Effect of Dosing Different Levels of Nano-Curcumin on Some Histological Traits of Testicles of Broiler Breeders Cocks

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Abstract:
This experiment was conducted in the poultry fields of the Department of Animal Production / College of Agriculture / Al-Qasim Green University from 11/1/2023 until 1/30/2024 for a period of 13 weeks. The experiment was conducted using 20 male broiler mothers and was randomly distributed into four treatments. Each treatment consisted of five replicates, one rooster for each replicate. The experimental treatments were as follows: the first treatment was control without dosing and addition, the second treatment was a dose of 20 mg/day, the third treatment was a dose of 25 mg/day, and the fourth treatment was a dose of 30 mg/day.

The study aims to know the changes that occur in the testicular tissue of male broiler breeders when dosed with nano-curcumin on a daily basis. The results of this experiment showed an improvement in some components of testicular tissue for patients dosed with nano-cumin. We conclude from the results of the experiment that nano-curcumin can improve the testicular tissue characteristics of male broiler breeders Ross308.

Keywords: Dosage, male broiler breeders, nanocurcumin, testicular tissue.

Introduction
Fertility and the safety of the reproductive organs are considered among the most important factors on which broiler breeder projects depend. Fertility decreases when the birds reach the age of 50 weeks, and the testicular tissue and its secretions begin to deteriorate when the birds reach this age (Jaber, 2023). The hormone testosterone is responsible for fertility and the maintenance of testicular tissue in general. When testosterone begins to decline after the birds grow older, part of it will turn into estrogen under the influence of the aromatase enzyme, which inhibits the pituitary gland's secretions of LH and F.S.H., which are responsible for the secretions, growth, and maintenance of testicular tissue (Ali, et al., 2017). A low level of testosterone within the testicular tissue will cause damage to the testicular tissue and also reduce the activities of natural antioxidant enzymes within the testicular tissue (Safari Asl, et al., 2018). This problem prompted the use of safe, cheap medicinal plants that act as antioxidants and an alternative to chemotherapy, which is associated with side effects (Ali, & Abdel-Zalmi, 2022). Among these medicinal plants is turmeric (Curcuma Longa), which is
found in Asian countries and belongs to the ginger family (Zingiberaceae). The turmeric plant contains Curcumin (which is the active ingredient in turmeric), which has an important role as an antioxidant, anti-free radical, and anti-inflammatory (Patel, et al., 2020). Curcumin is among a large group of phenolic compounds that have an important role as an antioxidant, anti-free radical, anti-inflammatory and anti-tumour (Noorafshan, & Ashkani-Esfahani, 2013). The use of nano-curcumin resulted in an improvement in the biological activity of curcumin inside the cells. Nano-curcumin consists of the same compounds as curcumin but in nanoscale sizes, and this matter will facilitate the process of its absorption and entry into cells (Yadav, et al., 2017). This experiment aims to determine the effect of dosing nano-curcumin daily at different doses on the testicular tissue of male Ross308 broiler breeders.

Materials and Methods

This study, which lasted 13 weeks, was conducted using 20 roosters from (Ross 308) broiler mothers, aged 54 weeks and average weight of 5 kg, in the poultry farm of the Department of Animal Production at the College of Agriculture - Al Qasim Green University for the period from 11/1/2023 to 1/30/2024. The roosters were randomly distributed in cages with an area of (1.5 m * 1.5 m) according to the ground-rearing system. Coarse wood sawdust with a thickness of 10 cm was used to cover the floor. The lighting system was 14 hours of light per day until the end of the experiment. Groundwater sources for drinking water were used. The birds were exposed to similar conditions of temperature, humidity, lighting, and feeding. The males in the cages were divided into four treatments, and each treatment contained five replicates, one rooster for each replicate. The treatments were distributed as follows: T1 control treatment without dosing, T2 dosing 20 mg/day nano-curcumin, T3 dosing 25 mg/day nano-curcumin, T4 dosing 30 mg/day. Males were dosed with nano-curcumin packed in a pharmaceutical capsule in doses according to the experimental parameters. Every morning of the 13-week trial period. The birds were fed daily with a feed ration of 140 grams per bird according to the company's recommendations (Ross, 2022); the roosters were fed a ration containing crude protein (14.73%) and a metabolic energy of 2873 kilocalories.

Preparation of Nanocurcumin

Nanocurcumin was prepared in the laboratories of the Iraqi Ministry of Science and Technology - Department of Environment and Water, where curcumin was prepared biologically (Elumalai, et al., 2015). 10 ml of curcumin at a concentration of 10% is added to distilled water (non-ionic water) 900 ml, after which the nano curcumin mixture is heated with Distilled water at a temperature of 80 degrees Celsius, then zinc sulphate is added in the form of an aqueous solution (2.5 grams of zinc sulphate with 100 ml of distilled water). The mixture is stirred using a special device. At the same time, the heating process continued, then sodium hydroxide (1 molar) was added to The mixture was mixed at a concentration of 2.1, and the stirring process continued for 12 hours in order to form the suspended precipitate solution. After that, the mixture was left for 24 hours in an air oven at a temperature of 60 degrees Celsius to complete the precipitation process. The sediment is then washed with ethyl alcohol and distilled water. The sediment is separated from the filtrate by a centrifuge for 10 minutes at a speed of 10,000 rpm. After the sediment is well separated, it is dried in an electric oven at a temperature of 400 degrees Celsius for 120 minutes for drying. The laboratory mill is then used to grind the dry precipitate, which is then stored in glass cylinders for transportation and use. It is then ground using a laboratory mill and stored in glass beakers for use and diagnosis (Daneshvar, Aber, & Dorraji, 2008).

