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Using Zinc Oxide Nanoparticles, Thyme Oil, and Their Combination to Improve the Reproductive Efficiency of Rabbits during the Summer Season

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ABSTRACT
The present research aims to evaluate the influence of Zn nanoparticles (ZnO-NPs) and/or thyme oil on the reproductive performance of rabbits during the summer season in Egypt. Eighty female New Zealand White rabbits (20 per diet) at approximately 6 months of age with an average live weight of 3500 g were used and divided into 4 groups as follows: in the first group, the animals were given the basic diet without supplements (control group). The animals in the second and third groups were fed a basal diet supplemented with ZnO-NPs at 100 mg/kg or thyme oil at 300 mg/kg diet, respectively. The animals in the fourth group were fed a basal meal that was supplemented with both ZnO-NPs at 100 mg/kg and thyme oil at 300 mg/kg. Rabbits fed ZnO-NPs, thyme, or both during heat stress showed no significant effects on TP, ALB, and GLB variables. In the treated groups, there was a significant ($P<0.05$) drop in the levels of plasma triglycerides (TG) and total cholesterol (TC). The ovulation rate, number of embryos, embryo recovery rate, and pregnancy rate of rabbit females exposed to heat stress were significantly increased when thyme and ZnO-NPs were incorporated into their diets. On the other hand, the number of hemorrhagic embryos and total abnormal follicles decreased compared with the control group. Hence, it is possible to conclude that adding ZnO-NPs and thyme oil to rabbits' diets considerably enhanced their physiological and reproductive performance when the rabbits were exposed to heat stress in the summer. Therefore, given the heat-stressed climatic circumstances in Egypt, it could be advised to feed rabbits with ZnO-NPs (100 mg/kg) and thyme oil (300 mg/kg diet).

INTRODUCTION
Rabbits are susceptible to the effects of heat stress correlated with the physiological and hormonal changes that take place during the reproductive cycle (Mady, et al., 2018). Therefore, dietary functional supplements could lessen the adverse impacts of heat stress on rabbits (El-Kholy *et al.*, 2021; Hashem *et al.*, 2021).

Zinc (Zn) is essential for proper physiological body functions, including normal growth, protein synthesis, and energy production, membrane protection from bacterial endotoxins, lymphocyte proliferation, and antibody production. Although rabbits supplemented with premix without Zn showed reduced growth and feed conversion rate, this still highlights the importance of Zn for normal growth (Case and Carlson, 2002). Dietary nano-minerals have positive effects due to their small particles which are reasonable to diffuse through mucous membranes, penetrate deeply into tissues through tiny capillaries, cross epithelial lining fenestration (such as the liver), and allow for efficient cell uptake and delivery of active compounds to target sites in the body. Additionally, nano-minerals, which have a larger surface area and are therefore used to improve the bioavailability of minerals in the livestock business, have been shown to boost growth performance parameters and overall health conditions.

Because of their protective effect against oxidative stress, phytogenics have garnered increasing interest in recent years as natural, safe antioxidants that are regularly utilized for animal feeding. Furthermore, phytogenic compounds can influence various physiological and biological processes within the animal body (El-Ratel et al., 2020; El-Essawy et al., 2021). Herbs and herbal extracts were added to rabbit diets to improve the animals' ability to productive and reproductive performance. According to Raskovic et al. (2015), phytogenic chemicals are widely utilized in the food, and feed industries, and are usually considered safe. Additionally, under hot environmental conditions, herb extracts may benefit animal welfare, health, and performance (Attia et al., 2016).

Similarly, rabbits' intestinal integrity and antioxidant status were markedly enhanced by supplementing with 0.5 g/kg thyme oil (Placha et al. 2013). Carvacrol, thymol, γ-terpinene, p-cymene, β-myrcene, and linalool are the principal constituents of thyme oil (Abdel-Wareth et al. 2018 and Lee et al. 2005). According to Rota et al. (2008), the active ingredients have antioxidant roles that lower body fat and blood cholesterol (Abdulkarimi et al. 2011). Placha et al. (2013) and Abdel-Wareth et al. (2018) suggested that dietary thyme oil supplementation may affect feed intake, body weight gain, meat quality, antioxidants, gut integrity, and feed conversion ratio overall.

On the other hand, there is no information available about the interaction between thyme compounds and Zn nanoparticles (ZnO-NPs) with regard to their efficiency and their impact on the reproductive performance of rabbits during the summer.

Thus, the current study's goals were to ascertain how dietary ZnO-NPs supplementation (100 mg/kg diet) and/or thyme oil (300 mg/kg diet) addition to diets affected the activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione (GSH), and total antioxidant capacity in addition to biochemical parameters in the plasma and reproductive performance of rabbits.

