MITIGATE HEAT STRESS IMPACTS ON SEMEN QUALITY AND ANTIOXIDANTS STATUS OF MALE RABBITS USING SOME NATURAL ANTIOXIDANTS

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SUMMARY

The present study was conducted to investigate the effects of feeding corn silk, orange peel, and pomegranate peel extracts on semen quality, blood biochemical constituents, antioxidants capacity of male New Zealand White rabbits under heat stress conditions. In an experiment that lasted 8 weeks, thirty male rabbits aged twenty-four weeks with an average body weight of 2.85±0.1kg were randomly distributed into five equal groups (6 rabbits/ group). Each rabbit was kept in an individual cage. The first group (control) was fed a basal diet. The second group (positive control) was fed the basal diet supplemented with vit E. at a level of 250 ppm. The third, fourth, and fifth groups were fed the basal diet supplemented with corn silk, orange peel, and pomegranate peel extracts at a level of 150 ppm, respectively. Experimental diets were offered *ad-libitum* and clean drinking water was freely available. The overall average of ambient temperatures and relative humidity were 31.1°C and 62.5%, respectively. Overall temperature-humidity index (THI) through the experimental period was 29.14 indicating severe heat stress conditions. According to the results of the HPLC screening for the different extracts: ferulic acid, cinnamic acid and coumaric acid were the most abundant polyphenols in orange peel extract, while gallic acid was the most abundant polyphenol in pomegranate peel extract. Semen quality parameters improved in rabbits of orange peel and corn silk groups, opposite to rabbits fed pomegranate peel extract. The activity of seminal catalase and glutathione peroxidase increased in rabbits fed orange peel and corn silk extracts. Blood total proteins, albumin, globulins and creatinine were not affected by different antioxidants supplements. Serum total antioxidants capacity, catalase and superoxide dismutase were significantly increased in all experimental groups compared with the control. In conclusion, the current results showed that using orange peel or corn silk extracts as feed supplements at a level of 150ppm could improve semen quality as well as antioxidants capacity of male rabbits reared under heat stress conditions.

Keywords: *Heat stress, male rabbits, antioxidants, cornsilk extract, orange peel extract, pomegranate peel extract*

INTRODUCTION

Heat stress is a serious issue in rabbit production, especially in tropical and sub-tropical regions of the world, since rabbits are very sensitive to high ambient temperature (Pei *et al.*, 2012). The negative effects of heat stress on rabbits extend to productive and reproductive performance. These effects include growth, feed consumption and utilization, immunity, health status, and semen quality (Oladimeji *et al.*, 2021). Reactive oxygen species (ROS) elevation appears to be one of the most detrimental impacts of heat stress, causing polyunsaturated fatty acids peroxidation. These are crucial for the integrity of sperm plasma membranes and consequent fertility disorders (Sahin *et al.*, 2001). During the rest phase, ROS is produced with low concentrations which are necessary for different sperm functions (Mathur and D'Cruz, 2011). ROS production increases (oxidative stress) as the environmental temperature rises, which impairs sperm integrity causing male infertility (Paul *et al.*, 2009). To overcome oxidative stress, managerial or nutritional techniques should be used. Since managerial techniques such as cooling the housing buildings are expensive, natural antioxidant sources could be used as feed supplements. Furthermore, vitamin E is one of the principal membrane protectors against ROS and lipid peroxidation,

and is thought to be the primary component of the spermatozoa's antioxidant system (Doostabadi *et al.*, 2021). In rabbit bucks, vitamin E has also been demonstrated to improve total sperm production and sperm concentration (Hashem *et al.*, 2013).

Corn (*Zea mays L.*) silk, is a collection of the fine, soft, yellowish threads from the female flowers of the maize plant. Corn silk extract has a wide range of bioactive compounds such as polyphenols and flavonoids, and subsequently had high free radicals scavenging potentiality as determined by DPPH assay (Rajeshwari and Sivapriya, 2021; Singh *et al.*, 2022). Maysin (a corn-specific flavonoid) is a flavone glycoside that contains luteolin, a physiologically active substance with antioxidant and anticancer properties (Rajeshwari and Sivapriya, 2021). Stressed mice showed low lipid peroxidation and high antioxidant enzymes levels when subjected to corn silk flavonoids administration (Zhang *et al.*, 2015). The presence of alkaloids, tannins, and phenolic components in corn silk extract significantly protected infected mice from the immune cells and platelet dysregulation (Antony, 2020).

