Effect of adding Manganese sulfate for Diet on the hormonal and Histological response of aged Roosters exposed to Heat stress

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Abstract

This study was conducted in poultry farm of the college of Agriculture - Al-Qasim Green University, the effect of adding manganese sulfate for diet on the hormonal and histological response of aged roosters exposed to heat stress, 30 of Hy-Line roosters 50 week old was used, roosters were arbitrarily divided into 5 treatments,: 1st treatment T1: without manganese sulfates (comparison group), other treatments T2, T3, T4 and T5 were added manganese sulfates at (10, 20, 30, 40 mg / kg) respectively, the experiment continued for six periods, each period lasting two weeks, the roosters were exposed to a temperature of 30 ºC during the experiment period, results of this experiment showed there was a significant improvement (P≤0.05) for T3 and T5 in testosterone levels, and noticed significant increase of FSH in T4 and T5. There was a significant difference (P≤0.05) for T2 and T3 in GPX enzyme. In the thickness of the interstitial tissue, a significant difference (P≤0.05) was found in T3, a significant decrease (P≤0.05) was observed in the thickness of the germinal epithelium in T2 and T3, a significant difference (P≤0.05) in T3 noticed for diameter of the seminiferous tubes, a significant in the number of Sertoli cells for T3,

and found a significant in the number of spermatozoa for treatments T2, T4 and T5. For the Vanguard of sperm, a significant increase in treatments T4 and T5. Significant increase of sperms was obtained in T2, T4 and T5 compared to T1.

Keywords: hormones, glutathione peroxidase, histological, roosters, manganese sulfate.

Introduction

Fertility is the first and most important condition in the roosters industry (1). It peaks early in the reproductive period (30-40 weeks of age) and declines shortly thereafter (~45 weeks of age) (2). The tests are the male reproductive organs that have two main functions: testosterone sperm synthesis and production. These functions are critical not only to memorization characteristic of males, but also to preserve the species (3). Supplements of antioxidant ingredients classified in herbs, vitamins, and minerals have been associated with benefits on breeding cocks. Supplementation of antioxidant components both in the diet and in semen extenders, has been shown to have a significant effect in mitigating the adverse effects of oxidative stress and improving fertility in roosters (4, 5, 6). The high environmental temperature hinders the growth of the poultry sector. Because heat stress changes the natural physiological and behavioral of birds (7, 8, 9). Among these trace, nutrition of minerals is vital for various biological functions such as normal growth, development and reproduction (10). The optimal reproductive efficiency of animals depends on many factors, such as genetics, nutrition, management and environmental determinants (11). In addition, even narrow differences in micronutrient levels (such as trace minerals) may have a significant impact on vital biological processes including reproductive health and performance (10, 11). Manganese (Mn) is a required element in nutrition because it is physiologically active in the enzyme systems involved in the metabolism of fats and carbohydrates. It plays an important role in the growth, development and maintenance of poultry performance. Manganese is added to the diet in the form of sulfate. Manganese sulfate, commonly referred to as the organic compound MnSO4, is a light pink, manganese salt (12). Thus, the study was meant to find out Heat-

stressed roosters' hormonal and histological response to dietary manganese sulfate supplementation.

Materials and methods

This study was conducted at the field of poultry farm of the College of Agriculture at Al-Qasim Green University , during the period from March 30 / 2021 to July 5 / 2021 for 12 weeks, to demonstrate of adding manganese sulfate for diet on the hormonal and histological response of aged roosters exposed to heat stress. In this study, total of 30 of Hy-Line roosters 50 weeks old was used, the roosters were exposed to a temperature of 30 ºC during the experiment period, roosters were randomly distributed into five treatment groups: Which were:

T1 (control) no manganese sulfate added .

T2: supplemental manganese sulfate at 10 mg/ Kg diet.

T3: supplemental manganese sulfate at 20 mg/ Kg diet.

T4: supplemental manganese sulfate at 30 mg/ Kg diet.

T5: supplemental manganese sulfate at 40 mg/ Kg diet.

Feed treatment

Manganese sulfate was added from the 1st day till ended of experiment to all treatments, and all treatments were given food and water during the experiment period, and the contents of diet were selected as the National Research Council (13) which contained 44% protein while the metabolic energy was 2788.44 kcal / kg feed**.**

Studied traits

Hormones:

The levels of Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), Testosterone and Estrogen in the blood were measured using a special detection kit (Kit) from the Chinese company (Bioassay Technology Laboratory) using ELISA technology and according to the method of work followed by the manufacturer.

Glutathione peroxidase (GPx) activity

The activity of glutathione peroxidase was determined according to the method of (14).

