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RECENT TECHNIQUES FOR AMELIORATION THE EFFECT OF HEAT STRESS CONDITIONS ON MALE RABBITS

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ABSTRACT: The present work was conducted to study the effect of selenium (Se) plus vitamin E (Vitam E) and AD_3E vitamins (Vitam AD_3E) mixture as two alleviation techniques on heat stressed rabbits during hot summer season of Egypt. In total 45 New Zealand white (NZW) male rabbits were used in this research. The animals were divided into three equal groups. The 1st group was kept without any treatment and served as control group. The 2nd and 3rd groups were supplemented daily 1m/l in drinking water with Se plus E and AD_3E vitamins mixture, respectively. The experiment lasted 7 weeks during hot summer season (from third week of July to the end of August, 2017). Results showed that adding both of Se plus vitamin E and AD_3E vitamins mixture in drinking water decreased ($P\leq0.001$) rectal, skin, ear temperature and respiration rate compared with the control group. Adding both of Se plus vitamin E and AD_3E vitamins mixture in drinking water improved ($P\leq0.05$) final live body weight and feed intake and decreased ($P\leq0.001$) daily water intake in comparative with control group. Adding both of Se plus vitamin E and AD_3E vitamins mixture in drinking water increased ($P\leq0.01$) serum total protein, albumin and globulin concentrations and decreased ($P\leq0.05$) urea-N and creatinine concentrations in male rabbits. Serum cortisol level was ($P\leq0.001$) lower while Triiodothyronine (T_3), Thyroxin (T_4) hormonal levels were ($P\leq0.001$) higher in both two treatment groups compared with control. Normal sperm, volume, count, motility and live sperm increased ($P\leq0.001$) while dead sperm was decreased ($P\leq0.001$) in the two treatment groups compared with control group. It can be concluded that the two techniques can alleviate the negative effects of heat stress on rabbits under hot summer season in Egypt.

Key words: Heat stress, selenium, vitamin E, AD_3E vitamins, rabbits.

INTRODUCTION

Heat stress mainly occurs when animals are exposed to high ambient temperatures, high humidity, low wind speed, and direct and indirect high solar radiation (**Willmer *et al.*, 2000**). Heat stress means that animals are not able to regulate their heat homeostasis passively **(Marai *et al.*, 1996).** In tropical and sub-tropical countries, climatic characteristic is the major constraint on animal productivity. Production and reproduction are impaired as a result of the drastic changes in biological functions caused by heat stress. Alleviations of heat stress syndrome during hot summer season can be reduce or even eliminate those losses in farm animals. The process of minimizing or reducing these effects is called amelioration process **(Habeeb *et al.*, 2018a).** High temperatures during summer of Egypt represent a major constraint factor for rabbit production (**Askar and Ismail, 2012**). Exposure of rabbits to < 30 THI (temperature-humidity index) units or more as severe heat stress during summer of Egypt, adversely affects their hormones and trace elements and these drastic changes that occur in

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biological functions may be responsible on that depression in vitamins and trace elements in blood of heat stressed rabbits **(Habeeb *et al.*, 2018b).** Amelioration of heat stress in heat stressed animals in order to improve its productivity; amelioration of heat stress has been attempted using different techniques including nutritional and physiological means. Vitamin A is required for normal reproductive process in males; deficiency of vitamin A causes decrease of sexual activity and spermatogenetic disorders (**Fennema, 2008)**. Vitamin D_3 stimulates the absorption of calcium and phosphorus in the intestine performing the function of carrier of these minerals; likewise, it regulates and corrects its metabolism in the blood. In addition, vitamin D_3 acts on the bone tissue, both on the osteodasts increasing the production of alkaline phosphatase and on osteoblasts stimulating the cell difference and multinucleate **(Institute of Medicine, 2010)**. Vitamin E is a fat soluble antioxidant prevents oxidation of unsaturated fatty acids and vitamin A in the intestine and acts as an important role in growth, fertility and increase body resistance (**National Institutes of Health, 2016**). Vitamin E removes the free radicals which are unstable compounds that damage the cell structure by combining with free oxygen and destroys free radicals (**Mayo Clinic, 2013**). In addition, immunity levels improve when vitamin E is consumed (**Institute of Medicine, 2013**). Vitamins A, D_3 and E as fat soluble vitamins are potent antioxidants and animals cannot produce these vitamins in their bodies. Therefore an exogenous regular supply is needed to cover the physiological requirements and to sustain high production performance (**Hafez, 2012**). In addition, supplementation of heat stressed animals with these vitamins resources is required to correct the protein and energy negative balances during hot weather season in Egypt (**Habeeb *et al.*, 2015, 2016**).

