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Research Article
Effect of Spirulina and Vitamin E on Reproduction and *in vitro* Embryo Production in Heat-stressed Rabbits

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Abstract

Background and Objective: High ambient temperature can cause heat stress and evokes a combination of change in blood biochemicals and reproduction of rabbit. This study targeted to investigate the effect of *Spirulina platensis*, vitamin E and their combination on *in vivo* and *in vitro* reproductive performance and some physiological and health indicators of heat stressed rabbit does. **Materials and Methods:** Nili-parous rabbit does (n = 80) were allocated to 4 groups. Does in the 1st group were fed commercial complete feed diet, while those in the 2nd, 3rd and 4th were fed complete feed diet with *Spirulina platensis* (300 mg kg^{-1}), vitamin E (100 mg kg^{-1} diet) and *Spirulina platensis*+vitamin E kg^{-1} diet, respectively. All does were naturally mated with fertile bucks (5 bucks/group). **Results:** The does in the 2nd group showed significantly ($p<0.05$) better reproductive performance (conception rate, kindling rate and litter size), lipid profile (total lipids, cholesterol, triglycerides, high and low density lipoproteins, antioxidant capacity (total antioxidant capacity, glutathione, malondialdehyde, glutathione peroxidase, glutathione S-transferase, superoxide dismutase and catalase), immunity (lysozyme, IgG and IgM), ovulatory response (corpora lutea number and ovulation rate), embryo quality and hatched blastocysts production with higher cell number and inner cell mass as compared to other groups. **Conclusion:** Dietary supplementation with *Spirulina platensis* (300 mg kg^{-1} diet), in comparing with vitamin E (100 mg kg^{-1} diet) or their combination at the same levels, had positive impact on reproductive performance of rabbit does used in breeding program under heat stress condition in Egypt.

Key words: Rabbit, heat stress, antioxidants, immunity, reproduction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In Egypt, rabbits are playing an important role in solving deficiency of meat production. Egypt is located within the humid tropics characterized by long period of high ambient temperature and humidity, particularly during summer months[1]. The comfortable zone for rabbits is ranging from 18-21°C[2]. High ambient temperature can cause heat stress and evokes a combination of change in blood biochemicals and reproduction of rabbit in Egypt[3,4]. Heat stress reduces immune function and increases free radical production and lipid peroxidation of cell membranes[5]. Also, respiratory rate and water intake increase and feed intake decreased in heat stressed rabbits[6]. Heat stress increases corticosteroid levels, decreases LH and FSH secretion, which affects ovarian development and ovulation[7,8].

Oxidative damage accumulation in distinct sub-cellular components that exert very toxic effects on DNA, proteins and lipids was observed as a result of heat stress exposure[9]. Various natural antioxidants, such as spirulina and vitamin E, as dietary additives, are beneficial to attenuate the detrimental effects of oxidative stress on fertility of females[5,10].

Spirulina is a photosynthetic, filamentous and blue-green algae and considered a promising dietary source due to its high contents from protein (65-70%), essential amino and fatty acids (α and γ-linolenic acid), vitamins (thiamine, nicotinamide, riboflavin, folic acid, pyridoxine, vitamins A, D and E), minerals (Ca, K, Cr, Cu, Mn, Fe, P, Mg, Na, Zn) and different natural carotene variety and photosynthetic pigments[11]. Several reports indicated the biological activities of spirulina, including antioxidant, anti-inflammatory and immune-modulatory properties and hypo-lipidemic action[12,13]. Spirulina strongly induces antioxidant enzyme activity, which in turn reducing free radical production, lipid peroxidation and damage of DNA, because spirulina contain flavonoids, alkaloids, phenolic compounds and steroids[14,15].

Vitamin E (α-tocopherol) is main natural antioxidant for scavenging free radicals generation through non-enzymatic defense system and considered as an excellent biological antioxidant, representing a 1st line of defense against lipid peroxidation and free radical cleaner in the cellular membranes[16-18]. Also, vitamin E may affect the impact and maintenance of immune function and it is necessary for normal reproduction of animals[19]. It is required for development of follicles and corpora lutea[20]. It was suggested that vitamin E could reduce the adverse effects of corticosterone induced by heat stress in hens, protects macrophages, lymphocytes and cell membrane against oxidative damage[18,20]. Addition of SP enhanced growth performance, immune response, of chickens under heat stress condition[21]. Also, vitamin E maintains and enhances reproductive performance in poultry[22]. Moreover, SP or vitamin E improved the ovulatory response and production in pigs[23].

