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**Effects of oral administration of graded doses of *Curcuma longa* rhizome (turmeric) powder on the testis, epididymis and blood levels of selected reproduction related biochemical parameters of rabbit bucks**

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**Abstract**

The aim of the study was to evaluate the effects of oral administration of graded doses of *Curcuma longa* (turmeric) rhizome powder on the testis, epididymis and reproduction related blood biochemical parameters of male rabbits. Thirty-two sexually mature rabbit bucks were used for the experiment. They were assigned to four groups (A – D) of eight bucks each. Groups A, B and C were given 250, 500 and 1000 mg/kg of turmeric rhizome powder reconstituted in distilled water, respectively. Group D (control) received distilled water as a placebo. All treatments were given *per os* daily for 135 days. Evaluations of all other testicular, epididymal and blood biochemistry parameters were done on days 45 and 135, but fasting blood sugar (FBS) was determined on days 15, 30, 45, 75, 105 and 135 of treatment. Results showed that the mean weights of the testis and epididymis of Groups A, B and C bucks were significantly ( $p < 0.05$ ) higher than that of Group D, in a dose dependent manner. The epididymal sperm reserves of bucks in Groups B and C were significantly ( $p < 0.05$ ) higher than those of Groups A and D. Serum levels of testosterone and luteinizing hormone (LH) were significantly higher ( $p < 0.05$ ) in the Group A, B and C bucks when compared to the Group D on day 135. Also on day 135, the serum triglyceride levels of Groups B and C bucks were significantly ( $p < 0.05$ ) lower than that of Group D, while the serum total protein of Groups B and C were significantly ( $p < 0.05$ ) lower than that of Group D. The FBS of Groups A, B and C bucks were significantly lower ( $p < 0.05$ ) than that of Group D all through. The Sertoli cells, primary spermatocytes and spermatids of Group C bucks showed better differentiation on day 135 with more sperm cells in the lumen of the seminiferous tubules. It was concluded that administration of *C. longa* rhizome powder as used in the study, especially at the dose of 1000 mg/kg, positively impacted on the testicular, epididymal, hormonal and other serum biochemical parameters of the rabbit bucks and significantly lowered blood sugar levels.

**Keywords:** *Curcuma longa* rhizome; Turmeric; Male rabbits; Hormones; Blood biochemistry; Testis; Epididymis.

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## Introduction

*Curcuma longa* (turmeric) rhizome is an extensively used spice for coloring, flavoring and medicinal purposes (Ammon and Wahl, 1991). The volatile oils found in the rhizomes of *C. longa* are mostly made up of monoterpenes, curcumin, minerals, vitamin C, and carotene (Leung and Foster, 1996). According to Heath *et al.* (2004), the flavonoid known as "curcuminoid," which is a combination of curcumin (diferuloylmethane), monodemethoxycurcumin, and bisdesmethoxycurcumin, is the major active component of turmeric. Curcumin is the most well studied active ingredient in turmeric, accounting for over 90% of its curcuminoid content. Curcumin has a variety of uses, and is frequently employed as a coloring agent in foods, medications, and cosmetics (Anto *et al.*, 1996). Earlier reports have shown that curcumin has anti-inflammatory and anti-arthritic (Miquel *et al.*, 2002), anti-mutagenic and anti-carcinogenic (Anto *et al.*, 1996), antioxidant (Majeed *et al.*, 1995) and immunomodulatory properties (Gao *et al.*, 1996). Curcumin is a lipophilic polyphenol that is almost insoluble in water but quite stable in the stomach's acidic pH (Wang *et al.*, 1997). The phenolic groups found in curcumin's structure account for its capacity to eradicate free radicals produced by oxygen (Sreejayan *et al.*, 1997). Curcumin has been reported to have the ability to remove the following free radicals: hydroxyl radical, singlet oxygen, superoxide radical, and nitrogen dioxide (Sreejayan *et al.*, 1997).

Asian cuisines have long benefited from the flavor and colour of turmeric rhizome, which is also used in Chinese and Ayurvedic medicine as an anti-inflammatory agent and to treat jaundice, menstruation problems, haematuria, bleeding, and colic (Kapoor, 1990). It is recognized for use in a broad range of medical applications and is included in the Chinese Pharmacopoeia as well as those of other Asian nations including Japan and Korea (Kapoor,

1990). It is taken internally and topically for urticaria, skin allergies, viral hepatitis, joint inflammation, sore throat and wounds in China (Kapoor, 1990). The rhizome of *C. longa* is primarily taken orally. It can also be inhaled or applied topically to treat a variety of conditions, including eczema, acne, wounds, boils, bruises, blisters, ulcers, insect bites, parasitic infections, haemorrhages and skin conditions such as zoster, herpes and pemphigus (WHO, 1999).

With regards to male reproduction, curcumin administration has been shown in laboratory studies to mitigate the adverse effects of metronidazole, including the Leydig cell hyperplasia of the testis and the reduction in tubule volume, length, width, and height of the germinal epithelium (Noorafshan *et al.*, 2011). Curcumin was believed to exercise an ameliorative effect on the aforementioned parameters because of its antioxidant and free radical-scavenging properties, as well as its capacity to raise testosterone levels in the bloodstream (Noorafshan *et al.*, 2011). Another study that assessed curcumin's therapeutic effects on testicular damage caused by di-n-butylphthalate (DBP) in rats reported that curcumin administration prevented peroxidative changes in the testicular membrane and sperm, improved sperm motility and reduced spermatozoa defects (Farombi *et al.*, 2007). Mathuria and Verna (2008) reported that treatment with curcumin ameliorated aflatoxin-induced decrease in sperm count, immobilisation and viability, and enhanced the morphological characteristics of the sperm. Reports by Giannessi *et al.* (2008) showed that curcumin administration shielded Leydig cells of mice from the harm brought on by long-term alcohol use. Also, earlier reports by Ilbey *et al.* (2009) showed that curcumin administration significantly prevented cisplatin induced hormonal, blood biochemical and histopathological alterations in the testis of rats.