Selenium (Se) is a micronutrient that is essential in several biological functions in the organism, particularly in protection of cell membranes. Se is important physiologically because it is an integral component of the enzyme glutathione peroxidase (GSHpx) **Miller *et al.* (1993)**. Se is known to be incorporated in the enzyme glutathione peroxidase performing the antioxidative defence of the body by eliminating hydrogen peroxides. Several selenoproteins have later been identified, with functions connected *e.g.* to the thyroid hormone metabolism, testes and sperm function and muscle metabolism (**Brown and Arthur, 2001**). The positive role of Se in the immune system is well documented, where it stimulates both humoral and cell-mediated immunity (**McKenzie *et al.*, 1998).** Vitamin E and Se are involved with cellular antioxidant status and increased intake of vitamin E can reduce responses to Se supplementation **(Hogan *et al.*, 1990).** Selenium is an essential compound of glutathione peroxide enzyme which involved in the detoxification of hydrogen peroxide and lipid peroxidation. Moreover, Se is a composition of selenoproteins and is involved in immune and Neuro-psychological functions in the nutrition of animals (**Meschy, 2000**). The existence of a synergistic action between selenium and vitamin E reported, while, both improved synergistic antioxidant effect (**Kalaba, 2012**).

The main objective of this study was to confirm the ability of the two techniques for amelioration the heat stress conditions of hot summer season of Egypt on NZW male rabbits.

MATERIALS AND METHODS

Experimental Location and Ethics

The experimental work was carried out in the Rabbitry of the Experimental Farms Project, Radioisotopes Application Division, Nuclear Research Centre, Atomic Energy Authority with cooperation with Animal Production Department, Faculty of Agriculture, Zagazig University, during the hot summer season of Egypt.

The experimental work was carried out in Rabbits Farm, Biological Application Department, Radioisotopes Applications Division, Nuclear Research Centre, Atomic Energy Authority, Inshas, Egypt (latitude 31º 12' N to 22 º 2' N, longitude 25 º 53' E to 35º 53' E) .

This study was reviewed and approved by the Animal Care and Welfare Committee of Zagazig University, Egypt (ANWD. 206). These ethics contain relevant information on the

endeavor to reduce animal suffering and adherence to best practices in veterinary care according to the International Council for Laboratory Animal Science guidelines.

Experimental Design

Forty five mature male healthy New Zealand white rabbits were used in this experiment. The experiment lasted 7 weeks from hot summer season (from 2nd week of July to end of August 2017). The rabbits were randomly divided into three groups, 15 animals in each. In first group, rabbits did not receive any treatment in drinking water and served as control group. In second and the third groups, rabbits received drinking water containing each of vitamin E plus Se and AD_3E vitamins mixture, respectively, at the rate of 1ml from each treatment per litter drinking water. Each litre of vitamin E plus Se contains 50,000 mg vitamin E, 100 mg selinate sodium, 50,000 mg probilin glycol in distilled water (Oral solution for veterinary uses, produced by Pure Toko Veterinary, Nour Elhuda, Egypt). Each litre of AD_3E vitamins mixture contains 10,000,000 unit vitamin A, 2000,000 unit vitamin D_3 and 2000 mg vitamin E in distilled water (Oral solution for veterinary uses, produced by international Ibex Co.).

Management and Feeding

The rabbits in three groups were vaccinated with clostridia enterotoxaemia bloat at weaning (Veterinary Research and Vaccines, Research Institute, Cairo, Egypt. The Rabbitry building was naturally ventilated through wired windows. The animals were individually housed in galvanized wired battery cages (50×55×39cm), and each cage was provided with a feeder, automatic nipple drinker and a crock. The crock was used to measure the water consumption after separating the automatic nipple drinker. Urine and faeces dropped from cages were cleaned daily. Animals in the three groups were fed the same diet during experimental period. The ingredients and chemical analysis of the commercial pelleted diet were as shown in Table 1.

Estimation of Metrological Data

Air temperature (^oC) and relative humidity (%) were measured inside the rabbitry building using automatic thermo-hygrometer (Table 2). Each value from air temperature and relative humidity was the average of three measurements recorded at 12.00, 13.00 and 14.00 hours once a day weekly.

The combined effect of the ambient air temperature and relative humidity as temperature humidity index (THI) was calculated using the equation of **Marai *et al.* (2001)** as follows: THI = db ^oC – [(0.31-0.31 RH%) (db^oC -14.4)], where db ^oC = dry bulb temperature in Celsius and RH= relative humidity percentage/100 and the obtained THI values were classified as follow: <27.8= absence of heat stress, 27.8 to < 28.9= moderate heat stress, 28.9 to <30.0 = severe heat stress and 30.0 or more = very severe heat stress. Data in Table 2 showed that rabbits exposed to very severe heat stress during experimental period due to that average THI during experimental period was more than 30.0.

