



## Protective Effects of Dietary Pomegranate Seeds Powder Supplementation against Oxidative Stress of Organochlorine Pesticides Residues in Growing Rabbits

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**Abstract** | The objective of this work was to evaluate the pomegranate seeds powder (PSP) as an effective antioxidant in growing rabbit's diets to alleviate the adverse effects of organochlorine pesticides residues (OCPs) on growth performance, antioxidant status and physiological parameters of growing rabbits. A total of 120 unsexed weaned V-Line male rabbits (4 weeks of age) were assigned at random to five experimental groups. The control group was fed a diet free of PSP. The negative control was fed a diet free of PSP and OCPs, while the other empirical groups were fed the same diet supplemented with 500, 750 and 1000 g of PSP/100 kg diet. The results indicated that PSP contained content of vitamin C and vitamin E; 3.13 and 1.45 mg/100g, respectively. The content of total phenols was 0.27% and total flavonoids were 0.21 %. The accumulation of OCPs was concentrated in the rabbit's liver, followed by the muscle. The control diet contaminated with Alpha Benzene hexachloride ( $\alpha$ BHC), Delta Benzene hexachloride ( $\Delta$ BHC), dieldrin and methoxychlor values. Dietary supplementation of PSP at levels of 750 and 1000 g tended to improve average daily gain and feed conversion ratio compared to the control group. Rabbits fed diet containing 1000g PSP had the lowest mortality %. Edible giblets were significantly increased in the treated groups. The values of Hemoglobin, Packed cell volume, White Blood Cell, lymphocyte, total protein, globulin, higher density lipoprotein (HDL), Total antioxidant capacity (TAC) and glutathione peroxidase (GPx) were improved, but plasma low density lipoprotein (LDL) and total lipids levels were decreased in the treated groups. A significant decrease of plasma and liver malondialdehyde (MDA) level and significant increase in liver (GPx) and catalase (CAT) were observed in rabbits fed diets supplemented with PSP groups. Conclusively, dietary pomegranate seed powder supplementation improved growth performance and enhanced the antioxidant status. So it eliminated the adverse effects of oxidative stress induced by organochlorine pesticides of growing rabbits diets.

**Keywords** | Pomegranate seeds, Antioxidants, Growth performance, Oxidative stress, Rabbits.

**Received** | February 22, 2021; **Accepted** | May 02, 2021; **Published** | July 01, 2021

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**Citation** | Hassan FA, El-Halim HA, Sdeek FA, Elghalid OA, El-Hady AMA (2021). Protective effects of dietary pomegranate seeds powder supplementation against oxidative stress of organochlorine pesticides residues in growing rabbits. *Adv. Anim. Vet. Sci.* 9(8): 1123-1134.

**DOI** | <http://dx.doi.org/10.17582/journal.aavs/2021/9.8.1123.1134>

**ISSN (Online)** | 2307-8316; **ISSN (Print)** | 2309-3331

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The increased use of pesticides in crop protection raises the possibility of feed contamination that contributing to in a variety of biological or toxicological effects in the production of animals, as well as the exposure to pesticides is one of the causes of the increased levels of oxidative stress (Jabłońska-Trypuć, 2017). Organochlorine, organofluorine, organophosphates, carbamates, pyrethroids, bipyridyl herbicides, triazine herbicides and chloroacetanilide herbicides that have been shown to induce oxidative stress (Temiz, 2020). The oxidative stress is the imbalance between the generation of free radicals and antioxidant defense of the body. Pesticides induce oxidative stress as a mechanism for their toxic action in the body (Medithi et al., 2020). Free radicals play a significant role in the toxicity of pesticides that can cause oxidative stress, leading to the production of free radicals, antioxidants alterations, oxygen free radicals, the scavenging enzyme system, and lipid peroxidation (Cortés-Iza and Rodríguez, 2018).

In recent years, due to their antioxidant properties, considerable emphasis has been placed on using of the naturally available botanicals. Therefore, some plants are considered to have antioxidant properties due to the existence of certain phytochemicals that have been shown to exhibit antioxidant activity in *in vitro* and *in vivo* studies against certain oxidative stress and pomegranate (*Punica granatum L.*) contained phytochemicals that has been widely referred in medical folklore for its antioxidant, antiproliferative, antimicrobial properties (Doostan et al., 2017). Pomegranate seed pulp is the by-product of the pomegranate juice industry and contains powerful antioxidants, anti-inflammatory compounds, vitamin E, sterols, phenols and natural estrogens (Hassan et al., 2020). Pomegranate seeds have a prospect preservative agent against dimethoate which induces hepatic poisoning induced by organophosphate pesticide. Egypt is the fifth largest producer of rabbit meat in the world (FAO, 2019). The quality of rabbit meat associated with a healthy and balanced diet (Trocino et al., 2019).

The objective of this work was to evaluate the productive effects of pomegranate seeds powder as an effective antioxidant in growing rabbit's diets to alleviate the adverse effects of organochlorine pesticides residues (OCPs) oxidative stress on growth performance, carcass traits, antioxidant status, hematological and some plasma biochemical parameters of growing rabbits.

## MATERIALS AND METHODS

The current experiment was performed at the nucleus breeding rabbit unit of the Poultry Research Center, De-

partment of Poultry Production, Faculty of Agriculture, Alexandria University, Egypt during the period from January to February 2018.

All animal care procedures were approved by the Institutional Animal Care and Use Committee in AU-IACUC, Alexandria University, Egypt. Authors adhere the procedures imposed on the animals have been implemented in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Authors also adhere to the EU regulations on feed legislation, the 'Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed.

## EXTRACTION, CLEAN-UP AND ANALYSIS OF ORGANOCHLORINE PESTICIDES RESIDUES

**Extraction of samples:** Extraction and partitioning 10.0g±0.1g of the comminuted homogenous and frozen sample are weighed into a 50 mL centrifuge tube, 10 mL acetonitrile and the ISTD solution (e.g. 100 µL of an ISTD-mixture, + 10 g – water amount in 10 g sample (diet). After that added a mixture of: 4 g ± 0.2 g Magnesium sulphate anhydrous; 1 g ± 0.05 g Sodium chloride; 1 g ± 0.05 g Trisodium citrate dihydrate and 0.5 g ± 0.03 g Disodium hydrogencitrate sesquihydrate. After that for extract: fat containing samples: freeze fat out, removed overnight in the refrigerator.

