

The Efficacy of Manganese in Improving Semen Quality of Heat-Stressed Male Layer Chickens

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ABSTRACT

The reproductive efficiency of breeder roosters is crucial due to the low male-to-female ratio, particularly under heat stress, which negatively impacts fertility. This study evaluated the effects of dietary manganese supplementation on semen quality and reproductive hormones in 45 Hi-Line breeder roosters (50 weeks old) over a 12-week period at the College of Agriculture, Al-Qasim Green University, Iraq. Roosters were assigned to five groups (three replicates per group, three birds per replicate) and fed either a control diet (basal diet, T5) or diets supplemented with 10, 20, 30, or 40 mg of manganese sulfate per kg of feed (T1–T4). Semen samples were analyzed biweekly for ejaculate volume, sperm motility, concentration, and plasma membrane integrity. Blood samples were collected to assess testosterone, LH, FSH, and oxidative stress markers (ALT, AST, GPx). Results showed significant differences ($P \leq 0.05$) among treatments. The control (T5) had the highest ejaculate volume, while T4 and T5 improved sperm motility. T3 and T5 increased sperm concentration, whereas T1 and T2 reduced the percentage of dead and abnormal sperm. These findings suggest that manganese supplementation enhances reproductive performance under heat stress.

Keywords: cocks, heat stress, manganese, semen quality.



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Received: 16/12/2024, Accepted: 12/3/2025, Published: 30/9/2026

INTRODUCTION

High ambient temperatures pose a significant challenge to poultry production, adversely affecting reproductive efficiency, semen quality, fertility, hatchability, and overall flock productivity (Karaca et al., 2002b). Heat stress (HS) is particularly detrimental to breeder roosters, leading to impaired sperm quality and reduced sperm-egg penetration, which ultimately compromises fertilization success (McDaniel et al., 2004). Studies have shown that HS increases the proportion of dead sperm, decreases the sperm quality index, and significantly reduces fertility rates (McDaniel et al., 2004). Moreover, elevated concentrations of chloride (Cl⁻) and sodium (Na⁺) ions in semen under HS conditions have been linked to reduced fertility and potential

sterility in roosters (Fernandes & Murakami, 2010). Manganese (Mn), an essential trace mineral, plays a vital role in various biological processes, particularly as a cofactor for metalloenzymes involved in oxidative stress regulation, metabolism, and reproductive function. Mn deficiency in poultry has been associated with skeletal abnormalities, cartilage malformations, and impaired antioxidant defense mechanisms (Bucak et al., 2010; Kim et al., 2013). Additionally, Mn-dependent enzymes such as arginase and pyruvate carboxylase contribute to key metabolic pathways, including amino acid metabolism and energy production (Bucak et al., 2010). Recent research has highlighted the importance of Mn in reproductive physiology, demonstrating that Mn supplementation

enhances the expression of follicle-stimulating hormone (FSH) and gonadotropin-releasing hormone-I (GnRH-I), both of which are essential for regulating reproductive function (Moomaw et al., 2009). Furthermore, Mn has been reported to improve sperm motility in a time- and dose-dependent manner, stabilize the sperm plasma membrane, and enhance sperm viability by reducing oxidative stress (Bansal, 2013). Although excessive Mn levels can be toxic, adequate supplementation has been shown to mitigate oxidative damage, improve sperm survival during capacitation, and support overall reproductive performance (Bakst & Dymond, 2013; Bansal & Bilaspuri, 2010). Given these findings, this study aims to evaluate the effects of dietary Mn supplementation on semen quality in laying breeder roosters exposed to heat stress. By investigating the role of Mn in enhancing sperm function and mitigating the adverse effects of HS, this research seeks to provide valuable insights into the potential benefits of Mn supplementation in improving reproductive efficiency in poultry production systems.

MATERIALS AND METHODS

Animal Ethics: This experiment was conducted in the Department of Animal Production, College of Agriculture, Al-Qasim Green University, Iraq, following approval from the institutional ethics committee. All procedures related to animal care, including

housing, feeding, euthanasia, and overall animal welfare, were carried out in accordance with ethical guidelines for poultry research.

