



## **Impact of Black Grape byproducts on Blood Picture in Common Carp (*Cyprinus carpio* L.)**

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**Abstract:** This experiment was conducted for 70 days on 165 common carp *Cyprinus carpio* fingerlings which were brought from local fish farms located in Daqooq, Haftaghar, Al-Tameem province. Fishes were distributed among experimental tanks with mean initial weight of 24.09 gm. They were pre-acclimated to laboratory conditions for 21 days prior to the feeding trials. The experiment was conducted by using 15 plastic tanks (100 L) representing five treatments with three replicates. Each tank was stocked with 11 fishes. Treatments included 0, 2.5, 5.0, 7.5 or 10 gm grape by-product/ kg diet. The result showed that red blood cell (RBC) data of the control group was significantly higher than other treatments. Hemoglobin of fishes in groups 2.5 and 10 gm/ kg diet was significantly different from other treatments. Mean corpuscular hemoglobin (MCH) values for 0.0, 5.0 and 10 gm/kg groups were significantly different, so mean corpuscular volume (MCV) values showed significant differences resulted from 0.0, 5.0, 7.5 and 10 gm/ kg diet compared to 2.5 gm/ kg diet group. The white blood cells (WBC) were significantly different in 2.5, 5.0 and 7.5 gm/ kg diet. Granulocyte percentages obtained in all treatments were significantly different from control. Lymphocyte percentages were significantly different in all treatments compared to 10 gm/ kg diet group. The Monocytes were significantly different in 2.5, 7.5 and 10 gm/ kg groups.

**Key words:** Black grape byproducts, blood picture, common carp, *Cyprinus carpio*.

### **Introduction**

Aquaculture is an increasingly important option in animal protein production. This activity requires high quality feeds with high protein content, which should contain not only necessary nutrients but also complementary additives to keep organisms healthy and favor growth (Arora *et al.*, 2010). The use of herbs is a time-honored approach

to treat a range of health problems and potentiate immunity of the body. As a result, the nutraceutical industry is one of the upcoming businesses in the modern world. Grapes, *Vitis vinifera* L., (Family Vitaceae) are widely consumed fruits all over the world and have considerable importance for their nutritive value for thousands of years.

Traditionally, grapes were used to treat throat infection and dried grapes (raisins) to treat constipation, skin, kidney, and liver diseases (Arora *et al.*, 2010).

Grapes are one of the world's leading fruit crops, with production rates at more than 50 million tons a year (Shan *et al.*, 2009). Grape seeds, which are by-products of wine and the grape juice industries, are shown to contain large quantities of phenolic compounds such as gallic acid and monomeric flavonoid catechin, epicatechin, gallic acid, epigallocatechin, and epicatechin-3-O-gallate, as well as dimeric, trimeric, and polymeric proanthocyanidins (Sehirli *et al.*, 2008).

Grape is one of the well-known plants with remarkable anti-carcinogenic and antioxidant properties (Bagchi *et al.*, 2000; Schieber *et al.*, 2001). Grape pomace is the residue left after juice extraction by pressing grapes. This by-product (constituted by seeds, skin, and stem) is used every year either as animal feed (with low nutritional value) or for ethanol production by fermentation and distillation. Grape seed extract is a heterogeneous mixture of polyphenols obtained from solvent extraction. Grape seed proanthocyanidins are polyphenolic bioflavonoids, present in lignified portions of grape clusters, especially in the seeds. Tannins are natural polyphenols (Al-Sowayan and Mahmoud, 2014). Grape seed and skin extract (GSSE) is a nutritional supplement exhibiting beneficial health effects (Yousef *et al.*, 2009). GSSE is a complex mixture of polyphenolics classified as flavonoid and non-flavonoid compounds (Khanal *et al.*, 2009). Grape seed pomace extract (GSPE) might be useful in ameliorating chemotherapy induced cytotoxicity in normal cells (Joshi *et al.*, 2001; Zhang *et al.*, 2005).

As far as we know, few data are available on the effect of grape seeds on health and immune responses in fishes. Such comprehensive study on the subject has not been performed on common carp. Therefore, we can summarize the objective of the present study in assessing the effect of certain levels of dietary grape by-products that are locally

available on some blood constituents and some immune responses.

## Materials and Methods

**Experimental Animals:** The experiment was conducted for 70 days on 165 common carp *C. carpio* fingerlings which were brought from local fish farms located in Daqoq, HaftaGar, and Al-Tameem province. The weight of fish varied between 22.45-25.72 gm. Fishes were distributed among experimental tanks with mean initial weight of 24.09 gm. They were pre-acclimated to laboratory conditions and fed with commercial pellets for 25 days prior to the feeding trials. Fifteen plastic tanks (100 L) were used in this trial representing five treatments with three replicates each. Each tank was provided with a proper continuous aeration. Each tank was stocked with 11 fishes. The tanks (replicates) were randomly allocated to minimize variation among treatments. In addition, a daily cleaning by siphoning method was applied to remove remained particles from the system.

The experimental trial included five treatments with three replicates; each contains 11 fishes per tank as follows:

T1: control without any supplement.

