Semen quality and histological changes in the testes of Noiler breeder cocks fed turmeric (*Curcuma longa*) rhizome powder supplemented diet

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Abstract

The study was conducted to evaluate the effect of turmeric-supplemented diets on semen quality and histological changes in the testes of Noiler breeder cocks. Sixty (60) breeder cocks (aged 24 weeks) were randomly allotted to 4 treatments (T_1, T_2, T_3 and T_4) and replicated thrice in a completely randomized design (CRD). Four levels of turmeric (0 g, 2 g, 5 g and 6 g), which constituted the respective treatments, were mixed per kilogram of diet. Semen samples were collected and evaluated for pH, volume, colour, consistency, sperm motility, normal and abnormal sperm, sperm concentration and morphologically abnormal sperm. Histological changes were studied in the testes. Semen pH and volume were significantly higher in T_4 (7.34 ± 0.05 and 6.85 ± 0.02 ml) compared to other treatments. Semen colour was better and similar in T_2 - T_4 (2.00 ± 0.00) than the control (1.00 ± 0.00). Semen consistency, mass motility, percentage live sperm proportion, percentage normal sperm proportion and sperm concentrations were significantly higher in T_2 and least in T_4. Percentage dead sperm proportion and abnormal sperm were significantly higher in T_4 (22.46 ± 1.53 %) and least in T_2 (6.06 ± 0.28 %). Total sperm per ejaculate was significantly higher and similar ($p > 0.05$) in T_2 –T_4 ($1.76 \pm 0.07 \times 10^{12}$) compared to the control ($1.31 \pm 0.01 \times 10^{12}$). Percentage of tapered sperm head was significantly higher and was comparable ($p > 0.05$) in T_4 (0.41 ± 0.10 %) and T_3 (0.20 ± 0.06%) and least in T_1 (0.09 ± 0.4 %). Testicular histology showed mild degeneration of spermatogenic cells only in T_4. It was concluded that turmeric rhizome powder improves semen quality and testicular ultrastructures. Daily turmeric supplementation at 2g and 5g/kg are recommended for Noiler breeder cocks.

Keywords: Noiler cocks, turmeric powder, semen, testes, histology

Effet de la poudre de rhizome de curcuma (Curcuma longa) sur la qualité du sperme et les modifications histologiques testiculaires chez les coqs reproducteurs Noiler

Résumé

Cette étude visait à évaluer l'impact de l'ajout de curcuma dans l'alimentation sur la qualité du sperme et les modifications histologiques testiculaires chez les coqs reproducteurs Noiler. Soixante (60) coqs reproducteurs âgés de 24 semaines ont été répartis aléatoirement en 4 groupes (T_1, T_2, T_3 et T_4) avec trois répétitions par groupe selon un dispositif entièrement randomisé (ER). Quatre teneurs en curcuma (0 g, 2 g, 5 g et 6 g) ont été incorporées dans l'alimentation respective de chaque groupe (par kilogramme d'aliment). Des échantillons de sperme ont été prélevés et analysés afin d'évaluer le pH, le volume, la couleur, la consistance, la motilité spermatique, la proportion de spermatozoïdes normaux et anormaux, la concentration spermatique et la morphologie spermatique. Les modifications histologiques des testicules ont également été étudiées. Le pH et le volume du sperme étaient significativement plus élevés dans le groupe T_4 (7,34 ± 0,05 et 6,85 ± 0,02 ml) par rapport aux autres groupes. La couleur du sperme était meilleure et comparable dans les groupes T_2 à T_4 (2,00 ± 0,00) par rapport au groupe témoin (1,00 ± 0,00). La consistance du sperme, la motilité de masse, la proportion