Bird Husbandry: A total of 45 Hi-Line laying breeder roosters, aged 50 weeks and with an average body weight of 2730 g, were used in this study. The birds were randomly assigned to five dietary treatment groups, each comprising three replicates with three birds per replicate. The experiment was conducted at the poultry farm of the College of Agriculture, Al-Qasim Green University, Iraq. The roosters were housed in individual cages under controlled environmental conditions, maintaining a temperature of 34–35 degrees Celsius and a photoperiod of 16 hours light and 8 hours dark (16L: 8D). The experimental period lasted 12 weeks, during which the birds were provided with one of five dietary treatments: a control diet (C) containing a basal feed without manganese supplementation, or one of four experimental diets supplemented with manganese sulfate at 10 mg/kg (T1), 20 mg/kg (T2), 30 mg/kg (T3), or 40 mg/kg (T4). All diets were formulated to meet the nutritional requirements of breeder roosters, and feed and water were provided *ad libitum* throughout the experimental period.

Experimental Diets: The experimental diet contained 16.53% crude protein and 2788.44 kcal/kg metabolizable energy, with a daily feed allowance of 104 g per rooster (Table 1).

Table 1. Diet Composition and Nutritional Analysis

Ingredients	%
yellow corn	37
wheat	14
barley	16.2
Soybean meal (44% protein)	20
Premix *	6.8
DCP Calcium Diphosphate	3
Sunflower oil	3
Total**	100%
Metabolic energy (kilocalories/kg feed)	2788.44
Crude protein (%)	16.53
Crud fiber	3.45
Calcium (%)	1.26
Available phosphorus (%)	0.74
Methionine + cysteine (%)	0.69
Lysine (%)	0.73

*Premix Mixture of Belgian origin Each 1 kg contains: crude protein 7.9%, lysine 2.4%, * Premix Composition (Belgian origin, per 1

kg):Crude protein (7.9%), lysine (2.4%), methionine (7.7%), methionine + cysteine (7.7%), calcium (23.1%), phosphorus (3.3%),

sodium (5.5%), representative energy (2903 kcal/kg), vitamin A (400,000 IU), vitamin D₃ (300,000 IU), vitamin D (20,000 IU), vitamin E (800 IU), vitamin K (80 ppm), vitamin B₁ (40 ppm), vitamin B₂ (1600 ppm), calcium pantothenate (320 ppm), niacin (600 ppm), biotin (1600 ppb), vitamin B₁₂ (1000 ppb), folic acid (40 ppm), vitamin B₆ (160 ppm), iron (2800 ppm), copper (600 ppm), zinc (2400 ppm), magnesium (4000 ppm), iodine (80 ppm), selenium (8 ppm).

Semen Assessments: Semen was collected three times per week using the abdominal massage method described by Al-Daraji (2013). Samples were collected in 1.5 mL microtubes and immediately incubated at 37 degrees Celsius before assessment. All subsequent evaluations were conducted using a light microscope.

Ejaculate Volume: The ejaculate volume was measured directly using a graduated 1.5 mL microtube.

Total Motility: Total sperm motility was assessed by preparing two slides per sample, examining two microscopic fields per slide, and counting 100 spermatozoa per field. Spermatozoa that absorbed the stain were classified as non-motile (dead), following the criteria of Al-Daraji (2007).

Progressive Motility: Progressive sperm motility was determined by placing a drop of semen on a glass slide and mixing it with three drops of 2.9% sodium citrate solution. A coverslip was placed over the sample, and sperm movement was evaluated under an optical microscope at 40× magnification, following the method described by Al-Daraji (2007).

Sperm Concentration: Sperm concentration was determined using a hemocytometer, as described by Al-Daraji (2007).