MATERIALS AND METHODS:

The current animal experiment was carried out during the summer at a private commercial rabbit farm in a village in Mansoura city, Dakahlia Province, Egypt in cooperation with the Animal, Poultry and Fish Production Department, Faculty of Agriculture, Damietta University, Egypt. ZnO-NPs, thyme oil, and their combination as a supplement were investigated for their effects on rabbits' productive performance using a completely randomized design. The procedures used in the experiments adhered to the Egyptian Research Ethics Committee's guidelines as well as the guidelines found in the 2011 Guide for the Care and Use of Laboratory Animals, which was updated and approved by the Institutional Animal Care and Use Committee (AU-IACUC) at Damietta University (approval number AU 08 21 07 26 2 82).

Preparation and description of ZnO nanoparticles in powder form:

ZnO nanoparticles were created via the wet chemical method by utilizing the naturally occurring polysaccharide, namely, sodium alginate, and the precursor, zinc nitrate, $Zn(NO_3)_2.4H_2O$ in the existence of alkaline solution of sodium hydroxide (NaOH) according to method Desai et al. (2019). To characterize ZnO nanoparticles, X-ray diffraction (XRD) was recorded under ambient conditions through a Siemens D-500 X-ray diffractometer (from 30 mA to 40 kV) bearing a copper (Cu) tube. The morphological description of the ZnO nanoparticles was discovered during transmission electron microscopy (TEM) on a JEOL (JEM-1230, Japan); the instrument had an acceleration voltage of 120 kV.

Component analysis of thyme oil:

Thyme oil was purchased from El Hawag Natural Oils Company, Cairo, Egypt. Components of essential oil were analyzed at the Department of Medicinal and Aromatic Plants Research, National Research Centre, Egypt, by gas chromatography as described by Juliano et al. (2000). Based on the analysis of thyme oil, it was found that it contains some active compounds such as thymol (46.1%), ρ-cymene (12.5%), γ-terpinene (18.5%), Carvacrol (3.5%), Eugenol (0.5%), and linalool (3.3%).

Four experimental diets were designed to provide all of the key dietary needs of rabbits according to De Blas and Mateos (1998). The following are the experimental groups: in the first group, the animals were given the basic diet without supplements (control group). The animals in the second and third groups were fed a basal diet supplemented with nano-zinc at 100 mg/kg or thyme oil at 300 mg/kg diet, respectively. The animals in the fourth group were fed a basal meal that was supplemented with both nano-zinc at 100 mg/kg and thyme oil at 300 mg/kg. The nutritional composition and formulation of diets are displayed in Table (1).

Table (1): Ingredients and chemical analysis of the diet used for feeding rabbits in different experimental treatments.

Ingredient	%
Clover hay	31.00
Barley grain	24.60
Wheat brain	28.00
Soybean meal	13.25
Di-calcium phosphate	1.60
Limestone	0.95
Sodium chloride	0.30
Mineral-vitamin premix[a]	0.30
Chemical analysis	**(as % on dry matter**
Crude protein	17.08
Crude fiber	12.55
Ether extract	2.20
Methionine (%)	0.23
Total phosphorus (%)	0.761
Metabolisable energy (ME, kcal/kg)	2219
Digestible energy (kcal/ kg)	2416

[a]One kilogram of minerals—vitamins premix provided as Vitamin E, 100 mg; Vitamin A,150,000 IU; Vitamin B1, 10 mg; Vitamin K3, 21 mg; Vitamin B2, 40 mg; Vitamin B6, 15 mg; Vitamin B12, 0.1 mg; Pantothenic acid, 100 mg; Niacin, 200 mg; Biotin, 0.5 mg; Folic acid, 10 mg; Cholinechloride, 5000 mg; Cu, 50 mg; Fe, 0.3 mg; Mn, 600 mg;; Co, 2 mg; Se, 1 mg; and Zn, 450 mg.

Animal procedure: Eighty female New Zealand White rabbits (20 female/group), weighing an average of 3500 g live, and around 6 months old, were used during the period from 1 July 2022 to 30 Septamber 2022. Semen was artificially inseminated into rabbit females at diluted (1 semen: 5 diluents) with Tris-extender (0.5 ml/doe). During the experiment, a cycle of 16 hours of light and 8 hours of darkness was used.