de spermatozoïdes vivants et normaux, ainsi que la concentration spermatique étaient significativement plus élevées dans le groupe T_2 et plus faibles dans le groupe T_4. La proportion de spermatozoïdes morts et anormaux était significativement plus élevée dans le groupe T_4 (22,46 ± 1,53 %) et plus faible dans le groupe T_2 (6,06 ± 0,28 %). Le nombre total de spermatozoïdes par éjaculat était significativement plus élevé et similaire ($p > 0,05$) dans les groupes T_2 à T_4 (1,76 ± 0,07 x 1012) par rapport au groupe témoin (1,31 ± 0,01 x 1012). Le pourcentage de spermatozoïdes à tête effilée était significativement plus élevé et comparable ($p > 0,05$) dans les groupes T4 (0,41 ± 0,10 %) et T_3 (0,20 ± 0,06 %) et plus faible dans le groupe T_1 (0,09 ± 0,4 %). L'analyse histologique des testicules n'a révélé qu'une légère dégénérescence des cellules spermatogènes dans le groupe T_4. L'étude démontre que la poudre de rhizome de curcuma améliore la qualité du sperme et l'ultrastructure testiculaire. Une supplémentation quotidienne en curcuma à des doses de 2 g et 5 g/kg d'aliment est recommandée pour les coqs reproducteurs Noiler.

Mots-clés: Coqs Noiler, poudre de curcuma, sperme, testicules, histologie

Introduction

The poultry industry in Nigeria is growing with increased capacity to solve the animal protein deficiency in the nation. Nevertheless, higher prevalence of infectious diseases and flock infertility are among the numerous factors that militate against the growth of the industry. Thus, feeding of antibiotics has become a routine management practice in poultry production to put down bacterial load, maintain good health and to improve growth and reproductive performance (Huyghebaert *et al.,* 2018). However, the risk of antibiotic residue in edible animal products and the development of resistant genes and cross infection between man and animals led to the ban on the use antibiotics in livestock and poultry production (Sugiharto and Turrini, 2022). Similarly, research reports on man and rats, indicated that most antibiotics are gonadotoxic and are capable of modifying the hypothalamic-pituitary - gonadal axis, resulting in oligospermia, cryptozoospermia and azoospermia (Schlegel *et al*., 1991). It was reported that neomycin, tylosin and furacin at therapeutic doses adversely affected sperm concentration, total sperm count, and spermatozoa motility; blocked spermatogenesis at the level of spermatogonia, and decreased the number of seminiferous tubules containing spermatocytes, spermatids and average number of spermatozoa per tubule (Schlegel *et al*., 1991). Yunda *et al*. (1973) and Kushuniruk (1976) found spermatogenic arrest at premeiotic stage of spermatogensis with attendant suppression of mitotic activity of spermatogonia with high doses of tylosin and neomycin. Also the withdrawal of antibiotic in poultry production is implicated for such problems as bacterial over growth in the gastrointestinal tract, increased water content in faces, thinning and ballooning of the small intestine as well as feed passage syndrome (Dibner and Richards, 2005). These have led to increased research on complementary and alternative medicines that are safer, effective and economical (Yun *et al*., 2021). A typical example of the alternative antimicrobial is turmeric **(***Curcuma longa*) (Borghei – Rad *et al*., 2017). Turmeric contains curcumin and ar-turmerone, which have potent antibiotic, antifungal, antiviral, antioxidant and anti-inflammatory properties (Angel *et al*., 2014; Sharifi-Rads *et al*., 2020). It was reported that dietary turmeric supplementation enhances meat quality and stability, liver enzymes activity, and immune response in broiler chickens (Zhang *et al.,* 2015); semen volume, sperm motility and viability, and sperm concentration in heat stressed broiler roosters (Yan *et al*., 2017), and in rabbits (Seadawy *et al*., 2014), fertility rates in broiler layers (Yan *et al*., 2017). It was also demonstrated that turmeric showed great protective effect on the spermatozoa membrane functional integrity, resulting in low percentage of total sperm abnormality when added to Tris-based bull and goat semen extender during freezing/thawing processes (Bucak *et al.,* 2010). Ali *et al*. (2020)

