

## Impacts of Nano encapsulated Moringa Leaf Ethanolic Extract on Health Status and Reproductive Performance of Rabbit Does under Heat Stress

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### ABSTRACT

This study explores the effect of Nano encapsulated Moringa leaf ethanolic extract (NMLEE) on blood metabolites, redox status, hormonal response, and reproductive performance of rabbit does APRI breed under severe heat stress conditions. A total of one hundred nulliparous does were randomly divided into five homogeneous groups of 20 does each. The basal diet fed to the experimental groups contained 0, 20, 40, 60, and 80 mg/kg NMLEE for the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> groups, respectively. The NMLEE treatments were administered over a period of 30 days in July. Thereafter, the reproductive performance of the doe rabbits was monitored and evaluated during the months of August and September. The results showed that NMLEE at a level of 80 mg/kg diet decreased the concentrations of total cholesterol, tri-glycerides, low-density lipoprotein, urea, and creatinine as well as the levels of lipid peroxidation (MDA) and liver enzymes (AST and ALT) activities. Contrarily, it increased ( $P < 0.05$ ) total proteins, albumin, globulin, high-density lipoprotein, blood hemoglobin, hematocrit, total antioxidant capacity, glutathione, glutathione peroxidase, and superoxide dismutase compared to the control group. The levels of thyroid hormones (T3 and T4), progesterone, and estradiol were significantly induced in the blood serum of rabbits co-treated by 60 or 80 mg NMLEE/kg diet. However, the concentration of cortisol was significantly ( $P < 0.05$ ) reduced in the aforementioned groups compared to the control. Both the ovulation and blastocyst hatching rates as well as the normal embryo percentage were significantly ( $P < 0.05$ ) improved by the 80 mg NMLEE/ kg diet supplementation. The productivity of heat-stressed rabbits does fed by NMLEE supplements was significantly higher than those in the untreated group. In conclusion, dietary NMLEE supplementation significantly mitigates the stress of heat which negatively affects the rabbit does physiology, metabolism, and reproduction and showed improvement of the blood redox balance and the rabbit does hormonal response.

### INTRODUCTION

In developing countries, rabbits are a viable alternative option for meat production as they offer multiple advantages over larger mammals (Oseni & Lukefahr, 2014). Nevertheless, rabbits exhibit high reproductive performance surpassing many other farm animals in this regard but, they are very sensitive to heat stress (Marai *et al.*, 2001 and Nourhan *et al.*, 2020), since they do not have enough sweat glands, which makes it challenging for them to disperse excess heat metabolized in the body (Rafel *et al.*, 2012). Rabbits' sensitivity to heat stress affects

their productivity and economic benefits negatively (Hashem *et al.*, 2013).

Nardone *et al.* (2010) mentioned that heat stress has been characterized by some environmental factors such as air temperature, airflow, humidity, and heat radiation as well as the metabolic heat produced by animals. Moreover, Kumar *et al.* (2011) indicated that this stress found when the ambient temperature surpasses the thermo neutrality range within which animals can preserve their normal body functions. Animals' physiology, particularly

reproduction is the first function significantly affected by heat stress (Hansen, 2009). It has been reported to have detrimental effects on embryo growth by raising mortality rates (Hansen, 2007). Additionally, this is accompanied by a decrease in fertility rate due to poor expression of estrus which is attributed to a reduction in estradiol secretion (De Rensis & Scaramuzzi, 2003). However, considering the social considerations and economic benefits of rabbits in the tropics (Mutwedu *et al.*, 2021a) to improve farmers' income, it becomes of importance to find out alternative solutions for mitigating the negative effects of heat stress on productive and reproductive performances of rabbits (Mutwedu *et al.*, 2021a and b). Svoradova *et al.* (2021) reported that phytogetic feed additives derived from plants used to be added in animal feeds to enhance agricultural livestock performance due to their high antioxidant capacity, low toxicity, and cheap availability. These characteristics in addition to the environment friendly nature of such resources makes them convenient for addressing several health issues (Svoradova *et al.*, 2021 and Chumark *et al.*, 2008).

Worldwide, *Moringa oleifera* (MO), as a perennial tree belonging to the family *Moringaceae*, is widely recognized as one of the most potent antioxidant plants (Wadhwa *et al.*, 2013). Every part of this tree (Stem barks, leaves, seeds, and pods) are found to be rich in bioactive compounds, e.g. phenols, alkaloids, glycosides, saponins, flavonoids, tannins, and terpenoids (El-Alfy *et al.*, 2011; Ayirezang *et al.*, 2020). Therefore, *Moringa oleifera* has been suggested to mitigate oxidative stress-induced damages (Sharma *et al.*, 2011; Chisholm, 2015). Odeyinka *et al.* (2008) showed that dietary addition of 10% *Moringa oleifera* leaves in rabbit diets significantly increased their litter weight, litter size, gestation length, milk yield, weaning weight, and survival rate. In Ajuogu *et al.* (2019) study on female rabbits fed diets contained 5, 10 and 15 g/kg *Moringa oleifera* leaf powder, the reproductive hormones (LH, FSH, estrogen, progesterone, and prolactin) levels were improved.

From another point of view, McClements (2015) and Embuscado (2015) mentioned that practical implementation of phytogetic substances that originated from active components is restricted by several factors, including absorption, cellular uptake, the stability of these molecules in the gastrointestinal tract, and stability during handling and storage.

Therefore, the nano-encapsulation of phytogetic bioactive components has emerged as a promising approach to overcome the aforementioned challenges associated with the use of phytogetic components (Kurozawa & Hubinger, 2017).

Accordingly, this study aimed to investigate the effects of Nano encapsulated Moringa leaf ethanolic extract (NMLEE) on the hematological-biochemical parameters, hormonal response, antioxidant indicators, immunity status, and reproductive performance of rabbits does breed under severe heat stress conditions.

## Materials and Methods:

This present experiment was carried out during the summer from 1/7 to 30/9/2022 at a private commercial rabbit farm, Mansoura, Dakahlia Province, Egypt in cooperation with the Animal, Poultry and Fish Production Department, Faculty of Agriculture, Damietta University, Egypt. This study was conducted to investigate the effect of Nano-encapsulated Moringa leaf ethanolic extract (NMLEE) as a supplement under heat stress on productive performance of rabbit does. Experimental steps have followed the guidelines of the Egyptian Research Ethics Committee and the instructions contained in the Guide for the Care and Use of Laboratory Animals (2011).

### 1. Plant Extraction and Nano-encapsulation:

The leaves of *Moringa oleifera* were dried naturally until they reached content of approximately 90% dry matter and then the dried sample were ground through a 1 mm screen. The powder of Moringa leaf (25 g/100 mL) was extracted by a 70% hydroethanolic solution at a temperature of 40 °C for 72 hours. The obtained extract was filtered using Whatman No. 1 filter paper (Camlab, Cambridge, UK). Thereafter, the collected filtrate was evaporated at a temperature of 45°C to achieve the complete dryness. Then the residues were stored at a temperature of -20°C pending use as outlined by El-Desoky *et al.* (2017).