Using an automatic thermo-hygrometer, the air temperature and relative humidity within the rabbit building were measured every day between 12:00 and 14:00 p.m. The following formula, which was altered by Marai et al. (2001), was used to calculate the temperature-humidity index (THI):

THI = db˚C – [(db˚C – 14.4) (0.31 – 0.31 RH)]

Where: db˚C = dry bulb temperature in ˚C and RH = relative humidity percentage/100.

The resulting THI values were then divided into four categories: moderate heat stress (27.8 to <28.9), severe heat stress (28.9 to <30.0), and very severe heat stress (30 and above).

The rabbit building's recorded air temperature varied between 30.7 and 37.6 degrees. The rabbit building has a relative humidity range of 50–70%. These numbers were used to calculate the temperature-humidity index (THI) range within the rabbit building, which was 28.17–35.44 (heat stress conditions).

Palpation of the abdomen is used to determine pregnancy ten days following insemination. The rabbits were housed in individual cages made of galvanized wire net, measuring 48 cm in width, 55 cm in length, and 38 cm in height. The cages had manual feeders and automatic drinkers. The experimental period lasted thirty days.

The female rabbits were housed in sanitary, well-managerial conditions during the experiment period. The physical condition and overall health of the animals were routinely assessed. Thereafter, the rabbits' reproductive performance was assessed.

Measurements: The temperature, moisture content, and TH index were determined and monitored. The used diet for the rabbit females of this study is displayed in Table (1) and the evaluation indicators of blood biochemical analysis, blood hematology, redox, cellular immunity status, and reproductive performance indicators including ovulatory response were investigated and evaluated as follows.

Blood Collection: Six rabbits were chosen at random from each treatment at the end of the treatment time so that blood could be drawn. Blood samples were extracted from the does' marginal ear veins, submerged into heparinized tubing, and then split into two distinct subsamples for various analyses. The second subsample was centrifuged for 15 minutes at 3000 rpm in the meantime. Following centrifugation, the blood plasma sections were separated and kept in 1.5 mL Eppendorf tubes at − 20 °C for further analysis.

Biochemical blood parameters: Using reagent kits from Spectrum Diagnostics Egyptian Company for Biotechnology, Egypt, blood profile parameters such as total protein (g/dL), albumin (g/dL), triglycerides (mg/dL), total cholesterol (mg/dL), high-density lipoprotein (mg/dL), urea, and creatinine levels were assessed. Concerning the globulin value (determined from the equation of total protein–albumin).

Reproductive performance (*In vitro* study): To assess the ovulatory response parameters, three conceived rabbit females from each group were collected, brought to the laboratory, and slaughtered 72 hours after insemination. The ovaries were taken as soon as they were slaughtered and placed in 60 x 15 mm plastic flacon tissue culture dishes containing saline solution at 38.5°C. For every female, the count of visible follicles on the ovarian surface measuring ≥2 mm in diameter (LF), corpora lutea (CLs), and hemorrhagic follicles (HF) was noted. The following formulas were used to determine the total follicles (TF) and ovulation rate (OR):

OR (%) = (Number of corpora lutea / Total follicles) × 100

TF = Number of LF + HF.

Using phosphate buffer saline that contained 10% fetal calf serum and 50 µg of gentamycin per milliliter, embryos were extracted from the reproductive tracts of animals that had been slain (all at the morulae stage). After embryo searching by stereoscopic microscope, the number of embryos found, and the embryo recovery rate (ERR) were calculated.

ERR = (number of embryos/number of corpora lutea) × 100.

The number of embryos at the morula stage (n = 459) was noted, and after three PBS washes, the morphology of the mucin coat, zona pellucidae, blastomeres, and refractive cytoplasm was examined under a stereoscopic microscope to determine which embryos were of good quality (n = 416) and poor quality (n = 43).

Reproductive performance of does (*In vivo* study): The rest number of rabbit females in each group was left to continue post-insemination, and pregnancy was diagnosed by abdominal palpation on day 10 post-insemination. The number of confirmed pregnancies was recorded, and the pregnancy rate (PR) was computed:

PR = (Number of pregnant females / number of mated females) × 100.

Nest boxes were added to the cages of the female rabbits two days before the anticipated date of delivery. The kindling rate (KR), following parturition, was computed.

KR = (Number of kindled females / number of pregnant females) × 100.

At birth, born (TB) and number born alive (NBA), after 12 hr of kindling per rabbit female were determined, and then viability rate (VRB) was computed

VRB = (number born alive / total born) × 100.

Also, the number weaned (NW) and viability rate were determined at weaning. Additionally, individual body weight and litter weight at birth and at weaning were recorded.

