JSAES
Journal of Sustainable Agricultural and Environmental Sciences
Print ISSN : 2735-4377
Online ISSN : 2785-9878
Homepage: https://jsaes.journals.ekb.eg/

Research Article

Effect of Curcumin Nanoparticles on Reproductive Performance, Haemato-Biochemical Parameters, Antioxidants, Immunity and *In Vitro* Embryo Production of Doe Rabbits

El-Ratel I. T. [1], Rizk R. M. [2], Hammad M. E. R [2] and Gabr A. A. [2,*]

[1] Department of Poultry Production, Faculty of Agriculture, Damietta University, Damietta, Egypt
[2] Department of Animal Production, Faculty of Agriculture, Tanta University, Tanta, Egypt
* Correspondence: Gabr, A.A.

Article info: -

- **Received:** 18 October 2024
- **Revised:** 31 December 2024
- **Accepted:** 7 January 2025
- **Published:** 12 January 2025

Keywords:
Rabbits; Immunity; Antioxidants; Reproduction; Curcumin; Nanoparticles.

Abstract:

The present trial investigated the efficacy of dietary curcumin nanoparticles on reproductive performance, haemato-biochemical parameters, antioxidants, immunity and in vitro embryo production of rabbit does. Mature rabbit does (APRI line) were distributed into 4 groups. Does in the first treatment were fed commercial diet (CP) as the control group. The rabbits in 2nd, 3rd, and 4th were fed CP with curcumin nanoparticles (CURNPs) at levels of 3,5, and 7 mg per kg diet respectively. All does were naturally mated with fertile APRI bucks (five bucks per treatment). Results showed that does in all treatments showed significantly ($P<0.05$) better in vivo reproductive efficiency in terms of rates of conception, kindling rate and litter size, as well as total cholesterol, triglycerides, high and low density lipoproteins, antioxidant status such as concentrations of total antioxidant capacity, glutathione, and malondialdehyde, as well as activity of glutathione peroxidase, glutathione S-transferase, superoxide dismutase and catalase, immunity, ovulatory response of CLs number and ovulation rate), in vitro embryo quality, and hatched blastocysts production as compared to other treatments. In conclusion, dietary supplementation with curcumin nanoparticles had positive effects on *in vivo and in vitro* reproductive efficiency of doe rabbits.

1. Introduction

Heat stress has become a widespread concern worldwide, which is a major environmental stress that causes substantial economic loss in the rabbit industry (Ebeid et al., 2023). Exposing rabbits heat stress (HS) for long periods, affects their productivity, causing large economic losses (Ayyat et al., 2018). It is well known that HS is the highest hazard factor deteriorating growing rabbits' growth performance and viability (Al-Sagheer et al., 2017; El-Ratel et al., 2020). Also, HS reduces immunity and increases free radicals (ROS) and cellular peroxidation of lipid were shown by Mirzaie et al. (2018). Heat stress increases corticosteroid levels and decreases reproductive performance of rabbit does in terms reduced reproductive hormones secretion, which affects ovarian development and ovulation (El-Ratel et al., 2020; Arabameri et al., 2017).

Under thermoneutral conditions, there is equilibrium between the generation and elimination of ROS by the antioxidative system. Enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) scavenge reactive oxygen species (ROS), which are neutralized by the antioxidative defense system (Ebeid et al., 2023). While under HS conditions, the redox balance is disturbed, and consequently, the ROS generation is elevated, leading to oxidative stress in rabbits (Mutwedu et al., 2021; Jimoh et al., 2018). Exposure to HS resulted in reducing the activities of antioxidative enzymes, including GSH-Px, SOD, and CAT, and increasing serum oxidative markers such as protein carbonyl (as an index of amino acids oxidation) and malondialdehyde (MDA, as an index of lipids peroxidation) in growing rabbits (Saghir et al., 2023).

Several realistic strategies were used to alleviate the detrimental effects of rising temperatures, including dietary manipulation, which is becoming increasingly important in various parts of the world. Feed additives of nutraceuticals, including vitamins, minerals, antioxidants, probiotics, prebiotics, symbiotic, enzymes, organic acids, fatty acids, medicinal plants, etc., gain a great concern nowadays as available approaches to relieve the unfavorable impacts of HS via maintaining the common biological situation, strengthening the immune responsiveness, and preventing the illness that ultimately led to an increase in productivity (Al-Sagheer et al., 2017; El-Ratel et al., 2020; Ebeid et al., 2023).

Phytogenic additives such as curcumin (CUR), the yellow pigment of turmeric (*Curcuma longa*), is considered chemopreventives and antioxidants agent (Rahmani et al., 2018). Turmeric is a combination of three molecules defined as curcuminoids. The CUR (60%–70%) is the most rep-resented component, followed by dimethoxy-curcumin (27%) and bisdemethoxycurcumin (15%). The CUR, as a polyphenolic compound, has been reported to possess antioxidant, antimicrobial, anti-inflammatory and hypoglycaemic properties (Raghavendhar and Devanand, 2019). The CUR in native form has low biological stability and bioavailability, which may decrease its absorption and increased its metabolism and elimination from the body (Anand et al., 2007).

Nanotechnology is used in feeding farm animals, and the most important applications in this area are nanoparticles (NPs) with1–100 nm dimension. Some of

these NPs are stable at high temperature and pressure. In addition, they can be easily assimilated in the digestive system (Damian and Konrad, 2018). The NPs of elements are characterized by increased bioavailability and less toxicity, exhibiting novel characteristics such as improving the specific surface area, and increasing activity of the surface centers, surface, catalytic efficiency and ability of adsorption (Shi et al., 2010). The bioavailability of CUR is suggested to be improved in form of NPs (CURNPs), which increases its resistance to metabolic processes (Ravichandran, 2013). Therefore, in vivo bioavailability, tissue distribution and biological half-life are higher for CURNPs than for native CUR (Rahimi et al., 2016).