The dried MLEE was used for the fabrication of a sodium alginate nanocomplex using calcium chloride (CaCl<sub>2</sub>) as a cross-linking agent by adopting the ionic-gelation method. Under continuous magnetic stirring, the MLEE (1.5 g) was first mixed with the sodium alginate solution (1%, w/v). Then, the mixture was added dropwise using a syringe into a CaCl<sub>2</sub> solution (2.2 mol/L) with a ratio of 2 sodium alginate and

MLEE mixture:1 CaCl<sub>2</sub> solution. The synthesized nanoparticles were centrifuged at 8000 rpm for 20 min, and the resultant nanoparticles were collected and stored at -80 °C. (El-Desoky et al., 2021).

## 2. Meteorological parameters:

Throughout the entire study duration, the ambient temperature (AT) and relative humidity (RH) were measured daily at 1400 hours using an automated Thermo hygrometer (Dostmann GmbH and Co. KG, Wertheim, Germany). The temperature-humidity index (THI), as proposed by Marai et al. (2002), was calculated based on the recorded data of relative humidity percentage (RH) and dry bulb temperature (AT) measured in Celsius using the following equation:

$$\text{THI} = \text{AT} - [(0.31 - 0.31(\text{RH})) \times (\text{AT} - 14.4)]$$

According to the classification established by Marai et al. (2002), the THI values were categorized as follows:

- THI less than 27.8 considered indicator for the absence of heat stress (HS).
- THI ranged from 27.8 to 28.9, considered indicator for moderate HS.
- THI ranged from 28.9 to 30.0, considered indicator for severe HS.
- THI exceeded 30.0, considered indicator for very severe HS.

## 3. Animals, experimental design and diets:

A total of one hundred clinically healthy adult APRI rabbit does, weighed approximately 3030 ± 43.29 g with approximately six months old were randomly divided into five homogenized groups (20 does each). The does were individually housed in galvanized wire cages with standard dimensions of 60 x 55 x 40 cm<sup>3</sup> and provided with a kindling nest-box measuring 43 x 26 x 26 cm<sup>3</sup>. In order to maintain proper nutrition and hydration, manual feeders and an automatic nipple drinkers system were installed in the rabbit cages, ensuring *ad libitum* access to fresh water.

The experimental groups were organized as follows: the first group served as the control and received the basal diet without any supplementation. The 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> groups were given the basal diet supplemented with Nano-encapsulated Moringa Leaf Ethanolic Extract (NMLEE) at the rates of 20 (NMLEE20), 40 (NMLEE40), 60 (NMLEE60), and 80 (NMLEE80) g/kg body weight, respectively. The NMLEE treatment lasted for a period of 30 days

prior to mating. The does were provided with a basal diet that fulfilled the nutrient requirements for nulliparous rabbit does, as described by De Blas & Mateos (2010). The chemical composition and ingredients of the basal diet for nulliparous rabbit does are outlined in Table 1. The feeds underwent chemical analyses following the methods outlined by Horwitz et al. (1970).

During the experimental period, the rabbits does were kept under unique managerial, hygienic, and management circumstances. Animals underwent regular examinations for health status as well as their body condition. The evaluation of the rabbits' body condition involved tactile assessment of the spine, pelvis, and ribs.

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

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

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.

## 4. Blood hematology:

At the end of the treatment period in July, six does were randomly selected from each group for blood collection. Blood samples were drawn into heparinized test tubes from the does' marginal ear vein after applying Xylocaine (4%) to induce relevant anesthesia and divided into two separate subsamples for different

analyses. One subsample was used for the evaluation of the hematological attributes, including the count of red blood cells (RBCs,  $10^6/\text{mm}^3$ ) and white blood cells (WBCs,  $10^3/\text{mm}^3$ ), hemoglobin concentration (HGB, mg/dl), platelets count (PLT) and hematocrit (HCT, %) via automated Cbc Hematology Analyzer Hb-7021, China. The other subsample was centrifuged at 700 g for 20 minutes using a T32c centrifuge (Janetzki, Wallhausen, Germany). After centrifugation, the sections of blood plasma were separated and stored at a temperature of  $-20^\circ\text{C}$  in 1.5 ml Eppendorf tubes for biochemical analysis.

### 5. Biochemical assessments:

Blood profile including total protein (TP; g/dl), albumin (Alb; g/dl), triglycerides (TG; mg/dl), total cholesterol (TC; mg/dl), high-density lipoprotein (HDL; mg/dl), low-density lipoprotein (LDL; mg/dl), urea and creatinine levels were colorimetric measured using commercial kits (BioSystem S.A., Barcelona, Spain) in plasma samples. The levels of globulin (Glo; mg/dl) were assessed by subtracting the values of Alb from the corresponding values of total protein. The liver enzymes (Alanine transaminase; ALT and aspartate transaminase; AST IU/L) activities were assessed via commercial kits (BioSystems, Barcelona, Spain).

### 6. Redox status:

Blood plasma was analyzed for antioxidant potent as well as cellular immunity, its capacity of total antioxidant (TAC), superoxide dismutase (SOD) activities, glutathione (GSH), glutathione peroxidase (GPx), and malondialdehyde (MDA) levels by a spectrophotometer (Shimadzu, Kyoto, Japan) using commercial kits according to instructions of the manufacturers.

### 7. Hormonal analysis:

Cortisol, triiodothyronine (T3;  $\mu\text{g}/\text{dl}$ ), thyroxine (T4;  $\mu\text{g}/\text{dl}$ ), Estradiol (E2;  $\mu\text{g}/\text{dl}$ ), and progesterone (P4;  $\mu\text{g}/\text{dl}$ ) hormones were analyzed using ELIZA kits obtained from Cusabio Technology LLC and Pointe Scientific Inc. a manufacturer's instructions according to manufacturer's instructions.

### 8. Reproductive performance:

Following the treatment period (30 days), a total of 20 rabbits does from each group were artificially inseminated with 0.5 ml straw of pooled semen collected from fertile APRI bucks and diluted at a ratio of 1:5 using a glucose yolk citrate diluent in addition to 50  $\mu\text{g}/\text{ml}$  gentamycin. To induce ovulation, artificially

inseminated does received an intramuscular injection of 0.25 ml GnRH in the form of Receptal, Intervet equivalent B.V. The pregnancies of does were confirmed through abdominal palpation according to the methods outlined by **Theau-Clement et al. (2005)** and **El-Ratel & Gabr (2019)**. The parturition rate was calculated according to the following equation (the number of delivered does divided by the number of pregnant does) by 100. Twelve hours after kindling (giving birth), total litter size (TLS) and live litter size at birth (LSB) were recorded. Litter size at weaning (LSW) was recorded after weaning kits at 28 d of age. Furthermore, viability rates at birth (VRB) and weaning (VRW) were recorded. Also, the body weights of kids were recorded at birth and weaning. For suckling purposes, the kits cages were opened once daily every morning (for a maximum of 5 min.) at a fixed time (7 am).