reported that turmeric suppressed the growth of several bacteria like *Streptococcus*, *Staphylococcus* and *lactobacillus.* Turmeric decreased the growth of *E coli* in broiler chickens (Sugiharto and Turrini, 2022). Although turmeric is considered as safe with wide a spectrum of biological activities, it is toxic to the liver, testis and ovary, and is not recommended in pregnancy (Sharifi – Rads *et al*., 2020). For instance, Garg (1974) reported that aqueous extract of turmeric rhizome powder showed 100% anti-infertility effects in rats. In hamsters, curcumin inhibited 5α-reductase and the growth of flank organs (Garg, 1978). Rithaporan *et al.,* (2003) observed that curcumin inhibited motility of human sperm and can be developed into normal intra-vaginal contraceptive. It therefore appears that mating between roosters and hens fed diets supplemented with turmeric may not result in a chick. Most of the research works conducted on turmeric in poultry were on broilers (Yan et al., 2017). Hence, information on Noiler is scanty. Similarly, previous works on turmeric were in poultry were on short durations, resulting in controversy over its safety. In this study, turmeric was fed to Noiler breeder cocks daily for as long as 24 weeks to reveal its possible toxic effect on the testes and semen quality parameters, which are predictors of spermatozoal fertilizing potential. This research is designed to provide useful information on the effect of turmeric supplementation on semen quality and histological changes in the testes of Noiler breeder cocks.

Materials and Methods

Experimental location

The research was carried out at the Poultry Unit of the Teaching and Research Farm, Michael Okpara University of Agriculture, Umudike, Abia State. Umudike lies on co-ordinates 05° 29' N and 07° 33' E, and an altitude of about 122 m above sea level. The average annual rainfall ranges from 1700 to 2100 mm. Minimum and maximum temperature are in the ranges 18 to 23°C and 26 to 36°C, respectively; while relative humidity is 57 to 97% (NRCRI, 2022). Semen samples and testicular tissues were analyzed and processed at pathology lab of the Department of Veterinary Anatomy, Michael Okpara University of Agriculture, Umudike.

Sourcing of experimental birds and test ingredient

Noiler birds were sourced from a reputable farm in Ogun State, Nigeria. Fresh turmeric rhizomes were purchased from National Root Crops Research Institute, Umudike, Abia State, Nigeria. The rhizomes were washed, sliced and dried at room temperature. The dried rhizomes were milled with a hammer mill, sieved and stored in air tight container and kept away from sun light.

Experimental birds and management

Sixty Noiler cocks were used for the study at 20-weeks of age with initial mean live weight of 2.19kg. The cocks were reared on well ventilated deep litter pens throughout the experimental period. A uniform layers' mash containing 16.5% CP and 2,600 Kcal metaboliseable energy/kg was formulated and used in all the treatments (T_1, T_2, T_3 and T_4). Four levels of turmeric (0g, 2g, 5g and 6g) were mixed per kilogram of feed and administered *ad libitum* throughout the experimental period which, lasted for 24 weeks. T1(control) received 0 g of turmeric/kg feed, T2 received 2 g of turmeric/kg feed, T3 received 5 g of turmeric/kg feed while T4 received 6 g of turmeric/kg feed. Throughout the study, only the routine vaccinations were given in all the treatments.

Experimental Design

The study adopted a Completely Randomized Design (CRD) to consider four dietary supplemental levels of turmeric (0, 2, 5 and 6 g/kg feed). Sixty cocks (60) were assigned to 4 treatments - T_1 T_2 T_3 and T_4. Each treatment had 15 birds with 3 replicates of 5 birds per replicate.

The model for Experiment is as stated below:

$Y_{ijk} = \mu + T_i + e_{ijk}$

Where:

Y_{ijk} = Individual observation

μ = Overall mean

T_i= Treatment effect of ith level of turmeric

e_{ijk} = Random error, assumed to be independently, identically and normally distributed with zero mean and constant variance.

Table 1: Percentage and proximate composition of experimental diet

Feed ingredients	%
Maize	43.0
Wheat offal	18.0
Soya bean cake	17.5
Palm kernel cake	9.0
Fish meal	2.5
Bone meal	3.0
Lysine	0.25
Methionine	0.25
Vitamin premix	0.25
Salt	0.25
Oyster shell	6.0
Total	100
Calculated:	
Crude protein (%)	16.5
Energy (Kcal ME/kg)	2,600