**Dispersive-SPE Cleanup:**—An aliquot of the extract is transported to a PP-single use centrifugation tube which includes each of 25 mg primary secondary amine (PSA) sorbent and 150 mg magnesium sulphate per mL extract (e.g.: for 8 mL extract 200 mg PSA and 1.2 g magnesium sulphate are needed). The tube is shaken forcibly for 30 s and centrifuged (e.g. for 5 min. 3000 U/min) to separate solids from solution, and transfer 0.5 mL extract into autosampler vial for GC/MS analysis.

## Gas chromatography/mass spectrometry (GC/MS)

**analysis:** Analysis of pesticides residues was carried out with Hewlett Packard GC model 6890 chromatograph equipped with Ni<sup>63</sup> electron capture detector (GC) was used for analysis of 14 OCPs conditions: Separation of pesticides was carried out with HP- 5MS capillary column (30 m X 0.32 mm internal diameter X 0.25 µm film thickness). The carrier gas is N<sub>2</sub> at a flow rate of 4 ml/min. the injector temperature was 300°C and the detector temperature was 320° respectively. First column temperature was for oven 180°C for 2 min and raised at 3°C/ min and then held at 220°C for 1 min then raised at 9°C/min to 280°C and then held at 2 min to a total time of 30 min had elapsed.

**Table 1:** Feed ingredients and chemical analysis of experimental diet of growing rabbits (% DM basis).

Feed ingredients (%)	Control diet	Chemical analysis (%)	(%)
Ground barley	15.00	Dry matter	89.65
Wheat bran	21.70	Organic matter	87.63
Yellow corn	7.00	Crude protein	17.87
Soybean Meal (44%)	19.0	Crude fiber	14.62
Straw	8.0	Ether extract	2.70
Clover hay Alfalfa	24.0	Neutral detergent fiber	37.49
Di-calcium phosphate	1.20	Nitrogen free extract	52.44
Limestone	0.20	Ash	12.37
Methionine	0.15	Calcium	0.83
Lysine	0.05	Available phosphorus	0.31
Salt	0.40	Calculated composition	
Premix <sup>1</sup>	0.30	Methionine	0.36
Molasses	3.0	Lysine	0.91
Total	100	Digestible energy <sup>2</sup> (Kcal/kg)	2727.25

<sup>1</sup>Each 3 kg of vitamin and Mineral as premix contains: Vitamin-mineral premix provide per kg of diet vit. A, 13,340 iu; vit. D3, 2680. i.u; vit. E, 10 .i.u; vit. K, 2.68 mg; Calcium pantothenate, 10.68 mg; vit. B12, 0.022 mg; folic acid, 0.668 mg; choline chloride, 400 mg; chlorotetracycline, 26.68 mg; manganese, 133.34 mg; iron, 66.68 mg; zinc, 53.34 mg; copper, 3.2 mg; iodine, 1.86 mg; cobalt, 0.268 mg; selenium, 0.108 mg.

<sup>2</sup>Digestible energy (DE) was calculated according to LEBAS (2013)

### PREPARATION OF POMEGRANATE SEEDS POWDER

Pomegranate seeds were separated from pomegranate by-products which were obtained from Fontana Company (Food industries, Kalyubia Governorate, Egypt) producing about 520 tons yearly of pomegranate by-products which include defective fruits, skins, seeds and peels. These by-products were in wet form with moisture content of 65–70%. Therefore, they were dried by sun drying to 9–10% moisture (for 3 days), and then the seeds were detached from by-products by rubbing the mixture over a coarse screen. The seeds were grounded by a hammer mill to gain a fine powder and were kept for subsequent processing.

### EXPERIMENTAL DESIGN AND ANIMALS

A total number of 120 unsexed weaned V-Line rabbits at an average age of four weeks with initial weight 570.37±7.12 g. Rabbits were randomly allocated into five groups (n=24 in each group). Each group was further divided into three equal replicates of eight rabbits each. The control group was fed a diet without pomegranate seeds powder (PSP) supplementation. The negative control was fed a diet without PSP and OCPs, while the other empirical groups were fed the same diet supplemented with 500, 750 and 1000 g of PSP/ 100 kg diet (500PSP, 750PSP and 1000PSP, respectively). The experimental duration continued for 6 weeks (from 4 to 10 weeks of age).

### MANAGEMENT AND DIET

The rabbits were housed in well ventilated in galvanized wire cage batteries with a one level design cages with di-

mensions 40 × 50 × 35 cm for length, width and height, respectively. Rabbits were managed under the same executive and environmental status. Ranges of ambient temperature, relative humidity and day-light length during the experimental period were from 20 to 22°C, 68%–74% and 14 hr 24 min to 15 hr 48 min, respectively. Each cage was equipped provided with manual feeders, and clean fresh water was excessively available through an automatic nipple system *ad libitum*. The experimental diets were pelleted and formulated to meet the recommended nutrient requirements of growing rabbits according to De Blas and Wiseman (2020) as shown in Table 1. Digestible energy (DE) was calculated according to LEBAS (2013).

### CHEMICAL ANALYSIS OF DIET AND PSP

Chemical analysis of the diet was completed as recommended by AOAC (2007) for determining moisture, crude protein, crude fiber, ether extract, ash, calcium and phosphorus. The fragments of phenolic complexes, flavonoid compounds, minerals and vitamins of pomegranate seeds powder were specified in the Micro Analysis Laboratory, Food Technology Research Institute, Cairo, Egypt. Total flavonoid compounds were specified by high-performance liquid chromatography (HPLC) according to the method described by (Mattila et al., 2000), while total phenolic compounds were determined by HPLC according to (Goupy et al., 1999). Minerals (Zn, Se) of pomegranate seeds powder were determined using Atomic Absorption Spectrophotometer (Perkin Elmer, Model 3300, Germany). Determination of vitamin C (L-Ascorbic acid) and

**Table 2:** Biologically active compounds pomegranate seeds powder (on DM basis).

Items (%)	Vitamins content		Minerals content		Polyphenols content	
	Vitamin C L-Ascorbic acid (mg/100g)	Vitamin E $\alpha$ - Tocopherol (mg/100g)	Zn (mg/100g)	Se (mg/100g)	Total phenols (%)	Total Flavonoids (%)
Pomegranate Seeds powder (PSP)	3.13	1.45	5.60	0.22	0.27%	0.21%