### 9. Ovulatory response:

The ovarian activity of rabbits does received NMLEE exposed to heat stress conditions, subsequent weaning, and after the end of the suckling period was evaluated using five does from each group. Those does were randomly selected and transported to the laboratory and slaughtered according to the Islamic methods at 46 – 60 h post-mating according to **El-Ratel & Gabr (2019)**. Immediately after slaughtering, ovaries were separated and then several haemorrhagic follicles (HFs), large follicles ( $> 2\text{mm}$  diameter), and total follicles (LH+HF), corpora lutea (CLs) on the superficial tissues of ovarian were recorded for all slaughtered dose. The equation of **El-Ratel et al. (2020)** ( $\text{OR} = \text{CLs} (n) / \text{TF} (n) \times 100$ ) was applied for calculating the ovulation rate (OR %).

### 10. Statistical procedures:

A MIXED procedure of statistical analysis system (**Proc. mixed; SAS 2012**) was used for assessing haemato-biochemical parameters, hormones, antioxidant capacity, cellular immunity, and fertility traits. The levels of NMLEE were introduced as fixed factor in the model. However, individual rabbit doe was presented as random factor. In case of significant effects, the multiple comparisons between means were performed by Duncan's Multiple Range Test according to **Duncan (1975)**. Results were expressed as means  $\pm$  SE. The statistical significance was accepted at  $P < 0.05$ . Normality check was performed according to Shapiro–Wilk and Levene tests (**Razali and Wah 2011**).

## Result and Discussion

### 1. Meteorological parameters:

During the entire experimental weeks, the average ambient temperature (AT), relative humidity (RH), and temperature-humidity index (THI) were recorded as  $31.45 \pm 0.16^\circ\text{C}$ ,

$74.29 \pm 1.63\%$ , and  $30.09 \pm 0.22$ , respectively as illustrated in Table 2. The aforementioned findings suggested that the rabbit does experience a severe heat stress condition (Marai *et al.*, 2002).

**Table (2):** The values of ambient temperature, relative humidity, and temperature-humidity index during July month in Dakahlia Province, Egypt,

Parameters	WK1	WK2	WK3	WK4	Overall average
AT	31.08	31.46	31.37	31.89	$31.45 \pm 0.16$
RH	69.45	76.33	75.16	76.22	$74.29 \pm 1.63$
THI	29.50	30.20	30.06	30.60	$30.09 \pm 0.22$

WK, week; AT, ambient temperature; RH, relative humidity; THI, temperature-humidity index.

### 2. Hematological attributes:

Hematological parameters represent good indicator for rabbits' health status, infectious diseases, immune capacity, and other environment challenges such as HS. High ambient temperatures can impair the hematological parameters, particularly the erythrocyte and leucocyte counts, making rabbits more sensitive to infection or diseases (Ismail *et al.*, 2023). In this study, there were considerable effects of dietary supplementation of rabbit does with Nano-encapsulated Moringa Leaf Ethanolic Extract (NMLEE) on the concentration of hemoglobin (HGB;  $P < 0.0001$ ), the number of red blood cells count (RBCs;  $P < 0.0001$ ) and hematocrit (%;  $P = 0.0171$ ), being significantly higher in the NMLEE60 and NMLEE80 treated groups compared to the control ( $P < 0.05$ ) as illustrated in Table 3. Interestingly, all aforementioned hematological parameters were in the normal physiological range of clinically healthy rabbits as described in former scientific research (Jenkins, 1993; Hillyer, 1994; David *et al.*, 2002; Ahemen *et al.*, 2013), indicating that NMLEE had no prejudicial effect on rabbit health status.

The present results were in general agreement with the findings of El-Desoky *et al.* (2021) who reported a significant increase in the concentration of HGB and RBCs in the blood serum of rabbits does treated with 10 or 25 mg Nano-encapsulated Moringa Leaf Ethanolic Extract compared to their counterparts in the control group. Also, Ahemen *et al.* (2013) and Aljohani and Abduljawad (2018) showed a significant increase ( $P < 0.05$ ) in RBCs and HGB concentration in the growing rabbits as a

response to the dietary supplementation with Moringa Oleifera Leaf compared to the control group.

Herein, the improvements in hematological parameters may enhance blood's ability to carry oxygen to different tissues, thus improving different physiological and metabolic functions under severe HS condition (Hashem *et al.*, 2013). This increase in RBCs and HGB concentration of rabbits does could be explained by the nutritional content of moringa leaves, which are known to be abundant in vitamins, amino acids, and minerals particularly iron (Faye *et al.*, 2011) and have strong antioxidants such as vitamin C (Morsy *et al.*, 2007). Moreover, Ahemen *et al.* (2013) reported that the increased RBC counts can be attributed to the high-quality dietary protein provided by Moringa leaves.

In context, non-significant differences that exist in the WBCs and PLT ( $P = 0.7571$  and  $0.2731$ , respectively) in the current study revealed that there were no negative effects on the general health status of the rabbit does, which in general agreement with the findings of Nikkon *et al.* (2009) who observed non-significant differences in leukocyte, erythrocyte counts, leukocyte differential counts and PLT counts of rats orally administered with Moringa bark extract. In contrast, Adedapo *et al.* (2009) indicated that oral administration of rats with aqueous *M. oleifera* leaf extract at 400, 800, and 1600 mg/kg caused varied significant effects on the values of PLT and WBCs which could be attributed to the higher concentration of the examined extract.

**Table (3):** Effect of different levels of Nano-encapsulated Moringa leaf ethanolic extract (NMLEE) on hematological parameters of rabbit does under severe heat stress conditions

Items <sup>#</sup>	Nano-encapsulated Moringa Leaf Ethanolic Extract (mg/kg diet) <sup>*</sup>					SEM	p-value
	NMLEE0	NMLEE20	NMLEE40	NMLEE60	NMLEE80		
HGB(g/dl)	10.912 <sup>d</sup>	11.760 <sup>c</sup>	12.328 <sup>c</sup>	13.208 <sup>b</sup>	13.912 <sup>a</sup>	0.232	<.0001
RBCs (10 <sup>6</sup> /μl)	5.936 <sup>c</sup>	6.190 <sup>bc</sup>	6.492 <sup>ab</sup>	6.548 <sup>a</sup>	6.742 <sup>a</sup>	0.114	<.0001
WBCs(10 <sup>3</sup> /mm <sup>3</sup> )	7.810	7.632	7.506	7.490	7.472	0.207	0.7571
PLT(10 <sup>3</sup> /mm <sup>3</sup> )	234.712	230.730	231.986	222.790	216.874	6.255	0.2731
HCT (%)	36.570 <sup>b</sup>	38.746 <sup>b</sup>	40.804 <sup>ab</sup>	41.100 <sup>ab</sup>	46.148 <sup>a</sup>	1.805	0.0171

\*NMLEE0, NMLEE20, NMLEE40, NMLEE60, and NMLEE80 indicate 0, 20, 40, 60 and 80 mg Nano-encapsulated Moringa Leaf Ethanolic Extract /kg diet, respectively. <sup>#</sup> HGB, hemoglobin; RBCs, red blood corpuscles; WBCs, white blood cells; PLT, platelet count; HCT, hematocrit. Mean values with different superscript letters in the same row are significantly different (P<.0.05).

