NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 49 Number 2, October 2018. Pp. 205-210
Available online at: http://www.ajol.info/index.php/naj

SEMEN CHARACTERISTICS AND SEMINAL PLASMA OF NIGERIAN INDEGENOUS COCKS FED DIET WITH GRADED LEVELS OF TUMERIC (*Cucurma longa*)

Urom, S.M.O.C., Inyang E.C., Abu B.E. and Onunkwo D.N.
College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
*Corresponding Author's email: scholtica@gmail.com

Abstract
This study was conducted to evaluate Semen characteristics and seminal plasma of Nigerian indigenous chicken (cock) fed diet with graded levels of Turmeric Rhizome Powder (TRP). One hundred and twenty (120) adult local breeding cocks were divided into 4 treatment groups with 30 birds per group; each group was further divided into 3 replicates of 10 birds per replicate. The birds in the experimental groups designated as T_1, T_2, T_3, and T_4 were fed with TRP supplemented diet at various inclusion levels of, 0.0%, 0.25%, 0.50 %, and 0.75% respectively for 8 weeks. Semen samples were collected using abdominal massage technique from all the experimental groups and analyzed. Parameters measured were; semen volume, colour, consistency, mass motility sperm cell viability (live proportion), sperm concentration, abnormal sperm proportion, and sperm morphology, While the Seminal plasma measured consisted of protein, fructose, sorbitol, citric acid, inocitol, glycerol, phosphoryl choline, ergothioneine, sodium, potassium, calcium, magnesium and chloride. From the results obtained, the group fed with 0.5 % inclusion had the highest semen volume (0.80 ± 0.06 ml) and were significantly ($p < 0.05$) better than the mean volume of 0.49±0.08 ml in the group without Turmeric powder. The semen consistency, motility, total number of viable spermatozoa, the total number of sperm cell/ejaculate, significantly ($p < 0.05$) improved in the TRP supplemented group and better than the control group. Sperm cell concentration increased from 3.35 ± 0.07 in the control group to the range 4.05 ± 0.18 – 4.75 ± 0.22×10^6/cell in the TRP groups. The TRP did not significantly ($p > 0.05$) affect the spermatozoa morphology in all the treatment groups. To improve their reproductive performance and semen characteristics using TRP, the inclusion is best at 0.25 and 0.5 %.

Keywords: Semen, seminal plasma, tumeric, cocks

Introduction

The productive potential of poultry birds (cocks) is determined to a large extent by the quality of the semen it produces. The assessment of semen quality characteristics of Nigerian local chicken gives excellent indices of its reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs (Peters *et al.*, 2004) .Bioactive plant substances which have been proved to be very effective in animal nutrition may include; the stimulation of appetite and feed intake, improvement of endogenous digestive enzyme secretion, activation of immune response, antibacterial, antiviral and antioxidant actions (Toghyani *et al* .,2010;). Turmeric (*Curcuma longa*) is a tropical plant native of Southern and South-eastern tropical Asia. The main yellow bio active substances isolated from the rhizomes of curcuma are *Curcumin demothoxycurcumin* and *Bisdemethoxy curcumin* which is present to the extent of 2-5% of the total spice in turmeric. Curcumin is the main important bio-active ingredient responsible for the biological activity of turmeric. Curcumin has been shown to have several biological effects exhibiting anti – inflammatory and antioxidant (Igbal *et al.*, 2003). It is used in gastrointestinal and respiratory disorders. The significant biological properties of turmeric powder makes it a potential substitute for feed antibiotics in livestock diets. A number of studies have been conducted to evaluate its effects on the performance of broiler chickens (Nayaka *et al.*, 2013; Abou-Elkhair *et al.*, 2014). However the results of these studies have been inconsistent keeping in view of the medicinal attributes of *Curcuma longa*, the purpose of the present study was to evaluate the effects of graded levels of Turmeric Rhizome Powder (TRP) on semen characteristic and, seminal plasma biochemistry of Nigerian indigenous cocks.

Materials and Methods

Experimental Site

The research was carried out in the Poultry Unit of the University Teaching and Research Farm of Michael

Okpara University of Agriculture, Umudike (MOUAU), Abia State in the South Eastern Nigeria. Umudike falls within Lattitude 5° 28' North and Longitude 07° 35' East and lies at an altitude of 112m above sea level. The location has an annual precipitation rainfall of 177-2,000cm per annum, (April–October) and a short period of dry season (November-March) with a relative humidity of about 50-90% and monthly temperature range of 17°C - 36°C. (Meteorological Station- NRCRI, Umudike, 2017).