Orange (*Citrus sinensis*) peels are considered to be an economic and renewable source for valuable compounds such as vitamin C, phenolic compounds and flavonoids such as neohesperidin and hesperidin (Addi *et al.*, 2021). High antioxidants content could be achieved through orange peel extraction to be used in pharmaceutical, nutraceutical, food and cosmetic industries (Shehata *et al.*, 2021). Under heat stress conditions, dietary orange peel extract could be used to boost antioxidant enzymes activity and improve metabolic functions in broiler chickens (Akbarian *et al.* 2015). Dietary orange peel extract has been shown to promote growth performance, antioxidative status, regulate ascorbic acid levels in plasma and meat, and lower plasma total cholesterol and LDL cholesterol in growing rabbits (Hassan *et al.*, 2021).

Pomegranate (*Punica granatum L.*) peel represents approximately half of the total weight of the fruit and is a rich source of bioactive components such as flavonoids, ellagitannins, and proanthocyanidin (Hadjadj *et al.*, 2018; Alexandre *et al.*, 2019). Dietary pomegranate peel at levels of 1.5, 3.0, and 4.5% improved total antioxidant capacity and lowered malondialdehyde levels in seminal plasma of heat stressed male rabbits (Zeweil *et al.*, 2013). Antioxidant, anti-cancer, anti-inflammatory, lipid-lowering, and anti-hypertensive properties have been associated with pomegranate peel extracts (Chaves *et al.*, 2020; Gullón *et al.*, 2020).

Therefore, the aim of this study was to investigate the beneficial effects of corn silk, orange peel, and pomegranate peel extracts on antioxidants status and semen quality of male rabbits reared under heat stress conditions.

MATERIALS AND METHODS

Extraction of corn silk, orange peel and pomegranate peel and HPLC screening:

Corn silk, pomegranate peel, and orange peel were collected from local foods factories. They washed separately multiple times in tap water before being air dried in the shade. Using an electric mill, the dry materials were ground into small particles and stored at room temperature for future processing. The crushed corn silk, pomegranate peel, and orange peel were extracted using a heat reflux extraction process with 70% ethanol at 40°C under vigorous stirring for two hours (Šavikin *et al.*, 2018; Castro-Vázquez *et al.*, 2021; Tian *et al.*, 2021). At 40°C, the extracts were separated using a rotary evaporator. The final step, calcium carbonate was used as a carrier material to separately load the extracts, allowing them to be used and incorporated with the other feed ingredients (Samy *et al.*, 2022).

Quantification of phenolic compounds was performed by High Performance Liquid Chromatography (HPLC), LC- 10AD, Shimadzu, Japan. Phenolic compounds were analyzed using a Luna RP-C18 (2) column (250×4.6 mm i. d, 5 µm, Phenomenx). Phenolic acids were extracted after hydrolysis with sodium hydroxide. Approximately 10 ml of each extract was added to 15 ml of 4 N NaOH, shaken for 2 h in the dark with a shaker and acidified with 6 N HCl to reduce pH to 2. Samples were centrifuged at 3000g, and the supernatant was decanted into separatory funnel. The supernatant was extracted with ethyl acetate (3 x 50ml) with shaking for 10 s, and the mixture was allowed to settle for 5 min between extractions. The ethyl acetate fraction was dried by adding anhydrous sodium sulfate and concentrated using rotary evaporator at 40°C to dryness. The residue was resolubilized in 3 ml of methanol and filtered through a 0.2 µm PTFE filter prior to analysis (El-Mergawi *et al.*, 2016).

The mobile phase consisted of a mixture of acetate buffer: acetonitrile (9:1, v/v). Acetate buffer was prepared by dissolving 6.35 g sodium acetate in 1 liter H_2O and 20 ml acetic acid. The detecting wavelength was 260 nm. The injection volume was 10 µL for all extracts. Standard phenolic acids (gallic, protocatechuic, p-hydroxybenzoic, syringic, ferrulic, caffeic, coumaric and cinnamic) were purchased from Sigma Aldrich.

Animals and feeding:

In an experiment that lasted 8 weeks, thirty male New Zealand White rabbits (NZW) aged twenty-four weeks with an average body weight of 2.85±0.1kg were randomly distributed into five equal groups (6 rabbits/ group). Rabbits were individually housed in galvanized wire mesh cages provided with feeders and automatic stainless steel nipple drinkers. The first group (control) was fed a basal diet (Table 1). The second group was fed the basal diet supplemented with vit E. at a level of 250 ppm equal to 0.5g/kg diet (as a powder with a concentration of 50%, positive control). The third, fourth and fifth, groups were fed the basal diet supplemented with corn silk, orange peel, and pomegranate peel extracts by 150 ppm, respectively. The level of supplemented extracts was determined as vitamin E equivalent. Experimental diets were offered *ad-libitum* and clean drinking water was freely available.

Table (1): Ingredients and calculated analysis of the basal diet.