Limited information is available for the impacts of CURNPs on *in vivo and in vitro* reproductive performance of doe rabbits under HS condition. Therefore, the present study targeted to investigate the effect of CURNPs on the reproduction, haemato-biochemical parameters, antioxidants, immunity and in vitro embryo production of doe rabbits under HS conditions.

2. Materials and Methods

The present study was carried out at a private rabbit farm. APRI doe rabbits were raised under similar managerial and environmental conditions. Values of Minimum and maximum of recorded ambient temperature and relative humidity percentage and calculated thermo-humidity index according Marai et al. (2001) were 27.50 and 33.62 oC, 49.0, and 81.20% and 25.22-32.22, respectively. 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 (1994) recommendations. Does were fed *ad-libitum* on the diet, which was provided twice daily (7 a.m. and 3 p.m.), while drinking water was available from the nipple of each cage. Ingredients and chemical composition of the diet are shown in Table 1.

Table 1. Ingredients and chemical analysis of experimental diet (% as DM basis).

Item	(%)
Ingredient:	
Clover hay	30.0
Soybean meal (44%)	18.0
Wheat bran	24.6
Barley grain	21.0
Molasses	3.0
Limestone	1.0
DL-Methionine	0.20
Common salt	0.50
Minerals[1]	0.15
Vitamins[2]	015
Di-Calcium phosphate	1.40
Total	100
Chemical analysis	
Organic matter	93.15
Crude protein	18.15
Crude fiber	10.19
Ether extract	2.60
Nitrogen free extract	62.21
Ash	6.85
Digestible energy (kcal/kg)	2700

[1]Each 1kg contains on Vitamin A (150, 000 UI), Vitamin E (100 mg), Vitamin B1(10 mg), Vitamin K3 (21mg), VitaminB2 (40mg), Vitamin B6 (15mg), Vitamin B12 (0.1mg), Pantothenic acid (100 mg) Niacin (200 mg), Biotin (0.5mg), Folic acid (10mg) and Choline chloride (5000 mg).

[2] Each 1kg contains manganese (800 mg), zinc (600mg), iron (300 mg), copper (40m g), iodine (500 mg), selenium (100 mg), and cobalt (100 mg).

The CURNPs were purchased from Sigma Company (Cairo, Egypt). Eighty doe rabbits, 3 months of age with an average live body weight of 3.35 ± 0.20 kg body weight. Animals were divided into four experimental treatments (20/treatment). Does in the 1st treatment were fed the CFD without supplementation as a control rabbit, while those in the 2nd, 3rd and 4th treatments were fed the CFD supplemented with 3, 5, and 7 mg of CURNPs per kg diets, respectively. The weekly CFD of each treatments were well mixed with their additives in homogenous form.

Does in all groups were naturally mated with fertile APRI rabbit bucks. Does were manually palpated 10-12 days post-mating to calculate conception rate. After parturition, kindling rate was recorded. Total and live litter size at birth were computed 12 h after kindling. Kits were weaned at 28 day of age, then litter size at weaning was determined. Also, viability rate at birth and weaning was recorded.

Following the suckling period (end of 1st parity), receptive does (with red vulva) were naturally mated, then five conceived does from each group were taken,

transported to Laboratory and slaughtered 60 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 (≤2 mm in diameter), hemorrhagic follicles (HF), antral follicles (≥2 mm in diameter), and corpora lutea (CLs) on the ovarian surface were recorded for each dose. Ovulation rate (OR) was calculated as the following: OR (%) = (Number of CLs/ number of HF and antral follicles) x 100.

Embryos were recovered from reproductive tract of does slaughter - in Petri dishes containing phosphate buffer saline (PBS) with 10% fetal calf serum (FCS) and 50 µg gentamycin/ml. After embryo searching by stereoscopic microscope, embryos were counted, then embryo recovery rate (ERR) was calculated as the following: ERR = Number of embryos/number of CLs. Number of morulae was recorded 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 and abnormal embryos

At the end of treatment period, five does from each group were carefully chosen randomly and subjected to anaesthesia by intramuscular injection of ketamine and xylazine, and then 10 ml blood was withdrawn from ear vein of each doe into a clean sterile tube heparinized and subdivided into two sub-samples. Hematological variables were determined in the first one, including hemoglobin concentration (Hb), count of red blood cells (RBCs) and white blood cells (WBCs), packed-cell volume (PVC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) using blood hematology analyzer (HB 7021). Also, boold 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 cholesterol, triglycerides, and high-density lipoproteins (HDL), 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 according to Lie et al. (1989).

Statistical analysis

Data were subjected to analysis of variance using general linear model procedure (GLM) of statistical analysis system SAS (2012).

The significance between the means was determined using Duncan's multiple ranges test (Duncan, 1955). The following statistical model was applied for analysis of all measurements $Y_{ij} = \mu+TRT_i+e_{ij}$.

Where, Y_{ij} = Observations, μ = Overall mean, TRT = effect of the antioxidant material (i, 1 to 6), e_{ij} = random error. The association between CURNPs supplementation and each of sexual receptivity, pregnancy rate and kindling rate was detected by Chi-Square test ($\chi 2$). The statistical significance was accepted at $p<0.05$. Shapiro-Wilk test was conducted in order to check for normality (Razali and Wah, 2011).