Statistical analysis: SAS (2009) was used to conduct statistical analysis. Analysis of variance was used to test the effect of treatment, and the differences between means were detected by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION:

Impacts of HS on Some Blood Constituents:

In rabbits subjected to heat stress, the blood biochemical index is a crucial sign of metabolic alterations and organ damage. When rabbits experience heat stress, their levels of triglycerides and total protein decrease, but their levels of cholesterol dramatically increase (Hen and Wang, 2004). These results are caused by increased glucocorticoid release, which activates the gluconeogenesis pathway (Gallagher and Mcdowell, 1990). According to a previous study (Amici et al., 2000), heat stress inhibits immunoglobulin synthesis and immune cell proliferation, which hurts immune cell differentiation and proliferation. Yang et al. (2012) showed that the cellular stress response, oxidative stress, apoptosis, and glucose metabolism during heat stress were all impacted by the differently expressed genes in immune cells.

When compared with the control group, the heat-stressed rabbit females that received ZnO-NPs, thyme oil, or both did not show any statistically significant variations in the concentrations of TP, ALB, or GLB (Table, 2).

The administration of thyme and/or ZnO-NPs increased TP, ALB, and GLB concentrations, but not significantly, according to the results. Consistent with our results, Attia et al. (2018) observed that dietary thyme powder significantly enhanced the protein metabolites of heat-stressed Arbor Acres broilers from one to twenty-eight days of age. According to Kucukgul Gulec et al. (2013), adding thyme-containing herbal essential oils to the diet raised albumin and a few other electrolyte markers. Conversely, the addition of thyme powder resulted in lower quantities of blood total protein and its fraction (albumin) (Hosseini et al., 2013). According to El-Faham et al. (2015), there was no discernible impact of dry thyme powder (1.5 and 3 g/kg diet) on blood protein levels in broilers.

Table 2. Effect of adding ZnO-NPs, thyme oil, and their combination on total protein, albumin and globulin of rabbits exposed to heat stress conditions.

Item	Treatments			
	T1	T2	T3	T4
TP (g/dL)	6.37±0.20	6.78±0.20	6.80±0.21	6.98±0.21
Albumin (g/dL)	3.33±0.07	3.20±0.07	3.36±0.07	3.34±0.06
Globulin (g/dL)	3.03±0.22	3.57±0.22	3.43±0.22	3.64±0.22

Mean values followed by different superscript letters in the same row are significantly different (p <0. .05); T1: Basal diet (B); T2: B + nano-zinc (100 mg/kg); T3: B + thyme oil (300mg/kg); T4: B + nano-zinc (100mg/kg) + Thyme oil (300 mg/kg).

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When compared to the control group, giving thyme aqueous extract increased serum TP and ALB levels, according to Swayeh et al. (2014) and Abu-Raghif et al. (2015). Attia et al. (2017) found that plasma total protein and globulin in boilers treated with thyme oil were higher than in the control group and decreased liver function (ALT and AST) in all treated groups. This suggests that phytogenic extracts, particularly thyme extract, which is rich in antioxidants and poly-unsaturated fatty acids, are improving overall health care and promoting liver functions (Assiri et al. 2016 and Attia et al., 2017). Furthermore, Bovera et al. (2015) found that treated groups with higher globulin values and lower Albumin/globulin ratio had enhanced resistance to illness and immunological response. According to Bozkurt et al. (2012) and Attia et al. (2017), the administration of ZnO-NPs and/or thyme to rabbits may have improved their overall health status due to enhanced feed digestion and absorption, as well as antibacterial and antioxidant effects and improved fatty acid profile. Thymol, carvacrol, and linalool are the main ingredients in thyme; these compounds have antimicrobial, increased digestibility, and antioxidant qualities (Cross et al., 2003 and Bozkurt et al., 2012).

The influence of ZnO-NPs and/or thyme on the lipid profile is seen in Table (3). When compared to the control group, the treated groups had considerably (p <0.05) lower levels of total cholesterol and plasma triglycerides, but significantly (p <0.05) greater levels of high-density lipoprotein. Furthermore, compared to groups 2 (treated with ZnO-NPs) and 3 (treated with thyme extract), group 4 (treated with a combination of nano-zinc and thyme) showed a significant reduction in total cholesterol and triglycerides.

ZnO-NPs and/or thyme may have the effect of lowering blood cholesterol levels by inhibiting the synthesis of cholesterol within cells. Plant thyme may help reduce some of the risk factors linked to the development of cardiovascular disease in both people and animals, as evidenced by reduced serum cholesterol (Ademola et al. 2009).

Table 3. Effect of adding ZnO-NPs, thyme oil, and their combination on cholesterol, triglycerides and HDL of rabbits exposed to heat stress conditions.