Ingredients	**%**
Barley grain	25.0
Clover hay	25.0
Soybean meal (44%)	20.0
Wheat bran	13.1
Yellow corn	9.0
Cane-molasses	5.0
Di-Ca-P	1.5
Limestone	0.6
Premix*	0.3
NaCl (salt)	0.3
DL-Methionine	0.2
Total	**100**
Calculated analysis,	
Crude protein, %	17.09
Crude fiber, %	12.12
Digestible energy (kcal/kg)	2601
Calcium, %	1.05
Available phosphorus, %	0.49
Lysine, %	0.87
Methionine, %	0.51

** Each 3 Kg contains: vit A 12000000 IU, vit D_3 1200000 IU, vit C 50mg, vit E 75g, vit K_3 2g, vit B_1 1.5g, vit B_2 5.5g, vit B_6 2.5g, vit B_{12} 12mg, Niacin 35g, Folic 1.5g, Biotin 0.2g, Pantothenic acid 13g, Copper 30g, Iodine 1.1g, Selenium 0.25g, Iron 70g, Manganese 30g, Zinc 80g, Cobalt 0.25g.*

Temperature-humidity index:

Throughout the experimental period, ambient temperature and relative humidity were daily recorded using two hydro-thermograph devices. The daily values of indoor ambient temperature and relative humidity were recorded and estimated as the average of maximum and minimum values of each parameter. Temperature-humidity index (THI) was calculated according to the following equation as stated by Marai *et al.*, 2001:

$$THI = db\ °C - [(0.31 - 0.31RH)(db\ °C - 14.4)]$$

Where db °C= dry bulb temperature in degrees Celsius and RH= relative humidity/100. The values obtained are then classified as follows: <27.8= absence of heat stress, 27.8–28.9= moderate heat stress, 28.9–30.0= severe heat stress and 30.0 and more= very severe heat stress.

In the present study, overall averages of temperature and relative humidity were 31.1°C and 62.5%, respectively. The weekly calculated values of THI were ranged from 28.09 as minimum to 30.69 as maximum (Table 2). Results of the calculated THI values indicate that the experimental rabbits were under heat stress conditions, being very severe during the first two weeks followed by severe heat stress through the third to the fifth week, then moderate heat stress till the end of the experimental period.

Table (2): Average of temperatures (°C), relative humidity (%) and temperature-humidity index (THI) through the experimental period.

Period	Temperature, °C	Relative humidity, %	THI
1st week	33.4	54	30.69
2nd week	32.9	59	30.55
3rd week	30.8	67	29.12
4th week	30.7	67	29.03
5th week	30.7	68	29.08
6th week	30.5	59	28.45
7th week	30.2	58	28.14
8th week	29.6	68	28.09
Overall average	**31.1**	**62.5**	**29.14**

Semen collection and evaluation:

During the first two weeks of the study period, rabbit bucks were trained for semen collection by artificial vagina prior to the main collection phase. Semen was collected once per week for six consecutive weeks from all the rabbit bucks (n = 6) in each group from the third to the eighth week. This led to 36 ejaculates being collected and assessed per group, after which the weekly data for each semen parameter for each rabbit over the course of the collection period were averaged. After removing the gel mass from each ejaculate during collection, the volume of semen was measured in a graduated collecting tube, and the pH of the semen was ascertained right away. A phase-contrast microscope (Leica DM 500, Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) equipped with a hot stage at 37°C was used to measure the percentage of sperm progressive motility in five microscopic fields per semen sample after diluting the semen with saline (0.9 percent Nacl) at a rate of 1 semen: 10 saline (El-Ratel *et al.*, 2021). To determine the percentages of vitality, abnormality, and normality in 200 spermatozoa in 5 microscopic fields using phase contrast microscopy at 400 magnification, aliquots of raw semen (5 µL) were fixed using a vital stain of eosin (5%) and nigrosine (10%). Both morphological abnormalities (defects in the head or tail) and vital sperm cells (sperm that had not been stained) were examined. The improved Neubauer hemocytometer slide (GmbH +Co., Brandstwiete 4, 2000 Hamburg 11, Germany) was used to measure the concentration of sperm cells (106/mL) just after semen dilution (1 semen: 99 saline) (El-Ratel *et al.*, 2021).

Seminal plasma collection and analysis:

During the last week of the semen collection period, seminal plasma was separated by centrifugation at 1500 rpm for 20 min at 4°C and stored at –20°C, pending analysis. Total antioxidant capacity (TAC), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) were assayed. The concentration of oxidative capacity and enzymes activity were determined using commercial kits (Bio-diagnostic Co., www.bio-diagnostic.com).