DM= dry matter; Zn= Zinc; Se= Selenium

**Table 3:** Validation parameters of organochlorine pesticide (OCPs) compounds ( $\mu\text{g/g}$ ).

Pesticides	RT	LOD	LOQ	$r^2$	RSD	Average Recovery	MRL (mg/kg)
$\alpha$ BHC	3.139	0.004	0.015	0.792	13	72.3	-
$\gamma$ BHC	3.778	0.004	0.015	0.791	15	76.45	-
$\Delta$ BHC	4.347	0.002	0.004	0.793	14	73.25	-
Heptachlor	5.421	0.01	0.02	0.993	12	71.46	0.2
Aldrin	6.329	0.01	0.02	0.991	17	73.52	0.2
Heptachlor epoxide	7.673	0.002	0.003	0.993	16	71.4	0.2
$\gamma$ Chlordan	8.273	0.003	0.005	0.851	13	70.23	0.05
Endosulfan	8.614	0.003	0.005	0.882	14	71.03	0.05
Endrin	9.047	0.001	0.003	0.992	12	70.92	0.05
Dieldrin	9.458	0.001	0.003	0.996	13	71.19	-
pp-DDE	10.125	0.001	0.003	0.994	14	73.39	Sum = 1
ppDDD	11.048	0.002	0.005	0.891	13	72.36	-
ppDDT	12.285	0.002	0.005	0.992	13	74.26	-
Methoxychlor	14.463	0.002	0.005	0.846	14	70.95	0.01

RT = Retention time (min); LOD = limits of detection; LOQ = limits of quantification;  $r^2$  = correlation coefficient; RSD% = relative standard deviation percent; MRL = Maximum Residue Limit (in tissue of farmed animal) according to EU (2005); BHC= Benzene hexachloride; DDE= dichlorodiphenyl- dichloroethylene; DDD= dichlorodiphenyldichloroethane; DDT= dichlorodiphenyltrichloroethane

vitamin E ( $\alpha$ -tocopherol) were determined according to the method described in the AOAC (2007) using HPLC.

**PERFORMANCE DATA**

Throughout the experimental period, individual live body weight (LBW) was determined weekly and daily body weight gain (DBWG) was calculated. Daily feed intake (DFI) was calculated as grams per rabbit per day. The unused feed from each cage was collected daily, weighed and taken into consideration for the calculation of feed intake, accordingly, feed conversion ratio (FCR) was easily calculated (g feed / g gain) and mortality rate were recorded weekly.

**SLAUGHTER PROCEDURE AND BLOOD SAMPLES**

At the end of the experimental period at (10 weeks of age), six rabbits from each group were randomly taken, fasted for 12 hours, weighted individually and slaughtered to complete bleeding. After bleeding, rabbits were weighted and skinned. Pelt and viscera were removed and then carcass was weighed. The hot carcass was weighed without giblets. The giblets are liver, heart and kidneys. The pancreas, abdom-

inal fat, full intestine and full caecum were separated and weighed. Intestine and caecum lengths were also measured. The edible giblets, total edible parts and dressing percentages were calculated, liver was removed, separately weighted, cut into small pieces and homogenized using glass homogenizer, with ice-cooled saline to prepare tissue homogenate. After centrifugation, supernatants were collected and frozen at  $-70^{\circ}\text{C}$  till estimation of enzymatic antioxidants activity. Samples of muscles *longissimus dorsi* (MLD) muscles and the whole brain were analyzed for organochlorine pesticide residues.

At slaughtering, blood samples were taken from six rabbits of each group in fair tubes with or without heparin, coagulated blood samples were centrifuged at 4000 rpm for 15 minutes and the obvious serum was detached and stored in a deep freezer at  $-20^{\circ}\text{C}$  until biochemical test. Non-coagulated blood was tested shortly after gathering for estimating blood picture. Red Blood Cells (RBCs), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration



**Table 4:** Determination of organochlorine pesticide (OCPs) residues in the control diet, brain, muscle, liver and kidney of rabbits fed on the control diet (µg/g).

Pesticides	Control diet	Negative control	Brain	Muscle	Kidney	Liver
αBHC	0.003 ± 0.001	ND	ND	0.001± 0.001	ND	0.001 ± 0.001
γ BHC	ND	ND	ND	ND	ND	ND
ΔBHC	0.002 ± 0.001	ND	ND	ND	ND	0.001 ± 0.001
Heptachlor	ND	ND	ND	ND	ND	ND
Aldrin	ND	ND	ND	ND	ND	ND
Heptachlor epoxide	ND	ND	ND	ND	ND	ND
γ Chlordan	ND	ND	ND	ND-	ND	ND
Endosulfan	ND	ND	ND	ND	ND	ND
Endrin	0.003 ± 0.001	ND	ND	ND	ND	0.002 ± 0.001
Dieldren	0.004 ± 0.001	ND	ND	0.001 ± 0.001	ND	0.002 ± 0.001
pp-DDE	ND	ND	ND	ND	ND	ND
ppDDD	ND	ND	ND	ND	ND	ND
ppDDT	ND	ND	ND	ND	ND	ND
Methoxychlor	0.003 ± 0.001	ND	ND	ND	ND	0.001 ± 0.001

BHC= Benzene hexachloride; DDE= dichlorodiphenyldichloroethylene;DDD= dichlorodiphenyldichloroethane;DDT= dichlorodiphenyltrichloroethane; ND = Not determined.

**Table 5:** Effect of dietary supplementation of SPS in diets containing OCPs residues on growth performance of growing rabbits.

