Egyptian J. Nutrition and Feeds (2012), 15 (2): 323-334

EFFECT OF POMEGRANATE PEEL AS ANTIOXIDANT SUPPLEMENTATION ON DIGESTIBILITY, BLOOD BIOCHEMICAL AND RABBIT SEMEN QUALITY.

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(Received 2 /4/ 2012, Accepted 15/5/ 2012)

SUMMARY

Thirty six healthy New Zealand White breed rabbit bucks aged 6 months with an average weight of 2.865 kg were used in a factorial experiment with 9 bucks per treatments and allotted into four dietary groups (n=15). First group served as control, and 2, 3, 4th groups fed control diet supplemental with 0.5, 1.0 and 1.5% pomegranate peel, respectively. The experimental period lasted eight weeks. Results indicated that rabbits fed diets containing pomegranate peel 0.5, 1.0 and 1.5% had lower digestible nutrients % (CP, EE and NFE) and nutritive value (TDN, DE and DCP) than those fed the control diet, while pomegranate peel 1.5% improved CF compared with the control. All treatments reduced final body weight gain and total feed intake compared with control. Also, results revealed better sexual activity and higher libido of pomegranate treated bucks. Bucks receiving 0.5, 1.0 or 1.5% dietary pomegranate peel had a higher semen volume, mass motility and sperm count. Dietary pomegranate peel reduced the percentage of dead sperm and abnormal sperm. Significant decrease in blood triglyceride (TG), low density lipoprotein (LDL) and very low density lipoprotein (vLDL) were observed. The activities of different blood and seminal plasma enzymes were significantly enhanced. Interestingly, pomegranate peel reduced seminal plasma thiobarbituric acid-reactive substances (TBARS) while increasing both the blood and seminal plasma antioxidant enzymes (TAC, SOD and GPx). These results may indicate that dietary supplementation of pomegranate peel could be used up to 1.5% to have a favorable effect on improved CF digestibility coefficient, blood level profile, the semen quality and antioxidant status. Addition of pomegranate peel may have extra protective effect according to its contents of natural antioxidants.

Keywords: *pomegranate peel; digestibility; blood lipid profile; semen quality; rabbits.*

INTRODUCTION

The shortage of animal protein facing Egypt cannot be solved by only large animals but by increasing the production of highly reproductive animals in the livestock unit. The increase in demand for animal protein necessitates the utilization of the potentials of small livestock species and stimulates their introduction into animal research and economic development programs especially in developing nations. Much research is needed in order to select raw materials; those of residual origin are especially promising due to their lower costs. Pomegranate peel (*Punica granatum L.*) family Punicaceae is cultivated around the world in subtropical and tropical regions such as in Iran, California, Turkey, Egypt, Italy, India, Chile and Spain. Pomegranate production amounts could reach to approximately 65,000 tons in Egypt (Faostat-Fao, 2010), where the peels (pericarp, rind or hull) amounts to approximately 60% of the pomegranate fruit weight (Lansky and Newman 2007). Pomegranate peels contains a substantial amount of polyphenols such as sugar-bound flavoniods quercetin and kaempferol, flavond, diglycoside, ellagic acid tannin and organic acids. Ellagitannins (ETs) are the predominant phenolics in pomegranate peel (Nasr *et al.*, 1996). Although polyphenolic compounds may improve animal health, they can also decrease proteolytic activity and, thus, compromise protein digestion (Oliveira *et al.*, 2010). Feizi *et al.*, (2005b) demonstrate the potential of pomegranate seed can be used in animal nutrition. They indicated that inclusion of pomegranate seed up to 25% of the diet has no negative effect on the nutrients intake and digestibility. Also, they showed that pomegranate peel tannins have a negative effect on in vitro rumen fermentation and increase the volume of gas produced with polyvinylpolypyrrolidone, revealed the inhibitory effects of tannins on fermentation (Feizi *et al.*, 2005a).