Data collection

Training of the cocks for semen collection

The cocks in each treatment were trained for semen collection three times weekly for two weeks using abdominal massage technique as described by Burrows and Quinn (1937). The semen colour was determined as described by Bearden *et al.* (2004) and graded on a scale of 1-2. Only creamy white samples were analyzed while soiled and blood-tainted samples were discarded. Semen consistency was evaluated according to Ukar *et al*. (2017) and graded on a scale of 1-4. Semen pH was measured with a pH meter (Brouwland) and graded in a scale of 0 to 14**.** Semen volume was determined with the aid of a 10ml beaker and 1ml tuberculin syringe. Each ejaculate was transferred into an insulated 5 ml beaker and then aspirated into the tuberculin syringe. Progressive motility was determined using the method described by Bearden *et al.* (2004) and Hafez (1984). The percentage motile sperm was determined by subjective scoring from a range of 0 - 100% (Bearden *et al.,* 2004). The percentage live and dead spermatozoa were determined by differential staining technique as described by Bearden *et al.* (2004). Sperm concentration was determined with a haemocytometer as described by Bearden *et al.* (2004). The number of spermatozoa per sample of semen was calculated with a formula as shown below:

$$\frac{Number\ of\ spermatozoa\ counted \times Df}{A \times D} \times 10^6$$

Where:

Df = Dilution factor (0.6)

A = Area of the haemocytometer $\left(\frac{1}{400}mm\right)$

D = Depth of the haemocytomter (0.1mm)

Total sperm count = Sperm concentration x total volume of ejaculate (Hafez, 1995)

Histological Procedures

Testicular histology was carried out according to Clayden (1967) as outlined below**:** Three longitudinal sections were obtained from the testis of each cock and immediately immersed in Bouin's fixative for 24 hours. Thereafter, a slice of each sample was put in a separate sample bottle with label and fixed in a 10% formal saline for 48 hours. Thereafter, the sample was processed by placing in ascending grades of alcohol viz: 70%, (1 hour), 95% twice (1 hour each), absolute alcohol twice (1 hour, 30 min each) and another absolute alcohol for 2 hours. The dehydrated sample was transferred to a mixture of equal volumes (50/50) of alcohol and xylene and kept overnight; then cleaned in two changes of xylene for 1 hour each. Paraffin wax infiltration was carried out with two changes of paraffin wax at 1 hour interval each in an electric hot air oven to ensure proper dehydration of the tissues. The dehydrated sample was transferred to a mixture of equal volumes of alcohol and xylene and leaving overnight; clearing with two changes of xylene for 1 hour each. Molten paraffin wax infiltration was carried out in the oven at 60°C. The sample was then embedded in paraffin

wax, trimmed and mounted on a wooden chuck and sectioned in a microtome at 5 µm thickness and floated on a floatation bath. The floated sections were picked - up with clean albumenized microscopic slides and placed in a staining dish. The sample was re-hydrated in descending grades of alcohol (95%, 70%). The sample was stained with Ehrlich haematoxylin, differentiated in 10% alcohol and blued in running water for 15 minutes. The slide was counter- stained with eosin for 2 minutes. Excess eosin was removed in ascending grades of alcohol (75%, 95%) and then absolute alcohol before cleaning in two changes of xylene. Each slide was placed on an optical microscope attached to a digital camera (Motic Image Camera - Moti-Cam) which was connected to a laptop. Cross-sections of seminiferous tubules from each slide were randomly selected and their images captured at two levels of magnification (X100 and X400), and the latter with oil immersion.

Data analyses

Data collected on semen quality parameters were subjected to one – way analyses of variance using SPSS. Significantly different means were separated using Tukey's test at 0.5% level of probability.

Results and discussion

Table 2: Semen quality parameters of Noiler breeder cocks fed diets supplemented with turmeric powder