Sourcing and Processing of Test Ingredient

The harvested rhizomes of turmeric plant used for this work was obtained from and identified by Genetic resource unit of National Root Crop Research Institute, Umudike. The rhizomes were carefully washed with clean water, peeled, air-dried and oven-dried at a temperature of 65°C. The dried rhizomes were polished to remove the rough surface by handpicking and finally milled into turmeric rhizome powder meal using hammer mill. The meal was then used to formulate the bird's experimental diet containing 0.00 %, 0.25 %, 0.50 %, 0.75% for treatments T_1, T_2, T_3,and T_4, respectively.

Management of Experimental Animals/Design

One hundred and twenty (120) weeks old matured normal feathered local cocks were used for this experiment. The birds were procured from Oriegbe Market in Ebonyi State, Nigeria, quarantined for 2weeks, weighed, and then randomly assigned to the four experimental groups of 30 birds per treatment group. Each treatment group was further divided into 3 replicates of 10 birds per replicate. The levels of Turmeric Rhizome Powder (TRP) that was included in the diet were 0.0%, 0.25%,0.50 %,and 0.75% represented as T_1, T_2, T_3,and T_4 respectively. Treatment 1 (T_1) which contained no (TRP) was used as the control. The birds were raised for 8 weeks in deep litter. Feed and water were supplied *ad-libitum*

Semen Collection / Semen Evaluation

Abdominal massage technique was used during semen collection. Evaluation of semen commenced immediately after semen collection per cock. Both macroscopic and microscopic evaluation were carried out. A graduated centrifugal tube (10ml) was used to collect the semen from which semen colour was taken, a pH paper (ROTA® MEB) was used in recording the pH. Sperm concentration, dead sperm, live sperm percentage, motility semen colour, and semen pH were done , semen volume, colour and consistency were evaluated macroscopically and recorded, while Seminal plasma consisting of protein, fructose, sorbitol, citric acid, inocitol, glycerol, phosphoryl choline, ergothioneine, sodium, potassium, calcium, magnesium and chloride were analysed . Seminal plasma was separated from the semen concentration at 3500g ×10 min and stored at 20°C before analysis.

Statistical Analysis

Data collected in all the parameters measured were subjected to Analysis of Variance (ANOVA) in a Completely Randomized Design (CRD) as outlined by Steel and Torrie, (1980). The treatment means were separated using Duncan's New Multiple Range Test at 5% probability as described by Obi, (1990).

Experimental Design

The design of the study was Completely Randomized Design (CRD). The statistical model is expressed thus;

Yij = μ+ Gi+eij

where:

Yij = Single observation,

μ = Treatment means,

Gi = Effect of the treatment (I=1, 2, 3, & 4), and

Eij = Random error.

Results and Discussion

The effect of tumeric on semen characteristics shows that apart from the percentage normal spermatozoa, which showed no significant ($p > 0.05$) difference, every other semen parameters evaluated were significantly ($p < 0.05$) improved in the turmeric groups compared with the control (non-turmeric) group (Table 2). The turmeric significantly ($p < 0.05$) increased the mean semen volume from 0.49ml in the control group to 0.80ml in the 0.50% inclusion group which is an equivalent of 63% increase as reported by Peters *et al*., (2008), reported ranges of semen volume of 0.34 – 0.59ml, 0.40 – 0.73ml, 0.28 – 0.61ml and 0.55ml were given in T1, T2,T3 and T4 respectively were higher than reported value of 0.28ml reported by Bah *et al*., (2001) for breeding cocks of the Sahel but compared favourably with 0.7ml documented by Tuncer *et al*., (2006) for Denizli cocks, the range of 0.37-0.73ml was reported by Peters *et al*., (2008b) for the same Nigerian indigenous strains in the humid tropics. The observed range of 0.64 – 0.80ml in the turmeric group obtained in this study compared favourably with the reported values of the above researchers and within the acceptable normal range of 0.25 – 0.80 ml in breeder cocks (Lao *et al.,* 2006). The mean semen volume from this study compared favorably with that from both exotic and other indigenous cocks of the tropics Semen from the treatment groups were significantly creamy white compared with the milky white semen obtained from the control cocks. There is a direct relationship between semen colour and sperm concentration. Semen with creamy white colour has greater number of spermatozoa than milky white semen (Peter *et al.,* 2002). The turmeric at the various inclusion levels

significantly ($p < 0.05$) increased the thickness (consistency) of the semen compared with the control group.