Blood sampling:

At the end of the experiment, blood samples were collected from six bucks in each group from the ear vein into non-heparinized tubes and were placed immediately in the ice box till complete coagulation. Serum was obtained by blood centrifugation at 3000 rpm for 20 min at 4°C. Obtained serum stored at –20°C until assay. Serum total proteins and creatinine were determined according to the method described by Henry (1974). Determination of serum albumin was carried out according to Doumas *et al.* (1997). Globulin concentration was calculated as the difference between total proteins and albumin. Total cholesterol and Tri-glycerides were measured according to the method proposed by Allain *et al.* (1974). Alanine aminotransferase (ALT) was measured using the method of Reitman and Frankel (1957). Total antioxidant capacity (TAC), catalase, superoxide dismutase (SOD) and malondialdehyde (MDA) were

assayed. Concentration of oxidative capacity and enzymes activity were determined using commercially kits (Bio-diagnostic Co., www.bio-diagnostic.com).

Statistical analyses:

Data were subjected to one-way analysis of variance. The General Linear Model of SAS (1994) for PC was applied, and significant differences among treatment means were separated by using Duncan's multiple range test (Duncan, 1955) at 5% level of probability.

RESULTS AND DISCUSSION

Phenolic acid screening:

The percentage of extracts yield were 16.5%, 50%, and 29% for corn silk, pomegranate peel, and orange peel, respectively. From the results of phenolic acids screening in Table 3, the most abundant compound of polyphenols in the pomegranate peel waste was gallic acid (gallotannins), while ferulic acid was found to be the most abundant polyphenolic compound in the extracts of orange peel, followed by cinnamic acid and coumaric acid. Whereas, no noticeable superiority was observed for any of the polyphenols in corn silk extract.

Table (3): Phenolic acids screening of different dietary supplement extracts using HPLC.

Phenolic compound	Retention time (min.)	Corn silk (mg/kg)	Orange peel (mg/kg)	Pomegranate peel (mg/kg) *
Gallic acid	4.01	58.83	40.34	508.24
Protocatechuic acid	5.64	15.62	44.54	62.48
p-Hydroxybenzoic acid	8.79	6.52	23.2	53.18
Syringic acid	9.99	36.44	51.7	36.28
Caffeic acid	10.97	37.08	61.92	82.63
Cinnamic acid	18.43	140.82	806.56	71.60
Coumaric acid	20.33	57.08	478.42	50.88
Ferulic acid	24.8	104.14	1815.44	19.98

* *mg phenolic compound per each kg of waste dry matter.*

Concerning the results of the HPLC, the present findings are in agreement with many previous studies. Many secondary metabolites are responsible for pomegranates wide-ranging effects. As for polyphenolic chemicals, gallic acid and punicalagin stand out as being the major polyphenolic compounds in pomegranate (Qu *et al.* 2012). Hydrolysable tannins, which are phenolic compounds having a central core of glucose or another polyol esterified with gallic acid (gallotannins), are found in abundance in pomegranates (Singh *et al.*, 2014). High-performance liquid chromatography (HPLC) was used to characterize and quantify various polyphenolic profiles in the orange peel extract, as ferulic acid is the most prevalent component, followed by coumaric acid and gallic acid (Ozturk *et al.*, 2018). da Hora *et al.* (2021) found that extracts of corn silk contain flavone glycosides, which help explain their antioxidant properties.

Semen quality:

Semen quality of heat-stressed male rabbits fed supplemental corn silk (CS), orange peel (OP), and pomegranate peel (PP) extracts is shown in Table 4. The ejaculate volume of OP group was increased ($P<0.01$) by 46% when compared with the control group. Also, CS and vit. E groups showed some improvements concerning ejaculate volume, while PP group decreased by 14.6%, as compared to the control group. Moreover, progressive motility, normal sperm morphology, live sperms, sperm cell concentration, packed sperm volume, total sperm output, total motile sperm, and total functional sperm fraction has been improved in groups fed vit. E, CS, and OP. All semen quality data showed many disorders concerning rabbits of PP group, especially for total functional sperm fraction. However, semen pH did not significantly change due to different supplemental antioxidants.

Table (4): Effect of corn silk, orange peel, and pomegranate peel extracts on semen quality of male rabbits under heat stress conditions.