Pomegranate peel, a waste product of the pomegranate industry with higher antioxidant levels than the juice itself, an attractive candidate as a nutritional supplement for rabbit feed. Pomegranate peel extract had high antioxidant capacity, considering the scavenging or preventive capacity against super oxide anion, hydroxyl and peroxyl radical as well as inhibiting. Pomegranate fruit peel exerted diverse pharmacological functions as antioxidant activity (Li, Yunfeng *et al.*, 2006 and Thring *et al.*, 2009). However, in recent years, many attempts have been made to study natural antioxidants, particularly those of plant origin. Pomegranate contains 2,92 ± 0,19 mg / 100gr total phenols and 0.2–1.0% (Aviram and Dornfeld, 2001) soluble phenols showing remarkable antioxidant activity and significant health properties. Great interest has recently been focused on the addition of polyphenols to foods and biological systems, due to their well-known abilities to scavenge free radical antioxidant power. Reactive oxygen species (ROS) play an important role in the animal reproduction. Spermatozoa are rich in polyunsaturated fatty acids as well as susceptible to be attacked by ROS or membrane lipid peroxide ion. The equilibrium between the amounts of ROS produced and scavenged is related to the gamete cell stability and damage. Free radicals have beneficial or detrimental effects upon sperm functions, which depend on their nature and concentration. Excessive generation of ROS in semen may be associated with reduced sperm fertilizing potentials. Polyunsaturated fatty acid in the phospholipids of the animal spermatozoa is highly susceptible to peroxidation. Numerous antioxidants are related with the ROS detoxification, including superoxide dismutase (SOD), catalase, malondialdehyde (MDA), and glutathione peroxidase (GPx). MDA, an end product of lipid peroxidation, represented the level of lipid peroxidation. Antioxidative activity has often been associated with a decreased risk of various diseases and mortality. A positive correlation between oxidative stress and illnesses is widely documented in cattle (Shabtay *et al.*, 2008).

Therefore, the current study is an attempt to study the inclusion effects of pomegranate peel as natural antioxidant on rabbits' digestibility, blood lipid profile, semen quality and antioxidant status.

MATERIALS AND METHODS

The experimental work was carried out at Borg El-Arab Poultry Research Station (Alexandria), Animal production Research Institute, Agricultural Research Center, Ministry of Agriculture during October to December, 2011.

A total number of 36 New Zealand White (NZW) rabbit bucks (at age 6 months) and averaged 2865±35.2 g body weight distributed into four experimental groups (n=9). Each group had three replicates, (3 each) were housed in galvanized batteries (60×40×24 cm) provided with feeders and automatic drinkers. First treatment served as a control (0% pomegranate peel), second, third and fourth treatments were fed dietary supplemented with 0.5, 1.0 and 1.5% pomegranate peel, respectively (Table 1). All the experimental diets were formulated to be isonitrogenous and isoenergetic containing approximately 16% CP and 2600 Kcal/Kg DE. The rabbits were individually housed in galvanized wire cages with a photoperiod of 12 hours light/day and a temperature ranging from 18–25°C., had free access to fresh water and feed ad-libitum. The chemical composition of pomegranate peel was presented in (Table 2). Daily feed intake and weekly body weight were recorded. Experimental period lasted 8 weeks.

Digestibility Trial:

A total number of 12 adult males (4 males in each group) were used in the digestible trial for determining nutrient digestibility of the tested diets. Animals were housed individually in cages that allowed the separation of feces and urine. All rabbits were kept under the same management, hygienic and environmental conditions. The experimental diets were offered twice daily at 9 a.m. and 15 p.m. and fresh water was provided ad Libitum. Survey of daily feed intake was recorded. Any possible feed contamination was removed from the feces.

The trial lasted for 15 days, 8 days as a preliminary period followed by 7 days for measurements of actual feed intake and feces output. Samples of daily feces (20%) of each rabbit were taken and oven dried at 70° C for 48h, then the bulked was ground and stored for chemical analysis. Samples of feed and feces were analyzed for dry matter (DM), crude protein (CP), crude ether extract (EE), crude fiber (CF), and crude ash

(CA) according to the classical A.O.A.C. methods (1990). The nutritive value of the experimental diets as TDN value and DE (Kcal/ Kg) were calculated according to Cheeke (1987).

Table (1): Formulation and chemical analyses (%) of the experimental diets.

Ingredients	Pomegranate peel in the diet (%)			
	Control (PP 0.0%)	PP 0.5%	PP 1.0%	PP 1.5%
Clover hay	33.00	33.00	33.00	33.00
Yellow corn	17.90	17.90	17.90	17.90
Wheat bran	11.00	10.50	10.00	9.50
Barley grain	17.30	17.30	17.30	17.30
Soybean meal (44%)	15.00	15.00	15.00	15.00
Molasses	3.00	3.00	3.00	3.00
Pomegranate peel	0.00	0.50	1.00	1.50
Limestone	1.20	1.20	1.20	1.20
Salt	0.50	0.50	0.50	0.50
Dl- Methionine	0.20	0.20	0.20	0.20
L-Lysine	0.10	0.10	0.10	0.10
Vit, and Min. mix.[1]	0.30	0.30	0.30	0.30
Total	100	100	100	100
Chemical analysis				
Dry Matter	90.15	90.14	90.03	90.10
Organic Matter	91.51	91.49	91.47	91.45
Crude Protein	16.01	16.07	16.03	16.00
Ether Extract	3.46	3.50	3.49	3.49
Crude Fiber	13.50	13.51	13.53	13.55
Nitrogen Free Extract (calculated)	58.54	58.41	58.42	58.41
NDF (calculated)[2]	37.79	37.80	37.81	37.83
Ash	8.49	8.51	8.53	8.55
DE(Kcal/Kg)*(calculated)[3]	2505	2504	2504	2503