Estimation of Body Weight and Feed and Water Intakes

Feed and water intakes were estimated individually daily for all animals, during the experimental period. Water intake was estimated by measuring the difference in the water volume in the crocks. The difference in water volume within the crock at 10.00 hr., was calculated. Water consumption was calculated by dividing the obtained total water by the number of animals. The evaporative water was considered in estimation of water intake. The animals were weighted every two weeks during experimental period.

Estimation of Thermoregulatory Parameters

Rectal temperature was recorded by inserting a digital clinical thermometer into the rectum for one minute. The skin temperature was measured at one location between the neck and loin on the body surface while the thermometer was fixed on the bare skin. The ear temperature was measured by placing the thermometer into direct contact with the central area of the auricle. Respiration rate was measured when the rabbit was at rest by counting the number of breaths for one minute by counting how many times the chest has risen. Thermoregulatory parameters were estimated once each two weeks for all animals during the experimental period.

Table 1. Ingredients and chemical composition of the commercial pelleted diet used in feeding experimental rabbits during the experimental period

Item	(%)
Ingredients of pelleted diet	
Clover hay	41.50
Wheat bran	25.0
Yellow corn	15.0
Soybean meal (44% CP)	10.0
Molasses	5.0
Bone meal	1.75
Calcium carbonate	0.70
Sodium chloride	0.55
Vitamins and minerals premix*	0.35
DL-Methionine	0.15
Chemical composition as DM (%)	
Crude protein	18.10
Crude fiber	12.35
Ether extract	3.17
Nitrogen free extract	57.20
Ash	9.18
Digestible energy (kcal/ kg DM)	2600

* Each kilogram of vitamins and minerals premix contained: 10000 IU Vitam A, 3900 IU Vitam D, 2 mg Vitam K, 50 mg Vitam E, 12 mg Vitam B_{12}, 6 mg Vitam B_2, 2 mg Vitam B_6, 20.01 mg Vitam B_1, 20 mg Panathonic acid, 50 mg Niacin, 5 mg Folic acid, 1.2 mg Biotin, 12000 mg Choline, 3 mg Copper, 0.2 mg Iodine, 75 mg Iron, 30 mg Manganese, 70 mg Zinc, 0.1 mg Selenium, 0.1 mg Cobalt and 0.04 mg Magnesium.

Table 2. Air temperature, relative humidity, and temperature-humidity index during experimental period (2017)

Experimental month	Temperature (^{o}C)	Relative humidity (%)	Temperature humidity index (THI)
July 2017			
2nd week	35.5	83.0	31.2
3rd week	32.4	82.0	31.2
4th week	34.0	80.5	32.6
Mean ±SE	33.9±0.64	81.8±0.75	31.7±0.70
August 2017			
I st week	35.0	70.0	33.1
2nd week	34.1	83.0	33.1
3rd week	34.7	83.0	28.9
4th week	36.5	86.0	30.7
Mean ±SE	35.07±0.51	80.50±3.75	31.45±1.02
Overall mean	34.5	81.2	31.6 very severe heat stress

Blood Samples and Estimation of Blood Biochemical Components and Hormonal Levels

Blood sample from all experimental groups were collected from ear vein into vacutainer tubes at the end of experimental period without any handling stressor. Serums were separated by centrifugation at 3,000 rpm for 15 min and were frozen and stored at -20°C until analysis. The concentrations of total protein, albumin, creatinine and urea-N were measured by quantitative enzymatic colorimetric methods using chemical commercial kits (Diamond Diagnostic Company, Egypt). The concentration of globulin calculated as the difference between total protein and albumin. The level of cortisol, T_3, triiodothyronine T_4, thyroxine and testosterone hormones were estimated in serum by Radioimmunoassay (RIA) using coated tubes kit (DI Asource- Immuno-Assays S.A. Rue du Bosquet, 2B-1348 Louvain-la-Neuve, Belgium) and counting using a computerized gamma counter (ISOCOMP1–MGM) in Biological Applications Department at Nuclear Research Centre.

Semen Collection and Estimation of Semen Characteristics

Semen was collected from each buck at the end of experimental by artificial vagina using a female teaser rabbit. The temperature of the inner rubber sleeve of the artificial vagina was adjusted to 40- 42°C and the lubrication of the inner sleeve was performed using white Vaseline. Physical semen characteristics, percentage of normal sperm, ejaculate volume, sperm-cell concentration ($x10^6$ /ml), percentage of sperm progressive motility, percentage of live and dead spermatozoa were estimated in semen samples.