Parameters	T_1 (0 g/kg)	T_2 (2 g/kg)	T_3 (5 g/kg)	T_4 (6 g/kg)
Semen pH (scale:1 - 14)	6.85±0.02^{b}	6.85±0.04^{b}	6.98±0.01^{b}	7.34±0.05^{a}
Semen volume (ml)	0.41±0.02^{b}	0.46±0.02^{ab}	0.46±0.02^{ab}	0.50±0.02^{a}
Semen color (scale:1 - 2)	1.00±0.00^{b}	2.00±0.00^{a}	2.00±0.00^{a}	1.67±0.21^{a}
Semen consistency (scale:1 - 4)	2.50±0.22^{bc}	4.00±0.00^{a}	3.00±0.00^{b}	2.333±0.21^{c}
Mass motility (%)	71.34±0.71^{b}	83.08±0.95^{a}	80.55±0.62^{a}	67.83±0.52^{c}
Live sperm proportion (%)	81.95±1.31^{c}	93.90±0.30^{a}	90.38±0.59^{b}	75.89±0.95^{d}
Dead sperm proportion (%)	18.22±1.24^{b}	6.06±0.28^{c}	9.63±0.59^{c}	22.46±1.53^{a}
Normal sperm proportion (%)	92.16±0.82^{ab}	95.020±0.51^{a}	93.77±0.81^{a}	89.97±1.32^{b}
Abnormal sperm (%)	7.85±0.82^{ab}	4.98±0.51^{b}	6.21±0.82^{b}	10.03±1.32^{a}
Total viable sperm(x 10^9)	8.456±0.09^{b}	13.77±0.90^{a}	13.09±0.38^{a}	10.14±0.54^{b}
Sperm concentration (x10^6)	3.29±0.02^{b}	3.75±0.06^{a}	3.67±0.05^{a}	3.41±0.07^{b}
Total sperm per ejaculate (x10^{12})	1.31±0.01^{b}	1.76±0.07^{a}	1.700±0.04^{a}	1.65±0.06^{a}
Headless sperm cell (%)	3.51±0.14	3.03±0.28	3.04±0.09	3.43±0.15
Proximal cytoplasmic droplet (%)	0.67±0.16^{ab}	0.17±0.07^{b}	0.19±0.06^{b}	0.98±0.19^{a}
Distal cytoplasmic droplet (%)	0.67±0.45	0.07±0.03	0.15±0.04	0.21±0.05
Tapered head (%)	0.092±0.041^{b}	0.142±0.014^{b}	0.198±0.057^{ab}	0.408±0.19^{a}
Twisted head (%)	2.31±0.21^{ab}	1.38±0.49^{b}	1.44±0.316^{b}	3.33±0.34^{a}
Broken neck (%)	0.49±0.05^{ab}	0.18±0.05^{b}	0.44±0.14^{ab}	0.87±0.27^{a}
Short tail sperm cell (%)	0.20±0.09	0.00±0.00	0.39±0.13	0.47±0.31

$^{a, b, c,}$ Mean ± Standard error in the same row with different superscripts are significantly ($p < 0.05$) different.

The result of semen quality parameters of Noiler breeder cocks fed diets supplemented with turmeric rhizome powder are shown in Table 2. Turmeric supplementation significantly ($p < 0.05$) affected all the parameters measured, except percentage headless sperm cells, percentage distal cytoplasmic droplet and short tailed sperm cells, which did not vary ($p > 0.05$) from the control. Semen pH was significantly higher in the birds fed 6 g/kg turmeric (7.34 ± 0.05) than those fed 0 g/kg (6.85 ± 0.02), 2g/kg (6.85 ± 0.04) and 5 g/kg (7.89 ± 0.01) turmeric. Semen volume was significantly higher in birds fed 6 g/kg turmeric (0.50 ± 0.02mL) and did not differ ($p > 0.05$) from those fed 0 g/kg (0.46 ± 0.02 ml) and 5g/kg (0.46 ± 0.02 ml). Semen colour was better in birds fed 2 g/kg (2.00 ± 0.00), 5g/kg (2.00 ± 0.00) and 6 g/kg turmeric (1.67 ± 0.21), than the control (1.00 ± 0.00). Semen consistency was significantly higher in birds fed 2 g/kg turmeric (4.00 ± 0.00) followed by 5 g/kg (3.00 ± 0.00) and least in birds fed 6 g/kg turmeric (2.33 ± 0.21). Mass motility was

significantly higher in birds fed 2 g/kg (85.08 ± 0.95%) and 5 g/kg (80.55 ± 6.62%) and least in birds fed 6 g/kg turmeric (67.83 ± 0.52%). Percentage live sperm proportion was significantly higher in birds fed 2 g/kg turmeric (93.90 ± 0.30 %) followed by 5 g/kg (90.38 ± 0.59 %) and least in birds fed 6 g/kg (75.89 ± 0.95%). Percentage dead sperm proportion was significantly higher in birds fed 6 g/kg turmeric (22.46 ± 1.53 %) followed by those fed control diet (18.95 ± 1.24 %) and least in birds 2 g/kg (6.06 ± 0.28 %) and 5 g/kg (9.63 ± 0.59 %).