The mean pH obtained in this study compared with the normal range of 7.10 – 7.40 reported by Peters *et al.,* (2008) for Nigerian/indigenous breeder cock. This means that the turmeric powder even at the highest inclusion did not adversely increase the semen pH. The spermatozoa mass motility result showed that the turmeric powder at the various inclusions, significantly enhanced the progressive mass motility of the spermatozoa in the treated birds compared with the untreated group and this increase in mass motility could be attributed to the effect of turmeric supplemented diet. The highest percentage motility of 81.83 ± 1.99 was observed in 0.50% inclusion group although, not significantly ($p > 0.05$) different from other inclusion groups. This increased spermatozoa motility recorded in 0.50% inclusion group could be as a result of increased glucose concentration (155.67 ± 8.68 mg/dl,) obtained in that inclusion group compared to other groups. The result of the mass motility compared shows that turmeric at the various inclusion levels significantly ($p < 0.05$) increased the thickness (consistency) of the semen compared with the control group. The mean pH obtained in this study compared with the normal range of 7.10 – 7.40 reported by Peters *et al.,* (2008); for Nigerian/indigenous breeder cock. This means that the turmeric powder even at the highest inclusion did not adversely increase the semen pH. The spermatozoa mass motility result showed that the turmeric powder at the various inclusions significantly enhanced the progressive mass motility of the spermatozoa in the treated birds compared with the untreated group and this increase in mass motility could be attributed to the effect of turmeric supplemented diet. The highest percentage motility of 81.83 ± 1.99 was observed in 0.50% inclusion group although, not significantly ($p > 0.05$) different from other inclusion groups. This increased spermatozoa motility recorded in 0.50% inclusion group could be as a result of increased glucose concentration (155.67 ± 8.68 mg/dl,) obtained in that inclusion group compared to other groups. The result of the mass motility compared favourably with 83.5% and within the range of 70% - 87.35% reported by Peters *et al.,* (2008) but higher than the 73% reported by Tabatabaei *et al.,* (2010), from turmeric treated indigenous cock breeders. The live proportion of the spermatozoa was significantly ($p < 0.05$) improved in the turmeric supplemented groups compared with the control. The significant ($p < 0.05$) increase recorded in the total number of viable spermatozoa, sperm concentration and the total number of sperm cell/ejaculate in this study could be attributed to the effect of turmeric supplemented diet at 0.25%, 0.50% and 0.75% inclusion compared with the 0.00% turmeric (control) group.The significant value of the sperm concentration, ranged 4.05 to 4.75 $\times 10^9$/cell obtained in turmeric groups was comparable to the normal range of 3.40 – 9.70$\times 10^9$/cell reported by Bilicik *et al.,* (2005), and above the range of 2.17 – 3.14$\times 10^9$/cell, and a mean of 2.26 $\times 10^9$/cell reported by Bah *et al.,* (2011), respectively on Nigerian local breeder cock treated with turmeric supplement. This significant increase in the sperm concentration is in consonant with the creamy colour of the semen obtained in from the turmeric groups.

Spermatozoa abnormalities reveals disturbances that occurs during spermatogenesis and this usually could be linked to age of animal, type of nutrition and pollution from the environment (Bah *et al.,* 2001). The result as presented in Table 3 showed no significant ($p > 0.05$) difference in most of the sperm morphological parameters as observed in the stained slides. The percentage defects was however, significantly ($p < 0.05$) more in the tail and mid-piece regions than in other parts of the spermatozoon. The higher percentage of mid-piece defect (0.41 ± 0.04%) recorded in 0.75% inclusion group did not significantly ($p > 0.05$) differ from T3 but differs significantly ($p < 0.05$) from all other treatment groups. The result also shows that the percentage normal sperm cell of the turmeric supplemented groups showed no significant ($p > 0.05$) difference compared with the control. This implies that the turmeric at the various inclusions do not have any deleterious effect on the sperm morphology.