(1)= Each 3 kilogram of Vit+Min mixture provides: Vitamin A, 12000 IU; Vitamin E, 20 IU; menadione, 1.3 mg; Vit. D_3, 2500 ICU; Riboflavin, 5.5 mg; Ca Pantothenate, 12 mg; nicotinic acid, 50 mg; Choline chloride, 600 mg; Vitamin B_{12}, 10 µg; Vitamin B_6, 3 mg; Thiamine, 3 mg; folic acid, 1.0 mg; d-biotin, 50 µg. Trace mineral (milligrams per kilogram of diet): Mn, 80; Zn, 60; Fe, 35; Cu, 8; Se, 0.60. ***Based on NRC (2001).***

(2, 3)= DE (Kcal/ Kg) = 4.36-0.0491 × NDF%, Where, NDF% = 28.924 + 0.657 × CF%. (Calculated according to Cheeke, 1987).

Table (2): Chemical composition of pomegranate peel (g/kg DM).

Nutrients	Pomegranate peel %
Dry matter	96.2
Organic matter	94.6
Crude Protein	3.60
Ether Extract	0.61
Crude Fiber	23.4
NDF	44.29
Ash	5.4
Nitrogen free extract	66.99
Digestible Energy (Kcal/ Kg) (calculated)	2185

Semen collection was weekly occurred over the 8 weeks of the study, so 72 ejaculates were obtained per treatment (9 rabbits buck × 8 weeks). Ejaculates were collected using an artificial vagina maintained at 45-46°C and a teaser doe. Semen was kept immediately after collection at 35 °C in a water bath in order to be evaluated. Semen volume of each ejaculate was recorded after removal of the gel mass. Two drops of fresh

semen were placed on a warmed slide and covered with a cover slip (20×20mm); mass motility from at least three fields was examined at 37°C under a phase microscope at 40× and assessed from 0 to 100%. A weak eosin solution was used at a rate of 1:99 before counting the cells, sperm concentration ($\times 10^6$/ml) was evaluated according to Smith and Mayer (1955) by the haemocytometer slide. Total sperm output was calculated by multiplying semen ejaculate volume by semen concentration. Assessment of live and abnormal spermatozoa was performed using an eosin-nigrosine blue staining mixture (Blom, 1950). The percentage of live spermatozoa was determined by using stains that penetrate cells with damaged membranes. Total number of motile sperm was calculated as multiplying percentage of motile sperm by total sperm outputs. Seminal plasma was obtained by centrifugation of semen samples at 860 rpm for 20 min at 4 °C, and was stored at -60 °C until analysis.

Blood samples were collected from the ear vein of each buck every week (9 rabbit's × 4 weeks) and immediately placed on ice in heparinized tubes. Plasma was separated from the blood by centrifugation at 860 rpm for 20 min and stored at -60 °C. Blood and seminal plasma samples were analyzed biweekly for total cholesterol, HDL- cholesterol, triglycerides (TG) calorimetrically using commercial kits (Diamond Diagnostics, Egypt). The concentration of very low density lipoprotein (VLDL-c) was estimated according to the Fridewald's equation (Fridewald et al. 1972).

Thiobarbituric acid-reactive substances (TBARS) was measured in the seminal and blood plasma using the method of Tappel and Zalkin (1959). Seminal and blood plasma glutathione peroxidase (GPx) activity assayed using the method of Chiu et al. (1976). Superoxide dismutase (SOD) activity was assayed according to Misra and Fridovich (1972). Total antioxidant capacity (TAC) was determined according to Diamond Biodiagnostic, Egypt.

Statistical analysis:

The obtained data were analyzed using one-way ANOVA of GLM Procedure of SAS® (SAS Institute, 2000). Significant differences between means were detected using New Duncan Multiple Range - Test (Duncan, 1955).