3. Results

3.1. Reproductive performance (*in vivo*)

Table 2 showed that litter size at birth (total and live) and weaning significantly (P<0.05) increased in CURNPs at a level of 3 and 5 mg/kg diets Conception rate significantly (P<0.05) improved in all treatment of CURNPs, while viability rate at birth and weaning significantly (P<0.05) improved only in CURNPs at a level of 3 mg/kg diets. However, all reproductive traits of does in CURNPs at a level of 7 mg/kg diets did not differ significantly from those in the control.

Table 2. Effect of curcumin nanoparticles on reproductive performance of heat-stressed APRI doe rabbits

Item	Control	Curcumin nanoparticles (mg/kg diets)			SEM	P –Value
		3	5	7		
Conception rate (%)	50^{c}	80^{a}	70^{b}	70^{b}		-
Kindling rate (%)	90	100	100	100		-
Total litter size at birth/doe (n)	6.10^{b}	8.50^{a}	6.80^{a}	6.77^{b}	0.53	0.015
Live litter size at birth/ doe (n)	4.58^{c}	7.00^{a}	6.00^{b}	5.85^{c}	0.30	0.001
Viability rate at birth (%)	75.20^{b}	90.25^{a}	87.20^{ab}	82.23^{ab}	3.28	0.0389
Litter size at weaning/doe	4.25^{c}	6.25^{a}	5.5^{b}	4.28^{c}	0.125	0.0001
Viability rate at weaning (%)	70.78^{b}	90.15^{a}	88.20^{b}	85.22^{b}	4.58	0.0324

a, b, c Means in the same row with different superscripts are significantly different (P<0.05).

3.2. Hematological blood

The effects of CURNPs levels on hematological parameters of heat-stressed APRI doe rabbits are presented in Table 3. All dietary supplementations levels of CURNPs significantly increased concentration of Hb and RBCs count, while decreased count of WBCs as compared to the control group. There were no significant differences in PVC, MCV, MCH, and MCHC values among the experimental treatments (Table 3).

Table 3. Effect of curcumin nanoparticles on hematological parameters of heat-stressed APRI doe rabbits

Item	Control	Curcumin nanoparticles (mg/kg diets)			SEM	P-Value
		3	5	7		
Hb (g/dl)	12.50 ^{b}	13.85^{a}	13.81^{a}	13.74^{a}	0.088	0.0001
RBCs (x106/ml)	5.35 ^{b}	6.00^{a}	5.90^{a}	6.10^{a}	0.254	000452
WBCs (x103/ml)	7.25^{a}	6.20^{b}	6.10^{b}	6.66^{b}	1.254	0.0001
PCV (%)	32.85	35.58	35.77	36.25	2.450	0.8547
MCV (µ3)	88.52	92.54	91.47	92.87	2.574	0.758
MCH (pg)	29.665	30.21	30.25	29.85	1.852	0.856
MCHC	33.25	32.45	32.88	33.055	4.124	0.5698

a, b, c Means in the same row with different superscripts are significantly different (P<0.05).

3.3. Lipid profile and blood constituents

The effects of curcumin nanoparticles levels on serum lipid profile and blood constituents of heat-stressed APRI doe rabbits are presented in Table 4. Feeding does on different levels of curcumin nanoparticles significantly (P<0.05) affect lipid profile in serum of doe rabbits under HS conditions. The lowest concentration of total cholesterol and triglycerides was recorded in rabbits that received 3 mg curcumin nanoparticles per kg diet, while this level gave the best HDL concentration, as compared to control group (Table 4). Serum urea and creatinine as a kidney functions as well as activity of AST and ALT as a liver functions significantly decreased by curcumin nanoparticles levels compared with the control group.

Table 4. Effect of curcumin nanoparticles on serum lipid profile and blood constituents of heat-stressed APRI doe rabbits

Item	Control	Curcumin nanoparticles (mg/kg diets)			SEM	P -Value
		3	5	7		
Total cholesterol (mg/dl)	125.50^{a}	90.20^{d}	9325^{c}	100^{b}	1.254	0.0052
Triglycerides (mg/dl)	100.90^{a}	80.10 ^{c}	85.22^{b}	90.75^{b}	0.258	0.0001
High density lipoproteins (mg/dl)	30.28^{c}	38.54^{a}	35.36^{ab}	33.58^{b}	2.547	0.0001
Urea (mg/dl)	35.20^{a}	28.22^{c}	30.50^{bc}	33.52^{b}	1.858	0.0012
Creatinine (mg/dl)	1.50^{a}	1.00^{c}	1.20^{bc}	1.29^{b}	0.0856	0.0001
AST (U/ml)	40.10^{a}	30.55^{c}	32.77^{bc}	36.55^{b}	1.258	0.0001
ALT (U/ml)	30.50^{a}	23.75^{b}	26.25^{b}	25.36^{b}	3.658	0.0001

a, b, c Means in the same row with different superscripts are significantly different (P<0.05).

3.4. Antioxidant capacity

The effects of CURNPs levels on serum antioxidant capacity and immunity of heat-stressed APRI doe rabbits are presented in Table 5. All CURNPs groups significantly (P<0.05) improved antioxidant capacity and immunity. The best value of TAC, GST, GSH, SOD, catalase, GPx and MDA was recorded in rabbits that received curcumin nanoparticles at a level of 3 mg per kg diet. Regarding the immune response, all treatment led to significant (P<0.05) increase in contents of IgG and IgM in serum of rabbits.