Rabbits, known for their prolificacy and adaptability, hold immense significance in various sectors. They serve as a valuable source of protein and fur, contributing to food security and economic growth (Aduku and Olukosi, 1990). Moreover, their sensitivity to environmental changes makes them crucial bioindicators of ecosystem health. However, to fully harness the potentials of rabbits, it is essential to enhance their reproductive performance. By optimizing breeding practices, increasing litter size, and reducing mortality rates, we can unlock their maximum productivity and contribute to sustainable animal protein supply. Improvement in the reproductive efficiency of rabbits will not only benefit rabbit farmers but also promote food security, fur production and alleviate poverty (Aduku and Olukosi, 1990).

The ability of *C. longa* rhizome to ameliorate experimentally induced reproductive disorders in laboratory rodents is fairly well documented in available literature; however, there is dearth of information regarding its impact on the reproductive health of apparently healthy animals and humans. The present study evaluated the effects of oral administration of graded doses of *Curcuma longa* rhizome (turmeric) powder on the testis, epididymis and blood levels of selected reproduction related biochemical parameters of rabbit bucks.

## Materials and Methods

The turmeric rhizome powder (Tiger® brand) used for this study was procured from Tiger Foods Limited, Obosi, Anambra, Nigeria. The turmeric powder was reconstituted in distilled water into different concentrations (250, 500 and 1000 mg/kg) as required. Doses were chosen based on information in existing literature (Pandey, 2011).

Thirty-two sexually mature Chinchilla breed rabbit bucks, of seven months of age, weighing between 987 to 1200 grams were

used for this experiment. They were acclimatized for two weeks prior to commencement of the experiment. This study protocol was approved by the Institutional Animal Care and Use, Faculty of Veterinary Medicine, University of Nigeria Nsukka. The rabbits were handled and cared for humanely all through the study.

Following acclimatization, the bucks were assigned to four groups (A – D) of eight (8) each. Group A, B and C bucks were given 250, 500 and 1000mg/kg body weight of turmeric powder reconstituted in 1 ml of distilled water, respectively, while Group D (control) bucks were given 1 ml of distilled water as placebo. All treatments were given *per os* daily for 135 days. Evaluations of all parameters were done on days 45 and 135, except fasting blood sugar assay that was done on days 15, 30, 45, 75, 105 and 135. The choice of days 45 and 135 for the evaluation of the reproductive parameters was based on the fact that across 45 days the process of spermatogenesis initiated from the beginning of the treatment will be almost complete in the rabbit buck. Evaluating reproductive parameters at this point therefore provides an indication of the efficiency of spermatogenesis and the quality of spermatozoa produced. The evaluation on day 135 will probably show whether or not the effect induced by treatment with turmeric is sustained across time (Lebas and Colin, 2010).

The weights of the testis and epididymis of each rabbit buck was measured using a digital weighing balance. Their length and width were also measured using a vernier caliper. The paired cauda epididymal sperm reserve was determined, as described by Olukole *et al.* (2010).

Blood samples were collected from the jugular veins into plain sample bottles and allowed to clot for 45 minutes. They were subsequently centrifuged at 3,000 revolutions per minute for 10 minutes. The serum supernatant was carefully aspirated and used for all

biochemical assays, except fasting blood sugar determination that was done using whole blood dropped on to a glucometer. At the end of the study (after day 135), three of the rabbit bucks in each group were euthanized and their testes were collected, fixed and processed for histopathology.

Serum levels of testosterone were assayed by the competitive inhibition enzyme immunoassay technique, while serum levels of luteinizing hormone (LH) were assayed by the quantitative sandwich ELISA technique (Beastall *et al.*, 1987). The serum cholesterol levels were determined by the enzymatic colorimetric method, while serum triglycerides level was determined by glycerol-phosphate oxidase method (Rifai *et al.*, 2008). Determination of serum calcium levels was done following the ortho-cresolphthalein direct method (Endres and Rude, 2008), and serum levels of inorganic phosphorus was determined by the Fiske-SubbaRow method (Fiske and SubbaRow, 1925). Serum levels of total protein were determined by the direct Biuret method (Johnson, 2008), while serum malondialdehyde (MDA) levels were assayed following the modified thiobarbituric acid method (Draper and Hadley, 1990).

One-way analysis of variance (ANOVA) followed by a post-hoc least significant difference (LSD) test was performed on data generated, using the IBM Statistical Package for Social Sciences (SPSS) statistics version 16.0 for Windows. Probability values less than 0.05 were considered statistically significant. Summary of the results were presented as means  $\pm$  standard deviation in tables.

## Results

At both days 45 and 135 of administration of turmeric rhizome powder, the mean epididymal and testicular weights of Group A, B and C bucks were significantly ( $p < 0.05$ ) higher than that of Group D bucks, in a dose dependent manner (Tables 1 and 2). The mean widths of the testis of bucks in all groups did not significantly ( $p > 0.05$ ) vary at day 45 (Table 1), but at day 135, the mean width of the testis of Group C bucks was significantly ( $p < 0.05$ ) higher than those of all other groups (Table 2). At both days 45 and 135 of administration, the mean testicular length of Group C bucks was significantly ( $p < 0.05$ ) higher than that of group A (Tables 1 and 2).