### 3. Blood biochemical analysis:

It is well known that the blood profile is considered an important biomarker of nutritional and physiological status in the entity (**Olorode et al., 2007**). The concentrations of blood protein and its fractions, total cholesterol (TC) and triglycerides (TG) are markedly increased under HS conditions in rabbits (**Ayyat & Marai, 1997; Li & Wang 2004**) which may be attributed to the increased secretion of glucocorticoid that promotes gluconeogenesis process (**Siegel & Van Kampen 1984**). In this study, co-treated rabbits does by NMLEE at a dose of 80mg/ kg diet resulted in a significant increase in serum total protein (TP), globulin (Glo), albumin (Alb), and high-density lipoprotein (HDL) and significant decreases in the concentrations of TC, TG, low-density lipoprotein (LDL) compared to the control group (Table, 4).

The current results corresponded with the results of **El-Wardany et al. (2015)**, **Abdella & Khalifah (2021)**, and **El-kashef (2022a)**, who showed significant increases in TP, Alb, and HDL and significant decreases in TC, TG, and LDL as a response to dietary supplementation of adult rabbit does with Moringa leaves. Similarly, **Samar et al. (2016)** and **Mehta et al. (2003)** reported a significant decrease in both TC and LDL and an increase in blood protein of rabbits which received moringa fruit compared to their corresponding control (P<0.05).

On the other hand, **El-Desoky et al. (2021)** reported a significant increase in levels of TP and Alb in the blood serum of rabbit does when they used moringa leaf. In contrast; **Yakubu et al. (2013)**, **Adeyemi (2018)**, and **Abdul-Azeem et al. (2022)** showed non-significant (P>0.05) differences in TP, Alb, TC, and TG of growing rabbits blood serum when fed diets supplemented

with Moringa extract compared to the untreated group.

Concerning liver function, the present study showed that the highest activities in liver enzymes (ALT and AST) were recorded in the control group compared to the other NMLEE-supplemented groups, and were minimized in the NMLEE60 or NMLEE80 groups (P<0.05; Table 4). This may refer to the ability of NMLEE to enhance the metabolism of protein and stimulate hepatic tissue regeneration which increases the synthesis of protein in rabbit does liver and thus improve the functional status of liver cells. In this concern, **El-kashef et al. (2022a)** attributed the health and safety of liver tissues to the improvement in functional status of the liver cells.

Additionally, the present results showed that the values of AST and ALT were within the normal physiological range of clinically healthy rabbits as outlined by **Melillo, (2007)**. This indicated that the rabbits were able to counter the anti-nutritional factors existing in Moringa leaves which can lead to possible toxic impacts when consume a large amounts. The present results agreed with the results of **Abdel-Latif et al. (2018)** and **El-kashef (2022b)** who showed a significant diminishing in levels of ALT and AST in the blood serum of rabbits received diets supplemented with *Moringa oleifera* extract under severe HS conditions. The contradictory results have been reported by **Ghomsy et al. (2017)** who recorded non-significant (P>0.05) differences in liver enzymes (AST and ALT) activities in blood serum of growing rabbits received *Moringa oleifera* leaf compared to the control group.

Concerning kidney function, the levels of serum urea and creatinine are known to reflect the state of kidney function and glomerular filtration rate (Kaneko *et al.*, 2008). In this study, the control group under severe HS showed elevated ( $P<0.05$ ) levels of serum urea and creatinine compared to NMLEE-treated groups (Table 4), indicating that the NMLEE can alleviate the negative effect of HS on renal function in rabbits does, with no deleterious effect on glomerular filtration rate, what in general agreement with the results of El-kashef (2022b) who reported significant decrease in the

levels of urea and creatinine in blood serum of heat stressed growing rabbits received *Moringa oleifera* leaves meal at levels of 5 or 7.5%. On the other side, Mohamed *et al.* (2020) reported a significant decrease in the levels of urea and creatinine in rabbits fed diets supplemented with *Moringa oleifera* leaf extract against lead toxicity. In contrast, Ghomsi *et al.* (2017) reported non-significant effects of *Moringa oleifera* leaf on kidney function represented by urea and creatinine levels in the blood serum of growing rabbits.

**Table (4):** Effect of different levels of Nano-encapsulated Moringa leaf ethanolic extract (NMLEE) on the blood profile of rabbits does under heat stress conditions.

Items <sup>#</sup>	Nano-encapsulated Moringa Leaf Ethanolic Extract (mg/kg diet) <sup>*</sup>					SEM	P-value
	NMLEE0	NMLEE20	NMLEE40	NMLEE60	NMLEE80		
TP (g/dl)	6.062 <sup>a</sup>	6.328 <sup>c</sup>	6.388 <sup>c</sup>	6.786 <sup>b</sup>	7.160 <sup>a</sup>	0.072	<.0001
Alb (g/dl)	3.156 <sup>c</sup>	3.454 <sup>ab</sup>	3.374 <sup>b</sup>	3.530 <sup>ab</sup>	3.598 <sup>a</sup>	0.053	0.0001
Glob (g/dl)	2.906 <sup>c</sup>	2.874 <sup>c</sup>	3.014 <sup>bc</sup>	3.256 <sup>b</sup>	3.562 <sup>a</sup>	0.100	0.0005
Alb/Glob ration	1.105	1.204	1.128	1.086	1.011	0.032	0.0907
TC (mg/dl)	98.270 <sup>a</sup>	89.946 <sup>b</sup>	87.541 <sup>bc</sup>	81.968 <sup>bc</sup>	80.228 <sup>c</sup>	2.657	0.0009
TG (mg/dl)	79.324 <sup>a</sup>	74.286 <sup>ab</sup>	70.178 <sup>bc</sup>	67.714 <sup>c</sup>	65.778 <sup>c</sup>	1.781	0.0002
HDL (mg/dl)	49.716 <sup>c</sup>	52.230 <sup>bc</sup>	52.330 <sup>bc</sup>	54.848 <sup>ab</sup>	57.546 <sup>a</sup>	1.421	0.0106
LDL(mg/dl)	30.404 <sup>a</sup>	26.150 <sup>b</sup>	24.286 <sup>b</sup>	24.392 <sup>b</sup>	22.754 <sup>b</sup>	1.427	0.0119
Urea (mg/dl)	39.980 <sup>a</sup>	35.294 <sup>ab</sup>	31.968 <sup>bc</sup>	30.628 <sup>bc</sup>	27.980 <sup>c</sup>	1.782	0.0013
Creatinine (mg/dl)	1.704 <sup>a</sup>	1.470 <sup>b</sup>	1.386 <sup>b</sup>	1.334 <sup>b</sup>	1.278 <sup>b</sup>	0.068	0.0026
AST (IU/l)	35.944 <sup>a</sup>	28.934 <sup>b</sup>	29.152 <sup>b</sup>	26.230 <sup>bc</sup>	23.410 <sup>c</sup>	1.357	<.0001
ALT (IU/l)	28.789 <sup>a</sup>	25.888 <sup>ab</sup>	24.338 <sup>ab</sup>	23.370 <sup>ab</sup>	20.822 <sup>b</sup>	1.792	0.0482