The seminal plasma biochemical parameters evaluated were significantly ($p<0.05$) affected by the turmeric supplemented diet (Table 4). The seminal total cholesterol was significantly ($p<0.05$) decreased in the 0.25 and 0.50 % groups, but was significantly elevated in the 0.75 % group compared with the control groups. Studies have shown that elevated seminal total cholesterol decreases the degree of membrane cohesion and permeability thereby decreasing semen out-put, fertility and the motility of spermatozoa (Al-Daraji *et al.,* 2011). This may explain the low performance of the semen obtained from 0.75 % group. This study strongly agreed with the observation made by Al-Daraji *et al.,* (2011) who reported that increased cholesterol level in seminal plasma may inhibit fertilization by inhibiting membrane fusion during acrosome reaction as a result of its incorporation into lipid bilayer. The seminal albumin, glucose. Potassium, calcium, and chloride were significantly ($p<0.05$) higher in the turmeric groups compared with the control group. There is a strong

relationship between semen characteristics and seminal plasma biochemical parameters (Karaca *et al*., 2000b).The improvement in the semen output more importantly the semen concentration and sperm motility recorded in the turmeric groups especially at 0.25 % and 0.50 % inclusions observed from this study could be attributed to the significant increase in seminal plasma albumin, glucose, Calcium, potassium, and chloride ions. This is in agreement with the report that Ca++, and glucose is very important to spermatozoa flagella while its interruption impede viability and fertility. Activation of motility also depends on both intra and extra cellular Ca++, and extracellular Na+ and K+.., glucose is also essential for immunoprotein and early gamete maturation and interactions also by providing energy as a substrate for the production of ATP (Peter,et al., 2002).

Conclusion

The reproductive potential of poultry birds (cocks) is often determined to a large extent by the quality of the semen they produce. Evaluation of semen quality and quantity of domestic fowls have been studied extensively (Bah *et al*., 2001; Tuncer *et al*., 2006; Peters *et al.*, 2008) in indigenous tropical chicken breeds, and it has been reported to be an excellent indicator of reproductive potentials and a major determinant of fertility and subsequent hatchability of eggs (Peters *et al*., 2004). The semen characteristic of Nigerian indigenous cocks fed with Turmeric powder supplemented diet as observed in this study compared favorably with exotic breeds such as Punjab Brown, This is an indication that Turmeric powder has a significant propensity to enhance the reproductive performance of our indigenous cock semen. To improve their reproductive performance and semen characteristics using TRP, the inclusion is best at 0.25 and 0.5 %.

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Table 1: Experimental Diet Level (%) of Turmeric Rhizome Powder

Ingredient	**T_1**	**Quality (kg/100kg)**	**T2**	**T3**	**T4**
Maize	45		42.50	40.00	38.50
Soya bean	7.5		7.5	7.5	7.5
Wheat offal	12		12	12	12
Turmeric	0		0.25	0.50	0.75
Bone meal	2.5		2.50	2.5	2.5
Palm kernel cake	12		12	12	12
Groundnut cake	15		15	15	15
Premix	0.25		0.25	0.25	0.25
Toxin Binder	0.15		0.15	0.15	0.15
Salt	0.3		0.3	0.3	0.3
Methionine	0.20		0.20	0.20	0.20
Lysine	0.10		0.10	0.10	0.10
Total	100		100	100	100
Crude protein	17.06		17.04	17.04	17.04
ME (kcal/kg)	3,210		3210	3180	3182