Table 5. Effect of curcumin nanoparticles on serum antioxidant capacity and immunity of heat-stressed APRI doe rabbits

Item	Control	Curcumin nanoparticles (mg/kg diets)			SEM	P -Value
		3	5	7		
Antioxidant capacity						
TAC (mmol/l)	0.30^{b}	0.40^{a}	0.37^{a}	0.39^{a}	0.052	0.0001
GST(IU)	1.10^{c}	1.28^{a}	1.20^{b}	1.15^{b}	0.032	0.0001
SOD (IU)	6.30^{c}	6.95^{a}	6.50^{b}	6.45^{b}	0.245	0.0001
GSH (mg/dl)	13.25^{b}	16.52^{a}	16.55^{a}	16.14^{a}	0.365	0.0001
GPx (mg/l)	4.04^{c}	6.52^{a}	6.38^{a}	5.14^{b}	0.028	0.0001
Catalase (U/ml)	110.25^{c}	140.23^{a}	138.45^{a}	145.25^{a}	0.456	0.0001
MDA (µmol/l)	8.35^{a}	6.20b	6.35^{b}	6.45^{b}	0.024	0.0001
Immunity response						
IgG (ng/ml)	52.55^{d}	89.20^{a}	72.77^{b}	65.44^{c}	1.524	0.0001
IgM (µg/ml)	6.10 ^{b}	7.55^{a}	7.50^{a}	7.00^{a}	1.586	0.0001

a, b, c, d Means in the same row with different superscripts are significantly different (P<0.05).

3.5. Ovulatory response and ovulation rate

Treatment of curcumin nanoparticles significantly (P<0.05) increased absolute or relative ovarian weight, which 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 stages of development at a level of 3 mg curcumin nanoparticles / kg diet of. Increasing CLs number and decreasing follicular number resulted in significant increase in ovula-

tion rate in rabbits received curcumin nanoparticles at a level of 3 and 5mg / kg diets as compared to the control group (Table 6).

Although the differences in embryo recovery rate among groups were not significant, embryo quality was significantly (P<0.05) better at a level of 3 mg curcumin nanoparticles / kg diet of than the control but did not differ from that in 5 mg / kg diet of curcumin nanoparticles, indicating beneficial effect of curcumin nanoparticles on quality of recovered embryos.

Table 6. Effect of curcumin nanoparticles on ovulatory response and ovulation rate of heat-stressed APRI doe rabbits

Item	Control	Curcumin nanoparticles (mg/kg diets)			SEM	P-Value
		3	5	7		
Absolute ovarian weight (g)	0.57^{c}	0.60^{a}	0.58^{ab}	0.57^{c}	0.007	0.0001
Relative ovarian weight (g/kg)	0.190^{c}	0.200^{a}	0.192^{ab}	0.190^{c}	0.002	0.0001
Antral follicles/doe (n)	20.25^{a}	16.24^{b}	18.10^{ab}	19.50^{a}	1.456	0.0001
Secondary follicles/doe (n)	25.24^{a}	19.20c	20.44^{bc}	23.25^{b}	0.785	0.0001
Bleeding follicles/doe (n)	4.20^{a}	2.85b	2.95^{ab}	3.10^{ab}	0.425	0.0001
Corpora lutea/doe (n)	11.25^{b}	14.35a	13.58^{ab}	13.85^{ab}	0.214	0.0001
Ovulation rate (%)	55.56^{c}	89.36a	75..03^{b}	71.03^{b}	4.25	0.0001
Embryo recovery rate	95.20	99.25	97.40	95.47	1.585	0.7452
Acceptable embryos (%)	66.52^{b}	90.25a	77.35^{ab}	70.00^{b}	1.456	0.0001
Poor embryos (%)	33.48^{a}	9.75b	22.65^{ab}	30.00^{a}	5.856	0.0001

a, b, c Means in the same row with different superscripts are significantly different (P<0.05).

4. Discussion

Heat stress has become a widespread concern worldwide, which is a major environmental stress that causes substantial economic loss in the rabbit industry. In the current study, rabbit does suffer from severe heat stress via the experimental period. In this way, Minimum and maximum values in the current study were recorded ambient temperature and relative humidity percentage and calculated thermo-humidity index according Marai et al. (2001) were 27.50 and 33.62 ºC, 49.0, and 81.20% and 25.22 and 32.22, respectively. Severe heat stress for does exposing to THI value ≥30 during summer season in Egypt were reported by Marai et al. (2002). Heat stress negatively affected reproduction in doe rabbits, which posed a danger to the rabbit business in hot and semi-hot climates (Marco-Jiménez et al., 2017). Therefore, the current work sought to assess the beneficial role CURNPs supplementation for elimination the negative effects of heat stress on reproduction of doe rabbits, so reproductive performance (*in vivo and in vitro*), hematological and biochemical parameters, antioxidant capacity and immune response of doe rabbits were studied. Marco-Jimenez et al. (2017) reported that maternal exposure to heat stress decreased reproduction in terms of reduced litter weight, litter size, and kit weight at birth, while the rates of stillborn was higher in does during pregnancy under heat stress conditions (Abdel–Moneim et al., 2013; Mousa-Balabel, 2017). Thus, usage of additional natural antioxidants had beneficial impacts on reproductive performance to attenuate the detrimental impacts of heat stress conditions (El-Ratel et al., 2020).

In the current study, the observed trend of enhancement in reproductive performance in vivo of doe rabbits such as conception rate, litter size and viability rate of bunnies in CURNPs at a level of 3 mg/kg diets. This means that these supplements improved antioxidant status and the immune response of heat-stressed doe rabbits. The productivity of rabbits breeding program is directly dependent on the reproductive performance of rabbits. Evaluating reproductive performance is essential for selecting ideal rabbit breeds and food supplements that enhance effective breeding (Ekere et al., 2024).