**Table 1.** Epididymal and testicular morphometric parameters of rabbit bucks that were given varied doses of turmeric powder, at day 45 of treatment.

| Parameters             | Means $\pm$ standard deviation of parameters at day 45 of treatment |                               |                               |                               |
|------------------------|---|-------------------------------|-------------------------------|-------------------------------|
|                        | Group A (250 mg/kg turmeric)  | Group B (500 mg/kg turmeric)  | Group C (1000 mg/kg turmeric) | Group D (Untreated Control)   |
| Epididymal Weight (g)  | 1.00 $\pm$ 0.01 <sup>a</sup>  | 1.06 $\pm$ 0.01 <sup>a</sup>  | 1.19 $\pm$ 0.04 <sup>b</sup>  | 0.90 $\pm$ 0.02 <sup>c</sup>  |
| Testicular Weight (g)  | 1.84 $\pm$ 0.04 <sup>a</sup>  | 1.97 $\pm$ 0.02 <sup>a</sup>  | 2.36 $\pm$ 0.07 <sup>b</sup>  | 1.37 $\pm$ 0.04 <sup>c</sup>  |
| Testicular width (cm)  | 1.35 $\pm$ 0.29 <sup>a</sup>  | 1.13 $\pm$ 0.01 <sup>a</sup>  | 1.22 $\pm$ 0.02 <sup>a</sup>  | 1.10 $\pm$ 0.01 <sup>a</sup>  |
| Testicular Length (cm) | 3.20 $\pm$ 0.02 <sup>a</sup>  | 3.25 $\pm$ 0.02 <sup>ab</sup> | 3.27 $\pm$ 0.02 <sup>b</sup>  | 3.26 $\pm$ 0.03 <sup>ab</sup> |

<sup>a, b, c</sup> Means with different alphabetical superscripts in a row indicate significant ( $p < 0.05$ ) difference.

**Table 2.** Epididymal and testicular morphometric parameters of rabbit bucks that were given varied doses of turmeric powder, at day 135 of treatment.

| Parameters             | Means ± standard deviation of parameters at day 135 of treatment |                              |                               |                             |
|------------------------|--|------------------------------|-------------------------------|-----------------------------|
|                        | Group A (250 mg/kg turmeric)                                     | Group B (500 mg/kg turmeric) | Group C (1000 mg/kg turmeric) | Group D (Untreated Control) |
| Epididymal Weight (g)  | 1.08 ± 0.01 <sup>a</sup>   | 1.14 ± 0.05 <sup>ab</sup>    | 1.23 ± 0.01 <sup>b</sup>      | 0.93 ± 0.02 <sup>c</sup>    |
| Testicular Weight (g)  | 2.08±0.03 <sup>a</sup>   | 2.42±0.04 <sup>b</sup>       | 2.70±0.04 <sup>c</sup>        | 1.72±0.04 <sup>d</sup>      |
| Testicular width (cm)  | 1.13±0.01 <sup>a</sup>   | 1.17±0.02 <sup>a</sup>       | 1.34±0.02 <sup>b</sup>        | 1.13±0.01 <sup>a</sup>      |
| Testicular Length (cm) | 3.25±0.01 <sup>a</sup>   | 3.30±0.02 <sup>b</sup>       | 3.33±0.01 <sup>b</sup>        | 3.29±0.01 <sup>ab</sup>     |

<sup>a, b, c</sup> Means with different alphabetical superscripts in a row indicate significant ( $p < 0.05$ ) difference.

The epididymal sperm reserve of bucks in Groups B and C were significantly ( $p < 0.05$ ) higher than those of Groups A and D on both days 45 and 135, in a dose dependent manner (Table 3 and 4). At day 45, the mean serum testosterone and LH levels of Group A and C bucks were significantly ( $p < 0.05$ ) higher than those of group B and D (Table 3), but on day 135 however, the mean serum testosterone and LH levels of all the treated groups (A, B and C) were significantly ( $p < 0.05$ ) higher than that of the Group D (Control), in a dose dependent manner (Table 4).

Bucks in Groups A and C had a significantly ( $p < 0.05$ ) higher mean serum total cholesterol at days 45 when compared to Group D (Table 3), but on day 135 the serum total cholesterol of Group C bucks alone was significantly ( $p < 0.05$ ) higher than those of groups A and D (Table 4). At day 45, there were no significant ( $p < 0.05$ ) variations among the groups in their serum levels of triglyceride, calcium, phosphorus, total protein and malondialdehyde (MDA) (Table 3), but at day 135, the serum triglyceride levels of rabbits in Groups B and C were significantly ( $p < 0.05$ ) lower than those of bucks in Group D (Table 4). There were no significant ( $p < 0.05$ ) variations among the groups in their serum calcium levels on day 135, but the serum

phosphorus levels of bucks in Group A was significantly ( $p < 0.05$ ) lower than that of Group D bucks on day 135 (Table 4). At day 135, also, the serum total protein of Group B and C bucks were significantly ( $p < 0.05$ ) lower than those of bucks in Groups A and D (Table 4). There were also no significant ( $p < 0.05$ ) variations in the serum MDA levels among the groups on day 135 (Table 4).

On all the days of the evaluation of fasting blood sugar, rabbit bucks in the treated groups (A, B and C) had significantly lower ( $p < 0.05$ ) blood sugar level when compared to the untreated control Group D (Table 5).

Stained sections of the testis of the four rabbit groups on day 45 and 135 are presented in Figures 1 and 2, respectively. Sections of the testis of the treated groups (A, B and C) did not show any histopathological lesions when compared to the control group (Group D) on both days 45 and 135 (Figures 1 and 2). The Sertoli cells, primary spermatocytes, early spermatids, late spermatids and the lumen of the seminiferous tubules of Groups A and B were not different from that of the control group, but sections of the testis of Group C bucks showed better cellular differentiation on day 135, with more sperm cells in the lumen of the seminiferous tubules (Figure 2).