Histological traits:

Four roosters were randomly sampled from each treatment at the end of the experiment at 62 weeks of age, slaughtered, testes and livers isolated and kept for each treatment in 10% formalin solution until tissue sections were clear in each treatment. - It is formed

as part of the liver and was covered with a thin layer of wax after that it was folded with formalin for 24 hours, after which the passage was passed in different concentrations of alcohol 60, 70, 80 and 90% for a quarter of an hour in each concentration and then it is placed in the wax mold at a temperature of 50 degrees Celsius for 24 hours, then the mold is placed in the freezer for 24 hours after placing it in a microtome device like this. With distilled water after staining the slides with hemotoxins and eosin (hemaloxylin and eosin) then slide I passed with different concentrations of Xylol 70, 80 and 90%. Then it is passed with different concentrations of alcohol 60, 70, 80 and 90%. After this process, the cover is placed over the slide and the slide is placed in the oven for one hour until it dries after that, the slides are examined microscopically to see the effect (15).

Statistical analysis

A complete randomized design (CRD) was used to examine the effect of different treatment on all traits, (16), and multiple scale tests were used to compare significant differences between means. Data were analyzed using the Statistical Analysis System (17).

Results and Discussion

Table (1) shows the effect of the treatment on testosterone levels, as a significant improvement ($P \leq 0.05$) was detected in T3 and T5 compared to the other treatments, and there was a significant decrease ($P \leq 0.05$) for T1 treatment compared to the rest of treatments, and a significant increase ($P \leq 0.05$) in T4 and T5 treatments in the level of FSH, there was no statistically significant difference between the treatments in the level of LH hormone. There was a significant increase ($P \leq 0.05$) in T2 and T3 compared to the rest of treatments in GPX enzyme, while there was no significant difference between the treatments of T5 and T4in the levels AST and ALT enzyme (table 2).

Table 1. Effect of adding manganese sulfate on the level of hormones in roosters exposed to heat stress at the age of 62 weeks

Hormones	Average± Stander error					
	T1	T2	T3	T4	T5	Significant
Testosterone	1.56 ±0.29 b	1.61 ±0.18 b	3.64 ±0.10 a	1.73 ±0.96 b	3.83 ±0.53 a	*
Estrogen	4.94 ±0.23 a	3.24± 0.22 b	3.05 ±0.55 b	3.10 ±0.19 b	3.34 ±0.60 b	*
FSH	2.05 ±0.38 b	2.02 ±0.64 b	2.46 ±0.91 b	3.33 ±0.27 a	3.46 ±0.33 a	*
LH	2.18 ±0.56	2.59 ±0.68	2.70 ±0.26	2.74 ±0.71	2.56 ±2.00	N.S

The means with different letters within the same rows are significantly between them (P<0.05) *, N.S: Not significant.

Table2. Effect of adding manganese sulfate on AST, AST and GPX levels in roosters exposed to heat stress at 62 weeks of age

Enzyme	Average± Stander error					
	T1	T2	T3	T4	T5	Significant
AST	32.66 ±0.65	32.78 ±0.21	32.53 ±0.32	32.91 ±0.41	32.86 ±0.57	N.S
ALT	8.42 ±0.10	8.38 ±1.01	8.31 ±0.47	8.44 ±0.53	8.35 ±0.62	N.S
GPX	522.13 ±0.25 b	530.16 ±0.58 a	531.22 ±0.31 a	5.23.18 ± 0.65 b	525.87 ±0.20 b	*

The means with different letters within the same rows are significantly between them (P<0.05) *, N.S: Not significant.

It was noticed from table (3) that there was a high significant increase (P≤0.05) in the thickness of the interstitial tissue for the T3 treatment compared to the control treatment T1 and the rest of the treatments. A significant decrease (P≤0.05) was observed in the

thickness of the germinal epithelium in T2 and T3 compared to the rest of the treatments, at the same time, significant differences (P≤0.05) appeared between T1 and T5, and with regard to the diameter of the seminiferous tubes a significant increase (P≤0.05) in T3 compared with control treatment and treatment T4, T5 a significant decrease (P≤0.05) was obtained in treatment T4, T5 compared to treatment T1, T2 and no significant differences appeared between treatments T2 and T3.

Table3. Effect of adding manganese sulfate on tests tissues in roosters exposed to heat stress at 62 weeks of age

Tissues characteristics	Average± Stander error					
	T1	T2	T3	T4	T5	Significant
The thickness of interstitial tissue	6.61±1.00 b	6.83±1.22 b	7.21±0.90 a	6.91±0.33 b	6.65±0.57 b	*
The thickness of the germinal epithelium	30.76±1.20 b	28.73±1.01 c	29.80±0.13 c	31.07±0.78 ab	32.56±0.45 a	*
Diameter of the seminiferous tubules	140.25±2.00 b	145.89±1.58 ab	146.13±0.27 a	139.09±2.22 c	138.35±1.98 c	*

The means with different letters within the same rows are significantly between them (P<0.05) *

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Table (4) shows a significant increase (P≤0.05) in the number of Sertoli cells for treatment T3 compared to the rest of the treatments. At the same time, treatment T2 was significantly (P≤0.05) greater to control, T4, T5 to the same trait. There was a significant (P≤0.05) in the number of spermatozoa in treatments T2, T4 and T5 compared to T1 and T3. There was a significant increase (P≤0.05) in Vanguard of sperm in treatments T4 and T5 compared to T1, T2 and T3. Significantly increased T2 on treatment T1 and T3. A significant increase (P≤0.05) of sperms was obtained in treatments T2, T4 and T5 compared to T1.