In accordance with the present results, Ekere et al. (2024) showed that at 500 mg/kg of turmeric powder had improved reproductive performance of rabbit does. Also, Majeed et al. (2019) who reported no treatment related effect on mean litter size after oral treatment of female rats with tetrahydro curcumin as compared to the control group. Under hot summer season, Habeeb et al. (2019) who observed significant improvement in litter size of does feed ginger and curcumin-supplemented diets. Mirzaie et al. (2018) reported significant improvement in performance traits of broiler chickens fed diet supplemented with antioxidants under HS as previously proved on reproductive parameters of does (El-Ratel, 2017) and mice (Pankaj, 2015) under normal conditions. Most compounds in natural sources of antioxidant are polyphenols, which have important physiological functions (Ebrahimzadeh et al., 2018). CURNPs have 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 (El-Ratel et al., 2020). It is worth noting that enhancing the in vivo reproductive performance of doe rabbits was in parallel with the ovulatory response of CURNPs treated groups. Does treated with CURNPs resulted in more stimulation of *in vitro* ovulatory response and embryo yield as well as subsequently increasing ovulation rate of rabbit does.

Does reproductive system is very sensitive to oxidative stress, then reduction in production and secretion of gonadotropins (LH and FSH) that are necessary for vital growth and development of ovarian follicles (Arabameri et al., 2017). The LH level reduction may impair rate of ovulation (Chatterjee and Chatterjee, 2009), fertility and developmental competence of embryos (Silva et al., 2013; Avila et al., 2016). Also, implantation failure, embryo fragmentation, impaired

placentation, and abortion may be associated with excessive ROS production (Agarwal et al., 2006). Furthermore, a reduction in GSH of embryos, and elevation of ROS level leading to DNA damage were observed in in vivo heat stressed mice (Ozawa et al., 2002). These findings provide both direct and indirect evidence of a close relationship between heat and oxidative stress in embryo development. The noticed tendency of increase in number of CLs in CURNPs treatment groups may be attributed to the effect of CUR as antioxidants on increasing LH surge as compared to the control group, reflecting higher ovulation rate. Natural plant derived antioxidants may exhibit beneficial effects on ovulation and ovary functions (Zhong and Zhou, 2010; El-Ratel et al., 2020). Interestingly, improving embryo quality in treatment groups may be due to direct effect of allicin on ovarian tissues and activity, and/or indirect effect on heathy status and immunity of doe rabbits.

Also, the improvement in conception rate in supplemented rabbits may be due to the increase in progesterone (P4) level compared to non-treated rabbits. The increase in P4 in treated groups may be owing to that CUR has strong phytoestrogenic properties and its action on the reproductive system is mediated by ovarian steroid hormone synthesis and/or steroid receptors in the ovaries (Tiwari-Pandey and Sairam, 2009). The ovaries of rabbits fed high doses of turmeric released more P4 in vitro than the ovaries of control does and suggest that ovarian follicular genesis, oogenesis and fertility are affected by turmeric through changes in the output of ovarian steroid hormone (Sirotkin et al., 2014). Supplementation with nano-curcumin in doe rabbits diet improved the reproductive performance (*in vivo and in vitro*) under heat stress conditions.

According to the results of hematological blood of rabbits, all dietary supplementations levels of CURNPs significantly increased concentration of Hb and RBCs count, while decreased count of WBCs as compared to the control group. Blood hematological parameters are physiologically, pathologically, and nutritionally indicators in rabbits and can be potential to exhibit the impact of dietary additives (Alagawany et al., 2016). The phytochemical contents (polyphenolic, alkaloids, and flavonoids) in curcumin have positive impacts on hematological measurements, which could be attached to the cellular plasma membranes to savage the oxidative stress by protecting the body cells from the produced free radicals, and/or activating the antioxidant enzymes (Alagawany et al., 2016). The contradictory results of curcumin and nano-curcumin on farm animals obtained in our study may be attributed to characteristics of curcumin in nano-form, bioactive components amount, the various doses of herbal plant, duration of experimental period, number of experimental animals and animal age (Alagawany et al., 2016).

In the current study, feeding does on different levels of CURNPs significantly ($P<0.05$) affect lipid profile in serum of doe rabbits under HS conditions, including decreasing of total cholesterol and triglycerides concentration being the lowest in CURNPs at a level of 3 mg per kg diets, and increasing HDL concentration, as compared to control group. Serum urea and creatinine as a kidney function as well as activity of AST and ALT as a liver functions significantly decreased by all diet's supplements with the CURNPs levels compared with the control group. The improved lipid profile in our study was similarly reported by several authors, who found that supplementation of CURNPs had positive effects on lipid profile, kidney and liver functions. The assessment of these parameters could be useful in determining rabbit health. Blood biochemicals are physiologically, pathologically and nutritionally indicators in rabbits and can be potential to exhibit the impact of dietary additives (Alagawany et al., 2016; El-Ratel et al., 2020). The benefits on blood parameters may be due to the phytochemical compounds of CUR, such as alkaloids, flavonoids and polyphenols. The observed reduction of CUR or CURNPs on lipid profile of growing rabbits was reported on turmeric (Alagawany et al., 2016). The CUR has anti-hypercholesteremic effects and plays a vital role in preventing atherosclerosis against oxidation of LDL (Rahmani et al., 2018). Under heat stress conditions, increasing the activity of AST by increased ROS production may result in damage in the hepatic cells of the liver (Fathi et al., 2011). Regarding the liver function of doe rabbits as affected by CURNPs serum activity of AST and ALT decreased, which may indicate an improved liver function in prevention of liver damage due to ROS as indicated by improved antioxidant capacity in blood rabbits in nano-curcumin treatments. Enhanced activity of AST and ALT is used widely accepted as a biomarker of hepatic damage (Rahmani et al., 2018). In healthy livers under basal conditions, low amounts of ROS are produced by hypatocytes, while in response to bacterial stimuli, Kupffer cells release free radicals.