**Table 3:** Epididymal sperm reserve, serum hormonal levels and levels of some serum biochemistry parameters on day 45 of administration of varied doses of turmeric powder to rabbit bucks.

| Parameters                                 | Means ± standard deviation of parameters at day 45 of treatment |                              |                               |                             |
|--|---|------------------------------|-------------------------------|-----------------------------|
|  | Group A (250 mg/kg turmeric)                                    | Group B (500 mg/kg turmeric) | Group C (1000 mg/kg turmeric) | Group D (Untreated Control) |
| Epididymal Sperm reserve ( $\times 10^6$ ) | 342.00 ± 2.58 <sup>a</sup>                                      | 373.25 ± 10.81 <sup>b</sup>  | 380.75 ± 7.04 <sup>b</sup>    | 333.25 ± 6.99 <sup>a</sup>  |
| Testosterone (ng/dl)                       | 5.57 ± 0.08 <sup>a</sup>  | 3.57 ± 0.21 <sup>b</sup>     | 4.35 ± 0.22 <sup>c</sup>      | 3.47 ± 0.14 <sup>b</sup>    |
| Leutinizing hormone (mIU/ml)               | 7.89 ± 0.13 <sup>a</sup>  | 5.80 ± 0.11 <sup>b</sup>     | 6.96 ± 0.25 <sup>c</sup>      | 5.56 ± 0.56 <sup>b</sup>    |
| Total Cholesterol (mg/dl)                  | 106.59 ± 4.19 <sup>a</sup>                                      | 89.77 ± 14.79 <sup>ab</sup>  | 103.36 ± 1.23 <sup>a</sup>    | 68.48 ± 2.29 <sup>b</sup>   |
| Triglycerides (mg/dl)                      | 48.44 ± 3.59 <sup>a</sup>                                       | 47.44 ± 5.49 <sup>a</sup>    | 53.34 ± 2.71 <sup>a</sup>     | 43.96 ± 0.86 <sup>a</sup>   |
| Calcium mg/dl)                             | 10.09 ± 0.37 <sup>a</sup>                                       | 9.77 ± 0.33 <sup>a</sup>     | 10.18 ± 0.14 <sup>a</sup>     | 9.98 ± 0.11 <sup>a</sup>    |
| Phosphorus (mg/dl)                         | 6.47 ± 0.84 <sup>a</sup>  | 5.50 ± 0.43 <sup>a</sup>     | 6.67 ± 1.29 <sup>a</sup>      | 5.07 ± 0.33 <sup>a</sup>    |
| Total Protein (g/dl)                       | 5.88 ± 0.08 <sup>a</sup>  | 5.74 ± 0.18 <sup>a</sup>     | 6.16 ± 0.22 <sup>a</sup>      | 5.92 ± 0.09 <sup>a</sup>    |
| MDA (nmol/ml)                              | 4.53 ± 0.74 <sup>a</sup>  | 4.42 ± 0.83 <sup>a</sup>     | 4.47 ± 0.56 <sup>a</sup>      | 4.35 ± 0.53 <sup>a</sup>    |

<sup>a, b, c</sup> Means with different alphabetical superscripts in a row indicate significant ( $p < 0.05$ ) difference.

**Table 4.** Epididymal sperm reserve, serum hormonal levels and levels of some serum biochemistry parameters on day 135 of administration of varied doses of turmeric powder to rabbit bucks.

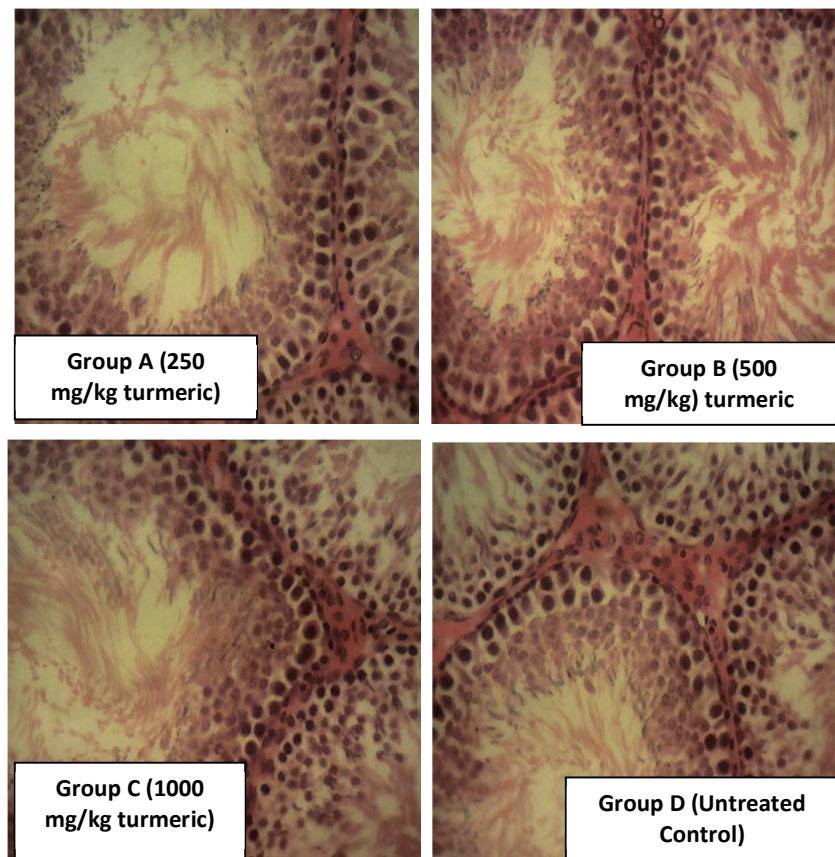
| Parameters                                 | Means ± standard deviation of parameters at day 135 of treatment |                              |                               |                             |
|--|--|------------------------------|-------------------------------|-----------------------------|
|  | Group A (250 mg/kg turmeric)                                     | Group B (500 mg/kg turmeric) | Group C (1000 mg/kg turmeric) | Group D (Untreated Control) |
| Epididymal Sperm reserve ( $\times 10^6$ ) | 353.75 ± 4.65 <sup>a</sup>                                       | 434.75 ± 3.30 <sup>b</sup>   | 454.25 ± 4.11 <sup>c</sup>    | 350.00 ± 2.58 <sup>a</sup>  |
| Testosterone (ng/dl)                       | 5.86 ± 0.04 <sup>a</sup>   | 6.73 ± 0.12 <sup>b</sup>     | 8.60 ± 0.12 <sup>c</sup>      | 3.81 ± 0.04 <sup>d</sup>    |
| Leutinizing hormone (mIU/ml)               | 8.46 ± 0.41 <sup>a</sup>   | 10.36 ± 0.06 <sup>b</sup>    | 11.55 ± 0.07 <sup>c</sup>     | 6.00 ± 0.14 <sup>d</sup>    |
| Total Cholesterol (mg/dl)                  | 62.23 ± 4.15 <sup>a</sup>  | 77.19 ± 1.47 <sup>ab</sup>   | 88.27 ± 9.18 <sup>b</sup>     | 70.53 ± 1.46 <sup>a</sup>   |
| Triglycerides (mg/dl)                      | 52.25 ± 3.05 <sup>ab</sup>                                       | 49.73 ± 4.12 <sup>a</sup>    | 46.82 ± 9.24 <sup>a</sup>     | 67.43 ± 2.10 <sup>b</sup>   |
| Calcium mg/dl)                             | 8.65 ± 0.12 <sup>a</sup>   | 8.82 ± 0.20 <sup>a</sup>     | 8.89 ± 0.18 <sup>a</sup>      | 8.89 ± 0.08 <sup>a</sup>    |
| Phosphorus (mg/dl)                         | 6.88 ± 0.62 <sup>a</sup>   | 8.17 ± 0.64 <sup>ab</sup>    | 7.90 ± 0.54 <sup>ab</sup>     | 8.91 ± 0.51 <sup>b</sup>    |
| Total Protein (g/dl)                       | 6.76 ± 0.06 <sup>a</sup>   | 5.98 ± 0.11 <sup>b</sup>     | 6.29 ± 0.22 <sup>b</sup>      | 6.80 ± 0.13 <sup>a</sup>    |
| MDA (nmol/ml)                              | 4.28 ± 1.18 <sup>a</sup>   | 3.49 ± 0.48 <sup>a</sup>     | 3.92 ± 0.43 <sup>a</sup>      | 3.97 ± 0.25 <sup>a</sup>    |

