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Changes in Hematological Parameters of Common Carp (*Cyprinus carpio*) Fingerlings Fed on Pomegranate (*Punica granatum*) Peel Supplement

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Abstract: The present study aimed to determine the effect on different blood parameters (WBCs, RBC, HGB, HCT, MCV, MCH, and MCHC) in fingerlings of common carp (*Cyprinus carpio*) weighing 13.5 ± 1 g with pomegranate peels (*Punica granatum*) in their different forms, raw (PPR), alcoholic (PPA) and water (PPW), which were added to their feed as 0.5% and 1% respectively, in addition to the control treatment (21 replicates). A commercial diet was used containing 35% crude protein, 6% lipid, 12% Ash and 50 TVN for ten weeks. At the end of the experiment, blood was drawn from the heart of the fish, and analyses were performed. The results showed that RBCs, HGB and HCT improved significantly (P< 0.05), while there were no significant differences (P> 0.05) in WBCs, MCH, and MCHC. In short, we recommend adding pomegranate peels or extracts (alcoholic or aqueous) to common carp food at a rate not exceeding 1%.

التغيرات في مصل دم إصبعيات أسماك الكارب الشائع (*Cyprinus carpio*) المغذاة على قشور الرمان (*Punica granatum*)

الكلمات المفتاحية :
قشور الرمان؛ خلايا الدم البيض؛ خلايا الدم الحمر؛ الكارب الشائع.

المستخلص : تهدف الدراسة الحالية إلى تحديد تأثر معاملات الدم المختلفة (WBC, RBC، HGB، HCT، MCV، MCH و MCHC) في إصبعيات الكارب الشائع (*Cyprinus carpio*) التي تزن 13.5 ± 1 غم بقشور الرمان (*Punica granatum*). حيث تمت إضافته مسحوقا خاما (PPR) ومستخلصا كحوليا (PPA) ومستخلصا مائيا (PPW) بنسب 0.5 %، و1 % على التوالي بالإضافة إلى معاملة السيطرة (21 مكرر). استخدمت عليقة تجارية تحتوي على 35٪ بروتين خام، و6٪ دهون، و12٪ رماد، و50 TVN لمدة 70 يومًا. في نهاية التجربة، تم سحب الدم من القلب، وإجراء التحليلات. أظهرت النتائج أن RBCs، HGB، وHCT تحسنت بشكل ملحوظ (P<0.05)، بينما لم تكن هناك فروق معنوية (P>0.05) في WBCs، HCT، MCH، وMCHC. باختصار، نوصي بإضافة قشور الرمان، أو أحد مستخلصاته إلى عليقة الكارب الشائع بمعدل لا يتجاوز 1٪.

INTRODUCTION

In the intensive aquaculture industry, several environmental stressors influence the fish species. Fish have stress reactions when exposed to harmful stimuli such as temperature fluctuations, pH variations, decreases in oxygen levels, increases in ammonia levels, handling, transport, and osmotic changes in water (Pickering, 1993; Everly and Lating, 2013). The fish individual's integrated stress response, which consists of behavioral, neurological, hormonal, and physiological components, can alter the fish's health status and diminish its resistance to diseases and stress. It

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also takes some time for the fish to get back to normal. (Lebelo et al., 2001; Suljević et al. 2016). Additionally, antibiotics are frequently used to treat fish infections, despite having adverse side effects. As a result, scientists have looked for cheaper, safer, and more efficient natural alternatives, such as herbs, vegetables, and other edible plants, to employ as growth stimulants or immunostimulants (Badrey et al., 2019). Pomegranate (*Punica granatum*) peel products have higher antioxidant levels than pomegranate juice, making them attractive candidates as a nutritional supplement for animal feed (Badawi and Gomaa 2016). However, Türkyılmaz et al., (2013) reported pomegranate peel is also a good source of flavonoids, phenolic acids, and tannins (ellagitannins as punicalin, punicalagin, gallic acid, and ellagic acid). Therefore, pomegranate peel's beneficial components make it a potential candidate for the discovery of new natural agents with a variety of biological functions, antibacterial activity, and potential health benefits (Kaderides et al., 2015). Pomegranate peel extract has been extensively studied, in particular, for its potent anti-microbial effect, high anti-oxidant activity, cytotoxic effect, hypoglycemic effect (Rajput et al., 2011) hypolipidemic effect (Belal et al., 2009), hepatoprotective effect, and anti-inflammatory activity (Ibrahim, 2010; Jurenka, 2008).