T2: with 2.5 gm grape by-product/ kg diet.

T3: with 5gm grape by-product/ kg diet.

T4: with 7.5gm grape by-product/ kg diet. T5:

with 10 gm grape by-product/ kg diet.

**Experimental Diets:** They were of a standard commercial diet type found in Kirkuk markets, enriched with grape by-product. The chemical composition of the different diets, based on NRC (1993) is shown in Table (1). The ingredients were mixed with water to obtain doughs. Doughs were converted into pellets using room temperature for few days and crushed to yield fine crumbles. Fishes were fed twice a day at 9:00 AM and 2:00 PM with a ratio of 3% of body weight but after a week, it was adjusted to 2% due to fish size. Fishes were individually weighed every two weeks. The feeding ratio was then recalculated according to new weights. The feeding trial continued for 10 weeks.

**Whole Blood Examination:** At the end of the experimental period, three fishes were

randomly taken from each experimental group. The blood samples from each fish in the different groups were collected by suctioning of the punctured vein. Whole blood samples were collected in small plastic vials containing heparin and stored under cooling condition. All CBC (complete blood count) tests were determined by using the Automatic Hematology Analyzer (USA origin) and prior to analysis for determination of: RBC (Red Blood Cells:  $10^{12}$  cells/ l), WBC (White Blood Cells:  $10^9$  cells/ l), PCV (Packed Cell Volume %), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration: g/ l), MCV (Mean Corpuscular Volume: fl), Hb (Hemoglobin: gm/ l), Granulocyte (%), Lymphocyte (%), Monocyte (%) and Platelet ( $10^9$  cells/ l).

The experiment was conducted using the RCBD design and general linear models (GLM) procedure of XLSTAT. Pro. 7.5 One way (ANOVA). Duncan's test was used to compare means of the experimental treatments.

## Results and Discussion

Hematological parameters are shown in Table (2). RBC data of the control group was significantly higher than other treatments. Hemoglobin concentrations of T2 and T5 were significantly different from other treatments. The results of MCH for T1, T3 and T5 were significantly ( $P < 0.05$ ) different among treatments. The data for CHC and HCT revealed no significant differences among treatments. Values of MCV observed in table (2) showed that T1, T3, T4 and T5 groups had significant ( $P < 0.05$ ) differences among treatments as compared to T2.

Effective protection of blood cells could be achieved by some synergism between polyphenols and fatty acids containing GSSE (Shi *et al.*, 2003) and this in general was observed in the main trend of the present results. Furthermore, the obtained enzymatic improvement by administration of GSPE may be due to protecting the cell membranes by the scavenging property of the free radical by

the antioxidative phytochemicals present in GSPE such as flavonoids (Suwannaphet *et al.*, 2010) and/ or due to the fact that GSPE improves hepatic ischemic injury (Sehirli *et al.*, 2008), significantly different than other treatment. The data of granulocyte percentage obtained for all treatment were significantly higher than the control. Lymphocyte percentage was significantly different ( $P \leq 0.05$ ) in all treatments as compared with T5. The monocytes were significantly different in T2, T4 and T5 groups. No significant differences were observed among the dietary treatments as related to platelets percentages. Abdulrahman *et al.* (2013) demonstrated that the inclusion of grape seed in fish diet at 8 gm/ kg affects significantly RBC ratio in comparison with other treatments while the platelet ratio of the control was significantly higher as compared with inclusion treatments. The WBC, HGB, and HCT were not affected by the treatments.

Lymphocytes and neutrophils are often used in studies evaluating general immune response due to ease of sampling and cost-efficiency. Lymphocytes are key white blood cells involved with coordination of specific and nonspecific immune components and their abundance in the blood is usually a long-term (chronic) response.

In another way, the results demonstrated that grape by-products induced a significant decrease in erythrocyte and leukocytes counts, hematocrit, platelet count and lymphocyte percentage which may be due to inhibition or defective hematopoiesis (Table 3). These findings are agreed with those obtained from other studies (Maddocks *et al.*, 1986; Amin and Hamza, 2005).

Antioxidant activity of grape seed extract is due to its polyphenolic capacity which is a mixture of proanthocyanidines and have been demonstrated to inhibit oxidative stress through modulation of metabolic functions, enhancement of detoxification pathways, and/ or prevention of the interaction of xenobiotics with biological molecules (Ray *et al.*, 1999; Yamakoshi *et al.*, 2002).

