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Semen and haematological characteristics of cocks drenched varying levels of Turmeric Powder (TP)

Amao, E. A.[1]*, Amao, O. D.[2], Busari, Z. O.[1], Adelegan, T. M.[1], Tiamiyu, W. A.[1] and Yunus, M. O.[1]

[1]Department of Animal Production and Technology, The Oke-Ogun Polytechnic, P.M.B 021 Saki (TOPS), Oyo State, Nigeria.
[2]National biotechnology Development Agency, Bioresources Development Centre, Ilorin, Kwara State, Nigeria.

*Corresponding author. Email: amaoemmanuelayodeleamao@gmail.com; Tel: +234 8059529136.

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ABSTRACT: Emerging evidence from *in vivo* as well as *in vitro* studies indicates that botanicals play noteworthy roles in the treatment, prevention and management of livestock diseases. Use of natural compounds in botanicals has been proposed as potential alternative to conventional therapeutic options. Therefore, this study aimed to evaluate the effect of varying levels of turmeric powder (*Curcuma longa*) on semen characteristics and haematological indices of cocks. Turmeric (*C. longa*) rhizomes were obtained from a local market in Saki in Oyo State, Nigeria, in March 2023. They were washed, skin scraped and air-dried at room temperature for 10 hours, and then oven-dried at 40°C for 12 hours. Afterwards, the dried turmeric rhizomes were ground into powder using a blender. The product was kept in an air-tight container until use. The experimental material was drenched at 0.00 g (T_1), 0.50 g (T_2), 1.00 g (T_3) and 1.50 g (T_4) after 2 weeks of acclimatization. Semen volume, sperm cell progressive motility, sperm cell liveability, acrosome integrity, sperm cell concentration and normal sperm cell were evaluated for semen characteristics. Haematological parameters measured were: PCV, RBC, WBC Hb, MCV, MCH, MCHC and white blood cell differentials. Data obtained were subjected to one-way analysis of variance. Semen volume (0.34 – 0.37 ml), sperm cell progressive motility (68.33 – 80%), sperm cell liveability (46.66 – 85.00%), acrosome integrity (50.00 – 85%) and normal sperm cell (66.66 – 90%) showed significant difference ($p<0.05$) in favour of cocks on highest level (1.50 g) of turmeric powder, while sperm cell concentration (28.33 - 40.00 $X10^9$/ml) showed no significant difference ($p>0.05$). Also PCV (36.00 – 40.33%), RBC (3.55 – 3.74 $X10^6$/ml), WBC (19.01 – 19.71 $X10^9$/ml), Hb (11.66 – 13.00 dl), MCV (100.53 – 109.53 η), MCH (32.57 – 35.31pg) and MCHC (32.00 – 32.37%) showed no significant differences ($p>0.05$) between treatments. In conclusion turmeric powder up to 1.50 g showed an improvement in all the semen characteristics measured without negative effect on haematological characteristics of cocks.

Keywords: Haematological indices, light ecotype cocks, phytogenics, semen characteristics, turmeric rhizomes.

INTRODUCTION

Infertility is one of the major concerns in poultry breeding with roughly 30% of the complications is being male related (Lee *et al*., 2012; Barkhordari *et al*., 2013). Effective production of chicks is reliant on the potency and reproductive performance of the cock, which is determined to a great extent by the excellence of the semen it produces (Ilori *et al*., 2012). The conservation of fertility in breeder cocks over many years has however been difficult especially in the humid tropics (Okoro *et al.,* 2016). In these tropical regions, absolute meteorological factors such as high ambient temperature and high relative humidity (which results in severe heat stress), in addition to other factors such as age, poor nutrition, and management, negatively affect semen production capacity