The hematological variables can be used as an indicator for assessing the health conditions of aquatic organisms. However, this can be very difficult due to many endogenous and exogenous factors. According to Fernandes and Mazon (2003), the blood traits of fish are closely related to environmental and biological factors. Physiological changes can be a result of stress, and several blood characteristics are used to determine these conditions. Adams et al., (1996) stressed the importance of using hematology as a tool to track changes in fish diet, water quality, and disease while also monitoring their biological state. Since fish are closely entwined with their environment, they are subject to physical and chemical changes that may be reflected in the components of blood cells. Other factors, including behavior, environment, and climate, can also affect blood values (Fazio, 2019). Therefore, the aim of the present study was to investigate how supplementing pomegranate peel to the diet of common carp (*Cyprinus carpio*) influenced hematological parameters.

MATERIALS AND METHODS

Preparation of pomegranate peels extract

Water 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 et al. (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 4 C° until use. The process was then repeated 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 gm of pomegranate peel powder was mixed with 250 ml of ethanol alcohol with a concentration of 96%. The mixture was stirred for 24 hours on a magnetic stirrer and then filtered through gauze two successive times, then using filter paper (Whatmann No.1). The filtrate was then 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. The process was repeated by following the same steps and conditions until a sufficient amount was reached from the extract.

Experimental fish: The fingerlings of common carp used in the experiments were obtained from the aquaculture station of the College 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). The fish were allowed to acclimate to the laboratory conditions for two weeks before the start of the experiment. 60 × 40 × 50 cm aquaria (experimental units) were used where fish were distributed.

Fish diets and feeding regime: An Iranian commercial diet of known chemical composition was used, as shown in Table (1). Seven experimental diets were supplemented by pomegranate peel powder (PP) at (0.5% and 1%) and its aqueous and alcohol extracts (0.5% and 1%), as well as the control diet (C). The fish were fed diets two times daily (9 am and 4 pm) at a rate of 2 % of body weight for ten weeks. Water quality parameters 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
Metabolizable 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.

Hematological analysis: In test tubes containing an anticoagulant, blood samples from the heart were collected using a 2-ml glass syringe. The Mindrary- BC-30S hematology analyzer was used to measure the hemoglobin concentration (HGB), hemocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cells (RBC) and white blood cells (WBC).

Statistical analysis: Data were presented as mean ±SD. The results were subjected to a one-way analysis of variance (ANOVA) to test the effect of treatment inclusion on fish performance. Data were analyzed using IBM SPSS (2013) program, Version 22. Differences between means were compared using LSD's multiple range tests at $P< 0.05$ level.

RESULTS AND DISCUSSION

Figure (1) of the results shows the values for RBCs, WBCs, MCV, and HGB. The inclusion of PP and PPE has been shown to improve blood hematological parameters. Except for PP1, the increase in RBC coefficients was not significant ($P>0.05$) when compared with the control (C) treatments. In terms of WBCs, no significant differences ($P>0.05$) were found between any of the treatments. There were no significant differences ($P>0.05$) in MCV. Both the PPA1 % and the water extract treatments enhanced HGB.

In aquaculture, hematology is gaining importance because of the ease and reliability of monitoring the health status of cultured species (Hrubec et al., 2000). WBCs are circulatory cells that function as a cellular defense, aid in acquired and inherent immunological responses, and are correlated with BMI. The thymus, spleen, and kidneys are additional organs where WBCs are made (Esmaeili, 2021). The findings revealed that the WBCs values in the blood of carp fish did not differ significantly ($P>0.05$). The active components in the pomegranate peel may have decreased free radicals compared to the control group, which may have decreased the vulnerability to stress brought on by the buildup of free radicals. Fish subjected to stress will have decreased blood WBC counts, especially lymphocytes (Esmaeili, 2021). Additionally, no bacterial infections were present in the fish used in the experiment. According to Hrubec et al., (2000) exposure to low-quality water with a high bacterial load affects the WBCs count and tends to lower the number of RBCs. The present findings are consistent with the study of Shafiei et al., (2016) who used PPE in the diets of common carp fingerlings. This result agrees with the findings of Badrey et al., (2019), which indicated that the increase in WBC in the blood of monosex tilapia (*Oreochromis niloticus*) fed on PPR is a result of a possible immune modification in its body. The results of Sönmez et al., (2022) on rainbow trout (*Oncorhynchus mykiss*), Avazeh et al., (2021) indicated an increase in WBC with increased levels of PPR in rainbow trout (*O. mykiss*) diets, which indicated the immunomodulatory effect of PPR, while the results of Harikrishnan et al., (2012) found that the olive flounder (*Paralichythys olivaceus*) had increased resistance against *Philasterides dicentrarchi* due to the increase in WBCs after feeding with pomegranate peels.