Traits	Experimental diets					SEM	P-value
	Control	N. control	500PSP	750PSP	1000PSP		
No. of rabbits	24	24	24	24	24		
Initial LBW, g	567.5	567.3	573.5	569.2	571.3	10.22	0.9779
Final LBW, g	1612.4 <sup>c</sup>	1645.39 <sup>bc</sup>	1645.5 <sup>bc</sup>	1677.3 <sup>ab</sup>	1697.9 <sup>a</sup>	15.91	0.0015
DBWG, g/ rabbit/ day	24.87 <sup>c</sup>	25.66 <sup>bc</sup>	25.52 <sup>bc</sup>	26.38 <sup>ab</sup>	26.80 <sup>a</sup>	0.39	0.0032
DFI, g/rabbit/day	67.49 <sup>c</sup>	72.90 <sup>b</sup>	72.89 <sup>b</sup>	79.60 <sup>a</sup>	80.52 <sup>a</sup>	1.27	0.0001
FCR, g feed/g gain	2.71 <sup>b</sup>	2.86 <sup>ab</sup>	2.86 <sup>ab</sup>	3.02 <sup>a</sup>	3.00 <sup>a</sup>	0.068	0.0078
Mortality, %	16.70 <sup>a</sup>	8.31 <sup>b</sup>	8.30 <sup>b</sup>	0.01 <sup>c</sup>	0.00 <sup>c</sup>	0.06	0.0001

<sup>a,b,c</sup> Means values with the same letter within the same row did not differ significantly

N. control= Negative control; LBW= Live body weight; DBWG= Daily body weight gain; DFI= daily feed intake; FCR= Feed conversion ratio.

(MCHC), Platelets (Plt), White Blood Cells (WBCs) and differentiation of WBCs (lymphocyte, L and neutrophils, N), hemoglobin concentration and Packed Cell Volume (PCV) percentages were enumerated by conventional methods.

Plasma total protein, albumin, glucose, total cholesterol, Low density lipoprotein (LDL-cholesterol) and High density lipoprotein (HDL-cholesterol), triglycerides and total lipids were measured calorimetrically using commercial kits (bought from Bio-diagnostic, Cairo, Egypt, www.bio-diagnostic.com) according to the manufacturers' instructions. Lipid peroxidation was examined by measuring the level of malondialdehyde (MDA, nmol/ ml), total antioxidant capacity (TAC, mmol/ L), superoxide dismutase enzyme (SOD, U/L), glutathione peroxidase enzyme

(GPx, mU/mL) and catalase (CAT, U/g) were determined by colorimetric techniques using a commercially obtainable kit (Bio-diagnostic., Egypt).

#### STATISTICAL ANALYSIS

The obtained data were statistically analyzed by using the GLM (General Linear Model) procedure of SAS software (2009) by applying one-way analysis of variance (ANOVA). Using the following model:  $Y_{ij} = \mu + T_i + E_{ij}$ , Where,  $Y_{ij}$  = An observation;  $\mu$  = Overall mean;  $T_i$  = the effect of treatment groups;  $E_{ij}$  = experimental random error. Differences between means were tested with Duncan's multiple range test at the level of  $\alpha = 0.05$  (Duncan, 1955).

GROWTH PERFORMANCE

Effect of feeding rabbits diets containing varied levels of dietary PSP on growth performance is summarized in Table 5. The obtained results indicated that dietary supplementation of PSP at levels of 750 and 1000 g tended to increase ( $P < 0.05$ ) final LBW and average DBWG compared to the control group. In addition to average DFI was significantly ( $p < 0.05$ ) increased with increasing of dietary PSP supplementation. Also, the control group tended to be the lower ( $P < 0.05$ ) in FCR compared to the other tested groups. Concerning the mortality%, it gradually reduced ( $P < 0.05$ ) with the increasing of the dietary PSP supplementation levels and the rabbits group fed diet supplemented with 1000g PSP had the lowest ( $P < 0.05$ ) mortality % when compared to the other experimental groups.

CARCASS TRAITS

As presented in Table 6, all of the carcass traits were insignificantly changed due to dietary supplementation levels of PSP compared to the rabbits group fed the control and negative control diets, except for edible giblets which were significantly ( $P < 0.05$ ) increased in the treated groups in comparison with the control one. While there was a significant difference ( $P < 0.05$ ) in the full cecum (g) between the rabbits group fed diet supplemented with 500 g PSP and the rabbits group fed diet contained 1000 g PSP.

HAEMATOLOGICAL AND PLASMA CONSTITUENTS

Findings of blood hematological and plasma profile as impacted by different levels of dietary supplementation of PSP are shown in Table 7. All of the hematological parameters studied were insignificantly differed, except for Hb (g/dl), PCV (%), WBCs ( $10^3/ml$ ) and lymphocyte% which were significantly elevated ( $P < 0.05$ ) with the increasing of PSP levels. In the meantime, rabbits group fed diet supplemented with 1000 g PSP had higher ( $P < 0.05$ ) Hb (g/dl), PCV (%), WBCS ( $10^3/ml$ ) and lymphocytes% and lower significantly N/L ratio ( $P = 0.034$ ) than the control and negative control groups. However there were insignificant improve of RBCs, N, MCV, MCH, MCHC and Plt values by treated groups in Table 7. With regard to plasma profile, the rabbits groups fed diets supplemented with PSP at levels of 500,750 and 1000 were higher ( $P < 0.05$ ) in total protein and globulin than the control and negative control groups. Rabbits group fed diet supplemented with 750 and 1000 g were the lowest ( $P < 0.05$ ) in A/G ratio compared to the control group. Rabbits group fed diet contained 1000g PSP tended to be lower ( $P < 0.05$ ) triglycerides level than the control and negative control groups. While, supplemented of SPS at levels of 500 and 750 g had lower ( $P < 0.05$ ) cholesterol value than the control group. It worthy to notice that the supplementation of PSP increased ( $P < 0.05$ ) HDL concentration and decreased ( $P < 0.05$ ) total lipids and LDL levels compared

BIOLOGICALLY ACTIVE COMPOUNDS OF PSP

The content of vitamins, minerals and polyphenols of PSP is presented in Table 2. Pomegranate seeds powder contained content of vitamin C (L-ascorbic acid) and vitamin E ( $\alpha$ -tocopherol), were being 3.13 and 1.45 mg/100g, respectively. Besides, PSP contained an appreciable amount of some microelements such as Zn and Se at levels of 5.54 and 0.23 mg/100g dry matter, respectively. Furthermore, the content of total phenols was 0.27% and total flavonoids were 0.21 %.