Sperm Plasma Membrane Integrity: To assess sperm plasma membrane integrity, a drop of semen was mixed with an equal

volume of eosin-nigrosine stain and spread into a thin smear. The smear was air-dried for one minute before evaluation under a microscope.

Statistical Analysis: Data were analyzed using the Statistical Analysis System (SAS) software (SAS Institute, 2012) based on a Completely Randomized Design (CRD). Mean differences among treatments were compared using Duncan's Multiple Range Test (DMRT) (Duncan, 1955). The statistical model used for data analysis was:

$$Y_{ij} = u + t_i + e_{ij}$$

Where **Y_{ij}** represents the observation *j* of treatment *i*; **u** is the overall mean; **t_i** is the effect of treatment *i* (the study included the impact of five treatments); and **e_{ij}** is the random error, which is normally distributed with an average of zero and a variance of sigma square *e*

RESULTS AND DISCUSSION

Table 2 presents the effects of manganese supplementation on ejaculate volume across different experimental periods. No significant differences were observed between treatments in the first period. However, in the second period, a significant ($P \leq 0.05$) increase in ejaculate volume was recorded in the T4 and T5 treatments compared to the other groups. During the third period, the T5 treatment exhibited a significantly ($P \leq 0.05$) higher ejaculate volume than the other treatments. In the fourth period, the T4 treatment showed significant ($P \leq 0.05$) superiority over the other groups, whereas in the fifth period, the T2 treatment demonstrated a significantly ($P \leq 0.05$) higher ejaculate volume. In the sixth period, the T3 and T4 treatments exhibited significant ($P \leq 0.05$) increases in ejaculate volume. When considering the overall average, the T5 treatment resulted in a significantly ($P \leq 0.05$) higher ejaculate volume compared to the T1, T2, and T3 treatments

Table 2. Effect of Different Levels of Manganese Supplementation on Ejaculate Volume in Roosters

periods	Average± Stander error					Significant
	T1	T2	T3	T4	T5	
1	0.45 ± 0.06	0.46 ± 0.01	0.45 ± 0.05	0.44 ± 0.05	0.45 ± 0.06	N.S
2	0.40 ± 0.09 c	0.45 ± 0.05 b	0.40 ± 0.05 c	0.50 ± 0.05 a	0.51 ± 0.01 a	*
3	0.52 ± 0.03 c	0.50 ± 0.02 c	0.53 ± 0.06 c	0.54 ± 0.05 b	0.59 ± 0.05 a	*
4	0.45 ± 0.07 b	0.47 ± 0.05 b	0.44 ± 0.02 b	0.52 ± 0.07 a	0.50 ± 0.01 ab	*
5	0.39 ± 0.05 c	0.43 ± 0.09 a	0.38 ± 0.07 c	0.41 ± 0.07 b	0.40 ± 0.09 b	*
6	0.46 ± 0.08 b	0.51 ± 0.03 ab	0.53 ± 0.01 a	0.52 ± 0.02 a	0.50 ± 0.05 ab	*
Average	0.44 ± 0.07 c	0.47 ± 0.15 b	0.45 ± 0.06 c	0.48 ± 0.01 ab	0.49 ± 0.02 a	*

Means with different letters indicate a significant difference in probability level 0.05, *($P \leq 0.05$). The treatment T1, T2, T3, T4, and T5 are control treatments without addition, adding 10, 20, 30, and 40 mg manganese/kg feed, respectively.

Mass Motility: Table 3 shows the effect of manganese supplementation on mass motility.

A significant ($P \leq 0.05$) improvement was observed in the T3 and T5 treatments during the first and second periods, respectively.

However, no significant differences were

detected among treatments in the third and fourth periods. Conversely, in the fifth and sixth periods, as well as in the overall average, T4 and T5 treatments exhibited significantly ($P \leq 0.05$) higher mass motility.