Item	Treatments			
	T1	T2	T3	T4
TC (mg/dL)	97.32±1.47[a]	87.95±1.46[b]	81.66±1.47[bc]	75.28±1.46[c]
Trig. (mg/dL)	73.70±2.52[a]	72.67±2.53[ab]	71.27±2.53[ab]	69.27±2.52[b]
HDL (mg/dL)	50.36±1.88[b]	51.48±1.89[b]	53.75±1.88[b]	58.69±1.89[a]

[a,b,c] Mean values followed by different superscript letters in the same row are significantly different (p <0. .05); T1: Basal diet (B); T2: B + nano-zinc (100 mg/kg); T3: B + thyme oil (300mg/kg); T4: B + nano-zinc (100mg/kg) + Thyme oil (300 mg/kg). TC: total cholesterol; Trig: triglycerides; HDL: high-density lipoprotein.

The presence of zinc in nanoparticle form gives it a potent hypolipidemic effect compared to the regular form, which has no important effect (Samman and Roberts, 1988). This is why the lipid profile in the serum of rabbits treated with ZnO-Nps showed a significant decline in total cholesterol and triglycerides and a rise in high-density lipoprotein. According to Al-Daraji and Amen (2011), zinc is essential for the function of many enzymes, or metalloenzymes, which are used in the digestion and absorption of lipids. There is a strong correlation between total cholesterol and HDL-cholesterol (TC/HDL-c), which indicates the risk of heart disease incidence; the higher the ratio, the higher the risk, by increasing the incidence of chronic heart disease and myocardial infarction. This is because total cholesterol is composed of small dense LDL particles, which are more atherogenic, and high-dense HDL particles, which are thought to be protective. So, ZnO-Nps supplementation in rabbit diets lowers the incidence of cardiac disease and, thus, lowers the death rate in rabbits. Alike findings were also stated by Din and Noha (2019), who found that the ZnO-NPs supplemented rabbit group demonstrated a highly significant rise in serum HDL-C levels and a highly significant diminish in serum TG, TC, and VLDL-C levels when compared with the control treatment. Furthermore, supplementing with a ZnO-NPs diet reduced serum total cholesterol, triglycerides, and LDL-cholesterol while there were no significant variations in cholesterol; conversely, HDL-cholesterol raised ($P<0.05$) when compared to the control diet, according to Ahmadi et al. (2013).

Khafar et al. (2019) observed a diminish in total cholesterol in broiler diets give thyme oil (150 and 200 mg/kg). According to Shamma et al. (2019), broiler chicken blood total cholesterol was decreased ($P<0.05$) when 0.4 ml of thyme oil was added per kilogram of food. Additionally, thyme supplementation in the meal (300 or 450 mg/kg) decreased total cholesterol in quail serum as compared with the control group (Gümüş et al., 2017). Feeding broilers meals containing 200 mg of thyme oil per kg reduced the broilers' serum cholesterol levels (Al-Mashhadani, et al., 2011). In a similar vein, numerous studies have shown that adding thyme and other phytogenic feed additives lowered the blood cholesterol values of chickens (El-Ghousein and AlBeitawi 2009; Manafi et al., 2016). The reduction in blood cholesterol levels might be ascribed to either enhanced bile acid release (Amad et al., 2011) or raised synthesis of digesting enzymes (Jang et al., 2004).

According to El-Ghousein and Al-Beitawi (2009), Abdulkarimi et al. (2011), Khan et al. (2012), and others, carvacrol in thyme exhibits hypocholesterolemic and antilipidemic actions on HMG-CoA (3-hydroxy-3-methylglutaryl) reductase, which may lessen the lipid catabolism for gluconeogenesis or the absorption of fat from the gut. Increased bile and digestive enzyme secretion improves lipid digestibility, which may be the cause of the decreased serum cholesterol (Manafi, 2015). According to Yalcin et al. (2020), this pattern suggested that thyme may have hypolipidemic and antioxidative effects on laying hens.

When compared to the control, the heat-stressed rabbit female which receive ZnO-NPs, thyme, and their combination show significant differences ($p<0.05$) in the values of creatinine and urea (Table, 4). The heat-stressed rabbits' renal functions considerably declined when ZnO-NPs, thyme, and their combination were added to their diets compared to the control group. The addition of thyme and ZnO-NPs to the meal significantly improved the kidney functions of heat-stressed rabbits.

Table 4. Effect of adding ZnO-NPs, thyme oil, and their combination on kidney functions of rabbits exposed to heat stress conditions.