and quality in cocks (Ayo *et al*., 2011). The chicken spermatozoa are known to own distinctive structural and chemical configurations. Polyunsaturated fatty acids are indispensable constituents of avian spermatozoa and these plays major roles both in maintaining the physical properties and roles of the spermatozoa and in sperm-egg fusion (Khan *et al*., 2011). The high level of polyunsaturated fatty acids presents in the chicken spermatozoa however, predisposes them to per-oxidative impairment and associated spermatozoa dysfunction. Therefore, lipid peroxidation constitutes the chief cause of infertility in males (Zaniboni *et al*., 2006) and its effects can be enhanced by the antioxidant system (Słowinska *et al*., 2011). Naturally, sperm cells are secured from oxidative harm by several enzymatic and non-enzymatic anti-oxidant defence mechanisms. The use of synthetic antioxidants in animal production to improve health, performance, and product quality has been discouraged due to their potential adverse effects (Lin *et al*., 2016). Studies on the use of natural antioxidants of plant derivation (such as flavonoids and other polyphenolic compounds) in animal production have demonstrated improved productivity and enhanced endogenous antioxidant system (Ansari *et al*., 2012; Lee *et al*., 2013). Turmeric *(Curcuma longa)* is a tropical plant native to Southern and South-eastern Asia. The main bio active substances extracted from the rhizomes of *Curcuma longa* are *Curcumin demothoxycurcumin* and *Bisdemethoxy curcumin* which are make-up of 2-5% of the total spice in turmeric. Curcumin is the main important bio-active constituent responsible for the biological activity of turmeric. Curcumin has been shown to have numerous biological effects such as anti – inflammatory and antioxidant effects (Iqbal *et al.*, 2003). Curcumin possesses many beneficial biological activities, e.g., anticancer, anti-inflammatory, antimicrobial, antiviral, antifungal, and antioxidant activities (Manikandan *et al.,* 2004; Aggarwal *et al.,* 2007). Curcumin hunts free oxygen radicals and prevent lipid peroxidation in membranes (Kuhad *et al.,* 2007). It is, therefore, useful for the treatment of many diseases, such as cardiovascular disorders (Ramirez-Tortosa *et al.*, 1999; Manikandan *et al.,* 2004) and reproductive issues (Oguzturk *et al.,* 2012; Głombik *et al.,* 2014). Effects of turmeric powder on sperm motility and viability in roosters are however unclear. Therefore, the present study was conducted to evaluate semen and haematology of cocks as affected by dietary levels of turmeric powder.

MATERIALS AND METHODS

Study area

The experiment was conducted at the poultry unit of the teaching and research farm of The Oke-Ogun Polytechnic, Saki, Oyo State, Nigeria. Saki is located on longitude 30.42 East and latitude 8.41 North. It is about 1,500 meters above sea level. Saki is about 184 Km North – West to Ibadan (The capital of Oyo State) and is the largest town on the boarder of Nigeria with the Republic of Benin.

Procurement and preparation of test ingredient

Turmeric (*C. longa*) was obtained from Sango market in Saki in Oyo State, Nigeria, in March 2023. The rhizomes were washed, its skin scraped and air-dried at room temperature for about 10 hours. and further oven-dried at 40°C for 12 hours. Afterwards, the dried turmeric was ground into powder using a blender to form turmeric powder (TP). The product was kept in an air-tight container for used.

Management of experimental animals

A total of 60 sexually matured and apparently healthy light ecotype Nigerian local cocks (weighing between 1.5 and 1.8 kg) were obtained from the Department of Animal Production Technology Teaching and Research Farm, The Oke - Ogun Polytechnic, Saki, were used for the study. The birds were randomly allotted into 4 treatments (T) groups. Birds in T_1 were drenched with 0.00 g TP and this served as control group. Birds in T_2, T_3, and T_4 were drenched with 0.50, 1.00 and 1.50 g TP, respectively. Each treatment had 15 cocks and were replicated 3 times with 5 birds per replicate. The birds were reared and managed intensively in cage housing system and were observed and acclimatized for 2 weeks before the commencement of the study. During the 2 weeks acclimation period, the birds were trained (abdominal massage) for semen collection. Drenching with experimental material commenced at the end of the acclimation period. Commercial feed (growers mash) and water were given to the birds *ad libitum.*

Data collection

Semen collection and evaluation

Semen collection and evaluation were carried out for a period of 8 weeks. Semen was collected by the abdominal massage techniques (Hafez, 1978). Semen collection was done twice a week (Mondays and Thursdays) between 07:0 3 am and 10:00 am. The birds responded to massage by partially averting their cloaca, and semen was collected from the ventral lip of the vents in calibrated tubes maintained at 35°C using insulated jackets. Individual ejaculates were collected into a 4 mL graduated collection tube, and ejaculate volumes were read to the nearest 0.1

mL. Following semen collection, the semen was maintained in a 35°C water bath for sperm motility assessment. The physical semen characteristics were analysed as described previously by Peters *et al*. (2008). For the sperm motility, a drop of semen was placed on a microscope slide using a rubber micropipette and then covered with a glass cover slip to spread the semen uniformly on the slide. The slides were placed under a microscope (x400 magnification) for observation. Several microscopic fields were examined for each sample. Motility was expressed as a percentage of the cells that were motile within the observed fields. Sperm concentration was measured with an improved Neubauer haemocytometer using direct cell count method. The haemocytometer consists of specially designed slides that contains 2 counting chambers and 2 dilution pipettes. The counting chambers are 0.1 mm in depth and have an area of 1.0 mm^2. The squares are further sub-divided into 25 smaller squares. One milliliter (1 ml) of the semen was diluted with 0.9 M normal saline at the rate of 1:250. The cover slip was moistened with water to enable adhesion and then affixed to the haemocytometer. A drop of the diluted semen was placed at both ends of the haemocytometer and allowed to settle. The loaded haemocytometer was then placed under the microscope (x400 magnification) for observation. Cells which have their heads within the subdivided smaller squares at the 4 edges and the centre of the haemocytometer were counted and the average taken for a bird.