Tissue Characteristics of the Testicles

After the end of the experiment period, which was 13 weeks, the roosters reached the age of 67 weeks. During the experiment, the males were dosed according to the treatments with nano-curcumin. Twelve roosters were slaughtered (3
roosters per treatment) and randomly. The birds were dissected, the testicles were taken out, they were weighed on a sensitive scale, and then they were placed in a plastic box and submerged with formalin at a concentration of 10%. The samples were then sent to Al-Qadisiyah University - College of Veterinary Medicine to make slides for histological examination. Then, the slides were sent to Anbar University - College of Agriculture - Department of Animal Production for reading. The slides were read according to Weible’s point distribution method (Weible, 1979). The results were then sent to statistical analysis, where the Statistical Analysis System program was used.

**Studied Characteristics**

Absolute testicle weight (g), relative testicle weight (%), seminiferous tubule diameter, seminiferous tubule lumen diameter, germ cell layer thickness, seminiferous tubule components, and interstitial tissue components.

**Statistical Analysis**

The Statistical Analysis System - S.A.S (2012) was used in data analysis to study the effect of different treatments on the traits studied according to a Completely Randomized Design (C.R.D.), and the mean differences among the treatments were compared according to Duncan multiple ranges test (S.A.S., 2012):

\[ Y_{ij} = \mu + \tau_i + \epsilon_{ij} \]  

(1)

**Results and Discussion**

Table 1 shows the effect of dosing different levels of nano-curcumin using a daily dosing method on male broiler breeders in calculating the absolute weight (g) and relative weight (%) of the testicles, the diameter of the seminiferous tubule, the diameter of the lumen, and the thickness of the germ cell layer (microns), where it is clear that there are no significant differences. (P≤0.05) among all treatments for absolute and relative testicle weight and lumen diameter. As for the diameter of the seminal tubule, the fourth and third treatments were significantly (P≤0.05) superior to the second and first treatments. As for the thickness of the germ cell layer, the third treatment was significantly superior (P≤0.05). On the rest of the experimental treatments, the fourth treatment was significantly (P≤0.05) superior to the second and first treatments.

**Table 1. Effect of Dosing Different Levels of Nano Curcumin on Average Absolute Weight (g), Relative Testicular Weight (%), Tubule Diameter, Tubule Lumen Diameter, and Germ Cell Layer Thickness (microns) for Male Broiler Breeders**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean±standard error</th>
<th>Absolute testicle weight (gm)</th>
<th>Relative testicle weight (%)</th>
<th>Seminiferous tubule diameter</th>
<th>Seminiferous tubule lumen diameter</th>
<th>Germ cell layer thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>11.00±1.52</td>
<td>0.22±0.03</td>
<td>0.75±0.04</td>
<td>0.57±0.18</td>
<td>0.23±0.03</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>11.67±1.20</td>
<td>0.23±0.02</td>
<td>0.87±0.11</td>
<td>0.45±0.19</td>
<td>0.24±0.05</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>12.33±1.45</td>
<td>0.26±0.03</td>
<td>1.41±0.19</td>
<td>0.82±0.12</td>
<td>0.42±0.06</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>13.00±1.52</td>
<td>0.24±0.04</td>
<td>1.39±0.20</td>
<td>0.52±0.06</td>
<td>0.31±0.02</td>
<td></td>
</tr>
<tr>
<td>Significant</td>
<td>N.S.</td>
<td>N.S.</td>
<td>*</td>
<td>N.S.</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

Means with different letters within one column differ significantly at* (P≤0.05), N.S.: not significant.

Table 2 shows the effect of dosing different levels of nano-curcumin to male broiler breeders in measuring the relative weight of the seminal tubule components of the testes of male broiler breeders. It is clear from the table that there are no significant differences for sperm progenitors, sperm cells, and sperm precursors between the treatments. As for the sperm, they excelled. The third treatment was significantly (P≤0.05) superior to the rest of the experimental treatments, and the second treatment was significantly superior (P≤0.05) to the fourth and first treatment. The second treatment was also significantly superior (P≤0.05) to the first
treatment. As for Sertoli cells, it was significantly superior \((P \leq 0.01)\). The third treatment outperformed all the experimental treatments, and the second treatment was significantly superior \((P \leq 0.01)\) to the fourth and first treatments.