Traits	Experimental groups					P- value
	Control	Vit. E	Corn silk	Orange peel	Pomegranate peel	
Ejaculate volume, ml	$0.48^{c}\pm0.01$	$0.65^{b}\pm0.02$	$0.66^{ab}\pm0.02$	$0.70^{a}\pm0.01$	$0.41^{d}\pm0.01$	**
pH	7.47±0.17	7.80±0.01	7.72±0.09	7.69±0.07	7.55±0.10	NS
Progressive motility, %	$71.90^{b}\pm0.12$	$81.30^{a}\pm0.35$	$82.30^{a}\pm0.23$	$79.40^{a}\pm0.12$	$68.15^{c}\pm0.61$	**
Normal sperm morphology, %	$80.60^{c}\pm0.35$	$90.85^{b}\pm0.78$	$90.40^{b}\pm0.40$	$93.50^{a}\pm0.17$	$78.65^{c}\pm0.89$	**
Abnormal sperm morphology, %	$19.40^{a}\pm0.35$	$9.15^{b}\pm0.78$	$9.60^{b}\pm0.40$	$6.50^{c}\pm0.17$	$21.35^{a}\pm0.89$	**
Live sperms, %	$72.65^{b}\pm0.81$	$86.63^{a}\pm0.88$	$88.40^{a}\pm0.12$	$88.20^{a}\pm0.29$	$74.70^{b}\pm0.81$	**
Dead sperms, %	$27.35^{a}\pm0.81$	$13.37^{b}\pm0.88$	$11.60^{b}\pm0.12$	$11.80^{b}\pm0.29$	$25.30^{a}\pm0.81$	**
Sperm cell concentration, 10^6/ml	$336^{b}\pm2.17$	$394^{a}\pm1.10$	$388^{a}\pm5.74$	$392^{a}\pm2.68$	$311^{c}\pm0.97$	**
Packed sperm volume, %	$40.45^{b}\pm0.03$	$45.80^{a}\pm1.10$	$47.25^{a}\pm0.09$	$45.85^{a}\pm0.61$	$41.70^{b}\pm0.92$	**
Total sperm output, 10^6/ejaculate	$161.24^{b}\pm0.90$	$256.44^{a}\pm9.82$	$254.32^{a}\pm11.60$	$274.60^{a}\pm0.39$	$126.15^{c}\pm1.29$	**
Total motile sperm, 10^6/ejaculate	$115.94^{b}\pm0.83$	$208.58^{a}\pm8.87$	$209.23^{a}\pm8.96$	$218.03^{a}\pm0.01$	$86.00^{c}\pm1.65$	**
Total functional sperm fraction, 10^6/ejaculate	$93.44^{c}\pm0.27$	$189.29^{b}\pm6.43$	$189.03^{b}\pm7.25$	$203.86^{a}\pm0.37$	$67.59^{d}\pm0.53$	**

*a, b, c and d: Means in the same row with different superscripts are significantly different; **= P≤0.01; NS= non-significant.*

The present findings are in agreement with many studies (Sharaf *et al.*, 2019; El-Kholy *et al.*, 2020; El-Ratel *et al.*, 2021). They reported an alteration in the sperm motility of rabbits' semen associated with high ambient temperature. Exposing NZW rabbits to elevated ambient temperature (36±3 °C for 12h.), negatively affected their internal homeostasis (Ondruska *et al.*, 2011). As a direct impact of heat stress, male rabbits suffer from many issues such as interruptions of libido, semen quality, and spermatogenesis (Marai *et al.*, 2003). The oxidative stress has deleterious impacts on sperm quality and function (Paul *et al.*, 2009). Many studies tried to use natural antioxidant sources as feed supplements to mitigate heat stress effects on different animal species (Zeweil *et al.*, 2013; Akbarian *et al.* 2015; Hassan *et al.*, 2016).

The results of the current study showed that semen quality parameters have been improved using orange peel and corn silk extracts as feed supplements to male rabbits under heat stress conditions. These positive impacts may be due to the presence of cinnamic acid, coumaric acid, and ferulic acid with high concentrations in orange peel extract as stated in Table 3. Coumaric acid's hydroxyl group on a benzene ring can react with ROS by donating hydrogen and creating a radical form of coumaric acid with an unpaired valence electron on the oxygen atom, breaking free radical chain reactions (Ulrih *et al.*, 2021). Another important role played by cinnamic and coumaric acids, is their ability to chelate transition metals like copper or iron, which act as catalysts in the generation of free radicals that cause lipid peroxidation, and DNA damage (Gaspar *et al.*, 2009). Moreover, corn silk has a wide range of flavonoids that have strong antioxidant potentiality. In H_2O_2-induced cells, maysin (the most abundant corn silk flavonoid) greatly reduced intracellular ROS generation and up-regulated intracellular antioxidant enzyme expression (Rajeshwari and Sivapriya, 2021). Also, vit. E protects the cellular plasma membrane from oxidative damage, allowing cells to proliferate and act its function properly (Doostabadi *et al.*, 2021). The previously mentioned roles may explain the positive impacts of orange peel and corn silk extracts, as well as vitamin E, on mitigating the heat stress on semen quality of rabbits reared under heat stress conditions. However, using pomegranate peel extract achieved semen quality disorders as shown in Table 4. Gallotannins, an abundant component of pomegranate peel extract, are hydrolysable tannins that act as anti-nutritional factors in monogastric nutrition, shown to reduce feed intake and palatability (Bee *et al.*, 2017).