Limited information is available for the effects of *Spirulina platensis* in comparing with vitamin E or their combinations on reproduction of rabbit does kept under heat stress condition in Egypt. Therefore, the present study targeted to investigate the effect of *Spirulina platensis*, vitamin E and their combinations on the reproductive performance, lipid metabolites, antioxidant, immune and ovulatory responses and embryos development of heat stressed rabbit does in Egypt.

MATERIALS AND METHODS

Study area: The present study was carried out at a private rabbit farm in Mansoura city, Egypt. The laboratorial work was carried out in the laboratory of Physiology and Biotechnology, Department of Animal Production, Faculty of Agriculture, Mansoura University, Egypt. The experimental work lasted three months from 1 July-30 September, 2017.

Animals and climate: Nili-parous NZW rabbit does (n = 80) were raised under similar managerial and environmental conditions. Does were fed commercial complete feed diet (CFD) in pelleted form to cover the nutritional and physiological requirements of mature rabbit does according to NRC recommendations[24]. Does were fed *ad libitum* on the diet, which was provided twice daily (7 am and 3 pm), while drinking water was available from nipple of each cage. Ingredients and chemical composition of the diet are shown in Table 1.

During the experimental period from July-September, 2017, averages of minimum and maximum values of recorded ambient temperature and relative humidity as well as calculated thermo-humidity index[25] were 27.41 and 32.91°C, 49.0 and 80.38% and 24.56-31.38, respectively.

Experimental design: Does were allocated into 4 experimental groups (20 per group), according to live body weight. Does in the 1st group (G1) were fed the control CFD without supplementation, while those in the 2nd, 3rd and 4th groups were fed the diet supplemented with SP (300 mg kg^{-1} diet, G2), vitamin E (100 mg kg^{-1} diet, G3) and their combination (300 mg SP+100 mg kg^{-1} vitamin E diet, G4), respectively. The weekly CFD of each treatment groups was well mixed with their additives in homogenous form. Does were fed the experimental diets during a feeding period of 3 months, 1 month pre-mating, pregnancy and suckling (weaning at 28 days of age).

Table 1: Ingredients and chemical composition of the diet fed to rabbit does in different experimental groups

Items	Percentage
Ingredient	
Clover hay	30.00
Soybean meal (44%)	18.00
Wheat bran	24.60
Barley grain	21.00
Molasses	3.00
Limestone	1.00
DL-Methionine	0.20
Common salt	0.50
Minerals	0.15
Vitamins	015.00
Di-calcium phosphate	1.40
Total	100.00
Chemical composition (Dry matter basis (%))	
Organic matter	93.15
Crude protein	18.15
Crude fiber	10.19
Ether extract	2.60
Nitrogen free extract	62.21
Ash	6.85

Table 2: Antioxidant compounds and chemical composition of *Spirulina platensis*

Item amount/100 g	Percentage
Antioxidant compounds	
Vitamin E (µg)	110.000
Total carotenoids (µg)	455.000
β-carotene (mg)	220.000
Chlorophyll (mg)	1.085
Phycocyanin (mg)	11.650
Superoxide dismutase (IU)	510.000
Chemical composition (Dry matter basis (%))	
Crude protein	55.800
Crude fat	6.200
Crude fiber	4.900
Nitrogen free extract	23.000
Ash	10.100

Spirulina platensis (SP) powder as a commercial product was prepared by was prepared in the National Institute of Oceanography and Fisheries, Egypt. Antioxidant compounds and chemical composition of SP according to the manufacturer (Regional Centre for Food and Feed, RCFF, Agricultural Research Center, Giza, Egypt), is presented in Table 2.

Doe reproductive measurements: Does in all groups were naturally mated with fertile NWZ rabbit bucks (5 bucks per group, 4 does for each buck). Does were manually palpated 10-12 days post-mating to calculate conception rate. After parturition, kindling rate was recorded and total and live litter size at birth was computed 12 h after kindling. Kits were weaned at 28 day of age and then litter size at weaning was determined. Also, viability rate at birth and weaning was recorded.

