



**HEMATOLOGICAL AND ANTIOXIDANT STATUS OF
PREGNANT RABBITS AT THIRD TRIMESTER AS AFFECTED
BY POMEGRANATE PEELS UNDER HEAT STRESS CONDITION**

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Received: 26 / 11 /2017

Accepted: 29 /01 /2018

ABSTRACT: It is assumed that pregnant rabbits under heat stress condition need to be supplemented with an antioxidant substance that help to keep pregnant does healthy. Various antioxidants are available in the natural like pomegranate peel (PP). The purpose of the current study is to understand the effect of different levels of pomegranate peel supplementation on the hematological and antioxidant status of heat-stressed doe rabbits pregnant at 21st day. Thirty-two V-line does were divided into 4 treatment groups (8 does/group): control, pomegranate peel (0.75%, 1.5% and 3.0%). All treatment diets were free of any antibiotics or added antioxidants. Rabbitry minimum and maximum ambient temperature, humidity and temperature-humidity index throughout the experimental period were between 27.5-33.5°C, 64-76% and 77.84-87.77, respectively. Significantly, all diets containing PP improved each of serum total antioxidants capacity, high density lipoprotein, high density lipoprotein/low density lipoprotein ratio and significantly reduced low density lipoprotein, malondialdehyde and cholesterol concentrations. Pomegranate peel at 3% inclusion level significantly recorded the highest count of red blood cell. Likewise, the two highest level of pomegranate peel (1.5 and 3%) recorded the highest concentration of hemoglobin. The present results indicate that the supplementation of diets with natural antioxidants as pomegranate peel is necessary to overcome some side effects of heat stress on pregnant does via enhancing their antioxidant status and modulating lipids metabolism during the 3rd trimester of pregnancy.

Key word: Antioxidant status - Heat stress - Hematology - Pomegranate peel - Rabbit.

INTRODUCTION

Hematological studies are specific diagnostic tools in health science; indicators of diseases and stress in living organism. Recently, climate changes and high temperatures are appearing in many world regions, with increasing changes in weather patterns have been shown. Rabbit production sector suffers significant losses every year because of heat stress. Oxidative-antioxidative status of rabbits are important indicators of measuring ability of heat stressed rabbits (Ruis, 2006). Besides, heat stress raises oxidation condition that is caused enlarged free radical generation, a condition that boosts reactive oxygen species (ROS) formation and lead to cellular oxidative stress. Also, pregnancy is a critical time at which different biochemical reactions eventually cause oxidative stress (Saikumar et al., 2013), e.g. the synthesis of hormones like prostaglandins, involved in embryo implantation (Jenes, 1984) cause formation of some free radical species (Hope et al., 1975). Heat stress affected negatively most of the blood metabolites and the effects were more deleterious during the 2nd half when compared to the 1st half of pregnancy (Haeb et al., 2007). Natural antioxidants could be used as protector of cellular components from oxidation caused by ROS (Su et al., 2007). Antioxidant compounds enhanced fertility and activated liver enzyme (Martin and Gilbert, 1968) with low rate of mortality, low of various diseases (Huxley and Neil, 2003). Li et al. (2006) reported that PP offers high yields of phenolic component such as gallic acid, cyanidin, quercetin and catechin; flavonoids and proanthocyanidin. Blood total antioxidant capacity of heat stressed does are low, and result in substantial

economic loss. Although several researchers studied the effect of different additives on improving pregnant rabbit's tolerance to heat stress, the role of pomegranate peel in this respect was not extensively studied. Thus, the present research was designed to estimate the effect of three levels of PP supplementation on blood composition and antioxidant condition of V-line pregnant does at 21st d. (third trimester, the most stressed period) under tropic condition such as Egyptian summer.

MATERIAL AND METHODS

The present research was executed by Animal and Fish Production Department, Faculty of Agriculture, Saba Basha, Alexandria University, Alexandria, Egypt, during the period from June to August 2014.

Thirty-two V-line does with average initial live body weight of 3.57- 3.61 Kg (2nd parity, 9 months old) were divided into 4 treatments (8 does / treatment). Does in the first treatment group were given the basal diet, while the second, third and fourth groups were fed the basal diet supplemented with 0.75, 1.5 and 3 % PP. Feed was offered ad libitum.