Seminal antioxidants:

Responses of rabbits' seminal antioxidants to different supplemental natural antioxidants are presented in Table 5. The seminal total antioxidants capacity of OP and PP groups were increased by 77 and 43%, respectively as compared with the control group. However, OP, CS and vit. E groups showed high improvements concerning catalase, SOD and GSH-Px activities among the experimental groups. On the other hand, rabbits of PP group showed great reductions in GSH-Px and catalase activities among the experimental groups. The present findings are in agreement with recent studies (Attia *et al.*, 2017; El-Ratel *et al.*, 2021). They reported improvements in the seminal TAC, SOD and GSH-Px of rabbits fed natural antioxidants supplements under heat stress conditions. In the present study, the superiority of rabbits, concerning TAC, that fed orange peel and pomegranate peel extracts may be due to its large contents of potent polyphenols as stated in Table 3. Concerning enzymes activity of PP group, our data are in disagreement with Al-Olayan *et al.*, 2014; Emam *et al.*, 2020. They found that pomegranate peel extract protects rats against oxidative stress via improving mRNA expression levels of SOD, catalase and GSH-Px. However, rabbits of vit. E, CS and OP groups showed clear improvements against heat stress condition regarding antioxidants status. Also, the low activity of testicular GSH-Px during oxidative stress might be due to the decreased availability of glutathione substance as well as the rising lipid peroxidation.

Table (5): Effect of corn silk, orange peel, and pomegranate peel extracts on simenal antioxidants of male rabbits under heat stress conditions.

Parameters	Experimental groups					P- value
	Control	Vit. E	Corn silk	Orange peel	Pomegranate peel	
TAC, (mM/L)	2.35^{c}±0.39	2.44^{c}±0.82	2.48^{c}±0.06	4.17^{a}±0.04	3.36^{b}±0.17	*
Catalase, (U/L)	328.2^{b}±6.41	343.6^{a}±18.4	347.2^{a}±10.2	353.6^{a}±31.2	208.3^{c}±9.5	*
SOD, (U/L)	275.8^{c}±6.06	332.8^{ab}±4.19	319.1^{b}±2.47	345.3^{a}±3.91	323.7^{b}±4.47	*
GSH-Px, (U/L)	52.3^{c}±2.61	78.6^{b}±0.86	75.9^{b}±1.39	87.5^{a}±0.92	18.16^{d}±0.06	*

*a, b, c and d: Means in the same row having different superscripts are significantly different, * P≤ 0.05*

After oxidative stress, alterations in the spermatogenic cycle, seminiferous tubules and germ cells degenerated, and the interstitium partially disappeared in some parts of the rats' testes (Horn *et al.*, 2006). However, orange peel and corn silk have a high percentage of polyphenols and flavonoids (as stated in Table 3), which may help to mitigate the effects of oxidative stress. These CS and OP extracts components are known to have a variety of anti-organ damaged properties (Hanafy *et al.*, 2021; Rajeshwari and Sivapriya, 2021; Singh *et al.*, 2022). As stated by Ebeid (2012), the beneficial effects of vitamin E supplementation (as a positive control group in the present study) may be attributable to its role in decreasing lipid peroxidation and increasing the antioxidant activity in the seminal plasma of the domestic fowl under heat stress conditions.

Blood biochemical constituents:

Table 6 shows the effects of corn silk, orange peel, and pomegranate peel extracts on some blood constituents in male rabbits. Supplemental corn silk and pomegranate peel extracts decreased serum total cholesterols of male rabbits as compared with the other experimental groups. An opposite trend was recorded concerning alanine aminotransferase. However, total proteins, albumin, globulins, triglycerides and creatinine did not significantly change among experimental groups.

Table (6): Effect of corn silk, orange peel, and pomegranate peel extracts on some blood constituents of male rabbits under heat stress conditions.