RESULTS AND DISCUSSION

Apparent digestibility of nutrients and Nutritive value of feeds:

It was noticed that incorporation of pomegranate peel (PP) at any level in the diets of rabbits was not resulted in any improvement of digestibility of the diet nutrients, except the CF digestibility when PP was added at 1.5 %. The control group (zero PP) had always the higher ($P<0.05$) digestion coefficients (Table3). Lebas, (1986) was recorded that certain fiber sources (beet-root pulp, fruit pulp in general) are highly in CF digestibility, it varied from 60 to 80 %. The present of polyphenolic compounds could decrease the proteslytic activity and these compromise protein digestion(Oliveira *et al.*, 2010). In the same concern, condensed tannins are considered to have negative effect on palatability and digestion, as it may reduce intake, protein digestibility and carbohydrate (Feizi *et al.*, 2005 a, b). However, these negative effect could affected on both of TDN, DCP and DE as these substances form insoluble complexes with proteins and carbohydrates lowering the nutritive value of any products contained tannins (Ferket and Middelton, 1999). In this respect, Akbar and Gupta (1985) reported that tannin formulate protein- tannin complexes in the gut which resulted in limiting dietary protein availability. In contrast, Oliveira *et al.* (2010) illustrated that feeding PP did not influence DM, OM, or starch digestibility, but it reduced CP and fat digestion of calves in the first 70 days of age.

Performance of growing rabbits:

Feeding rabbits diets contained any level of PP (0.5, 1.0, and 1.5%) resulted in reducing the live body weight at the end of the experiment. The decrease was reached 2.2, 2.6 and 2.77 %, respectively, which indicated that as far as PP level increase, LBW could be decreased. (Table 3)

The decrease in body weight may be attributed to the effect of condensed tannins or plyphenols in the PP which reflected on the significant decrease in feed intake and crude protein digestibility. Tanninates are known to reduce mucosal secretion and make the intestinal mucosa more resistant (Scalbert, 1991; Tripathi, 1994). However the decreased rate of final body weight of rabbit bucks fed on diets containing different levels of PP does not reflect on any health hazards on rabbit bucks.

Table (3): Digestible nutrients, Nutritive value (%) and Performance characteristics of male rabbits fed diet supplemented with different levels of pomegranate peel.

Item	Pomegranate peel				P value
	0	0.5	1.0	1.5	
	Digestible nutrients (%)				
CP	$82.69^{a}\pm0.93$	$78.17^{b}\pm1.12$	$77.01^{b}+0.73$	$76.42^{c}\pm0.83$	0.05
EE	$85.69^{a}\pm0.43$	$80.17^{b}\pm0.37$	$79.87^{bc}\pm0.39$	$78.13^{c}\pm0.70$	0.0001
Fiber	$74.91^{b}\pm4.77$	$74.89^{b}\pm1.37$	$74.79^{b}\pm0.61$	$78.69^{a}\pm0.43$	0.01
NFE	$86.32^{a}\pm0.46$	$84.84^{b}\pm0.68$	$81.94^{c}\pm0.40$	$80.54^{c}\pm0.38$	0.05
	Nutritive value (%)				
TDN	$56.09^{a}\pm0.32$	$54.91^{b}\pm0.24$	$51.98^{c}\pm0.34$	$51.86^{c}\pm0.11$	0.01
DE	$2.569^{a}\pm0.04$	$2.533^{ab}\pm0.06$	$2.499^{c}\pm0.05$	$2.494^{c}\pm0.03$	0.01
DCP	$12.96^{a}\pm0.15$	$12.76^{ab}\pm0.21$	$12.34^{b}\pm0.12$	$12.14^{c}\pm0.12$	0.01
	Performance				
IBW, (g)	2865	2870	2866	2871	NS
FBW, (g)	3245^{a}	3173^{b}	3161^{b}	3155^{b}	0.01
TFI, (g)	2866^{a}	2801^{b}	2778^{c}	2775^{c}	0.005

$^{a-c}$ Means within a column not sharing similar superscripts are significantly different ($P \leq 0.05$). NS: Not significant ($P > 0.05$).
IBW, (g) = Initial body weight, FBW, (g) = Final body weight, TFI, (g) = Total feed intake.

These results are in accordance with those reported by Mahmoud, *et al.* (2011) who found that rats fed on diets contained dry PP recorded the lowest final live weight, while those fed on control diet recorded the highest one. On the other hand, Labib (2009) found that rats administrated with different levels of pomegranate peel powder (5, 10 and 15%) had a significant decrease in body weight gain than the control group. With conflict results had been found by Shabaty et al. (2008); dietary supplementation with fresh pomegranate peels promoted significant increases in feed intake with a positive tendency toward increased weight gain of bull calves.