\*NMLEE0, NMLEE20, NMLEE40, NMLEE60, and NMLEE80 indicate 0, 20, 40, 60 and 80 mg Nano-encapsulated Moringa Leaf Ethanolic Extract /kg diet, respectively <sup>#</sup> TP, total protein; Alb, albumin; Glob, globulin; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Mean values with different superscript letters in the same row are significantly different ( $P<0.05$ ).

#### 4. Hormonal response:

Several former studies have observed that the using of *Moringa oleifera* leaves in diets of heat-stressed rabbits can improve the ability of animals to face HS and its deleterious effects on the hormonal system (Mutwedu *et al.*, 2022; El-kashef *et al.*, 2022a). In this study, the supplementation of rabbit does diets with NMLEE affected thyroid hormones (Triiodothyronine; T3 and Thyroxine; T4) significantly ( $P<0.0001$ ), being significantly ( $P<0.05$ ) higher in the NMLEE60 and NMLEE80 groups than the control group (Table 5). These results are in general agreement with the findings of El-kashef *et al.* (2022a) who showed a significant increase in the levels of T3 and T4 in heat-stressed growing rabbits supplemented with *Moringa oleifera* leaves meal.

Cortisol is a hormone that is commonly associated with the body's response to stress.

Mazlomi *et al.* (2017) showed that there was a direct association between HS and elevated levels of cortisol in the blood serum of animals. Moreover, it has also been observed that HS can induce inflammatory reactions in animal's body (Yun *et al.*, 2012). The current study revealed decreased ( $P<0.05$ ) levels of serum cortisol in all NMLEE-treated groups, minimizing in the NMLEE80 group compared to the control (Table 5). The present results agreed with the results of Abdel-Latif *et al.* (2018) who showed that the dietary addition of *Moringa oleifera* can improve the ability of rabbits to confront HS by shrinking the levels of cortisol compared to the control group. Also, Khalid *et al.* (2020) found the same results when they added *Moringa oleifera* leaf powder in heat-stressed rabbit diets at a dose of 200 mg/kg body weight.

Under severe HS, the low levels of cortisol and high levels of thyroid hormones

presents better adaptation to chronic HS, reduced oxidation rate of glucose, as well as increase metabolic heat production (Kumari & Nath, 2018). Therefore, the low levels of cortisol and high levels of T3 and T4 in the NMLEE-treated groups indicate the ability of rabbits to heat resistance. The present results could be attributed to its higher contents of antioxidant vitamins such as vitamins C and E as well as polyphenols and  $\beta$ -carotene (Kidmose *et al.*, 2006). These components can increase the antioxidant activity of *Moringa Oleifera* more than traditional antioxidants such as ascorbic acid (Yang *et al.*, 2006). Additionally, *Moringa Oleifera* leaves contain anti-inflammatory compounds, vitamin A, iron, simple sugar, and vitamin B1 & B2 (Yang, *et al.*, 2006; Ferreira *et al.*, 2008; Konmy *et al.*, 2016) which can support body functions in addition to safeguarding the health of body tissues.

When it comes to reproduction, female rabbits are thought to reproduce best in the temperature range of 15 to 20°C. When the outside temperature rises above this range, the rabbits become ill and experience health problems that impair their ability to reproduce (Marco Jiménez *et al.*, 2017). According to Upadhyay *et al.* (2009), raising the outside temperature by more than 2°C causes the pineal-hypothalamo-hypophyseal-gonadal axis and other endocrine systems to become desynchronized or to function less normally. This leads to a decline in the reproductive hormones' respective functions. Additionally, according to Ozawa *et al.* (2005), HS impairs the synthesis of follicular estradiol and interferes with LH action by lowering its receptor level, which causes a significant drop

in the fertilization rate. Low secretion of estradiol has been linked to ovulation weakness and a reduction in gonadotropin levels in response to the regulation of estrus symptoms (Wolfenson *et al.*, 2000). Furthermore, it has been noted that heat-stressed rabbits have decreased progesterone secretion, which affects embryonic development by interfering with endometrial function (Jimoh *et al.*, 2021).

Results in Table 5 indicated that serum levels of progesterone (P4) and estradiol (E2) significantly decreased in the group submitted to heat stress and increased in heat-stressed rabbits does which co-treated by 60 or 80 mg NMLEE/ kg diet. The significant increases in P4 and E2 hormones in this study could be explained by the presence of several bioactive compounds in *Moringa oleifera* which have been reported to stimulate the secretion of reproductive hormones. For instance, phytosterols were found to have a chemical structure similar to cholesterol that can be used as precursors of steroid hormones (estradiol progesterone and testosterone). Isoflavones are considered one of the flavonoid compounds that have estrogenic activities, which can bind with the receptors of estrogen such as ER- $\alpha$  and ER- $\beta$  (Setiasih *et al.*, 2021). Alkaloids and phenolic compounds have been shown to protect embryonic tissue from the impairment of reactive oxidative stress by promoting the secretion of ovarian hormones in blood serum (Grzanna *et al.*, 2005). Saponins, phytosterols, flavonoids, and polyphenols in *Moringa oleifera* seeds have been previously linked to stimulate the production of female reproductive hormones (Estrada *et al.*, 2001 and Khan *et al.*, 2005).

**Table (5):** Effect of different levels from Nano-encapsulated *Moringa* leaf ethanolic extract (NMLEE) on blood hormones of rabbit does under severe heat stress conditions.