GAS CHROMATOGRAPHIC ANALYSIS OF ORGANOCHLORINE PESTICIDES RESIDUES

Table 3 shows the validation parameters of fourteen organochlorine pesticides (OCPs) compounds (RT, LOD, LOQ,  $r^2$ , RSD, average Recovery and MRL). To confirm that the developed method is suitable for its intended use, and to fulfill the aim of this study, the validation process was carried out. All 14 OCPs were qualitatively determined in spiked blank samples, as the results from all replications were averaged. Correlation coefficients ( $r^2$ ) values varied from 0.791 to 0.996, as the highest  $r^2$  was obtained for dieldrin, and the lowest was for  $\gamma$ -Benzene hexachloride ( $\gamma$ -BHC). The recoveries of the individual compounds varied from 70.23 in  $\gamma$  Chlordan to 76.45% in  $\gamma$  BHC. Most of the compounds obtained results near to 70 % priority in first validation level.

Table 4 shows that the residues of OCPs expressed as the mean values of  $\alpha$ BHC,  $\Delta$ BHC, Endrin, Dieldrin, Methoxychlor ( $\mu$ g/g) in the examined samples of the control rabbits diet, negative control, brain, muscle, kidney and liver, respectively. It is clear to note that the control diet, muscle and liver samples were contaminated with  $\alpha$ BHC at levels of  $0.003 \pm 0.001$ ;  $0.001 \pm 0.001$  and  $0.001 \pm 0.001$   $\mu$ g/ g, respectively. Additionally,  $\Delta$ BHC level was  $0.002 \pm 0.001$  in the control diet and  $0.001 \pm 0.001$  in the liver. The control diet and liver contained  $0.003 \pm 0.001$  and  $0.002 \pm 0.001$  of Endrin  $\mu$ g/ g, respectively. Dieldrin levels were  $0.004 \pm 0.001$ ,  $0.001 \pm 0.001$  and  $0.002 \pm 0.001$   $\mu$ g/ g in the control diet, muscle and liver, respectively. Methoxychlor values were  $0.003 \pm 0.001$  and  $0.001 \pm 0.001$   $\mu$ g/ g in the control diet and liver, respectively. On the other hand, it is clear to notice that  $\gamma$  BHC (benzene hexachloride), Heptachlor, Aldrin, Heptachlor epoxide,  $\gamma$  Chlordan, Endosulfan, pp-DDE (dichlorodiphenyldichloro- ethylene), pp-DDD (dichlorodiphenyldichloro ethane) and pp-DDT (dichlorodiphenyl- trichloroethane) did not find in the examined samples. Furthermore, there were non-significant alterations observed in the studied pesticide residues among the experimental groups.

**Table 6:** Effect of pomegranate seed powder supplementation on carcass traits of growing rabbits

Traits	Experimental diets						SEM	P-value
	Control	N. control	500PSP	750PSP	1000PSP			
Live body weight, g	1667.5	1670.0	1696.3	1715.0	1727.5	34.19	0.638	
Hot carcass weight, g	901.0	915.0	927.50	952.50	965.0	26.37	0.367	
Dressing, %	54.01	54.79	54.67	55.55	55.85	0.98	0.551	
Spleen,%	0.054	0.055	0.054	0.07	0.05	0.006	0.251	
Pancreas, %	0.31	0.33	0.33	0.34	0.33	0.01	0.469	
Abdominal fat, %	0.75 <sup>a</sup>	0.75 <sup>a</sup>	0.67 <sup>ab</sup>	0.66 <sup>ab</sup>	0.60 <sup>b</sup>	0.04	0.047	
Full intestine, %	6.35	6.38	6.76	6.77	6.86	0.16	0.180	
Intestine length, cm	319.50	322.0	319.0	326.50	328.50	3.55	0.190	
Full cecum, g	103.69 <sup>ab</sup>	103.06 <sup>ab</sup>	112.62 <sup>a</sup>	107.69 <sup>ab</sup>	102.55 <sup>b</sup>	2.87	0.008	
Cecum length, cm	48.50	48.75	49.20	51.55	51.95	1.38	0.260	
Edible giblets <sup>1</sup> , %	3.81 <sup>b</sup>	3.81 <sup>b</sup>	4.92 <sup>a</sup>	4.97 <sup>a</sup>	5.04 <sup>a</sup>	0.23	0.007	
Total edible parts <sup>2</sup> , %	57.82	57.82	59.59	60.53	60.89	1.12	0.263	

<sup>a,b,c</sup> Means values with the same letter within the same row did not differ significantly (P>0.05).

Hot carcass including head as percentage of pre-slaughter weight; N. control= negative control

<sup>1</sup> Edible giblets [%] = (liver, g + kidney, g + heart, g)/pre-slaughter weight, g \*100;

<sup>2</sup>Total edible parts [%] = (carcass weight, g+ weight of edible giblets, g)/ pre-slaughter weight, g \*100.

**Table 7:** Effect of pomegranate seed powder supplementation on hematological parameters and plasma constituents of rabbits.