The reduction in feed intake may be due to the lower intestinal motility which led to a higher retention time of the digest in the gut as reported by Garcia *et al.* (1999). Li *et al.* (2006) demonstrated that the PP contained some compounds that influence palatability and consequently nutritive value, which include tannins, phenols, steroids, cyanogenic and alkaloids compounds. Makled et al. (2003) reported that bucks fed 0.25 and 0.50% tannic acid consumed less ($P<0.01$) amount of feed than that of the control group (lowered by 33.13 and 23.50%, respectively). Moreover, feed intake per buck was markedly decreased at the lower level of dietary tannic acid (0.25%) than at the higher level (0.50%). The difference between the current study and that the reported one by Shabtay et al. (2008) that PP intake up to 20% of the total feed intake does not possess deleterious or positive effects on fattening ration intake of feedlot calves.

Semen characteristics:

The changes in semen characteristics in rabbit bucks fed diet supplemented with pomegranate peel are presented in Table (4). It showed that ejaculate volume (ml), reaction time (Sec.), mass motility (%), sperm concentration ($\times10^{6}$/ml), total sperm output ($\times10^{6}$), the total motile sperm ($\times10^{6}$), live sperm (%) and abnormal sperm (%) of rabbit bucks were significantly increased for bucks received 0.5, 1.0 or 1.5% pomegranate peel, while additional increase did not make an improvement over 1% pomegranate peel. In contrast, the opposite development was shown in the reaction time and abnormal sperm while difference between different levels of supplementing pomegranate peel was not significant for reaction time where abnormal sperm linearly decreased up to 1.5% pomegranate peel. But was not difference between 0.5 and 1% significant. The semen volume of the bucks recorded within the range 0.3 to 0.6 ml for rabbit, and also the sperm concentration ranging from 223.9 to 327.43 ($\times10^{6}$ ml). The data obtained in the study were in agreement with concentration of rabbit sperm cells of 150 to 500 ($\times10^{6}$ ml) as reported by Lebas, (1986). The higher sperm concentration recorded with 1.0 and 1.5% compared to 0.5% pomegranate peel could be

attributed to the ingredients formed the tested data since that was the only varying factor in the feed. Also, the overall higher performance of 1.0 and 1.5% groups could be attributed to the additional mineral elements contributed to their diet as pomegranate peel was found to be rich in mainly potassium, nitrogen, calcium, phosphorus, magnesium, and sodium (Mirdehghan and Rahemi 2007), and complex polysaccharides (Jahfar *et al.*, 2003). The effects of trace element biochemistry and physiology on parameters of fertility are presented for zinc, selenium, iodine, copper and manganese (Leonhard–Marek, 2000). It has been reported that sodium and potassium ions maintain equilibrium in different fluids (Tortora and Grabowski, 1996). Sodium plays vital roles in cellular hydration and helps the maintenance of acid-alkaline equilibrium. The additional minerals from the test ingredients may have contributed to the metabolic regulation of sperm cells, an enhanced enzyme activity of the semen and increased spermatogenic activities. The increase of sperm concentration, live sperm cells and motility indicated that pomegranate peel could improve and enhance the fertilizing capacity of semen. The same results were included by El-Damrawy (2011) who studies the effects of olive leave extracts (OLE) supplementation (0.5 g/Kg body weight daily) in alleviating the changes in semen quality parameters and enzyme activities in seminal plasma of rabbit bucks. The same author found significant increase in sperm concentration, sperm motility; also, OLE supplementation could decrease dead and abnormal sperms.

Seminal plasma antioxidant constituents:

The data presented in Table (4) showed the effect of different pomegranate peel concentrations on seminal plasma antioxidant status. Seminal plasma total antioxidant capacity (TAC), super oxidase dismutase (SOD), and glutathione peroxidase (GPx) activities significantly increased with increasing pomegranate peel content, while it decreased TBARS activity of blood plasma. These improvements of antioxidant constituents were maximized at 1.5% in total antioxidant capacity (TAC), super oxidase dismutase (SOD) and glutathione peroxidase (GPx) in seminal plasma. Our data showed that pomegranate peel significantly reduced the level of TBARS and increased the level of SOD and GST in seminal plasma which may be due to its free radical scavenging ability as an antioxidant. Ochoa *et al.* (2011) reported that antioxidant supplementation reduced lipid peroxidation and lengthen life span in rodents.

Table (4): Effect of pomegranate peel on semen characteristics and biochemical semen plasma of rabbit males.