Increasing lipid peroxidation and decreasing antioxidant enzymes activities (SOD and GPx) in cell membrane, is an imbalance in the antioxidants system and incidence of oxidative stress under heat stress (Georgieva et al., 2006). In the current study, the active compound of Curcuma longa, such as flavones, can activate SOD and catalase (Gagandeep et al., 2003), phycocyanin and β-carotene, which have strong activity of scavenging acting, individually or together, directly on superoxide radicals (Kurd and Samavati, 2015).

Phenolic chemicals have the ability to increase antioxidant enzyme activity (Farag et al., 2024). All CURNPs groups significantly improved antioxidant capacity and immunity including increased TAC, GST, GSH, SOD, catalase and GPx contents and reduced MDA level, which indicated inconsistent trend of improvement in antioxidant capacity of doe rabbits being the best in CURNPs at a level of 3 mg per kg diet.

In general, CUR phytochemical compounds (alkaloids, flavonoids and polyphenols) bind with the cytoplasmic membrane to remove ROS, activate antioxidant enzymes and inhibit oxidative stress (Alagawany et al., 2016). The CUR, as an antioxidant, is used for protecting against ROS and increasing the animal antioxidant

status (Gao et al., 2013), whereas it contains beneficial phenols (inhibitors of lipid peroxidation), as strong antioxidant activity. Within the cellular antioxidant defense system, SOD is the [1]st enzyme that prevents the cellular damage caused by ROS generation via improving the steady state of antioxidant system of rabbits (Alagawany et al., 2016). Besides SOD, intracellular antioxidants namely GSH act to eliminate lipid peroxidation and enhancing antioxidant status via the enzyme reactions catalyzed by GSH-Px (Fang et al., 2002). It is worth noting that CUR supplementation may lead to inhibition of lipid peroxidation in rabbit liver microsomes (Reddy and Lokesh, 1994). The MDA is the primary ROS marker, which produces by lipid peroxidation, and H_2O_2 is well known for its negative impacts on different components within the cells (Lee et al., 2006). The nano-curcumin treatments reduced level of MDA in blood serum of doe rabbits.

The current study showed that dietary supplementation of CURNPs improved immune responses, in terms of increase in contents of IgG and IgM in serum of rabbits. It is of interest to note that improving antioxidant status via antioxidant properties of nano-curcumin was in association with increasing immune response of doe rabbits. As such we found a marked increase in serum IgG, IgM and IgA concentrations of heat-stressed rabbits fed diets supplemented with nano-curcumin at different levels. Flavonoids in curcumin extend vitamin C activity, antioxidants, and may increase immunity (Acamovic and Brooker, 2005). The impacts of CUR may be attributed to their activity as anti-inflammatory, antioxidant and antibacterial agents (Raghavendhar and Devanand, 2019). Turmeric supplementation improves the immunity of growing rabbits (Alagawany et al., 2016) via limiting the proliferation of the gut pathogenic and non-pathogenic bacterial count in the animal, leading to improving the feed efficiency (Nouzarian et al., 2011).

5. Conclusions

The obtained results indicated beneficial effects of dietary supplementation curcumin nanoparticles on reproductive efficiency, lipid profile, liver function, antioxidants status, immunity, yield, quality and developmental competence of rabbit embryos under heat stress conditions

Data Availability Statement: All data associated with this article is embedded and presented

Conflicts of Interest: the authors declare that there is no confect of interest.

6. Reference

Abdel-Moneim, E. A.; Attia, A. I.; Askar, A. A.; Abu-Taleb, A. M.; and Mahmoud, M. H. (2013). Response of growing rabbits supplemented with copper sulfate, ascorbic acid or drinking cooled water under Egyptian summer conditions. Zagazig Journal of Agricultural Research, 3, 511-523

Acamovic, T.; and Brooker, J. D. (2005). Symposium on “plants as animal foods: A case of catch 22?” biochemistry of plant secondary metabolites and their effects in animals. Proceedings of the Nutrition Society, 3, 403–412.

Agarwal, A.; Said, T. M.; Bedaiwy, M. A.; Banerjee, J.; and Alvarez, J. G. (2006). Oxidative stress in an assisted reproductive techniques setting,” Fertility and Sterility, 86:50

Alagawany, M.; Elwy, A. A.; and Fayez, M. R. (2016). Effect of dietary supplementation of garlic (Allium sativum) and turmeric (Curcuma longa) on growth performance, carcass traits, blood profile and oxidative status in growing rabbits. Annals of Animal Science, 16, 489–505.

Al-Sagheer, A.A.; Daader, A.H.; Gabr, H.A.; and Abd El-Moniem, E.A. (2017). Palliative Effects of Extra Virgin Olive Oil, Gallic Acid, and Lemongrass Oil Dietary Supplementation on Growth Performance, Digestibility, Carcass Traits, and Antioxidant Status of Heat-Stressed Growing New Zealand White Rabbits. Environmental Science and Pollution Research, 24, 6807–6818.

Anand, P.; Kunnumakkara, A.B.; Newman, R.A.; and Aggarwal, B.B. (2007). Bioavailability of curcumin: problems and promises. Molecular Pharmacology. 4:807–818.

Arabameri, A.; Sameni, H.; and Bandegi, A. (2017). The effects of propolis extract on ovarian tissue and oxidative stress in rats with maternal separation stress. International Journal of Reproductive BioMedicine, 8, 509-520.

Avila, J.; Gonzalez-Fernandez, R.; Rotoli, D.; Hernandez, J.; and Palumbo, A. (2016). Oxidative stress in granulosa-lutein cells from in vitro fertilization patients, Reproductive Sciences, 23: 1656.