Ovulatory response: Following the suckling period (end of 1st parity), receptive does (with red vulva) were naturally mated, then 5 conceived does from each group were taken, transported to Laboratory and slaughtered 60-46 h post-mating. Immediately after slaughtering, blood was collected and ovaries were removed, weighed to determine relative ovarian weight (ROW), then number of secondary follicles ($\leq$2 mm in diameter), hemorrhagic follicles (HF), antral follicles ($\geq$2 mm in diameter) and corpora lutea (CLs) on the ovarian surface was recorded for each dose. Ovulation rate (OR) were calculated as the following:

$$\text{OR (\%)} = \frac{\text{Number of CLs}}{\text{Number of HF and antral follicles}} \times 100$$

Recovery and culture of embryos: Embryos were recovered from reproductive tract of does slaughtered in petri dishes containing phosphate buffer saline (PBS) with 10% fetal calf serum (FCS) and 50 µg gentamicin mL^{-1}. After embryo searching by stereoscopic microscope, embryos were counted, then embryo recovery rate (ERR) was calculated as the following:

$$\text{ERR} = \frac{\text{Number of embryos}}{\text{Number of CLs}}$$

Number of morulae was recorded (n = 273) and morphologically evaluated based on abnormality in mucin coat, intact zona pellucidae, blastomeres and refractive cytoplasm after washing for 3 times in PBS into acceptable (n = 202) and abnormal (n = 71) embryos.

Only acceptable morulae in G1 (n = 41), G2 (n = 64), G3 (n = 52) and G4 (n = 45) were cultured, with TCM-199 (100 µL) supplemented with 10% FCS and 50 µg mL^{-1} gentamicin, in CO_2 incubator (38.5°C, high humidity and 5% CO_2 in air) under mineral oil for 3 days for development to embryos at blastocyst, expanded and hatched blastocyst stages.

Blastocyst characteristics: Embryos at blastocyst stage from each group (n = 5) were randomly taken on the 2nd day of culture and stained[26].Briefly, embryos were incubated in 0.5% sodium nitrate solution at 37°C for 30 min, placed on a clean by a pasteur pipette and the excessed sodium nitrate was removed, then embryos were fixed in mixture of acetic acid and methanol (1:3). After air drying, the slides were stained with 5% Giemsa in PBS for 30 min and washed with distilled water. Nuclei of the blastomeres were counted using a light microscope at 100 x.

Blood sampling: Blood samples of slaughtered does were collected into sterile tubes and left for clotting, then centrifuged at 3500 rpm for 20 min and blood serum was isolated for determination of total lipids, total cholesterol, triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL) concentrations using commercial kits (Biomerieux, Poains, France).

Concentration of total antioxidant capacity (TAC), glutathione (GSH) and malondialdehyde (MDA) as well as activity of glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase were assayed in blood serum by commercial kits (Bio Diagnostic Research, Egypt) according to manufacturer's instructions.

Concentration of immunoglobulins (IgG and IgM) in blood serum was determined by commercial ELISA kits (Kamiya Biomedical Company, USA), while serum lysozyme activity was determined[27].

Statistical analysis: Data was statistically analyzed by one-way ANOVA design using a software package (SAS)[28]. Completely randomized design was used based on the following model:

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

where, μ is the overall mean, G_i is the group (1-4) and e_{ij} is the residual error. Rate of conception, kindling, ovulation, embryo recovery, normality and blastocyst production were statistically analyzed using Chi-Square test. The group significant differences were tested by Duncan's multiple range test (Duncan)[29] and set at $p<0.05$.

RESULTS

Reproductive performance: Reproductive performance, in terms of kindling rate and litter size at birth (total and live) and weaning significantly ($p<0.05$) increased in G2 and G3. Conception rate significantly ($p<0.05$) improved in all treatment groups, while viability rate at birth and weaning significantly ($p<0.05$) improved only in G2. However, all reproductive traits of does in G4 did not differ significantly from those in G1 (control, Table 3).