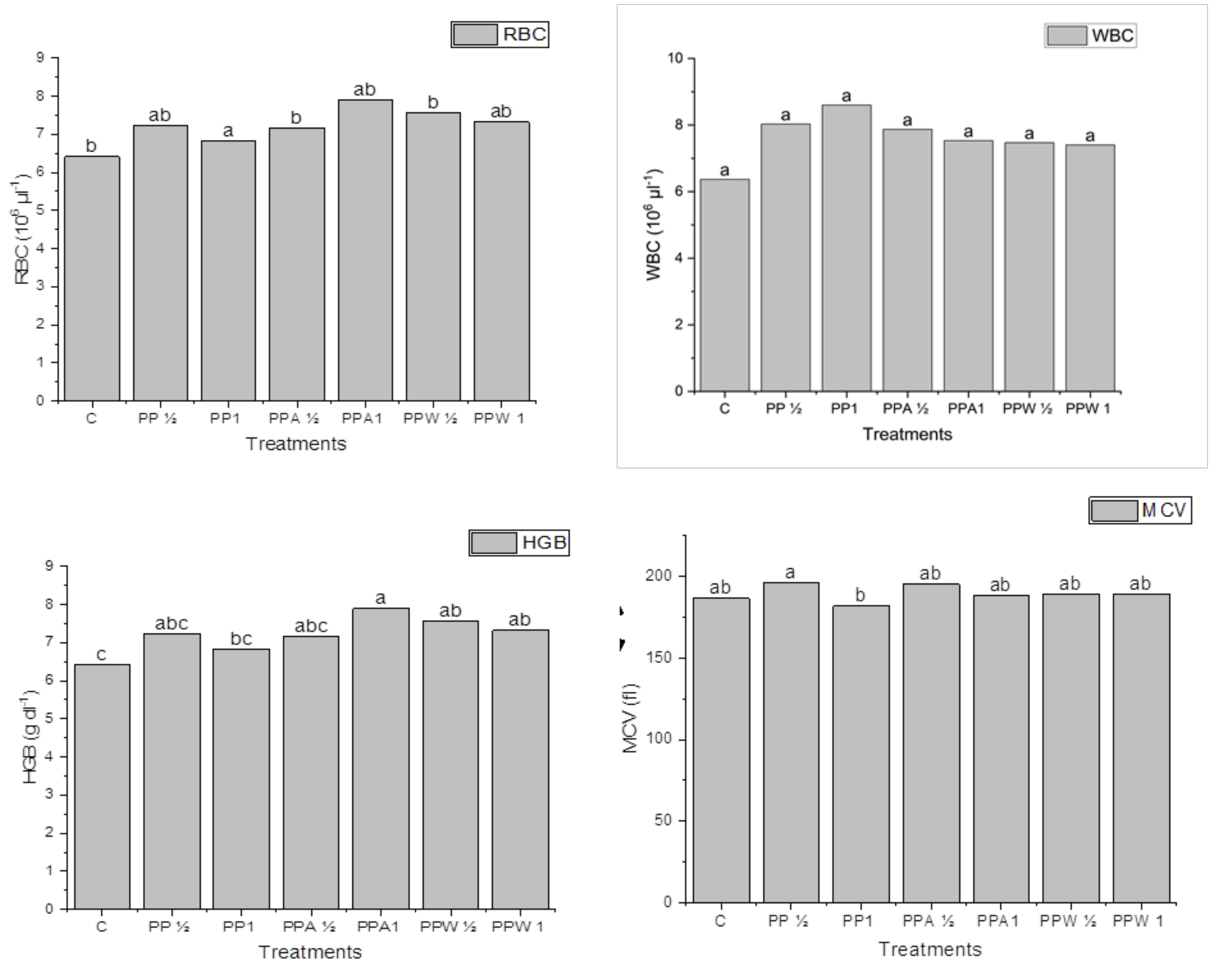

Figure: (1). Changes in the white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB) and mean corpuscular volume (MCV) in treatments fed supplementation with (PPR) and (PPE) pomegranate diets. Data are represented as mean±SD. (n = 3, in replicate). The **ab** Mean in the same columns with different superscripts are significantly different at (P<0.05).

RBCs, HGB, and HCT are measures of oxygen-carrying capacity and general health. PVC, RBCs, and HGB levels in the blood are absolutely necessary for MCV, MCH, and MCHC to function (Tavares-Dias 2006; Singh et al., 2020). The absorption of iron, that the body needs, due to the compounds in pomegranate peels, or the effect of antioxidants in the peels that can reduce the dissolution of blood cells due to hydrogen peroxide H_2O_2 caused by oxidative stress in RBCs, could be the reasons why HGB was improved by the aqueous extract treatments in addition to PPA at 1% (Ma et al., 2000). The polyphenolic compounds found in plant components have the capacity to form complexes with metal ions like iron and obstruct physiological processes involving iron and other minerals (Satyakeerthy, 1999). When several polyphenolic compounds interact with the cell membrane's surface, they can act as a barrier against soluble free radicals (Shafiei et al., 2016).