Diets:

Pomegranate fruits (*Punica granatum*) were obtained from local farm (Borg El-Arab, 30.848889 N^o- 29.611667 E^o). Pomegranate peels were washed well in running water, dried in the sun for 96 h. then the dried peels were grounded using coffee grinder. Total phenolics concentrations of PP extracts were measured by colorimetric method of (Singleton and Rossi, 1965), using the Folin-Ciocalteu reagent. The composition of the basal diet (%) was: Yellow corn, 7.5; Clover hay, 22; Soybean meal 44% CP, 23.5; Wheat bran, 24; Barley, 20; Limestone, 1.15; Di-calcium phosphate,

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0.5; DL-Methionine, 0.2; Anti-aflatoxin+Anti-coccidia, 0.5; Vitamin and minerals Velamax- premix, 0.3; NaCl, 0.35; (CP, 20.2%; CF, 12.6% and DE= 2831.6 kcal/kg DM).

Animals and housing:

Female rabbits were kept under 16 h light photoperiod and exposed to high ambient temperature ranged between 27.5-33.5°C with relative humidity 64-76% and temperature humidity index 76.84-87.77. Rabbits were kept individually in flat-deck cages with feeders and automatic drinkers. Mating with untreated bucks was carried out after a period of 4 weeks of does feeding the experimental diets. Buck/doe ratio was 1:4. The mating was between 8.00 and 9.00 a.m. Pregnancy was diagnosed by abdominal palpation 15 d after mating. After palpation, females failed to conceive were returned to the buck for another mating.

Blood sampling and analysis data:

The does were prevented to eat for some hours before blood sampling. Serum samples were individually collected in late pregnancy (21st day) from marginal vein. Serum was separated by centrifugation for 20 minutes (700 g) and stored in vials at -20°C for later analysis. Frozen serum was thawed and assayed calorimetrically for total lipids, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, alkaline phosphatase, total antioxidant capacity (TAC) and malondialdehyde (MAD) contents following the recommendations of the manufacturer (Bio-diagnostic, Egypt). Moreover, fresh blood samples were collected from each rabbit in heparinized tubes for red blood cells (RBCs) count and hemoglobin (Hb, g/dl) as reported by Natt and Herrick (1952). Packed cell volume (PCV %) was determined by

using micro hematocrit tubes, and mean cell volume (MCV); mean cell hemoglobin (MCH) and Mean cell hemoglobin concentration (MCHC) were then calculated (Bauer et al., 1976).

White blood cells (WBCs) count was done by Hepler (1966) method. For WBCs differentiation, blood film was prepared according to Lucky (1977) and leucocytes were differentiated according to Schalm and Jain (1986).

Statistical Analysis:

Data were analyzed by using the general linear model procedure (SAS Institute Inc., Cary, 2011) using one way ANOVA. Differences between treatments means were compared using Duncan's multiple range test (Duncan, 1955). Percentage numbers were changed to Arc-Sin values to approximate normal distribution before statistical analysis.

RESULTS

Total phenolic compounds:

Total phenolic compounds of pomegranate peel were 275 mg of GAE/100 g dry weight.

Blood Biochemical Analysis:

The blood components are particularly sensitive to changes in ambient temperature, being an important indicator of physiological responses in rabbit to stressing agents. In Table 1, PP significantly ($p<0.01$) lower both serum cholesterol and LDL by about 19.75, 21.22, 24.72 and 41.66, 45.95, 51.57% for 0.75, 1.5 and 3% PP treatment as compared with control. While, PP significantly increased HDL ($p<0.01$; 21.01, 28.93 and 29.40%) and the ratio of HDL/LDL in the blood of doe rabbits treated with 0.75, 1.5 and 3% PP as compared with control does, respectively. These results indicate that PP plays a positive role in the treatment of lipid metabolic disorder and obesity.

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Moreover, serum alkaline phosphatase activity, as an indicator for better kidney and liver function, was numerically reduced in rabbits from treatment groups than the control one.

Significant and positive linear correlations were found between total antioxidant capacities, used as a good indicator to describe the dynamic equilibrium between pro-oxidants and anti-oxidants in serum compartment and phenolic contents. Our results in Fig. 1 showed that TAC significantly increased ($p < 0.01$) by increasing level of PP in the diet. While, the value of MDA, as indicator of oxidative stress, was significantly decreased by increasing PP level in the diet.