Table 3. Effect of Different Levels of Manganese on Mass Motility (%) in Roosters

periods	Average± Stander error					Significant
	T1	T2	T3	T4	T5	
1	84.50 ± 2.92 b	80.50 ± 2.87 c	87.00 ± 2.57 a	85.00 ± 3.18 b	83.50 ± 2.84 bc	*
2	81.30 ± 2.76 c	81.00 ± 2.55 c	84.50 ± 3.10 b	86.00 ± 4.00 ab	87.50 ± 2.39 a	*
3	86.45 ± 3.11	88.41 ± 3.25	86.30 ± 2.50	85.50 ± 2.41	86.10 ± 2.65	N.S
4	81.00 ± 2.61	83.50 ± 2.30	82.50 ± 2.17	82.20 ± 3.27	81.00 ± 3.60	N.S
5	83.00 ± 2.55 b	84.00 ± 2.47 b	87.50 ± 2.63 ab	91.00 ± 2.71 a	88.50 ± 3.00 ab	*
6	85.00 ± 3.50 b	83.00 ± 2.65 c	86.50 ± 2.13 b	89.00 ± 1.88ab	90.45 ± 1.67 a	*
Average	83.54 ± 2.15 b	83.40 ± 3.52 b	85.71 ± 3.06 ab	86.45 ± 2.94 a	86.17 ± 3.12 a	*

Means with different letters indicate a significant difference in probability level 0.05, N. S: Not significant. *($P \leq 0.05$). The treatment T1, T2, T3, T4, T5 are control treatments without addition, adding 10, 20, 30, and 40 mg manganese/kg feed, respectively

Individual Motility: Table 4 presents the effects of manganese supplementation on individual sperm motility across different experimental periods. In the first period, a significant ($P \leq 0.05$) increase in motility was observed in the T3 and T4 treatments compared to the other groups. During the second period, the T4 treatment showed a significant ($P \leq 0.05$) improvement compared to T1 and T2. In the third, fourth, and fifth

periods, a significant ($P \leq 0.05$) increase in motility was recorded for the T2 and T5 treatments. Similarly, in the sixth period, the T2 treatment exhibited a significant ($P \leq 0.05$) improvement in individual motility compared to the other treatments. Considering the overall average, the T4 and T5 treatments showed significantly ($P \leq 0.05$) higher individual motility than the rest of the treatments.

Table 4. Effect of Different Levels of Manganese on Progressive Motility (%) in Roosters

periods	Average± Stander error					Significant
	T1	T2	T3	T4	T5	
1	76.00 ± 1.16 c	79.50 ± 1.32 c	84.00 ± 1.54a	85.00 ± 6.61a	82.00 ± 2.02 b	*
2	81.50 ± 3.14 b	76.50 ± 2.24 c	84.50 ± 3.55 ab	86.00 ± 2.80 a	84.00 ± 2.11 ab	*
3	86.00 ± 3.56 a	84.50 ± 4.37 b	83.00 ± 4.00 b	85.50 ± 3.75 ab	86.00 ± 4.20 a	*
4	85.00 ± 2.51 b	86.16 ± 2.00 b	90.00 ± 2.17 ab	92.41 ± 2.91 ab	94.00 ± 2.63 a	*
5	83.00 ± 1.92 c	85.50 ± 1.25 b	86.70 ± 2.16 b	85.00 ± 2.47 b	88.50 ± 2.50 a	*
6	87.00 ± 3.20 c	93.00 ± 3.61 a	87.00 ± 3.01 c	90.78 ± 3.13 b	90.00 ± 2.85 b	*
Average	83.08 ± 2.51 c	84.19 ± 2.75 bc	85.86 ± 2.27 b	87.44 ± 2.34 a	87.41 ± 2.81 a	*

Means with different letters indicate a significant difference in probability level 0.05, *($P \leq 0.05$). The treatment T1, T2, T3, T4, T5 are control treatments without addition, adding 10, 20, 30, and 40 mg manganese/kg feed, respectively

Sperm concentration: Table 5 illustrates the effects of manganese supplementation on sperm concentration. A significant ($P \leq 0.05$) improvement was observed in T4 and T5 treatments in the first period, while T3 treatment demonstrated significant ($P \leq 0.05$) superiority in the second, third, and fourth periods. In the fifth period, both T3 and T4 treatments resulted in significantly ($P \leq 0.05$)

higher sperm concentrations. Similarly, in the sixth period, the T5 treatment showed a significant ($P \leq 0.05$) improvement in sperm concentration compared to the other treatments. In the overall average, the T3 and T5 treatments exhibited significantly ($P \leq 0.05$) superior sperm concentrations compared to the other treatments.