Statistical Analysis

Data were statistically analyzed using procedure of **SPSS (2012)** version19 according the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where: μ = the overall mean, T_i = the fixed effect of treatments (1= control, 2=Se plus vitamin E, 3= vitamins AD_3E mixture, e_{ij} = residual error. The significant differences among means were compared using Duncan's new multiple-range test (**Duncan, 1955**).

RESULTS

Effect of the Two Techniques to Ameliorate Heat Stress on Male Rabbits

Physiological and thermoregulatory parameters

Adding vitamin E plus Se in drinking water decreased significantly the physiological thermoregulatory parameters of heat stressed rabbits. Rectal, skin, ear temperature and respiration rate values in rabbits drinking water containing Se plus vitamin E were lower than control rabbits.

Rectal, skin, ear temperature and respiration rate of rabbits drinking water containing AD_3E vitamins mixture were lower ($p< 0.001$) than in males control rabbits. Skin temperature and respiration rate values in rabbits treated with AD_3E mixture were lower than in rabbits treated with Se plus vitamin E.

Body Weight, Feed and Water Intake

Adding vitamin E plus Se in drinking water improved ($P\leq0.03$) final live body weight, feed intake and feed/water ratio of heat stressed rabbits. The improvement percentages were 6.0, 13.8 and 53.8%, respectively, in comparison with the rabbits not received vitamin E plus Se in drinking water. Daily water intake of heat stressed males rabbits decreased ($P\leq0.03$) by 26.4% due to adding vitamin E plus Se as in drinking water compared with control heat stressed rabbits (Table 4).

Adding AD_3E vitamins mixture in drinking water of heat stressed male rabbits lead to increases ($P\leq0.03$) in final live body weight, feed intake and feed/water ratio by 3.45%, 14.7% and 53.8%, respectively. Daily water intake decreased ($P\leq0.03$) by 24.3% compared with heat stressed male rabbits drinking water without AD_3E vitamins.

Final body weight of rabbits treated with Se plus vitamin E was higher ($P<0.05$) than body weight of rabbits treated with AD_3E vitamin mixture. Water intake in rabbits treated with Se plus vitamin E was lower ($P<0.05$) than water intake in rabbits treated with AD_3E vitamin mixture.

Table 3. Effect of different techniques to ameliorate heat stress on thermoregulatory parameters of male rabbits

Thermoregulatory parameter	Control	Vitamin E plus Se	Vitamins AD_3E mixture	P value
Rectal temperature (˚C)	$41.0^{a} \pm 0.07$	$39.8^{b} \pm 0.03$	$39.7^{b} \pm 0.06$	0.001
Skin temperature (˚C)	$40.1^{a} \pm 0.03$	$39.6^{b} \pm 0.03$	$39.3^{c} \pm 0.09$	0.001
Ear temperature (˚C)	$39.3^{a} \pm 0.03$	$38.6^{b} \pm 0.06$	$38.4^{b} \pm 0.05$	0.001
Respiration rate, rpm	$117.2^{a} \pm 0.80$	$106.4^{b} \pm 0.90$	$99.9^{c} \pm 1.60$	0.001

rpm= No of respiration per minute.
a,b…Means in the same row within each classification having different superscripts per each item differ significantly.

Table 4. Effect of different techniques to ameliorate heat stress on body weight, feed and water intakes of male rabbits

Body weight, Feed and water intake	Control	Vitamin E plus Se	Vitamins AD_3E mixture	P value
Initial live body weight/g	2672±35.13	2712±36.2	2706±43.08	0.730
Final live body weight (g)	2884^{c}±34.2	3058^{a}±54.9	2984^{b}±43.2	0.030
Feed intake (g/d)	119.0^{b}±1.13	135.3^{a}±1.59	136.6^{a}±1.30	0.0001
Water intake (ml/d)	904.7^{a}±2.90	665.8^{c}±7.40	784.2^{b}±5.90	0.0001
Feed/water ratio	0.13^{c}	0.20^{a}	0.17^{b}	0.0001

a,b… Means in the same row within each classification having different superscripts per each item differ significantly.

Although feed intake was the same in the two treated groups, feed/water ratio was lower ($P<0.05$) in rabbits treated with AD_3E vitamin mixture than in rabbits treated with Se plus vitamin E (Table 4).

Blood Biochemical Components

Total protein, albumin and globulin concentrations increased ($P\leq0.01$) due to adding vitamin E plus Se or vitamin AD_3E mixture in drinking water of male rabbits. Opposite trend was observed in urea and creatinine concentrations. Adding vitamin E plus Se decreased ($P\leq0.05$) urea-N and creatinine concentrations in the two experimental groups compared with control rabbits. Total protein, albumin and globulin concentrations in rabbits treated with vitamin AD_3E mixture were higher ($p<0.05$) than in rabbits treated with vitamin E plus Se. Differences between the two treated groups in urea and creatinine concentrations were insignificant.