<sup>a, b, c</sup> Means with different alphabetical superscripts in a row indicate significant ( $p < 0.05$ ) difference.

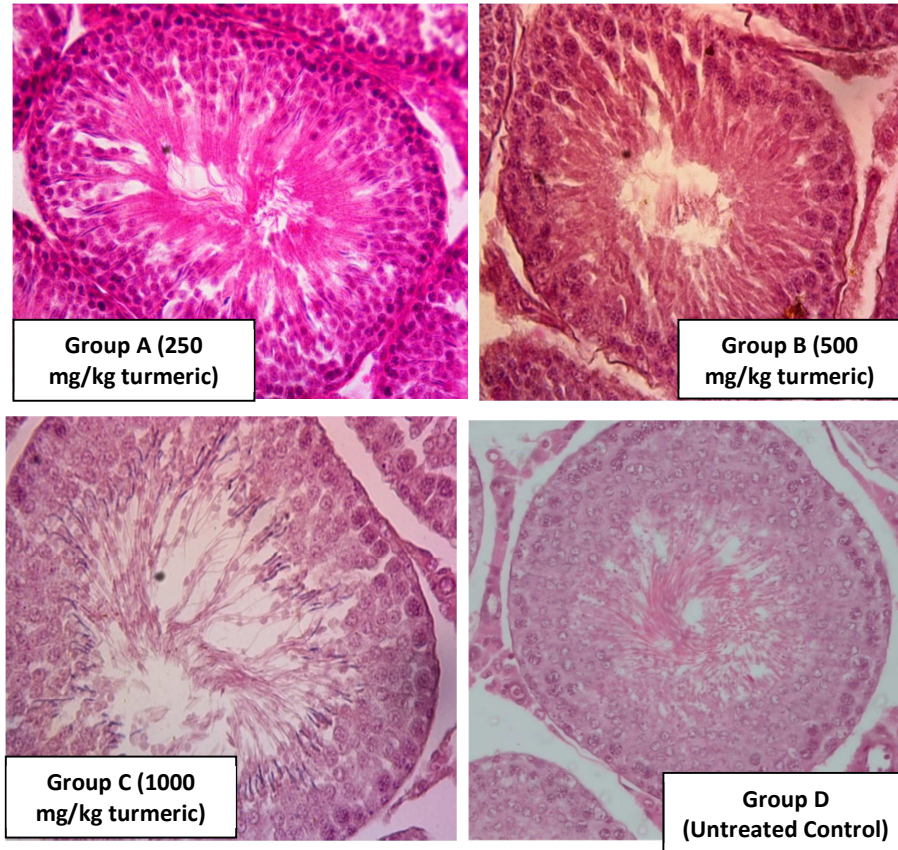
**Table 5.** Fasting blood sugar levels of rabbit bucks that were given graded doses of turmeric for 135 days.