Table 5. Effect of Different Levels of Manganese on Sperm Concentration ($\times 10^9$) in Roosters

periods	Average $\pm$ Stander error					Significant
	T1	T2	T3	T4	T5	
1	9.21 $\pm$ 0.36 b	10.02 $\pm$ 0.23 b	8.22 $\pm$ 0.80 c	11.41 $\pm$ 0.45 a	11.35 $\pm$ 0.79 a	*
2	10.43 $\pm$ 0.13 c	9.74 $\pm$ 0.56 c	14.30 $\pm$ 0.75 a	13.43 $\pm$ 0.81 b	13.09 $\pm$ 0.80 b	*
3	10.14 $\pm$ 0.27 b	9.87 $\pm$ 0.33 c	11.38 $\pm$ 0.18 a	10.58 $\pm$ 0.70 b	10.67 $\pm$ 0.18 b	*
4	11.81 $\pm$ 0.50 c	14.13 $\pm$ 0.25 ab	15.33 $\pm$ 0.37 a	11.62 $\pm$ 0.22 c	12.97 $\pm$ 0.92 b	*
5	11.21 $\pm$ 0.41 c	12.90 $\pm$ 0.39 b	13.15 $\pm$ 0.47 a	13.10 $\pm$ 0.45 a	12.92 $\pm$ 0.16 b	*
6	10.35 $\pm$ 0.52 c	11.05 $\pm$ 0.34 b	10.70 $\pm$ 0.29 c	11.12 $\pm$ 0.40 b	12.90 $\pm$ 0.25 a	*
Average	10.52 $\pm$ 0.71 c	11.28 $\pm$ 0.20 b	12.18 $\pm$ 0.75 a	11.87 $\pm$ 0.82 b	12.31 $\pm$ 0.32 a	*

Means with different letters indicate a significant difference in probability level 0.05, *($P \leq 0.05$). The treatment T1, T2, T3, T4, T5 are control treatments without addition, adding 10, 20, 30, and 40 mg manganese/kg feed, respectively

Dead sperm : Table 6 shows that in the first period, the T1 and T5 treatments exhibited significantly ($P \leq 0.05$) higher dead sperm percentages. In the second period, significant ($P \leq 0.05$) increases were noted in the T1, T4, and T5 treatments. During the third period, T1

and T4 treatments exhibited significantly ($P \leq 0.05$) higher dead sperm percentages. Similarly, the T1 treatment showed significantly ($P \leq 0.05$) higher dead sperm percentages in the fourth, fifth, and sixth periods, as well as in the overall average.

Table 6. Effect of Different Levels of Manganese on Dead Sperm Percentage (%) in Roosters

periods	Average $\pm$ Stander error					Significant
	T1	T2	T3	T4	T5	
1	4.00 $\pm$ 0.65 a	3.94 $\pm$ 0.77 b	3.85 $\pm$ 0.64 b	3.93 $\pm$ 0.75 b	4.00 $\pm$ 1.10 a	*
2	5.56 $\pm$ 0.69 a	3.54 $\pm$ 0.18 c	4.10 $\pm$ 0.60 b	5.35 $\pm$ 0.50 a	5.86 $\pm$ 0.61 a	*
3	6.10 $\pm$ 0.34 a	4.55 $\pm$ 0.25 c	5.74 $\pm$ 0.47 b	6.40 $\pm$ 0.21 a	4.72 $\pm$ 0.33 c	*
4	5.30 $\pm$ 0.63 a	3.73 $\pm$ 1.25 b	3.40 $\pm$ 0.96 b	3.10 $\pm$ 0.82 b	3.57 $\pm$ 1.00 b	*
5	5.00 $\pm$ 0.51 a	4.50 $\pm$ 0.48 b	4.30 $\pm$ 0.37 b	3.31 $\pm$ 0.76 c	3.61 $\pm$ 0.91 c	*
6	5.50 $\pm$ 0.95 a	5.08 $\pm$ 0.35 a	4.11 $\pm$ 0.27 b	4.10 $\pm$ 0.69 b	4.04 $\pm$ 0.53 b	*
Average	5.24 $\pm$ 0.39 a	4.22 $\pm$ 0.40 b	4.20 $\pm$ 0.50 b	4.36 $\pm$ 0.87 b	4.30 $\pm$ 0.51 b	*