Traits	Experimental diets					SEM	P-value
	Control	N. control	500PSP	750PSP	1000PSP		
Hematological parameters							
RCBs, 10 <sup>6</sup> /mm <sup>3</sup>	5.26	5.31	5.38	5.48	5.62	0.17	0.643
Hb, g/dl	10.50 <sup>b</sup>	10.75 <sup>ab</sup>	10.99 <sup>ab</sup>	11.30 <sup>ab</sup>	11.37 <sup>a</sup>	0.25	0.096
PCV, %	37.30 <sup>b</sup>	37.30 <sup>b</sup>	41.05 <sup>ab</sup>	42.65 <sup>a</sup>	44.22 <sup>a</sup>	1.27	0.006
WBCs, 10 <sup>3</sup> /mm <sup>3</sup>	6.95 <sup>b</sup>	6.97 <sup>b</sup>	7.20 <sup>ab</sup>	7.47 <sup>ab</sup>	7.95 <sup>a</sup>	0.27	0.111
Neutrophils,%	52.21	52.04	52.07	51.87	51.50	2.13	0.534
Lymphocytes,%	27.28 <sup>b</sup>	27.53 <sup>b</sup>	29.12 <sup>ab</sup>	30.67 <sup>ab</sup>	33.92 <sup>a</sup>	1.630	0.043
N/L ratio	1.91 <sup>a</sup>	1.89 <sup>a</sup>	1.79 <sup>b</sup>	1.69 <sup>bc</sup>	1.52 <sup>c</sup>	0.07	0.034
MCV, um <sup>3</sup>	70.86	71.11	76.32	78.46	78.82	3.35	0.298
MCH	19.96	20.21	20.44	20.64	20.24	0.39	0.794
MCHC	28.19	28.44	26.83	26.73	25.79	1.08	0.419
Plt *10 <sup>3</sup>	306.0	306.25	316.75	340.75	359.0	17.69	0.193
Plasma constituents							
Total Protein, g/dl	6.66 <sup>c</sup>	6.91 <sup>bc</sup>	7.67 <sup>a</sup>	7.83 <sup>a</sup>	7.39 <sup>a</sup>	0.16	0.002
Albumin, g/dl	3.20	3.35	3.52	3.30	3.39	0.13	0.498
Globulin, g/dl	3.46 <sup>c</sup>	3.56 <sup>bc</sup>	4.15 <sup>ab</sup>	4.53 <sup>a</sup>	4.00 <sup>b</sup>	0.18	0.003
A/G ratio	0.93 <sup>a</sup>	0.94 <sup>ab</sup>	0.85 <sup>ab</sup>	0.73 <sup>b</sup>	0.86 <sup>b</sup>	0.06	0.014
Triglycerides, mg/dl	52.79 <sup>a</sup>	53.29 <sup>a</sup>	49.00 <sup>ab</sup>	44.00 <sup>ab</sup>	42.46 <sup>b</sup>	2.92	0.012
Cholesterol, mg/dl	93.14 <sup>a</sup>	92.39 <sup>ab</sup>	84.81 <sup>c</sup>	88.77 <sup>b</sup>	89.38 <sup>ab</sup>	1.19	0.060
HDL, mg/dl	37.33 <sup>c</sup>	38.08 <sup>c</sup>	46.39 <sup>b</sup>	48.06 <sup>ab</sup>	55.04 <sup>a</sup>	2.51	0.001
LDL, mg/dl	49.07 <sup>a</sup>	49.57 <sup>a</sup>	42.99 <sup>b</sup>	43.09 <sup>b</sup>	43.89 <sup>b</sup>	1.87	0.047
Total lipids, mg/dl	339.45 <sup>a</sup>	342.05 <sup>a</sup>	291.36 <sup>b</sup>	277.64 <sup>b</sup>	279.63 <sup>b</sup>	12.17	0.002

<sup>a,b,c...</sup> Means values with the same letter within the same row did not differ significantly (P>0.05).

N. control= negative control; RCBs = Red blood cell; Hb= Hemoglobin; PCV=Packed cell volume; MCHC=Mean Corpuscular Hemoglobin Concentration; Plt= Platelets; WBCs= White Blood Cell; MCV=Mean Cell Volume; MCH=Mean Corpuscular

**Table 8:** Effect of dietary SPS supplementation on antioxidant enzymes and antioxidant markers of rabbit plasma and liver.

Traits	Experimental diets					SEM	P-value
	Control	N. control	500PSP	750PSP	1000PSP		
Plasma antioxidant markers							
MDA, nmol/ ml	17.10 <sup>a</sup>	16.48 <sup>a</sup>	14.12 <sup>b</sup>	13.57 <sup>b</sup>	13.75 <sup>b</sup>	0.69	0.011
TAC, mmol/ L	1.06 <sup>b</sup>	1.03 <sup>b</sup>	1.24 <sup>ab</sup>	1.27 <sup>ab</sup>	1.34 <sup>a</sup>	0.07	0.099
Plasma antioxidant enzymes							
SOD, U/L	1.03 <sup>b</sup>	1.17 <sup>ab</sup>	1.16 <sup>ab</sup>	1.27 <sup>a</sup>	1.29 <sup>a</sup>	0.06	0.047
GSH, mU/mL	3.92 <sup>b</sup>	3.97 <sup>b</sup>	4.37 <sup>ab</sup>	4.79 <sup>ab</sup>	5.11 <sup>a</sup>	0.28	0.053
CAT, U/g	559.28 <sup>b</sup>	644.98 <sup>a</sup>	646.99 <sup>a</sup>	639.01 <sup>a</sup>	658.48 <sup>a</sup>	15.30	0.002
Liver antioxidant marker							
MDA, nM/g tissue	6.78 <sup>a</sup>	6.79 <sup>b</sup>	4.44 <sup>b</sup>	3.61 <sup>c</sup>	3.01 <sup>d</sup>	0.11	0.0001
Liver antioxidant enzymes							
SOD, U/g tissue	72.92 <sup>b</sup>	73.41 <sup>b</sup>	83.61 <sup>ab</sup>	83.24 <sup>a</sup>	92.86 <sup>a</sup>	3.65	0.018
GPx, U/g tissue	22.07 <sup>c</sup>	22.08 <sup>c</sup>	26.25 <sup>b</sup>	28.56 <sup>a</sup>	27.97 <sup>a</sup>	0.26	0.0001
CAT, mM/g tissue	6.34 <sup>c</sup>	6.40 <sup>c</sup>	7.06 <sup>b</sup>	7.68 <sup>a</sup>	7.93 <sup>a</sup>	0.14	0.0001

<sup>a,b,c</sup> Means values with the same letter within the same row did not differ significantly (P>0.05).

N. control= Negative control; MDA= Malondialdehyde; TAC= Total antioxidant capacity; SOD= Superoxide dismutase enzyme; GP<sub>x</sub>= Glutathione peroxidase enzyme; CAT= Catalase

to the control and negative control groups.

#### ANTIOXIDANT STATUS

Results concerning the effects of dietary SPS supplementation on antioxidative status presented in Table 8. The current results revealed that a significant (P<0.05) decrease (p<0.05) of plasma and liver MDA level was observed in rabbits fed diets supplemented with PSP at tested levels of 500,750 and 1000 g. At the same time, the rabbits group fed diet supplemented with 1000 g PSP was higher (P<0.05) in plasma TAC and GPx levels than the control and the negative groups. Additionally, dietary SPS supplementation resulted in a significant (P<0.05) increase in liver GPx and CAT compared to rabbits fed the control and the negative diets. While, the rabbits fed the negative control diet and diets supplemented with dietary SPS were significantly higher (P<0.05) in plasma CAT than the rabbits fed the control group. It could be observed that the rabbits group fed diet supplemented with 750 and 1000 g PSP achieved the highest (P<0.05) plasma and liver SOD as compared to the control one. Moreover, the present data revealed a significant increase in malondialdehyde (MDA) level, the biomarker of lipid peroxidation, in both control and negative control groups in comparison to the other experimental groups.

