digestion and enzymatic secretions in the intestine. This increases the efficiency of utilizing nutrients because it contains biologically active compounds **(Akuru *et al.*, 2021)**. Fish survival rate is a crucial component of fish production; it is influenced by factors such as the availability and kind of feed, as well as the physical and chemical characteristics of the water **(Kumar *et al.*, 2017)**.

Biochemical composition analysis of fish body clearly showed that the percentage of body water is a good indicator of the relative contents of energy, proteins and fats in fish bodies because a lower percentage of water indicates that there is an inverse relationship between it and the contents of protein, fat and higher energy **(Dempson *et al.*, 2004)**. With a drop in moisture before the experiment, the body's protein, fat, and ash contents improved, and the treatments didn't show any significant differences ($P>0.05$) from one another. The findings demonstrated that, with the exception of the control (C), protein levels rose across all treatments. Because of the tannins in pomegranate peels, animals can keep more protein in their body tissues **(Graingerd *et al.*, 2009)**. Additionally, herbal additives modulate the secretionof pancreatic enzymes, the main factors in nutrientdigestion and assimilation, lead to increase muscleprotein **(Yılmaz, 2012)**. According to the findings improved crude lipid because of the polyphenols found in pomegranate peels, may prevent the development of fat tissue through their anti-obesity effect and by altering adipocytes' metabolism **(Baile *et al.*, 2011)**. The present are in line with those of **Hussein *et al*. (2022)**. The value of ash in tissues is a measurement of their overall mineral content. According to **Stoyanova (2016)** reported fish meat has high mineral content, particularly calcium, phosphorus, magnesium, and potassium, as well as significant amounts of vitamin D. The findings demonstrated that the ash content did not differ significantly ($P>0.05$). The present findings are support those of other studies **Zakeri *et al.*, (2018)** and **Toutou *et al.*, (2019)**.

A marked effect of PPE on fish liver functions that decreased ALT, AST and ALP. According to certain research, pomegranate peel preserves the integrity of the structural membrane of the liver and the hepatocytes , therefore it appears that pomegranate peels and extracts have preserved the liver **(Friedman, 2000; Chattopadhyay, 2003)**. Studies have shown that the liver's defense mechanism is linked to antioxidants' capacity to lower ROS **(Cao *et al.*, 2016)**. This is evident from the pomegranate peels' presence of antioxidants,

which were found in the form of phenols and flavonoids. These protect the liver against aggravating factors and free radical-induced fibrosis. The present findings concurred with **Badawi & Gmaa (2016); Shafiei *et al*., (2016); Acar *et al*., (2018)** and **Badrey *et al*., (2019)**. A number of researchers explained that the roles of urea, uric acid and creatine were not clearly understood physiologically, but their levels indicated the general health of the gills and kidneys **(Campbell, 2004)**.

The measurement of creatinine is important and is an indicator of kidney health because it is an easy-to-measure by-product of muscle metabolism that is secreted by the kidneys without changes. If there is a deficiency in glomerular filtration, the level of creatinine in the blood will rise. In any case, because creatinine is a simple-to-measure by-product of muscle metabolism that is continuously released by the kidneys, its measurement is significant and serves as a sign of renal health. Blood levels of creatinine will increase if glomerular filtration is inadequate **(Rastiannasab *et al*., 2016)**.

CONCLUSION

In accordance with the present findings, it is possible to add pomegranate peel (raw, aqueous or alcoholic extract) in a ratio of 0.5 and 1% to the food of common carp, which fosters development and nutritional transformation and raises the body's protein and fat content. Additionally, antioxidants increase kidney function and lower blood serum levels of liver enzymes. More investigation is required to ascertain whether pomegranate peel supplementation at higher levels may be used in intensive systems for confined animals without affecting growth performance, efficient feeding, or survival rates and both kidney and liver efficiency.

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