Means with different letters indicate a significant difference in probability level 0.05, *($P \leq 0.05$). The treatment T1, T2, T3, T4, T5 are control treatments without addition, adding 10, 20, 30, and 40 mg manganese/kg feed, respectively.

Abnormal sperm: As presented in Table 7, a significant ($P \leq 0.05$) increase in abnormal sperm percentage was recorded for the T1 and T2 treatments in the first, second, and fifth periods, as well as in the overall average. In

the third period, T1, T2, and T3 treatments exhibited significantly ($P \leq 0.05$) higher abnormal sperm percentages. Additionally, in the fourth period, the T1, T4, and T5 treatments showed significantly ($P \leq 0.05$) increased abnormal sperm percentages compared to the other treatments. Similarly, in the sixth period, the T1 treatment exhibited significantly ($P \leq 0.05$) higher abnormal sperm percentages than the other treatments.

Table 7. Effect of Different Levels of Manganese on Abnormal Sperm Percentage (%) in Roosters

periods	Average± Stander error					Significant
	T1	T2	T3	T4	T5	
1	5.06 ±1.06 a	5.30 ±1.12 a	4.60 ±1.14 b	3.84 ±1.25 c	3.60 ±1.35 c	*
2	4.25 ±0.34 a	4.05 ±0.52 a	2.75 ±1.07 c	2.25 ±0.85 c	2.46 ±0.95 c	*
3	5.17 ±1.58 a	5.81 ±0.24 a	5.21 ±0.37 a	4.81 ±1.00 b	4.76 ±2.14 b	*
4	5.60 ±0.63 a	4.27 ±0.40 b	4.33 ±0.55 b	5.05 ±0.71 a	5.32 ±0.25 a	*
5	4.32 ±0.87 a	4.23 ±1.12 a	3.73 ±0.47 b	3.74 ±0.98 b	2.66 ±0.91 c	*
6	4.44 ±0.90 a	3.62 ±0.28 b	2.84 ±0.54 c	2.98 ±1.63 c	2.12 ±0.82 c	*
Average	4.80 ±0.73 a	4.54 ±0.39 a	3.91 ±0.60 b	3.77 ±1.22 b	3.48 ±0.76 b	*

Means with different letters indicate a significant difference in probability level 0.05, *($P \leq 0.05$). The treatment T1, T2, T3, T4, T5 are control treatments without addition, adding 10, 20, 30, and 40 mg manganese/kg feed, respectively.