Hormonal levels

Level of cortisol was lower ($P\leq0.001$) due to adding vitamin E plus Se or AD_3E vitamins mixture in drinking water than in control rabbits. However, level of cortisol hormone was lower ($P\leq0.05$) in treated rabbits with AD_3E vitamins mixture than the level of cortisol hormone in rabbits treated with vitamin E plus Se.

Levels of T_3 and T_4 hormones were higher ($P\leq0.001$) in male rabbits drinking water containing vitamin E plus Se or AD_3E vitamins mixture than control rabbits. No significant difference between the two hormonal levels in rabbits treated with vitamin E plus Se or AD_3E vitamins mixture.

Level of testosterone increased ($P\leq0.001$) due to adding vitamin E plus Se or AD_3E vitamins mixture without significant difference between the hormonal levels in two treated groups (Table 6).

Table 5. Effect of different techniques to ameliorate heat stress on blood biochemical components of male rabbits

Blood parameter	Control	Vitamin E plus Se	Vitamins AD_3E mixture	P value
Total protein (g/dl)	5.1^{c}±0.12	5.7^{b}±0.20	6.2^{a}±0.13	0.01
Albumin (g/dl)	2.7^{c}±0.09	3.1^{b}±0.01	3.4^{a}±0.02	0.01
Globulin (g/dl)	2.4^{c}±0.06	2.6^{b}±0.04	2.8^{a}±0.05	0.01
Urea (mg/dl)	13.0^{a}±0.47	11.06^{b}±0.47	11.77^{b}±0.49	0.02
Creatinine (mg/dl)	1.12^{a}±0.10	0.91^{b}±0.56	0.80^{b}±0.50	0.02

a,b…Means in the same row within each classification having different superscripts per each item differ significantly.

Table 6. Effect of different techniques to ameliorate heat stress on hormonal levels in male rabbits

Hormone level (ng/ml)	Control	Vitamin E plus Se	Vitamins AD_3E mixture	P value
Cortisol	25.19^{a}±0.67	18.36^{b}±0.27	15.55^{c}±0.71	0.001
T_3	1.16^{b}±0.45	1.35^{a}±0.02	1.39^{a}±0.38	0.001
T_4	35.15^{b}±2.03	46.30^{a}±0.62	44.60^{a}±1.07	0.001
Testosterone	2.94^{b}±0.08	3.86^{a}±0.12	3.78^{a}±0.16	0.001

a,b…Means in the same row within each classification having different superscripts per each item differ significantly.

Semen characteristics

Normal sperm percentage, volume, concentration, motility percentage and live sperm percentage as physical semen characteristics are improved ($p \leq 0.001$) in heat stressed rabbits drinking water containing vitamin E plus Se compared with control rabbits.

Live sperm percentage value was higher ($p \leq 0.001$) in rabbits drinking water containing vitamin E plus Se than in control rabbits. Opposite trend was found in dead sperm percentage. Dead sperm percentage value was lower ($p \leq 0.001$) in rabbits drinking water containing vitamin E plus Se than in control rabbits.

Adding AD_3E vitamins in drinking water for heat stressed rabbits improved ($P \leq 0.0001$) semen characteristics (normal sperm, volume, count, motility and live sperm) and decreased significantly percentage of dead sperm in heat stressed NZW males rabbits in comparison with rabbits drinking water without AD_3E vitamins.

The improvement in Semen characteristics in rabbits treated with drinking water containing vitamin E plus Se were better than Semen characteristics in rabbits treated with drinking water containing AD_3E vitamins (Table 7).

Table 7. Effect of different techniques to ameliorate heat stress on semen characteristics in male rabbits

Semen characteristic	Control	Vitamin E plus Se	Vitamins AD_3E mixture	P value
Normal sperm (%)	85.9^{c}±0.45	94.7^{a}±0.26	91.5^{b}±0.31	0.001
Volume (ml)	0.40^{c}±0.013	0.53^{a}±0.01	0.46^{b}±0.01	0.001
Concentration×10^6/ml	77.7^{c}±1.21	155.9^{a}±2.35	93.2^{b}±1.44	0.001
Motility (%)	70.7^{c}±1.27	80.6^{a}±1.00	76.6^{b}±0.51	0.001
Live (%)	74.0^{c}±0.42	95.9^{a}±0.52	92.1^{b}±0.43	0.001
Dead (%)	26.0^{a}±0.40	4.1^{c}±0.52	7.9^{b}±0.43	0.001

a,b….Means in the same row within each classification having different superscripts per each item differ significantly.