#### DISCUSSION

##### BIOLOGICALLY ACTIVE COMPOUNDS OF PSP

The results of the current study showed that PSP contained adequate levels of vitamin C (L-ascorbic acid; 3.13 mg/100g) and vitamin E (α-tocopherol; 1.45 mg/100g). The chemical composition of PSP was similar to that reported by Rowayshed et al. (2013) who found that PSP contained 3.02, 1.35 mg/100g for vitamin C (L-ascorbic acid) vitamin E (α-tocopherol). The determined vitamins naturally occurred in pomegranate peels and seeds are considered one of the most important phytochemicals having the anti-oxidants. In the present study, PSP contained suitable amount of Zn and Se. In this connection, Dadashi et al. (2013) reported that variations of pomegranate seeds contained concentrations of Zn ranged between 29.173-70.95 mg/kg. Regarding polyphenols, PSP had a high content of total polyphenols were similar to those observed in the present study. Gözlekçi et al. (2011) further reported that the total phenolic content of pomegranate seeds extract ranged from 117.0 to 177.4 mg GAE/L. As well Doostan et al. (2017) stated that the total phenolic and total flavonoid compounds of pomegranate peel extract were 41.1 mg GAE /g extract and 0.42%, respectively and as reported by Bibi et al. (2019) the predominant polyphenol and flavonoids fractions in pomegranate seed extract were catechins, gallic acid, ellagic acid, gallic acid pentoside, gallic acid deoxyhexoside pinocembrin and phloretin. So, it could be seen that PSP is considered a



good source of the determined vitamins (C and E), minerals (Zn and Se) polyphenols and flavonoids.

### GAS CHROMATOGRAPHIC ANALYSIS OF PESTICIDES RESIDUES

As presented in Table 4. Gas chromatographic analysis of OCPs for the control diet, brain, muscle, kidneys and liver of rabbits fed the control diet assumes greater significance in the quantitative and qualitative especially for  $\alpha$ BHC,  $\Delta$ BHC, Endrin, Dieldrin, and Methoxychlor ( $\mu\text{g/g}$ ). These findings showed that the accumulation of organochlorine pesticides was concentrated in the rabbit's liver, followed by the muscle. This could be regarded as the fact that OCPs are highly persistent pesticides to environmental conditions, remaining a long time in soils and sediments. As well they tend to accumulate in tissues of living organisms with devastating toxic effects (Olişah et al., 2020). The OCPs detected in the rabbit diet, several authors have reported the pattern of accumulation of OCPs in poultry feeds from contaminated feed to poultry (Noble, 1990). These present findings showed that the OCPs are retained in the liver and muscle of growing rabbits. Organochlorine compounds (insecticides, e.g., aldrin, DDT, HCH, heptachlor, chlordane, endosulfan) are in general very effective contact insecticides, and they are structurally related to steroid hormones and act on the respective hormone receptor (Tebourbi et al., 2011).

OCPs impacted heavily the top predators in terrestrial food chains, like birds of prey, and accumulate in adipose tissues of animals and humans, being transferred to newborns with the milk fat, and act as an endocrine disruptor (Gee et al., 2013). Further studies should be designed to monitor OCPs levels in the animal's tissues and feed ingredients. Therefore, the need for more studies on these matrices to investigate the potential risk they pose on the health status of animals.

### GROWTH PERFORMANCE

In the present work, there are favorable impacts due to dietary supplementation of PSP on most of growth performance traits studied. Dietary PSP improved DBWG and reduced mortality percentage. This might be assigned to the powerful antioxidants such as polyphenols, total phenols and total flavonoids which found in high levels in pomegranate seeds as well as enhancing the immunity by activation of antioxidant enzymes glutathione and glutathione peroxidase, where PSP is considered a rich source of macro and microelements (Rowayshed et al., 2013). The increase in FI as an increasing dietary PSP level might be attributed to its palatability and a further increase in the nutrients and active sustenance's intake of PSP diets. It is worthy to note that compounds of OCPs such as aldrin and dieldrin caused the loss in body weight and increased

mortality of rabbits (Jayaraj et al., 2016).

The present findings are in the same line as those found by Zeweil and El-Gindy (2016) who concluded that pre-weaning mortality of rabbits was decreased when supplementing diets of rabbits does with pomegranate peel. Additionally, dietary supplementation of pomegranate pomace impaired broiler growth performance indices as compared to those fed with the un-supplemented diet or diets supplemented with pomegranate pomace extract (Ahmadipour et al., 2018). These results were consistent with the findings obtained by Hassan et al. (2020) who stated that rabbits fed diets supplemented with pomegranate by-product extract at 100, 150, and 200 mg/kg diet significantly ( $P < 0.05$ ) enhanced the average final body weight and FCR.

### CARCASS TRAITS

The present results are in accordance with those obtained by Ahmed and Yang (2017) showed that the liver (as relative weight) of broilers was not differed by dietary supplementation with *Punica granatum* L. by-products at levels of 0.5 and 1%. The same authors added that dietary supplementation of 1% pomegranate led to a linear increase in the spleen's relative weight. In this regard, Ahmadipour et al. (2018) indicated that the liver and heart of broilers at 40 days of age fed 1% pomegranate peel were significantly lower than the control group. In view of these results, Hassan et al. (2020) reported that dietary supplementation of pomegranate by-product extract at 100, 150, and 200 mg did not significantly affect all carcass traits studied except kidneys% which was higher ( $P < 0.05$ ) than the control group.

### HEMATOLOGICAL AND PLASMA CONSTITUENTS

The addition of SPS had no significant changes in most of the hematological constituents. However, supplementation of PSP at 1000 g had higher ( $P < 0.05$ ) HB and WBCS levels. These findings agreed with those reported by El-Gindy, (2018) who found that rabbits fed diet supplemented with 3% pomegranate peel recorded the highest count of red blood cell, while rabbits fed diet contained with 1.5 and 3% levels of pomegranate peel had the highest concentration of hemoglobin. The enhancement of some blood hematological constituents such as HB and WBCS levels may be attributed to the bioactive ingredients in PSP like phenolic, flavonoids, vitamins (C, E) and micro minerals (Zn, Se) as mentioned in Table 2.