The sperm concentration was calculated using the formula: C = 50, 000 × N × D. Where C = concentration of semen per volume (ml), N = Number of spermatozoa counted, D = dilution rate (Uzochukwu *et al*., 2019). Total spermatozoa were calculated as the concentration of sperm cell in the total volume of ejaculate collected from a cock. The sperm viability was determined by placing a drop of semen on a microscope slide with a micropipette, and a drop of eosin-nigrosine stain was added, smeared, immediately air-dried, and viewed under a microscope (×400 magnification). The proportions of live (eosin-impermeable) and dead (eosin-permeable) spermatozoa in a sample were assessed on the basis of 200 counted cells. Percentage (%) normal cells were determined as the percentage of cells with intact and normal morphological features (head and tail).

Blood collection and haematology

Haematological indices such as Red Blood Cell Count (RBC), Packed Cell Volume (PCV), White Blood Cell Count (WBC), Haemoglobin Concentration, and WBC differentials were determined. Haemoglobin concentration was determined Spectrophoto-metrically using the cyano-haemoglobin method (Elarabany, 2018), while PCV was determined by Hacksley hematocrit centrifuge (UK) and micro haematocrit reader according to the procedure by Morris *et al*. (2001). The WBC counts and its differentials were determined using the Neubaer counting chamber following procedure described in previous studies (Fudge, 2000; Cray and Zaias, 2004).

Statistical analysis

At the end of the trial, data were analysed in accordance with one-way analysis of variance (ANOVA) in completely randomized design (CRD) using SAS (1999) computer analytical package and means were separated with Duncan multiple range test of the same software.

RESULTS AND DISCUSSION

Table 1 shows the result of semen characteristics of cocks drenched with varying levels of turmeric powder (TP). There existed significant differences ($p<0.05$) across the treatment groups with semen volume ranging from 0.34 ml in the control to 0.37 ml for T_4 (1.50 g TP). The result shows a progressive increase in the semen volume with the increasing levels of turmeric powder. The result deviates from the report of Uzochukwu *et al*. (2019) who reported a decrease in semen volume of cocks with increased level of Ethiopian pepper fruit meal, though the range obtained (0.34 - 0.37ml) in this work is greater than the range (0.18 - 0.34 ml) reported by Uzochukwu *et al*. (2019). Sperm cell progressive motility showed significant difference ($p<0.05$) with 0.50 g TP having the lowest value of 68.33%, followed by control (80%) while 1.00 g TP and 1.50 g TP had statistically similar sperm progressive motility value of 83.33% and 91.66%, respectively. This result corroborates the finding of Okoro *et al*. (2016) who reported increase in progressive motility of cocks with increased levels of onions and garlic mixture. Sperm cell liveability showed significant differences across the treatment ($p<0.05$) with values ranging from 46.66 - 85.00%. It showed the same trend as sperm cell motility. Sperm cell liveability showed decreased value (46.66%) in 0.50 g TP while the result of other treatments showed similar result, though the value of control is numerically higher than 1.00 g TP (85%) and 1.50 g TP (76%), nevertheless, 1.00 g TP and 1.50 g TP compete favourably with the control. Acrosome integrity revealed significant differences ($p<0.05$) across the treatments and shows the same trend with sperm liveability. Acrosome integrity value (50%) was lowered in 0.50 g TP and had higher values in 1.00 g TP (85%) and 1.50 g TP (76.66%) that compete favourably with the control. The results showed that the acrosome integrity of spermatozoa from cocks drenched with 1.00 g TP and 1.50 g TP were well protected compared to that of the control. There existed no significant differences in sperm cell concentration ($p>0.05$) between the treatment groups with values ranging from

Table 1. Semen characteristics of cocks drenched with varying levels of turmeric powder.

Parameters	0.00g TP	0.50g TP	1.00g TP	1.50g TP	SEM
Semen volume (ml)	0.34[b]	0.36[a]	0.37[a]	0.37[a]	0.00
Sperm progressive motility (%)	80.00[b]	68.33[c]	83.33[a]	91.66[a]	17.30
Sperm livability (%)	90.00[a]	46.66[b]	85.00[a]	76.66[a]	15.90
Acrosome integrity (%)	83.33[a]	50.00[b]	85.00[a]	76.66[a]	4.71
Sperm concentration (x 10^8 ml^{-1})	40.00	31.33	28.33	33. 33	72.25
Normal sperm cell (%)	83.33[a]	66.66[b]	83.33[a]	90.00[a]	9.38

[a, b, c] means with different superscript within a row are significantly different.

Table 2. Haematological indices of cocks drenched with varying levels of turmeric powder.