Lipid profile: Feeding does on SP, Vitamin E or their combination significantly ($p<0.05$) affect lipid profile in blood serum of does, in term of reducing concentration of total cholesterol and triglycerides, being the lowest in G2 and increasing HDL concentration, being the highest in G2 as compared to control group (G1). However, LDL concentration was not affected by treatment (Table 4).

Antioxidant capacity and immune response: All treatments significantly ($p<0.05$) increased GST and GPx contents and decreased MDA concentration, while both SP and vitamin E treatments significantly ($p<0.05$) increased SOD, GSH and catalase contents, but only SP significantly ($p<0.05$) increased

Table 3: Effect of *Spirulina platensis*, vitamin E and their combination on reproductive performance of NZW rabbit does

	Experimental groups				
Items	G1 (control)	G2 (SP)	G3 (Vitamin E)	G4 (SP+Vitamin E)	p-value
Conception rate (%)	55.00^{c}	85.00^{a}	75.00^{ab}	70.00^{b}	-
Kindling rate (%)	73.00^{b}	94.00^{a}	86.00^{a}	85.00^{ab}	-
Total litter size at birth/doe (n)	5.13±0.44^{b}	8.19±0.28^{a}	7.15±0.34^{a}	5.67±0.43^{b}	0.0000
Live litter size at birth/doe (n)	4.13±0.29^{c}	7.81±0.26^{a}	6.38±0.31^{b}	4.92±0.36^{c}	0.0001
Viability rate at birth (%)	80.50±4.34^{b}	95.36±1.77^{a}	89.23±3.45^{ab}	86.77±3.58^{ab}	0.0389
Litter size at weaning/doe	3.13±0.23^{c}	7.50±0.33^{a}	5.15±0.37^{b}	3.92±0.34^{c}	0.0001
Viability rate at weaning (%)	75.78±7.19^{b}	96.03±2.01^{a}	80.72±4.99^{b}	79.67±5.72^{b}	0.0324

^{a,b,c}Means in the same row with different superscripts are significantly different ($p<0.05$)

Table 4: Effect of *Spirulina platensis*, vitamin E and their combination on lipid profile in blood serum of NZW rabbit does

	Experimental groups				
Items	G1 (control)	G2 (SP)	G3 (Vitamin E)	G4 (SP+Vitamin E)	p-value
Lipid profile (mg dL^{-1})					
Total lipids	408.75±8.26^{a}	317.25±3.68^{c}	365.00±11.90^{b}	388.25±3.12^{ab}	0.0001
Total cholesterol	128.75±1.31^{a}	93.50±1.94^{d}	101.50±1.32^{c}	119.25±0.85^{b}	0.0002
Triglycerides	103.50±2.53^{a}	83.25±1.376^{c}	91.25±1.25^{b}	96.75±2.84^{b}	0.0001
High density lipoproteins	29.75±0.85^{c}	41.25±1.11^{a}	38.75±1.11^{ab}	35.50±1.71^{b}	0.0002
Low density lipoproteins	79.75±2.06	77.00±2.04	77.25±2.09	79.50±0.65	0.6053

^{a,b,c}Means in the same row with different superscripts are significantly different ($p<0.05$)

Table 5: Effect of *Spirulina platensis*, vitamin E and their combination on serum antioxidant compounds and immune response of NZW rabbit does

	Experimental groups				
Items	G1 (control)	G2 (SP)	G3 (Vitamin E)	G4 (SP+Vitamin E)	p-value
Antioxidant compounds					
TAC (mmol L^{-1})	0.36±0.02^{b}	0.39±0.01^{a}	0.36±0.01^{ab}	0.37±0.01^{ab}	0.0109
GST(IU)	1.01±0.01^{d}	1.26±0.02^{a}	1.18±0.01^{b}	1.08±0.01^{c}	0.0001
SOD (IU)	5.72±0.18^{c}	6.78±0.04^{a}	6.34±0.19^{b}	5.89±0.07^{c}	0.0007
GSH (mg dL^{-1})	14.43±0.29^{b}	17.23±0.10^{a}	16.97±0.28^{a}	15.18±0.13^{b}	0.0001
GPx (mg L^{-1})	4.040±0.02^{c}	6.52±0.14^{a}	6.38±0.17^{a}	5.14±0.02^{b}	0.0001
Catalase (U mL^{-1})	100.50±1.55^{c}	144.50±2.10^{a}	134.25±2.17^{b}	106.25±1.75^{c}	0.0001
MDA (µmol L^{-1})	8.133±0.05^{a}	7.19±0.03^{d}	7.93±0.03^{b}	7.41±0.07^{c}	0.0001
Immunity status					
Lysozyme (µ mL^{-1})	3.94±0.03^{d}	6.77±0.07^{a}	5.75±0.09^{b}	5.09±0.03^{c}	0.0001
IgG (ng mL^{-1})	41.60±1.30^{d}	84.03±1.96^{a}	73.90±1.51^{b}	52.50±1.04^{c}	0.0001
IgM (µg mL^{-1})	5.62±0.09^{b}	6.03±0.02^{a}	5.89±0.02^{a}	5.65±0.04^{b}	0.0006