| Experimental period (Days) | Means of fasting blood sugar levels $\pm$ standard deviation |                               |                               |                                |
|----------------------------|--|-------------------------------|-------------------------------|--------------------------------|
|                            | Group A (250 mg/kg turmeric)                                 | Group B (500 mg/kg turmeric)  | Group C (1000 mg/kg turmeric) | Group D (Untreated Control)    |
| 15                         | 91.23 $\pm$ 0.64 <sup>a</sup>                                | 91.63 $\pm$ 0.81 <sup>a</sup> | 90.76 $\pm$ 1.43 <sup>a</sup> | 122.56 $\pm$ 1.78 <sup>b</sup> |
| 30                         | 92.29 $\pm$ 0.92 <sup>a</sup>                                | 90.73 $\pm$ 0.48 <sup>a</sup> | 91.53 $\pm$ 0.50 <sup>a</sup> | 122.32 $\pm$ 1.40 <sup>c</sup> |
| 45                         | 91.80 $\pm$ 0.74 <sup>a</sup>                                | 90.75 $\pm$ 0.33 <sup>a</sup> | 90.65 $\pm$ 0.27 <sup>a</sup> | 122.77 $\pm$ 1.33 <sup>b</sup> |
| 75                         | 90.91 $\pm$ 1.07 <sup>a</sup>                                | 90.92 $\pm$ 0.57 <sup>a</sup> | 90.69 $\pm$ 0.44 <sup>a</sup> | 123.09 $\pm$ 0.18 <sup>b</sup> |
| 105                        | 91.36 $\pm$ 0.97 <sup>a</sup>                                | 92.79 $\pm$ 1.42 <sup>a</sup> | 90.63 $\pm$ 0.17 <sup>a</sup> | 123.01 $\pm$ 1.76 <sup>b</sup> |
| 135                        | 92.33 $\pm$ 0.88 <sup>a</sup>                                | 91.26 $\pm$ 0.81 <sup>a</sup> | 90.68 $\pm$ 0.28 <sup>a</sup> | 123.40 $\pm$ 1.24 <sup>b</sup> |

<sup>a, b</sup> Means with different alphabetical superscripts in a row indicate significant ( $p < 0.05$ ) difference.



**Figure 1.** Photomicrograph of sections of the testes of rabbit bucks treated with varied doses of turmeric, on day 45 of treatment (H & E;  $\times 400$ ).



**Figure 2.** Photomicrograph of sections of the testes of rabbit bucks treated with varied doses of turmeric, on day 135 of treatment (H & E;  $\times 400$ ).

### Discussion

Nutritional factors have been reported to have various impacts on sexual and reproductive health of both male and female mammals (Silva *et al.*, 2019). The male gonads (the testes) has two main functions: (i) an endocrine function to produce testosterone, responsible for secondary sexual characteristics including erection, and (ii) an exocrine function to produce sperm (Tijani *et al.*, 2014). A knowledge of the ability of testes to produce and store active spermatozoa as well as mating soundness of the bucks is of particular importance in evaluating breeding soundness in all animal breeding programs.

The epididymis is an organ in which spermatozoa undergoes final maturation and storage prior to ejaculation. Consequently, the storage capacity of the epididymis is found to

greatly influence semen production and fertility in mammals. As observed in this study, administering graded doses of *Curcuma longa* (especially the higher doses – 500 and 1000 mg/kg) led to positive effects on epididymal weights of the bucks, and the effects was found to be dose dependent at both day 45 and 135 of treatment. The findings in this study of significantly higher epididymal weights in bucks given turmeric powder when compared to untreated controls concurs with earlier reports by Jeber and Tawfeek (2013) of a significant increase in epididymal weights of rats after oral turmeric supplementation, following exposure to potassium dichromate, and that of Taba *et al.* (2018) that showed a significant increase in the epididymal weights of mice after oral supplementation of curcumin. Creasy (2001) opined that measurement of male reproductive organ



weights is generally a more reliable method than histopathology for the detection of decreased secretory activity and content. Meanwhile, it has been stressed that weights of prostate and epididymis is most strongly correlated with ejaculate volume and percentage of mobile spermatozoa (Paris *et al.*, 2005), whereas, sperm mobility is among the factors found to determine the inseminating capacity of particular semen (Okoro *et al.*, 2016). Hence, the findings in this study of higher weight of the testis and epididymis might be an indication that turmeric supplementation at graded doses for 135 days was not toxic to these reproductive organs. The observed significantly higher epididymal weights of the bucks recorded in groups that were given graded doses *Curcuma longa* powder suggest that semen from the treated bucks is likely to have a better fertilizing ability compared to those in the control group.

It has been reported that testicular size is a good indicator of the present and future spermatozoa production capacity of an animal (Hyacinth *et al.*, 2016). Punab (2017) opined that larger (longer and wider) testicles are able to produce more spermatozoa when compared to smaller ones, since seminiferous tubules make up 80% to 90% of the testis. In this regard, findings in this study suggests that the bucks in group C which received highest dose of turmeric would have greater fertility potential compared to other groups after 45 days of treatment, while on day 135 of the treatment, the bucks in groups B and C are supposed to have a greater spermatozoa production potential than those in other groups. In general, the results suggest that dietary *Curcuma longa* supplementation at the graded doses used in this study was safe in terms of fertility potentials of the treated rabbits, since deleterious and/or regressive effects were not observed in the total testicular volume. The study further showed that dose and duration of treatment

significantly affected the testicular weight of the bucks given *C. longa*. The findings in this present study is in agreement with the reports of Jeber and Tawfeek (2013) who recorded a significantly higher testicular weight of bucks fed diets supplemented with *Curcuma longa*.

It is thought that the significantly higher testicular weights recorded in this study may be as a result of the enhanced protein synthetic capability of *Curcuma longa* constituents (El-Wakf *et al.*, 2011). Testicular weight had been reported to be positively correlated to daily sperm production and sperm quality (Kastelic, 2013). Consequently, the dose dependent higher testicular weights recorded on day 135 of this study would suggest that these testes contained larger and healthier seminiferous tubules and other cells responsible for spermatogenesis, and this may ultimately result in comparatively better fertility.