Heat stress represents a major environmental hazard that severely impairs poultry production, particularly in breeder roosters, where it disrupts spermatogenesis, reduces semen quality, and decreases fertility rates (SAS Institute, 2012). The physiological impact of heat stress is primarily driven by oxidative stress, which induces cellular damage, lipid peroxidation, and mitochondrial dysfunction. These pathological changes ultimately compromise sperm viability, motility, and fertilization potential. The present study demonstrates that dietary manganese (Mn) supplementation significantly mitigates the detrimental effects of heat stress, leading to a marked improvement in crucial semen quality parameters—such as ejaculate volume, sperm motility, sperm concentration, and membrane integrity—while concurrently reducing the percentages of dead and abnormal spermatozoa. The profound impact of heat stress on testicular function often triggers apoptosis-induced loss of germ cells, resulting in testicular atrophy and a subsequently depressed sperm output (Al-Ghurairi et al., 2023). This suppression explains the decreased sperm count and lower ejaculate volume observed in the control group (C). Conversely, Mn supplementation significantly improved ejaculate volume in the T3, T4, and T5 groups, particularly during the later phases of the study. This protective effect may be attributed to the vital role of Mn in preserving testicular function; prior literature indicates that Mn upregulates the expression of follicle-stimulating hormone (FSH) and gonadotropin-releasing hormone-I (GnRH-I), both of which act as critical endocrine drivers of spermatogenesis (Ahmed & Salem, 2018; Al-

Ghurairi et al., 2023). The enhanced sperm motility observed in the Mn-supplemented treatments aligns with previous investigations demonstrating that Mn stimulates sperm movement in a time- and dose-dependent manner (Al-Hassani & Al-Suhail, 2017). Mechanistically, manganese sustains motility by safeguarding mitochondrial function, suppressing oxidative stress, and maintaining robust ATP production (Bakst & Dymond, 2013). Because sperm motility heavily relies on mitochondrial respiration to generate the energy required for flagellar propulsion, heat stress-induced mitochondrial disruption directly reduces motility, as evidenced by the diminished values in the control group (C). However, the significantly higher motility scores in the Mn-supplemented cohorts (T3, T4, and T5) strongly suggest that Mn plays an indispensable role in maintaining mitochondrial structural integrity and preventing radical-mediated damage. Sperm concentration is equally vital to male fertility, as it dictates the baseline pool of viable spermatozoa available for fertilization. In this study, Mn supplementation significantly elevated sperm concentration in the T3, T4, and T5 groups. These results corroborate earlier evidence showing that Mn promotes spermatogenesis by optimizing Sertoli cell function and accelerating testosterone synthesis (Bansal & Bilaspuri, 2010). Given that heat stress suppresses circulating testosterone and compromises sperm output, Mn acts as a buffer (Duncan, 1955; Shahat et al., 2020). By supporting overall endocrine pathways and shielding testicular tissues from oxidative stress, Mn directly aids in sustaining stable sperm concentrations under high

ambient temperatures. Furthermore, the plasma membrane integrity of spermatozoa is essential for cell survival, capacitation, and successful fertilization. Thermal damage to this membrane increases permeability, causing premature capacitation, loss of motility, and complete fertilization failure. Our study found that Mn supplementation significantly decreased the percentages of dead and abnormal sperm, which underscores its contribution to plasma membrane stability. This outcome matches reports that Mn administration curtails lipid peroxidation within sperm membranes, thereby protecting against structural breakdown (Cheema et al., 2009; Miriyala et al., 2012). Additionally, Mn assists in regulating cellular ion transport and maintaining osmotic equilibrium, both of which are required to preserve membrane architecture (Huang et al., 2006). Similar protective patterns have been documented in mammalian models; for instance, Mn supplementation improves bull semen quality by minimizing reactive oxygen species (ROS) production during liquid storage at 4 degrees Celsius (Bakst & Dymond, 2013). This defense mechanism stems from the role of Mn as an essential cofactor for manganese superoxide dismutase (MnSOD)—a frontline antioxidant enzyme that neutralizes superoxide radicals and prevents oxidative damage (Eidan et al., 2015). The current findings indicate that Mn exerts an identical protective function in avian species, countering the heat-induced oxidative pathways that undermine rooster sperm performance. In summary, the beneficial actions of Mn on sperm function operate through several distinct pathways: Mn functions as a mandatory cofactor for MnSOD, scavenging mitochondrial ROS and blocking oxidative lesions (Bakst & Dymond, 2013; A. et al., 2022). It stabilizes mitochondrial membranes, ensuring uninterrupted ATP production for flagellar movement (Duncan, 1955; Eidan et al., 2015). It preserves sperm plasma membrane integrity by suppressing lipid peroxidation (Hassan & Ali, 2014). It enhances the expression of upstream reproductive hormones like FSH and GnRH-I, driving spermatogenesis (Ahmed & Salem,