Criteria	Pomegranate peel (%)				
	0	0.5	1.0	1.5	P value
Semen quality					
Volume of semen (ml)	0.435^{c} ±0.017	0.497^{b} ±0.02	0.569^{a} ±0.02	0.58^{a} ±0.019	0.01
Reaction time (sec.)	9.9^{a} ±0.019	6.6^{b} ±0.02	6.9^{b} ±0.021	6.8^{b} ±0.019	0.01
Mass motility (%)	69.1^{c} ±0.59	75.1^{b} ±0.63	80.7^{a} ±0.60	80.9^{a} ±0.66	0.0001
Sperm concentration ($\times 10^{6}$/ml)	223.9^{c}± 3.12	294.8^{b}±3.01	319.5^{a} ±3.19	327.43^{a}±3.22	0.05
Total sperm output ($\times 10^{6}$)	156.73^{c}±5.30	250.58^{b}±5.60	284.63^{a}±4.9	301.24^{a}±5.46	0.05
Total motile sperm ($\times 10^{6}$)	108.3^{c}±4.19	188.19^{b}±4.35	229.7^{a}±4.38	243.7^{a}±4.64	0.005
Live sperm (%)	71.9^{c}±0.60	77.1^{b}±0.61	81.9^{a}±0.67	82.4^{a}±0.66	0.05
Abnormal sperm (%)	19.6^{a}±0.35	14.9^{b}±0.36	13.7^{b}±0.32	13.6^{c}±0.32	0.0.001
Seminal plasma antioxidant constituents					
TAC[1], (mmol/L)	1.25^{c} ±0.02	1.79^{b} ±0.02	2.02^{ab}±0.04	2.22^{a} ±0.05	0.0001
SOD[2], (u/l)	24.5^{d} ±1.63	33.68^{c} ±2.43	35.09^{b} ±1.96	36.48^{a} ±2.64	0.0001
GPx, (u/l)	455^{d}±40.1	673^{c}±45.6	791^{b} ±48.7	896^{a} ±42.2	0.001
TBARS[4], (µmol/ml)	1.37^{a} ±04	1.09^{b}±02	0.87^{c}±01	0.71^{d} ±03	0.0001

[a-d] Means within a column not sharing similar superscripts are significantly different ($P \leq 0.05$). NS: Not significant ($P > 0.05$).

(1) TAC = Total antioxidant capacity. (2) SOD = Superoxide dismutase. (3) GSH-Px = Glutathion peroxidase. (4) TBARS= Thiobarbituric acid.

These results could go parallel to the investigation of Yousef (2005) on the acasia saligna leaves fed for 8 weeks old white New Zealand male rabbits until maturity. He concluded that up to 40% acacia leaves could be successfully and safely used in the diet of rabbits without adversely affecting on their reproductive performance as their semen quality and characteristics were improved, in addition the lower of thiobarbituric acid reactive substances in seminal plasma. Furthermore, the decreased of TBARS could decrease incidence

of dead sperm and that may propose an association between lipid peroxidation and sperm quality. In addition, this finding suggests that the lower concentrations of TBARS found in seminal plasma were apparently related to a lower antioxidant capacity in the present results. About 50% of the total fruit weight corresponds to the peel, which is an important source of bioactive compounds such as phenolics, flavonoids, ellagitannins (ets), and proanthocyanidin compounds (Li *et al*, 2006).In parallel of our studies El-Damrawy (2011) found that supplementation of olive leave extracts 0.5 g/Kg body weight rabbit's bucks daily increased seminal plasma of glutathione s-transferase activity and superoxide dismutase (SOD) activity, but it decreased seminal plasma of thiobarbituric acid-reactive substances (TBARS).

Blood biochemical constituents:

Li *et al*., (2006) who reported that Pomegranate peel extract appeared to have more potential as a health supplement richer in natural antioxidants than the pulp extract. It could be observed that buck rabbits fed on different levels (0.5, 1.0 and 1.5% pomegranate peel) in diets had significant increase in high density lipoprotein (HDL) cholesterol comparing with rabbits fed on basal diet (control group) (Table5). Moreover, all groups administrated with different level of pomegranate peel powder (0.5, 1.0 and 1.5%) had a significant decrease in plasma total cholesterol; triglycerides low density lipoprotein (LDL) and very low density lipoprotein (VLDL), compared with the control group. Blood plasma cholesterol and low density lipoprotein were significantly decreased when 0.5% pomegranate peel was supplemented compared to the control group. A high consumption of phenolic compounds has already been found to decrease serum cholesterol and triacylglycerol concentrations in rat (El-Ansary *et al*., 2000). Meanwhile, groups supplemented with 1.0 and 1.5% had intermediate values. Dietary supplementation with nutrients rich in antioxidants is associated with inhibition of atherogenic modifications to LDL, macrophage foam cell formation, and atherosclerosis. Aviram *et al*., (2000) reported that Dietary supplementation with nutrients rich in antioxidants is associated with inhibition of atherogenic modifications of LDL, macrophage foam cell formation and atherosclerosis. These results are agreed with Esmaill Zadeh *et al*. (2006) who reported that consumption of concentrated pomegranate juice for diabetic patient with hyperlipidemia significantly decreased in total cholesterol ($P<0.006$) and had no significant changes in serum HDL. Also, Labib (2009) reported that all hypercholestrolemic groups administrated with different level of pomegranate peel powder (5, 10 and 15%) or administrated with pomegranate peel extracted (1, 2 and 3%) had a significant decrease in serum low density lipoprotein LDL, very VLDL, lipid peroxidation and atherogenic index compared with hypercholesterolemic rats (control positive).