Hematology:

Results in Table 2 present the effect of PP on some hematological parameters at the 21st day of pregnancy. Data revealed that PP treatments significantly ($p < 0.05$) increased RBCs count, but the 3% PP-fed does recorded the best value. Also, both 1.5 and 3% PP groups significantly recorded the highest Hb concentration. White blood cells count non-significantly decreased by treatments; however, the lymphocytes counts tend to increase by increasing level of PP in the diets.

DISCUSSION

Blood Biochemical Analysis:

The pomegranate peel treatments significantly lowered cholesterol and LDL as compared with control, that may be due to mediated motivation of hepatic cholesterol-7- hydroxylase activity (Babu and Srinivasan, 1997). While, PP significantly ($p < 0.01$) increased HDL and the ratio of HDL/LDL in the blood of doe rabbits treated with PP, thus play a positive role in the treatment of lipid metabolic disorder and obesity. Our results agree with Aviram and Rosenblat

(2013) who found that pomegranate is better protecting for high-density lipoprotein from oxidation compared to other antioxidants. Additionally, pomegranate has the ability to stimulate HDL-associated paraoxonase 1, which depredated harmful oxidized lipids in lipoproteins (Fuhrman et al., 2005; Rosenblat et al., 2006). Our results agree with Al-Moraie et al. (2013) who indicated that oral treatment with pomegranate juice to hypercholesterolemic rats significantly increased levels of HDL and antioxidant enzymes as compared with the control positive group.

Dietary pomegranate peels modulate fat metabolism (Medjakovic and Jungbauer, 2013). Indeed, decreased levels of blood cholesterol were demonstrated following pomegranate consumption (Aviram et al., 2008). Many action mechanisms might explain these effects. Among them one can that include metabolic effects such as: (1) suppression of energy intake and inhibition of pancreatic lipase activity, leading to decreased absorption of fat (Lei et al., 2007), (2) changing the interplay between the metabolic hormones leptin and insulin (decrease of both) and adiponectin (increase) (McFarlin et al., 2009).

Significant and positive linear correlations were found between total antioxidant capacities and phenolic contents (Surveswaran et al., 2007). Our results cleared that TAC was significantly increased by increasing level of PP in the diet. While, the value of MDA was significantly decreased by increasing PP level. Pomegranate flavonoids has antioxidant activity by eliminating free radicals (Suo et al., 2009). Generally, higher levels of free radicals increase the level of MDA (Yardibi and Türkay,

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2008). Pomegranate juice significantly decreased plasma MDA levels (Yardibi and Türkay, 2008), by their antiperoxidative activity. The results agree with Rosenblat et al. (2006) who reported that PP had a favorable effect by scavenging free radicals, decrease macrophage oxidative stress and peroxidation in animal and moreover, increase antioxidant capacity in humans. Also, studies in rats and mice approve the antioxidant properties of a pomegranate by-product extract with 19% reduction in oxidative stress, 42% decrease in cellular lipid peroxides content, and 53% increase in reduced glutathione levels (Rosenblat et al., 2006).

Phenolic compounds attain their active anti-oxidant activity through free-radical scavenging activity (Andjelkovic' et al., 2006), transition-metal-chelating activity (Nagai et al., 2005) and/or singlet-oxygen quenching capacity (O'Grady et al., 2006), these mechanisms may explain our results.

Hematology:

Our results revealed that PP treatments (1.5 and 3 %) significantly increased RBCs at the 21st day of pregnancy. The increase in RBCs could be due to lipid peroxidation inhibition in erythrocyte membranes due to the antioxidant effect of PP. Statistically non-remarkable increase in lymphocytes percentage could be due to the small number of animals used in the experiment. Our result disagrees with Gracious Ross et al. (2001) and Oliveira et al. (2010) who reported that high consumption of polyphenol rich foods from fruit enhanced lymphocyte functions in mice, rabbit and human subjects.

CONCLUSION

All in all, the antioxidant capacity of PP turns it into a favorable health-promoting constituent of pregnant rabbit diet and a current study indicates that the active biological compounds present in PP may be involved in the control of some homeostasis of heat stressed pregnant does.