Percentage normal sperm proportion was significantly higher in birds fed 2 g/kg turmeric (95.02 ± 0.51 %) and 5 g/kg (92.77 ± 0.81 %), which did not vary ($p > 0.05$) from the control (92.16 ± 0.82 %) and least in birds 6 g/kg (89.97 ± 1.32 %). Percentage abnormal spermatozoa was significantly higher in birds fed 6g/kg (10.03 ± 1.32 %) which was comparable with the control (7.85 ± 0.82 %) and least in birds fed 2 g/kg (4.98 ± 0.51%) and 5 g/kg (6.21 ± 0.82%). Total viable spermatozoa were significantly higher in birds fed 2 g/kg (8.46 ± 0.09 x 10^9) compared to 0 g/kg (8.46 ± 0.09 x 10^9) and 6 g/kg (10.14 ± 0.54 x 10^9). Sperm concentrations were significantly higher in birds fed 2 g/kg (1.76 ± 0.07 x 10^6) and 5 g/kg (3.67 ± 0.05 x 10^6) turmeric.

Total sperm per ejaculate was significantly higher and similar ($p > 0.05$) in birds fed 2 g/kg (1.76 ± 0.07 x 10^{12}), 5 g/kg (1.70 ± 0.03 x 10^{12}) and 6 g/kg turmeric (1.65 ± 0.06 x 10^{12}) compared to the control (1.31 ± 0.01 x 10^{12}). Percentage headless and short-tailed spermatozoa did not differ ($p > 0.05$) between the control and the treated groups of cocks. Percentage of tapered sperm head was significantly higher are comparable ($p > 0.05$) in birds fed 6 g/kg (0.41 ± 0.10 %) and 5 g/kg (0.20 ± 0.06%) than those fed 0g/kg (0.09 ± 0.4 %) and 2 g/kg (0.14 ± 0.01 %) turmeric. The percentage of spermatozoa with proximal cytoplasmic droplets was significantly higher in cocks fed 6g/kg turmeric (0.98 ± 0.19 %) compared to the other treatments. Percentages of spermatozoa with twisted head and broken neck were significantly higher in birds fed 6 g/kg turmeric (3.33 ± 0.34 %) and 0.87 ± 0.27 %) than the other treatments. The result of this study indicated that turmeric at the supplemental levels used in this study improved testicular function resulting increased semen quality. This increase in semen quality could be attributed to androgenic effect of turmeric on spermatogenesis.

The result of this study is in line with those of Akklaghi *et al.* (2014a), Borghei – Rad *et al.* (2017) and Yan *et al.* (2019) who reported significantly increased semen volume, sperm progressive motility, sperm concentration and viability in broiler roasters fed turmeric rhizome powder. The pH of a semen sample determines the survivability, motility and metabolic rate of spermatozoa (Donoghue and Wishart, 2000). Chicken and turkey semen can tolerate a pH range of 6.0 – 8.0 (Van Wambeke and Huyghebaert, 1989). A low pH reduces motility, metabolic rate, lactic acid production and oxygen uptake, while a high pH increases metabolic rates (Bogdonoff and Schaffner, 1954). The semen pH range recorded in this study 6.85 ± 0.02 – 7.34 ± 0.05 fell within the range 6.92 – 7.06 reported by Iskander *et al.* (2006), Urom *et al.* (2018) and Majid *et al.* (2021) for Arabian Kedu-Lingen, Bangkok and Nigerian local roosters respectively.