Mean testicular length and width usually reflects mean testicular volume/size, which had been found to be significantly correlated with semen parameters such as sperm density, total sperm count, total mobile spermatozoa, volume, sperm count, serum follicle-stimulating hormone, as well as spermatogenic function (Sakamoto *et al.*, 2008; Tijani *et al.*, 2014; and Ehala-Aleksejev, and Punab, 2017). The results obtained in this study showed no significant difference between the testicular widths of the bucks at day 45 after administration of graded doses of turmeric powder. Significantly higher testicular length was also observed in bucks in Group C on days 45 and 135 of treatment. In view of these findings, and considering that both testicular width and length constitute testicular volume, it would make logical sense to suggest that the effect of *Curcuma longa* on testicular volume of bucks was dose- as well as treatment duration-dependent. At a relatively small dose and short treatment duration (45 days) the *Curcuma longa* did not significantly influence testicular total volume of the bucks,

while there was significantly higher testicular volume after 135 days.

Epididymal sperm concentration is a vital indicator of male fertility. It is usually used in male reproductive assessment, since it indicates the possible number of sperm cells to reach and fertilize the eggs. As such, evaluating the quantity of spermatozoa stored in the epididymis is crucial in detecting bucks with probable good fertilizing potential. This present study showed a significantly higher epididymal sperm concentration of bucks in Groups B and C that were given higher doses of *Curcuma longa* powder compared to bucks fed the control diet. Contrary to our findings, Mishra and Singh (2009) reported a significant decrease in the number of spermatozoa in cauda epididymis after administering aqueous rhizome extract of *Curcuma longa* orally (600 mg/kg daily for 84 days) to male mice. Also, Ashok and Meenakshi (2004) observed a significant reduction in cauda epididymal sperm density as well as in the number of spermatocytes after the administration (500 mg/kg for 60 days) of aqueous and alcoholic extracts of *Curcuma longa* to male albino rats. Our findings are consistent with the reports of Jeber and Tawfeek (2013) who recorded significantly higher total sperm count of adult male rats after oral treatment with turmeric. It is thought that the reason for variations in these results may be attributed to difference in animal species treated, types of extract used, as well as dose and duration of administration.

Increased sperm production and concentration can be linked to improved growth and development of testicular glands. For instance, bucks with larger testicular weight are reported to possess more seminiferous tubules, Leydig and Sertoli cells (Emmanuel et al., 2019). Meanwhile, curcumin which is known as the most active component of *Curcuma longa* (Taba et al., 2018; Mohebbati, 2017) has earlier been reported to improve histopathology of the testis as well as

spermatogenesis in male animals (Chandra et al., 2007; Khorsandi et al., 2013). The, significantly higher epididymal sperm concentration recorded in this study may be attributed to ability of the curcumin content in *Curcuma longa* to actively enhance spermatogenesis by stimulating production of more seminiferous tubules, Leydig and Sertoli cells. The significantly higher epididymal sperm reserve recorded in this study in the groups treated with 500 and 1000 mg/kg *C. longa* concurs with their recorded higher testicular morphometric parameters (epididymal weight, testicular weight, length and width).

Studies have shown that the hormonal profile can influence epididymal sperm characteristics of bucks. Earlier reports have shown some adverse effects of aqueous extract of *Curcuma longa* on the spermatogenesis, motility, morphology, viability, and number of spermatozoa in the cauda epididymides and these effects were attributed to the effects on function of the testes and deficiency of testosterone (Mohebbati et al., 2017). However, Jeber and Tawfeek (2013) recorded significantly higher serum testosterone concentration in adult male rats after dietary supplementation of turmeric oil for 60 days. Similarly, Akinyemi et al. (2015) reported that dietary supplementation with turmeric and ginger significantly increased testosterone levels in hypertensive male rats. In agreement with these earlier reports, the findings in the present study showed significantly higher testosterone and luteinizing hormone levels of the bucks fed graded doses of *Curcuma longa* powder. There was however no significant difference between the testosterone and LH levels in the group fed 500 mg/kg *Curcuma longa* powder supplement and the untreated control on day 45.

In rats (as well as rabbits), LH acts on Leydig cells in the testis and is the primary regulator of testosterone secretion; the major stimulus for testosterone production comes from blood

levels of LH from the pituitary (Creasy and chapin, 2013). It has been reported that dietary administration of turmeric oil stimulates a significant expansion of both thickness and diameter of seminiferous tubules of male rats (Jeber and Tawfeek, 2013) which in turn trigger Leydig cells' steroidogenesis and stimulate male reproductive activity. It is therefore believed that the dose dependent significantly higher serum levels of testosterone and LH in the turmeric treated groups in this study can be attributed to the reported ability of turmeric to induce seminiferous tubule expansion. The observed high testosterone and LH levels might be the signal maintaining a quantitatively maximum spermatogenic potential of rabbits in the treated groups.

Serum total cholesterol and triglyceride concentrations are viewed as diagnostic markers in lipid metabolism (Reda et al., 2020). Cholesterol is reported to be an obligatory precursor in the synthesis of testosterone, and is supplied to the Leydig cells (Creasy and Chapin, 2013) as a necessity for sperm production. This present study recorded significantly higher levels of mean total cholesterol of bucks fed graded doses of *Curcuma longa* powder for 45 and 135 days, compared to control group. However, Ogbuewu et al., (2017) reported that turmeric powder supplementation at 2 and 4 mg/kg dose for 84 days had no significant influence on total cholesterol on the treated rabbits compared to control group. Similarly, Majeed et al, 2019 reported no significant change in serum cholesterol levels in male rats administered with tetrahydrocurcumin. Meanwhile, Purohit, (1999) and Reda et al., (2020) reported a significant decrease in serum cholesterol level of male rats and quails after oral administration of turmeric extract and nano-curcumin, respectively. The differences between our findings in this present study and the cited studies can as well be attributed to variation in the doses of

turmeric supplement, the animal species used for the study, as well as duration of study. It was however observed that the degree of increase on day 45 was higher compared to that of day 135. This may suggest that administration of turmeric as used in this study for relatively shorter duration could stimulate biosynthesis of serum total cholesterol more effectively compared to exposure for lengthy duration.