Table 2: Effect of Turmeric Powder on Semen Characteristics

	Treatment (Turmeric)			
	T_1 0.00%	**T_2 0.25%**	**T_3 0.50%**	**T_4 0.75%**
Parameters				
Semen volume (ml)	0.49 ± 0.08^{b}	0.73 ± 0.06^{ab}	0.80 ± 0.06^{a}	0.64 ± 0.08^{ab}
Semen colour (Score: 1-2)	1.00 ± 0.00^{b}	2.00 ± 0.00^{a}	2.00 ± 0.00^{a}	2.00 ± 0.00^{a}
Semen consistency (Score: 1-4)	3.00 ± 0.16^{b}	4.00 ± 0.00^{a}	4.00 ± 0.00^{a}	4.00 ± 0.00^{a}
Semen pH (Scale: 1 – 14)	7.06 ± 0.04^{c}	7.11 ± 0.02^{bc}	7.20 ± 0.05^{b}	7.38 ± 0.16^{a}
Spermatozoa mass motility (%)	58.00 ± 2.17^{b}	79.70 ± 0.25^{a}	81.83 ± 1.99^{a}	76.13 ± 2.99^{a}
Spermatozoa live proportion (%)	73.38 ± 2.43^{b}	83.61 ± 0.50^{a}	87.35 ± 3.44^{a}	84.03 ± 2.73^{a}
Sperm Cell Concentration ($\times 10^9$/cell)	3.35 ± 0.07^{c}	4.75 ± 0.22^{a}	4.51 ± 0.07^{ab}	4.05 ± 0.18^{b}
Total no. of sperm cell/ejaculate($\times 10^9$/ml)	1.64 ± 0.29^{b}	3.47 ± 0.15^{a}	3.63 ± 0.25^{a}	2.66 ± 0.44^{a}
Total viable spermatozoa ($\times 10^{12}$/ml)	8.94 ± 1.91^{c}	26.57 ± 1.07^{ab}	27.90 ± 2.36^{a}	18.81 ± 3.75^{b}
Percentage Normal Sperm Cell (%)	92.34 ± 1.96	96.02 ± 1.03	93.70 ± 1.25	92.08 ± 2.38

Note: Values are presented as means ± S.E.M, where a, b, and c represent significant differences. Semen score: 1= milky white, 2= creamy white. Semen consistency score: 3=slightly thick, 4= very thick. Values of $p<0.05$ is considered significant

Table 3: Effect of Turmeric Powder on Differential Abnormalities

	Treatment (Turmeric)			
	T_1 0.00%	**T_2 0.25%**	**T_3 0.50%**	**T_4 0.75%**
Parameters				
Headless spermatozoa (%)	0.76 ± 0.21	1.37 ± 0.35	2.14 ± 0.58	2.41 ± 1.24
Double head spermatozoa (%)	0.00 ± 0.00	0.06 ± 0.06	0.13 ± 0.13	0.06 ± 0.03
Twisted tail spermatozoa (%)	3.67 ± 0.63	2.22 ± 0.77	3.40 ± 0.69	4.60 ± 1.27
Tailless spermatozoa (%)	1.74 ± 0.93^{a}	0.00 ± 0.00^{b}	0.34 ± 0.28^{ab}	0.23 ± 0.08^{ab}
Broken neck spermatozoa (%)	0.28 ± 0.28	0.21 ± 0.05	0.04 ± 0.04	0.18 ± 0.18
Presence of Cytoplasmic droplet (%)	1.06 ± 0.80	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Bent mid-piece sperm cell (%)	0.13 ± 0.88^{b}	0.10 ± 0.10^{b}	0.23 ± 0.04^{ab}	0.41 ± 0.04^{a}
Total abnormality (%)	7.65 ± 1.96	3.97 ± 1.03	6.30 ± 1.25	7.91 ± 2.38

Note: Values are presented as means ± S.E.M, where a, & b represents significant differences. Values of $p < 0.05$ is considered significant

Table 4: Effect of turmeric supplemented diet on seminal plasma biochemical parameters

Treatment	**T_1 0.00%**	**T_2 0.25%**	**T_3 0.50%**	**T_4 0.75%**
Total protein	1.44 ± 0.04^{c}	1.64 ± 0.05^{ab}	1.73 ± 0.06^{a}	1.54 ± 0.05^{bc}
Albumin	0.55 ± 0.01^{b}	0.69 ± 0.00^{a}	0.74 ± 0.02^{a}	0.59 ± 0.04^{a}
Glucose	152.45 ± 4.05^{c}	190.53 ± 6.40^{a}	196.39 ± 5.46^{a}	172.60 ± 4.24^{b}
Total cholesterol	56.75 ± 0.71^{b}	53.03 ± 0.66^{c}	50.21 ± 0.63^{d}	60.36 ± 0.75^{a}
Potassium	15.66 ± 0.03^{b}	16.34 ± 0.17^{a}	17.06 ± 0.28^{a}	15.53 ± 0.01^{a}
Calcium	6.41 ± 0.71^{b}	9.08 ± 0.37^{a}	9.39 ± 0.21^{a}	8.08 ± 0.59^{a}
Chlorine	86.72 ± 2.57^{b}	96.93 ± 1.20^{a}	97.92 ± 0.94^{a}	93.86 ± 1.48^{a}

Note: Values are presented as means ± S.E.M, where a, b and c represent significant differences. Values of $p<0.05$ is considered significant.