Ayyat, M.S.; Al-Sagheer, A.A.; Abd El-Latif, K.M.; and Khalil, B.A. (2018). Organic Selenium, Probiotics, and Prebiotics Effects on Growth, Blood Biochemistry, and Carcass Traits of Growing Rabbits During Summer and Winter Seasons. Biological Trace Element Research, 186, 162–173.

Chatterjee, A.; and Chatterjee, R. (2009). How stress affects female reproduction: An overview. Biomedical Research, 20: 79.

Damian, K.; and Konrad, W. (2018). The use of nanominerals in animal nutrition as a way to improve the composition and quality of animal products. Review Article. Journal of Chemistry, 1–7.

Duncan, D. B. (1955). Multiple range and multiple F test. Biometrics, 11, 1–42.

Ebeid, T.A.; Aljabeili, H.S.; Al-Homidan, I.H.; Volek, Z.; and Barakat, H. (2023). Ramifications of Heat Stress on Rabbit Production and Role of Nutraceuticals in Alleviating Its Negative Impacts: An Updated Review. Antioxidants, 12, 1407.

Ebrahimzadeh, S.K.’ Navidshad, B.’ Farhoomand, P.’ Mirzaei Aghjehgheshlagh, F. (2018). Effects of grape pomace and vitamin E on performance, antioxidant

status, immune response, gut morphology and histopathological responses in broiler chickens. South African Journal of Animal Science, 48, 324.

Ekere, S. O.; Ezema, A. S.; Njoku, A. L.; Anya, O. K.; Obidike, I. R.; and Ihedioha, J. I. (2024). Effects of curcuma longa rhizomes (*turmeric*) on reproductive performance in rabbits in the humid tropics. Animal Research International, 21(2), 5495 – 5502.

El-Ratel, I.T. (2017). Reproductive performance, oxidative status and blood metabolites of doe rabbits administrated with spirulina alga. Egyptian Poultry Science Journal, 37, 1153-1172.

El-Ratel, I. T.; Tag El-Din, T. E. H.; and Bedier, M. M. (2020). Beneficial Effects of Curcumin as A Native or Nanoparticles Form on Productive Efficiency, Liver and Kidney Functions, Antioxidative Status and Immunity of Heat-Stressed Growing Rabbits. Journal of Animal Physiology and Animal Nutrition, 104, 1778–1787.

Farag, S. A.; El-Keredy, A.; Abd El Gawad, S. A.; Swelum, A. A.; Tellez-Isaias, G.; and Abouzeid, A.E. (2024). Impacts of willow (*Salix babylonica* L.) leaf extract on growth, cecal microbial population, and blood biochemical parameters of broilers. Poultry Science, 103: 103386

Fang, Y. Z.; Yang, S.; and Wu, G. (2002). Free radicals, antioxidants, and nutrition. Nutrition, 18, 872–879.

Fathi, M.; Tanha, T.; Nazer-Adl, K.; Ebrahi-Nezhad, Y.; Aghdam-Shahryar, H.; and Daneshyar, M. (2011). The role of oxidative stress in the de velopment of congestive heart failure (CHF) in broilers with pulmonary hypertension syndrome (PHS). Journal of Animal and Veterinary Advances, 10, 2724–2729.

Gagandeep, D. S.; Mendiz, E.; Rao, A. R.; and Kale, R. K. (2003). Chemopreventive effects of Cuminum cyminum in chemically in duced forestomach and uterine cervix tumors in murine model systems. Nutrition and Cancer, 47, 171–180

Gao, S.; Duan, X.; Wang, X.; Dong, D.; Liu, D.; Li, X.; and Li, B. (2013). Curcumin attenuates arsenic-induced hepatic injuries and oxidative stress in experimental mice through activation of Nrf2 path way, promotion of arsenic methylation and urinary excretion. Food and Chemical Toxicology, 59, 739–747.

Georgieva, N.V.; Koinarsk, V.; and Gadjeva, V. (2006). Antioxidant status during the course of Eimeria tenella infection in broiler chickens. The Veterinary Journal, 172, 488-92.

Habeeb, A. A.; El-Darawany, A. A.; Abdel-Mageed S. N.; and Sharaf, A. K. (2019). Impact of Some Medicinal Plants Supplement on Pregnant Rabbits Diet During Hot Summer Season. Research Journal of Medicinal Plants, 13: 145-154.

Jimoh, O. A.; Ayedun, E. S.; Oyelade, W. A.; Oloruntola, O. D.; Daramola, O. T.; Ayodele, S. O.; and Omoniyi, I. S. (2018). Protective Effect of Soursop (Annona Muricata Linn.) Juice on Oxidative Stress in Heat Stressed Rabbits. Journal of Animal Science and Technology, 60, 28.

Lee, D. H.; Lim, B. S.; Lee, Y. K.; and Yang, H. C. (2006). Effects of hydrogen peroxide (H_2O_2) on alkaline phosphatase activity and matrix min eralization of odontoblast and osteoblast cell lines. Cell Biology and Toxicology, 22, 39–46.

Majeed, M.; Natarajan, S.; Pandey, A.; Bani, S.; and Mundkur, L. (2019). Subchronic and reproductive/developmental toxicity studies of tetrahydrocurcumin in rats. Toxicological Research, 35, 65 – 74

Marai, I. F. M.; Habeeb, A. A. M.; and Gad, A. E. (2002). Rabbits' productive, reproductive and physiological performance traits as affected by heat stress: A review. Livestock Production Science. 2, 71-90.

Marai, I.F.M.; Ayyat, M.S.; and Abd El-Monem, U.M. (2001). Growth Performance and Reproductive Traits at First Parity of New Zealand White Female Rabbits as Affected by Heat Stress and Its Alleviation under Egyptian Conditions. Tropical Animal Health and Production, 6, 451-462.