Some semen parameters such as sperm motility, sperm concentration and morphology have been reported to be influenced by the blood lipid profile. It has been reported that sperm concentration is positively correlated to triglyceride levels, while sperm motility increases with increase in cholesterol levels (Liu et al., 2017). The higher serum total cholesterol concentration observed in the present study may indicate higher inseminating potential of spermatozoa in epididymis of the *Curcuma longa* treated bucks compared to the control group, while decreased triglyceride levels observed in groups B and C on day 135 may suggest higher sperm concentration in epididymis of bucks in the treated groups when compared to the control group. Also, significantly lower serum levels of triglyceride, reported in the treated groups on day 135 may suggest regressive effect of *Curcuma longa* on these parameters.

Inorganic minerals (including calcium and phosphorus) are important for the maintenance of optimal osmolality and activity of spermatozoa (Ogbuewu et al, 2017). In line with this assertion, Shahzad et al. (2016) reported that mineral deficiency (calcium and phosphorus imbalance) may result in infertility in male animals. On the other hand, serum proteins play vital role in maintaining healthy sperm motility as well as in spermatogenesis. Creasy and Chapin (2013) opined that over 90% of protein is reabsorbed during sperm transportation from rete testis to the epididymis. Results obtained from this study showed no significant changes in serum levels

of triglyceride (TG), calcium, phosphorus, as well as total protein of bucks after oral administration of graded doses of *Curcuma longa* powder for 45 days. The lack of significant changes in these parameters in the treated groups suggests non-interference of *Curcuma longa* in digestion and absorption of those minerals from the gastrointestinal tract as well as in hepatic protein synthesis. In agreement with the above findings on the serum biochemical parameters, Majeed et al. (2019) reported no significant variation in serum levels of total protein, calcium, TG and phosphate in rats orally treated with tetrachlorocurcumin (an extract of turmeric) in comparison to the control group. Also, El-Wakf et al. (2011) reported no significant change in serum total protein and triglyceride in male rats fed *Curcuma longa*. However, results of the present study is not in agreement with the findings of Reda et al., (2020) who reported that supplementation of various doses of nano-curcumin significantly increased serum total protein levels in quails.

Biochemical assay of malondialdehyde, which is a by-product of lipid peroxidation process, is said to be essential in understanding impaired sperm function in terms of motility and capacity for sperm oocyte fusion (Oral et al., 2006). Results of our study showed no significant variation in the serum levels of malondialdehyde of bucks that were given graded doses of *Curcuma longa* throughout the study, in comparison with control. Contrary to this finding, Jeber and Tawfeek (2013) recorded significant increase in serum malondialdehyde levels of male rats after orally administering turmeric oil, while El-Wakf et al., (2011) reported significant decrease in malondialdehyde levels in male rats fed diets supplemented with turmeric. Variations in the species of animal used for a study and duration of the study may be responsible for the differences in the effects reported for serum malondialdehyde levels. High malondialdehyde levels in seminal plasma had

been reported to negatively correlate with sperm viability, motility, morphology and concentration (Atig et al., 2012). Similarly, El-Wakf et al., (2011) attributed reduction in sperm number in testes of rabbits to increased lipid peroxidation. However, the lack of significant difference in serum malondialdehyde levels observed in this study may suggest non-deleterious effect or no significant interference of the *Curcuma longa* constituents on lipid peroxides generation in blood of the treated rabbits.

Abnormalities in blood glucose levels of mammals can cause anomalies in reproductive dysfunction. Diabetes mellitus, which has been attributed to glucose dysregulation in blood of affected animals and humans (Maresc et al., 2017), has been associated with a number of fertility problems including erectile and ejaculatory dysfunction, reduced semen volume and sperm count as well as sperm motility and morphological abnormalities (Omolaoye and Plessin, 2020). In this present study, results obtained showed significantly lower fasting blood glucose of rabbit bucks treated with graded doses of *Curcuma longa* powder throughout the duration of the treatment, in comparison to control treated group. This finding is consistent with the results of Hodaei (2019) who reported that daily administration of 5000 mg curcumin had a positive effect of reducing fasting blood glucose in patients with type-2 diabetes. Reduced blood sugar levels observed in bucks of the turmeric treated groups may be attributed to the reported ability of turmeric components to stimulate more insulin sensitivity through three possible pathways, which may be through modulating glucose homeostasis by triggering glucokinase activity in the liver; through lipid metabolism by raising lipoprotein lipase activity to reduce triglyceride levels; and lastly, sugar levels can be reduced by affecting the insulin pathway independently through inducing glucose transporter-4 (GLUT4) expression to increase

peripheral glucose uptake (Jimenez-Osorio et al., 2016).

Observing the direction of structural change of testes can be essential in understanding the kind of influence that treatment exerts on quality of spermatozoa produced, as well as reproductive performance of male animals. Emmanuel et al. (2019) opined that histological soundness of the entire testes is fundamental to quality spermatozoa production; as such any treatment that damages the testis would result in production of sub-fertile spermatozoa. The finding in the present study that Group C bucks had ductus epididymis with wider diameter and seminiferous tubules with greater lumens containing more sperm cells and better differentiated cells is in agreement with the earlier reports of Jeber and Tawfeek (2013) who recorded a significant increase in diameter and thickness of seminiferous tubules in the group of adult male rats orally administered with turmeric oil. The relatively better testicular architecture in the treated groups, especially in Group C bucks, suggests better reproductive health status of bucks in that group treated with 1000 mg/kg *C. longa* powder.

**Conclusion:** Administration of *C. longa* powder as used in the study, especially at the higher dose of 1000 mg/kg, led to significantly higher weights of the testis and epididymis of the rabbit bucks, higher epididymal sperm reserves and serum testosterone and LH levels and much better differentiated testicular histomorphology. It also significantly lowered fasting blood sugar levels.

#### Conflict of interests

The authors declare no conflict of interest.

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