Items <sup>#</sup>	Nano-encapsulated <i>Moringa</i> Leaf Ethanolic Extract (mg/kg diet)*					SEM	P-value
	NMLEE0	NMLEE20	NMLEE40	NMLEE60	NMLEE80		
Cortisol (ng/ml)	12.150 <sup>a</sup>	10.838 <sup>b</sup>	10.042 <sup>b</sup>	9.432 <sup>c</sup>	9.338 <sup>c</sup>	0.143	<.0001
T3 ( $\mu$ g/dl)	0.524 <sup>c</sup>	0.744 <sup>b</sup>	0.772 <sup>ab</sup>	0.832 <sup>ab</sup>	0.868 <sup>a</sup>	0.036	<.0001
T4 ( $\mu$ g/dl)	1.750 <sup>d</sup>	2.658 <sup>c</sup>	3.730 <sup>b</sup>	4.282 <sup>ab</sup>	4.984 <sup>a</sup>	0.249	<.0001
E2 ( $\mu$ g/dl)	9.698 <sup>c</sup>	10.940 <sup>b</sup>	11.406 <sup>b</sup>	11.540 <sup>ab</sup>	12.694 <sup>a</sup>	0.413	0.0012
P4 ( $\mu$ g/dl)	3.200 <sup>c</sup>	3.732 <sup>b</sup>	3.780 <sup>b</sup>	3.934 <sup>b</sup>	4.654 <sup>a</sup>	0.120	<.0001
E2/P4 ratio	3.032	2.936	3.027	2.949	2.734	0.133	0.5348

\*NMLEE0, NMLEE20, NMLEE40, NMLEE60, and NMLEE80 indicate 0, 20, 40, 60 and 80 mg Nano-encapsulated *Moringa* Leaf Ethanolic Extract /kg diet, respectively #T3, triiodothyronine hormone; T4, thyroxine hormone; E2, Estradiol; P4, progesterone. Mean values with different superscript letters in the same row are significantly different (P<0.05).



**5. Redox balance:**

Moringa oleifera is a plant that has been known for its multiple antioxidants, including phenolic acids such as chlorogenic acid, ellagic acid, gallic acid, and ferulic acid. It also contains flavonoids and glucosinolate such as kaempferol, rutin, and quercetin, as well as significant levels of vitamins B-complex, β-carotene (a precursor of vitamin A), vitamin C, D, and K (Mbikay, 2012 and Sodamade et al., 2013). These antioxidants found in Moringa oleifera are documented to be effective in countering the induced oxidative damage by enhancing the activities of antioxidant enzymes. This resulted in a reduction in the peroxidation of lipids and protein as well as the production of harmful free radicals, which can lead to cellular damage (Sreelatha & Padma, 2009).

Results in Table 6 showed significant effects of NMLEE on serum total antioxidant capacity (TAC; P<0.001), glutathione content (GSH; P=0.0038), and the activities of glutathione peroxidase (GPX; P<0.0001) and superoxide dismutase (SOD; P=0.0250), maximizing in the NMLEE80 treated group compared to the control group (P<0.05). Meanwhile the levels of malondialdehyde (MDA;P<0.0001) showed the opposite trend (P<0.05). In general, HS causes a negative change in the redox balance of rabbits,

inducing oxidative stress, which raises lipid peroxidation (MDA) and decreases the endogenous antioxidant enzymes (Jimoh et al., 2018; Kuang et al., 2021) as observed in the current study in the control group which submitted to severe heat stress. However, the dietary supplementation of rabbits does with NMLEE can mitigate their adverse impacts.

The current results corresponded with the results of El-Desoky et al. (2021) who reported that the rabbit does treated with Nano-encapsulated Moringa Leaf Ethanolic Extract at a level of 10 mg/kg BW showed a significant increase in levels of TAC and the activity of GPX. Also, Salem et al. (2022) indicated that rabbits supplemented with 20% Moringa leaves showed a significant decrease in the levels of malondialdehyde (MDA) while the concentration of total antioxidants capacity increased significantly compared to the control group (P<0.05). Moreover, Olusiyi et al. (2022) reported that 5% Moringa leaf meal can improve animal performance and increased the expressions of mRNA for CAT, and SOD. In rats, several former studies revealed that Moringa leaves or extract can increase the activities of GSH, GPx, and catalase and reduce the levels of MDA (Sutalangka et al., 2013; Oseni & Idowu, 2014; Oparinde & Atiba, 2014 and Lamou et al., 2016).

**Table (6):** Effect of different levels from Nano-encapsulated Moringa leaf ethanolic extract (NMLEE) on redox status of rabbit does under severe heat stress conditions

Items <sup>#</sup>	Nano-encapsulated Moringa Leaf Ethanolic Extract (mg/kg diet) <sup>*</sup>					SEM	p-value
	NMLEE	NMLEE2	NMLEE4	NMLEE60	NMLEE8		
	0	0	0	0	0		
<b>Redox balance</b>							
TAC (mmol/L)	0.954 <sup>c</sup>	1.031 <sup>c</sup>	1.366 <sup>b</sup>	1.514 <sup>ab</sup>	1.644 <sup>a</sup>	0.080	<.0001
GSH (mg/dl)	11.222 <sup>b</sup>	13.042 <sup>b</sup>	14.772 <sup>ab</sup>	17.232 <sup>a</sup>	17.868 <sup>a</sup>	1.194	0.0038
GPX (mg/dl)	2.210 <sup>d</sup>	2.460 <sup>c</sup>	2.740 <sup>b</sup>	2.712 <sup>b</sup>	2.964 <sup>a</sup>	0.056	<.0001
SOD (IU)	18.216 <sup>c</sup>	21.652 <sup>bc</sup>	24.700 <sup>ab</sup>	25.596 <sup>ab</sup>	28.284 <sup>a</sup>	2.063	0.0250
MDA(nmol/ml)	5.965 <sup>a</sup>	3.738 <sup>b</sup>	3.442 <sup>b</sup>	3.384 <sup>b</sup>	3.184 <sup>b</sup>	0.340	<.0001

<sup>\*</sup>NMLEE0, NMLEE20, NMLEE40, NMLEE60, and NMLEE80 indicate 0, 20, 40, 60, and 80 mg Nano-encapsulated Moringa Leaf Ethanolic Extract /kg diet, respectively <sup>#</sup>TAC, total antioxidant capacity; GSH, glutathione content; GPx, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde. Mean values with different superscript letters in the same row are significantly different (P<.05).

## 6. Ovarian activity:

Results in Table 7 illustrate the effect of different levels of NMLEE on ovarian activity. Herein, the dietary supplementation of NMLEE resulted in a significant increase in the percentage of normal embryos ( $P=0.0377$ ) and ovulation rate ( $P=0.0033$ ), being significantly higher in all NMLEE-treated group compared to the control group ( $P<0.05$ ). However, there was a significant decrease ( $P=0.0377$ ) in the number of abnormal embryos, minimizing in the NMLEE60 and NMLEE80 treated group when compared to the control ( $P<0.05$ ). Regarding the blastocyst hatching rate of blastocysts, it was significantly improved by the dietary treatment ( $P=0.0226$ ), being significantly higher in the aforementioned treated groups than the control group ( $P<0.05$ ). Non-significant differences were observed in the total ( $P=0.3747$ ), large ( $P=0.5363$ ) and haemorrhagic follicles ( $P=0.4803$ ), and the numbers of corpora lutea ( $P=0.1469$ ), blastocysts percentage ( $P=0.8706$ ). The present results could be attributed to the positive energy of NMLEE-supplemented rabbits does before mating as Moringa leaf is rich in fatty acids which may be related to improved body weight and metabolism due to changes in the feed intake. **El-Desoky, et al. (2022)** reported that the NMLEE contained 30 fatty acids, including 54.27% total unsaturated fatty acids. The positive effect of NMLEE on the energy status of female rabbits is required to achieve better reproductive efficiency. Several former studies showed a significant association between the better artificial insemination outputs and higher body weight of rabbits does around mating time and early pregnancy (**Benayad et al., 2021 and Balthazar et al., 2021**). In addition to the crucial role of Moringa oleifera leaf extract on metabolism and body weight, the unique fatty acid composition of Moringa oleifera might have other positive impacts. Previous studies have observed that fatty acid-rich diets can enhance fertility and pregnancy outcomes in rabbits (**Rebollar et al., 2014 and Rodríguez et al., 2019**). Fatty acids are important molecules for