Item	Treatments			
	T1	T2	T3	T4
Urea (mg/dL)	39.28±1.64^{a}	32.58±1.65^{b}	30.25±1.65^{b}	27.24±1.65^{c}
Creatinine (mg/dL)	1.33±0.06^{a}	1.22±0.05^{a}	1.18±0.06^{b}	1.16±0.06^{b}

^{a,b,c} Mean values followed by different superscript letters in the same row are significantly different (p <0. .05); T1: Basal diet (B); T2: B + nano-zinc (100 mg/kg); T3: B + thyme oil (300mg/kg); T4: B + nano-zinc (100mg/kg) + Thyme oil (300 mg/kg).

According to this study, there were notable changes in the concentrations of urea and creatinine between the experimental treatments. Furthermore, it was demonstrated that thyme and/or nano-zinc had a substantial ($p<0.05$) impact on lowering the concentrations of creatinine and urea in the blood. Thus, in comparison with the control group, we may conclude that nano-zinc and/or thyme may have a beneficial effect on the elimination of urea and creatinine from plasma. The T4 rabbits had the lowest urea and creatinine levels.

As for the addition of thyme, in rabbits, thyme extracts (100 mg/kg) dramatically lowered creatinine (Abdel-Gabbar et al., 2019). When thyme oil (150, 300, and 450 mg/kg) was added, the quails' serum creatinine concentration was lower than in the control treatment (Gümüş et al., 2017). When compared to a control group exposed to heat stress conditions, Abdelnour et al. (2020) discovered that thyme essential oil (100 mg/kg diet) resulted in the lowest blood creatinine levels in rabbits after 30 days. Additionally, thyme oil dramatically reduced the serum creatinine levels in rabbits (Abdel-Wareth et al., 2020). Thyme leaf addition reduced serum creatinine in comparison with the control group (Ezzat et al., 2020). When compared to a control group, the creatinine value was lowered when rabbits received diets added with thyme oil at doses of 60, 120, and 180 mg/kg (Abdel-Wareth and Metwally, 2020). Blood creatinine concentrations in rats fed diets supplemented with 2.5 percent thyme leaves were found to be significantly lower (Salem, 2015).

According to El-Kholy et al. (2021), organic zinc (75 mg Zn picolinate/kg diet) or inorganic zinc (75 mg $ZnSO_4$/kg diet) might lessen the negative effects of heat stress on the renal and liver functions, testosterone levels, and antioxidative qualities, in the blood of NZW rabbit bucks suffering from heat stress conditions.

Dietary antioxidants and naturally occurring phenolic compounds may have an impact on kidney function because of their capacity to stimulate the endogenous antioxidant defense system by scavenging free radicals and promoting the synthesis of various antioxidants such as glutathione, thioredoxin, SOD, and catalase through the upregulation of NrF-2 (nuclear factor erythroid 2-related factor). The renal antioxidant system protects every part of the kidney, including the glomeruli, tubules, and renal arteries, against oxidative damage. As a result, the kidney continues to function normally (Ratliff et al., 2016). The latest findings corroborate our earlier research, which suggested that feeding rabbits an organic zinc supplement may enhance their kidney's antioxidant status (Cobanová et al., 2018).

Reproductive performance:

Hot stress has an impact on the pregnancy rate, growth of embryos, milk production, litter size and litter weight, in female rabbits (Marai et al., 2002). High temperatures can lead to abnormal morphology of egg cells, including cytoplasmic shrinkage and rupture of the transparent membrane, which prevents egg cells from fertilizing and affects the reproduction of rabbits. They also significantly reduce estrogen secretion and cause irregular estrus (Garcíal and Argente, 2017). Rabbits who are expecting to distribute a lot of blood to their skin to dissipate more heat. However, with heat stress, the amount of blood in the uterus and umbilical cord significantly decreases, which leaves the fetus and embryo sac with a critical blood shortage. Following that, the embryo shrinks, which hinders the fetus's growth and raises the mortality rate (Marco-Jiménez et al., 2017). Litter and pregnant rabbits lose a large amount of weight while under heat stress (Marai et al., 2002). In pregnant rabbits, mutant heat stress can result in abortion, neurological problems, and salivation. Pregnant rabbits are particularly vulnerable to this kind of heat stress, according to Song et al. (2006). Furthermore, compared to thermoneutral temperature, elevated ambient temperature during late pregnancy negatively affected the breastfeeding of female rabbits (Hen Li and Wang, 2004).