Parameters	Experimental groups					P- value
	Control	Vit. E	Corn silk	Orange peel	Pomegranate peel	
Total proteins, (g/dl)	5.81±0.11	6.01±0.30	6.52±0.15	6.38±0.14	6.13±0.10	NS
Albumin, (g/dl)	2.39±0.02	2.63±0.14	2.49±0.10	2.63±0.16	2.53±0.23	NS
Globulins, (g/dl)	3.41±0.09	3.38±0.18	4.03±0.23	3.75±0.14	3.60±0.15	NS
Tri-glycerides, (mg/dl)	51.79±0.79	44.03±3.12	40.92±1.21	53.97±4.26	57.77±6.50	NS
Cholesterols, (mg/dl)	31.27^{a}±1.5	32.54^{a}±0.16	24.13^{b}±1.70	34.05^{a}±0.59	26.51^{b}±0.73	*
ALT, (U/L)	7.68^{b}±0.09	7.63^{b}±0.59	9.81^{a}±0.99	6.99^{b}±0.46	11.35^{a}±0.63	*
Creatinine, (mg/dl)	1.43±0.15	1.35±0.12	1.21±0.05	1.37±0.05	1.29±0.05	NS

*a and b: Means in the same row having different superscripts are significantly different, * P≤ 0.05, NS= non-significant.*

The present results are in agreement with other studies (Sadeghipour *et al.*, 2014; Arafat *et al.*, 2021). They found that giving rats a hydroethanolic extract of pomegranate peel had significant antihyperlipidemic effects when they were fed a high-fat diet. Also, the extract attenuated liver damage including fatty change in hepatocytes, and consequently lowered ALT secretion as recorded in the present study. Gallic acid, as the most abundant polyphenol in pomegranate peel, can reduce the hyperlipidemia and fatty liver that a high-fat diet induces in mice (Jang *et al.*, 2008). Moreover, Lee *et al.*, 2017 stated the hypolipidemic effect of corn silk extract. They suggest that maysin (a corn-specific flavonoid) has a strong potential as an anti-adipogenic agent based on its ability to reduce lipid accumulation in the cell and adipocyte differentiation.

Regarding the positive control group, vitamin E has no effects on blood biochemical parameters in the current study, which is in contrast to Hashem *et al.* (2013) who confirmed that vitamin E can reduce the negative effects of heat stress by promoting greater animal adaptation to hot climates and stimulating crucial physiological processes like the incorporation of cholesterol in steroidogenesis, primarily the synthesis of testosterone.

Serum antioxidants:

Effects of corn silk, orange peel, and pomegranate peel extracts on blood antioxidants profile of male rabbits reared under heat stress conditions are presented in Table 7. Rabbits fed supplemental orange peel extract showed the most superior total antioxidants capacity followed by rabbits of CS and PP groups, since recorded increases by 176, 101 and 78%, respectively, as compared with the control group. An opposite trend was achieved for malondialdehyde concentrations, in which rabbits of the different experimental groups recorded high reductions in

comparison with the control group, especially for OP rabbits group that reduced by 49%. However, catalase and superoxide dismutase activities were raised in vit. E, CS, OP, and PP groups as compared with the control group, since the best improvements were achieved for OP rabbits' group.

Table (7): Effect of corn silk, orange peel, and pomegranate peel extracts on serum antioxidants of male rabbits under heat stress conditions.

Parameters	Experimental groups					P- value
	Control	Vit. E	Corn silk	Orange peel	Pomegranate peel	
TAC, (mM/L)	0.91^{d}±0.02	1.26^{c}±0.09	1.83^{b}±0.06	2.51^{a}±0.07	1.62^{b}±0.04	*
Catalase, (U/L)	298.3^{c}±9.9	351.8^{a}±14.3	338.5^{b}±15.6	360.3^{a}±11.3	354.6^{a}±14.3	*
SOD, (U/L)	219.9^{c}±10.3	257.9^{b}±12.4	249.4^{b}±11.9	280.7^{a}±13.5	276.9^{a}±4.75	*
MDA, (nmol/ml)	1.42^{a}±0.07	1.27^{b}±0.06	1.33^{ab}±0.04	0.72^{c}±0.02	1.19^{b}±0.06	*

*a, b, c and d: Means in the same row having different superscripts are significantly different, * P≤ 0.05, NS= non-significant.*

Antioxidant enzymes (such as SOD, catalase, and GST) have been reported to form a mutually supporting defence system against reactive oxygen species (ROS) (Gusti *et al.,* 2021). In the present study, we demonstrated that stressed rabbits fed diets supplemented with natural antioxidants sources showed high levels of blood antioxidants as compared with stressed rabbits fed a diet without any supplement. Antioxidant enzyme suppression following heat stress is most likely due to protein inactivation by reactive oxygen species (ROS). The losing function of a specific protein is often occurred after oxidative damage (Pizzino *et al*., 2017). The present results are in agreement with other studies (Zeweil *et al.*, 2013; Faiz *et al.*, 2017; Emam *et al.*, 2020; Hassan *et al.*, 2021).