Parameters	0.00gTP	0.50gTP	1.00gTP	1.50gTP	SEM
PCV (%)	40.00	40.33	40.33	36.00	2.98
RBC (X10^6/ml)	3.74	3.68	3.67	3.55	0.07
WBC (X10^9/ml)	19.10	19.71	19.48	19.01	8.28
HB (dl)	12.86	12.93	13.00	11.66	0.99
MCV (η)	106.95	109.45	109.53	100.53	5.66
MCH (pg)	34.40	35.09	35.31	32.57	1.90
MCHC (%)	32.16	32.00	32.24	32.37	0.01
L(%)	70.66	70.66	69.33	66.33	2.50
N (%)	23.00	23.00	24.33	26.66	2.11
M (%)	3.66	2.00	2.00	3.00	0.51
E(%)	2.33[b]	4.00[a]	4.33[a]	4.00[a]	0.36
B(%)	0.66	0.66	0.00	0.00	0.01

[a, b, c] means with different superscript within a row are significantly different. **Key:** PVC – Packed Cell Volume, RBC – Red Blood Cell, WBC – White Blood Cell, MCV –Mean corpuscular volume, MCH - Mean corpuscular haemoglobin, MCHC- Mean corpuscular haemoglobin concentration, Hb – Determination of Hemoglobin, L – Lymphocytes, N – Neutrophils, M – Monocytes, E – Eosinophil, B – Basophi.

28.33 - 40.00 (X10^9/ml), since the result is statistically similar, turmeric powder had no detrimental effect on sperm cell concentration. This result deviates from the report of Okoro *et al*. (2016) who observed significant difference in sperm cell concentration of cocks fed mixture of onion and garlic. Normal sperm cell was higher in 1.00 g TP (83.33%) and 1.50 g TP (90%) were statistically similar with the control while normal sperm cell was lowered in 0.50 g TP (66.66%).

Table 2 shows the results for the haematological evaluation of cocks drenched with varying level of turmeric powder. PCV values showed no significant difference (p>0.05) across the treatments. The value of PCV ranged from 36.00 - 40.33%, which fall within the normal physiological value of 35.9 - 41.00% (Chicken Vet, 2023). This could mean that turmeric rhizome powder could be drenched in cocks up to 1.50 g without any detrimental effect on their packed cell volume. This finding is in agreement with that of Daramola *et al*. (2020), who reported that turmeric had no effect on broiler birds fed at the rate of 0%, 0.5% and 1.0% for eight (8) weeks and Emadi *et al*. (2007) who also reported that addition of turmeric had no effect on PCV level of broiler birds measured at day-21 and day-42. Turmeric powder had no significant effect on RBC across the treatment (p>0.05) with value ranging from 3.55 - 3.74 X10^6/ml, though lower than the recommended physiological range of 4.21 - 4.84 X10^6/ml reported by Chicken Vet (2023). Turmeric powder had no negative effect on RBC of cocks up to 1.50 g. The WBC showed no significant differences (p>0.05) with values ranging from 19.01 - 19.71 (X10^9/ml) which is higher than the value reported by Jiddah *et al*. (2023) who reported 7.55 - 9.15 X10^6/ml. This finding strongly agrees with the study by Daramola *et al*. (2020) and Eko *et al*. (2020) who reported that the WBC values of broilers fed diets with turmeric level of 1.5%, 3.0% and 4.5% were statistically not significantly different (p>0.05). All the white blood cell differentials showed no significant differences (p>0.05) between treatments, except the eosinophil that had higher values (4.00%, 4.33% and 4.00%) in birds drenched with 0.50, 1.00 and 1.50 g TP respectively . The MCV showed no significant differences (p>0.05) with value ranging from 100.5 η (1.50 g TP) -109.53 η (1.00 g TP) which is higher than (81.6 - 89.1 η) reported by Chicken

Vet (2023). The MCH showed no significant differences (p>0.05) with value ranging from 32.57 pg (1.50 g TP) - 35.09 pg (0.50 g TP) which is higher than the reported values (27.2 - 28.9 pg) by Chicken Vet (2023). The MCHC also showed no significant difference (p>0.05) with value ranging from 32.00% (0.50 g TP) - 32.37% (1.50 g TP) and is within the range of 32.41 - 33.37% reported by Chicken Vet (2023).

Conclusion and Recommendation

From the results of this work, it was observed that drenching cocks with turmeric powder (*Curcuma longa*) up to 1.50 g as supplement, had detrimental effect on the haematology of the cocks, also there is an improvement in semen characteristics such as semen volume, sperm cell liveability, acrosome integrity and normal sperm cells. It was therefore suggested that further research is needed with more emphasis on large sample size and higher turmeric powder levels to better understand the effect of turmeric powder on cock semen characteristics and their haematological indices.

CONFLICT OF INTEREST

The authors declare that they have no conflict interest.

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