^{a,b,c,d}Means in the same row with different superscripts are significantly different (p<0.05)

Table 6: Effect of *Spirulina platensis*, vitamin E and their combination on ovulatory response and ovulation rate of NZW rabbit does slaughtered at 60-64 h after mating

	Experimental groups				
Items	G1 (control)	G2 (SP)	G3 (Vitamin E)	G4 (SP+Vitamin E)	p-value
Doe weight (kg)	3.000±0.00111	3.000±0.006	3.000±0.002	3.000±0.001	0.7567
Absolute ovarian weight (g)	0.558±0.005^{c}	0.591±0.002^{a}	0.577±0.006^{ab}	0.569±0.003^{c}	0.0011
Relative ovarian weight (g kg^{-1})	0.186±0.001^{c}	0.197±0.001^{a}	0.192±0.002^{ab}	0.189±0.001^{c}	0.0012
Antral follicles/doe (n)	20.200±0.58^{a}	16.600±1.02^{b}	18.200±0.58^{ab}	19.600±0.5^{a}	0.0112
Secondary follicles/doe (n)	27.800±0.86^{a}	18.800±1.16^{c}	21.400±1.03^{bc}	22.200±0.86^{b}	0.0001
Bleeding follicles/doe (n)	4.400±0.51^{a}	2.600±0.40^{b}	3.200±0.37^{ab}	3.600±0.51^{ab}	0.0448
Corpora lutea/doe (n)	12.800±0.16^{b}	15.400±0.19^{a}	14.200±0.29^{ab}	13.400±0.23^{ab}	0.0148
Ovulation rate (%)	51.670±2.99^{c}	80.590±3.41^{a}	66.550±4.12^{b}	57.680±3.65^{bc}	0.0002

^{a,b,c}Means in the same row with different superscripts are significantly different (p<0.05)

Table 7: Effect of *Spirulina platensis*, vitamin E and their combination on recovery rate and quality of NZW doe embryos

	Experimental groups				
Items	G1 (control)	G2 (SP)	G3 (Vitamin E)	G4 (SP+Vitamin E)	p-value
Embryo recovery rate	96.93±1.93	98.66±1.33	98.33±1.67	97.32±1.65	0.8639
Acceptable embryos (%)	67.00±3.89^{b}	84.22±3.56^{a}	75.15±4.58^{ab}	67.11±6.74^{b}	0.0111
Poor embryos (%)	33.00±3.88^{a}	15.78±3.56^{b}	24.85±4.58^{ab}	32.89±6.74^{a}	0.0111

^{a,b}Means in the same row with different superscripts are significantly different (p<0.05)

TAC contents in serum of does as compared to control (G1). These results indicated inconsistent trend of improvement in antioxidant status of does being the best in G2 treated with SP. Regarding the immune response, all treatment led to significant (p<0.05) increase in contents of lysozyme and IgG in serum of does, while IgM significantly (p<0.05) improved only in G2 and G3 as compared to G1 (Table 5).

Ovulatory response and ovulation rate: Treatment of SP or vitamin E significantly (p<0.05) increased ovarian weight as absolute or relative weight. This was trend was in association with significant (p<0.05) increase in number of CLs and significant (p<0.05) decrease in number of follicles at different stage of development in G2 only. Increasing CLs number and decreasing follicular number (antral and bleeding) resulted in significant (p<0.05) increase in ovulation rate only in G2 and G3 compared with G1. It is of interest to note that SP in combination with vitamin E (G4) had no effect on ovulatory response of does (Table 6).