Heat-stressed rabbit females' ovulation rate, embryo number, embryo recovery rate, and pregnancy rate were all significantly increased when ZnO-NPs, thyme, or both were added to their diets. In contrast to the control group, there was a drop in the percentage of abnormal embryos (%), hemorrhagic follicles, and lambing rate. Supplementing with dietary ZnO-NPs, thyme, or both boosted ovulation and the percentages of normal embryos significantly ($p<0.05$) when compared with the control (Tables 5–8). Overall, the heat-stressed rabbit does' embryo quality was significantly improved by the addition of thyme and/or ZnO-NPs to their diet.

The addition of ZnO-NPs, thyme, or their combination improved reproductive performance (Tables 5–8), with a significant ($p<0.05$) rise. Equally, the pregnancy and lambing rates are expected to rise considerably ($p<0.05$). Table 8 shows how the administration of thyme, ZnO-Nps, or their combination can affect the ovulatory response. Thyme in conjunction with ZnO-NPs considerably reduced the number of follicles (large, hemorrhagic, and total follicles) per female. Antioxidants can counteract oxidants and other cellular protection to boost the effectiveness of the antioxidant defense system and prevent cellular damage under heat stress (Mittler et al., 2004). Natural sources of antioxidants are vital for improving the immune system, reproductive effectiveness, and overall health of rabbits (El-Ratel et al., 2017). Furthermore, in animals and fowl, thyme enhances the digestive system's capacity to produce nutrients that are available in the intestine for the body's needs (Windisch et al., 2008). Furthermore, essential oils may raise ovarian activity and intestinal nutritional digestibility (Olgun, 2016). Many biological activities for a healthy state have been linked to thyme, a natural antioxidant: hypoglycemic, anti-microbial, immunomodulatory, and anti-inflammatory (Indrasanti, et al., 2017).

Table 5. Effect of adding ZnO-NPs, thyme oil, and their combination on reproductive traits of rabbits exposed to heat stress conditions.

Item	Treatments			
	T1	T2	T3	T4
CL (n)	16.67±1.15	15.33±1.14	16.00±1.15	18.33±1.14
OR (%)	68.17±7.88^{ab}	64.60±7.89^{b}	76.17±7.88^{ab}	98.73±7.88^{a}
EN (n)	13.67±1.36^{b}	14.33±1.35^{ab}	15.33±1.36^{ab}	17.33±1.35^{a}
ER (%)	81.73±5.44^{b}	93.93±5.45^{a}	95.57±5.44^{a}	94.70±5.45^{a}

^{a,b,c} Mean values followed by different superscript letters in the same row are significantly different (p <0. .05); T1: Basal diet (B); T2: B + nano-zinc (100 mg/kg); T3: B + thyme oil (300mg/kg); T4: B + nano-zinc (100mg/kg) + Thyme oil (300 mg/kg); CL: corpora lutea; OR: Ovulation rate.

Table 6. Effect of adding ZnO-NPs, thyme oil, and their combination on ovarian activity of rabbits exposed to heat stress conditions.

Item	Treatments			
	T1	T2	T3	T4
LF	20.67±1.65^{a}	19.00±1.66^{a}	17.67±1.65^{ab}	15.33±1.65^{b}
HF	4.67±1.00^{a}	5.00±1.00^{a}	3.67±1.00^{b}	3.33±1.00^{b}
TF	25.33±2.29^{a}	24.00±2.28^{a}	21.33±2.29^{ab}	18.66±2.29^{b}

^{a,b,c} Mean values followed by different superscript letters in the same row are significantly different (p <0. .05); T1: Basal diet (B); T2: B + nano-zinc (100 mg/kg); T3: B + thyme oil (300mg/kg); T4: B + nano-zinc (100mg/kg) + Thyme oil (300 mg/kg); LF: Number of visible follicles with ≥2 mm in diameter; HF: hemorrhagic follicles; TF: Total follicles.

Table 7. Effect of adding ZnO-NPs, thyme oil, and their combination on conception (pregnancy rate) of rabbits exposed to heat stress conditions.

Item	Treatments				
	T1	T2	T3	T4	Total
Pregnant	14	17	18	18	67
Non-pregnant	6	3	2	2	13
Total	20	20	20	20	80

Chi square= 3.94 DF= 3, p-value= 0.26.

Table 8. The effect of adding Zno-NPs, thyme oil, and their combination on the lambing rate of rabbits exposed to heat stress conditions.

Item	Treatments				
	T1	T2	T3	T4	Total
Lambing	11	15	16	17	59
Non-lambing	3	2	2	1	8
Total	14	17	18	18	67

Chi square= 1.90, DF= 3, p-value= 0.59.