Blood plasma antioxidant constituents:

It is clear that, the high cholesterol diet used in this experiment could induce many of the health hazards reported by different investigators. This shows how important it is to find a way or a mean to avoid these health complications. The beverages produced in this study which was prepared from the vegetable or fruit wastes can be that mean. These beverages proved to contain considerable number and quantities of the polyphenolic antioxidants (El-Shobaki et al., 2011) which are believed to participate in the prevention of these health hazards. Pomegranate peels contain of (3,164 % total phenols, w/ w.) could be a valuable source of natural phenolic antioxidants. Blood plasma TAC, SOD, and GPx activities significantly increased with increasing pomegranate peel content while it decreased TBARS activity of blood plasma (Table5). Chang-Sook Choil, *et al*. (2010) found that the level of malondialdehyde (MDA) was lower in the serum of rabbits fed grape seed extract or grape peel powder plus cholesterol than in the serum of rabbits fed cholesterol alone.

Higher activity of these indicators in plasma suggested that pomegranate peel could be increased the antioxidant status in rabbits fed higher levels (1.5) pomegranate peel diet.

These results showed a direct correlation between the ellagic acid (EA) content in pomegranate extracts and its ability in quenching free radicals. The contents of total phenolics in the pomegranate peel extract was reported to be 10-fold as much as its content in the pulp extract, which causes its stronger antioxidant ability (Li, *et al*, 2006). The antioxidant enzymes, mainly superoxide dismutase and catalase are first-line defensive enzymes against free radicals (Parathasarathy *et al*. 1986). Antioxidants reduce the oxidation of LDL and decrease the concentration of free radicals, which inactivate nitric oxide and be effective in reversing endothelial function associated with hypercholesterolemia (Bok *et al*., 1999). Now beneficial health effects of

edible phytochemicals is now considered to be an inexpensive, readily applicable, acceptable, and accessible approach to control and management a wide variety of diseases related to oxidative stress Tachibana, 2011.

Seham Kassem. *et al.* (2011) found that the activities of each of the antioxidant enzymes superoxide dismutase (SOD). Catalase and glutathione peroxidase all were decreased due to consumption of the high cholesterol diet. The values obtained were 453.0 ± 12.9 U/ml, 521.9 ± 17.69 U/L, 806.2 ± 43.3 mu/ml, respectively relative to values of 502.5 ± 13.0 U/ml, 739.3 ± 15.81 U/L, 11138.9 ± 41.65 mu/ ml for control rats. When each of the (Artichoke leaves, pomegranate peel and orange peel) 20% of the dry matter of each of these fruit or vegetable wastes was added to the diet, the activities of these enzymes were within the normal control range.

Table (5): Some blood constituents of male New Zealand White rabbits as affected by the experimental diets.

Criteria	Pomegranate peel (%)				
	0	0.5	1.0	1.5	P value
	Blood plasma constituents				
Total cholesterol, (mg/dl)	69.33±4.89^{a}	67.33±4.76^{b}	65.00±4.11^{c}	64.67±4.52^{c}	0.005
Triglycerides, (mg/dl)	66.10±3.54^{a}	64.00± 3.46^{b}	62.01± 3.69^{c}	52.90±3.99^{d}	0.0001
HDL- cholesterol, (mg/dl)	31.27±5.30^{c}	35.40±5.18^{b}	36.93±5.53^{b}	39.50±5.98^{a}	0.0001
LDL – cholesterol, (mg/dl)	24.84± 1.89^{a}	19.13±1.85^{b}	15.67±1.97^{c}	14.59±2.13^{c}	0.005
VLDL (mg/dl)	13.22±0.68^{a}	12.80±0.69^{ab}	12.40±0.84^{b}	10.58±0.59^{c}	0.005
	Blood plasma antioxidant constituents				
TAC[1], (mmol/l)	1.89^{d}±0.02	2.28^{c}±0.02	2.54^{b} ±0.04	2.62^{a} ±0.05	0.0001
SOD[2], units / L(u/l)	24.5^{b} ±1.63	35.68^{a} ±2.43	36.00^{a} ±1.96	36.48^{a} ±2.64	0.0001
GPx, (u/l)	455^{c} ±40.1	773^{b}±45.6	798^{b} ±48.7	896^{a} ±42.2	0.001
TBARS[4], (µmol/ml)	1.17^{a} ±04	0.89^{b} ±02	0.82^{c}±01	0.75^{d} ±03	0.0001