DISCUSSION

Effect of Amelioration Heat Stress Techniques on Male Rabbits Exposed to Hot Summer Season

Thermoregulatory parameters

Results in Table 3 show that the two amelioration heat stress techniques both vitamin E plus Se and vitamin AD_3E mixture decreased the physiological thermoregulatory parameters of male rabbits exposed to hot summer season. Skin temperature and respiration rate in heat stressed male rabbits were significantly lower with using of AD_3E mixture technique than with using of vitamin E plus Se technique.

Using of vitamin E and selenium in rabbits exposed to heat stress (35^{o}C) for 35 days reduced rectal and skin temperature comparative with control group **(AL-Zafry and Medan, 2012)**. Antioxidant and mineral supplementation in heat stressed ewes have a greatest positive effect on physiological parameters **(Sejian *et al.*, 2014)**. Combined Se and vitamin E supplementation has previously been reported to reduce negative effects of heat stress on respiration rates and rectal temperature in sheep **(Chauhan *et al.*, 2014)** and in goats **(Sivakumar *et al.*, 2010)**.

Vitamin E and C directly alters thermal set point by decreasing prostaglandin output especially of prostaglandin E series, whose turnover increase during stress, which has a direct effect on the hypothalamic thermoregulatory zone **(Hadden, 1987).** In addition, these vitamins may have an ameliorating effect upon heat stressed animals by affecting the prostaglandin output **(Ganong, 2001).** However, skin, rectal, ear temperature and respiration rate values in bucks goat were not affected significantly due to injection of vitamins AD_3E under hot summer season in Egypt (**Habeeb *et al.*, 2016)**.

Final body weight, feed and water intake

Adding vitamin E plus Se or vitamin AD_3E mixture in drinking water of heat stressed rabbits affected positively ($P\leq0.030$) body weight, feed ($P\leq0.001$) and water intake ($P\leq0.001$) of male rabbits. However, at the end of experimental period, final body weight of heat stressed rabbits drinking water containing vitamin E plus Se was significantly higher than body weight of heat stressed rabbits drinking water containing AD_3E mixture. Concerning feed intake, the tow techniques improved feed intake of heat stressed rabbits drinking water containing vitamin E plus Se and rabbits drinking water containing AD_3E mixture by 13.7 and 14.8% without significant difference between the two techniques. Concerning water intake, the two techniques lowered significantly water intake of heat stressed rabbits. However, water intake of heat stressed rabbits drinking water containing vitamin E plus Se was significantly lower than water intake of heat stressed rabbits drinking water containing AD_3E mixture by 118.4 ml. Drinking water containing vitamin E plus Se or drinking water containing

AD_3E mixture improved feed/water ratio in heat stressed rabbits. The differences in feed/water ratio values between the two techniques (0.20 vs. 0.17) are due to the higher water intake in rabbits drinking water containing vitamins AD_3E mixture than in water intake of heat stressed rabbits drinking water containing vitamin E plus Se.

Selenium or folic acid and their combination caused significant improvement in body weight and feed intake in male rabbits (**Kamel, 2012**). Similar results about increased feed intake and higher performance rate of treated groups with Se or Se plus vitam E combination were reported by **Sahin and Kucuk (2001**) in Japanese quails and by **Attia *et al.* (2010**) in hens. In lambs, supplementation of selenium either organic or inorganic sources resulted in improved performance particularly in respect of their growth rate with more effective for organic S than inorganic S (**Kumar *et al.*, 2009**).

AD_3E vitamins with yeast diet under summer season improved daily body weight gain, daily dry matter intake of native bovine calves (**Habeeb *et al.*, 2018a**). Injections of AD_3E a month prior to expect date of calving a higher monthly body gain was noted in new-born of Murrah buffaloes calves of supplemented dams with AD_3E (**Sikka *et al.*, 2002**). Same authors found enhanced the total immunoglobulins secretion in colostrum of buffaloes and concluded that antioxidant and fat soluble vitamins in combination with minerals have a passive immunity and growth in buffalo calves. Improvement of gain in heat stressed rabbits drinking water containing Se plus vitamin E or drinking water containing AD_3E vitamins mixture may be due to the fact that these vitamins are involved in the synthesis of important coenzymes (NAD and FAD), which are responsible for the biological oxidative process that produces the necessary ATP for protein, fat and carbohydrate biosynthesis (**Habeeb *et al.*, 2018b).**

Concerning the decrease in water intake in both treated groups as compared to control one may be due to the tow treatments ameliorate the heat load on heat stressed rabbits and consequently lowered drinking water intake.

Blood biochemical components

In the present study, both two treatments improved protein fractions and at the same time decreased concentrations of urea and creatinine.