**Table (1): Chemical composition of the different components of fish diets.**

Ingredients	Crude protein (%)	Crude fat (%)	Dry matter (%)	Crude fiber (%)	Energy (kg/kg)
Animal protein concentrate	40	5	92.9	2.2	2107
Yellow corn	8.5	3.6	89	2.2	3350
Soybean meal	44	1.1	89	7	2230
Barely	11	1.9	89	5.5	2640
Wheat bran	15.7	4	89	11	1300

**Table (2): Effect of adding black grape by-products on common carp blood parameters (T1: control, T2: 2.5 gm grape/ kg, T3: 5 gm grape/ kg, T4: 5 gm grape/ kg, T5: 10 gm grape/ kg).**

Treatment	RBC ( $10^{12}$ cells/l)	Hb (gm/l)	MCH (p/g)	MCHC (gm/l)	MCV (fl)	HCT
T1	1.670±0.042 <sup>a</sup>	10.100±0.059 <sup>b</sup>	59.700±0.002 <sup>ab</sup>	28.900±0.010 <sup>a</sup>	214.950±0.000 <sup>ab</sup>	35.900±0.042 <sup>a</sup>
T2	1.705±0.032 <sup>b</sup>	10.350±0.121 <sup>ab</sup>	58.050±0.016 <sup>b</sup>	29.300±0.041 <sup>a</sup>	213.050±0.013 <sup>b</sup>	36.300±0.047 <sup>a</sup>
T3	1.655±0.057 <sup>b</sup>	9.450±0.037 <sup>b</sup>	60.700±0.007 <sup>a</sup>	28.250±0.002 <sup>a</sup>	223.250±0.001 <sup>a</sup>	36.950±0.058 <sup>a</sup>
T4	1.690±0.012 <sup>b</sup>	9.900±0.010 <sup>b</sup>	58.600±0.002 <sup>b</sup>	27.300±0.026 <sup>a</sup>	214.600±0.027 <sup>ab</sup>	37.150±0.009 <sup>a</sup>
T5	2.030±0.069 <sup>b</sup>	12.100±0.041 <sup>a</sup>	60.575±0.014 <sup>a</sup>	28.050±0.048 <sup>a</sup>	219.750±0.010 <sup>ab</sup>	41.750±0.131 <sup>a</sup>

Mean values with different superscripts within a column differ significantly ( $P \leq 0.05$ ).

**Table (3): Effect of adding black grape by-products in young carp diet on differential WBC count (T1: control, T2: 2.5 gm grape/ kg, T3: 5 gm grape/ kg, T4: 5 gm grape/ kg, T5: 10 gm grape/ kg).**

Treatment	WBC ( $10^9$ cells/l)	Granulocytes (%)	Lymphocytes (%)	Monocytes (%)	Platelets (%)
T1	108.400±0.00 <sup>ab</sup>	9.000±0.089 <sup>b</sup>	88.850±0.037 <sup>ab</sup>	12.550±0.02 <sup>b</sup>	48.500±0.010 <sup>a</sup>
T2	109.900±0.02 <sup>a</sup>	9.600±0.010 <sup>ab</sup>	86.250±0.028 <sup>ab</sup>	14.650±0.00 <sup>a</sup>	44.000±0.114 <sup>a</sup>
T3	111.750±0.005 <sup>a</sup>	10.250±0.054 <sup>ab</sup>	85.600±0.007 <sup>a</sup>	11.650±0.03 <sup>b</sup>	42.500±0.506 <sup>a</sup>
T4	110.750±0.005 <sup>a</sup>	10.250±0.083 <sup>ab</sup>	85.050±0.003 <sup>a</sup>	14.300±0.04 <sup>a</sup>	36.000±0.167 <sup>a</sup>
T5	105.267±0.022 <sup>b</sup>	11.100±0.018 <sup>a</sup>	79.050±0.041 <sup>b</sup>	14.750±0.05 <sup>a</sup>	53.000±0.118 <sup>a</sup>

Mean values with different superscripts within a column differ significantly ( $P \leq 0.05$ ).

The study of Al-Sowayan and Mahmoud (2014) was carried out to examine anti-oxidant potential of GSPE along with Vitamin E on DOX induced cardiotoxicity. Since the free radicals produced during the metabolism of the drug are considered to be responsible for alteration in various cellular enzyme activities, lipid peroxidation, antioxidant and antioxidant enzymes; the effect of antioxidant GSPE together with Vitamin E (which intercepts the toxic free radicals) was investigated in rats.

Simonetti *et al.* (2002) and Shi *et al.* (2003) clearly revealed that polyphenols, mainly catechins, have anti-oxidative properties by the inhibition of low-density lipoproteins (LDL) oxidation. Furthermore, epicatechin also protects endothelial cells against oxidized LDL by scavenging free radicals and maintaining nitric oxide synthase (Steffen *et al.*, 2005). Although the anti-dyslipidemic activities of the major polyphenols in grape seed have been well investigated, studies regarding their effects on cholesterol digestion and absorption have not been undertaken (Sathaporn *et al.*, 2011).

The action of oligomeric proantho-cyanidins (OPC), the active ingredient of grape seed extract, serves to increase blood flow to all areas of the body; in particular, OPCs have the ability to improve microcirculation by strengthening delicate capillary walls. This result in increased circulation to peripheral areas of the body served by fine blood vessels, such as the liver, testes, spleen, pancreas, and eyes (Lowry *et al.*, 2003) and all these may be the reasons of variation in blood parameters observed in the present study.

## Conclusions

The obtained results confirmed that adding grape byproducts were able to enhance the fish immunity parameters by using WBC, RBC counts and hemoglobin ratio

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