However, the semen pH values recorded in this study for the control (6.85 ± 0.02) and the treated groups of cocks fed 2 g/kg, (6.85 ± 0.04) and 5 g/kg turmeric (6.90 ± 0.01) were lower than the values reported for Nigerian local cocks fed control diet (7.06 ± 0.04), 0.25 % (7.11 ± 0.02), 0.50 %, (7.20 ± 0.05) and 0.75 % turmeric (7.38 ± 0.16) diets. Agbalaya *et al.* (2021) also reported mean pH values of 7.70 for FUNAAB Alpha cocks fed control diet and 7.68 for those fed 15 g/kg turmeric supplemented diet. The differences in the pH values could be due to breed variation (Streezek *et al.,* 1995). In this study, the pH increased as the dosage of turmeric increased. Urom *et al.* (2018) also reported this scenario in Nigerian local cocks fed diets with increasing concentrations of turmeric. King and Macpherson (2005) reported that high semen

pH at the point of collection is associated with low semen quality and infections agent. Compared to the control, it appears that 2, 5, and 6 g/kg turmeric increased semen pH, with higher increase at 6 g/kg. This could be the reason for the reduced semen quality of the cocks which, led to the significant decrease in spermatozoamotility recorded in this study.

In this study, semen volume increased with dosage of turmeric. This is in agreement with Urom *et al.* (2018), but disagrees with those of Agbalaya *et al.* (2021) who reported a decrease in the semen volume of FUNAAB Alpha cocks from 0.90 ml in control to 0.53 ml in cocks fed 15g/kg turmeric supplemented diet. The range of semen volume recorded in this study was lower 0.41 ± 0.02 to 0.50 ± 0.02 ml than the range of 0.58 – 0.84 ml reported by Agbalaya *et al.* (2021) for different plumage phenotypes of Noiler breeder cocks raised in South Western part of Nigeria. Although semen volume was higher in the cocks fed 6 g/kg turmeric, its consistency was significantly lower and watery, resulting in lower sperm concentration. Tesfay *et al* (2020) reported a positive correlation between sperm concentration and fertility. Thus, lower sperm concentration could be implicated for the lower fertility. Semen from most males contains some abnormally formed spermatozoa, which may not be associated with low fertility rate until the proportion of abnormal spermatozoa exceeds 20% (Hafez, 1985) and 25% (Bearden *et al.,* 2004). The sperm abnormalities recorded in this study were within the range that may not affect fertility rate (10%). However, the percentage of abnormal spermatozoa was significantly higher at 6 g/kg inclusion level, indicating a negative effect on testicular functions. It therefore appears that 6g/kg turmeric supplementation for as long as 24 weeks could be toxic to the testes of Noiler breeder cocks. This result of this study appears to indicate that 6g/kg daily supplementation of turmeric induces a change from antioxidation to prooxidation of the spermatozoa which, reduced semen consistency, sperm motility, live sperm proportion, and increased spermatozoal abnormality, due to lipid peroxidation by build- up of reactive oxygen species (ROS). The result of this study corroborated those of Chattopadhyay *et al.* (2004) and Sharifi – Rads *et al.* (2020) who reported the ability of curcumin to switch from antioxidant to prooxidant. Histological sections of the testes of cock fed turmeric-supplemented diets are shown in Plates 1-4 below:

Plate 1: Photomicrograph of the testis of Noiler cocks fed 0g/kg turmeric showing seminiferous tubules surrounded by a tunica propria containing Leydig (interstitial) cells (LC), blood and lymphatic vessels, spermatogonia (Sg), Sertoli cells (Sc) recognized by their vesicular nuclei with prominent nucleoli, primary spermatocytes (PS), and late spermatids (LS) near the lumen. H&E, X400.

Plate 2: Photomicrograph of the testis of Noiler cocks fed 2g/kg turmeric showing marked structural integrity characterized by diffuse hyperplasia of the Sertoli and spermatogenic cell and active spermatogenesis H&E, X400.

Figure legend: LC = Leydig cell, blood and lymphatic vessels, Sg = Spermatogonia, SC = Sertoli cells, PS = Primary spermatocytes and LS = late spermatids.