Table 4. Effect of adding manganese sulfate on sperm cells types in roosters exposed to heat stress at 62 weeks of age

Sperm cells type	Average± Stander error					
	T1	T2	T3	T4	T5	Significant
Sertoli cells	11.32±0.21c	12.11±0.30b	13.24±0.57a	11.74±0.87c	11.91±0.17c	*
Leydig cells	4.11±0.14	4.00±0.11	4.21±0.17	4.63±0.10	4.42±0.20	N.S
spermatozoa	9.35±0.30b	10.50±0.09a	9.75±0.41b	10.33±0.18a	10.75±0.16a	*
Vanguard of sperm	12.75±0.24c	13.50±0.10b	12.50±0.07c	14.00±0.08a	14.25±0.13a	*
Sperms	6.55±0.71b	8.22±0.50a	7.33±0.25ab	8.66±0.19a	8.33±0.31a	*

The means with different letters within the same rows are significantly between them (P<0.05) *, N.S: Not significant.

Dietary manganese supplementation affects gene expression of GnRH-I in the brain and FSH in the pituitary, thus controlling the rise in levels of sex hormones in the body, as the formation of steroid hormones begins with cholesterol. Manganese is one of the cofactors of enzymes in cholesterol biosynthesis (18), and thus regulates the synthesis of steroidal hormones. It has been speculated that as the level of manganese increases, the level of sex hormones in the blood will rise. The formation of testosterone in the follicles is generally controlled by LH, however FSH stimulates the development of granulosa cells and the cells' ability to respond to LH, FSH, and other signals (19) and this explains the improvement in the level of sex hormones in manganese treatments, as well as, the improvement of histological characteristics in birds of those treatments, where Xie et al. (20) indicated that the nutritional supplement of 240 mg manganese/kg increased significantly. manganese content in the blood in addition to the abundance of FSH mRNA in the pituitary gland where FSH mRNA expression is regulated sequentially and a diet high in manganese can regulate the production of Mn in the blood. Sex hormones by several mechanisms including manganese can directly target FSH-producing cells. It can also regulate prolactin release and activate gene expression for

regulating antioxidant status (21), or it can regulate Mn and FSH secretion by affecting the dopaminergic system (22). Regulatory regulation of Mn on GnRH-I, which is the primary regulation of the hypothalamic-pituitary-gonadotropic axis. Mn can cross the blood-brain barrier, which makes it possible that manganese may have a role in regulating the synthesis and secretion of GnRH-I in the pituitary gland. And it increased the cellular volume density of the different stages of sperm formation as well as the supporting cells (Leydig and Sertoli cells), and studies that dealt with comparisons between organic and inorganic manganese indicated that MnSO4 manganese sulfate had more profound stimulatory effect on the gene expression of GnRH-I than manganese species. Other studies in broilers also found that the absorption of organic forms of Mn was higher than that of inorganic forms in the intestine (23). It is worth noting that the size of the testicle closely follows the changes in the levels of FSH, and it increases in mass with an increase in FSH and decreases in mass with a decrease in FSH. The weight of the testis in birds is closely related to the daily sperm production, and therefore there is a direct relationship between the size of the testicles and the concentration of FSH. Being a stimulator of sperm synthesis (24), indicates the clear role of FSH in improving testicular tissue.

As for the first treatment, the deterioration of the traits studied above may be due to the effect of heat stress, as the exposure of birds to heat stress causes a deterioration in immunity and the production of the cytokine IL-1, which in turn affects the pituitary-adrenal axis and inhibits the functions of the pituitary-pituitary-glands reproductive traits, implying that the cytokine IL-1 may also mediate some behavioral responses due to heat stress and inhibit the reproductive traits of birds as well. This mechanism appears to be the closest explanation for star infertility from exposure to high temperatures in pets including birds (25). The stress caused by high temperatures also causes a disturbance in the generation of hormones secreted by the gonads, which in turn causes the deterioration of the reproductive characteristics of birds and causes weakness in the tissue cells of the tests (21). It has also been shown that an ambient temperature of 30 -35°C causes impaired fertility by impairing sperm penetration, utero-sperm storage, seminal plas-

ma, and intracellular ion concentrations (26, 27).

Conclusion

The current study found that the addition of manganese sulfate to the diet improved the level of reproductive hormones, which led to an increase in fertility, and this was accompany by an improvement in the condition of the testicular tissue through the indicators that were studied.

Conflict of interest

The authors declare no conflict of interest.

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