Marco-Jiménez F.; García-Diego F.J.; and Vicente J.S. (2017). Effect of Gestational and Lactational Exposure to Heat Stress on PERFORMANCE in Rabbits. World Rabbit Science, 25:17–25.

Mirzaie, S.; Zirak-Khattab, F.; Hosseini, S. A.; and Donyaei-Darian, H. (2018). Effects of dietary Spirulina on antioxidant status, lipid profile, immune response and performance characteristics of broiler chickens reared under high ambient temperature. Asian-Australas Journal of Animal Science, 4, 556-563.

Mousa-Balabel, T. M.; El-Sheikh, R. A.; and Esraa H. Moustafa (2017). New strategies for controlling heat stress in New Zealand White (NZW) Rabbits in Egypt. Life Science Journal, 11, 64-70.

Mutwedu, V.; Nyongesa, A.; Oduma, J.; Kitaa, J.; and Mbaria, J. (2021). Thermal Stress Causes Oxidative Stress and Physiological Changes in Female Rabbits. Journal of Thermal Biology, 95, 102780.

Nouzarian, R.; Tabeidian, S. A.; Toghyani, M.; Ghalamkari, G.; and Toghyani, M. (2011). Effect of turmeric powder on performance, carcass traits, humoral responses, and serum metabolites in broiler chickens. Journal of Animal and Feed Sciences, 20, 389–400.

NRC, (1994). Nutrient requirements of poultry (9^{th} ed.). National Academics Press.

Pankaj, P.P. (2015). Efficacy of Spirulina platensis in improvement of the reproductive performance and easing teratogenicity in hyperglycemic albino mice. Indian Journal of Pharmacology, 47, 430-435.

Ozawa, M.; Hirabayashi, M.; and Kanai, Y. (2002). Developmental competence and oxidative state of mouse zygotes heat-stressed maternally or *in vitro*. Reproduction, 124, 683–689.

Kurd, F.; and Samavati, V. (2015). Water soluble polysaccharides from Spirulina platensis: Extraction and in

vitro anti-cancer activity. International Journal of Biological Macromolecules, 74: 498-506.

Raghavendhar, R. K.; and Devanand, L. L. (2019). Curcumin: Biological, pharmaceutical, nutraceutical, and analytical aspects. Molecules, 24, 2930–2957.

Rahimi, H. R.; Mohammadpour, A. H.; Dastani, M.; Jaafari, M. R.; Abnous, K.; Ghayour Mobarhan, M.; and Oskuee, K. R. (2016). The effect of nano-curcumin on HbA1c, fasting blood glucose, and lipid profile in diabetic subjects: A randomized clinical trial. Avicenna Journal of Phytomedicine, 6, 567–577.

Rahmani, M.; Goliana, A.; Kermanshahia, H.; and Bassamib, M. R. (2018). Effects of curcumin or nanocurcumin on blood biochemical parameters, intestinal morphology and microbial population of broiler chickens reared under normal and cold stress conditions. Journal of Applied Animal Research, 46, 200–209.

Ravichandran, R. (2013). Studies on dissolution behavior of nanoparticulated curcumin formulation. Advances in Nanoparticles, 2, 51–59

Razali, N. M.; and Wah, Y. B. (2011). Power comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling tests. Journal of Statistical Modeling and Analytics, 2, 2133.

Reddy, B. S.; Yuvaraj, N.; Babitha, V.; Ramnath, V.; Philomina, P. T.; and Sabu, M. C. (2004). Antioxidant and hypolipidemic effects of Spirulina and natural carotenoids in broiler chicken. The Indian Veterinary Journal, 81, 383-386.

Saghir, S. A. M.; Hroob, A.M.; Majrashi, K.A.; Jaber, F.A.; Abduh, M.S.; Al-Gabri, N.; Albaqami, N.M.; Abdelnour, S.A.; Alqhtani, A.H.; and Abd El-Hack, M.E. (2023). Effects of Alginates on the Growth, Haematological, Immunity, Antioxidant and Pro-inflammatory Responses of Rabbits under High Temperature. Research in Veterinary Science, 155, 36–43.

SAS, (2012). SAS Institute Inc. SAS User's Guide, Statistics. Cary, NC.

Shi, L. G.; Yang, R. J.; Yue, W. B.; Xun, W. J.; Zhang, C. X.; Ren, Y. S.; and Lei, F. L. (2010). Effect of elemental nano-selenium on semen quality, glutathione peroxidase activity, and testis ultrastructure in male Boer goats. Animal Reproduction Science, 118, 248–254.

Silva, C. F.; Sartorelli, E. S.; Castilho, A. C. S.; Satrapa, R. A.; Puelker, R. Z.; Razza, E. M.; Ticianelli, J. S.; Eduardo, H. P.; Loureiro, B.; and Barros, C. M. (2013). Effects of heat stress on development, quality and survival of Bos indicus and Bos taurus embryos produced in vitro. Theriogenology, 79: 351

Sirotkin, A.V.; Chrenek, P.; Kolesarova, A.; Parillo, F.; Zerani, M.; and Boiti, C. (2014). Novel regulators of rabbit reproductive functions. Animal Reproduction Science, 148, 188-196

Tiwari-Pandey, R.; and Sairam, M. R. (2009). Modulation of ovarian structure and abdominal obesity in curcumin - and flutamide-treated aging FSH-R haploinsufficient mice. Reproductive Sciences, 16, 539-550.

Zhong, R.; and Zhou, D. (2010). Oxidative stress and role of natural plant derived antioxidants in animal reproduction. Journal of Integrative Agriculture, 12, 1826–1838.