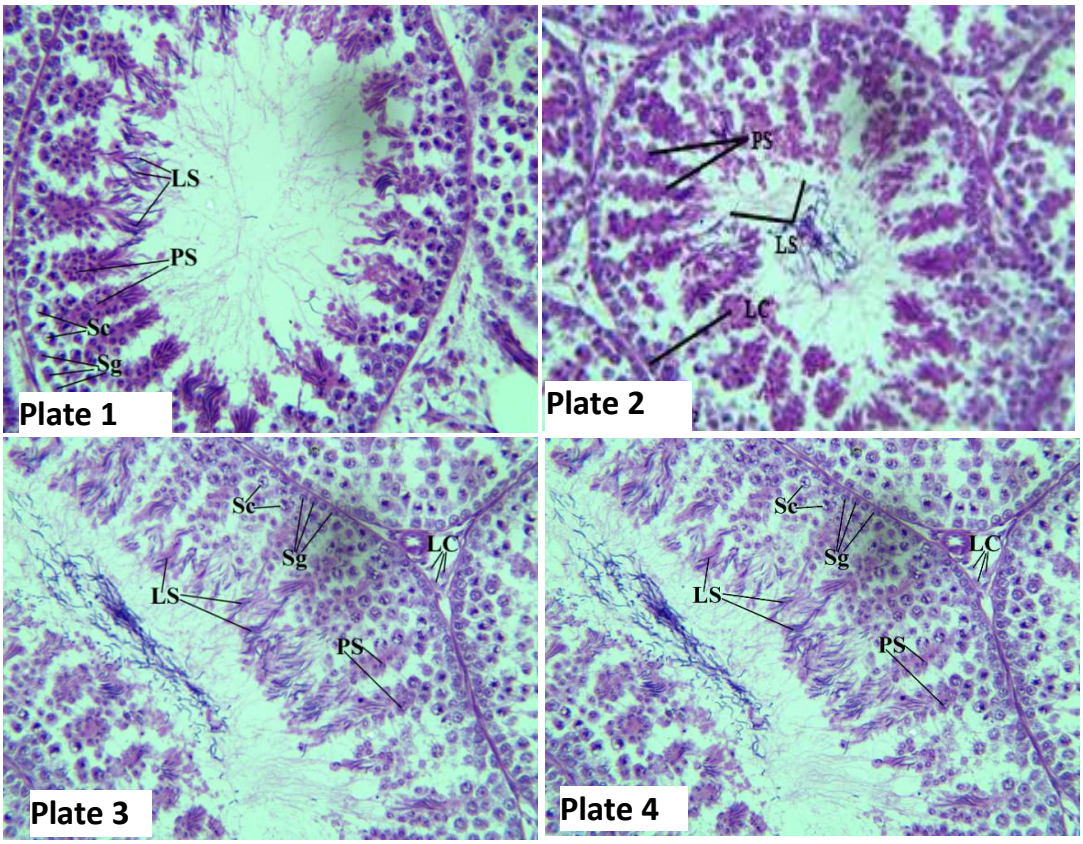
Plate 3: Photomicrograph of the testis of Noiler cocks fed 5g/kg turmeric showing marked structural integrity characterized by diffuse hyperplasia of the Sertoli and spermatogenic cell and active spermatogenesis H&E, X400.

Plate 4: Photomicrograph of the testis of Noiler cocks fed 6g/kg turmeric showing marked structural integrity characterized by diffuse hyperplasia of the Sertoli and spermatogenic cell and active spermatogenesis, mild degeneration of the spermatogenic cell H&E, X400.

The result above appears to indicate that daily intake of 2g and 5g of turmeric/kg feed did not affect testicular ultrastructure and spermatogenesis. The mild degeneration of the spermatogenic cell in seminiferous tubules as observed in the testicular histology of the cocks fed 6g/kg turmeric is an indication toxicity. This could be due to a shift in physiological equilibrium of ROS in the testes by turmeric at

6g/kg. This result is in consonance with those of Fujisawa *et al*. (2002) and Sharifi-Rads *et al*. (2020) who reported that most antioxidants are dose-dependent.

Plates 1-4 show the photomicrograph of the testes of Noiler breeder cocks fed 3g turmeric supplemented diets



Conclusion/ recommendation

Based on the result of this study, it was concluded that 2 g/kg and 5 g/kg daily supplemental levels of turmeric for 24 weeks could be used to improved semen quality indices and testicular structural integrity of Noiler cocks. Daily intake of 6 g/kg turmeric resulted in marked increases in sperm abnormalities, dead sperm proportion, and decreases in sperm motility, semen consistency as well as damage to the testicular ultrastructural integrity, indicating toxicity in the cock. Therefore, turmeric supplementation at 2 g/kg and 5g/kg therefore recommended for Noiler breeder cocks, while 6 g/kg should be given with caution.

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