Recovery rate and embryo quality: Although the differences in embryo recovery rate among groups were not significant (96.93-98.66%), embryo quality was significantly (p<0.05) better in G2 than in G1, but did not differ from that in G3, indicating beneficial effect of SP on quality of recovered embryos. Also, the differences in embryo quality in each of G3 and G4 with G1 were not significant (Table 7).

Table 8: Effect of *Spirulina platensis*, vitamin E and their combination on developmental competence, total cell number/blastocyst and number of inner cell mass of NZW rabbit does

	Experimental groups				
Items	G1 (control)	G2 (SP)	G3 (Vitamin E)	G4 (SP+Vitamin E)	p-value
Total acceptable morulae (n)	41.00	64.00	52.00	45.00	-
Undeveloped morulae (%)	9.51±1.51	1.67±1.66	8.89±2.22	6.67±3.85	0.1864
Blastocyst (%)	7.43±0.62	6.11±1.11	8.89±2.22	8.99±2.32	0.6199
Expanded blastocyst (%)	9.51±1.52^{b}	19.16±3.63^{a}	13.33±3.85^{ab}	13.43±0.10^{ab}	0.0139
Hatched blastocyst (%)	43.86±1.29^{b}	61.67±6.67^{a}	48.89±2.22^{ab}	51.52±2.45^{ab}	0.0537
Degenerated (%)	29.70±2.46^{a}	11.39±3.61^{b}	20.00±3.85^{b}	19.39±0.61^{b}	0.0151
Inner cell mas (n)/blastocyst	114.67±3.1^{c}	129.33±2.3^{a}	122.33±1.45^{ab}	118.67±1.45^{bc}	0.0091
Total cell (n)/blastocyst	127.67±0.8^{c}	141.33±0.6^{a}	131.67±1.45^{b}	130.00±1.00^{bc}	0.0001

^{a,b,c}Means in the same row with different superscripts are significantly different ($p<0.05$)

Developmental competence *in vitro*: After co-culture of embryos at morula stage for 5 days, percentage of expanded and hatched blastocysts significantly ($p<0.05$) increased only in G2, while percentage of degenerated embryos significantly ($p<0.05$) decreased in all treatment groups as compared to control (G1). However, percentage of undeveloped morulae was not affected by treatments. Despite the observed insignificant improve in embryo development in G3, number of trophoblast cells or inner cell mass/hatched blastocyst significantly ($p<0.05$) increased in G2 and G3 as compared to G1 and G4. On the other hand, combination treatment had no effects on embryo development, but significantly ($p<0.05$) decreased percentage of degenerated embryos as compared to control (Table 8).

DISCUSSION

Exposure of rabbit does to high ambient temperature has well recognized negative consequences on the rabbit industry worldwide. In the current study, rabbit does suffer from severe heat stress throughout the experimental period, as reflected by high average values of THI (31.78)[30]. Under these conditions, reproductive and physiological traits of does were adversely affected[31]. Therefore, the current work sought to assess the beneficial role of SP, vitamin E or their combination for elimination the adverse effects of heat stress on reproductive efficiency, lipid metabolism, antioxidant status, immunity, ovulatory response and embryo development of does.

Increasing free radical production and lipid peroxidation under heat stress lead to marked reduction in reproductive traits NZW does in Egypt[1,32]. The usage of additional natural antioxidants had beneficial effects on reproduction to attenuate the detrimental effects of oxidative stress[5,10]. The observed improvement in reproductive efficiency in treatment groups, in particular, those in G2 treated with SP may be due t o the synergetic effect of various compounds, such as vitamin E, total carotenoids, β-carotene, chlorophyll, phycocyanin, superoxide dismutase in SP (Table 2), total phenolic and flavonoid compounds[21]. Also, SP had all essential amino acids, essential fatty acids, vitamins such as thiamine, nicotinamide, riboflavin, folic acid, pyridoxine, vitamins A, D and E and trace elements[11]. These compounds characterized SP to be strong antioxidant, anti-inflammatory and antiviral properties[31,33].