Values are means ± S.D. Values in a row with unlike superscripts differ, P< 0.05.
a, b, c, d: different superscripts within a row indicate significant differences (P<0.05).

CONCLUSION

These results indicate that the addition of pomegranate peel (0.5 and 1.0 and 1.5% of the feed) in the diet of New Zealand White male rabbits improved the viability, oxidants and decrease triglycerides and LDL cholesterol.

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تاثير إضافة قشر الرمان كمصدر مضاد للاكسدة على الهضم والخصائص البيوكيميائية للدم وجودة السائل المنوى.

أمل محمد عبد المجيد فايد [1] و أبو بكر أحمد عبد الله عزوز [2] و عفاف حسن زيدان [1] و محمد بسيونى [3]

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اجريت هذة التجربة بمحطة بحوث الانتاج الحيوانى ببرج العرب – معهد بحوث الانتاج الحيوانى. تم استخدام عدد 36 من ذكور الارانب النيوزيلاندى بمتوسط وزن 2865 جرام وتم توزيعهم الى اربع مجاميع متوازنة. المجموعة الاولى مجموعة المقارنة، المجموعة الثانية والثالثة والرابعة تم تغذيتها على عليقة مضاف اليها 0.5 و 1.0 و1.5% قشر الرمان على التوالى، استمرت التجربة لمدة 8 اسابيع.

اوضحت النتائج الاتى

الارانب التى تم تغذيتها على علائق تحتوى على 0.5 و 1.0 1.5 % قشر الرمان ادت الى حدوث انخفاض معنوى لكل من معامل هضم البروتين والمستخلص الايثيرى والكربوهيدرات الذائبة مقارنة بمجموع المقارنة، فى حين انة حدث تحسن معنوى لمعامل هضم الالياف مقارنة بمجموعة المقارنة. ايضا اظهرت كل المعاملات الغذائية انخفاض معنوى لكل من الزيادة الوزنية المكتسبة النهائية ومجموع الغذاء المأكول مقارنة بمجموعة المقارنة.

واوضحت النتائج انة حدث تحسن معنوى للنشاط الجنسي والشهوة الجنسية للمجاميع المضاف اليها قشر الرمان. هذا بالاضافة الى ان ذكور الارانب التى اضيف الى غذائها قشر الرمان عند المستويات 0.5 و1.0 و1.5% اعطت تحسن معنوى فى كل من حجم السائل المنوى وحركة السائل المنوى والعد الكلى للنطف. هذا بالاضافة الى ان تغذية ذكور الارانب النيوزيلاندى على المستويات المختلفة من قشر الرمان ادت الى انخفاض كل من الجلسيريدات الثلاثية والكوليستيرول منخفض الكثافة والكوليستيرول منخفض الكثافة جدا بالدم مقارنة بمجموعة المقارنة.

هذا كما اوضحت النتائج الى ان النشاط الانزيمى لكل من بلازما الدم والسائل المنوى ادى الى حدوث انخفاض معنوى فى (TBARS) ولكن زاد النشاط الانزيمى لكل من القدرة التاكسدية الكلية (TAC) و السوبر اوكسيداز ديسميوتاز (SOD) والجلوتاثيون بيروكسيداز (GPx) مقارنة بالمجموعة المقارنة.

هذه النتائج توضح ان اضافة قشر الرمان 0.5 و1.0 و1.5% لاعلاف ذكور الارانب النيوزيلاندى لها تاثير مميز فى السائل المنوى ومستوى الدم من المركبات القابلة للتاكسد (الجلسيريدات الثلاثية والكوليستيرول المنخفض الكثافة). هذا بالاضافة الى ان قشر الرمان لة فعل التاثير الوقائى لما يحتوية من مركبات طبيعية مضادة للاكسدة.