In that respect, El-Sheikh et al. (2015) reported that the continuous exposure of rabbits to insecticides induced hematological alterations and RBCs count was significantly decreased after 28 days of exposure. Concerning the plasma constituents, the results of this study showed an

increase in total protein, globulin and HDL levels. Also, lower ( $P < 0.05$ ) total cholesterol, LDL and total lipids levels which may be due to SPS supplementation. Similar findings reported by Ibrahim et al. (2017) who stated that the supplementation of pomegranate by-products at levels of 1.0, 1.5 and 2.0 % increased ( $P < 0.05$ ) plasma total protein, albumin and globulin levels. In addition, the plasma content of total lipids, total cholesterol and LDL was observed to move down compared to those of rabbits fed the control diet. Also, Hassan et al. (2020) demonstrated that feeding rabbits on diets supplemented with pomegranate by-product extract at levels 100, 150, and 200 mg/kg diet increased ( $P < 0.05$ ) total protein, albumin and globulin levels as well decreased the total cholesterol, LDL and total lipids concentrations.

The rise in the level of cholesterol in the control group of rabbits might be attributed to an increased composition of cholesterol in the liver. The increase in blood cholesterol could be referred to as the impact of pesticides on the permeability of liver cell membrane (Abd Elmonem, 2014). In the current work, PSP lowered plasma cholesterol and LDL concentrations due to mediated motivation of hepatic cholesterol-7-hydroxylase activity (El-Gindy, 2018). The later author concluded that all diets containing pomegranate peel improved each of serum HDL levels and significantly reduced LDL and total cholesterol concentrations. In this regard, the current findings agree with those obtained by (El-Gindy, 2018).

#### ANTIOXIDANT STATUS

The current findings of antioxidants markers and enzymes of rabbits revealed that the supplementation of SPS had the ability to increase antioxidants marker (TAC) as an index of oxidation and improve the activity of SOD, GPx and CAT enzymes associated with a decreased activity of MDA as lipid peroxidation index (Naji et al., 2017) in plasma and liver. So the increases in SOD, GSH and CAT activity enhance the ability to eliminate free radicals resulting from the oxidative stress of OCPs residues in rabbit's diet. Therefore, a decrease of the MDA content in the tissues is associated with an enhancement of antioxidant enzymatic activity that results from the provision of supplemental dietary PSP.

The protective efficacy of PSP could be attributed to the biologically active compounds as mentioned in Table 2 like vitamins (C, E), Zn, Se, total phenols and flavonoids. It is well known that vitamin C and E are powerful antioxidants which recognized as protective agents that could repress the oxidative stress initiated by the pesticides (Harapanhalli et al., 1996). The determined vitamins such as C, E and A occurred in pomegranate peels and seeds are considered one of the most important anti-oxidants

agents (Rowayshed et al., 2013). Selenium contributes to the protection of cells from oxidative damage via the activity of GSH-Px, which is an enzyme that catalyzes the reduction of hydrogen peroxide and organic peroxides. This is in turn, helps to prevent lipid peroxidation, (Behne et al., 2009; Bratovic, 2020). Antioxidant efficiency of pomegranate seeds has been assigned to the existence of ascorbic acid and phenols, inclusive punicalagin, punicalin, gallic acid, ellagic acid, and anthocyanins (Parashar et al., 2018). Pomegranate tannins are the most considerable polyphenols that have shown powerful free radical scavenging activities (Packova et al., 2015). Flavonoids are an antioxidant set like catechin, catechin-gallate, quercetin and kaempferol, they stimulate antioxidant enzymes and minimize a-tocopherol radicals (Procházková et al., 2011). May be dietary PSP rich of antioxidant phenolic are proposed as a tool to prevent liver damage. It is clear to notice that the opposite trend observed with the lower antioxidant status of rabbits fed the control diet that associated with the higher ( $P < 0.05$ ) MDA and lower ( $P < 0.05$ ) TAC and antioxidant enzymes which means that organochlorine pesticides might induce oxidative stress for the rabbits fed these polluted diets. This confirmed by Jabłońska-Trypuć (2017) and Medithi et al. (2020) who stated that the pesticides is one of the causes of the increased oxidative stress level due to its ability to disrupt cell metabolic dysfunctions which may lead to permanent changes in the DNA, RNA, protein, lipid, and sugar structures. Previous reports revealed that PSP had the protective effects against the oxidative stress generated by organochlorine pesticides via the prevention of free radicals which imbalance the antioxidant defense system to cell oxidative stress (Temiz, 2020; Minisy et al., 2020).

#### CONCLUSION

In conclusion, the present study demonstrates that dietary pomegranate seed powder supplementation has protective effects against oxidative stress generated from organochlorine pesticide residues particularly when rabbit's diets supplemented with PSP at levels of 500, 750 and 1000 g/100 kg diet. Moreover, SPS improved growth performance, decreased mortality% and enhanced the antioxidant status of rabbits due to bioactive components that act as powerful antioxidant agents.

#### ACKNOWLEDGEMENTS

Fawzia Amer Hassana designed the experiment, wrote and published the manuscript; Hassan Abd El-Halim conducted the data analysis and collected review. Fayza Ahmed Sdeek directed the laboratory analysis. Ahmed Mohamed Abd El-Hady and Osama Ahmed Elghalid preparation of the research protocol, help in experimental

work, wrote and published the manuscript. The authors wish to thank «nucleus breeding rabbit unit» of the Poultry Research Center, Poultry Department, Faculty of Agriculture, Alexandria University, Egypt for providing the rabbit line and part of the laboratory tools; also Department of By-products Utilization Research, Animal Production Research Institute, Agricultural Research Center, Giza, Egypt for providing part of the laboratory tools, chemicals and supervising the experiment. This research did not receive any specific funding.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## AUTHORS CONTRIBUTION

Fawzia Amer Hassana designed the experiment, wrote and published the manuscript; Hassan Abd El-Halim conducted the data analysis and collected review. Fayza Ahmed Sdeek directed the laboratory analysis. Ahmed Mohamed Abd El-Hady and Osama Ahmed Elghalid preparation of the research protocol, help in experimental work and participated in writing the manuscript.

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