In the early stages of heat stress, the body's antioxidant enzymes temporarily increase their activity by accelerating the removal of oxygen free radicals and decreasing the production of lipid peroxidation products; however, long-term and/or high-intensity heat stress increases ROS and induces oxidative stress (Liang *et al.*, 2022). So, the concentration of serum MDA increased as recorded for the control group of the present study. However, exogenous antioxidant supplements play an important role in lowering lipid peroxidation besides rising the antioxidants capacity as recorded in rabbits of CS, OP, and PP groups. As shown in Table 3, the potent phenols of orange peel may be the main reason for the superiority of rabbits in OP group concerning the high capacity of antioxidants besides the low content of lipid peroxides.

As observed in the current study, vitamin E has a potent role in increasing antioxidants activity as well as lowering MDA concentration in rabbit's blood. The current findings are in agreement with Packer *et al.*, 2001 who showed that vitamin E can promote the synthesis of immunoglobulin to improve the body's disease resistance and decrease mortality, as well as alleviate the immunosuppression brought on by the release of adrenal cortex hormone at high ambient temperatures.

CONCLUSION

The results clearly demonstrate that orange peel and corn silk extracts enhance the male rabbits' ability to mitigate the negative impacts of heat stress via improving semen quality properties and antioxidants capacity. So, using orange peel, or corn silk extracts as feed supplements at a level of 150ppm could be useful for male rabbits under heat stress conditions.

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التخفيف من تأثير الإجهاد الحراري على جودة السائل المنوي وحالة مضادات الأكسدة فى ذكور الأرانب باستخدام بعض مضادات الأكسدة الطبيعية

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أجريت الدراسة الحالية لمعرفة تأثير إضافة مستخلصات حريرة الذرة ، قشر البرتقال ، قشر الرمان على جودة السائل المنوي، المكونات الكيميائية الحيوية للدم، وقدرة مضادات الأكسدة لدى ذكور الأرانب النيوزيلندية البيضاء تحت ظروف الإجهاد الحراري. في تجربة استمرت 8 أسابيع، تم توزيع ثلاثين أرنباً ذكرًا أعمارهم أربعة وعشرين أسبوعًا بمتوسط وزن جسم 2.85 ± 0.1 كجم بشكل عشوائي إلى خمس مجموعات متساوية (6 حيوانات / مجموعة). تم تربية كل أرنب في قفص منفرد. المجموعة الأولى (مجموعة المقارنة) كانت تتغذى على عليقة أساسية. تم تغذية المجموعة الثانية على العليقة الاساسية مضافا إليها فيتامين هـ (كمسحوق بتركيز 50٪، مجموعة مقارنة موجبة) بمستوى 250 جزء في المليون. تم تغذية المجموعات الثالثة والرابعة والخامسة على العليقة الاساسية مضافا إليها مستخلصات حريرة الذرة وقشر البرتقال وقشر الرمان بمعدل 150 جزء في المليون على الترتيب. تم اتاحة الغذاء ومياه الشرب بشكل حر طوال مدة التجربة. بلغ المتوسط العام لدرجات الحرارة المحيطة والرطوبة النسبية 31.1 درجة مئوية و 62.5٪ على التوالي. كان مؤشر درجة الحرارة والرطوبة الكلي (THI) خلال فترة التجربة 29.14 مما يشير إلى ظروف إجهاد حراري شديدة. كان حمض الفيروليك هو البوليفينول الأكثر وفرة في مستخلص قشر البرتقال ، بينما كان حمض الجاليك هو البوليفينول الأكثر وفرة في مستخلص قشر الرمان. تحسنت معايير جودة السائل المنوي في الأرانب التي تغذت على مستخلصات قشر البرتقال وحريرة الذرة بعكس الأرانب التي تم تغذيتها على مستخلص قشر الرمان. زاد نشاط انزيم الكاتاليز والجلوتاثيون بيروكسيديز في السائل المنوى للأرانب التي تم تغذيتها على مستخلصات قشر البرتقال وحريرة الذرة. لم تتأثر بروتينات الدم الكلية، الألبيومين، الجلوبيولين، والكرياتينين بإضافات مضادات الأكسدة المختلفة. ارتفعت السعة الكلية لمضادات الأكسدة، وانزيمات الكاتاليز و SOD في الدم بشكل معنوي في جميع المجموعات التجريبية مقارنة بمجموعة المقارنة. أظهرت النتائج الحالية أن استخدام مستخلصات قشر البرتقال أو حريرة الذرة كإضافات علفية بمستوى 150 جزء في المليون يمكن أن تحسن جودة السائل المنوي وكذلك سعة مضادات الأكسدة لدى ذكور الأرانب التي تربى تحت ظروف الإجهاد الحراري.