**Table 2. Effect of Dosing Different Levels of Nano Curcumin on the Relative Weight of the Seminiferous Tubule Components of the Testes of Male Broiler Breeders**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean ± standard error</th>
<th>Sperm precursors</th>
<th>Sperm cells</th>
<th>Spermatids</th>
<th>Sperm</th>
<th>Sertoli cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>23.66±0.99</td>
<td>7.89±1.16</td>
<td>8.50±0.99</td>
<td>3.50±0.25  c</td>
<td>1.56±0.13 c</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>27.77±1.09</td>
<td>8.10±1.22</td>
<td>8.91±0.38</td>
<td>6.18±0.71  ab</td>
<td>3.07±0.04 b</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>27.94±1.52</td>
<td>9.18±0.65</td>
<td>10.37±0.77</td>
<td>6.45±0.81  a</td>
<td>3.64±0.19 a</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>23.38±2.95</td>
<td>6.60±1.22</td>
<td>6.26±2.25</td>
<td>4.07±0.80  bc</td>
<td>1.31±0.25 c</td>
<td></td>
</tr>
<tr>
<td>Significant</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>*</td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

Means with different letters within one column differ significantly at* \((P \leq 0.05)\), ** \((P \leq 0.01)\), N.S.: not significant.

It is shown in Table 3: the effect of dosing different levels of nano-curcumin using a daily dosing method on male broiler breeders in calculating the relative weight of the components of the interstitial tissue of the testicle. It is clear from the table when calculating the relative weight of Leydig cells that there are no significant differences \((P \leq 0.05)\) between the third and second treatments. Moreover, the first was significantly superior \((P \leq 0.05)\) to the fourth treatment. With regard to calculating the relative weight of blood vessels, the third and second treatments were significantly superior \((P \leq 0.01)\) to the first and fourth treatments. The fourth treatment was significantly reduced \((P \leq 0.01)\) compared to the control treatment. When calculating the relative weight of the inter-distances, it was found that there were no significant differences \((P \leq 0.05)\) between the treatments. However, there were mathematical differences in favour of the third treatment.

**Table 3: Effect of Dosing Different Levels of Nano Curcumin on Relative Weight (%) of the Components of the Testicular Seminiferous Tubule**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean ± standard error</th>
<th>Leydig cells</th>
<th>Blood vessels</th>
<th>Testes interspaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>2.01±0.32 a</td>
<td>0.53±0.17 ab</td>
<td>0.08±0.07</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>2.11±0.40 a</td>
<td>0.88±0.01 a</td>
<td>0.40±0.25</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>2.54±0.23 a</td>
<td>0.94±0.03 a</td>
<td>0.44±0.25</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>0.57±0.14 b</td>
<td>0.39±0.22 b</td>
<td>0.14±0.14</td>
<td></td>
</tr>
<tr>
<td>Significant</td>
<td>**</td>
<td>*</td>
<td>N.S.</td>
<td></td>
</tr>
</tbody>
</table>

Means with different letters within one column differ significantly at** \((P \leq 0.01)\), * \((P \leq 0.05)\), N.S.: not significant.

The improvement that occurred in some characteristics of testicular tissue with the dosing treatments may be due to the intracellular drug delivery property of nano curcumin, as well as increasing the efficiency of the work of Sertoli cells, which causes improvement in testicular tissue and improvement in the daily production of sperm. There is a correlation between the reproductive activity of roosters with the size of the seminal tubule, as well as a Correlation with germ cell thickness (Mostafa, et al., 2019). The improvement in some testicular histological examinations of the experimental parameters may be due to the effect on the hypothalamic axis and, thus, the secretion of the hormone GnRH, which stimulates the pituitary gland to secrete FSH, which works to increase the size of the testicular tissue and also has a role in sperm production. The pituitary gland also secretes the hormone LH, which works to increase The level
of testicular tissue secretion of testosterone (Al-Jebory, et al., 2024; Vizcarra, Kirby, & Kreider, 2010; ACOG, 2013). High testosterone in male blood causes an increase in the growth of testicular tissue and the maintenance of the reproductive organs in general, in addition to an increase in sperm production and semen volume (Shanoun, 2011; Taha, 2008). Antioxidants have a role in protecting testicular tissue, protecting it from tissue damage, and increasing the lifespan of cells. A relationship has also been recorded between the live weight of males with the weight of the testicles and the components of the testicular tissue (Al-Dhalmi, 2022; Escorcia, et al., 2020). The results of this research are consistent with the study above in terms of calculating the relative weight of the components of the seminiferous tubule and the components of the interstitial tissue of the testicle. As for the dosage of nano-curcumin, the addition coefficients showed the growth of testicular tissue since nano-curcumin is considered an excellent antioxidant, which granted the perpetuation of the components of the seminiferous tubule and tissue. Interstitial testicles. Studies have shown that testosterone has a role in testicular size, meaning that the higher the testosterone hormone in male blood, the greater the weight of the testicle, as well as the protection of testicular tissue from ageing. (Rahim, et al., 2013).

**Conclusion**

The conclusion of this study may be, in the long term and at present, that the histological characteristics of the ageing testicles improve. This, in turn, improves the qualitative characteristics of the roosters' semen, and thus fertility and hatching. This leads to an improvement in the characteristics of the semen in the long term, and this may be a solution not to replace ageing roosters. Therefore, more study on nanocurcumin is needed to clarify more what the results of this are.

**References**