2018). It improves overall sperm survival rates during critical physiological events like capacitation and the acrosome reaction (Bansal & Bilaspuri, 2010). These results are highly consistent with cross-species literature. Mn has been shown to boost motility, membrane integrity, and mitochondrial output during semen preservation in bulls (Fernandes & Murakami, 2010; Huang et al., 2006; Karaca et al., 2002a) and turkeys (Karaca et al., 2002b; Ognik & Krauze, 2016). Collectively, these parallel findings establish Mn supplementation as an effective dietary strategy to counteract heat stress-induced reproductive failure in poultry. Therefore, identifying optimal Mn inclusion rates is crucial to maximize fertility benefits while avoiding toxic overdosage. Future research should evaluate the long-term carryover effects of dietary Mn on field fertility, hatchability, and subsequent chick quality to fully integrate this mineral into commercial poultry breeding programs.

CONCLUSION

The present study demonstrated that dietary Mn supplementation significantly improves semen quality in breeder roosters exposed to heat stress. Manganese supplementation enhanced ejaculate volume, sperm motility, sperm concentration, and sperm membrane integrity while reducing the percentage of dead and abnormal spermatozoa. These effects can be attributed to Mn's role in oxidative stress mitigation, mitochondrial protection, membrane stabilization, and hormonal regulation. Given the increasing global challenge of heat stress in poultry production, Mn supplementation represents a promising strategy for enhancing reproductive performance and maintaining fertility in breeder roosters. Future studies should explore the long-term effects of Mn supplementation on fertility rates, hatchability, and chick production to further validate its role in optimizing poultry reproduction.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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فعالية المنجنيز في تحسين خصائص السائل المنوي لديوك الدجاج البياض المعرضة للإجهاد الحراري

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المستخلص

تُعد كفاءة التكاثر لدى ديوك التربية ضرورية بسبب النسبة المنخفضة بين الذكور والإناث، خاصةً في ظل الإجهاد الحراري الذي يؤثر سلبيًا على الخصوبة. هدفت هذه الدراسة إلى تقييم تأثير مكملات المنجنيز الغذائية على جودة السائل المنوي والهرمونات التناسلية في 45 ديكًا من سلالة Hi-Line بعمر 50 أسبوعًا، على مدى 12 أسبوعًا، في كلية الزراعة، جامعة القاسم الخضراء، العراق. تم توزيع الديوك على خمس مجموعات (ثلاث تكرارات لكل مجموعة، ثلاثة طيور لكل مكرر) وتغذيتها إما على النظام الغذائي الضابط (T5) أو على وجبات تحتوي على 10، 20، 30، أو 40 ملغم من كبريتات المنجنيز لكل كغم من العلف (T1-T4). تم تحليل عينات السائل المنوي كل أسبوعين لقياس حجم القذف، حركة الحيوانات المنوية، تركيزها، وسلامة غشاء البلازما. كما تم جمع عينات دم لتحليل مستويات التستوستيرون، LH، FSH، ومؤشرات الإجهاد التأكسدي (ALT، AST، GPx). أظهرت النتائج فروقًا معنوية ($P \leq 0.05$) بين المعاملات. حيث سجلت المجموعة الضابطة (T5) أعلى حجم للقذف، بينما حسنت المجموعتان T4 و T5 من حركة الحيوانات المنوية. كما أدت المعاملتان T3 و T5 إلى زيادة تركيز الحيوانات المنوية، في حين خفضت T1 و T2 نسبة الحيوانات المنوية الميتة والمشوهة. تشير هذه النتائج إلى أن مكملات المنجنيز تعزز الأداء التناسلي تحت تأثير الإجهاد الحراري.

الكلمات المفتاحية: الديوك، الإجهاد الحراري، المنجنيز، جودة السائل المنوي.