several reproductive events through many potential modes. Numerous fatty acids can positively improve reproduction performance by altering the functions of ovarian follicles and corpus luteum in response to promote the general energy status as well as inducing precursor levels for the synthesis of prostaglandins and reproductive steroids (**De Mattos et al., 2000**). Moreover, they can boost embryo development and improve the competence of oocytes (**Cerri et al., 2009**). According to these findings, NMLEE supplementation in female rabbits exposed to heat stress (HS) could promote their reproductive performance by enhancing immune response and redox balance, whereas NMLEE molecules may act as a potential feed supplement that can be applied in the rabbit industry to protect against the adverse effects of HS. Thus, these impacts of dietary NMLEE addition may be associated with sexual neurotransmitter signaling pathways in addition to blood flow throughout the genital tract, resulting in significant improvements in the general sexual performance of rabbits does. Besides, NMLEE like other natural antioxidants contributes to multiple signaling pathways that are involved in the embryo development and molecular regulation of folliculogenesis in rabbits does (**Abdelnour et al., 2022**). During the follicular phase of the estrous cycle, **Mutweddu et al. (2022)** found that Moringa oleifera aqueous seed extracts enhanced the synthesis of estradiol-  $17\beta$  (E2) and induced the secretion of progesterone (P4) during the luteal phase. Despite the high ambient temperature decreases the secretion of estrogen, causing irregular estrus and abnormal oocyte morphology, such as rupture of the transparent membrane and cytoplasmic shrinkage, making oocyte cells unable to fertilize and ultimately affecting the reproduction performance of rabbits does (**García & Argente, 2017**). But the dietary addition of NMLEE particularly at high doses resulted in significant effects on the blastocyst hatching rate.

**Table (7):** Effect of different levels of Nano-encapsulated Moringa leaf ethanolic extract (NMLEE) on ovarian activity of rabbit does under severe heat stress conditions.

Items <sup>#</sup>	Nano-encapsulated Moringa Leaf Ethanolic Extract (mg/kg diet)*					SEM	p-value
	NMLE	NMLEE2	NMLEE4	NMLEE6	NMLEE8		
	E0	0	0	0	0		
LF (n)	23.200	20.600	20.200	19.800	19.600	1.627	0.5363
HF (n)	4.200	3.600	3.200	3.000	2.400	0.707	0.4803
TF (n)	27.400	24.200	23.400	22.800	22.000	1.969	0.3747
CL (n)	12.600	13.600	16.800	17.600	17.800	1.741	0.1469
OR (%)	47.287 <sup>b</sup>	58.561 <sup>b</sup>	74.016 <sup>a</sup>	79.686 <sup>a</sup>	87.911 <sup>a</sup>	4.597	0.0033
Embryos (n)	12.600	13.600	16.800	17.600	17.800	1.741	0.1469
Normal E (%)	66.545 <sup>b</sup>	75.921 <sup>ab</sup>	81.488 <sup>a</sup>	83.619 <sup>a</sup>	85.976 <sup>a</sup>	4.384	0.0377
Abnormal E (%)	33.455 <sup>a</sup>	24.079 <sup>ab</sup>	18.512 <sup>b</sup>	16.381 <sup>b</sup>	14.024 <sup>b</sup>	4.384	0.0377
BL (%)	44.727	44.889	38.475	38.611	41.850	5.667	0.8706
BL hatching rate (%)	20.303 <sup>b</sup>	29.921 <sup>ab</sup>	37.614 <sup>a</sup>	39.937 <sup>a</sup>	40.244 <sup>a</sup>	4.486	0.0226

\*NMLEE0, NMLEE20, NMLEE40, NMLEE60, and NMLEE80 indicate 0, 20, 40, 60 and 80 mg Nano-encapsulated Moringa Leaf Ethanolic Extract /kg diet, respectively # LF, Large follicles; HF, Haemorrhagic follicles; TF, Total follicles; CL, Corpora lutea; OR, Ovulation rate; BL, Blastocysts. Mean values with different superscript letters in the same row are significantly different (P<0.05).

### 7. Reproductive performance:

The present results demonstrated significant effects of NMLEE supplementation on total litter size at birth (TLS, P=0.0136), live litter size at birth (LSB; P=0.0025), and weaning (LSW; P=0.0167), being significantly higher in the NMLEE40, NMLEE60, and NMLEE80 groups than those in the control and NMLEE20 group. Moreover, both individual and cumulative body weight at birth and weaning showed significant improvements, taking the same previous trend (P<0.001) as presented in Table 8. The present results were in line with the findings of **El-Desoky et al. (2022)** who reported that the supplementation of 25 mg of nano-encapsulate *Moringa oleifera* leaf extract significantly increased both pregnancy and delivering rates, total litter size at birth and weaning, and live litter size at birth

and at weaning. **Mutwedu et al. (2022)** observed a significant decrease in litter size from birth to weaning in rabbits does submitted to severe heat stress which is similar to that observed in the control group in the present study. **El-Desoky et al. (2021)** reported that the body weights of kits born to encapsulate MLEE-treated does were significantly higher than those shown for does received nano-encapsulated MLEE and control diets. This phenomenon may refer to enhanced accessibility of MLEE bioactive components to developing fetuses through improved transportation by the placenta, as Nano-encapsulation form may facilitate the transfer of active components, specifically those with large molecular weight and limited solubility, across the fetal-placental circulating system (**Hashem and Gonzalez-Bulnes, 2020**).