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Table (1): Mean values of some blood consistence of heat stressed doe rabbits treated with 0.75, 1.5 and 3% pomegranate peel (PP)

Item	Pomegranate peels %				SEM	P Value
	0	0.75	1.5	3		
Total lipid (mg/dl)	309.28	289.98	293.54	286.33	7.26	0.713
cholesterol (mg/dl)	80.50 ^a	64.60 ^b	63.42 ^b	60.60 ^b	1.74	<0.001
LDL (mg/dl)	44.70 ^a	26.08 ^b	24.16 ^b	21.65 ^b	2.01	<0.001
HDL (mg/dl)	16.80 ^b	20.33 ^a	21.66 ^a	21.74 ^a	0.62	0.008
HDL/LDL	0.38 ^b	0.76 ^a	0.85 ^a	0.88 ^a	0.08	0.001
Triglycerides (mg/dl)	95.00	90.93	87.98	86.05	1.63	0.239
Alkaline phosphatase (IU/L)	34.18	29.06	30.70	28.25	0.86	0.065

Different letters (a, b) within a row denote significant differences between treatments (p<0.05). LDL = low density lipoprotein; HDL= High density lipoprotein.

Table (2): Mean values of hematological parameters of heat stressed doe rabbits treated with 0.75, 1.5 and 3% pomegranate peel (PP)

Items	Pomegranate peels%				SEM	P Value
	0	0.75	1.5	3		
WBCs*10 ³	5.20	4.63	4.96	4.93	0.15	0.637
RBCs *10 ⁶	3.93 ^b	4.29 ^{ab}	4.26 ^{ab}	4.56 ^a	0.08	0.026
PCV%	36.26	37.74	38.90	39.15	1.03	0.720
Hb (g/dl)	11.40 ^b	12.25 ^b	13.15 ^a	13.59 ^a	0.21	<0.001
Monocytes %	3.13	2.25	2.75	2.63	0.15	0.242
Basophils%	1.00	0.88	0.75	0.63	0.18	0.438
Eosinophils%	2.00	2.00	1.75	1.88	0.13	0.898
Neutrophils%	38.76	35.80	37.30	34.77	0.81	0.269
Lymphocytes%	55.14	59.26	57.58	60.18	0.99	0.127
MCV (fl)	92.57	88.52	91.88	85.89	2.42	0.763
MCH (pg)	29.31	28.74	31.16	29.86	0.57	0.504
MCHC (g/l)	31.86	33.70	34.19	35.07	0.85	0.618

Different letters (a, b) within a row denote significant differences between treatments (p<0.05). WBC= white blood cell; RBC= red blood cell; PCV= packed cell volume; Hb= hemoglobin; MCV= mean cell volume; MCH= mean cell hemoglobin; MCHC= mean cell hemoglobin concentration.

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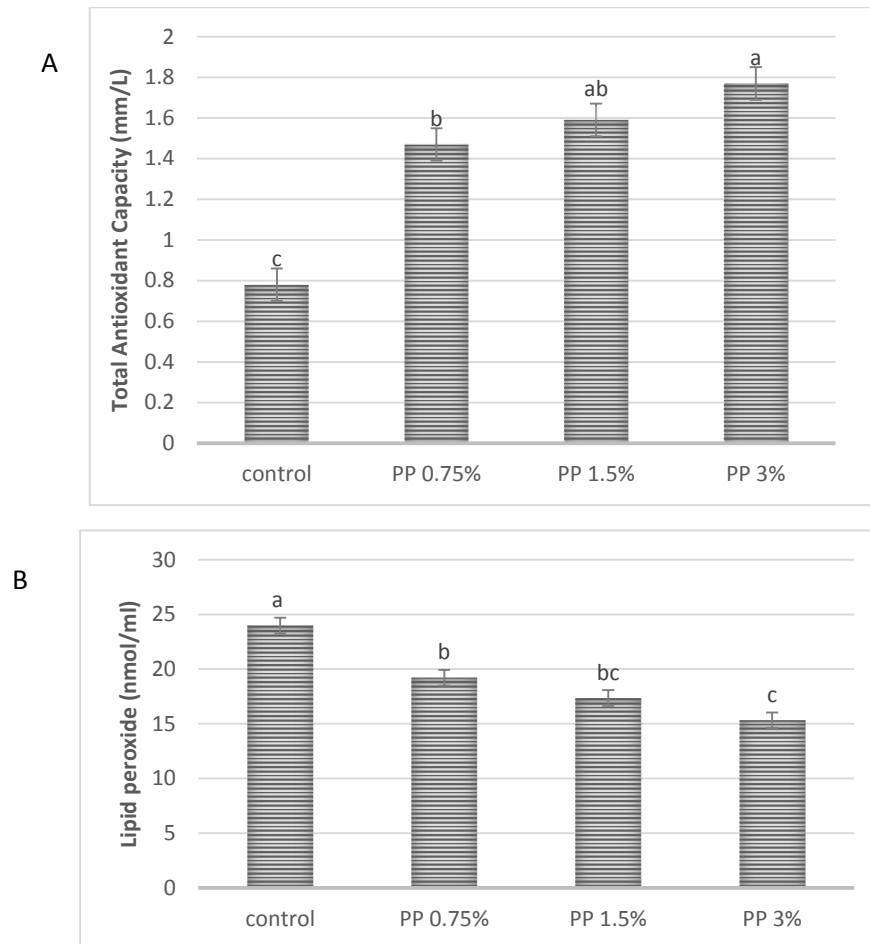