In accordance with the present results, dietary supplementation of SP significantly improved growth performance of broiler chickens under heat stress[5] and reproductive performance of does under normal conditions[34]. In addition, vitamin E maintained and enhanced reproductive performance in poultry[22]. Most compounds in natural sources of antioxidant were polyphenols, which have important physiological functions[35]. Vitamin E (α-tocopherol) had a vital role in antioxidant defense system of animal body via reducing the cellular free radical damage, protecting cellular plasma membrane against oxidative damage, leading to improvement of proliferation and functions of the cells[36]. Improved lipid profile in the present study by SP and vitamin E, in terms of decreasing concentrations of total lipids, total cholesterol and triglycerides and increasing HDL in blood of rabbits under normal condition[34] and rats[37] under heat stress. Hypolipidemic effect of SP has been reported to be due to the C-phycocyanin which inhibits the activity of pancreatic lipase in a dose-dependent manner[12]. Also, vitamin E affects lipoprotein enzyme that increase lipolysis activity[38].

Under heat stress, lipid peroxidation increased and antioxidant enzyme activity (SOD and GPx) decreased occurring oxidative stress[39]. The SP contains α-tocopherol, phycocyanin and β-carotene, which had strong activity of scavenging acting, individually or together, directly on superoxide radicals[40]. Also, SP maintained the activity of cellular antioxidant enzymes and increased the levels of reduced GSH in these cells. Intriguingly, the antioxidant capacity of SP could be enhanced when exposed to additional

environmental stress[41]. Treatment with SP improved antioxidants capacity of does under normal condition[34] and in heat stressed rats[42]. This impact was proved in the current study by increasing contents of TAC, GPx, GSH, catalase and SOD and decreasing MDA in blood serum of does treated with SP and vitamin E. Moreover, vitamin E administration decreased the physiological response to stress by enzyme defense mechanisms against lipid peroxidation and enhances stability of the cell membrane[17]. Thus, vitamin E supplementation was essential under heat stress[21,43].

Dietary supplementation of SP or vitamin E in this study improved immune responses under heat stress because SP was reported to increase macrophages phagocytic activity, stimulate the production of antibodies and cytokines and activation and mobilization of T and B cells[44]. Similarly, SP increased IgG antibody in heat stressed rats and vitamin E promoted macrophages phagocytic activity[45].

It is worthy noting that enhancing *in vivo* reproductive performance, in term of increasing litter size was in association with increasing in ovulation rate, embryo quality and embryo development beside improvement in antioxidant capacity and immunity of heat stressed treated does, particularly, those treated with SP. This was critical for maintaining the redox balance in the ovaries to support normal ovarian function and development of embryos[9,46]. Similar results of SP or vitamin E was reported on enhancing the ovulatory response and production in pigs[23].

It is of interest to note that SP supplementation had better results than vitamin E, because SP contained several antioxidant compounds (carotenoids, chlorophyll, phycocyanin and superoxide dismutase) beside VE content (Table 2). On the other hand, The results of SP and VE combination in G4 surprised us and no reasons were available to be explained, but may be attributed to overdose of vitamin E from SP as a source of this vitamin as well as vitamin E as a supplement.

CONCLUSION

According to the foregoing results, dietary supplementation with *Spirulina platensis* at a level of 300 mg kg^{-1} diet, in comparing with vitamin E at a level of 100 mg kg^{-1} diet or their combination at the same levels, had positive impact on reproductive performance, lipid profile, antioxidant capacity, immunity status, yield, quality and developmental competence of rabbit embryos under heat stress. In addition, the present study may suggest that no vitamin E supplementation is required if diet of doe rabbits contained *Spirulina platensis.* The physiological efficiencies of antioxidant compounds depend on many different factors and more research will be needed in the future to establish appropriate dosages of *Spirulina platensis* to optimize health benefits and reduce possible negative effects.

SIGNIFICANCE STATEMENT

This study discovered the vital role of antioxidants in natural form for reducing the oxidative stress resulting from high ambient temperature that can be beneficial for improving *in vivo* and *in vitro* reproductive efficiency and healthy status of heat stressed rabbit does. This study will help the researchers to uncover further types, levels and the combined effects of antioxidants. Thus a new theory on the effect of different antioxidants on reproductive hormones profiles, in particular the hormones responsible for ovulation and implantation. Also, the pathologically possible side effects of using antioxidant on a long term treatment period must be studied.

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