This result may be due to increase of protein anabolism and decrease of protein catabolism. In addition the increase in the T_4 and T_3 hormonal levels could be ascribed to the improvement of feed efficiency by vitamin E and Se which improve by the way the overall animal health and/or reproductive performance

Supplementation of vitamin E increased plasma protein concentration while markedly decreased blood glucose and cholesterol concentrations in heat-stressed (34C) Japanese quails (**Sahin *et al.*, 2001).** Similar effects of vitamin E and selenium existed as evidence that proteins fractions concentrations increased whereas urea and creatinine concentrations decreased. **Balicka-Ramsisz *et al.* (2006)** reported an improvement in blood metabolites in sheep administrated with selenium. In addition, total proteins, albumin, and globulin concentrations increased progressively with increased the level of vitamins AD_3E injection **(Habeeb *et al.*, 2016).** Same authors found that total protein, globulin concentrations increased by 8.35% and 11.36% over control when bucks injected with 2 ml vitamins and by 18.16% and 26.18% in bucks injected with 4 ml AD_3E vitamins. However, albumin level increased by 10.90% over control in bucks injected with 4 ml vitamins and was not affected significantly by injection 2 ml vitamins **(Habeeb *et al.*, 2016).**

Blood hormones levels

Adding vitamin E plus Se or vitamin AD_3E mixture in drinking water of heat stressed rabbits affect positively on T_3, T_4 and testosterone hormonal levels. The two amelioration techniques increased T_3 level by 16.4 and 19.8% and T_4 level by 31.7 and 26.9% and testosterone level by 31.3 and 28.6%, respectively. Opposite trend was found in cortisol hormonal level. Adding vitamin E plus Se or vitamin AD_3E mixture in drinking water of heat stressed rabbits decreased cortisol level by 27.1 and 38.3%, respectively.

The increases of T_3 and T_4 levels may be due to that these tow techniques improved thyroid gland activity. The increase in testosterone level

may be due to that the amelioration techniques affect positively on sex gonads. The decrease in cortisol level due to treatments may be due to that techniques affect positively on adrenal cortex and ACTH hormone to decrease secretion of cortisol.

Se and vitamin E improve thyroid hormone activity (**Sivakumar *et al.,* 2010**). Se is needed to hepatic conversion of T_4 to T3 and catalyses de-iodination of T_4 to T_3 (**El-Shahat and Abdel-Monem, 2011).** In goats, T_3 and T_4 hormones increased in antioxidant (Se and vitam. E) treatment group (**Sivakumar *et al.,* 2010**). Injection AD_3E vitamins in goats during summer season increased T_3 and T_4 hormones by increased levels of vitamins injection **(Habeeb *et al.,* 2016).** AD_3E plus yeast increased levels of T_3 and T_4 hormones in native bovine calves (**Habeeb *et al.,* 2018b**). Sexual activity of Awassi rams increased after treatment with vitam E alone or in combination with Se due to increase of testosterone level which led to a direct effect on semen volume and at the same time the level of testosterone was significantly reduced during Se deficiency **(Ali *et al.,* 2009)**. Treatment with vitam E or/and Se led to increases in testosterone level that has a direct effect on secondary sexual activity **(Bearden and Fuquay, 1997)**. **El-Sisy *et al.* (2008)** revealed that male goats fed a basal diet supplemented with selenium enriched yeast resulted in a significant increase in testosterone concentration.

Cortisol as a representative glucocorticoid is produced in the zona fasciculata of the adrenal cortex and is necessary in times of stress to maintain blood glucose levels and prevent shock. Secretion of cortisol is regulated by the negative feedback effect on the hypothalamic-pituitary-adrenal axis **(Burtis *et al.,* 2006).** In consistence with the present results, **Gupta *et al.* (2005)** reported vitamin E supplementation decreased concentrations of plasma cortisol in crossbred dairy cattle.

Semen characteristics

Adding vitamin E plus Se or AD_3E vitamin mixture in drinking water of heat stressed rabbits improved significantly semen characteristics as shown in Table 7. The improvement in semen characteristics of treated groups may be due to the increase in testosterone hormonal level in treated rabbits. However, adding vitamin E plus Se in drinking water produced semen with higher quality than semen produced when adding AD_3E mixture in drinking water of heat stressed rabbits.