Fig (1): Mean values of total antioxidant capacity (A) and Lipid peroxide (B) of heat stressed doe rabbits treated with 0.75, 1.5 and 3% pomegranate peel (PP). Different letters (a, b, c) within a column denote significant differences between treatments ($p < 0.001$).

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الملخص العربي

الحالة الهيموتولوجية والتأكسدية لإناث الأرانب العشر (في الثلث الأخير من الحمل) المغذاه على قشور الرمان تحت ظروف الإجهاد الحراري

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تحتاج إناث الأرانب العشر المرباه تحت ظروف الإجهاد الحراري إلى اضافة مواد مضادة للأكسدة وذلك للحفاظ على الحالة الصحية لتلك الإناث العشر. نجد أن هناك العديد من المصادر الطبيعية لمضادات الأكسدة، يعتبر قشر الرمان واحد من تلك المصادر. تهدف الدراسة إلى تقييم تأثير اضافة مستويات مختلفة من قشور الرمان إلى العليقة على كلاً من الحالة الهيموتولوجية والتأكسدية لإناث الأرانب سلالة الفي- لين العشر عند اليوم 21 من الحمل المرباه تحت ظروف الإجهاد الحراري ، قسمت عدد 32 أرنبه عشر إلى 4 معاملات (8 إناث / معاملة) و هم مجموعة الكنترول، مجموعة قشر الرمان 0,75%، مجموعة قشر الرمان 1,5% ، مجموعة قشر الرمان 3%. وكانت جميع العلائق التجريبية خالية من أي مضادات حيوية أو أي مضادات أكسدة. وكان متوسط درجات الحرارة والرطوبة النسبية ودليل الحرارة والرطوبة خلال الفترة التجريبية ما بين 27,5-33,5 درجة مئوية، 64-76% و 76,84 - 87,77 على التوالي، أوضحت النتائج أن تغذية إناث الأرانب العشر على علائق تحتوي على مستويات مختلفة من قشر الرمان حسنت وبشكل ملحوظ كلاً من تركيز الدم من مجموع المواد المضادة للأكسدة ، والكوليستيرول مرتفع الكثافة، والنسبة بين الكوليستيرول مرتفع الكثافة والكوليستيرول منخفض الكثافة كما سجلت إنخفاض معنوي في تركيز سيرم الدم من كلاً من بيروكسيد الدهون والكوليسترول والكوليسترول منخفض الكثافة. وسجلت مجموعة قشر الرمان 3% معنوياً أعلى قيمة لعدد كرات الدم الحمراء كما وجد أيضاً أن استخدام قشر الرمان بنسبتي 1,5 و 3% سجلت بشكل كبير أعلى تركيز للهيموجلوبين. ونستخلص من البحث أن اضافة مضادات الأكسدة إلى علائق الإناث العشر ضروري للتغلب على بعض الآثار الجانبية للإجهاد الحراري وعلى الحالة التأكسدية وصورة دهون الدم لأرانب الإناث العشر خلال الثلث الأخير من الحمل.