According to Gallaugher et al., (1992), MCV and MCHC are indicators of RBCs size. The present results showed no significant (P>0.05) differences in the values between the treatments. Therefore, it appears that the concentration of pomegranate peels had an impact on that; when it was increased in the ration, it decreased MCV and MCHC. Pomegranate peels contain tannic acid, which lowers the bioavailability of iron and results in a drop in RBCs. According to Lee et al., (2010), consuming 125, 250, 500, and 1000 TA/kg of tannic acid lowers iron levels in the blood. As a result, RBCs, HGB, and HCT counts will fall. Tannin levels also lower RBCs, HGB, and PCV quantities (Delimont et al., 2017).

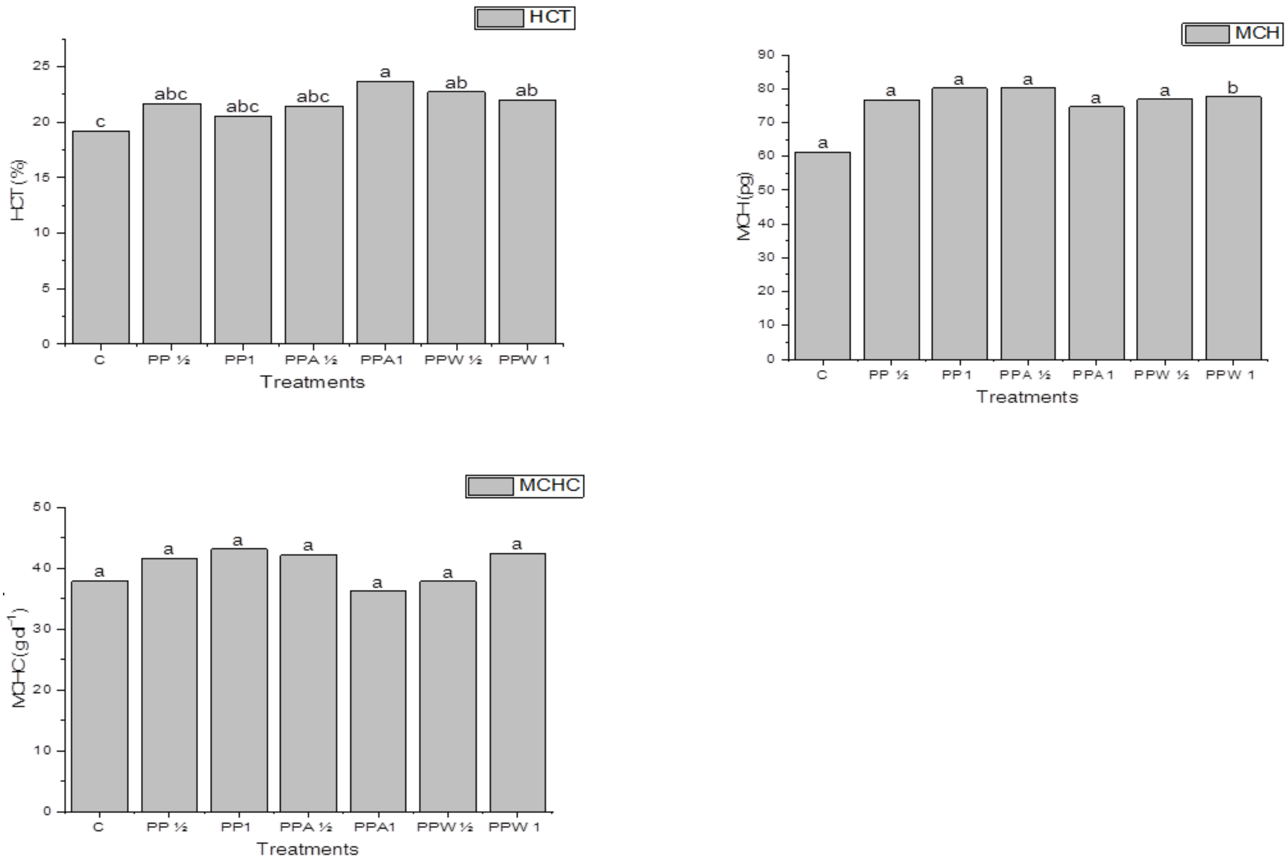

Figure: (2). Changes in the hematocrit (HCT), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in treatments fed supplementation with (PPR) and (PPE) pomegranate diets. Data are represented as mean±SD. (n = 3, in replicate). See Figure. (1) for statistical information.

CONCLUSION

The present study showed that adding pomegranate peels, whether raw or extracts (alcoholic or aqueous), improved hematological parameters of common carp without exceeding 1%. In addition, more studies should be conducted to verify the ideal ratios in fish diets to improve immunity and health of fish cultured using plant additives rather than resorting to antibiotics.

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ETHICS

The authors declare that they have no conflicts of interest associated with this manuscript**.**

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