Libido was delayed in NZW rabbit bucks exposed to heat stress and this delay may be due to the decrease in testosterone hormone concentration and minimal spermatogenesis, so, the low quality semen occurring in a hot climate (**El-Kelawy *et al.,* 1997**). Spermatozoa are very sensitive cells in regard to high temperature, and morphological abnormalities could be assumed as indicators of heat stress conditions suffered by the rabbits (**Finzi *et al.,* 1995).** Sexual activity of Awassi rams increased after treatment with Vitam E alone or in combination with Se due to increase of testosterone level which led to a direct effect on semen volume **(Ali *et al.,* 2009)**. Addition of selenium to balanced feed mixtures fed to rams improved libido and positively influenced both quantitative and qualitative of seminal traits (**Baiomy *et al.,* 2009**). In addition, **Spring (2006**) indicated significant improvements in spermatozoa concentration and activity, when fed diets supplemented with Se. Supplemented of vitamin E in diet of male zaraibi goats can improve density of spermatogenic cell, Sertoli cells, and diameter of seminiferous tubule and thickness of germinal epithelium (**Habeeb and Teama, 2013**). Combination of vitamin E plus Selenium had beneficial effects on alleviation of adversely effect of heat stress on the reproductive performance of doe rabbits in terms of increasing kindling rate litter size and weight of kits at birth **(Habeeb *et al.,* 2010 and 2011)**.

Conclusion

The important finding from this study supported that Se plus Vitamin E or AD_3E Vitamins mixtures can ameliorate the negative effects of heat stress on physiological thermoregulatory parameters, semen characteristics, blood biochemical components and thyroid hormones as well as testosterone in New Zealand rabbits bucks under Egyptian hot summer condition.

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التقنيـــات الحـــديثـــة لتخفيـف تـأثيـــر ظـــروف العبء الحـــرارى علـــى ذكـــور الأرانب

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أجريت هذه الدراسة فى مزرعة الأرانب التابعة لمركز البحوث النووية هيئة الطاقة الذرية بإنشاص بالتعاون مع قسم الإنتاج الحيوانى بكلية الزراعة جامعة الزقازيق لبحث تأثير إضافة السلينيوم (Se) مع فيتامين E أو إضافة خليط فيتامينات AD_3E إلى ماء الشرب بمعدل ١ مل لكل واحد لتر من ماء الشرب كتقنيتين جديدتين لتخفيف تأثير ظروف العبء الحراري لفصل الصيف الحار في مصر علي ذكور الأرانب النيوزلندية، تم استخدام خمسة وأربعين من ذكور الأرانب البيضاء النيوزيلندية (NZW) حيث تم تقسيم الحيوانات إلى ثلاث مجموعات متساوية فى العدد و الوزن وقد تم الاحتفاظ بالمجموعة الأولى تشرب الماء بدون أي إضافة كمجموعة كنترول أما المجموعة الثانية تم إضافة Se مع فيتامين E إلى ماء الشرب بمعدل ١ مل لكل واحد لتر من ماء الشرب وفى المجموعة الثالثة تم إضافة خليط فيتامينات AD_3E الى ماء الشرب بمعدل ١ مل لكل واحد لتر من ماء الشرب وقد استغرقت التجربة سبع أسابيع خلال موسم الصيف الحار (يوليو وأغسطس، ٢٠١٧)، وأهم نتائج الدراسة هى: إضافة Se مع فيتامين E وكذلك إضافة مخلوط فيتامينات AD_3E الى مياه الشرب أدى إلى تقليل العبء الحراري على ذكور الأرانب بدليل انخفاض معنوى في درجات حرارة المستقيم والجلد والأذن وانخفاض فى معدل التنفس بالمقارنة بمجموعة الكنترول، إضافة Se مع فيتامين E وكذلك إضافة مخلوط فيتامينات AD_3E إلى مياه الشرب أدى إلى تحسن معنوى فى وزن الجسم الحي النهائي و زيادة معنوية فى كمية الغذاء المأكول وانخفاض معنوى فى كمية ماء الشرب مقارنة بمجموعة الكنترول، إضافة Se مع فيتامين E وكذلك إضافة مخلوط فيتامينات AD_3E الى مياه الشرب ادي الي زيادة معنوية في مستويات كلا من البروتين الكلي والالبيومين والجلوبيولين وانخفاض في تركيز كلا من اليوريا والكرياتنين في الدم، إضافة Se مع فيتامين E وكذلك إضافة مخلوط فيتامينات AD_3E إلى مياه الشرب أدى إلى انخفاض معنوى فى مستوى هرمون الكورتيزول في الدم وارتفاع معنوى لتركيز هرمونات الغدة الدرقية (T3 و T4) مقارنة بمجموعة الكنترول، إضافة Se مع فيتامين E وكذلك إضافة مخلوط فيتامينات AD_3E الى مياه الشرب ادي الي تحسن معنوى فى نسبة الحيوانات المنوية الطبيعية وفى حجم السائل المنوى وتركيز الحيوانات المنوية وسرعة حركة الحيوانات المنوية الحية مع انخفاض معنوى فى نسبة الحيوانات المنوية الميتة مقارنة بمجموعة الكنترول، يمكن الاستنتاج أن تلك التقنيات المقترحة فى الدراسة يمكن أن تخفف من الآثار السلبية للإجهاد الحراري على ذكور الأرانب خلال موسم الصيف الحار في مصر.

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