Table 5 indicates that does treat with ZnO-NPs and thyme had more stimulation of the ovulatory response, as evidenced by the decrease in total follicles and the increase in CLs and embryo yield, which in turn improved the rate of ovulation. It is possible to ascribe the observed trend of increased CLs (p<0.05) in treatment T4 that consumed ZnO-NPs in combination with thyme to the thyme's ability to raise LH surge in comparison to the control group, which indicates a greater ovulation rate. According to Dufy-Barbe et al. (1973), the rabbit is a reflexively ovulating animal in which sensory and neuro-endocrine cues work in concert to cause an LH pre-ovulatory surge and record the ovulatory response. Thyme extract has been shown to activate the anterior pituitary, which in turn increases the release of gonadotropins (Obochi et al., 2009). Antioxidants derived from natural plants may have positive impacts on ovarian functioning and ovulation (Zhong & Zhou, 2010). Remarkably, thyme or zinc may have a direct impact on ovarian tissues and activity, as well as an indirect influence on rabbits' immunity and health state, which could account for the improved embryo quality in treatment groups. Thus, in rabbits subjected to oxidative stress in conditions of heat stress, thyme and zinc, as antioxidants, may alleviate gonadal-pituitary hormone disruption (Al-Masri, 2015).

These results suggest that the good heat tolerance and antioxidant effects of ZnO-NPs and thyme oil, alone or in combination, contributed to the protective benefits observed during hot climates. Consequently, the reproductive efficiency of the female rabbits was enhanced.

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الملخص العربي

استخدام جزيئات أكسيد الزنك النانوية وزيت الزعتر ومزيجهما لتحسين الكفاءة التناسلية للأرانب خلال موسم الصيف

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أجريت هذه التجربة لتقييم استخدام جزيئات الزنك النانونيه (ZnO-NPs) وزيت الزعتر ومزيجهما على الأداء الإنتاجي والتناسلي للأرانب ومواليدها. تم عمل أربع معاملات تجريبية على النحو التالي: في المعاملة الأولى تلقت مجموعة الحيوانات الغذاء الأساسي بدون أي مكملات وتم فصلها كمجموعة ضابطة (كنترول). في المعاملة الثانية، تلقت الحيوانات غذاء أساسيًا مكملًا بـ ZnO-NPs بجرعات 100 مجم / كجم من الغذاء، والمعاملة الثالثة، تلقت الحيوانات غذاء أساسيًا مكملًا بزيت الزعتر بجرعات 300 مجم / كجم من الغذاء، بينما في المعاملة الرابعة، تلقت الحيوانات غذاء أساسيًا مكملًا بـ 100 مجم نانو زنك / كجم وزيت الزعتر بجرعات 300 مجم / كجم من الغذاء. تم استخدام ثمانين أرنبًا من إناث النيوزلندي الابيض (20 أنثى لكل معاملة) يبلغ عمرها حوالي 6 أشهر بمتوسط وزن حي يبلغ 3500 جرام، لتقييم تأثير الأنظمة الغذائية التجريبية على الأداء التناسلي للأرانب. استمرت التغذية بالتجربة لمدة شهر واحد. لم تظهر الأرانب التي تعرضت لإجهاد حراري أي اختلافات معنوية في تركيزات TP وALP وGLB عند تلقيها ZnO-NPs والزعتر ومزيجهما مقارنة بمجموعة الكنترول. أدى تناول ZnO-NPs وزيت الزعتر ومزيجهما في وجبات الأرانب التي تعرضت لإجهاد حراري إلى انخفاض كبير في وظائف الكلى مقارنة بمجموعة الكنترول. أدى إضافة ZnO-NPs وزيت الزعتر ومزيجهما إلى وجبات إناث الأرانب المعرضة للإجهاد الحراري إلى زيادة كبيرة في معدل التبويض وعدد الأجنة ومعدل تعافي الأجنة ومعدل الحمل. بينما كان هناك انخفاض في عدد الأجنة النزفية والإجمالية غير الطبيعية (%) وكذلك معدل الولادة مقارنة بمجموعة الكنترول. ومن ثم يمكن أن نستنتج أن المكملات الغذائية من ZnO-NPs وزيت الزعتر ومزيجهما أدت إلى تحسين الأداء الفسيولوجي والتناسلي بشكل ملحوظ للأرانب المعرضة للإجهاد الحراري خلال فصل الصيف. لذلك، يمكن التوصية بتزويد الأرانب بالعلائق المحتوية على ZnO-NPs (100 مجم / كجم من الغذاء) وزيت الزعتر بمعدل (300 مجم / كجم من الغذاء) ومزيجهما هو أمر مستحسن في ظل الظروف البيئية المصرية المجهدة بالحرارة.