**Table (8):** Effect of different levels of Nano-encapsulated Moringa leaf ethanolic extract (NMLEE) on reproductive performance of rabbit does exposed to severe heat stress conditions

Items <sup>#</sup>	Nano-encapsulated Moringa Leaf Ethanolic Extract (mg/kg diet) <sup>*</sup>					SEM	P-value
	NMLEE0	NMLEE20	NMLEE40	NMLEE60	NMLEE80		
TMD (n)	20	20	20	20	20	--	--
PR (n)	13	16	18	18	19	--	--
DR (n)	11	15	18	18	18	--	--
TLS (n)	6.154 <sup>b</sup>	7.000 <sup>ab</sup>	7.556 <sup>a</sup>	7.778 <sup>a</sup>	8.211 <sup>a</sup>	0.467	0.0136
LSB (n)	5.231 <sup>c</sup>	6.000 <sup>bc</sup>	6.556 <sup>ab</sup>	6.833 <sup>ab</sup>	7.368 <sup>a</sup>	0.420	0.0025
VRB (%)	87.549	86.892	87.533	89.195	91.053	4.365	0.9355
LSW(n)	4.615 <sup>b</sup>	5.313 <sup>ab</sup>	5.833 <sup>a</sup>	6.111 <sup>a</sup>	6.474 <sup>a</sup>	0.438	0.0167
VRW (%)	89.505	88.889	89.083	88.931	89.254	3.762	1.000
BWB (g)	53.533 <sup>c</sup>	54.656 <sup>bc</sup>	56.873 <sup>abc</sup>	58.218 <sup>ab</sup>	59.760 <sup>a</sup>	1.358	0.0033
BWW (g)	281.257 <sup>c</sup>	330.691 <sup>bc</sup>	370.506 <sup>b</sup>	395.234 <sup>ab</sup>	439.778 <sup>a</sup>	24.948	<.0001
CBWB (g)	494.192 <sup>c</sup>	558.737 <sup>a</sup>	533.909 <sup>b</sup>	546.119 <sup>ab</sup>	554.445 <sup>a</sup>	5.480	<.0001
CBWW(g)	2278.046 <sup>b</sup>	2971.396 <sup>a</sup>	3110.604 <sup>a</sup>	3339.828 <sup>a</sup>	3554.531 <sup>a</sup>	196.248	0.0017

\*NMLEE0, NMLEE20, NMLEE40, NMLEE60, and NMLEE80 indicate 0, 20, 40, 60, and 80 mg Nano-encapsulated Moringa Leaf Ethanolic Extract /kg diet, respectively <sup>#</sup> TMD, total mated dose; PR, pregnant rabbit does, DR, delivered rabbit does; TLS, total litter size at birth; LSB, live litter size at birth; VRB, Viability rate at birth; LSW, litter size at weaning, VRW, Viability rate at weaning; BWB, body weight at birth; BWW, body weight at weaning; CBWB, cumulative body weight at birth, CBWW, cumulative body weight at weaning. Mean values with different superscript letters in the same row are significantly different (P<0.05).

## CONCLUSION

The present study confirms the beneficial impacts of NMLEE at a dose of 80 mg/kg diet as a supplement in enhancing heat-stress tolerance and reproductive performance in rabbits does exposed to natural heat-stress conditions. Thus, NMLEE supplements are a valuable tool for enhancing rabbit health. The rich composition of bioactive components present in MLEE were found to mend the adverse effects of heat stress by improving digestion, feed use, and enhancing antioxidant capacity. *Moringa oleifera* effectively counters oxidative damage by enhancing the activities of antioxidant enzymes, making it a valuable plant for health.

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## AUTHORS CONTRIBUTION:

All authors developed the concept of the manuscript, achieved the experiments, and wrote and revised the final manuscript.

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### الملخص العربي

تأثير مستخلص النانو مورينجا على الحالة الصحية والأداء التناسلي لإناث الأرناب تحت ظروف الإجهاد الحراري

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تم إجراء هذا البحث بهدف دراسة تأثير اضافة أربع مستويات من المستخلص الإيثانولي لأوراق نبات المورينجا في صورة نانو كبسول (NMLEE) علي خصائص الدم وحالة الأوكسدة بالجسم والاستجابة الهرمونية ونشاط المبيض والاداء التناسلي لإناث الأرناب تحت ظروف الإجهاد الحراري. تم اجراء التجربة بمزرعة خاصة بمحافظة الدقهلية بالتعاون مع قسم الإنتاج الحيواني بكلية الزراعة جامعة دمياط. تم تقسيم 100 أنثى لم تلد من قبل من سلالة الأبري عشوائياً على 5 مجاميع تجريبية (20 في كل مجموعة)، تم تغذيتها على العليقة الأساسية مضاف إليها مستويات 0، 20، 40، 60، 80 ملجرام NMLEE لكل كيلو جرام عليقة على التوالي لمدة 30 يوم كفترة معاملة من إجمالي 90 يوم كفترة تجريبية كلية خلال فصل الصيف. أظهرت النتائج ان اضافة 80 ملجرام من NMLEE أدى إلى انخفاض تركيز الكوليسترول والجليسريدات الثلاثية والمواد الدهنية منخفضة الكثافة واليوريا والكرياتينين و معدل اكسدة الدهون وتركيز انزيمات الكبد في الدم. بينما كان هناك زيادة في مستويات بروتينات الدم (الاليومين والجلوبولين) والمواد الدهنية مرتفعة الكثافة والهيموجلوبين والهيماتوكريت والكفاءة التأكسدية الكلية وكذلك نشاط انزيمات الجلوتاثيون والجلوتاثيون بيروكسيديز والسوبر اكسيد ديسموتيز مقارنة بالمجموعة الكنترول. كان هناك زيادة في تركيز هرمونات ال T3 و T4 وكذلك هرمون البروجسترون والاستروجين في المجموعة المعاملة بمستويات 60 او 80 ملجرام NMLEE لكل كيلو جرام عليقة بينما انخفض مستوى هرمون الكورتيزول في المجموعتين سالفتا الذكر مقارنة بالمجموعة الكنترول. كلا من معدل التبويض والبلاستوسيسست المفقسه وكذلك نسبة الأجنة الطبيعية قد تحسنتا معنويا في المجموعة المعاملة بمستوي 80 ملجرام NMLEE لكل كيلو جرام عليقة مقارنة بالكنترول. ارتفع الأداء التناسلي في المجموعات المغذاه على NMLEE مقارنة بالمجموعة الكنترول. في الخاتمة ادت اضافة NMLEE الى تخفيف الإجهاد الحراري على اناث الأرناب كما ادت إلى تحسن معنوي في ميتابوليزم الدم والأداء التناسلي وحالة الأوكسدة بالجسم والاستجابة الهرمونية خلال فصل الصيف.

### الخلاصة

أكدت الدراسة الحالية على الدور الإيجابي للمستخلص الإيثانولي لأوراق المورينجا في صورة نانو كبسول، في تعزيز الوظائف الميتابوليزمية، وتخفيف تأثير الإجهاد الحراري على إناث الأرناب، ويعزى ذلك إلى وجود المواد الفعالة التي من شأنها تخفيف الإجهاد الحراري من خلال تحسين الحالة المناعية وحالة الأوكسدة والاستجابة الهرمونية خلال فترة الحمل خلال فصل الصيف عند التعرض للإجهاد الحراري.

**الكلمات المفتاحية:** نانو كبسول، مستخلص أوراق المورينجا، الأرناب، الأوكسدة، الهرمونات، التناسل.